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RECONSTRUCTION AND ANALYSIS OF THE NF-κB PATHWAY INTERACTOME: A SYSTEMS BIOLOGY APPROACH

Presentata da **Dott. Paolo Tieri**

Coordinatore Dottorato **Chiar.mo Prof. Sandro Grilli**

Relatore **Chiar.mo Prof. Claudio Franceschi**

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RECONSTRUCTION AND ANALYSIS OF THE NF-κB PATHWAY INTERACTOME: A SYSTEMS BIOLOGY APPROACH

Abstract

Background. One of the phenomena observed in human aging is the progressive increase of a systemic inflammatory state (Sansoni 2008), a condition referred to as "inflammaging" (Franceschi 2000), negatively correlated with longevity (Franceschi 2007). A prominent mediator of inflammation is the transcription factor NF-κB, that acts as key transcriptional regulator of many genes coding for pro-inflammatory cytokines. Many different signaling pathways activated by very diverse stimuli converge on NF-κB, resulting in a regulatory network characterized by high complexity (Perkins 2007). NF-κB signaling has been proposed to be responsible of inflammaging (Salminen 2008). Scope of this analysis is to provide a wider, systemic picture of such intricate signaling and interaction network: the NF-κB pathway interactome.

Methods. The study has been carried out following a workflow for gathering information from literature as well as from several pathway and protein interactions databases, and for integrating and analyzing existing data and the relative reconstructed representations by using the available computational tools (Tieri 2010). Strong manual intervention has been necessarily used to integrate data from multiple sources into mathematically analyzable networks. The reconstruction of the NF-κB interactome

pursued with this approach provides a starting point for a general view of the architecture and for a deeper analysis and understanding of this complex regulatory system (Cevenini 2009).

Results. A “core” and a “wider” NF-κB pathway interactome, consisting of 140 and 3146 proteins respectively, were reconstructed and analyzed through a mathematical, graph-theoretical approach. Among other interesting features, the topological characterization of the interactomes shows that a relevant number of interacting proteins are in turn products of genes that are controlled and regulated in their expression exactly by NF-κB transcription factors. These “feedback loops”, not always well-known, deserve deeper investigation since they may have a role in tuning the response and the output consequent to NF-κB pathway initiation, in regulating the intensity of the response, or its homeostasis and balance in order to make the functioning of such critical system more robust and reliable. This integrated view allows to shed light on the functional structure and on some of the crucial nodes of thet NF-κB transcription factors interactome.

Conclusion. Framing structure and dynamics of the NF-κB interactome into a wider, systemic picture would be a significant step toward a better understanding of how NF-κB globally regulates diverse gene programs and phenotypes. This study represents a step towards a more complete and integrated view of the NF-κB signaling system.

1. Introduction

1.1. Aging as a complex trait

The study on human aging and longevity has become a very hot topic in the last years because of the so-called revolution in demography, which led to the remarkable increase in the number of people over the age of 65 or 80 years living in Western countries but also in some emerging countries such as China and India (Franceschi 2008). The data collected during the last 20 years in different models suggest that the picture of the aging phenotype is fragmented and above all qualitative and that we are far from an exhaustive and quantitative scenario. This incomplete knowledge comes out from several bias: 1. few studies evaluate many parameters at the same time in the same individual; 2. the collected data are not of high-dimensionality; 3. longitudinal studies, which are the most informative, are scanty. Another factor contributing to the complexity of the problem is that human aging and longevity are complex and multi-factorial traits, generally considered as the result of the combination of environmental factors, genetics, epigenetics and stochasticity, each making variable contributions to the overall phenotype (Salvioli 2006a, De Benedictis 2006, Fraga 2005). It seems that the importance of each component changes with the passing of time: the age of 60 years appears as a discriminatory point after which the role of environmental factors, genetics, epigenetics and also stochasticity increases, contributing to reaching very old ages. The rate of the age-related modification of the importance of each component is difficult to be quantified at present. Moreover, these different components interact with each other, in particular genetics and environment.

The studies on human aging and longevity are further complicated by the fact that human populations are heterogeneous from the point of view of genetic pool, life style, cultural habits, education, economic status and social network. All these components are different from population to population, and each population is characterised by a unique combination of them. This fact renders the studies difficult to compare and the results very often discordant. Finally, all these considerations also apply to gender difference, since gender appears to be a crucial player in the cross-talk between genes, environment and health (Ordovas 2007). The development of effective and realistic strategies for aging intervention, prevention and therapies may be facilitated by this integrated and multi-faced view. Indeed, it has been proposed that the manipulation of both genes and environment at the same time can open up novel possibilities of aging intervention and prevention (Rattan 2007).

1.2. Aging as a mosaic

It is conceivable that longevity could be achieved by different strategies and by different combinations of genetics, epigenetics, environment and stochasticity and the result, i.e. the aging phenotype, is very heterogeneous. Moreover, it is emerging that the multi-factorial process of aging does not occur only at organism level, but it also acts differently in each organ system, organ, tissue and even in each single cell of the body, determining a different aging rate for each of them. In mice it has been observed that different tissues age in a coordinated fashion (Zahn 2007), while in humans this is still to be ascertained. As a consequence of these different aging rates, the aged body could be considered as a mosaic of tissues and organs displaying a different level of senescence, a situation we proposed to indicate as “aging mosaic” (Cevenini 2008), as exemplified in Fig. 1.1. In this figure, we represented the human body as a mosaic of 12 organ systems

(indicated as rectangles) according to Hunter and Borg (Hunter 2003), each of them displaying a different level of senescence (represented by black dots). Thus, even in the same individual, the aging process appears to follow different trajectories in different organs, tissues and cells, which are variably affected and accumulate unrepaired damages at different rates. An example, among many others, is the brain where different regions, such as cortex, hippocampus and cerebellum, show different levels of neurodegeneration and inflammation in the same subject (Mishto 2006, Mishto 2009). In mice, a great heterogeneity in the amount of transcriptional changes with age in different tissues was found (Zahn 2007).

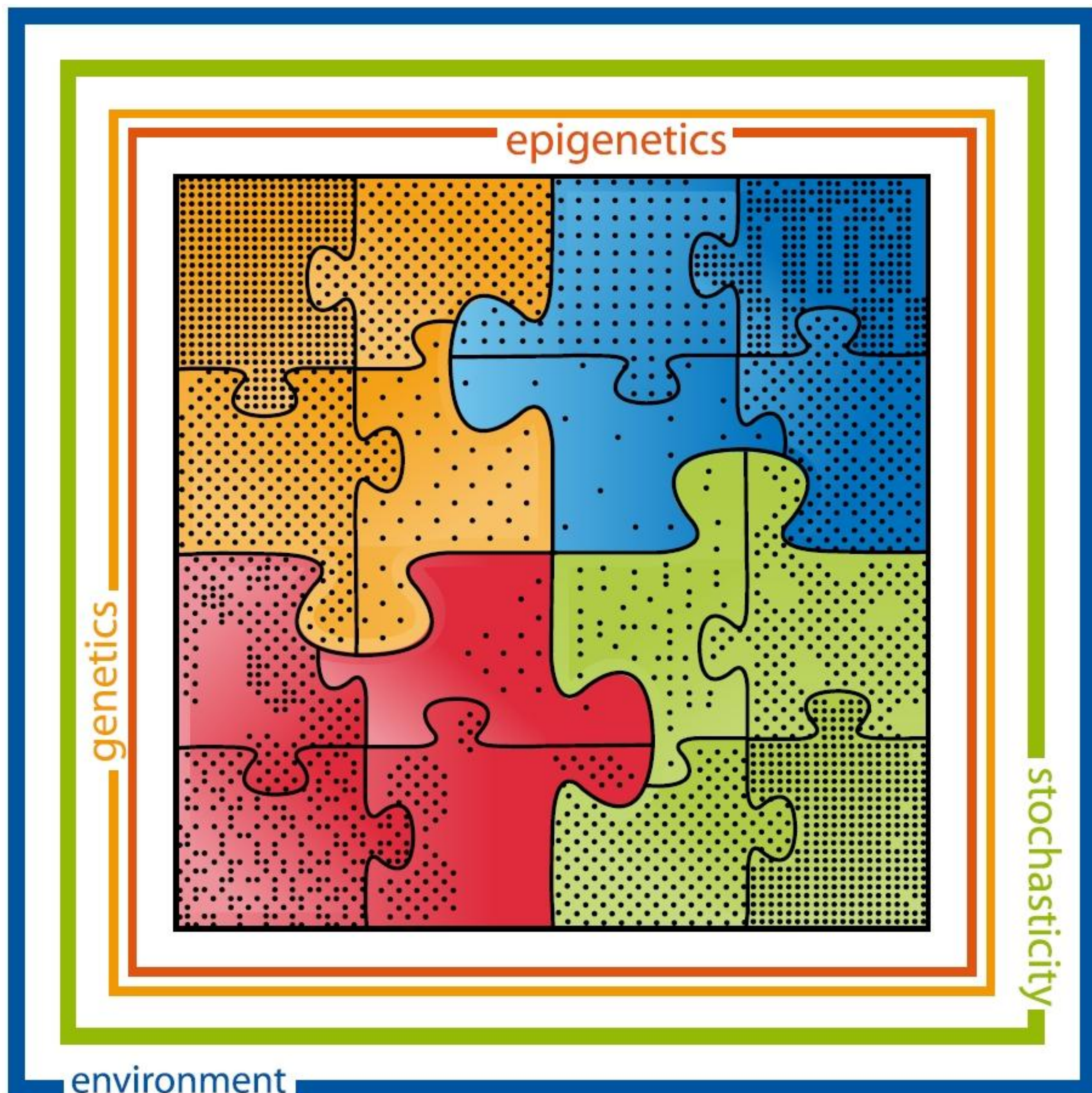


Fig. 1.1 The aging mosaic. The human body is represented as a mosaic of 12 organ systems (indicated as rectangles), each of them displaying a different level of senescence (represented by black dots). Even in the same individual, the aging process appears to follow different trajectories in different organs, tissues and cells, which are variably affected and accumulate unrepaired damages at different rates. Adapted by Cevenini 2009.

Based on the pattern of age-related transcriptional changes, three aging patterns have been proposed: (a) a pattern common to neural tissues, (b) a pattern for vascular tissues, and (c) a pattern for steroid-responsive tissues (Zahn 2007).

Moreover, this mosaic is dynamic, meaning that it changes with time, owing to the complex non linear interactions among the different components, resulting in a final phenotype that is not easily predictable by the study of its single sub-components.

Changes in this mosaic derive from different forms of stressors to which the body is exposed lifelong. The adaptive response to these perturbations can follow two trajectories: 1. in case of exposure to a repeated low grade stimulus, the mosaic is able to positively remodel the mechanisms of maintenance which are up-regulated and the systemic result is the resistance to stress (hormesis); 2. conversely, in case of exposure to a stronger stimulus (over a specific threshold) in terms of intensity and persistence, the remodelling process still occurs, but the mechanisms of maintenance are less efficient and the global result is negative and detrimental for health and survival (Gems 2008). Therefore, a strategy to increase healthy aging and longevity could be to favour the hormetic response by transforming intense and persistent stressors into low-grade ones. In this perspective it is also interesting to note that low-grade stressors can be assimilated to what is referred to as functional or constructive noise. The role of noise in biological systems is under investigation, and, for example, it is now evident that the stochastic or inherently random nature of the biochemical reactions of gene expression may contribute to the phenotypic variability in individuals (Paulsson 2004, Raser 2005). Thus, noise, which was often considered "unimportant" by traditional statistical methods and models, should be taken into account during data analysis, since it may hide un-analyzed information (Kaput 2007). For example, in gene expression analysis, only genes whose expression profile differs from an established threshold were evaluated and the others were classified as "noise" and erroneously excluded (Paulsson 2004). It is also important to note that noise-induced phenomena have been experimentally detected in many levels of biological functionality, i.e. in plankton detection by paddle fish (Russell

1999), in the retrieval processes of the human memory (Usher 2000), and in human brain waves (Mori 2002). How can noise potentially play a constructive role in the system dynamics of main components and compartments of a biological organism? As regards the immunoproteasome (Kloetzel 2001), it has been addressed whether the protein translocation inside the proteasome chamber can be driven by fluctuation, and a toy-model has been derived to show the probable mechanism of translocation (Zaikin 2005). Even if at the moment there is no experimental verification of the proposed hypothesis, however, it can be expected that noise-induced behaviour could play a major role in the immunoproteasome functioning, making this aspect worth of deeper investigation.

Currently, most of the studies focused on human aging still refer to a single district of the mosaic (generally the most easily available tissues, see for example the studies on human diploid fibroblasts, Franceschi 1999, Chondrogianni 2004, among many others). A more comprehensive approach to understand the complexity of this mosaic would be the simultaneous study of several organs at once; at present, this strategy has been applied only to animal models, as reported in mice by Schumacher and co-workers (Schumacher 2008). However it should be taken into account that extrapolation of results from experimental animals (mice) to humans is not always straightforward, mainly due to the quantitative differences in maintenance and reproduction (Demetrius 2006).

Within the complex scenario of such an interaction of different tissues and organs, each of them having intrinsic regulatory mechanisms as well as different aging rates, we proposed to go beyond the simplistic assumption of longevity genes playing the same role all along human life. The interpretation of antagonistic pleiotropy (Williams 1957, Leroi 2005) paved the way for a wider interpretation of genes (and their polymorphisms)

functioning: the reality is even more complex than antagonistic pleiotropy is capable to describe. Several experimental evidences suggest that the role of genes on physiology and pathology can change at different phases of human life. In genetics, these evidences are represented by complex trajectories in the frequencies of functional polymorphisms in population cohorts of different ages (De Benedictis 1998, Invidia 2009). The presence of such trajectories suggests that the same polymorphism can have different biological effects in young, middle-aged and extreme long-living individuals, in a phenomenon named Complex Allele Timing (De Benedictis 2006). It is noteworthy that this conceptualization is supported by the use of demographic information, that, together with data on genetic markers, allowed us to calculate hazard rates, relative risks, and survival functions for candidate genes or genotypes, providing a powerful tool for analyzing their influence on longevity and survival (Yashin 1999). This modelling approach allowed us to predict trajectories in genetic variation frequencies that have been subsequently confirmed by experimental data (Franceschi 2005).

Moreover, the role of epigenetics (DNA methylation, histone post-transcriptional modifications, including methylation, acetylation, ubiquitination and phosphorylation) in the aging process should also be considered within this scenario because it includes regulatory mechanisms that could play a pivotal role in cellular homeostasis, age-related diseases, such as human cancer, as well as lifespan. To this regard, "aging epigenetics" became an emerging discipline (Fraga 2007, Lee 2008). However, no data are available on the epigenetic of human (extreme) longevity and the global DNA methylation and the methylation status of specific candidate loci for longevity is at present lacking, as well as epigenetics applied to population genetics studies.

In this complex scenario, where a unique model for the study of human aging and longevity does not exist, a systems biology approach that combines and quantify

genetics, genomics, proteomics and other *–omics* fields is necessary to handle the ever increasing amount of experimental data made available by new high-throughput technologies. The final aim we are pursuing is to use a systems biology perspective to grasp the complexity of "aging" and "longevity"; in order to have a healthy old age, a systems perspective of medicine could promote the arrangement of advanced personalized therapies specifically aimed, for example, to target the individual patient's genetic defects (Kirkwood 2008, Cevenini 2008, Salvioli 2008b).

1.3. Methodological approaches to aging as a complex mosaic

1.3.1. Systems biology, aging and longevity

Aging can be studied at several levels of increasing complexity: molecular, organelle, cellular, organ, organ systems and organism level. For a long time the cellular level was used as the most integrate level to study aging and longevity, generating a huge amount of data, directly transferred to tissues, organs, organ systems and the whole organism. Despite an enormous literature regarding physiological changes during aging in different organs, only few studies have been performed at organ system and organism level and it is emerging that many findings obtained from studies at cellular level often are not informative about the scenario of higher levels of complexity. Only now scientists are trying to integrate the findings obtained by studies performed at different levels into a coherent framework. In this new perspective, systems biology represents a modern science whose aim is to operate at a systemic level, by using the most integrated models (organ and organism). Thus, it moves biology from a traditional microscopic/qualitative perspective to a macroscopic/quantitative one, allowing researchers to integrate and quantify the huge amount but fragmented data that nowadays can be obtained by high-throughput technologies (Doyle 2006). In addition, it also offers tools to develop

predictive mathematical models and networks (West 2009), able to evaluate and compare potential explanations for biological data (Cedersund 2009). Thus, systems biology represents a strategy to integrate and quantify the existing knowledge and data from different sources into models, to be later tested and then supported with experimental data for validation and refinement, in a recursive process of “wet and dry” experiments, that will be discussed in the last section of this review. The ultimate goal is to “compact” the new acquired knowledge into a single picture, ideally able to characterize the phenotype at systemic/organism level.

High-throughput technologies generate complex and high dimensionality data that need appropriate statistical analysis, such as nonlinear methods (Kaput 2007), and above all a sophisticated interpretative approach in order to get insight on their biological meaning. Visualization techniques, interaction maps and pathway diagrams can be of great value in order to understand how all molecular and cellular components and modules are intertwined and work together to determine the basic structures and the functions of the organism's complex machinery. Among many interesting examples (Jeong 2000, Pieroni 2008, Tieri 2005), this approach has been used by Raza and colleagues (Raza 2008) to reconstruct a logically represented pathway map, integrating four pathways central to macrophage activation (interferon signalling, NF-κB, apoptosis, toll-like receptor pathways), and representing them as one integrated pathway due to their strongly overlapping interactions. These new methods represent a step further, but we are still far from having powerful tools able to capture the complexity of the problem and to reach the ultimate goal, that is combining and quantifying the bulk of available data in specific fields, including multifactorial diseases, such as neurodegenerative disorders (Noorbakhsh 2009, Villoslada 2009), aging and longevity (Salvioli 2008a), vaccinology, preventive medicine, proteolytic systems (proteasome).

In spite of this complex scenario, immunological studies and studies on centenarians revealed that common mechanisms occurred both in very different age-related diseases, such as diabetes and neurodegeneration, and in healthy long-living subjects. One is the persistent, low grade inflammatory process which develops with advancing age called "inflammaging" (Franceschi 2000). Therefore, anti-inflammatory therapies could efficiently contribute to slow down aging and age-associated pathologies, thus increasing the possibility to reach longevity. In a general perspective, the prevention or the postponement of the aging process could have much greater benefit than those targeted at individual disease (Butler 2008). In addition, combining the already known empirical methods of anti-aging medicine with unique genetic profile of each human (gene-pass) may render new awarding opportunities for the further advancements of human longevity programs (Baranov 2007).

1.3.2. Inflammation, inflammaging and systems biology

Inflammation is a systemic physiological process fundamental for survival, which involves a variety of cells, organs and organ systems. Despite the well-recognized systemic character of inflammation, our knowledge of this multi-factorial phenomenon is highly fragmented and its comprehensive understanding is still in its infancy (Salvioli 2006b).

In the aging field, this consideration is particularly important for "inflammaging", (Franceschi 2000, Franceschi 2007b, Franceschi 2007a), which appears to be important in the pathogenesis of cardiovascular diseases (CVD), type 2 diabetes (T2D), metabolic syndrome (MS), neurodegeneration, sarcopenia and cancer, among others.

An age-related increase in the production of inflammatory compounds occurs in the immune system (Vescovini 2007, Larbi 2008), brain (Licastro 2003), adipose tissue (Hotamisligil 2007) and muscle (Barbieri 2003), but the possible contribution to

inflammaging of other organs or districts, such as gut microbiota and liver, is largely unexplored.

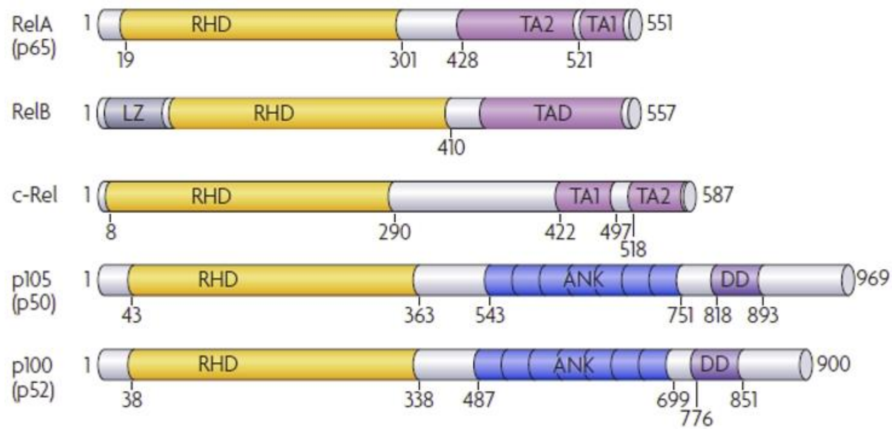
At present a major unsolved problem in biology and medicine is the source(s) of the inflammatory stimuli underpinning and sustaining inflammaging. Moreover, the signaling circuitry and the cross-talk among the various tissues and organs involved in inflammaging are far from being clear. Therefore, systems biology represents the most powerful and comprehensive approach to characterize the systemic nature of inflammaging, for example by developing predictive models of inflammaging, urgently needed to set up strategies for the modulation of inflammation, by acting on strategic targets with global, systemic effect on the whole process.

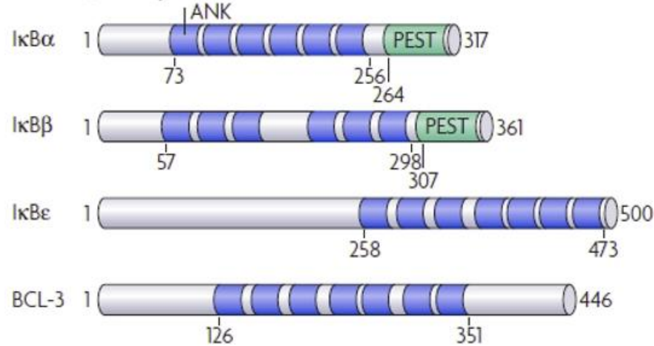
A key point in such a complex framework is the dynamics of signaling pathways crucial to inflammation, such as those related to NF-κB transcription factor activation. NF-κB regulates genes that in turn control cell proliferation, cell survival, as well as innate and adaptive immune response. The sheer complexity of such crucial signalling system is intuitively evident from the intricate network of interactions among input and output signals, mediator proteins and regulated genes. Framing structure and dynamics of the NF-κB interactome in a wider, systemic picture will be a significant step toward a better understanding of how it globally regulates diverse gene programs and phenotypes. This fact gives rise to the necessity of a systemic, multiscale approach able to take into consideration all the crucial levels of complexity into an unique framework, from protein-protein interactions to gene expression, from cellular to tissue response and finally to the dynamics of organs.

1.4. NF-κB: a key player in inflammation

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells, discovered in 1986 by Nobel laureate D. Baltimore) is a protein complex that both induces and represses gene expression by binding to discrete DNA sequences, known as κB elements, in promoters and enhancers. NF-κB is found in almost all animal cell types (Capri 2009) and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens, thus playing a key role in regulating the immune response to infection. Conversely, incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development.

In mammalian cells, there are five NF-κB family members, RelA (p65), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2), and different NF-κB complexes are formed from their homo and heterodimers (fig. 1.2). All proteins of the NF-κB family share a Rel homology domain in their N-terminus. A subfamily of NF-κB proteins, including RelA, RelB, and c-Rel, have a transactivation domain in their C-termini. In contrast, the NF-κB1 and NF-κB2 proteins are synthesized as large precursors, p105, and p100, which undergo processing to generate the mature NF-κB subunits, p50 and p52, respectively. The processing of p105 and p100 is mediated by the ubiquitin/proteasome pathway and involves selective degradation of their C-terminal region containing ankyrin repeats. Whereas the generation of p52 from p100 is a tightly-regulated process, p50 is produced from constitutive processing of p105.





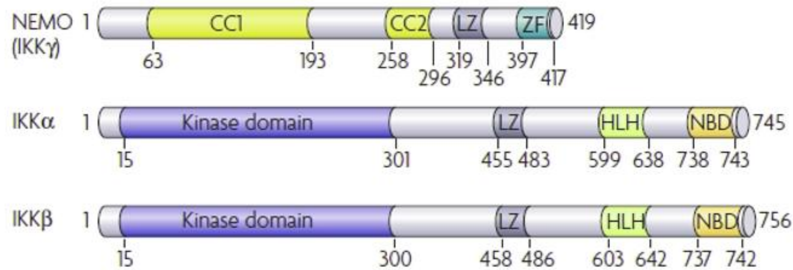


Fig 1.2 The five nuclear factor (NF)-κB family members (a), the inhibitor of NF-κB (IκB) family consists of IκBα, IκBβ, IκBε and BCL-3 (b), and the three core subunits of the IκB kinase (IKK) (c). Adapted by Perkins 2007.

In most cell types, NF-κB complexes are retained in the cytoplasm by a family of inhibitory proteins known as inhibitors of NF-κB (IκBs). Activation of NF-κB typically involves the phosphorylation of IκB by the IκB kinase (IKK) complex, which results in IκB degradation. This releases NF-κB and allows the free translocation in the nucleus.

There are several distinct NF-κB activation pathways (see fig. 1.3). Here there will be briefly summarized. Readers should refer to (3) and cited references for an exhaustive treatise.

The canonical pathway is induced by tumor necrosis factor-α (TNFα), interleukin-1 (IL-1) and many other stimuli, and is dependent on activation of IKKβ. This activation results in the phosphorylation of IκBα, leading to its ubiquitylation (Ub) and subsequent degradation by the 26S proteasome. Release of the NF-κB complex allows it to relocate to the nucleus.

IKK-dependent activation of NF-κB can occur following genotoxic stress. Here, NF-κB essential modifier (NEMO) localizes to the nucleus, where it is sumoylated and then ubiquitylated, in a process that is dependent on the ataxia telangiectasia mutated (ATM) checkpoint kinase. NEMO relocates back to the cytoplasm together with ATM, where activation of IKKβ occurs.

IKK-independent atypical pathways of NF-κB activation have also been observed and described, which include casein kinase-II (CK2) and tyrosine-kinase-dependent pathways.

The non-canonical pathway results in the activation of IKKα by the NF-κB-inducing kinase (NIK), followed by phosphorylation of the p100 NF-κB subunit by IKKα. This results in proteasome-dependent processing of p100 to p52, which can lead to the activation of p52–RelB heterodimers that target distinct κB elements.

Phosphorylation of NF-κB subunits by nuclear kinases, and modification of these subunits by acetylases and phosphatases, can result in transcriptional activation and repression as well as promoter-specific effects. Moreover, cooperative interactions with heterologous transcription factors can target NF-κB complexes to specific promoters,

resulting in the selective activation of gene expression following cellular exposure to distinct stimuli.

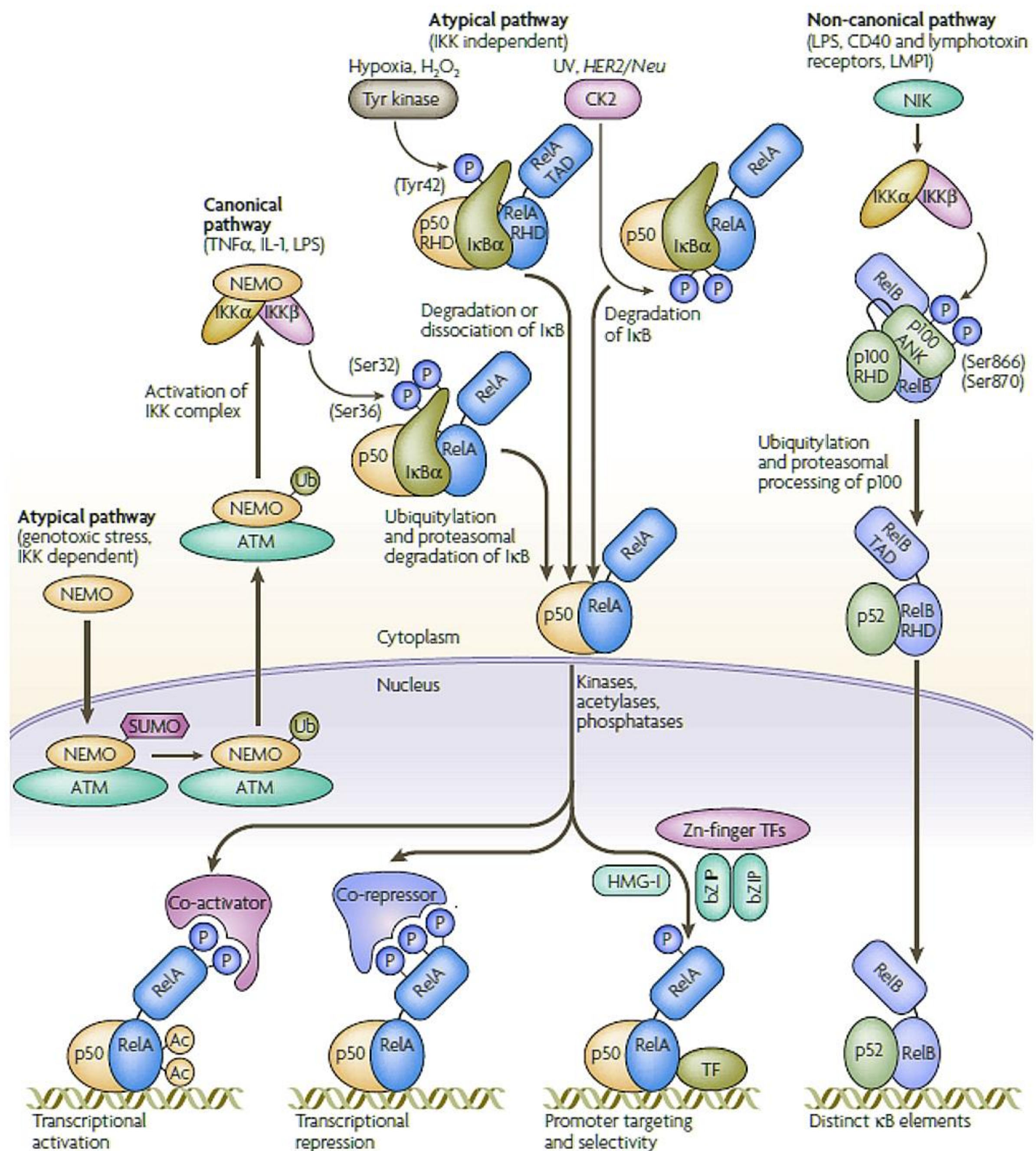


Fig 1.3 Distinct NF-κB activation pathways: canonical, atypical IKK dependent and IKK independent, and non canonical pathways. See text for a description. Legend: Ac, acetylation; bZIP, leucinezipper-containing transcription factor; HMG-I, high-mobility-group protein-I; IκB, inhibitor of κB; IKK, IκB kinase; LMP1, latent membrane protein-1; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; RHD, Rel-homology domain; TAD, transcriptnal activation domain; TF, transcription factor; UV, ultraviolet; Zn-finger TF, zinc-finger-containing transcription factor. Adapted by Perkins 2007.

In vertebrates, NF-κB is induced by over 150 different stimuli (Pahl 1999). In turn, there is evidence that active NF-κB participates in the control of transcription of a number of target genes (already 150 reported by Pahl 1999). These genes express cytokines, chemokines and their modulators, immunoreceptors, proteins involved in antigen presentation, cell adhesion molecules, acute phase proteins, stress response proteins, cell-surface receptors, regulators of apoptosis, growth factors, ligands and their modulators, early response proteins, transcription factors and regulators, and enzymes, among others. Updated information (Gilmore 2010) indicated that NF-κB may regulated the expression of more than 400 genes. These data thus suggest that NF-κB functions more generally as a central regulator of stress responses. In addition, NF-κB activation blocks apoptosis in several cell types. Indicatively, coupling stress responsiveness and anti-apoptotic pathways through the use of a common transcription factor may result in increased cell survival following stress insults.

This non exhaustive description (the reader should refer to the rich and day-by-day growing literature available online for a more complete depiction of the NF-κB system and functioning) briefly illustrates the sheer complexity of the NF-κB pathway system, which, despite the consistent efforts dedicated to its study, has only begun to be investigated. With these premises, NF-κB represents a perfect target for a systems biology study. Here following in this work, one among the first attempts to face its complexity providing a wider picture of the system is presented.

2. Materials and methods

2.1. Network biology: a functional approach to complex biological systems

Graph theory, a branch of mathematics that from its birth, due to Leonhard Euler in the XVIII century, has been recently rejuvenated, is the study of “graphs”, mathematical structures used to model relations between objects. A “graph” in this context refers to a collection of “vertices” or “nodes”, and a collection of “edges”, or “links”, that connect pairs of nodes. Network theory concerns itself with the study of graphs as a representation of relations between any kind of discrete objects.

Network abstractions and graph-theoretical approaches are today common in science. This approach has been applied for the representation of complex systems, and has achieved a certain success, from social studies (Travers 1969) to engineering (Alderson 2005) and biology (Watts 1998, Albert 2000, Jeong 2000, Newman 2000, Jeong 2001, Barabasi 2004, Goh 2007, Pieroni 2008).

Despite its intrinsically limited perspective, such conceptualization makes complex biological systems able to be considered as a whole and to undergo mathematical analysis, aiming to the discovery of salient systemic features and providing an accurate and analytic view at glance of entities, relations and functions that characterize them. This approach also allows to highlight how the qualities and behavior of single elements influence the network topology and dynamics, how network structure impinges upon processes spreading over the network, or the effect of perturbations on network performance (Boccaletti 2006, Tieri 2005). In this regard, the network abstraction of

biochemical signaling pathways can represent a useful functional view that can complement analyses and approaches from molecular biology and genomics.

Biochemical pathways are usually referred to as intracellular processes which scale can in some way be placed between small events, such as protein complexes formation or enzyme catalysis, and cell-wide or larger events, such as cell death or inflammation. These processes can be divided into separate steps, which seldom follow a linear and unambiguous succession. It is not yet simple to define a pathway in terms of its components, steps, dynamics and function, given its manifold, hazy and intricate nature. Actually, pathways and signaling cascades are not isolated entities. A signaling pathway can be triggered by many different extra- or intra-cellular events, cover different parallel paths and branches, intersect, be competitive or cooperative or interdependent with other events, every step can have diverging functions, and so on. Pathways, in conclusion, are processes characterized by high complexity (Bhalla 1999, Bhalla 2003, Ivakhno 2007). Abstractions and models of biological networks and pathways discussed here are mainly protein interaction networks (PINs) and protein-signaling networks (PSNs). PINs represent protein-protein binding events on a proteome-wide scale. Nodes and undirected edges represent proteins and binding events among them. In PSNs, nodes and directed edges represent phosphoproteins and phosphorylation reactions. The two models can be combined and enriched with additional layers, such as transcriptional regulatory networks, among others.

2.1.1 A workflow for integrative pathway and interactome reconstruction and analysis

Omics data and computational approaches are today providing a key to disentangle the complexity of objects like signaling pathways, assisted by dedicated online databases and specific software tools. Through such methodology, it is possible to integrate data of

different nature to extract meaningful representations and useful information, finally leading to an increased understanding of the biochemical process under examination. Nevertheless, the workflow for the integrated reconstruction and analysis of signaling pathways, interactomes and biological networks is hampered by difficulties of diverse nature, such as lack of data, shortcomings, annotation differences or multiple interpretations, data integration problems and other difficulties (Adriaens 2008, Bauer-Mehren 2009, Gardy 2009). Materials and workflow described here want to show a general approach for gathering information of interest from some of the existing pathway and protein interactions databases, for integrating and analyzing data and reconstructed representations by using the available tools, and to understand which kind of knowledge is possible to extract from the combination of existing information. The characteristics of some of the many pathway and protein interactions online resources and databases will be shortly described, together with the Cytoscape software platform and other online analysis tools able to greatly help in reconstructing and analyzing pathways and interactomes.

2.2. Materials: online databases and tools

2.2.1 Overview of databases and online data sources

Signaling cascades and pathways information is more and more often systematically collected and organized into publicly available databases. Such kind of resources lay the foundations for the systems level approach, allowing a workflow consisting in the reconstructive process of the pathway/interactome networks, that generally consists in the manual or automated retrieval of pathway data, their integration, merging, comparison and enrichment with other forms of data, and then the analytical process (simulation, mathematical modeling, statistical analysis). Iterative cycles of such

procedures, modeling, and prediction, combined with experimental validation (the systems biology cycle, fig. 2.1, Kitano 2002), can result in the improvement of the knowledge of cell signaling and responses.

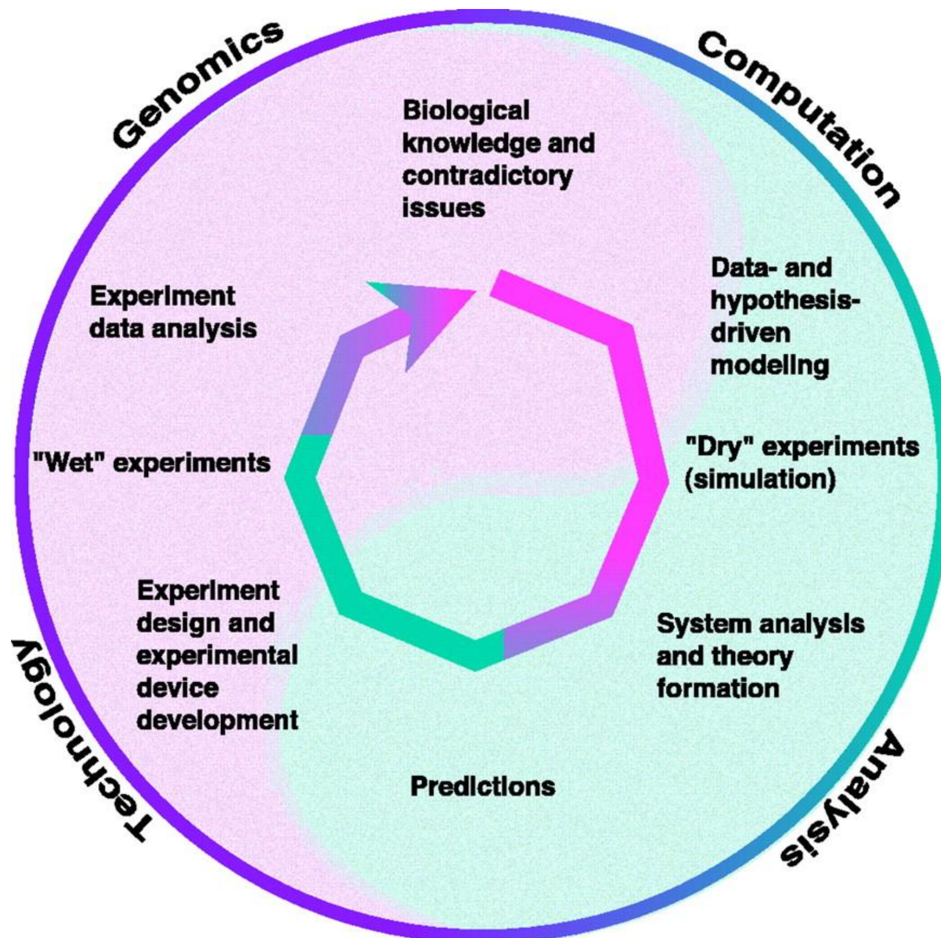


Fig. 2.1 The systems biology cycle: iterative cycles of modeling and prediction, combined with experimental validation may result in the improvement of the knowledge of cell signaling and responses. Adapted by Kitano 2002.

Online dedicated databases usually store cell signaling data in exchangeable formats (often BioPAX – Biological Pathway Exchange-, or SBML –Systems Biology Markup Language; see Notes section) accessible by diverse software platforms and tools, allowing for their retrieval, visualization and analysis. The following list should by no means considered as exhaustive; links and URLs can be found in the Notes section.

The Pathguide (the Pathway Resource List, fig 2.2, Bader 2006) is a resource useful as a starting point for biological pathway analysis, since it is a content aggregator for

integrated biological information systems. It is a meta-database that provides an overview of current pathway and other systems biology-oriented databases. Pathguide currently enlists and provides details and links to more than 300 web-accessible biological pathway and network databases. These include databases on metabolic pathways, signaling pathways, transcription factor targets, gene regulatory networks, genetic interactions, protein-compound interactions, and protein-protein interactions. The listed databases are curated and maintained by diverse scientific groups in different worldwide locations and the information in them is derived either from the scientific literature or from systematic, high-throughput experiments.

Fig 2.2 Screenshot of the Pathguide website (www.pathguide.org), a content aggregator for pathway analysis.

Reactome (Matthews 2009, Note 2) is a pathway database covering a very wide set of biological processes, organized in a hierarchical manner: lower levels for smaller reactions, higher levels for pathways and larger processes. Data are extracted from

literature and biomedical experiments, are human-curated and are represented as chains of chemical reactions (including transcription, catalysis, binding). Data can be physical entities (DNA, RNA, protein complexes, phosphorylated and unphosphorylated proteins, small molecules…) or events (reaction-like event, for smaller reactions, or pathway-like event, clustering many reaction-like events). It is possible to search and browse data and representations in remote, or to download them in local in the most common formats or in graphical representation. The website also provides some useful statistical and graphical tools and can be accessed through a SOAP (Simple Object Access Protocol) web service for automated data queries.

KEGG (the Kyoto Encyclopedia of Genes and Genomes, Kanehisa 2000, Kanehisa 2010, fig 2.3) consists of a number of interlinked databases devoted to several domains in the cell, the organism and the biosphere (genes, genomes, proteins, chemical compounds, pathways, diseases, drugs, ontologies). The pathways section covers many organisms including human. Data are categorized into the different processes (metabolic, genetic information, signaling etc) and are coded in its own XML format (KGML), or also in BioPAX and SBML through the use of additional available coding tools.

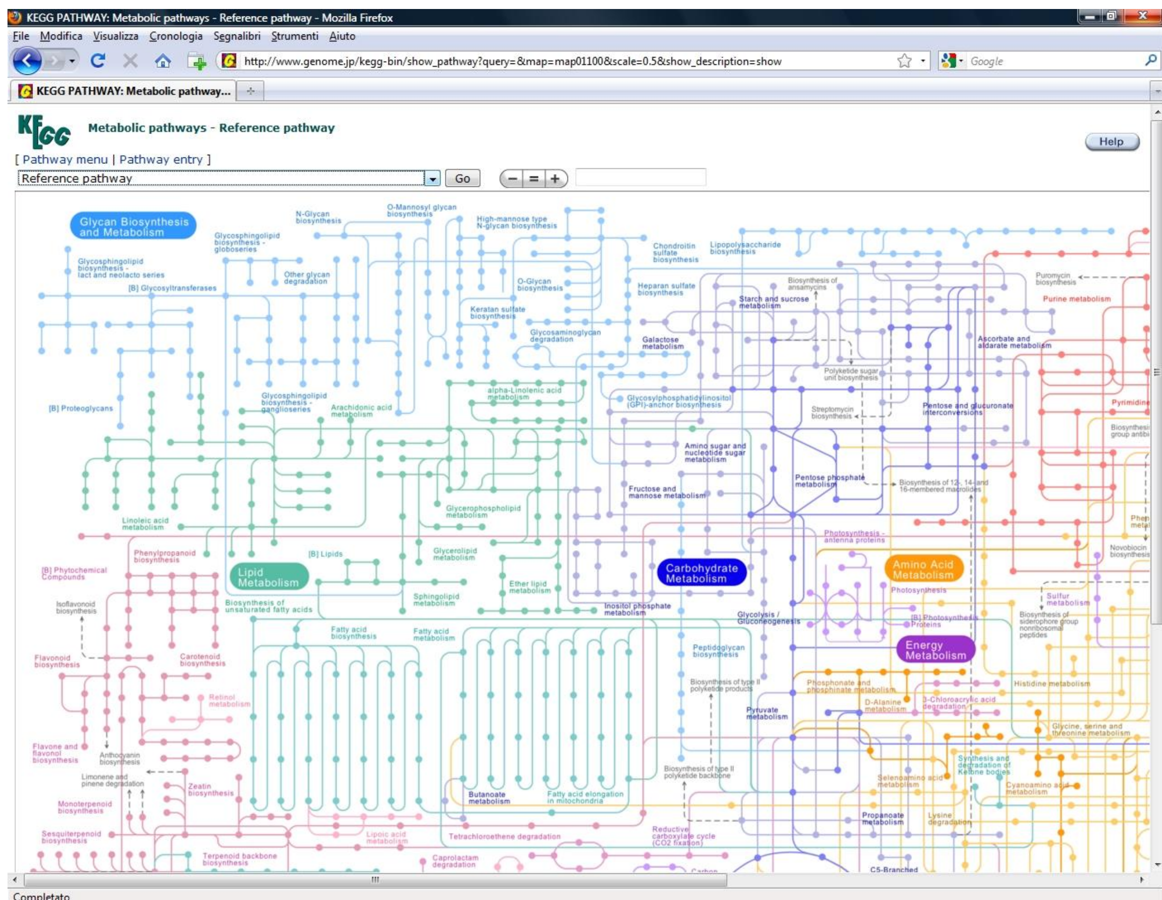


Fig 2.3 Screenshot of a comprehensive metabolic map in KEGG (www.genome.jp/kegg/).

The Nature Pathway Interaction Database (PID) (Schaefer 2009) is hierarchically organized in a way similar to Reactome and hosts pathway data (available in BioPAX or XML) obtained from peer reviewed literature or imported from other databases such as Reactome or BioCarta (a biotech commercial supplier reagents and assays for biopharmaceutical and academic research, see Notes). DNA and RNA are not part of the PID pathways but active/inactive, phospho/unphosphorylation states are annotated, the pathways can be browsed starting from UniProt, Entrez Gene (see Notes) or other types of identifiers, and statistical or query tools are provided.

Pathway Commons is based on already existing databases such as Reactome, PID, and other protein interactions databases, and provides an integrated access point and a compilation of such databases, thus conserving their structure and data hierarchies.

However, this kind of integration is not a simple task and this can result in overlapping, discordant and/or redundant information. A useful feature is the complete accessibility through the dedicated Pathway Commons plugin from the Cytoscape platform (see later in the chapter).

WikiPathways (Pico 2008) is an open source and collaborative platform for biological pathway information, storage and curation, in the wake of the Wikipedia style. Data are categorized by species and processes (f.i. metabolic process, molecular function, etc) and are coded in the GenMAPP (an application designed to visualize gene expression and other genomic data on maps representing biological pathways and groupings of genes) Pathway Markup Language (GPML) that can be compatible with applications such as PathVisio (a visualization tool, see Notes), Cytoscape and GenMAPP.

APID (Agile Protein Interaction DataAnalyzer) (Prieto 2006, fig 2.4) is an interactive web-based platform devoted to the exploration and analysis of diverse information about protein interactions, integrated and unified in a common and comparative environment. APID provides an open access frame where all known experimentally-validated protein-protein interactions (obtained by the most known protein interactions databases such as BIND, BioGRID, DIP, HPRD, IntAct and MINT, see Notes) are unified in a unique web application that allows the exploration and analysis of networks and interactomes. APID provides some embedded online tools to query and browse data and, most useful, a Cytoscape plugin (APID2NET, Hernandez-Toro 2007) that allows to extract, visualize and analyze unified interactome data by directly quering APID servers, including all the annotations and attributes associated to the retrieved PPIs.

164 interactions for NFKB1_HUMAN

PROTEIN INTERACTORS		EXPERIMENTS	PROVENANCE	More Info
TF65_HUMAN	NFKB1_HUMAN	12	IntAct MINT DIP BioGRID BIND HPRD	+info_inter
NFKB1_HUMAN	BCL3_HUMAN	12	MINT BioGRID BIND HPRD	+info_inter
NFKB1_HUMAN	NFKB1_HUMAN	11	IntAct MINT DIP BioGRID BIND HPRD	+info_inter
NFKB1_HUMAN	IKBA_HUMAN	6	IntAct MINT DIP BioGRID HPRD	+info_inter
NFKB1_HUMAN	NFKB2_HUMAN	5	IntAct MINT DIP BioGRID HPRD	+info_inter
NFKB1_HUMAN	M3K8_HUMAN	5	IntAct MINT DIP BioGRID HPRD	+info_inter
REL_HUMAN	NFKB1_HUMAN	5	IntAct MINT DIP BioGRID HPRD	+info_inter
IKKA_HUMAN	NFKB1_HUMAN	5	MINT DIP BioGRID HPRD	+info_inter
NFKB1_HUMAN	TNIP2_HUMAN	4	IntAct MINT DIP BioGRID	+info_inter
IKBE_HUMAN	NFKB1_HUMAN	4	IntAct MINT DIP BioGRID HPRD	+info_inter
NOTC1_HUMAN	NFKB1_HUMAN	4	MINT BioGRID HPRD	+info_inter
NFKB1_HUMAN	E2F1_HUMAN	4	DIP BioGRID HPRD	+info_inter
IKKB_HUMAN	NFKB1_HUMAN	3	BioGRID HPRD	+info_inter
NFKB1_HUMAN	FBW1A_HUMAN	3	BioGRID HPRD	+info_inter
FBW1B_HUMAN	NFKB1_HUMAN	3	HPRD	+info_inter
NFKB1_HUMAN	HDAC1_HUMAN	2	IntAct MINT BioGRID HPRD	+info_inter
RRP5_HUMAN	NFKB1_HUMAN	2	IntAct HPRD	+info_inter
COMD1_HUMAN	NFKB1_HUMAN	2	IntAct HPRD	+info_inter
RELB_HUMAN	NFKB1_HUMAN	2	IntAct MINT DIP BioGRID HPRD	+info_inter
IKBB_HUMAN	NFKB1_HUMAN	2	IntAct MINT DIP HPRD	+info_inter
NFKB1_HUMAN	LYL1_HUMAN	2	MINT BioGRID HPRD	+info_inter
NFKB1_HUMAN	RUVB2_HUMAN	2	MINT BIND	+info_inter
MEN1_HUMAN	NFKB1_HUMAN	2	DIP BioGRID HPRD	+info_inter

Fig 2.4 Screenshot of the APID database showing interaction of a NF-κB subunit (http://bioinfow.dep.usal.es/apid/index.htm).

TRED (Transcriptional Regulatory Element Database) (Zhao 2005, Jiang 2007) is a manually curated database of regulatory elements (promoters, transcription factor binding sites, both cis- and trans-) with experimental evidence in mammalian genomes. Currently it enlists a total of 36 transcription factors families (most of which are involved in cancer), more than 7000 target genes and around 15000 target promoters, with the goal to assist detailed functional studies and to help in obtaining a panoramic view of gene regulatory networks in a cancer research perspective.

TRANSPATH (Choi 2004), together with the more famous TRANSFAC (Matys 2003), that stores transcription factors and their DNA binding sites, is a wide and powerful knowledge base system about gene regulatory networks that comprises and integrates information on signal transduction and tools for visualization and analysis. It allows

obtaining complete signaling pathways from ligand to target genes and their products. Its access requires a license purchase, even if a version dating back to years ago can be accessed for free.

NetPath (Keshava Prasad 2009) is a curated compendium of human signaling pathways which currently contains annotations for several cancer and immune signaling pathways. Pathway data are available for browsing and download in the most common formats (included Proteomics Standards Initiative-Molecular Interaction –PSI-MI- format), and listing of up- and down-regulated genes for each pathway is provided, based on experiments and literature.

It is quite normal that users spend an amount of time in browsing many databases in search for the data and models that meet the requirements of the research project.

Notwithstanding the quantity and quality of the publicly available resources, information automatically extracted from a single pathway database is usually not yet exhaustive. Given the often complementary nature of data in different databases, they should be retrieved, integrated and combined, and we feel the quality of the result still strongly relies upon a sharp manual curation effort (Adriaens 2008, Bauer-Mehren 2009, Gardy 2009). The integration process itself, however, can present several problems, not least those of interchangeability of the different formats and data models, but also in terms of reaction annotation, or of significant differences in other key biological factors, such as cellular state and type (Adriaens 2008). Thus, the process of literature extraction of data (also possibly aided with text-mining techniques) together with combination of information from databases under expert supervision and curation probably remains a good choice in order to get an accurate pathway reconstruction. A complete and deep curation process can last months and employ many experts, and yet yield controversial results. Conversely, manual integration of data extracted from online pathway resources

–under expert review- can be decently performed in days, allowing to create a sufficiently accurate (also depending on the scope) representation of a given pathway, or part of it, able to undergo further functional enrichment and analysis.

2.3. Materials: computational analysis software

2.3.1. Main platforms and tools

Since the purpose of the interactome or pathway reconstruction process is to have an "object" can be further elaborated, enriched and analyzed step by step, we will need to access and store data in local machines, and not only to browse them online. As described before, most of available database allow downloading the relevant data in diverse formats (BioPAX, SBML, PSI-MI, among others). At this point, the choice of one or more tools for network editing and analysis is up to the user. Some of them are directly embedded or available inside the different databases, such as Reactome, WikiPathways, BioCarta, GenMAPP. Others are commercial suites, such as Ingenuity or Pathway Studio, with special visualization features (see Notes).

Among the open source applications, Cytoscape (Shannon 2003, fig. 2.5) is a very powerful software platform, available for all the major operating systems, designed for biological research, but versatile enough to be used in many other fields where network editing, visualization and analysis are key features. The core tool has been developed to visualize molecular interaction networks and biological pathways, and to integrate these networks with annotations, gene expression profiles and other state data. Many more additional features, such as advanced network and molecular profiling analyses, new layouts, additional file format support, scripting, and connection with databases, are available as plugins. It supports many different standard network and annotation file

formats, including SIF (Simple Interaction Format), BioPAX, PSI-MI, SBML, tab-delimited text files and MS Excel.

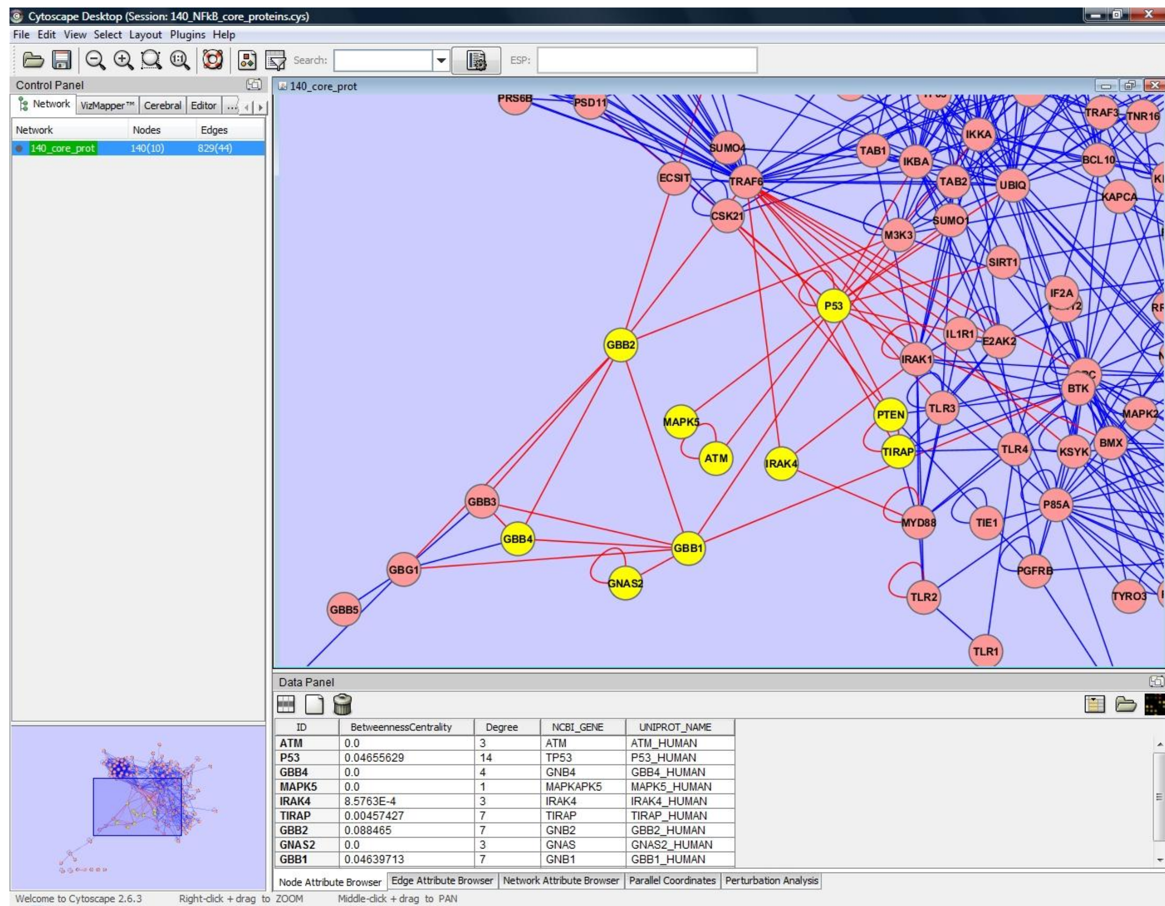


Fig. 2.5 Screenshot of a working window in the Cytoscape visualizing and analysis tool (www.cytoscape.org).

BiologicalNetworks (Baitaluk 2006) is an integrated research environment for biological sciences that allows querying and integrating molecular interaction networks, metabolic and signaling pathways with a large number of biological features related to transcriptional regulation, microarray and proteomic experiments, 3D structures ontologies, taxonomies and other types of data. It is based on a database currently integrating over 100 curated and publicly contributed data sources for thousands of eukaryotic, prokaryotic and viral genomes.

CellDesigner (Funahashi 2003) is a structured diagram editor for drawing gene-regulatory and biochemical networks. Networks are drawn based on the process

diagram, with a particular graphical notation system, and are stored using the SBML format. Networks are able to link with simulation and other analysis packages through a wider software platform named Systems Biology Workbench (SBW).

We will focus on a workflow mainly based on the Cytoscape platform given its free availability, diffusion in biology research, upgradeability and versatility.

2.3.2 Other specific analysis tools and plugins

Powerful standalone packages specific for network analysis are freely available. Pajek (Batagelj 2003) ("spider" in Slovene, the nationality of the developers), for instance, is able to visualize and analyze networks of millions of nodes. Specific add-on modules can be used inside the well-known R statistical package (www.r-project.org).

Other packages have direct web-based functionality: GraphWeb (Reimand 2008) is a public web server for graph-based analysis that has been designed for extensive analyses of directed and undirected, weighted and unweighted heterogeneous networks of genes, proteins and microarray probesets for many eukaryotic genomes, and is able to integrate multiple diverse datasets into global networks.

Among the many available Cytoscape plugins (for an exhaustive list and references see the Cytoscape.org website), NetworkAnalyzer (Assenov 2008) requires no expert knowledge in graph theory from the user. It is able to compute, display and shows charts for a quite complete set of topological parameters for undirected and directed networks, which includes the number of nodes, edges, and connected components, the network diameter, radius, density, centralization, heterogeneity, clustering coefficient, and the characteristic path length.

ClusterMaker (Cytoscape plugin, see Notes) unifies different clustering techniques and displays into a single interface. It uses specific algorithms for clustering expression or

genetic data, and similarity networks to look for protein families and putative functional similarities.

The Hub Objects Analyzer (Hubba) (Lin 2008) is both a web-based service and a Cytoscape plugin for exploring networks to discover hubs and important nodes in an interactome network generated from specific small- or large-scale experimental methods.

2.4. Methods: general retrieval and reconstruction procedures

2.4.1. Data retrieval

The process of manual literature mining for data extraction is labor-intensive and time consuming but typically gives back high-quality data and models. It is evident that, given the broadness and importance of this topic, it cannot be exhaustively treated here and we refer to Jensen and colleagues (Jensen 2006) for a comprehensive review on the field of manual and machine-aided extraction of biomedical facts from scientific literature.

In the first step of retrieving the pathway data of interest through Cytoscape, it is possible to use one of the many existing plugins, each one designed to query and retrieve data from many different databases. It is evidently advisable that the user has previously browsed the candidate databases to understand which type, model and format data have been stored in.

Among the many Cytoscape plugins, BioNetBuilder (Avila-Campillo 2007) can be used to build networks for many different species, including most common model organisms and human, retrieving data from currently supported databases that include DIP, BIND, KEGG, HPRD, BioGrid, among others. The interface offers different options to specify a set of initial genes/gene products for which to find molecular interactions (including

loading a text file, finding genes with specified Gene Ontology annotations, and finding genes whose common name match a given string). Biological networks for whole organisms can also be created and displayed.

Another very useful plugin is the aforementioned APID2NET, linked to the APID database. It is possible to specify a list of proteins and get the network of their interactions, at the desired connection level (level 0 considers only the interactions among the listed proteins, level 1 considers all their neighbors in APID, level 2 considers also the neighbors of the neighbors, etc.) and validated by the chosen number of different experimental methods. The system also displays additional information on node, edge and network attributes.

The user can also start a Cytoscape session with the embedded *import network from web service* function to connect directly to the Pathway Commons or WikiPathways servers and get the data. It is also possible to retrieve the data from each single database simply by downloading a formatted file and then upload and open it in the Cytoscape client that will visualize the relative network.

It is not always possible to retrieve data following a plugin-automated or semi-automated process as described above. For some databases, not specifically designed for systems biology but containing useful and well arranged information, as for instance the transcriptional regulatory element database TRED, it can be necessary to make the query, to extract the data with copy/paste operations in text format and make adaptation to import and incorporate them into a network in a very manual fashion.

2.4.2. Data merging and combination

As said, combination of data from different pathway databases is highly desirable. The user can for instance download the same pathway data as provided by two or more databases and try to confront and combine them in order to make it as complete as

possible. For this purpose, again, suitable Cytoscape plugins (f.i. AdvancedNetworkMerge) or embedded function in the tool may be used. This is typically a very critical point, since very often molecular and reaction data have been encoded and modeled in different manner according to the originating database, so that the network resulting from the merging of such two or more networks can disappointingly result as a simple sum of the originating objects, or anyway as an inconsistent network, without any -although expected- overlap, or any other shared information or link. There is no trivial solution to this kind of issues, since from database to database there are no uniquely defined identifiers for each of the entities that compose the pathways or the networks. Accurate filtering and expert curation performed before the merging process could purge the data from undesired or redundant information. This will also usually make it quite easy to build improved versions of the networks based on additional and different types of data. In figure 2.6 a schematic representation of the whole workflow is presented.

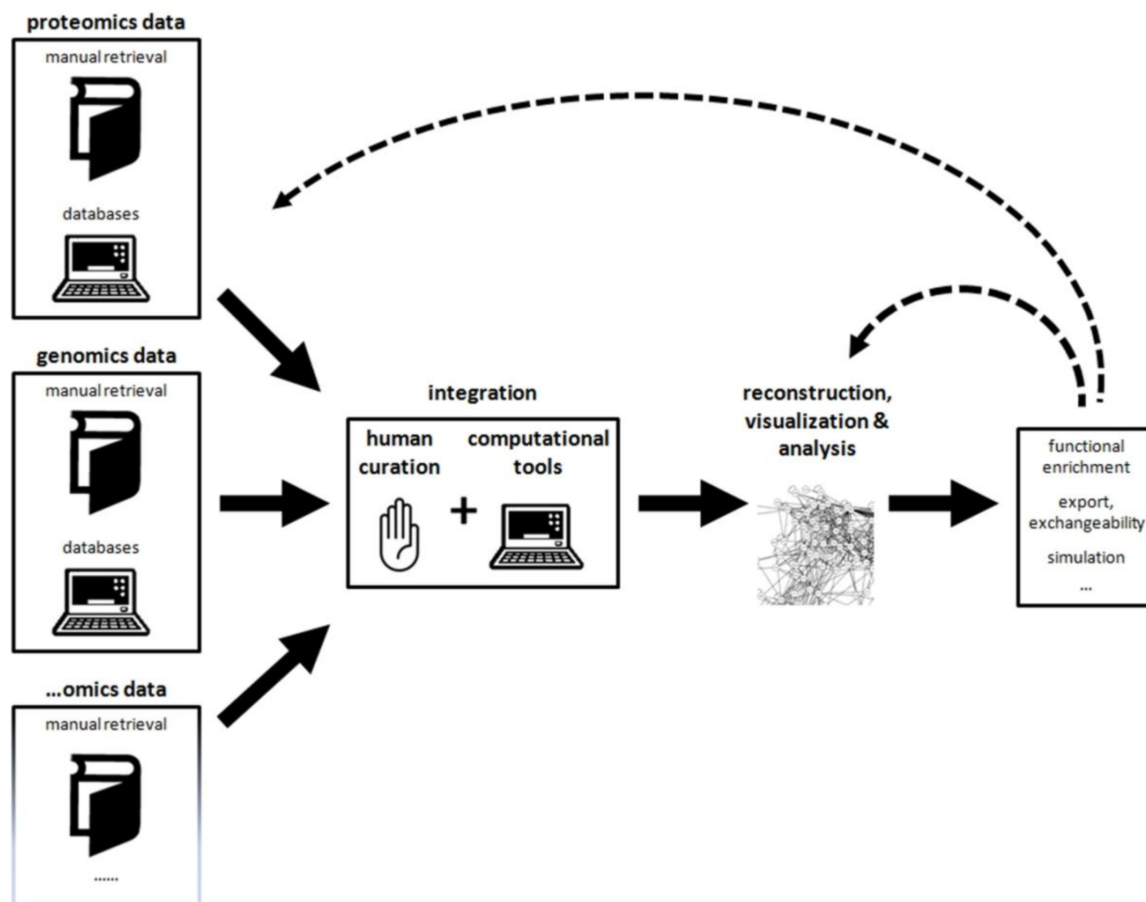


Fig. 2.6 Schematic representation of the workflow. From manual and automated data retrieval, through human curation and software platforms, data are integrated to reconstruct coherent objects able to undergo mathematical analysis. Results can feed back in the pipeline for further enrichment, analysis, simulations or improvement of existing models and representations.

2.4.3. Functional enrichment

Obtained networks can be functionally enriched, i.e. can be integrated and superimposed with data of different type, such as gene expression data or Gene Ontology (GO) categories to verify if statistically overrepresented features are linked to topological characteristics. Some plugins are available for Cytoscape and many others are accessible on the internet. Among many, we just mention BiNGO (Maere 2005) and ClueGO (Bindea 2009), plugins able to determine which Gene Ontology categories are overrepresented in sets of genes, (in the present context corresponding to subgraphs of a given biological network), to map the predominant functional themes of a given gene set

on the GO hierarchy as a graph, and to perform cluster analysis and comparison of clusters. GOrilla (Eden 2009) is a tool for identifying and visualizing enriched GO terms in ranked lists of Human genes.

2.5. Methods: network analysis

2.5.1. Topological measures

Once that the user has performed the reconstruction steps and considers the "object" pathway or interactome in some way complete and stable (for the subjective purpose of the study to be carried out), it is time to proceed with the subsequent network analysis. All cited computational platforms are precisely designed to perform such analyses that can be easily implemented through embedded or add-on features.

The goal of topological analysis of protein networks is to discern organizational 'design' principles, relate those to dynamical properties, and establish connections to biological functions. The detection of interesting topological properties occurs by comparing the network under study with a "null model"; that is, a set of networks that reflect what is expected by random chance. If a network under study possesses certain characteristics different from what is expected by chance alone, then these might be related to the function of the network: they could have been selected by evolution for their advantageous properties.

Topological measures have demonstrated their usefulness in uncovering the organizing principles that rule the development and the evolution of networks of different nature (Barabasi 2004). Several observations led to conclude that the classical degree distribution, and the well-investigated scale-free characteristic, of nodes in PINs, for instance, correlates with biological meaningful features, such as importance, lethality, robustness and dynamics of perturbations. Hierarchical topology, sub-graphs, modular

structures, clusters are, among others, strongly characterizing features of networks that a focused analysis can reveal (Pieroni 2008). In some fields, such as cancer research, extensive and deep meta-analyses have shown how some specific measures, such as betweenness and stress centrality, among others, are particularly relevant in characterizing pathological states and malignant tissues (Platzer 2007).

Among the many interesting network analysis techniques, clustering can help in the identification of functional groups and in heuristic discovery of un-annotated functions of some proteins. Since proteins tend to function in groups, or complexes, an important goal is to reliably identify protein complexes from graphs and networks derived from genome scale data on protein interactions. This task is commonly executed using clustering procedures, which aim at detecting densely connected regions within the interaction graphs. One of the most successful clustering procedures in this context has been the Markov Cluster algorithm (MCL), often specifically applied for partitioning protein interactions graphs. considers the connectivity properties of the underlying network. MCL has been used to derive complexes from protein interaction data in comprehensive analyses of the yeast interactome, and was shown to be especially effective for clustering protein interactions in that it possesses a high degree of noise-tolerance.

The most common and important topological parameters are briefly described below (Bollobàs 2002; or, for further information and an useful browsable online help, see http://med.bioinf.mpi-inf.mpg.de/netanalyzer/help/2.6.1/index.html).

Number of connected components

In undirected networks, two nodes are connected if there is a path of edges between them. Within a network, all nodes that are pairwise connected form a connected

component. The number of connected components indicates the connectivity of a network – a lower number of connected components suggests a stronger connectivity.

Parameters related to shortest paths

The length of a path is the number of edges forming it. There may be multiple paths connecting two given nodes. The shortest path length, also called distance, between two nodes *n* and *m* is denoted by *L*(*n*,*m*). The network diameter is the largest distance between two nodes. If a network is disconnected (some nodes are isolated), its diameter is the maximum of all diameters of its connected components. The average shortest path length, also known as the characteristic path length, gives the expected distance between two connected nodes.

Parameters related to neighborhood

The neighborhood of a given node *n* is the set of its neighbors. The connectivity of *n*, denoted by k_n, is the size of its neighborhood. The average number of neighbors indicates the average connectivity of a node in the network. A normalized version of this parameter is the network density. The density is a value between 0 and 1. It shows how densely the network is populated with edges (self-loops and duplicated edges are ignored). A network which contains no edges and solely isolated nodes has a density of 0.

The number of isolated nodes may provide insight how the network density is distributed. Another related parameter is the network centralization. Networks whose topologies resemble a star have a centralization close to 1, whereas decentralized networks are characterized by having a centralization close to 0. The network heterogeneity reflects the tendency of a network to contain hub nodes.

Clustering coefficient

In undirected networks, the clustering coefficient C_n of a node n is defined as $C_n = 2e_n/(k_n(k_n-1))$, where k_n is the number of neighbors of n and e_n is the number of connected pairs between all neighbors of n. In directed networks, the definition is slightly different: $C_n = e_n/(k_n(k_n-1))$.

In both cases, the clustering coefficient is a ratio N / M, where N is the number of edges between the neighbors of n, and M is the maximum number of edges that could possibly exist between the neighbors of n. The clustering coefficient of a node is always a number between 0 and 1. The network clustering coefficient is the average of the clustering coefficients for all nodes in the network. Here, nodes with less than two neighbors are assumed to have a clustering coefficient of 0.

Degree distributions

In undirected networks, the node degree of a node n is the number of edges linked to n. A self-loop of a node is counted like two edges for the node degree. The node degree distribution gives the number of nodes with degree k for $k = 0,1,\ldots$.

In directed networks, the in-degree of a node n is the number of incoming edges and the out-degree is the number of outgoing edges. Similar to undirected networks, there are an in-degree distribution and an out-degree distribution.

The node degree distribution may be used to distinguish between random (as defined by Erdős and Rényi 1959) and scale-free network topologies.

Neighborhood connectivity

The connectivity of a node is the number of its neighbors. The neighborhood connectivity of a node n is defined as the average connectivity of all neighbors of n. The neighborhood connectivity distribution gives the average of the neighborhood connectivities of all nodes n with k neighbors for $k = 0,1,\ldots$. Figure 2.6 shows a simple network and the relative neighborhood connectivity distribution. In analogy to the in-

and out-degree, every node n in a directed network has in- and out-connectivity. Thus, in directed networks, a node has the following types of neighborhood connectivity:

- *only in* - the average out-connectivity of all in-neighbors of n;
- *only out* - the average in-connectivity of all out-neighbors of n;
- *in and out* - the average connectivity of all neighbors of n (edge direction is ignored).

Based on the three definitions given above, there are three neighborhood connectivity distributions - "only in", "only out" and "in and out".

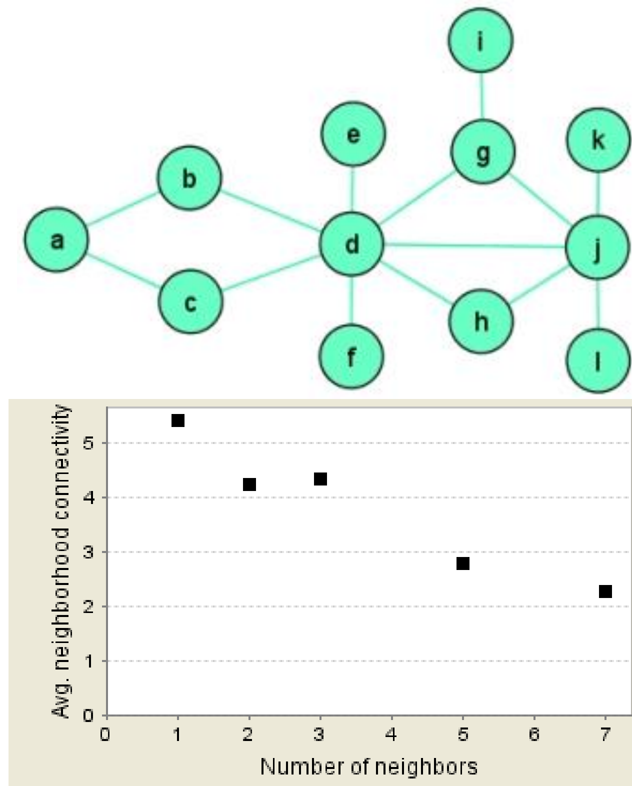


Fig. 2.6 Simple network formed by 12 nodes and 14 edges (left), and relative neighborhood connectivity distribution (right chart).

If the neighborhood connectivity distribution is a decreasing function in k, edges between low connected and highly connected nodes prevail in the network.

Shortest paths

The length of the shortest path between two nodes *n* and *m* is $L(n,m)$. The shortest path length distribution gives the number of node pairs (n,m) with $L(n,m) = k$ for $k = 1,2,\ldots$.

The network diameter is the maximum length of shortest paths between two nodes. If a network is disconnected, its diameter is the maximum of all diameters of its connected components.

The network diameter and the shortest path length distribution may indicate small-world properties of the analyzed network.

Clustering coefficients

A clustering coefficient is a measure of degree to which nodes in a graph tend to cluster together. In undirected networks, the clustering coefficient C_n of a node *n* is defined as $C_n = 2e_n/(k_n(k_n\text{-}1))$, where k_n is the number of neighbors of *n* and e_n is the number of connected pairs between all neighbors of *n*. In directed networks, the definition is slightly different: $C_n = e_n/(k_n(k_n\text{-}1))$.

In both cases, the clustering coefficient is a ratio N / M, where *N* is the number of edges between the neighbors of *n*, and *M* is the maximum number of edges that could possibly exist between the neighbors of *n*. The clustering coefficient of a node is always a number between 0 and 1.

The average clustering coefficient distribution gives the average of the clustering coefficients for all nodes *n* with *k* neighbors for $k = 2,\ldots$.

The clustering coefficient of a node is the number of triangles (3-loops) that pass through this node, relative to the maximum number of 3-loops that could pass through the node.

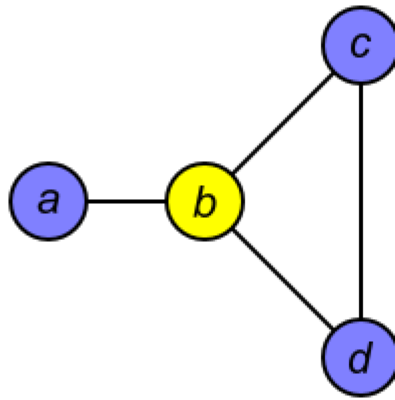


Figure 2.7 Example network with four nodes and four edges (see text).

For example, in Figure 2.7, there is one triangle that passes through node *b* (the triangle *bcd*). The maximum number of triangles that could pass through *b* is three (in this case, the pairs (*a*, *c*) and (*a*, *d*) would be connected additionally). This yields a clustering coefficient of $C_b = 1 / 3$.

The average clustering coefficient distribution may be used to identify a modular organization of metabolic networks (Ravasz 2002).

Shared neighbors

P(*n*,*m*) is the number of partners shared between the nodes *n* and *m*, that is, nodes that are neighbors of both *n* and *m*. The shared neighbors distribution gives the number of node pairs (*n*,*m*) with $P(n,m) = k$ for $k = 1, \ldots$. If a motif like the one presented in figure 2.8 is over-represented in a network, this can be inferred from the shared neighbors distribution.

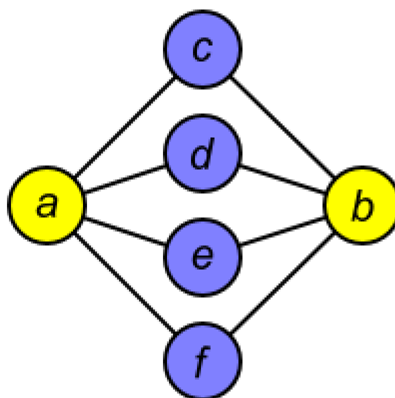


Figure 2.8 Motif of two nodes sharing exactly four neighbors.

Topological coefficients

The topological coefficient T_n of a node n with k_n neighbors is computed as follows:

$$T_n = avg\ (\ J(n,m)\ )\ /\ k_n.$$

Here, $J(n,m)$ is defined for all nodes m that share at least one neighbor with n. The value $J(n,m)$ is the number of neighbors shared between the nodes n and m, plus one if there is a direct link between n and m. For example, in figure 2.9, $J(b,c) = J(b,d) = J(b,e) = 2$. Therefore, $T_b = 2 / 3$.

The topological coefficient is a relative measure for the extent to which a node shares neighbors with other nodes. Nodes that have one or no neighbors are assigned a topological coefficient of 0 (zero).

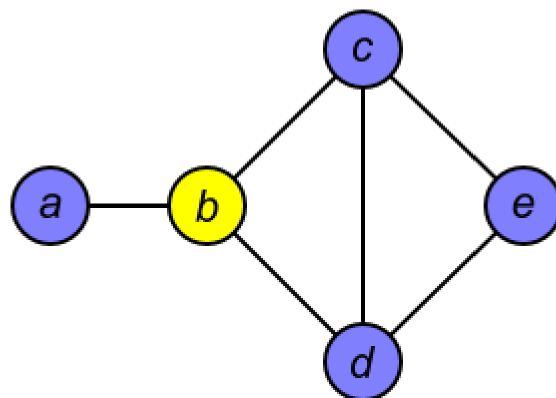


Figure 2.9 Example network with five nodes and six edges: the topological coefficient here is T_b = 2 / 3.

The chart of the topological coefficients can be used to estimate the tendency of the nodes in the network to have shared neighbors.

Stress distribution

The stress of a node n is the number of shortest paths passing through n . A node has a high stress if it is traversed by a high number of shortest paths. This parameter is defined only for networks without multiple edges. The stress distribution gives the number of nodes with stress s for different values of s.

Betweenness centrality

The betweenness centrality $C_b(n)$ of a node *n* is computed as follows:

$$C_b(n) = \sum_{s \neq n \neq t} (\sigma_{st}(n) / \sigma_{st}),$$

where *s* and *t* are nodes in the network different from *n*, σ_{st} denotes the number of shortest paths from *s* to *t*, and $\sigma_{st}(n)$ is the number of shortest paths from *s* to *t* that *n* lies on.

Betweenness centrality is computed only for networks that do not contain multiple edges. The betweenness value for each node *n* is normalized by dividing by the number of node pairs excluding *n*: $(N\text{-}1)(N\text{-}2)/2$, where *N* is the total number of nodes in the connected component that *n* belongs to. Thus, the betweenness centrality of each node is a number between 0 and 1.

For example, the betweenness centrality of node *b* in figure 2.10 is computed as follows:

$C_b(b) = ((\sigma_{ac}(b) / \sigma_{ac}) + (\sigma_{ad}(b) / \sigma_{ad}) + (\sigma_{ae}(b) / \sigma_{ae}) + (\sigma_{cd}(b) / \sigma_{cd}) + (\sigma_{ce}(b) / \sigma_{ce}) + (\sigma_{de}(b) / \sigma_{de})) / 6 = ((1 / 1) + (1 / 1) + (2 / 2) + (1 / 2) + 0 + 0) / 6 = 3.5 / 6 \approx 0.583$

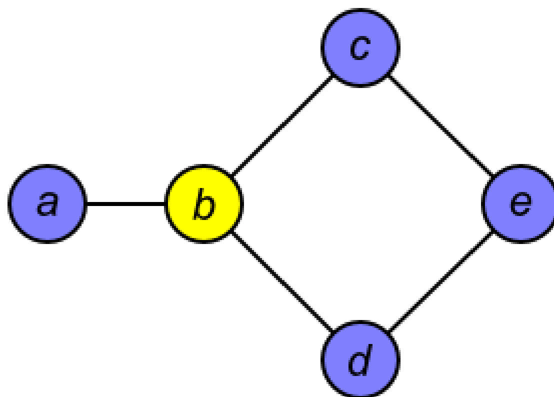


Figure 2.10 Example network with five nodes and five edges (see text).

The betweenness centrality of a node reflects the amount of control that this node exerts over the interactions of other nodes in the network. This measure favors nodes that join communities (dense subnetworks), rather than nodes that lie inside a community.

Closeness centrality

The closeness centrality $C_c(n)$ of a node n is defined as the reciprocal of the average shortest path length and is computed as follows:

$$C_c(n) = 1 / avg(L(n,m)),$$

where $L(n,m)$ is the length of the shortest path between two nodes n and m. The closeness centrality of each node is a number between 0 and 1. The closeness centrality of isolated nodes is equal to 0. Closeness centrality is a measure of how fast information spreads from a given node to other reachable nodes in the network. For example, the closeness centrality of node b in figure 2.10 is computed as follows:

$$C_c(b) = 1/ ((L(b, a) + L(b, c) + L(b, d) + L(b, e)) / 4) = 4/ (1 + 1 + 1 + 2) = 4/5 = 0.8$$

2.5.2. Dynamical models

Owing to the intricacy of signal transduction, computational analysis is necessary to obtain understanding of dynamical properties of PSNs. Even for very small, relatively simple PSNs, it has been shown that a wide variety of complex dynamical properties could be attained (Bhalla 1999, Bray 1995, Sauro 2004, Tyson 2003) and parallels were drawn between signaling circuits and man-made control systems to explaining important biological properties, such as amplification, robustness, homeostasis, and adaptation; particularly highlighting the importance of feedbacks in PSNs (Sauro 2004, Alon 1999, Ferrell 1996, Goldbeter 1981, Levin 1998, Yi 2000). Several larger mathematical models based on Ordinary Differential Equations have been formulated for signal PSNs, and their parameters were optimized in order to fit experimental observations (Chen 2004, Chen 2000, Kholodenko 2006, Tyson 2001). Although studies with such models provide many detailed insights into the dynamics and function of signaling pathways, formulating such models is a difficult problem that requires a huge amount of specific quantitative experimental data, which are not expected to be available on proteome-wide

scale in the near future. Dynamical models of proteome-wide PSNs, although lacking precise quantitative information of the kinetic dependencies, can still be used to discover principles of global dynamical organization. For example, a qualitative approach to the dynamic modeling of PSNs is the use of Boolean logic, in which each protein is ‘off’ or ‘on’ at a given time-step depending on the states of its inputs. Recently, it was shown that a PSN formalized with Boolean logic can classify sets of inputs into distinct output patterns – an ability that arises through the complex wiring pattern among the proteins in the PSNs (Helikar 2008). This ability is an emergent dynamical property determined by the structure of the PSN, because the authors showed that randomizing the network results in loss of this ability. Interesting metaphors have been drawn between PSNs and computational networks (Bhalla 2003). Back in 1990, Dennis Bray highlighted the similarity between PSNs and ‘artificial neural networks’ (Bray 1990). Rather than signal transduction as just a mechanism to transmit information from the cell surface to the nucleus and other functions, this analogy suggests a complex process of turning complex input signals (environments) into complex output signals (biological responses). Similar to artificial neural networks, where the parameters are adjusted through mathematical optimization to obtain required input-output relationships, evolution has tweaked the parameters in PSNs to obtain the ability to generate appropriate responses to the wide variety of complex environmental signals that organisms are subjected to (Bray 1990).

It has become clear that the proteome forms a complex system with many emergent properties yet to be discovered and understood (Pieroni 2008, Bhalla 2003). Topological and dynamical studies of PSNs that take explicitly the INPUT → CENTRAL NETWORK → OUTPUT structure (Helikar 2008, Cui 2006, de la Fuente 2008, Liu 2006, Ma’yan 2005) into account most certainly will yield many insights into the functional organization these intricate protein networks.

2.6. Methods: NF-κB interactome data retrieval and reconstruction

2.6.1. Workflow

The main steps of the workflow followed for the NF-κB pathway interactome reconstruction and analysis are briefly summarized here. More information can be found in chapter 3, Results.

1. The starting point consists in the manual literature mining and review: such approach took several months of expert work, and guarantees a quite complete list of proteins that take part with different roles and importance to the whole signaling cascade. This basic list has been increased, enriched and refined step by step confronting and complementing preliminary results with data browsed and downloaded manually or with Cytoscape from several pathway and PIN databases. The reference list is available in the Notes section. The result at the end of this manual process is a "core list" consisting of 140 proteins. It should be noted that such list can continuously change due to new knowledge and updates in the relative data and biological information. In the Notes section the list of articles and reviews examined for the determination of the core proteins list is provided.

2. Protein interactions data are added to build the first version of the "core interactome". The main tool used in this step is the APID database, automatically accessed through the dedicated plugin in Cytoscape. Parameters are setup in order to consider interactions that have been tested with one experimental method. It should be considered that it is also possible to retrieve interaction tested with more than one experimental method, but in this way, due to shortage of data, the percentage of false negatives is too high to be considered reliable. Conversely, using the "one experimental method" search parameter, it is possible to include in the interactome a acceptably low number of false positives.

The result is a network consisting of 140 nodes and 829 non-directional interactions, including self-interactions.

3. By using automated retrieval tool and databases (APID2NET, BioNetBuilder in Cytoscape, main PIN databases) a "wider interactome" is built, taking into account all the proteins with evidence of interaction with at least one protein present in the "core interactome". Search parameters are set to include interactions validated with one experimental method, as above for the core interactome. At the end of the process, the whole "wider interactome" consists of 3146 proteins accounting for a total of more than 42600 protein-protein interactions.

4. Data elaborated from a manually curated list of NF-κB-downstream genes (Gilmore 2010), from the TRED database (manually extracted) and integrated with results from TRANSFAC allow to constitute a relatively comprehensive list of more than 400 genes that result to be up- or down-regulated from NF-κB. Gene products and relative UniProt identifiers are obtained directly through the ID mapping functions available on the UniProt web interface, allowing to compile the list of proteins which expression can be regulated by NF-κB.

5. The whole interactome now consisting of core proteins (those that directly participate to signaling cascades that activate NF-κB), wider interactome proteins (their direct interactors), regulated genes and relative expressed proteins now undergoes functional enrichment and analysis: topological characterization, GO enrichment, clustering are performed thanks to the several standalone analysis tools, Cytoscape and web-based services. Results from the analysis include, among others, a wider, integrated overlook of the NF-κB signaling system and its main topological characteristics, the detection of specific hubs or central proteins, the discovery of feedback loops and cross controls among proteins and genes that can be candidates for further in-depth studies.

2.6.2. Pitfalls and issues encountered

Some of the most common pitfalls in the procedure shown as well as some general considerations on the proposed workflow and relative problems encountered are briefly taken into account here.

One of the major concerns in pathway and PSN reconstruction is the lack of clear and comprehensive data about reactions and subsequent directionality. Directional information is still a rare thing. As said, the user will unlikely find that the same pathway has been represented in similar ways on different databases. This poses the problem of the necessity to choose one among different data models and content, or to engage in the non trivial effort of integrating and complementing the various data and data types. The lack of undisputable data about a number of reactions and proteins in the NF-κB interactome reconstruction and the obvious existence of time constraints forced - at least provisionally- to omit the relative dynamical information in the obtained representation. Without directional information it is impossible to implement dynamical models and simulation, even a simple model based on Boolean dynamics, unless willing to make the assumptions are made that each edge A-B corresponds to influences in both directions, i.e. A → B and B → A, which is very unrealistic indeed.

Automation of procedures able to integrate different pathways in a coherent and biological meaningful way is a critical point. Currently, there is no practical, coherent and effective way to integrate data from multiple sources into a single object other than manual intervention. Even if data from single pathways in the different databases are often very close to be precise, comprehensive and satisfying to serve as a starting base, it is the integrative process and subsequent elaboration to hopefully bring valuable information and new knowledge. Actually, in this regard, data models and

representations, and annotation are key points in the discussion about these hot topics (Ceol 2008, Leitner 2008, Gerstein 2007, Tieri 2008).

It should be finally reminded that protein-protein interactions databases frequently store and provide PIN data without distinguish among cellular types, states and other biologically relevant conditions. Advanced filtering on such parameters thus remains a desirable but not often implemented query function. Commonly, pathologically relevant states of proteins, cells and tissues are reported, even if data query cannot always be adequately filtered and displayed. This could represents an additional hurdle for the biologically coherent reconstruction of interactomes.

3 Results and discussion

3.1. “Core” and “wider” NF-κB pathway interactomes

Following the workflow described in the Methods section for data retrieval, we started collecting relevant data from manual literature mining and review. This approach guaranteed a satisfactorily complete list of proteins that take part with different roles and importance to the whole NF-κB signaling cascade, from cell receptors to regulated genes and expressed proteins. This basic list has been increased, enriched and refined step by step confronting and complementing preliminary, manually retrieved results with data browsed and downloaded from several pathway and PIN databases. The result at the end of this manual process, a “core” list consisting of 140 proteins participating in the NF-κB signaling cascade, is the first step for further information integration and reconstruction. The list has been double checked, but –once again- it can be modified, augmented or enhanced on the basis of the new knowledge gained in time. Protein interactions data are added to build the first version of the “core interactome”. While, as a second step, and by using an automated retrieval procedure instead of the manual one, a “wider interactome” is built, taking into account all the proteins which show evidence of interaction with at least one protein present in the “core interactome”. At the end of the process, the whole “wider interactome” (that includes the core interactome) consists of 3146 proteins accounting for a total of more than 42600 interactions.

The third step allows to determine the NF-κB-downstream genes, providing a relatively comprehensive list of more than 400 genes that result to be up- or down-regulated from NF-κB complexes. Gene products and relative UniProt identifiers are obtained directly

through the ID mapping functions available on the UniProt web interface, allowing to compile the list of proteins which expression can be regulated by NF-κB.

The whole system, now consisting of core interactome proteins (those that directly participate to signaling cascades that activate NF-κB), wider interactome proteins (their direct interactors), downstream genes and their expressed proteins, undergoes topological characterization, GO enrichment analysis and clustering.

3.2. Analysis of the interactomes

3.2.1. Core interactome, structure and network analysis

The list of 140 manually retrieved proteins (see table 3.1) includes cell surface receptors, kinases, ubiquitination proteins, proteasome proteins, caspases, adaptor/mediator proteins and molecules, direct NF-κB regulators and inhibitors, the NF-κB family components and other transcription factors. Given the very small dimension of this network in terms of number of nodes and interactions, the topological analysis is very fast but poorly significant.

As said, all these proteins have been manually chosen on the basis of literature evidence for their involvement and role in the NF-κB pathway cascade (see Notes).

The 140 proteins have been divided in functional groups as in the following table 3.1: (for further details of each protein see table 3.2):

Table 3.1 Core interactome protein list divided in the main functional groups

Adaptor molecules
BCL10
ECSIT
MYD88
RRAS2
TIRAP
TRADD
TRAF2
TRAF3
TRAF5
TRAF6

Caspases
BIRC3
BIRC2
CASP3

Cell surface receptors
ERBB2
ERBB3
GP143
GPR1
IL1R1
MET
NTRK1
P2RY2
PGFRA
PGFRB
RET
TIE1
TLR1
TLR2
TLR3
TLR4
TNR16
TNR1A
TNR21
TR10A
TR10B
TYRO3

G Proteins
GBB1
GBB2
GBB3
GBB4
GBB5
GBG1
GNAI1
GNAS1
GNAS2

Main kinases
AKT1
ATM
BMX
BTK
CAR11
CSK21
E2AK2
FAK2
IRAK1
IRAK4
ITK
KAPCA
KPCT
KSYK
M3K1
M3K3
MALT1
MAPK2
MAPK5
P85A
PDE3B
PDE4D
PDK1
PLCG1
PLCG2
PTEN
RIPK1
SIRT1
SRC
TAB1
TAB2
TAB3
TAK1L
TEC
TXK

NF-κB family
NFKB1
NFKB2
REL
RELB
TF65

NF-κB family inhibitors
IKBA
IKBB
IKBE

NF-κB regulators
BCL3
IKKA
IKKB
IKKE
M3K14
NEMO
PIDD
SUMO1
SUMO2
SUMO3
SUMO4
TANK

Proteasome
PRS10
PRS6A
PRS6B
PRS7
PRS8
PSA1
PSA2
PSA3
PSA4
PSA5
PSA6
PSA7
PSB1
PSB10
PSB2
PSB3
PSB4
PSB5
PSB6
PSB7
PSB8
PSB9
PSD11
PSD13
PSDE
PSMD2
PSMD4
PSMD6

TCR-CD3 complex
FYN
LCK
TCR-CD3
ZAP70

Transcriptional activity
FOXO3
IF2A
NKRF
P53

Ubiquitination
RBX1
SKP1
UBIQ

In figure 3.1 the complete core interactome is depicted with its components, main functional roles and interactions.

Some considerations about the presence of given proteins in the core interactome: due to the importance of the 26S proteasome complex (composed by one 20S core particle structure and two 19S regulatory caps) in the dynamics of the activation of NF-κB, the complete set of proteins composing the complex appears in the list, each with its own interaction, as from evidenced by experimental data retrieved.

All the surface receptors listed appear able to trigger one or more adaptor molecules downstream and thus initiate –at least preliminarily and/or often complementarily with other receptors and proteins- the first steps of NF-κB activation cascade.

The TCR-CD3 complex appears as a small separate group given the very peculiar dynamics of this kind of receptor.

G proteins, short for guanine nucleotide-binding proteins, are a family of proteins involved in second messenger cascades. G proteins function as "molecular switches", alternating from 'inactive' guanosine diphosphate (GDP) to 'active' guanosine triphosphate (GTP), which is a binding state, and which proceeds to regulate downstream cell processes. G proteins are important signal transducing molecules in cells. There are evidences that diseases such as diabetes, blindness, allergies, depression, cardiovascular defects and certain forms of cancer, among other pathologies, arise due to derangement of G protein signaling. The human genomes encodes roughly 350 G protein-coupled receptors (GPCRs), which can detect photons (light), hormones, growth factors, drugs, and other endogenous ligands. Up to now, approximately 150 of the GPCRs found in the human genome have unknown functions.

In conclusion, the presence of the proteins in the list is justified by the evidence of a relevant direct role in the integrative dynamics of NF-κB activation, or otherwise by their evident capability of interaction with one or more proteins of the pathway, thus qualifying them as having a likely relevant role.

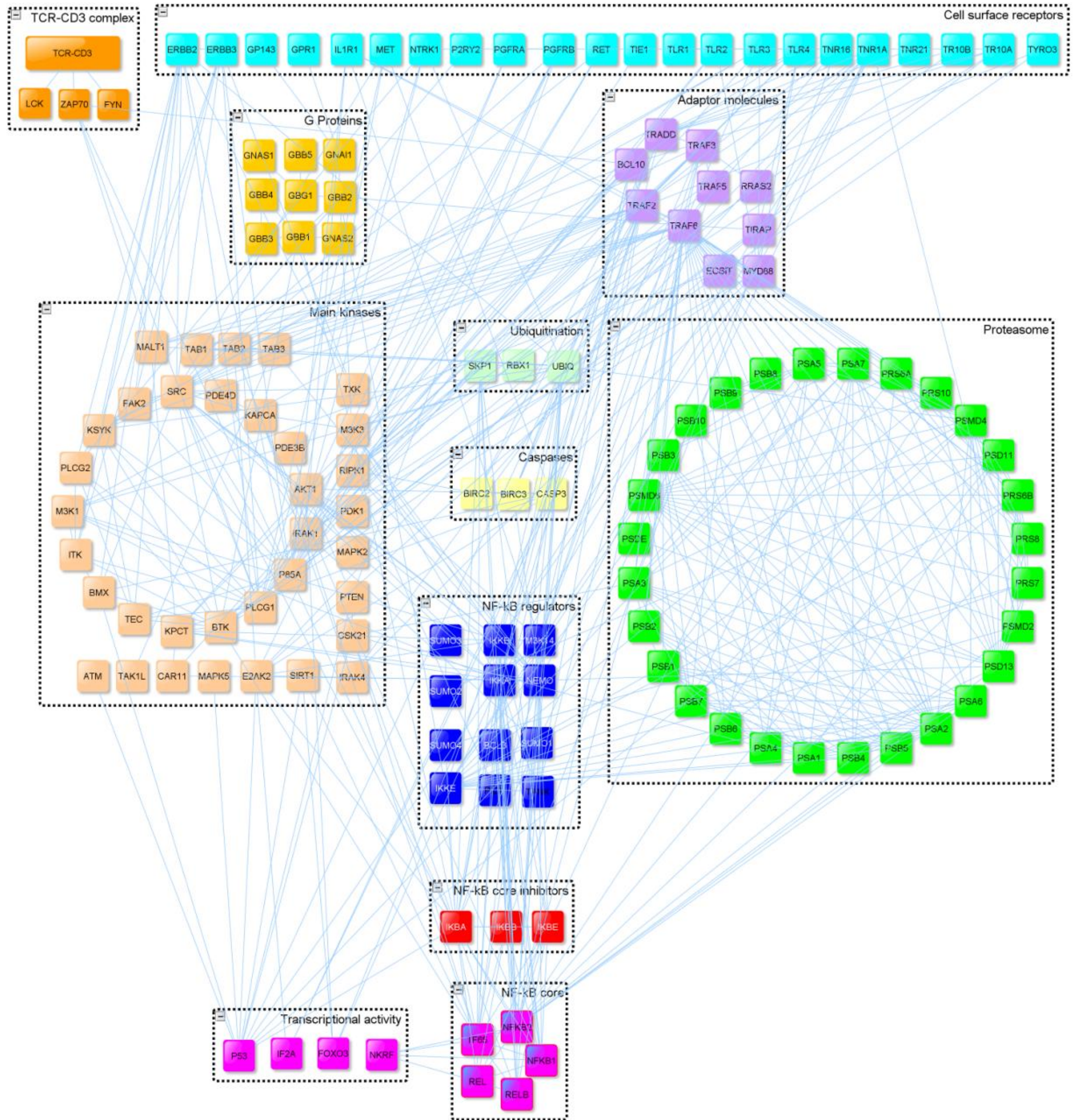


Fig. 3.1 Integral view of the NF-κB pathway core interactome consisting of 140 proteins. Proteins are grouped following 12 main functional roles (see Table 3.1 and 3.2 for a detailed description of the proteins present).

Table 3.2 Complete alphabetical list (UniProt name) of the 140 proteins that compose the core interactome

Accession	*UniProt name*	*Protein name*	*Gene name*
Q08828	ADCY1_HUMAN	Adenylate cyclase type 1	ADCY1
P31749	AKT1_HUMAN	RAC-alpha serine/threonine-protein kinase	AKT1
Q13315	ATM_HUMAN	Serine-protein kinase ATM	ATM
O95999	BCL10_HUMAN	B-cell lymphoma/leukemia 10	BCL10
P20749	BCL3_HUMAN	B-cell lymphoma 3-encoded protein	BCL3
Q13490	BIRC2_HUMAN	Baculoviral IAP repeat-containing protein 2	BIRC2
Q13489	BIRC3_HUMAN	Baculoviral IAP repeat-containing protein 3	BIRC3
P51813	BMX_HUMAN	Cytoplasmic tyrosine-protein kinase BMX	BMX
Q06187	BTK_HUMAN	Tyrosine-protein kinase BTK	BTK
Q9BXL7	CAR11_HUMAN	Caspase recruitment domain-containing protein 11	CARD11
P42574	CASP3_HUMAN	Caspase-3	CASP3
P68400	CSK21_HUMAN	Casein kinase II subunit alpha (CK II)	CSNK2A1
P19525	E2AK2_HUMAN	Interferon-induced, double-stranded RNA-activated protein kinase	EIF2AK2
Q9BQ95	ECSIT_HUMAN	Evolutionarily conserved signaling intermediate in Toll pathway, mitochondrial	ECSIT
P04626	ERBB2_HUMAN	Receptor tyrosine-protein kinase erbB-2	ERBB2
P21860	ERBB3_HUMAN	Receptor tyrosine-protein kinase erbB-3	ERBB3
Q14289	FAK2_HUMAN	Protein tyrosine kinase 2 beta	PTK2B
O43524	FOXO3_HUMAN	Forkhead box protein O3	FOXO3
P62873	GBB1_HUMAN	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	GNB1
P62879	GBB2_HUMAN	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	GNB2
P16520	GBB3_HUMAN	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3	GNB3
Q9HAV0	GBB4_HUMAN	Guanine nucleotide-binding protein subunit beta-4	GNB4
O14775	GBB5_HUMAN	Guanine nucleotide-binding protein subunit beta-5	GNB5
P63211	GBG1_HUMAN	Guanine nucleotide-binding protein G(T) subunit gamma-T1	GNGT1
P63096	GNAI1_HUMAN	Guanine nucleotide-binding protein G(i), alpha-1 subunit	GNAI1
Q5JWF2	GNAS1_HUMAN	Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	GNAS

P63092	GNAS2_HUMAN	Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	GNAS
P51810	GP143_HUMAN	G-protein coupled receptor 143	GPR143
P46091	GPR1_HUMAN	Probable G-protein coupled receptor 1	GPR1
P05198	IF2A_HUMAN	Eukaryotic translation initiation factor 2 subunit 1	EIF2S1
P25963	IKBA_HUMAN	NF-kappa-B inhibitor alpha (I-kappa-B-alpha)	NFKBIA
Q15653	IKBB_HUMAN	NF-kappa-B inhibitor beta (I-kappa-B-beta)	NFKBIB
O00221	IKBE_HUMAN	NF-kappa-B inhibitor epsilon (I-kappa-B-epsilon)	NFKBIE
O15111	IKKA_HUMAN	Inhibitor of nuclear factor kappa-B kinase subunit alpha (I kappa-B kinase alpha)	CHUK
Q14164	IKKE_HUMAN	Inhibitor of nuclear factor kappa-B kinase subunit epsilon (I kappa-B kinase epsilon)	IKBKE
P14778	IL1R1_HUMAN	Interleukin-1 receptor type I	IL1R1
P15260	INGR1_HUMAN	Interferon-gamma receptor alpha chain	IFNGR1
P51617	IRAK1_HUMAN	Interleukin-1 receptor-associated kinase 1	IRAK1
Q9NWZ3	IRAK4_HUMAN	Interleukin-1 receptor-associated kinase 4	IRAK4
Q08881	ITK_HUMAN	Tyrosine-protein kinase ITK/TSK	ITK
P17612	KAPCA_HUMAN	cAMP-dependent protein kinase catalytic subunit alpha	PRKACA
Q04759	KPCT_HUMAN	Protein kinase C theta type	PRKCQ
P43405	KSYK_HUMAN	Tyrosine-protein kinase SYK	SYK
Q99558	M3K14_HUMAN	Mitogen-activated protein kinase kinase kinase 14	MAP3K14
Q13233	M3K1_HUMAN	Mitogen-activated protein kinase kinase kinase 1	MAP3K1
Q99759	M3K3_HUMAN	Mitogen-activated protein kinase kinase kinase 3	MAP3K3
Q9UDY8	MALT1_HUMAN	Mucosa-associated lymphoid tissue lymphoma translocation protein 1	MALT1
P49137	MAPK2_HUMAN	MAP kinase-activated protein kinase 2	MAPKAPK2
Q16644	MAPK3_HUMAN	MAP kinase-activated protein kinase 3	MAPKAPK3
Q8IW41	MAPK5_HUMAN	MAP kinase-activated protein kinase 5	MAPKAPK5
P08581	MET_HUMAN	Hepatocyte growth factor receptor (HGF receptor)	MET
Q99836	MYD88_HUMAN	Myeloid differentiation primary response protein MyD88	MYD88
Q9Y6K9	NEMO_HUMAN	NF-kappa-B essential modulator (NEMO)	IKBKG
P19838	NFKB1_HUMAN	Nuclear factor NF-kappa-B p105 subunit	NFKB1

Q00653	NFKB2_HUMAN	Nuclear factor NF-kappa-B p100 subunit	NFKB2
O15226	NKRF_HUMAN	NF-kappa-B-repressing factor	NKRF
P04629	NTRK1_HUMAN	High affinity nerve growth factor receptor	NTRK1
P47900	P2RY1_HUMAN	P2Y purinoceptor 1	P2RY1
P41231	P2RY2_HUMAN	P2Y purinoceptor 2	P2RY2
P04637	P53_HUMAN	Cellular tumor antigen p53	TP53
P27986	P85A_HUMAN	Phosphatidylinositol 3-kinase regulatory subunit alpha (PI3K)	PIK3R1
Q13370	PDE3B_HUMAN	cGMP-inhibited 3',5'-cyclic phosphodiesterase B	PDE3B
Q08499	PDE4D_HUMAN	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	PDE4D
Q15118	PDK1_HUMAN	[Pyruvate dehydrogenase [lipoamide]] kinase isozyme 1, mitochondrial	PDK1
P16234	PGFRA_HUMAN	Alpha-type platelet-derived growth factor receptor	PDGFRA
P09619	PGFRB_HUMAN	Beta-type platelet-derived growth factor receptor	PDGFRB
Q9HB75	PIDD_HUMAN	Leucine-rich repeat and death domain-containing protein (p53-induced protein with a death domain)	LRDD
P19174	PLCG1_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-1	PLCG1
P16885	PLCG2_HUMAN	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2	PLCG2
P62333	PRS10_HUMAN	26S protease regulatory subunit S10B	PSMC6
P17980	PRS6A_HUMAN	26S protease regulatory subunit 6A	PSMC3
P43686	PRS6B_HUMAN	26S protease regulatory subunit 6B	PSMC4
P35998	PRS7_HUMAN	26S protease regulatory subunit 7	PSMC2
P62195	PRS8_HUMAN	26S protease regulatory subunit 8	PSMC5
P25786	PSA1_HUMAN	Proteasome subunit alpha type-1	PSMA1
P25787	PSA2_HUMAN	Proteasome subunit alpha type-2	PSMA2
P25788	PSA3_HUMAN	Proteasome subunit alpha type-3	PSMA3
P25789	PSA4_HUMAN	Proteasome subunit alpha type-4	PSMA4
P28066	PSA5_HUMAN	Proteasome subunit alpha type-5	PSMA5
P60900	PSA6_HUMAN	Proteasome subunit alpha type-6	PSMA6
O14818	PSA7_HUMAN	Proteasome subunit alpha type-7	PSMA7
P40306	PSB10_HUMAN	Proteasome subunit beta type-10	PSMB10

P20618	PSB1_HUMAN	Proteasome subunit beta type-1	PSMB1
P49721	PSB2_HUMAN	Proteasome subunit beta type-2	PSMB2
P49720	PSB3_HUMAN	Proteasome subunit beta type-3	PSMB3
P28070	PSB4_HUMAN	Proteasome subunit beta type-4	PSMB4
P28074	PSB5_HUMAN	Proteasome subunit beta type-5	PSMB5
P28072	PSB6_HUMAN	Proteasome subunit beta type-6	PSMB6
Q99436	PSB7_HUMAN	Proteasome subunit beta type-7	PSMB7
P28062	PSB8_HUMAN	Proteasome subunit beta type-8	PSMB8
P28065	PSB9_HUMAN	Proteasome subunit beta type-9	PSMB9
O00231	PSD11_HUMAN	26S proteasome non-ATPase regulatory subunit 11	PSMD11
Q9UNM6	PSD13_HUMAN	26S proteasome non-ATPase regulatory subunit 13	PSMD13
O00487	PSDE_HUMAN	26S proteasome non-ATPase regulatory subunit 14	PSMD14
Q13200	PSMD2_HUMAN	26S proteasome non-ATPase regulatory subunit 2	PSMD2
P55036	PSMD4_HUMAN	26S proteasome non-ATPase regulatory subunit 4	PSMD4
Q15008	PSMD6_HUMAN	26S proteasome non-ATPase regulatory subunit 6	PSMD6
P60484	PTEN_HUMAN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	PTEN
P62877	RBX1_HUMAN	RING-box protein 1	RBX1
Q04864	REL_HUMAN	C-Rel proto-oncogene protein	REL
Q01201	RELB_HUMAN	Transcription factor RelB	RELB
P07949	RET_HUMAN	Proto-oncogene tyrosine-protein kinase receptor ret	RET
Q13546	RIPK1_HUMAN	Receptor-interacting serine/threonine-protein kinase 1	RIPK1
P62070	RRAS2_HUMAN	Ras-related protein R-Ras2	RRAS2
Q96EB6	SIRT1_HUMAN	NAD-dependent deacetylase sirtuin-1	SIRT1
P63208	SKP1_HUMAN	S-phase kinase-associated protein 1	SKP1
P12931	SRC_HUMAN	Proto-oncogene tyrosine-protein kinase Src	SRC
P63165	SUMO1_HUMAN	Small ubiquitin-related modifier 1	SUMO1
P61956	SUMO2_HUMAN	Small ubiquitin-related modifier 2	SUMO2
P55854	SUMO3_HUMAN	Small ubiquitin-related modifier 3	SUMO3

Q6EEV6	SUMO4_HUMAN	Small ubiquitin-related modifier 4	SUMO4
Q15750	TAB1_HUMAN	Mitogen-activated protein kinase kinase kinase 7-interacting protein 1	MAP3K7IP1
Q9NYJ8	TAB2_HUMAN	Mitogen-activated protein kinase kinase kinase 7-interacting protein 2	MAP3K7IP2
Q8N5C8	TAB3_HUMAN	Mitogen-activated protein kinase kinase kinase 7-interacting protein 3	MAP3K7IP3
P57077	TAK1L_HUMAN	TAK1-like protein	TAK1L
Q92844	TANK_HUMAN	TRAF family member-associated NF-kappa-B activator	TANK
P01848	TCA_HUMAN	T-cell receptor alpha chain C region	TRAC
P01850	TCB_HUMAN	T-cell receptor beta chain C region	TRBC1
P42680	TEC_HUMAN	Tyrosine-protein kinase Tec	TEC
Q04206	TF65_HUMAN	Transcription factor p65	RELA
P35590	TIE1_HUMAN	Tyrosine-protein kinase receptor Tie-1	TIE1
P58753	TIRAP_HUMAN	Toll/interleukin-1 receptor domain-containing adapter protein	TIRAP
Q15399	TLR1_HUMAN	Toll-like receptor 1	TLR1
O60603	TLR2_HUMAN	Toll-like receptor 2	TLR2
O15455	TLR3_HUMAN	Toll-like receptor 3	TLR3
O00206	TLR4_HUMAN	Toll-like receptor 4	TLR4
P08138	TNR16_HUMAN	Tumor necrosis factor receptor superfamily member 16 (NGF receptor)	NGFR
P19438	TNR1A_HUMAN	Tumor necrosis factor receptor superfamily member 1A	TNFRSF1A
O75509	TNR21_HUMAN	Tumor necrosis factor receptor superfamily member 21	TNFRSF21
O00220	TR10A_HUMAN	Tumor necrosis factor receptor superfamily member 10A	TNFRSF10A
O14763	TR10B_HUMAN	Tumor necrosis factor receptor superfamily member 10B	TNFRSF10B
Q96RJ3	TR13C_HUMAN	Tumor necrosis factor receptor superfamily member 13C	TNFRSF13C
Q15628	TRADD_HUMAN	Tumor necrosis factor receptor type 1-associated DEATH domain protein	TRADD
Q12933	TRAF2_HUMAN	TNF receptor-associated factor 2	TRAF2
Q13114	TRAF3_HUMAN	TNF receptor-associated factor 3	TRAF3
O00463	TRAF5_HUMAN	TNF receptor-associated factor 5	TRAF5
Q9Y4K3	TRAF6_HUMAN	TNF receptor-associated factor 6	TRAF6
P42681	TXK_HUMAN	Tyrosine-protein kinase TXK	TXK

Q06418	TYRO3_HUMAN	Tyrosine-protein kinase receptor TYRO3	TYRO3
P62988	UBIQ_HUMAN	Ubiquitin	RPS27A
Accession	***Entry name***	***Protein name***	***Gene name***

Table 3.3 List of the 829 couples of interactions among the 140 proteins of the core interactome (legend: interacting protein UniProt name [number of literature sources/evidences stating the interaction] interacting protein UniProt name; proteins are listed in alphabetical order; data downloaded from the APID database).

AKT1 [1] AKT1
AKT1 [1] IRAK1
AKT1 [1] MAPK2
AKT1 [2] IKKA
AKT1 [2] SRC
AKT1 [2] UBIQ
AKT1 [3] BCL10
ATM [4] ATM
ATM [8] P53
BCL10 [1] BCL10
BCL10 [1] TLR4
BCL10 [1] TR10A
BCL10 [1] TRADD
BCL10 [5] CAR11
BCL10 [6] MALT1
BCL3 [1] REL
BCL3 [2] BCL10
BCL3 [2] RELB
BIRC2 [1] M3K14
BIRC2 [1] TRADD
BIRC2 [2] CASP3
BIRC2 [9] TRAF2
BIRC3 [1] RIPK1
BIRC3 [1] TNR1A
BIRC3 [1] UBIQ
BIRC3 [2] CASP3
BIRC3 [2] NEMO
BIRC3 [2] TRADD
BIRC3 [9] TRAF2
BMX [1] BTK
BMX [1] ERBB2
BMX [1] ITK
BMX [1] SRC
BMX [1] TEC
BTK [1] GBB1
BTK [1] IRAK1
BTK [1] PLCG1
BTK [1] TF65
BTK [2] ITK
BTK [2] KPCT
BTK [2] KSYK
BTK [2] TEC
BTK [3] BTK
BTK [3] PLCG2
CAR11 [2] MALT1
CAR11 [2] NEMO
CASP3 [1] BMX
CASP3 [2] CASP3
CASP3 [2] MET
CASP3 [2] TRAF3
CASP3 [3] AKT1
CSK21 [1] CSK21
CSK21 [1] PSA4
CSK21 [2] PTEN
CSK21 [2] TF65
E2AK2 [1] IKKA
E2AK2 [1] PGFRB
E2AK2 [1] TIRAP
E2AK2 [2] CASP3
E2AK2 [3] E2AK2
ERBB2 [1] UBIQ
ERBB2 [3] P85A
ERBB2 [4] ERBB2
ERBB2 [5] ERBB3
ERBB3 [1] ERBB3
ERBB3 [1] FAK2
ERBB3 [1] ITK
ERBB3 [1] KSYK
ERBB3 [1] PLCG1
ERBB3 [1] SRC
ERBB3 [1] TXK
ERBB3 [4] P85A
FAK2 [1] ERBB2
FAK2 [3] FAK2
FAK2 [3] SRC
FOXO3 [1] SIRT1
FOXO3 [2] AKT1
GBB1 [1] GBB2
GBB1 [1] GNAS2
GBB1 [4] GBG1
GBB2 [1] GBG1
GBB3 [1] GBB1
GBB3 [1] GBB2
GBB4 [1] GBB1
GBB4 [1] GBB2
GBB4 [1] GBB3
GBB4 [1] GBG1
GBG1 [1] GBB5
GBG1 [1] GNAS1
GBG1 [3] GBB3
GNAI1 [1] GNAI1
GNAI1 [1] GPR1
GNAS1 [1] GNAI1
GNAS1 [1] GNAS1
GNAS2 [1] GNAS2
GP143 [2] GNAI1
IF2A [1] CASP3
IF2A [4] E2AK2
IKBA [1] IKBB
IKBA [1] IKKE
IKBA [1] SUMO1
IKBA [2] CSK21
IKBA [2] E2AK2
IKBA [2] IKBA
IKBA [2] NEMO
IKBA [2] SKP1
IKBA [2] SRC
IKBA [5] UBIQ
IKBA [8] IKKA
IKBB [1] IKBB
IKBB [1] NEMO
IKBB [1] NFKB2
IKBB [1] SKP1
IKBB [2] NFKB1
IKBB [2] TNR16
IKBE [1] IKBA
IKBE [1] IKBB
IKBE [1] IKBE
IKBE [1] IKKA
IKBE [1] NEMO
IKBE [1] PSA5
IKBE [1] SKP1
IKBE [4] NFKB1
IKBE [4] NFKB2
IKBE [4] TF65
IKKA [1] BCL10
IKKA [1] FOXO3
IKKA [1] IKKE
IKKA [1] M3K1
IKKA [1] M3K3
IKKA [1] REL
IKKA [10] M3K14
IKKA [16] NEMO
IKKA [2] IKBB
IKKA [3] IKKA
IKKA [4] TF65
IKKA [5] NFKB1
IKKE [1] GBB2
IKKE [1] IKKE
IKKE [1] PRS10
IKKE [1] PRS6B
IKKE [1] PRS7
IKKE [1] PSA1
IKKE [1] PSA2
IKKE [1] PSA6
IKKE [1] PSA7
IKKE [1] PSB1
IKKE [1] PSB3
IKKE [1] PSB4
IKKE [1] PSB6
IKKE [1] PSDE
IKKE [1] PSMD2
IKKE [1] PSMD6
IKKE [1] TRAF2
IKKE [3] TANK
IL1R1 [1] M3K14
IL1R1 [1] TRAF6
IL1R1 [2] P85A
INGR1 [1] INGR1
IRAK1 [1] IKKA
IRAK1 [1] IRAK1
IRAK1 [1] TAB2
IRAK1 [2] IL1R1
IRAK1 [5] TRAF6
IRAK4 [3] IRAK1
IRAK4 [3] MYD88
ITK [1] ERBB2
ITK [1] SRC
ITK [2] ITK
ITK [3] PLCG1
KPCT [1] BCL10
KPCT [1] CAR11
KPCT [1] CASP3
KPCT [1] IKKA
KPCT [1] MALT1
KPCT [1] NEMO
KPCT [2] AKT1
KPCT [2] KPCT
KSYK [1] ERBB2
KSYK [1] FAK2
KSYK [1] TRAF6
KSYK [3] P85A
KSYK [3] PLCG1
KSYK [4] KSYK
M3K1 [1] M3K1
M3K1 [2] TRAF2
M3K1 [2] UBIQ
M3K14 [1] M3K14
M3K14 [1] REL
M3K14 [1] RIPK1
M3K14 [1] TRAF5
M3K14 [2] NEMO
M3K14 [2] TRAF3
M3K14 [4] TRAF2
M3K3 [1] GBB1
M3K3 [1] GBB2
M3K3 [1] NEMO
M3K3 [1] RIPK1
M3K3 [1] TRAF6
M3K3 [1] UBIQ
M3K3 [2] IKBA
M3K3 [2] KAPCA
M3K3 [2] M3K3
MALT1 [1] MALT1
MALT1 [1] TAB2
MALT1 [3] TRAF6
MALT1 [3] UBIQ
MAPK2 [3] MAPK2
MAPK5 [1] P53
MET [3] MET
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MYD88 [2] IL1R1
MYD88 [2] MYD88
MYD88 [3] IRAK1
MYD88 [4] TIRAP
MYD88 [4] TLR2
MYD88 [5] TLR4
NEMO [1] NFKB1
NEMO [1] PSD13
NEMO [1] REL
NEMO [1] TANK
NEMO [2] MALT1
NEMO [2] SRC
NEMO [5] UBIQ
NEMO [6] BCL10
NEMO [8] NEMO
NFKB1 [1] KAPCA
NFKB1 [11] NFKB1
NFKB1 [12] BCL3
NFKB1 [5] NFKB2
NFKB1 [6] IKBA
NFKB2 [1] IKBA
NFKB2 [1] IKKA
NFKB2 [1] IKKE
NFKB2 [1] NEMO
NFKB2 [1] PRS8
NFKB2 [1] PSA3
NFKB2 [1] PSA6
NFKB2 [1] PSB5
NFKB2 [1] PSD13
NFKB2 [1] PSMD2
NFKB2 [1] SKP1
NFKB2 [1] TRAF3
NFKB2 [1] UBIQ
NFKB2 [2] M3K14
NFKB2 [2] NFKB2
NFKB2 [3] PSD11
NFKB2 [4] TF65
NFKB2 [5] BCL3
NKRF [1] NFKB1
NKRF [1] NFKB2
NKRF [1] REL
NKRF [1] TF65
NTRK1 [1] UBIQ
NTRK1 [2] NTRK1
NTRK1 [3] PLCG1
P53 [1] BMX
P53 [1] IKKA
P53 [13] P53
P53 [2] CSK21
P53 [2] IKBA
P53 [3] E2AK2
P53 [4] SUMO1
P85A [1] AKT1
P85A [1] FAK2
P85A [1] IKBA
P85A [1] MET
P85A [1] RRAS2
P85A [2] NTRK1
P85A [2] P85A
P85A [2] TIE1
PDE3B [2] AKT1
PDE3B [2] KAPCA
PDE4D [1] KAPCA
PDE4D [1] SRC
PDE4D [3] PDE4D
PDK1 [1] AKT1
PDK1 [1] PDK1
PGFRA [1] P85A
PGFRA [2] PGFRB
PGFRA [2] PLCG1
PGFRA [5] PGFRA
PGFRB [1] SRC
PGFRB [6] P85A
PGFRB [6] PGFRB
PGFRB [8] PLCG1
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PIDD [1] RIPK1
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PLCG1 [1] PLCG2
PLCG1 [1] SRC
PLCG1 [2] RET
PLCG1 [3] ERBB2
PLCG2 [1] ERBB2
PLCG2 [1] ITK
PLCG2 [1] NTRK1
PLCG2 [1] P85A
PLCG2 [1] PLCG2
PLCG2 [3] KSYK
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PRS10 [1] PSA3
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PRS10 [1] PSA6
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PRS10 [1] PSB7
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PRS6A [2] PRS7
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PRS6B [1] PSB4
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PRS6B [1] PSB7
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PRS6B [2] PSMD4
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PRS6B [4] PRS6A
PRS6B [4] PRS8
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PRS7 [1] PSB2
PRS7 [1] PSB4
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PRS8 [1] PSB5
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PRS8 [2] PRS7
PRS8 [2] PSD13
PRS8 [2] PSMD4
PRS8 [3] PRS6A
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PSA1 [1] PRS7
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PSA1 [1] PSB9
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PSA1 [1] PSMD4
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PSA2 [7] PSB3
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PSA7 [2] PSMD4
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PSA7 [4] PSB4
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PSB1 [2] PSMD4
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PSB1 [4] PSA7
PSB1 [4] PSB4
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PSB10 [1] PSB9
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PSB2 [1] PSB9
PSB2 [1] PSD13
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PSB5 [4] PSA7
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PSB9 [1] PSA5
PSB9 [2] PSB7
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PSD11 [1] PSB4
PSD11 [1] PSB5
PSD11 [1] PSB7
PSD11 [1] PSMD2
PSD11 [1] SUMO2
PSD11 [2] PSD13
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PSD13 [1] PSA2
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PSMD4 [1] PSB4
PSMD4 [1] PSB5
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PSMD4 [2] PSA2
PSMD4 [2] PSA3
PSMD4 [2] PSB7
PSMD4 [2] PSMD2
PSMD4 [3] PSD13
PSMD4 [4] UBIQ
PSMD6 [1] PSA5
PSMD6 [1] PSB1
PSMD6 [1] PSB2
PSMD6 [1] PSB4
PSMD6 [1] PSDE
PSMD6 [2] PRS10
PSMD6 [2] PRS6B
PSMD6 [2] PRS8
PSMD6 [2] PSA1
PSMD6 [2] PSA2
PSMD6 [2] PSA3
PSMD6 [2] PSA6
PSMD6 [2] PSB3
PSMD6 [2] PSB7
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PSMD6 [2] PSMD2
PSMD6 [3] PRS6A
PSMD6 [3] PRS7
PSMD6 [3] PSB5
PSMD6 [3] PSD13
PSMD6 [3] PSMD4
PTEN [1] P53
PTEN [1] PGFRB
PTEN [2] AKT1
REL [1] IKKE
REL [2] IKBA
REL [2] IKBB
REL [2] RELB
REL [3] REL
REL [4] NFKB2
REL [5] IKBE
REL [5] NFKB1
REL [9] TF65
RELB [1] IKBA
RELB [1] IKBB
RELB [1] IKBE
RELB [1] IKKA
RELB [1] NEMO
RELB [1] RELB
RELB [1] UBIQ
RELB [2] NFKB1
RELB [2] TF65
RELB [4] NFKB2
RET [2] P85A
RET [2] RET
RIPK1 [1] TR10A
RIPK1 [1] TRAF5
RIPK1 [2] RIPK1
RIPK1 [4] NEMO
RIPK1 [5] TRAF2
RRAS2 [2] TRAF3

SIRT1 [3] P53
SKP1 [1] IKKA
SKP1 [1] IKKE
SKP1 [1] NEMO
SKP1 [1] NFKB1
SKP1 [1] REL
SKP1 [1] TF65
SKP1 [12] RBX1
SKP1 [3] SKP1
SRC [1] KSYK
SRC [1] MET
SRC [1] RET
SRC [2] ERBB2
SRC [2] KAPCA
SRC [2] P2RY2
SRC [2] P85A
SRC [3] IKKA
SRC [4] SRC
SUMO1 [1] IRAK1
SUMO1 [2] SUMO1
SUMO2 [1] SUMO3
SUMO2 [2] SUMO2
SUMO3 [1] SUMO3
SUMO4 [2] IKBA
TAB1 [1] IRAK1
TAB1 [1] TAB1
TAB1 [1] TRAF2
TAB1 [1] UBIQ
TAB1 [2] TAB3
TAB2 [1] E2AK2
TAB2 [1] TLR3
TAB2 [1] TRAF2
TAB2 [1] UBIQ
TAB2 [2] NFKB1
TAB2 [2] TAB2
TAB2 [3] TAB1
TAB3 [1] NEMO
TAB3 [1] TAB3
TAB3 [1] TRAF2
TAB3 [2] TAB2
TAK1L [1] TAB3
TANK [1] IKKA
TANK [1] TANK
TANK [5] TRAF2
TCB [3] TCA
TEC [1] ERBB2
TEC [1] P85A
TEC [1] PLCG1
TEC [1] PLCG2
TEC [1] TEC
TF65 [1] BCL3
TF65 [1] IKKE
TF65 [1] M3K14
TF65 [1] NEMO
TF65 [1] SIRT1
TF65 [12] NFKB1
TF65 [15] IKBA
TF65 [4] TF65
TF65 [4] UBIQ
TF65 [5] KAPCA
TF65 [8] IKBB
TIE1 [1] TIE1
TIRAP [1] TIRAP
TIRAP [1] TRAF6
TIRAP [2] BTK
TLR1 [1] TLR2
TLR2 [1] IRAK1
TLR2 [1] P85A
TLR2 [1] TLR2
TLR3 [1] E2AK2
TLR3 [1] MYD88
TLR3 [1] TRAF6
TLR3 [2] P85A
TLR3 [2] SRC
TLR3 [3] TLR3
TLR4 [1] BTK
TLR4 [1] IRAK1
TLR4 [1] KSYK
TLR4 [1] TLR1
TLR4 [3] TIRAP
TNR16 [1] TRADD
TNR16 [1] TRAF2
TNR16 [1] TRAF3
TNR16 [1] TRAF5
TNR16 [2] TRAF6
TNR16 [5] NTRK1
TNR1A [1] BCL10
TNR1A [1] IKKA
TNR1A [1] NEMO
TNR1A [1] TR10B
TNR1A [1] UBIQ
TNR1A [3] BIRC2
TNR1A [3] SUMO1
TNR1A [4] PSMD2
TNR1A [4] TRAF2
TNR1A [6] RIPK1
TNR1A [6] TNR1A
TNR21 [1] TRADD
TR10A [1] BTK
TR10A [2] TR10A
TR10B [1] RIPK1
TR10B [1] TR10A
TR10B [1] TRADD
TR13C [2] TR13C
TRADD [1] TRAF3
TRADD [1] UBIQ
TRADD [16] TNR1A
TRADD [2] RIPK1
TRADD [3] TR10A
TRADD [4] TRADD
TRADD [8] TRAF2
TRAF2 [1] MALT1
TRAF2 [1] UBIQ
TRAF2 [2] BCL10
TRAF2 [2] IKKA
TRAF2 [3] TRAF6
TRAF2 [6] TRAF2
TRAF3 [1] SRC
TRAF3 [1] TRAF3
TRAF3 [2] BIRC3
TRAF3 [2] RIPK1
TRAF3 [3] TANK
TRAF3 [3] TRAF2
TRAF5 [2] TRAF2
TRAF5 [2] TRAF3
TRAF5 [2] TRAF5
TRAF5 [2] TRAF6
TRAF6 [1] BCL10
TRAF6 [1] E2AK2
TRAF6 [1] GBB2
TRAF6 [1] IF2A
TRAF6 [1] PRS10
TRAF6 [1] PRS6A
TRAF6 [1] PRS6B
TRAF6 [1] PRS7
TRAF6 [1] PSA2
TRAF6 [1] PSA3
TRAF6 [1] PSA4
TRAF6 [1] PSA6
TRAF6 [1] PSB2
TRAF6 [1] PSB3
TRAF6 [1] PSB4
TRAF6 [1] PSB6
TRAF6 [1] PSD11
TRAF6 [1] PSDE
TRAF6 [1] PSMD4
TRAF6 [1] SRC
TRAF6 [2] IRAK4
TRAF6 [2] PRS8
TRAF6 [2] PSA1
TRAF6 [2] PSB5
TRAF6 [2] PSD13
TRAF6 [2] PSMD2
TRAF6 [2] PSMD6
TRAF6 [2] TRAF6
TRAF6 [3] ECSIT
TRAF6 [3] M3K14
TRAF6 [3] TAB1
TRAF6 [3] TAB3
TRAF6 [4] TAB2
TRAF6 [4] UBIQ
TXK [1] ERBB2
TXK [2] SRC
TXK [2] TXK
TYRO3 [1] SRC
TYRO3 [2] P85A
UBIQ [1] IKBB
UBIQ [1] IRAK1
UBIQ [1] KSYK
UBIQ [1] SRC
UBIQ [1] SUMO3
UBIQ [19] P53
UBIQ [4] BCL10
UBIQ [4] UBIQ

From a purely topological point of view, the analysis of the core interactome does not unveil big surprises, being so small (140 nodes and 829 interactions). Some interesting topological parameters show that there are few proteins that are central in the network, while the NF-κB family performs on average as for what concern node degree and betweenness centrality.

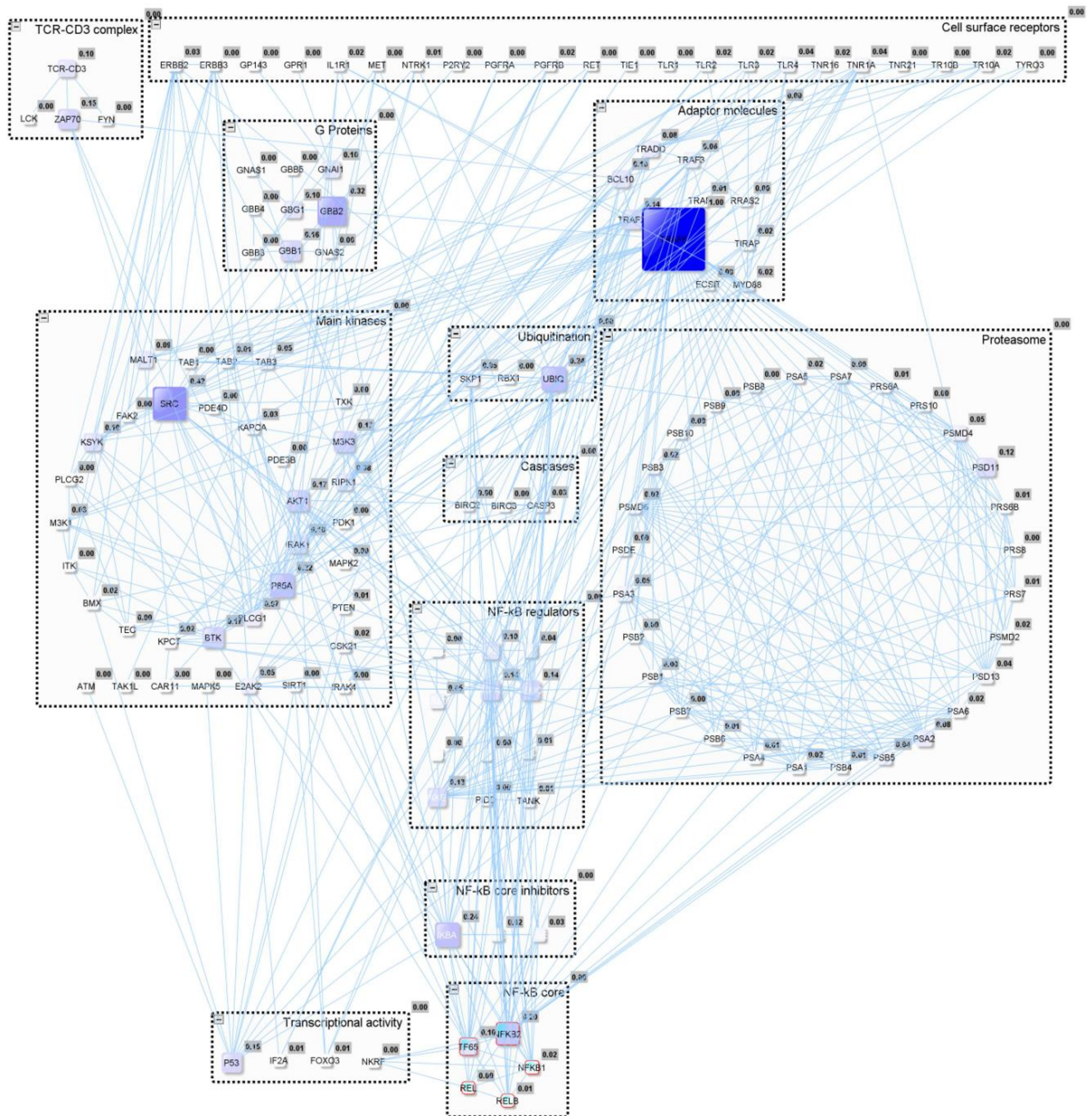

Figure 3.2 Core interactome, betweenness centrality chart: among the most central proteins, there are the signaling protein TRAF6, the kinase SRC, Ubiquitin, the phosphoinositide 3-kinase regulatory subunit P85A, the G proteins GBB2 and GBG1,

and the NF-κB inhibitors IKKE and IKKA (bigger and darker: more central; normalized values [0-1] for betweenness centrality are shown in the figure).

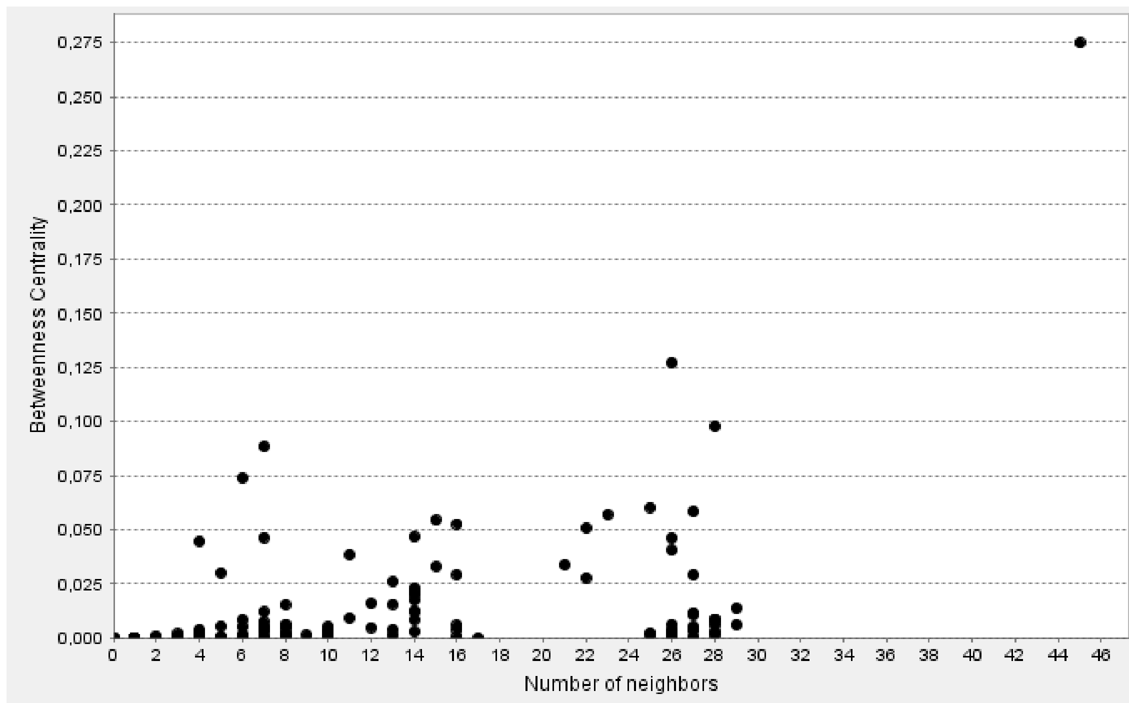

Fig 3.3 Core interactome, betweenness centrality vs number of neighbors

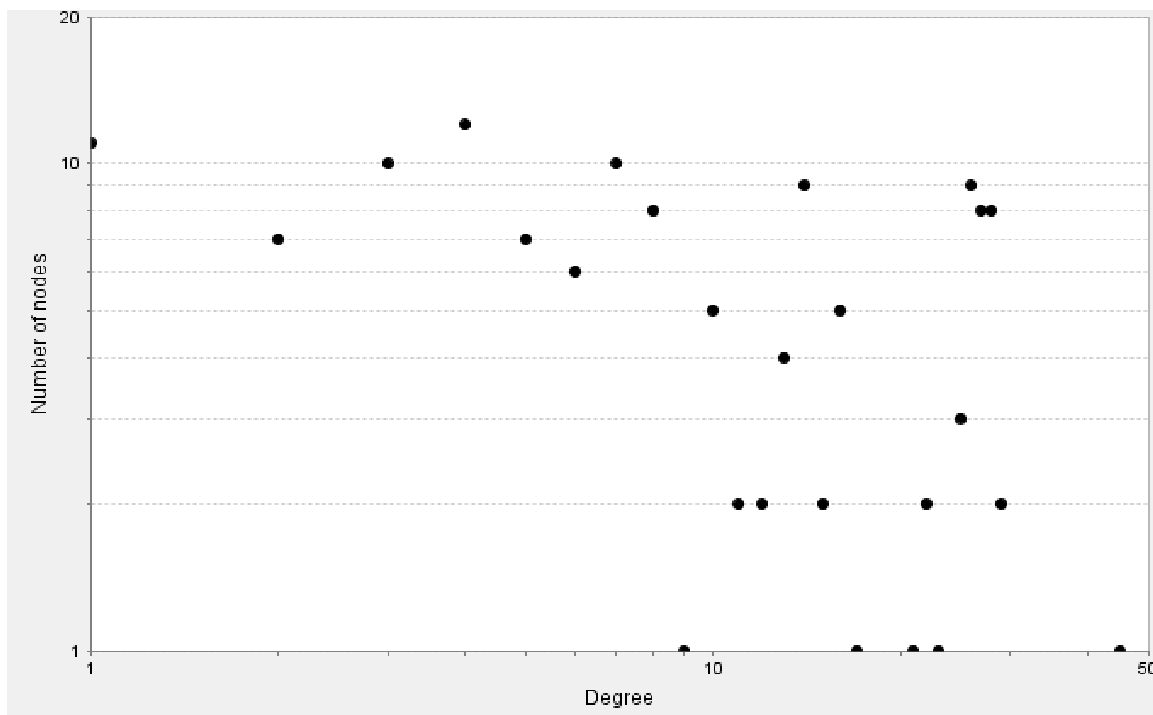

Fig 3.4 Core interactome, node degree distribution

Table 3.4 Core interactome, list of the 20 best ranking out of 140 core proteins for betweenness centrality.

Protein ID	Betweenness Centrality	Node Degree	Gene	Uniprot name
TRAF6	0.27481073	45	TRAF6	TRAF6_HUMAN
SRC	0.12720539	26	SRC	SRC_HUMAN
UBIQ	0.09787445	28	RPS27A	UBIQ_HUMAN
GBB2	0.088465	7	GNB2	GBB2_HUMAN
GBG1	0.07390701	6	GNGT1	GBG1_HUMAN
IKKE	0.06033168	25	IKBKE	IKKE_HUMAN
IKKA	0.05834001	27	CHUK	IKKA_HUMAN
P85A	0.05695809	23	PIK3R1	P85A_HUMAN
AKT1	0.05432327	15	AKT1	AKT1_HUMAN
BTK	0.052642	16	BTK	BTK_HUMAN
IKBA	0.05076037	22	NFKBIA	IKBA_HUMAN
P53	0.04655629	14	TP53	P53_HUMAN
GBB1	0.04639713	7	GNB1	GBB1_HUMAN
NEMO	0.04621614	26	IKBKG	NEMO_HUMAN
GNAS1	0.04476058	4	GNAS	GNAS1_HUMAN
NFKB2	0.04055352	26	NFKB2	NFKB2_HUMAN
M3K3	0.03858709	11	MAP3K3	M3K3_HUMAN
TF65	0.03384585	21	RELA	TF65_HUMAN
IRAK1	0.03297771	15	IRAK1	IRAK1_HUMAN
GNAI1	0.03018737	5	GNAI1	GNAI1_HUMAN

Table 3.5 Betweenness centrality value and ranking, and node degree of the members of the NF-κB family in the core interactome.

Protein	Betweenness Centrality	Rank	Degree
NFKB2	0.04055352	16°	26
TF65	0.03384585	18°	21
NFKB1	0.00587078	50°	16
REL	0.0040526	61°	16
RELB	0.00108828	86°	13

Among the core proteins, some of them show a prominent topological position in the network, position that may indicate a particular role in ruling the signaling cascade. Betweennees centrality has be chosen as a key parameter because there is evidence of its particularly relevant significancy in biological systems (Platzer 2007). A couple of significant cases are briefly discussed.

As shown from above data, the most central protein is the mediator molecule TRAF6. It is a member of the TNF receptor associated factor (TRAF) protein family. Its extraordinary betweenness centrality can be explained by the fact that TRAF proteins are associated with, and mediate signal transduction from members of the TNF receptor superfamily, but also from the members of the Toll/IL-1 family. There is evidence that TRAF6 also interacts with various protein kinases including IRAK1/IRAK, SRC and PKC-zeta, which provides a link between distinct signaling pathways. An more important, prominent role in inflammation processes for of this mediator protein has been very recently proposed: in mice, deficient TRAF6 signaling -but not TRAF2/3/5- in leukocytes prevents atherosclerosis by skewing the immune response toward an anti-inflammatory profile (Lutgens 2010). These data seem unveil a role for the TNF receptor superfamily costimulating protein CD40 and TRAF6 interactions in atherosclerosis and establish that targeting specific components of the CD40-CD40 Ligand pathway harbors the potential to achieve therapeutic effects in atherosclerosis.

Quite below the TRAF6 very high score, high rankings as well are achieved by the Proto-oncogene tyrosine-protein kinase Src (SRC), one of the most important non-receptor protein tyrosine kinases that plays a multitude of roles in cell signaling. The clinical importance in tumor signaling of this kinase is highlighted by the fact that several Src family kinase inhibitors have recently entered clinical trials based on their direct effects against tumor cells. Recent findings indeed indicate that Src kinase inhibitors (such as

dasatinib) possess a previously unrecognized anticancer mechanism of action by targeting key cell compartments of the tumor microenvironment (Liang 2010).

In the same manner, a help in getting clues about a particular protein role in signaling cascades may be derived by its topological scores.

3.2.2. Wider interactome, structure and network analysis

Following the reconstruction process detailed in the Methods section, the wider interactome results composed by a total of 3146 interacting proteins that include the 140 already present in the core interactome plus 3006 further proteins that show evidence of interaction with at least one belonging to the core interactome. The APID2NET system retrieves a total of 42638 protein-protein interactions. The complete list of proteins and interactions won't be reported here for lack of space. Data are available upon request.

The general structure of the wider interactome appears to be far from random, as from the comparison with simulated networks (Erdos-Renyi and Barabasi-Albert models; Barabasi 2002; see table 3.6). in particular, clustering coefficient of the real interactome greatly differs from those relative to two model networks of the same size. These data indicate that the interactome possesses its own peculiar structure, and not a random one, sign of a highly organized architecture.

Table 3.6 Basic network parameters of the wider interactome, compared with two different simulated randomized model networks (average values for 20 simulations).

	Wider interactome, 3146 nodes, real data	Simulated Erdos-Renyi model, 3146 nodes	Simulated Barabasi-Albert model, 3146 nodes
Number of edges	42638	42638	6289
Clustering coefficient	0.258	0.009	0.008
Network centralization	0.126	0.005	0.043
Characteristic path length	2.957	2.781	4.589
Network density	0.008	0.009	0.001

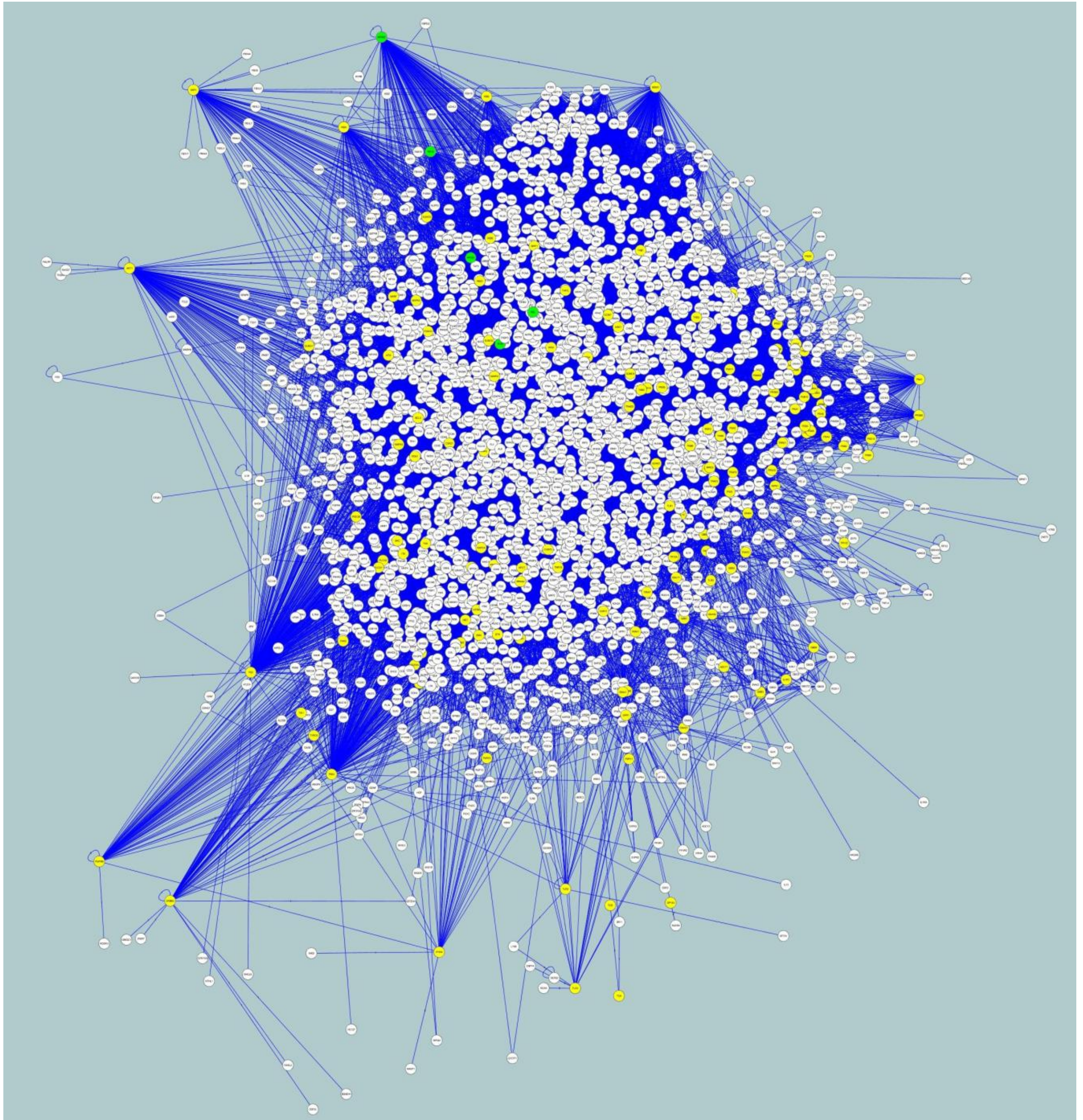

Fig. 3.5 Whole view of the wider interactome, accounting for a total of 3146 proteins. In yellow, the proteins of the core interactome, in green the five NF-κB family members. According to data shown, the general structure of the wider interactome appears to be far from random, as from the comparison with simulated networks. Data indicate that the interactome possesses its own peculiar structure, sign of a highly organized architecture Data elaborated and visualized with Cytoscape.

Existing clustering techniques allow the partitioning of protein interactions graphs to consider the connectivity properties of the underlying network. As said, Markov

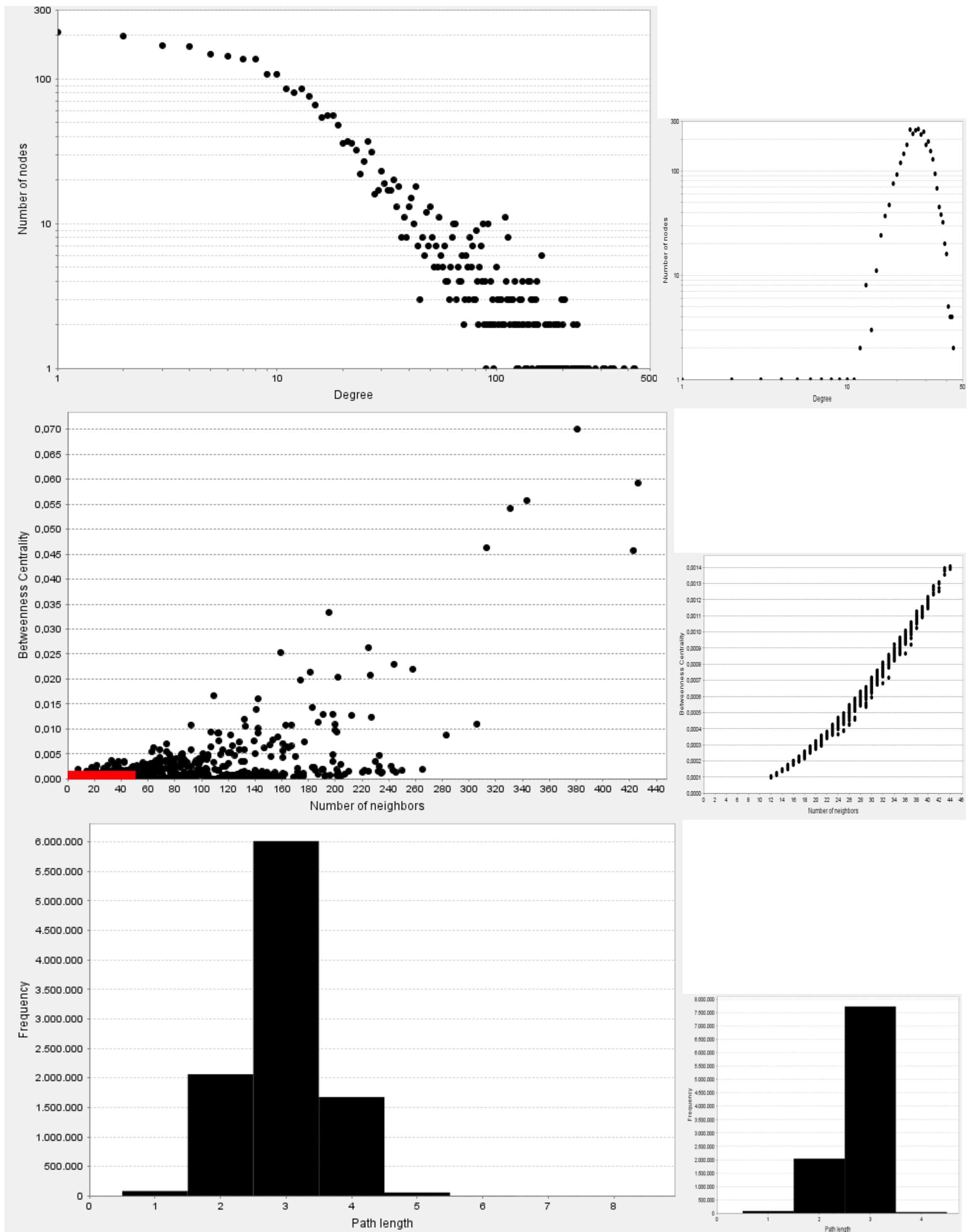

Fig 3.6 Comparison of (from above) node degree distribution, betweenness centrality and average path lengths in the wider interactome (left, large charts) vs simulated Erdos-Renyi model networks (right, small charts, not in scale). The red area in the betweenness centrality chart (second from above) is for comparison with the area taken by the betweenness centrality distribution of the Erdos-Renyi random network (small chart on the right). Wider interactome parameters significantly differ from those of random networks, thus confirming its inherently organized nature.

Clustering (MCL), for example, is a technique that may help in individuating clusters of protein interactions that possess a high degree of noise-tolerance.

MCL run on the dataset evidenced five main modules, highlighting the modular nature of the wider interactome. GO enrichment analysis identified main functions, biological processes, compartments and other relevant annotations of each of the clusters, as in table 3.7.

Table 3.7 Wider interactome, first five main modules isolated by Markov Clustering and GO enrichment analysis

Module	#Nodes	#Edges	Density	Profiler annotations		
				significance	*annotation reference*	*process/function/compartment*
# 1	**962**	**19002**	4.1 %	*6.15e-13*	**GO:BP**	cellular macromolecule metabolic processes
				5.70e-36	**GO:CC**	intracellular part
				4.17e-48	**GO:MF**	protein binding
				2.08e-07	**KEGG**	Proteasome
				1.43e-08	**REACTOME**	Activated TLR4 signalling pathway
# 2	**623**	**3796**	2.0 %	*4.63e-82*	**GO:BP**	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process
				1.32e-95	**GO:CC**	nucleus
				1.37e-72	**GO:MF**	transcription regulator activity
				4.57e-20	**KEGG**	Cell cycle
				3.77e-13	**REACTOME**	DNA Repair
# 3	**606**	**4052**	2.2 %	*5.69e-77*	**GO:BP**	cell communication
				5.43e-40	**GO:CC**	plasma membrane
				2.16e-82	**GO:MF**	protein binding
				2.42e-23	**KEGG**	ErbB signaling pathway
				1.97e-15	**REACTOME**	Down-stream signal transduction
# 4	**192**	**514**	2.8 %	*3.93e-14*	**GO:BP**	regulation of apoptosis
				8.08e-09	**GO:CC**	cytoplasm
				2.07e-08	**GO:MF**	protein binding
				1.01e-06	**KEGG**	Small cell lung cancer
				2.17e-07	**REACTOME**	TRADD:TRAF2:RIP1 complex formation and binding
# 5	**140**	**321**	3.3 %	*4.12e-06*	**GO:BP**	regulation of multicellular organismal process
				2.90e-06	**GO:CC**	sarcoplasmic reticulum
				5.30e-11	**GO:MF**	ligand-gated channel activity
				8.68e-05	**KEGG**	Calcium signaling pathway
				6.30e-05	**REACTOME**	Release of calcium from intracellular stores

As expected -and interestingly-, the first module results highly enriched in proteins related to key protein binding processes and belonging to functions that involve the proteasome. It comprises all cytoplasmic mediator proteins and kinases participating in the various steps of signaling cascades, as well as those that participate to proteasomal degradation. Moreover, proteins belonging to the Toll-Like Receptors (TLRs) signaling pathways -and TLR4 in particular- are overrepresented, fact that indicates a deeper intertwining of NF-κB and TLRs signaling pathways.

The module n. 2 incorporates proteins able to translocate in the nucleus and with remarkable transcriptional activity, while module n. 3 seems to be markedly linked to surface receptors and relative activity as well as in downstream signal transduction.

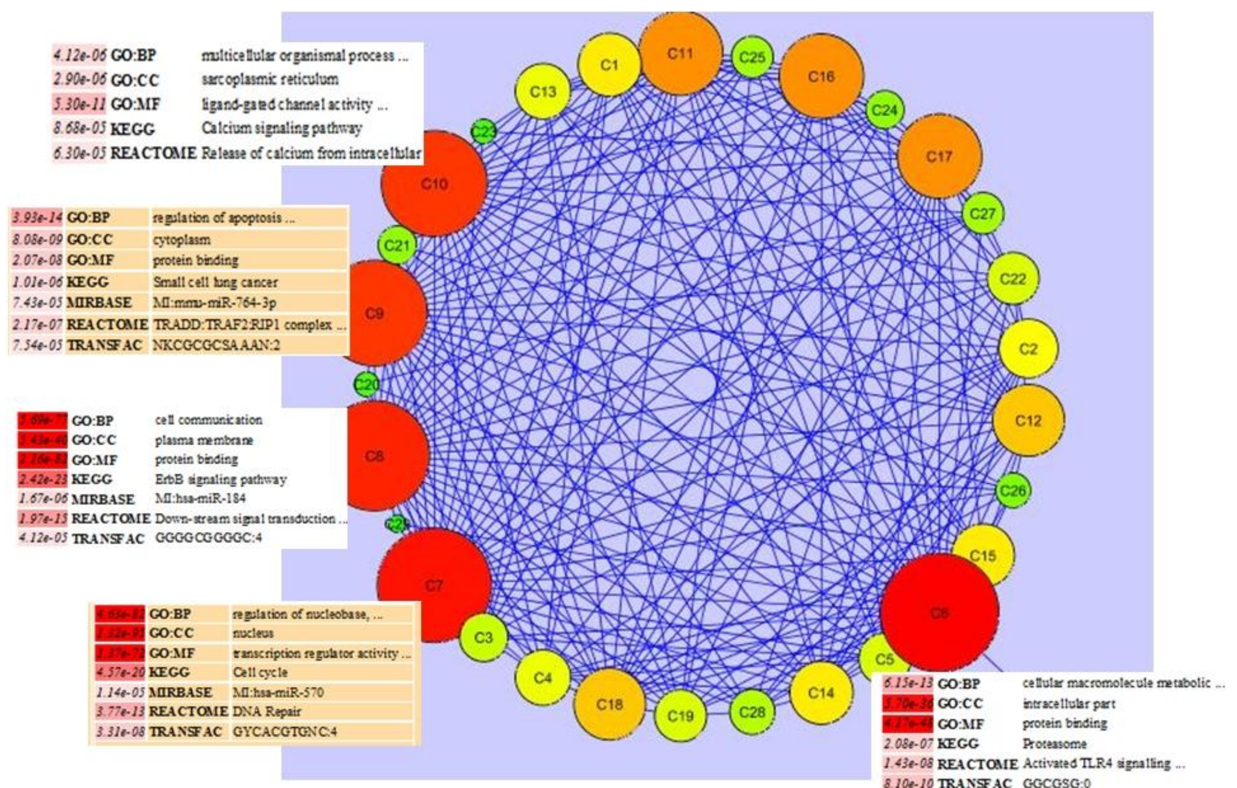

Fig 3.7 Wider interactome, MCL and GO enrichment, pictorial view showing the five main modules (bigger red cicles) and overrepresented GO categories.

The universe of proteins immediately around NF-κB (i.e. interacting with components of its pathway) thus appears to influence and to be influenced by processes related to

metabolism, calcium release, uptake and storage, protein binding and proteasomal activity, and to the function of several important cell compartments such as the nucleus, the membranes and the sarcoplasmic reticulum. As a proof, on the contrary, the NF-κB interactome does not seem particularly involved in energy and scavenging activities, such as mithocondrion and lysosome functioning.

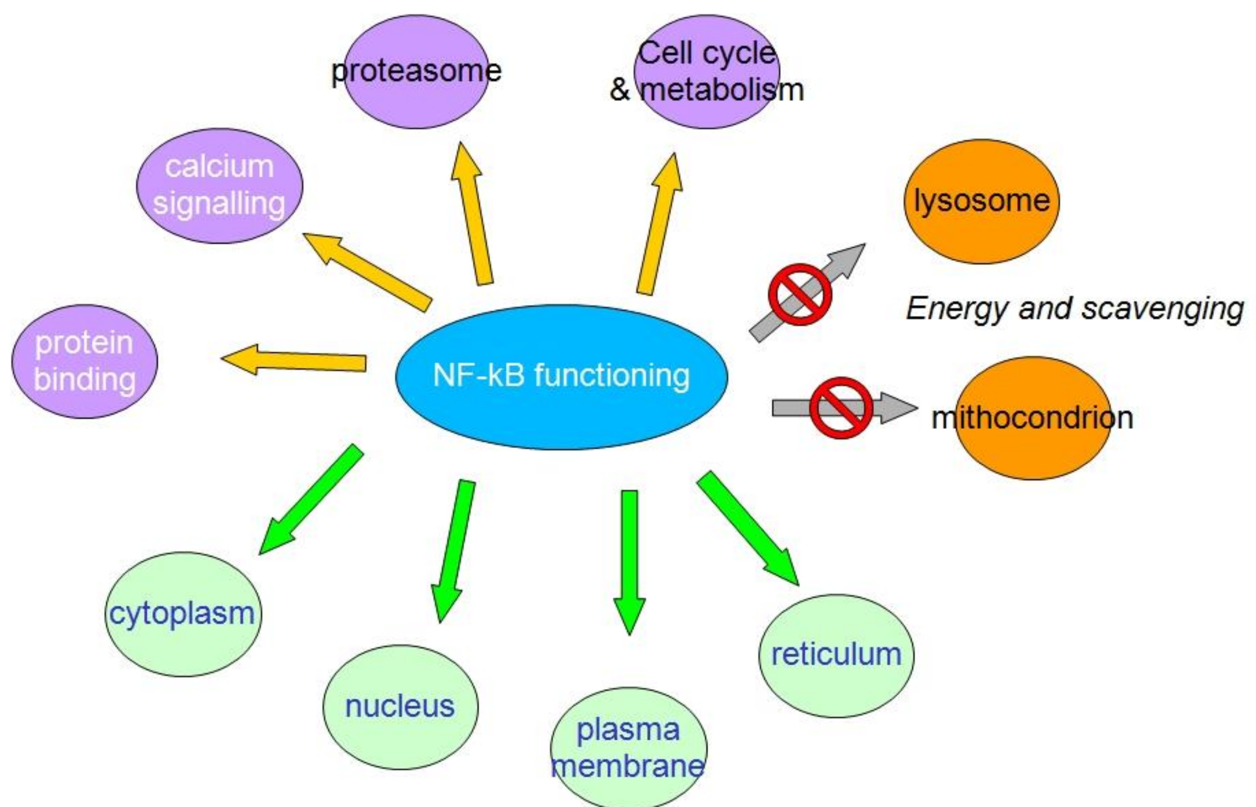

Fig 3.8 Wider interactome, MCL and GO enrichment analysis, NF-κB functioning appears to influence and to be influenced by processes related to metabolism and to the function of several important cell compartments such as cytoplasm, nucleus, membranes and reticulum, but not particularly involved in mithocondrion and lysosome functioning.

Since the wider interactome is reconstructed starting from the core interactome (as detailed in the Methods section) it is normal that the proteins belonging to the core have higher possibility to be more central (see table 3.8). Nevertheless, several proteins that do not appear in the core seem to have a relevant role in the wider interactome structure ranking high for betweenness centrality, as in the case of Protein kinase C inhibitor

protein 1 (1433Z) (see table 3.9 for description); RAC-alpha serine/threonine-protein kinase (AKT1), a general protein kinase capable of phosphorylating several known proteins, and of having complex effects on glucose metabolism and antiapoptotic signals; the multifunctional Myc proto-oncogene protein (MYC) that participates in the regulation of gene transcription binding DNA both in a non-specific manner and also specifically; and the Caspase-3 (CASP3), involved in the activation cascade of caspases responsible for apoptosis execution, among others.

Table 3.8 Wider interactome, most central proteins. First 15 proteins ranking for betweenness centrality.

Protein name	Betweeness centrality	Node degree	Gene name
TRAF6	0.06997543	381	TRAF6
GRB2	0.05919005	426	GRB2
IKKE	0.05579592	343	IKBKE
P53	0.0541714	331	TP53
SRC	0.04624446	313	SRC
UBIQ	0.04574077	423	RPS27A
TRAF2	0.03332032	195	TRAF2
EGFR	0.02631206	225	EGFR
KAPCA	0.02535598	159	PRKACA
TF65	0.02298955	244	RELA
1433Z	0.0219932	258	YWHAZ
CSK21	0.02141286	181	CSNK2A1
P85A	0.02076988	226	PIK3R1
NEMO	0.02030402	202	IKBKG

Table 3.9 Wider interactome, description of the 15 most central proteins according to betweenness centrality (alphabetically order by UniProt name).

Protein name	Description
1433Z_HUMAN	*14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1)*: Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathway. Binds to a large number of partners, usually by recognition of

	a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.
CSK21_HUMAN	*Casein kinase II subunit alpha*: Casein kinases are operationally defined by their preferential utilization of acidic proteins such as caseins as substrates. The alpha and alpha' chains contain the catalytic site.Participates in Wnt signaling. CK2 phosphorylates 'Ser-392' of p53/TP53 following UV irradiation.
EGFR_HUMAN	*Epidermal growth factor receptor (Receptor tyrosine-protein kinase ErbB-1)*: The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation. Mutations in this gene are associated with lung cancer.
GRB2_HUMAN	*Growth factor receptor-bound protein 2 (Adapter protein GRB2)*: The protein encoded by this gene binds the epidermal growth factor receptor and contains one SH2 domain and two SH3 domains. Its two SH3 domains direct complex formation with proline-rich regions of other proteins, and its SH2 domain binds tyrosine phosphorylated sequences. This gene is similar to the Sem5 gene of C. elegans, which is involved in the signal transduction pathway. Two alternatively spliced transcript variants encoding different isoforms have been found for this gene.
IKKE_HUMAN	*Inhibitor of nuclear factor kappa-B kinase subunit epsilon (I kappa-B kinase epsilon)*: Phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. May play a special role in the immune response.
KAPCA_HUMAN	*cAMP-dependent protein kinase catalytic subunit alpha (PKA C-alpha):* cAMP is a signaling molecule important for a variety of cellular functions. cAMP exerts its effects by activating the cAMP-dependent protein kinase, which transduces the signal through phosphorylation of different target proteins. The inactive kinase holoenzyme is a tetramer composed of two regulatory and two catalytic subunits. cAMP causes the dissociation of the inactive holoenzyme into a dimer of regulatory subunits bound to four cAMP and two free monomeric catalytic subunits. Four different regulatory subunits and three catalytic subunits have been identified in humans. The protein encoded by this gene is a member of the Ser/Thr protein kinase family and is a catalytic subunit of cAMP-dependent protein kinase. Alternatively spliced transcript variants encoding distinct isoforms have been observed.
NEMO_HUMAN	*NF-kappa-B essential modulator (NEMO) (NF-kappa-B essential modifier)*: Regulatory subunit of the IKK core complex which phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. Also considered to be a mediator for TAX activation of NF-kappa-B. Could be implicated in NF-kappa-B-mediated protection from cytokine toxicity.
P53_HUMAN	*Cellular tumor antigen p53 (Tumor suppressor p53)*: Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. Implicated in Notch signaling cross-over.
P85A_HUMAN	*Phosphatidylinositol 3-kinase regulatory subunit alpha (PI3-kinase p85 subunit alpha) (PI3K)*: Binds to activated (phosphorylated) protein-Tyr kinases, through its SH2 domain, and acts as an adapter, mediating the association of the p110

	catalytic unit to the plasma membrane. Necessary for the insulin-stimulated increase in glucose uptake and glycogen synthesis in insulin-sensitive tissues.
SRC_HUMAN	*Proto-oncogene tyrosine-protein kinase Src (pp60c-src) (p60-Src) (c-Src):* The protein encoded by this gene is a tyrosine-protein kinase whose activity can be inhibited by phosphorylation by c-SRC kinase. Mutations in this gene could be involved in the malignant progression of colon cancer. Two transcript variants encoding the same protein have been found for this gene.
TF65_HUMAN	*Transcription factor p65 (Nuclear factor NF-kappa-B p65 subunit)*: subunit of NF-kappa-B transcription complex.
TRAF2_HUMAN	*TNF receptor-associated factor 2 (Tumor necrosis factor type 2 receptor-associated protein 3)*: Adapter protein and signal transducer that links members of the tumor necrosis factor receptor family to different signaling pathways by association with the receptor cytoplasmic domain and kinases. Association to the receptor is also mediated by the interaction with TRADD. Mediates activation of NF-kappa-B and JNK and is involved in apoptosis. The TRAF1/TRAF2 complex recruits the apoptotic suppressors BIRC2 and BIRC3 to TNFRSF1B/TNFR2. Seems to be involved in IL-15 signaling.
TRAF6_HUMAN	*TNF receptor-associated factor 6 (Interleukin-1 signal transducer) (RING finger protein 85)*: Adapter protein and signal transducer that links members of the tumor necrosis factor receptor family to different signaling pathways by association with the receptor cytoplasmic domain and kinases. Also involved in the IL-1 signaling pathway via MYD88 and IRAK kinases. Seems to be involved in IL-17 signaling (By similarity). Mediates activation of NF-kappa-B and JNK. May function as an E3 ubiquitin ligase.
UBIQ_HUMAN	*Ubiquitin*: Highly conserved protein that has a major role in targeting cellular proteins for degradation by the 26S proteosome, is synthesized as a precursor protein consisting of either polyubiquitin chains or a single ubiquitin fused to an unrelated protein. It is a protein modifier which can be covalently attached to target lysines either as a monomer or as a lysine-linked polymer. Attachment to proteins as a Lys-48-linked polymer usually leads to their degradation by proteasome. Attachment to proteins as a monomer or as an alternatively linked polymer does not lead to proteasomal degradation and may be required for numerous functions, including maintenance of chromatin structure, regulation of gene expression, stress response, ribosome biogenesis and DNA repair.

Table 3.10 NF-κB subunits ranking for betweenness centrality in the wider interactome

Rank	Protein	Between centr	Node degree	Gene name
11°	TF65	0.02298955	244	RELA
31°	NFKB1	0.0107782	163	NFKB1
46°	NFKB2	0.00743673	177	NFKB2
78°	RELB	0.00464105	86	RELB
258°	REL	0.00141736	87	REL

Table 3.11 Wider interactome, proteins ranking from 16 to 100 for betweenness centrality

Protein	Between centr	Node degree	Gene name
M3K3	0.01978339	174	MAP3K3
SUMO1	0.01672142	109	SUMO1
AKT1	0.01601919	142	AKT1
ABL1	0.01433885	183	ABL1
CASP3	0.01398608	141	CASP3
MYC	0.01300149	198	MYC
CRCM	0.01286892	191	MCC
1433G	0.01269315	212	YWHAG
FYN	0.01242612	227	FYN
TNR1A	0.01196486	132	TNFRSF1A
HS90A	0.01133857	187	HSP90AA1
EF1A1	0.01101538	306	EEF1A1
HSP7C	0.01086117	200	HSPA8
PLCG1	0.0108359	167	PLCG1
SKP1	0.01083512	92	SKP1
NFKB1	0.0107782	163	NFKB1
KPCA	0.01050862	133	PRKCA
ESR1	0.01007701	142	ESR1
1B42	0.00972535	200	HLA-B
ARRB2	0.00945463	201	ARRB2
IKBA	0.00942124	107	NFKBIA
GNAI2	0.00923252	112	GNAI2
ERBB2	0.00922718	113	ERBB2
MK01	0.00920674	142	MAPK1
SUMO3	0.00880842	122	SUMO3
HS90B	0.00871235	283	HSP90AB1
VIME	0.00844735	157	VIM
EP300	0.00779088	153	EP300
CTNB1	0.00768794	113	CTNNB1
M3K14	0.0075835	139	MAP3K14
NFKB2	0.00743673	177	NFKB2
IKKA	0.00734046	128	CHUK
VHL	0.00711999	161	VHL
SUMO4	0.00698174	74	SUMO4
CUL1	0.00674148	151	CUL1
BRCA1	0.00670832	123	BRCA1
LCK	0.00654623	167	LCK
ACTB	0.00648923	164	ACTB
GRP78	0.00636037	107	HSPA5
NCK1	0.00634203	165	NCK1
GNAI1	0.0063114	64	GNAI1
CBP	0.00615844	147	CREBBP
PAXI	0.00592148	132	PXN
ANDR	0.00589548	128	AR
ERBB3	0.00588372	69	ERBB3
UBC9	0.00585242	92	UBE2I
SMAD3	0.00580522	116	SMAD3
1433F	0.00562166	161	YWHAH
CALM	0.00553478	74	CALM1
BTK	0.00538402	63	BTK
ARRB1	0.00524068	122	ARRB1

Protein	Between centr	Node degree	Gene name
M3K1	0.00519011	120	MAP3K1
STAT3	0.00508126	101	STAT3
CRK	0.00505448	140	CRK
RAF1	0.00501893	86	RAF1
KSYK	0.00496613	85	SYK
IKKB	0.00494709	114	IKBKB
PRS8	0.00487421	76	PSMC5
KU70	0.00483331	198	XRCC6
CDC2	0.00482632	105	CDC2
GBLP	0.00476892	125	GNB2L1
NPM	0.00476585	233	NPM1
RELB	0.00464105	86	RELB
JUN	0.00454575	105	JUN
CH60	0.00450869	170	HSPD1
TBB5	0.00450428	152	TUBB
RUVB2	0.00434977	161	RUVBL2
CAV1	0.00433428	69	CAV1
RB	0.00430932	102	RB1
PRKDC	0.00427342	77	PRKDC
CASP8	0.00416809	81	CASP8
MK03	0.00405329	98	MAPK3
LYN	0.00385829	104	LYN
SP1	0.00383641	87	SP1
CFTR	0.00376504	76	CFTR
IKBB	0.00375868	85	NFKBIB
KPCZ	0.00371273	73	PRKCZ
PTEN	0.00367281	33	PTEN
HDAC1	0.00363935	100	HDAC1
NDKB	0.00362893	66	NME2
SMAD2	0.00360967	96	SMAD2
RET	0.00355832	38	RET
CDC42	0.00355019	70	CDC42
EIF1B	0.00352336	130	EIF1B
MET	0.00351246	42	MET

3.3. Downstream genes and feedback cycles

NF-κB-downstream genes data have been extracted and elaborated from a manually curated list (Gilmore 2010), from the TRED database and integrated with results from TRANSFAC database (see Materials and Methods). This procedure allowed to constitute a relatively comprehensive original list of 444 genes that result to be up- or down-regulated from NF-κB. Gene products and relative UniProt identifiers are obtained

directly through the ID mapping functions available on the UniProt web interface, allowing to compile the list of proteins which expression can be regulated by NF-κB.

A summary of data retrieved can be found in table 3.12:

Table 3.12 Sequential steps, method and result for NF-κB-downstream genes and protein list retrieval.

Step 1	Manual and automated retrieval from cited sources	**444** genes names and identifiers, original list
Step 2	Database mapping services (Genecards, Geneàlacarte)	**441** valid gene ENSEMBL identifiers retrieved
Step 3	Database mapping services (Uniprot, Genecards)	**422** valid reviewed Uniprot protein identifiers
Step 4	Database mapping services (APID, APID2NET plugin)	**384** protein identifiers present in the APID protein interactions database

The 444 gene names were mapped in a new list consisting of 441 valid ENSEMBL gene identifiers that, again, mapped into 422 gene products, identified as reviewed proteins by means of the Uniprot database mapping services. 384 out of these 422 protein identifiers have been successfully found in the APID database.

Table 3.13 shows the list of the 422 gene identifiers and relative reviewed protein identifiers retrieved.

Table 3.13 List of the 422 genes downstream NF-κB and mapping with 422 reviewed proteins

Gene names (aliases)	*Protein name*
SLC3A2 (MDU1)	4F2_HUMAN
ORM1 (AGP1)	A1AG1_HUMAN
SERPINA1 (AAT) (PI) (PRO0684)	A1AT_HUMAN
SERPINA2 (ARGS) (ATR) (PIL)	A1ATR_HUMAN
APP (A4) (AD1)	A4_HUMAN
ADORA1	AA1R_HUMAN
ADORA2A (ADORA2)	AA2AR_HUMAN
ADORA2B	AA2BR_HUMAN
SERPINA3 (AACT) (GIG24) (GIG25)	AACT_HUMAN
ABCA1 (ABC1) (CERP)	ABCA1_HUMAN
ABCB9 (KIAA1520)	ABCB9_HUMAN
ABCG5	ABCG5_HUMAN
ABCG8	ABCG8_HUMAN
APOBEC2	ABEC2_HUMAN
ADAM19 (MLTNB) (FKSG34)	ADA19_HUMAN
ADH1A (ADH1)	ADH1A_HUMAN
AHCTF1 (ELYS) (TMBS62) (MSTP108)	AHTF1_HUMAN
AICDA (AID)	AICDA_HUMAN
AKR1C1 (DDH) (DDH1)	AK1C1_HUMAN
AMACR	AMACR_HUMAN
AR (DHTR) (NR3C4)	ANDR_HUMAN
ANGPT1 (KIAA0003)	ANGP1_HUMAN
AGT (SERPINA8)	ANGT_HUMAN

APOC3	APOC3_HUMAN
APOD	APOD_HUMAN
APOE	APOE_HUMAN
AQP4	AQP4_HUMAN
ARFRP1 (ARP1)	ARFRP_HUMAN
PYCARD (ASC) (CARD5) (TMS1)	ASC_HUMAN
ASPH	ASPH_HUMAN
ASS1 (ASS)	ASSY_HUMAN
ATP1A2 (KIAA0778)	AT1A2_HUMAN
BCL2L1 (BCL2L) (BCLX)	B2CL1_HUMAN
BCL2A1 (BCL2L5) (BFL1) (GRS)	B2LA1_HUMAN
B2M (CDABP0092) (HDCMA22P)	B2MG_HUMAN
BACE1 (BACE) (KIAA1149)	BACE1_HUMAN
BAX (BCL2L4)	BAX_HUMAN
PIK3AP1 (BCAP)	BCAP_HUMAN
BCL2	BCL2_HUMAN
BCL3 (BCL4) (D19S37)	BCL3_HUMAN
BDNF	BDNF_HUMAN
BDKRB1 (BRADYB1)	BKRB1_HUMAN
BLNK (BASH) (SLP65)	BLNK_HUMAN
BMI1 (PCGF4) (RNF51)	BMI1_HUMAN
BMP2 (BMP2A)	BMP2_HUMAN
BMP4 (BMP2B) (DVR4)	BMP4_HUMAN
BNIP3 (NIP3)	BNIP3_HUMAN
BRCA2 (FACD) (FANCD1)	BRCA2_HUMAN
BTK (AGMX1) (ATK) (BPK)	BTK_HUMAN
C4BPA (C4BP)	C4BPA_HUMAN
CALCA (CALC1)	CALC_HUMAN
CALCA (CALC1)	CALCA_HUMAN
CALCB (CALC2)	CALCB_HUMAN
CASP4 (ICH2)	CASP4_HUMAN
CTSB (CPSB)	CATB_HUMAN
CTSL1 (CTSL)	CATL1_HUMAN
CAV1 (CAV)	CAV1_HUMAN
CCL11 (SCYA11)	CCL11_HUMAN
CCL15 (MIP5) (NCC3) (SCYA15)	CCL15_HUMAN
CCL17 (SCYA17) (TARC)	CCL17_HUMAN
CCL19 (ELC) (MIP3B) (SCYA19)	CCL19_HUMAN
CCL1 (SCYA1)	CCL1_HUMAN
CCL20 (LARC) (MIP3A) (SCYA20)	CCL20_HUMAN
CCL22 (MDC) (SCYA22) (A-152E5.1)	CCL22_HUMAN
CCL23 (MIP3) (MPIF1) (SCYA23)	CCL23_HUMAN
CCL28 (SCYA28)	CCL28_HUMAN
CCL2 (MCP1) (SCYA2)	CCL2_HUMAN
CCL3 (G0S19-1) (MIP1A) (SCYA3)	CCL3_HUMAN
CCL4 (LAG1) (MIP1B) (SCYA4)	CCL4_HUMAN

CCL5 (D17S136E) (SCYA5)	CCL5_HUMAN
CCND1 (BCL1) (PRAD1)	CCND1_HUMAN
CCND2	CCND2_HUMAN
CCND3	CCND3_HUMAN
CCR5 (CMKBR5)	CCR5_HUMAN
CCR7 (CMKBR7) (EBI1) (EVI1)	CCR7_HUMAN
CD209 (CLEC4L)	CD209_HUMAN
CD38	CD38_HUMAN
CD3G (T3G)	CD3G_HUMAN
CD40LG (CD40L) (TNFSF5) (TRAP)	CD40L_HUMAN
CD44 (LHR) (MDU2) (MDU3) (MIC4)	CD44_HUMAN
CD48 (BCM1) (BLAST1)	CD48_HUMAN
CD69	CD69_HUMAN
CD80 (CD28LG) (CD28LG1) (LAB7)	CD80_HUMAN
CD83	CD83_HUMAN
CD86 (CD28LG2)	CD86_HUMAN
CDK6	CDK6_HUMAN
CDKN1A (CAP20) (CDKN1) (CIP1)	CDN1A_HUMAN
CDX1	CDX1_HUMAN
CFB (BF) (BFD)	CFAB_HUMAN
CFLAR (CASH) (CASP8AP1) (CLARP)	CFLAR_HUMAN
CHI3L1	CH3L1_HUMAN
CLDN2 (PSEC0059) (SP82)	CLD2_HUMAN
COL1A2	CO1A2_HUMAN
C3 (CPAMD1)	CO3_HUMAN
C4A (CO4) (CPAMD2)	CO4A_HUMAN
C4B (CO4) (CPAMD3)	CO4B_HUMAN
POMC	COLI_HUMAN
CYP19A1 (ARO1) (CYAR) (CYP19)	CP19A_HUMAN
CYP27B1 (CYP1ALPHA) (CYP27B)	CP27B_HUMAN
CYP2E1 (CYP2E)	CP2E1_HUMAN
CYP7B1	CP7B1_HUMAN
CR2 (C3DR)	CR2_HUMAN
CREB3 (LZIP)	CREB3_HUMAN
CRP (PTX1)	CRP_HUMAN
CSF1	CSF1_HUMAN
CSF2 (GMCSF)	CSF2_HUMAN
CSF3 (GCSF)	CSF3_HUMAN
GJB1 (CX32)	CXB1_HUMAN
CXCL2 (GRO2) (GROB) (MIP2A) (SCYB2)	CXCL2_HUMAN
CXCL3 (GRO3) (GROG) (SCYB3)	CXCL3_HUMAN
CXCL5 (ENA78) (SCYB5)	CXCL5_HUMAN
CXCL9 (CMK) (MIG) (SCYB9)	CXCL9_HUMAN
IL8RA (CMKAR1) (CXCR1)	CXCR1_HUMAN
IL8RB (CXCR2)	CXCR2_HUMAN
CXCR5 (BLR1) (MDR15)	CXCR5_HUMAN

CXCL10 (INP10) (SCYB10)	CXL10_HUMAN
CXCL11 (ITAC) (SCYB11) (SCYB9B)	CXL11_HUMAN
GAD1 (GAD) (GAD67)	DCE1_HUMAN
DCTN4	DCTN4_HUMAN
DEFB4 (DEFB102) (DEFB2)	DEFB2_HUMAN
HSD17B8 (FABGL) (HKE6) (RING2)	DHB8_HUMAN
HSD11B2 (HSD11K)	DHI2_HUMAN
DMP1	DMP1_HUMAN
DNASE1L2 (DHP1) (DNAS1L2)	DNSL2_HUMAN
REV3L (POLZ) (REV3)	DPOLZ_HUMAN
DPYD	DPYD_HUMAN
DUSP1 (CL100) (MKP1) (PTPN10) (VH1)	DUS1_HUMAN
E2F3 (KIAA0075)	E2F3_HUMAN
EDN1	EDN1_HUMAN
EGFR (ERBB1)	EGFR_HUMAN
ENG (END)	EGLN_HUMAN
EGR1 (ZNF225)	EGR1_HUMAN
PI3 (WAP3) (WFDC14)	ELAF_HUMAN
ELF3 (ERT) (ESX) (JEN)	ELF3_HUMAN
ENO2	ENOG_HUMAN
ERVWE1	ENW1_HUMAN
EPHA1 (EPH) (EPHT) (EPHT1)	EPHA1_HUMAN
EPO	EPO_HUMAN
ERBB2 (HER2) (NEU) (NGL)	ERBB2_HUMAN
F8 (F8C)	FA8_HUMAN
FABP6 (ILBP) (ILLBP)	FABP6_HUMAN
FCER2 (CD23A) (FCE2) (IGEBF)	FCER2_HUMAN
FCGRT (FCRN)	FCGRN_HUMAN
AFP (HPAFP)	FETA_HUMAN
FGF8 (AIGF)	FGF8_HUMAN
FN1 (FN)	FINC_HUMAN
FTH1 (FTH) (FTHL6) (OK/SW-cl.84))	FRIH_HUMAN
FSTL3 (FLRG) (UNQ674/PRO1308)	FSTL3_HUMAN
G6PC (G6PT)	G6PC_HUMAN
G6PD	G6PD_HUMAN
GADD45B (MYD118)	GA45B_HUMAN
GATA3	GATA3_HUMAN
GNB2L1 (HLC7) (PIG21)	GBLP_HUMAN
GBP1	GBP1_HUMAN
GCNT1 (NACGT2)	GCNT1_HUMAN
NR3C1 (GRL)	GCR_HUMAN
GUCY1A2 (GUC1A2) (GUCSA2)	GCYA2_HUMAN
SERPINE2 (PI7) (PN1)	GDN_HUMAN
GNAI2 (GNAI2B)	GNAI2_HUMAN
GNRH2	GON2_HUMAN
GZMB (CGL1) (CSPB) (CTLA1) (GRB)	GRAB_HUMAN

GRM2 (GPRC1B) (MGLUR2)	GRM2_HUMAN
CXCL1 (GRO) (GRO1) (GROA) (MGSA)	GROA_HUMAN
GCLM (GLCLR)	GSH0_HUMAN
GCLC (GLCL) (GLCLC)	GSH1_HUMAN
GSTP1 (FAEES3) (GST3)	GSTP1_HUMAN
SLC2A5 (GLUT5)	GTR5_HUMAN
HAS1 (HAS)	HAS1_HUMAN
HBZ (HBZ2)	HBAZ_HUMAN
HBE1 (HBE)	HBE_HUMAN
HAMP (HEPC) (LEAP1) (UNQ487/PRO1003)	HEPC_HUMAN
CD74 (DHLAG)	HG2A_HUMAN
HGF (HPTA)	HGF_HUMAN
HIF1A (MOP1)	HIF1A_HUMAN
HLA-G (HLA-6.0) (HLAG)	HLAG_HUMAN
HMGN1 (HMG14)	HMGN1_HUMAN
HMOX1 (HO) (HO1)	HMOX1_HUMAN
HPSE (HEP) (HPA) (HPA1) (HPR1)	HPSE_HUMAN
HSP90AA2 (HSPCAL3)	HS902_HUMAN
HSP90AA1 (HSP90A) (HSPC1) (HSPCA)	HS90A_HUMAN
HOXA9 (HOX1G)	HXA9_HUMAN
IL15RA	I15RA_HUMAN
IGFBP1 (IBP1)	IBP1_HUMAN
IGFBP2 (BP2) (IBP2)	IBP2_HUMAN
ICAM1	ICAM1_HUMAN
ICOS (AILIM)	ICOS_HUMAN
IER2 (ETR101)	IER2_HUMAN
IER3 (DIF2) (IEX1) (PRG1)	IEX1_HUMAN
IFI44L (C1orf29) (GS3686)	IF44L_HUMAN
IFNB1 (IFB) (IFNB)	IFNB_HUMAN
IFNG	IFNG_HUMAN
IGHE	IGHE_HUMAN
IGHG1	IGHG1_HUMAN
IGHG2	IGHG2_HUMAN
IGHG3	IGHG3_HUMAN
IGHG4	IGHG4_HUMAN
IGKC	IGKC_HUMAN
NFKBIA (IKBA) (MAD3) (NFKBI)	IKBA_HUMAN
NFKBIE (IKBE)	IKBE_HUMAN
NFKBIZ (IKBZ) (INAP) (MAIL)	IKBZ_HUMAN
IL10	IL10_HUMAN
IL11	IL11_HUMAN
IL12A (NKSF1)	IL12A_HUMAN
IL12B (NKSF2)	IL12B_HUMAN
IL13 (NC30)	IL13_HUMAN
IL15	IL15_HUMAN
IL17A (CTLA8) (IL17)	IL17_HUMAN

IL1A (IL1F1)	IL1A_HUMAN
IL1B (IL1F2)	IL1B_HUMAN
IL1RN (IL1F3) (IL1RA)	IL1RA_HUMAN
IL23A (SGRF) (UNQ2498/PRO5798)	IL23A_HUMAN
IL27 (IL27A)	IL27A_HUMAN
EBI3 (IL27B)	IL27B_HUMAN
IL2	IL2_HUMAN
IL2RA	IL2RA_HUMAN
IL32 (NK4) (TAIF)	IL32_HUMAN
IL6 (IFNB2)	IL6_HUMAN
IL8 (CXCL8)	IL8_HUMAN
IL9	IL9_HUMAN
SERPINB1 (ELANH2) (MNEI) (PI2)	ILEU_HUMAN
INHBA	INHBA_HUMAN
DIO2 (ITDI2) (TXDI2)	IOD2_HUMAN
IRF1	IRF1_HUMAN
IRF2	IRF2_HUMAN
IRF4 (MUM1)	IRF4_HUMAN
IRF7	IRF7_HUMAN
F11R (JAM1) (JCAM) (UNQ264/PRO301)	JAM1_HUMAN
JMJD3 (KDM6B) (KIAA0346)	JMJD3_HUMAN
JUNB	JUNB_HUMAN
KRT15 (KRTB)	K1C15_HUMAN
KRT3	K2C3_HUMAN
KRT5	K2C5_HUMAN
KRT6B (K6B) (KRTL1)	K2C6B_HUMAN
KCNK5 (TASK2)	KCNK5_HUMAN
KCNN2	KCNN2_HUMAN
KISS1 (PP5098)	KISS1_HUMAN
KLF10 (TIEG) (TIEG1)	KLF10_HUMAN
KLK3 (APS)	KLK3_HUMAN
PRKCD	KPCD_HUMAN
LAMB2 (LAMS)	LAMB2_HUMAN
LBP	LBP_HUMAN
LEF1	LEF1_HUMAN
LGALS3 (MAC2)	LEG3_HUMAN
LIPG (UNQ387/PRO719)	LIPE_HUMAN
ALOX12 (LOG12)	LOX12_HUMAN
ALOX5 (LOG5)	LOX5_HUMAN
SELE (ELAM1)	LYAM2_HUMAN
SELP (GMRP) (GRMP)	LYAM3_HUMAN
LYZ (LZM)	LYSC_HUMAN
MAP4K1 (HPK1)	M4K1_HUMAN
MADCAM1	MADCA_HUMAN
MBP	MBP_HUMAN
ABCB1 (MDR1) (PGY1)	MDR1_HUMAN

ABCB4 (MDR3) (PGY3)	MDR3_HUMAN
AMH (MIF)	MIS_HUMAN
MDK (MK1) (NEGF2)	MK_HUMAN
MMP1 (CLG)	MMP1_HUMAN
MMP3 (STMY1)	MMP3_HUMAN
MMP9 (CLG4B)	MMP9_HUMAN
SLC16A1 (MCT1)	MOT1_HUMAN
ABCC6 (ARA) (MRP6)	MRP6_HUMAN
MT3	MT3_HUMAN
MTHFR	MTHR_HUMAN
MUC2 (SMUC)	MUC2_HUMAN
MX1	MX1_HUMAN
MYB	MYB_HUMAN
MYC	MYC_HUMAN
MYLK (MLCK)	MYLK_HUMAN
MYOZ1 (MYOZ)	MYOZ1_HUMAN
NLRP2 (NALP2) (NBS1) (PAN1) (PYPAF2)	NALP2_HUMAN
ART1	NAR1_HUMAN
NCAM1 (NCAM)	NCAM1_HUMAN
NFKB1	NFKB1_HUMAN
NFKB2 (LYT10)	NFKB2_HUMAN
LCN2 (HNL) (NGAL)	NGAL_HUMAN
NGF (NGFB)	NGF_HUMAN
TACR1 (NK1R) (TAC1R)	NK1R_HUMAN
FAM148A (NLF1)	NLF1_HUMAN
GRIN2A (NMDAR2A)	NMDE1_HUMAN
GRIN1 (NMDAR1)	NMDZ1_HUMAN
NOD2 (CARD15) (IBD1)	NOD2_HUMAN
NOS1	NOS1_HUMAN
NOS2 (NOS2A)	NOS2_HUMAN
NPY1R (NPYR) (NPYY1)	NPY1R_HUMAN
NQO1 (DIA4) (NMOR1)	NQO1_HUMAN
NR4A2 (NOT) (NURR1) (TINUR)	NR4A2_HUMAN
SLC11A2 (NRAMP2) (OK/SW-cl.20)	NRAM2_HUMAN
NRG1 (GGF) (HGL) (HRGA) (NDF)	NRG1_HUMAN
NUAK2 (SNARK)	NUAK2_HUMAN
OLR1 (LOX1)	OLR1_HUMAN
OPRD1 (OPRD)	OPRD_HUMAN
OPRM1 (MOR1)	OPRM_HUMAN
OPN1SW (BCP)	OPSB_HUMAN
SPP1 (BNSP) (OPN) (PSEC0156)	OSTP_HUMAN
OXTR	OXYR_HUMAN
TP53 (P53)	P53_HUMAN
PLA2G4C	PA24C_HUMAN
PAFAH2	PAFA2_HUMAN
SERPINE1 (PAI1) (PLANH1)	PAI1_HUMAN

PAX8	PAX8_HUMAN
CD274 (B7H1) (PDCD1L1) (PDCD1LG1)	PD1L1_HUMAN
PDE7A	PDE7A_HUMAN
PDGFB (PDGF2) (SIS)	PDGFB_HUMAN
PDYN	PDYN_HUMAN
PENK	PENK_HUMAN
PRF1 (PFP)	PERF_HUMAN
PTGS2 (COX2)	PGH2_HUMAN
PGK1 (PGKA) (MIG10) (OK/SW-cl.110)	PGK1_HUMAN
PGLYRP1 (PGLYRP) (PGRP) (TNFSF3L)	PGRP_HUMAN
BGN (SLRR1A)	PGS1_HUMAN
PIGF	PIGF_HUMAN
PIGR	PIGR_HUMAN
PIM1	PIM1_HUMAN
PIK3CA	PK3CA_HUMAN
PLCD1	PLCD1_HUMAN
PLK3 (CNK) (FNK) (PRK)	PLK3_HUMAN
PPP5C (PPP5)	PPP5_HUMAN
PRDM1 (BLIMP1)	PRDM1_HUMAN
PGR (NR3C3)	PRGR_HUMAN
PRL	PRL_HUMAN
PSMA2 (PSC3)	PSA2_HUMAN
PSMB9 (LMP2) (RING12)	PSB9_HUMAN
PSME1 (IFI5111)	PSME1_HUMAN
PSME2	PSME2_HUMAN
PTAFR (PAFR)	PTAFR_HUMAN
PTEN (MMAC1) (TEP1)	PTEN_HUMAN
PTGDS (PDS)	PTGDS_HUMAN
PTGES (MGST1L1) (PGES) (PIG12)	PTGES_HUMAN
PTGIS (CYP8) (CYP8A1)	PTGIS_HUMAN
PTHLH (PTHRP)	PTHR_HUMAN
PTPN13 (PNP1) (PTP1E) (PTPL1)	PTN13_HUMAN
PTPN1 (PTP1B)	PTN1_HUMAN
PTS	PTPS_HUMAN
PTX3 (TNFAIP5) (TSG14)	PTX3_HUMAN
RAG1 (RNF74)	RAG1_HUMAN
RAG2	RAG2_HUMAN
AGER (RAGE)	RAGE_HUMAN
RBBP4 (RBAP48)	RBBP4_HUMAN
RDH5 (RDH1)	RDH1_HUMAN
REL	REL_HUMAN
RELB	RELB_HUMAN
RIPK2 (CARDIAK) (RICK) (RIP2)	RIPK2_HUMAN
S100A4 (CAPL) (MTS1)	S10A4_HUMAN
S100A6 (CACY)	S10A6_HUMAN
S100A10 (ANX2LG) (CAL1L) (CLP11)	S10AA_HUMAN

SAA3P (SAA3)	SAA3_HUMAN
SAA1; SAA2	SAA_HUMAN
SAT1 (SAT)	SAT1_HUMAN
SLC6A6	SC6A6_HUMAN
KITLG (MGF) (SCF)	SCF_HUMAN
SCNN1A (SCNN1)	SCNNA_HUMAN
SDC4	SDC4_HUMAN
SELS (VIMP) (AD-015) (SBBI8)	SELS_HUMAN
SH3BGRL	SH3L1_HUMAN
ST8SIA1 (SIAT8) (SIAT8A)	SIA8A_HUMAN
ST6GAL1 (SIAT1)	SIAT1_HUMAN
SKP2 (FBXL1)	SKP2_HUMAN
NRG1 (GGF) (HGL) (HRGA) (NDF)	SMDF_HUMAN
SNAI1 (SNAH)	SNAI1_HUMAN
SOD1	SODC_HUMAN
SOD2	SODM_HUMAN
SOX9	SOX9_HUMAN
SP7 (OSX)	SP7_HUMAN
SPATA19 (SPERGEN1)	SPT19_HUMAN
STAT5A (STAT5)	STA5A_HUMAN
SUPV3L1 (SUV3)	SUV3_HUMAN
TAP1 (ABCB2) (PSF1) (RING4) (Y3)	TAP1_HUMAN
TICAM1 (PRVTIRB) (TRIF)	TCAM1_HUMAN
TNC (HXB)	TENA_HUMAN
TERT (EST2) (TCS1) (TRT)	TERT_HUMAN
F3	TF_HUMAN
TFEC (TCFEC) (TFECL)	TFEC_HUMAN
TFF3 (ITF) (TFI)	TFF3_HUMAN
TFPI2	TFPI2_HUMAN
TGM1 (KTG)	TGM1_HUMAN
TGM2	TGM2_HUMAN
TIFA (T2BP)	TIFA_HUMAN
TLR2 (TIL4)	TLR2_HUMAN
TLR9 (UNQ5798/PRO19605)	TLR9_HUMAN
TNFSF13B (BAFF) (BLYS) (TALL1)	TN13B_HUMAN
TNFAIP2	TNAP2_HUMAN
TNFAIP3	TNAP3_HUMAN
TNFSF10 (APO2L) (TRAIL)	TNF10_HUMAN
TNFSF15 (TL1) (VEGI)	TNF15_HUMAN
TNF (TNFA) (TNFSF2)	TNFA_HUMAN
LTA (TNFB) (TNFSF1)	TNFB_HUMAN
LTB (TNFC) (TNFSF3)	TNFC_HUMAN
FASLG (APT1LG1) (FASL) (TNFSF6)	TNFL6_HUMAN
TNIP1 (KIAA0113) (NAF1)	TNIP1_HUMAN
TNIP3 (ABIN3) (LIND)	TNIP3_HUMAN
TNFRSF1B (TNFBR) (TNFR2)	TNR1B_HUMAN

TNFRSF4 (TXGP1L)	TNR4_HUMAN
CD40 (TNFRSF5)	TNR5_HUMAN
FAS (APT1) (FAS1) (TNFRSF6)	TNR6_HUMAN
TNFRSF9 (CD137) (ILA)	TNR9_HUMAN
TPMT	TPMT_HUMAN
TRAF1 (EBI6)	TRAF1_HUMAN
TRAF2 (TRAP3)	TRAF2_HUMAN
TREM1	TREM1_HUMAN
TF (PRO1400)	TRFE_HUMAN
LTF (LF)	TRFL_HUMAN
TRPC1 (TRP1)	TRPC1_HUMAN
THBS1 (TSP) (TSP1)	TSP1_HUMAN
THBS2 (TSP2)	TSP2_HUMAN
TWIST1 (TWIST)	TWST1_HUMAN
YY1 (INO80S)	TYY1_HUMAN
UBE2M (UBC12)	UBC12_HUMAN
UCP2 (SLC25A8)	UCP2_HUMAN
UGCGL1 (GT) (UGGT) (UGT1) (UGTR)	UGGG1_HUMAN
UPK1B (TSPAN20)	UPK1B_HUMAN
UPP1 (UP)	UPP1_HUMAN
PLAU	UROK_HUMAN
VCAM1 (L1CAM)	VCAM1_HUMAN
VEGFC	VEGFC_HUMAN
VIM	VIME_HUMAN
VPS53 (PP13624)	VPS53_HUMAN
WNT10B (WNT12)	WN10B_HUMAN
WT1	WT1_HUMAN
CX3CL1 (FKN) (NTT) (SCYD1)	X3CL1_HUMAN
XDH (XDHA)	XDH_HUMAN
XIAP (API3) (BIRC4) (IAP3)	XIAP_HUMAN
ZNF366	ZN366_HUMAN

At the end of this step, the NF-κB system reconstructed consists in 3146 interacting proteins (wider interactome that includes the subset of the core interactome) and more than 400 NF-κB-regulated genes, coding for 384 reviewed proteins (figure 3.9). It is worthy to be underlined once again that these data are referred to the situation of the various databases at the date of February-May 2009 (depending on the database) and that newly added/modified data can change shape or structure of the system.

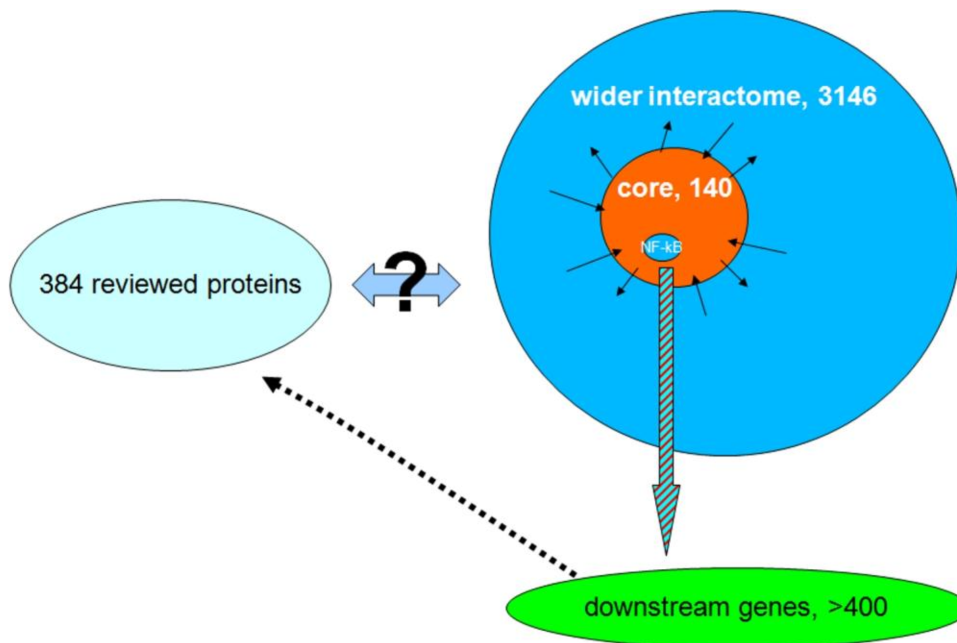

Fig 3.9 Representation of the retrieved data and reconstructed system: the core and wider NF-κB pathway interactomes are composed by a total of 3146 proteins, while the set of the NF-κB-downstream genes is composed by more than 400 genes whose products consist of 384 reviewed proteins. At this stage, analysis should carry on determining which are the feedback loops among regulated genes and NF-kB interactome.

3.3.1. Interactome feedback loops

Once that the gene products have been identified, it is possible to check if there are and how many of the NF-κB regulated proteins play a role in the interactome, thus establishing "feedback loops" that may deserve further consideration and analysis.

Cross-checking the interactomes proteins with the regulated proteins through the use of Cytoscape, it has been possible to identify the set of proteins present both in the interactomes set as well as in the NF-κB regulated set.

The result of this analysis raises some points of interest. As much as 150 out of 384 NF-κB regulated proteins are also present in the wider interactome set. This means that 43% of the identified NF-κB-regulated genes express proteins that play a direct role in the wider interactome. The implication of these data in the dynamics of the NF-κB interactome and in its regulation may thus be remarkable. The ability of NF-κB to 'self-

control' a non negligible part of its directly interacting proteins surely deserves a deeper attention.

The proteins present in both the core interactome subset and in the regulated gene set are 15. It is to be noted that several of these 'feedback loops' are already well-known and studied in the literature, while many others have never been taken under consideration, at least for what concerns the possible implications in the regulation of the NF-κB pathway dynamics.

Table 3.14 Number of proteins present in the interactome sets and in the NF-κB regulated genes set.

	Number of proteins (nodes)	Number of interactions (edges)	Number of proteins in the interactome which gene is regulated by NF-κB ('feedback')	Percentage of 'feedback' proteins
Core interactome	140	829	15	11%
Wider interactome	3146	42638	150	5%

The expression of ~11% (15 out of 140) of the proteins present in the core interactome and of ~5% (150 out of 3146) of the proteins present in the wider interactome can be directly regulated by members of the NF-κB family. Moreover, the expression of four out of five NF-κB subunits (NFKB1, NFKB2, REL and RELB, plus several inhibitors and directly regulating proteins) can be regulated directly by themselves. Conversely, the expression of TF65 does not seem to be regulated by a member of the NF-κB system itself. Since NF-κB is a constitutively expressed protein complex, and since it has a crucial importance in the regulation of fundamental tasks such as the immune responses, its direct control of 11% of the its core interactome may be an indication of how much the system need to be tightly self-regulated to prevent malfunctions and subsequent potentially destructive damage. On the contrary, a deeper investigation and experimental proofs are needed to speculate on the 'independence' of the expression of TF65, that may

be hypothetically related to the necessity to maintain an external control on the NF-κB activation process and relative gene expression.

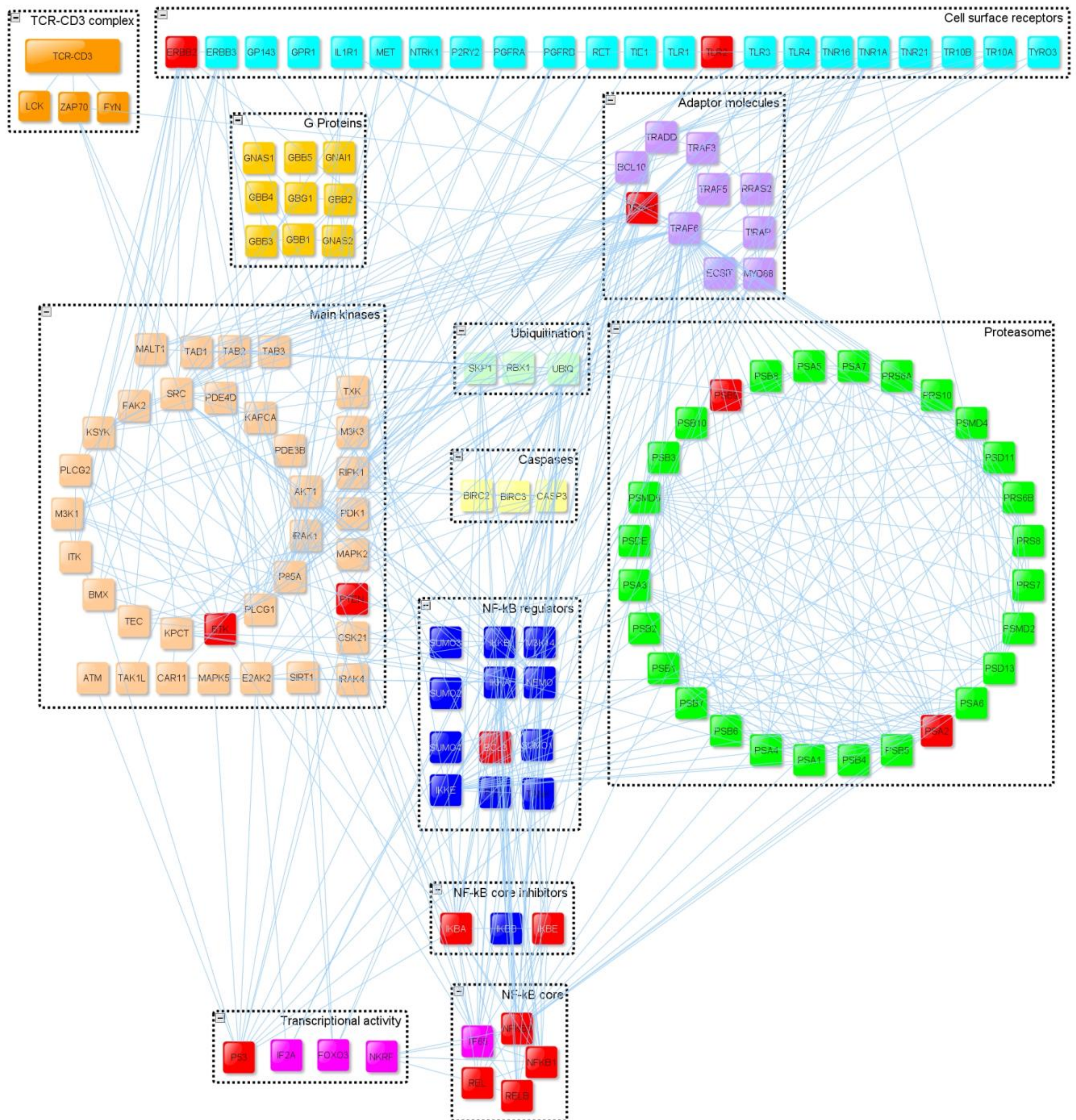

Fig 3.10 Core interactome, proteins whose expression is regulated by NF-κB are depicted in red.

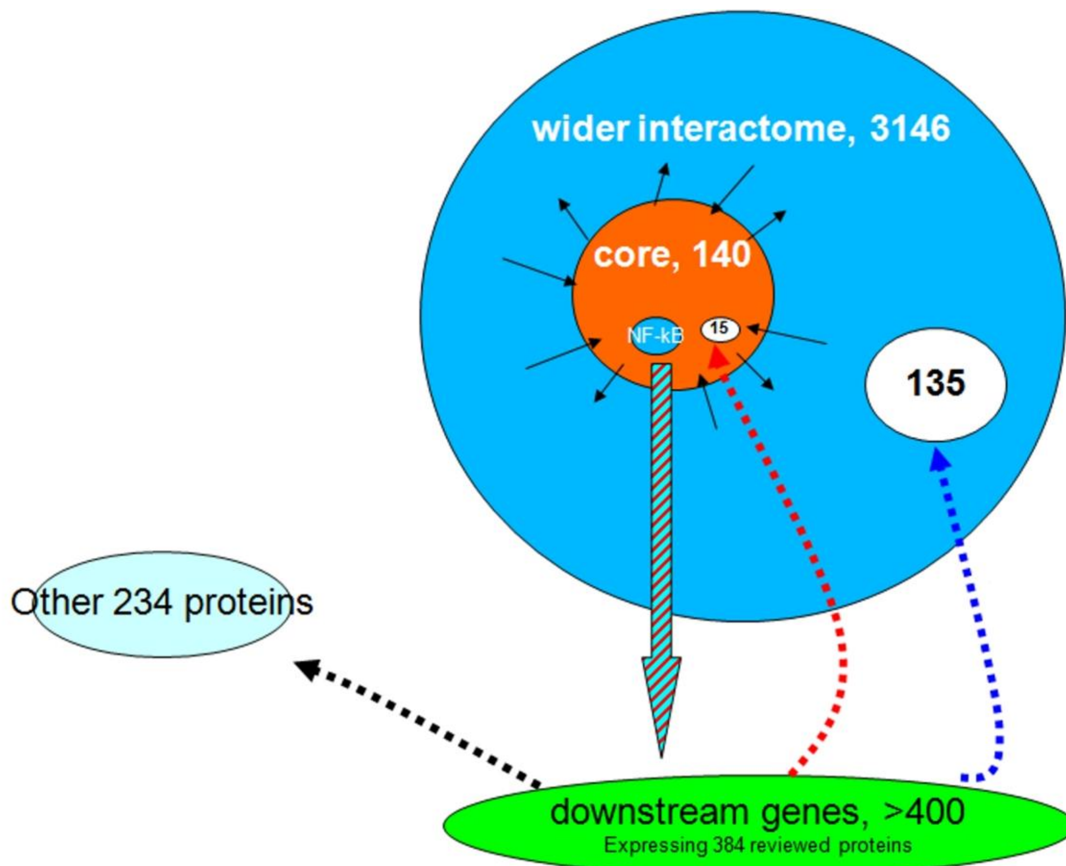

Fig 3.11 Representation of the NF-κB pathway system including interactomes and downstream gene set. The core interactome is composed by 140 proteins, 15 of which are expressed by genes controlled by NF-κB. In the wider interactome, composed by 3146 proteins, 150 are expressed by genes regulated by NF-κB. Many of the 234 proteins not in the interactome can interact with proteins belonging to the core and the wider interactomes (partial data shown).

Table 3.15 shows the 150 genes regulated by NF-κB that express proteins that are also present in the wider interactome and thus playing a relevant role (or, at least, deserving further attention) in the NF-κB system. A systematic analysis of these feedback loops has not yet been run, but a deeper biological interpretation of many of these interactions may also give some hints in terms of the understanding the dynamics and the regulation of NF-κB-dependent responses.

Table 3.15 List of the 150 proteins expressed by genes regulated by NF-κB and appearing in the wider interactome (lines in red: proteins appearing in the core interactome).

Node degree	Uniprot entry name	Protein name	Gene name (aliases)
55	A4_HUMAN	Amyloid beta A4 protein	APP (A4) (AD1)
4	AA1R_HUMAN	Adenosine receptor A1	ADORA1
7	AACT_HUMAN	Alpha-1-antichymotrypsin (ACT)	SERPINA3 (AACT) (GIG24)
11	ABCA1_HUMAN	ATP-binding cassette sub-family A member 1	ABCA1 (ABC1) (CERP)
2	ABCG5_HUMAN	ATP-binding cassette sub-family G member 5 (Sterolin-1)	ABCG5
4	ADA19_HUMAN	Disintegrin and metalloproteinase domain-containing protein 19 (ADAM 19)	ADAM19 (MLTNB) (FKSG34)
128	ANDR_HUMAN	Androgen receptor (Dihydrotestosterone receptor)	AR (DHTR) (NR3C4)
3	AQP4_HUMAN	Aquaporin-4 (AQP-4)	AQP4
21	B2CL1_HUMAN	Bcl-2-like protein 1 (Bcl2-L-1)	BCL2L1 (BCL2L) (BCLX)
58	BAX_HUMAN	Apoptosis regulator BAX (Bcl-2-like protein 4)	BAX (BCL2L4)
33	BCL2_HUMAN	Apoptosis regulator Bcl-2	BCL2
38	BCL3_HUMAN	B-cell lymphoma 3 protein (BCL-3)	BCL3 (BCL4) (D19S37)
25	BLNK_HUMAN	B-cell linker protein (Cytoplasmic adapter protein)	BLNK (BASH) (SLP65)
24	BMI1_HUMAN	Polycomb complex protein BMI-1 (RING finger protein 51)	BMI1 (PCGF4) (RNF51)
12	BRCA2_HUMAN	Breast cancer type 2 susceptibility protein (Fanconi anemia g D1 protein)	BRCA2 (FACD) (FANCD1)
23	BTK_HUMAN	Tyrosine-protein kinase BTK	BTK (AGMX1) (ATK) (BPK)
63	CASP4_HUMAN	Caspase-4 (CASP-4)	CASP4 (ICH2)
34	CAV1_HUMAN	Caveolin-1	CAV1 (CAV)
69	CCL20_HUMAN	C-C motif chemokine 20 (Small-inducible cytokine A20)	CCL20 (LARC) (MIP3A)
2	CCND1_HUMAN	G1/S-specific cyclin-D1 (PRAD1 oncogene)	CCND1 (BCL1) (PRAD1)
37	CCND2_HUMAN	G1/S-specific cyclin-D2	CCND2
9	CCND3_HUMAN	G1/S-specific cyclin-D3	CCND3
19	CCR5_HUMAN	C-C chemokine receptor type 5	CCR5 (CMKBR5)
23	CD40L_HUMAN	CD40 ligand (CD40-L)	CD40LG (CD40L) (TNFSF5)
5	CD44_HUMAN	CD44 antigen (Phagocytic glycoprotein I)	CD44 (LHR) (MDU2) (MDU3)
25	CDN1A_HUMAN	Cyclin-dependent kinase inhibitor 1 (p21)	CDKN1A (CAP20) (CDKN1)
46	CFLAR_HUMAN	CASP8 and FADD-like apoptosis regulator	CFLAR (CASH) (CASP8AP1) (
38	CR2_HUMAN	Complement receptor type 2 (Cr2)	CR2 (C3DR)
7	CXB1_HUMAN	Gap junction beta-1 protein (Connexin-32)	GJB1 (CX32)
4	DCE1_HUMAN	Glutamate decarboxylase 1	GAD1 (GAD) (GAD67)
11	DEFB2_HUMAN	Beta-defensin 2 (BD-2)	DEFB4 (DEFB102) (DEFB2)
3	DUS1_HUMAN	Dual specificity protein phosphatase 1	DUSP1 (CL100) (MKP1)
13	EGFR_HUMAN	Epidermal growth factor receptor	EGFR (ERBB1)
225	EGR1_HUMAN	Early growth response protein 1 (EGR-1)	EGR1 (ZNF225)
11	ELF3_HUMAN	ETS-related transcription factor Elf-3 (E74-like factor 3)	ELF3 (ERT) (ESX) (JEN)
26	ENOG_HUMAN	Gamma-enolase	ENO2
6	ERBB2_HUMAN	Receptor tyrosine-protein kinase erbB-2	ERBB2 (HER2) (NEU) (NGL)
113	FRIH_HUMAN	Ferritin heavy chain (Ferritin H subunit)	FTH1 (FTH) (FTHL6)
11	G6PD_HUMAN	Glucose-6-phosphate 1-dehydrogenase (G6PD)	G6PD

3	GBLP_HUMAN	Guanine nucleotide-binding protein subunit beta-2-like 1 (HLC-7)	GNB2L1 (HLC7) (PIG21)
125	GCR_HUMAN	Glucocorticoid receptor (GR)	NR3C1 (GRL)
88	GNAI2_HUMAN	Guanine nucleotide-binding protein G(i), alpha-2 subunit	GNAI2 (GNAI2B)
112	GRAB_HUMAN	Granzyme B	GZMB (CGL1) (CSPB)
15	GSTP1_HUMAN	Glutathione S-transferase P	GSTP1 (FAEES3) (GST3)
12	HGF_HUMAN	Hepatocyte growth factor	HGF (HPTA)
5	HIF1A_HUMAN	Hypoxia-inducible factor 1 alpha	HIF1A (MOP1)
41	HMGN1_HUMAN	Non-histone chromosomal protein HMG-14	HMGN1 (HMG14)
9	HMOX1_HUMAN	Heme oxygenase 1 (HO-1)	HMOX1 (HO) (HO1)
4	HS902_HUMAN	Putative heat shock protein HSP 90-alpha A2	HSP90AA2 (HSPCAL3)
5	HS90A_HUMAN	Heat shock protein HSP 90-alpha (HSP 86)	HSP90AA1 (HSP90A)
187	HXA9_HUMAN	Homeobox protein Hox-A9 (Hox-1G)	HOXA9 (HOX1G)
8	I15RA_HUMAN	Interleukin-15 receptor subunit alpha (IL-15R-alpha)	IL15RA
4	ICAM1_HUMAN	Intercellular adhesion molecule 1 (ICAM-1)	ICAM1
9	IFNG_HUMAN	Interferon gamma (IFN-gamma)	IFNG
6	IGHG1_HUMAN	Ig gamma-1 chain C region	IGHG1
17	IGHG3_HUMAN	Ig gamma-3 chain C region (Heavy chain disease protein) (HDC)	IGHG3
1	IKBA_HUMAN	NF-kappa-B inhibitor alpha (I-kappa-B-alpha)	NFKBIA (IKBA) (MAD3)
107	IKBE_HUMAN	NF-kappa-B inhibitor epsilon (I-kappa-B-epsilon)	NFKBIE (IKBE)
56	IKBZ_HUMAN	NF-kappa-B inhibitor zeta (I-kappa-B-zeta)	NFKBIZ (IKBZ) (INAP) (MAIL)
1	IL13_HUMAN	Interleukin-13 (IL-13)	IL13 (NC30)
1	IL1A_HUMAN	Interleukin-1 alpha (IL-1 alpha) (Hematopoietin-1)	IL1A (IL1F1)
9	IL1B_HUMAN	Interleukin-1 beta (IL-1 beta) (Catabolin)	IL1B (IL1F2)
8	IL1RA_HUMAN	Interleukin-1 receptor antagonist protein (IL-1ra)	IL1RN (IL1F3) (IL1RA)
1	IL2_HUMAN	Interleukin-2 (IL-2) (T-cell growth factor) (TCGF) (Aldesleukin)	IL2
7	IL2RA_HUMAN	Interleukin-2 receptor alpha chain (IL-2 receptor alpha subunit)	IL2RA
9	IRF1_HUMAN	Interferon regulatory factor 1 (IRF-1)	IRF1
9	IRF2_HUMAN	Interferon regulatory factor 2 (IRF-2)	IRF2
11	IRF4_HUMAN	Interferon regulatory factor 4 (IRF-4)	IRF4 (MUM1)
7	IRF7_HUMAN	Interferon regulatory factor 7 (IRF-7)	IRF7
13	JAM1_HUMAN	Junctional adhesion molecule A (JAM-A)	F11R (JAM1) (JCAM)
9	K1C15_HUMAN	Keratin, type I cytoskeletal 15 (Cytokeratin-15)	KRT15 (KRTB)
18	KLK3_HUMAN	Prostate-specific antigen (PSA)	KLK3 (APS)
10	KPCD_HUMAN	Protein kinase C delta type (nPKC-delta)	PRKCD
80	LEF1_HUMAN	Lymphoid enhancer-binding factor 1 (LEF-1)	LEF1
19	LEG3_HUMAN	Galectin-3 (Galactose-specific lectin 3)	LGALS3 (MAC2)
10	LOX5_HUMAN	Arachidonate 5-lipoxygenase (5-lipoxygenase) (5-LO)	ALOX5 (LOG5)
4	LYAM2_HUMAN	E-selectin (Endothelial leukocyte adhesion molecule 1) (ELAM-1)	SELE (ELAM1)
10	LYSC_HUMAN	Lysozyme C (1,4-beta-N-acetylmuramidase C)	LYZ (LZM)
7	M4K1_HUMAN	Mitogen-activated protein kinase kinase kinase kinase 1	MAP4K1 (HPK1)
25	MBP_HUMAN	Myelin basic protein (MBP)	MBP
30	MOT1_HUMAN	Monocarboxylate transporter 1 (MCT 1)	SLC16A1 (MCT1)
4	MYB_HUMAN	Myb proto-oncogene protein (C-myb)	MYB
13	MYC_HUMAN	Myc proto-oncogene protein (c-Myc) (Transcription factor p64)	MYC
198	MYLK_HUMAN	Myosin light chain kinase, smooth muscle (MLCK)	MYLK (MLCK)
12	NFKB1_HUMAN	Nuclear factor NF-kappa-B p105 subunit	NFKB1

163	NFKB2_HUMAN	Nuclear factor NF-kappa-B p100 subunit	NFKB2 (LYT10)
177	NGF_HUMAN	Beta-nerve growth factor (Beta-NGF)	NGF (NGFB)
9	NMDE1_HUMAN	Glutamate [NMDA] receptor subunit epsilon-1	GRIN2A (NMDAR2A)
16	NMDZ1_HUMAN	Glutamate [NMDA] receptor subunit zeta-1	GRIN1 (NMDAR1)
33	NOS1_HUMAN	Nitric oxide synthase, brain	NOS1
13	NOS2_HUMAN	Nitric oxide synthase, inducible	NOS2 (NOS2A)
8	NQO1_HUMAN	NAD(P)H dehydrogenase [quinone] 1	NQO1 (DIA4) (NMOR1)
7	NRG1_HUMAN	Pro-neuregulin-1, membrane-bound isoform (Pro-NRG1)	NRG1 (GGF) (HGL)
20	OPRD_HUMAN	Delta-type opioid receptor (DOR-1)	OPRD1 (OPRD)
14	OPRM_HUMAN	Mu-type opioid receptor (Mu opioid receptor) (MOP)	OPRM1 (MOR1)
9	OSTP_HUMAN	Osteopontin (Bone sialoprotein 1)	SPP1 (BNSP) (OPN)
11	P53_HUMAN	Cellular tumor antigen p53 (Tumor suppressor p53)	TP53 (P53)
331	PDGFB_HUMAN	Platelet-derived growth factor subunit B (PDGF subunit B)	PDGFB (PDGF2) (SIS)
8	PGH2_HUMAN	Prostaglandin G/H synthase 2	PTGS2 (COX2)
6	PGK1_HUMAN	Phosphoglycerate kinase 1	PGK1 (PGKA) (MIG10)
7	PIM1_HUMAN	Proto-oncogene serine/threonine-protein kinase Pim-1	PIM1
10	PK3CA_HUMAN	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	PIK3CA
24	PLK3_HUMAN	Serine/threonine-protein kinase PLK3	PLK3 (CNK) (FNK) (PRK)
6	PRGR_HUMAN	Progesterone receptor (PR)	PGR (NR3C3)
28	PSA2_HUMAN	Proteasome subunit alpha type-2	PSMA2 (HC3) (PSC3)
61	PSB9_HUMAN	Proteasome subunit beta type-9	PSMB9 (LMP2) (RING12)
22	PSME2_HUMAN	Proteasome activator complex subunit 2	PSME2
4	PTEN_HUMAN	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase PTEN	PTEN (MMAC1) (TEP1)
33	PTN13_HUMAN	Tyrosine-protein phosphatase non-receptor type 13	PTPN13 (PNP1) (PTP1E) (PTPL1)
45	PTN1_HUMAN	Tyrosine-protein phosphatase non-receptor type 1	PTPN1 (PTP1B)
14	PTPS_HUMAN	6-pyruvoyl tetrahydrobiopterin synthase (PTP synthase)	PTS
5	RAG1_HUMAN	V(D)J recombination-activating protein 1 (RAG-1) (RING finger protein 74)	RAG1 (RNF74)
7	REL_HUMAN	C-Rel proto-oncogene protein (C-Rel protein)	REL
87	RELB_HUMAN	Transcription factor RelB (I-Rel)	RELB
86	RIPK2_HUMAN	Receptor-interacting serine/threonine-protein kinase 2	RIPK2 (CARDIAK)
60	S10A4_HUMAN	Protein S100-A4 (S100 calcium-binding protein A4)	S100A4 (CAPL) (MTS1)
11	SAT1_HUMAN	Diamine acetyltransferase 1	SAT1 (SAT)
34	SH3L1_HUMAN	SH3 domain-binding glutamic acid-rich-like protein	SH3BGRL
2	SKP2_HUMAN	S-phase kinase-associated protein 2 (F-box protein Skp2)	SKP2 (FBXL1)
35	SOX9_HUMAN	Transcription factor SOX-9	SOX9
7	STA5A_HUMAN	Signal transducer and activator of transcription 5A	STAT5A (STAT5)
50	TCAM1_HUMAN	TIR domain-containing adapter molecule 1 (TICAM-1)	TICAM1 (PRVTIRB) (TRIF)
7	TERT_HUMAN	Telomerase reverse transcriptase	TERT (EST2) (TCS1) (TRT)
21	TGM2_HUMAN	Protein-glutamine gamma-glutamyltransferase 2	TGM2
26	TIFA_HUMAN	TRAF-interacting protein with FHA domain-containing protein A	TIFA (T2BP)
10	TLR2_HUMAN	Toll-like receptor 2	TLR2 (TIL4)
21	TLR9_HUMAN	Toll-like receptor 9	TLR9 (UNQ5798/PRO19605)
8	TN13B_HUMAN	Tumor necrosis factor ligand superfamily member 13B	TNFSF13B (BAFF) (BLYS)
6	TNAP3_HUMAN	Tumor necrosis factor, alpha-induced protein 3	TNFAIP3 (OTUD7C)
21	TNF10_HUMAN	Tumor necrosis factor ligand superfamily member 10	TNFSF10 (APO2L) (TRAIL)

11	TNFA_HUMAN	Tumor necrosis factor (TNF-alpha)	TNF (TNFA) (TNFSF2)
14	TNFB_HUMAN	Lymphotoxin-alpha (LT-alpha) (TNF-beta)	LTA (TNFB) (TNFSF1)
10	TNFC_HUMAN	Lymphotoxin-beta (LT-beta) (Tumor necrosis factor C)	LTB (TNFC) (TNFSF3)
6	TNFL6_HUMAN	Tumor necrosis factor ligand superfamily member 6 (Fas antigen ligand)	FASLG (APT1LG1) (FASL
26	TNIP1_HUMAN	TNFAIP3-interacting protein 1 (Nef-associated factor 1)	TNIP1 (KIAA0113) (NAF1)
16	TNIP3_HUMAN	TNFAIP3-interacting protein 3 (Listeria-induced gene protein)	TNIP3 (ABIN3) (LIND)
1	TNR1B_HUMAN	Tumor necrosis factor receptor superfamily member 1B	TNFRSF1B (TNFBR) (TNFR2)
92	TNR4_HUMAN	Tumor necrosis factor receptor superfamily member 4	TNFRSF4 (TXGP1L)
7	TNR5_HUMAN	Tumor necrosis factor receptor superfamily member 5 (CD40L receptor)	CD40 (TNFRSF5)
36	TNR6_HUMAN	Tumor necrosis factor receptor superfamily member 6 (FASLG receptor)	FAS (APT1) (FAS1) (TNFRSF6)
48	TNR9_HUMAN	Tumor necrosis factor receptor superfamily member 9 (4-1BB ligand recpt)	TNFRSF9 (CD137) (ILA)
7	TRAF1_HUMAN	TNF receptor-associated factor 1	TRAF1 (EBI6)
69	TRAF2_HUMAN	TNF receptor-associated factor 2	TRAF2 (TRAP3)
195	TWST1_HUMAN	Twist-related protein 1 (H-twist)	TWIST1 (TWIST)
8	TYY1_HUMAN	Transcriptional repressor protein YY1	YY1 (INO80S)
20	UBC12_HUMAN	NEDD8-conjugating enzyme Ubc12	UBE2M (UBC12)
83	UGGG1_HUMAN	UDP-glucose:glycoprotein glucosyltransferase 1	UGGT1 (GT) (UGCGL1)
6	VIME_HUMAN	Vimentin	VIM
157	WT1_HUMAN	Wilms tumor protein (WT33)	WT1
11	XIAP_HUMAN	Baculoviral IAP repeat-containing protein 4	XIAP (API3) (BIRC4) (IAP3)

Similarly, the 234 proteins expressed by NF-κB regulated genes not present in the interactomes show many interactions with the interactome proteins (data not shown and available upon request). Some of these interactions have been interestingly related to specific pathologies, while many others remain to be investigated. To name but a few, malfunctions of the androgen receptor ANDR have been related to prostate cancer, and together cross-modulation between androgen receptor and NF-κB proteins have been reported. Interestingly, it has been also reported that collectively these data suggest that the crucial interactions between ANDR and NF-κB complexes are partly due to another partner or to competition for a coactivator required for transcription (Péant 2007, Nelius 2007, Cloke 2008, Palvimo 1996).

Collective and complex dynamics of similar type has been reported between NF-κB complexes and other key proteins such as glucocorticoid receptors GCR (Davies 2008),

or between Receptor-interacting serine/threonine-protein kinase 2 (RIPK2), Ubiquitin, NEMO and IKKs (Hasegawa 2008), among several other examples. It is worthy to be noted that all mentioned interactions have been duly evidenced in the network analysis performed on the NF-κB pathway system here reconstructed. It may be thus surmised that the integrative work performed here can act as a heuristic basis for the discovery and the deeper understanding of collective dynamics of complex systems such as the NF-κB pathway system.

4. Conclusion

4.1. Overview of obtained results

Considering the wide variety of extra- and intracellular events contributing to the activation and the dynamics of NF-κB signaling, the complexity of its entangled signaling cascade, and the number and diversity of controlled genes, it has been a challenging task to reconstruct and get a global view of its interactome, and to finally point out critical proteins and feedback cycles that can potentially affect, impinge upon or determine alterations in the intricate regulatory mechanisms of the NF-κB signaling system.

Starting from literature and database-stored available data, a map of NF-κB pathway-related protein interactions has been reconstructed. This map is composed by a total of 140 proteins accounting for 829 protein interactions, including self-interactions. The structure of this preliminary system is clearly depicted and includes many cell surface receptors (including the TCR-CD3 complex) and several adaptor molecules immediately downstream, many caspases, many G proteins, the main kinases, the NF-κB family as well as the NF-κB family inhibitors and their direct regulators, all the proteins composing the proteasome complex, some transcriptional activity proteins, and finally few proteins with ubiquitination activity. All these proteins have been included in the NF-κB pathway interactome because they actively participate with different roles and at various stages to the signaling cascade that leads to the NF-κB transcription factor activation and translocation.

On the basis of this "core interactome", and using computational tools for automated data retrieval, a "wider interactome" has been reconstructed, taking into account all the proteins that show evidence of interaction with at least one belonging to the "core

interactome". Running this process, the NF-κB pathway wider interactome has been reconstructed, resulting in a network of 3146 proteins accounting for more than 42000 interactions.

Accessing online available data, the list of the downstream NF-κB regulated genes has been compiled. The search gave back 422 valid ENSEMBL gene identifiers that map onto a 384 reviewed proteins set. This set has been compared with the reconstructed NF-κB interactomes for the discovery of "protein expression → signal cascade → transcriptional activation → protein expression" feedback loops.

Network analysis and other *omics* analyses helped in determining and characterizing the salient features of the networks, their peculiar architecture and the key nodes in terms of the main topological parameters. Overrepresentations of particular Gene Ontology categories gave some interesting clues on the composition of the interactomes, in terms of belonging to given cellular compartment, or specific molecular functions and biological processes, or also to evidence the participation of many proteins to other signaling pathways than NF-κB.

The feedback loops discovery analysis helped in determining a number of "self-regulated" proteins, that is to say proteins that participate to the NF-κB activation signaling cascade and whose expression is in turn regulated by NF-κB itself. While some of these feedbacks were already well-known and characterized, many others remain not yet studied and analyzed. It can be speculated that a number of these non exhaustively studied feedback signals may be key controls for the NF-κB dynamics and downstream gene expression. Undoubtedly, in this sense, a simple and non exhaustive analysis of several feedback cycles has evidenced promising hints about their role in ruling NF-κB signaling, and many others surely deserve more attention.

4.2. Feedback controls

As said, a major relevant result of this study is the determination of the so-called feedback loops or controls. Their number and the nature of the proteins whose expression is controlled by NF-κB seem to suggest a distinctive structure of the whole NF-κB system. Conversely, a fundamental question should concern if number and "quality" of the feedback loops appear by chance or if it is a peculiar characteristic of the NF-κB signaling system. Unfortunately, up to now, there is no further easily available data that can help to solve this thesis. As explained, and notwithstanding the quantity and quality of information at disposal on the internet, there is no plain solution for the integrated view of such data nor for the reconstruction of complex objects such as signaling pathways starting from *omics* data. As a consequence, the answer to the request for the comparison of structures and architectures of complex pathways remains a non trivial question.

In the same manner, without the possibility to compare different pathways and without a deeper analysis of the feedback, it is still difficult to answer to the question if the nature of the feedback loops observed is in some way trivial or if they hide a specific cellular function and a meaningful biological interpretation.

In this view, and speaking on a purely speculative plane, it is possible to consider the ability of NF-κB to self-regulate the expression of its subunits NFKB1, NFKB2, REL and RELB as a form of self-control of such a complex system, while, at the same moment, to regard the independence of the TF65 expression (it is the only non-self-regulated subunit) from NF-κB as a sort of counterbalanced need for external control. In this perspective, it is worthy to be noted that TF65 hold a prominent topological position being the most central subunit of the NF-κB family in the wider interactome. Undoubtedly, further integrative and experimental studies are needed to exhaustively respond to the questions that arise from the study of the interactomes and the collective analysis of such data.

In the same way, from the deeper investigation of any single feedback loop, possibly integrated with wet-lab experimental setup, as in the virtuous systems biology cycle, many other similarly interesting results may be hopefully arise.

4.3. Further perspectives

As it can easily guessed, this kind of work is continuously in progress, since the available knowledge and data grow and are modified practically on a daily basis. Thus, information on proteins, interactions, network and pathways are highly dynamic and rapidly changing. Therefore, a necessary task for the updated reconstruction and correct interpretation of these complex objects is the continuous integration of newly created and modified data. Automation of these kinds of tasks by means of computational tools and databases should be made in the near future much easier, together with the much desirable improvement of the quality and quantity of the available information. In special way, the meaningful structure, arrangement and management of *omics* data is today a very topical problem, since they still need to be dramatically improved and made more accessible.

From a technical point of view, another interesting perspective consists in the deeper integration and further investigation of interaction data among the remaining 234 NF-κB regulated proteins and the interactomes, task that seems not to pose particular problems but the time needed.

A (necessary) comparison of the such reconstructed NF-κB interactomes with other pathway interactome is timely and absolutely interesting. As said, since the reconstruction and analysis procedures are still strewed with many hurdles, and thus structural and topological differences and general comparative analyses should be postponed after the completion of integrative work on other available pathway datasets. In this view, the progress of methodological and strictly scientific perspectives are tightly joined together.

Being this study of a highly integrative and systemic nature, the combination of its analytical steps and results with different approaches and methodologies is also desirable. A nice addition would be an integrative co-expressed gene network analysis by means of microarrays technology, that could be performed in a relatively easy way on the basis of already available data or specific and suitable experimental setup.

Another fundamental type of information worthy to be integrated in such netowrks is that relative to protein post-translational modifications (PTMs). PTMs are an extremely important cellular control mechanism because it may alter proteins' physical and chemical properties, folding, conformation, spatial distribution, stability, activity and consequently, their biological functions. Biological effects of PTMs include phosphorylation for signal transduction, glycosylation for changing protein half-life, targeting substrates, and promoting cell-cell and cell-matrix interactions, among others. Biological knowledgebases containing PTM information are not yet very common, but they may play key roles in cell regulation and cellular signal elaboration research. More, this kind of information may result fundamental for the implementation of dynamical and predictive models of cellular information processing networks.

As said, complex biochemical intracellular signal transduction networks are basically information processing network. The integrated characteristics of such networks confers nontrivial decision-making ability to the cellular systems and finally to the cell. Likely, decision-making functions are an emergent property of the entire system working in concert. Thus, starting from static connectivity maps and interactomes of signal transduction networks, a major step would consist in extending these initial and fundamental results to the study of actual dynamics of large-scale systems (Helikar 2008). In order to simulate and observe the dynamics of a system, the complete logic of each node in the system, i.e. the set of the outputs in function of the set of the inputs, must be

taken into account. Unfortunately, today this kind of valuable information remains very difficult to collect, store and make publicly available. On the other hand, the nature itself of this kind of information is often ambiguous and unclear. Think for example how many states (and function) could be taken by a single protein on the basis of its phosphorylation sites, cellular location, time, and other key biological parameters. The full output description of a single entity thus becomes a very hard task. As a consequence, the implementation of even a simple dynamical model as a Boolean model (with only two "on" or "off" output states) is hampered by non trivial difficulties. Again, enabling methodologies and computational technologies for high-throughput and automated information processing will facilitate and speed up this particularly interesting integrative effort, leading to more and more accurate and potentially predictive models.

5 Notes

5.1 Notes on existing information representation stardards

Standards for representation of information about pathways are necessary for integration and analysis of data from various sources.

XML (Extensible Markup Language) is a set of rules for encoding any kind of document and information (and not only limited to the biological field) electronically. It is thus suitable for very general purposes. It is defined in the XML 1.0 Specification produced by the World Wide Web Consortium (W3C) and several other related specifications; all are fee-free open standards. XML's design goals emphasize simplicity, generality, and usability over the Internet. It is a textual data format, with strong support via Unicode for the languages of the world. Although XML's design focuses on documents, it is widely used for the representation of arbitrary data structures, for example in web services.

BioPAX (Biological Pathway Exchange - http://www.biopax.org) is a biological pathway data exchange format. It enables the integration of diverse pathway resources by defining an open file format specification for the exchange of biological pathway data. Widespread adoption of BioPAX for data exchange will facilitate access to uniformity of pathway data from varied sources, thereby increasing the efficiency of computational pathway research.

The Systems Biology Markup Language (SBML - http://www.sbml.org) is a computer-readable format for representing models of biological processes. It is mostly used for dynamical models of metabolism, cell-signaling, and many other topics.

PSI-MI (Proteomics Standards Initiative-Molecular Interactions format – http://www.psidev.info) is a standard proposed for improving the annotation and

representation of molecular interaction data wherever it is published, i.e. in journal articles, authors' web-sites or public domain databases, and for improving the accessibility of molecular interaction data to the user community.

The Gene Ontology project (http://www.geneontology.org/) is a major bioinformatics initiative with the aim of standardizing the representation of gene and gene product attributes across species and databases. The project provides a controlled vocabulary of terms for describing gene product characteristics and gene product annotation data from GO Consortium members, as well as tools to access and process this data.

5.2 List of cited online resources URLs

The following is an alphabetical and non-exhaustive list of the resources cited and used in the described reconstruction and analysis process.

- APID Agile Protein Interaction DataAnalyzer - http://bioinfow.dep.usal.es/apid/index.htm
- Ariadne Genomics Pathway Studio - http://www.ariadnegenomics.com/products/pathway-studio/
- BIND Biomolecular Interaction Network Database - http://bond.unleashedinformatics.com/
- BioCarta Pathways - http://www.biocarta.com/genes/index.asp
- BioGRID The Biological General Repository for Interaction Datasets - http://www.thebiogrid.org/
- BiologicalNetworks - http://biologicalnetworks.net/
- CellDesigner - http://www.celldesigner.org/
- ClusterMaker - http://www.cgl.ucsf.edu/cytoscape/cluster/clusterMaker.html

- Cytoscape - http://www.cytoscape.org/
- DIP Database of Interacting Proteins - http://dip.doe-mbi.ucla.edu/dip/Main.cgi
- Entrez Gene - http://www.ncbi.nlm.nih.gov/gene/
- GenMAPP Gene Map Annotator and Pathway Profiler - http://www.genmapp.org/
- Gorilla - http://cbl-gorilla.cs.technion.ac.il/
- GraphWeb - http://biit.cs.ut.ee/graphweb/
- HPRD Human Protein Reference Database - http://www.hprd.org/
- HUBBA Hub objects analyzer - http://hub.iis.sinica.edu.tw/Hubba/
- Ingenuity Systems - http://www.ingenuity.com/
- IntAct - http://www.ebi.ac.uk/intact/
- KEGG Kyoto Encyclopedia of Genes and Genomes - http://www.genome.jp/kegg/
- MINT the Molecular INTeraction database - http://mint.bio.uniroma2.it/mint/
- NCI-Nature Pathway Interaction Database - http://pid.nci.nih.gov/
- NetPath - http://www.netpath.org/
- NetworkAnalyzer - http://med.bioinf.mpi-inf.mpg.de/netanalyzer/
- Pajek - http://vlado.fmf.uni-lj.si/pub/networks/pajek/
- Pathguide: the pathway resource list - http://www.pathguide.org/
- PathVisio - http://www.pathvisio.org/
- Pathway Commons - http://www.pathwaycommons.org/
- R Project for Statistical Computing - http://www.r-project.org/
- Reactome - http://www.reactome.org/
- SBW Systems Biology Workbench - http://sbw.sourceforge.net/
- TRANSFAC & TRANSPATH - http://www.gene-regulation.com/
- TRED Transcriptional Regulatory Element Database - http://rulai.cshl.edu/cgi-bin/TRED/

- UniProt - http://www.uniprot.org/
- WikiPathways - http://www.wikipathways.org/index.php/WikiPathways

5.3 Bibliographic references for the compilation the core interactome protein list

- Barkett M, Gilmore TD - Control of apoptosis by Rel/NF-kappaB transcription factors – Oncogene 18(49):6910-24 (1999)
- Bell S, Matthews JR, Jaffray E, Hay RT - I(kappa)B(gamma) inhibits DNA binding of NF-kappaB p50 homodimers by interacting with residues that contact DNA - Molecular and cellular biology 16(11):6477-85 (1996)
- Ben-Neriah Y. Regulatory functions of ubiquitination in the immune system. Nat Immunol, 2002, 3, 20-26.
- Campbell KJ, O'Shea JM, Perkins ND. Differential regulation of NF-kappaB activation and function by topoisomerase II inhibitors. BMC Cancer. 2006 Apr 21;6:101.
- Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. Nat Rev Mol Cell Biol. 2004 May;5(5):392-401.
- Chen Zj. Ubiquitin Signaling in the NF-κB Pathway. Nat Cell Biol. 2005 August ; 7(8): 758–765.
- Cohen S, Achbert-Weiner H, Ciechanover A. Dual effects of IkappaB kinase beta-mediated phosphorylation on p105 Fate: SCF(beta-TrCP)-dependent degradation and SCF(beta-TrCP)-independent processing. Mol Cell Biol. 2004 Jan;24(1):475-86.
- Dobrzanski P, Ryseck RP, Bravo R - Differential interactions of Rel-NF-kappa B complexes with I kappa B alpha determine pools of constitutive and inducible NF-kappa B activity - The EMBO Journal - 13(19):4608-16 (1994)
- Donzé O, Deng J, Curran J, Sladek R, Picard D, Sonenberg N. The protein kinase PKR: a molecular clock that sequentially activates survival and death programs. EMBO J. 2004 Feb 11;23(3):564-71.
- Elliott PJ, Zollner TM, Boehncke WH. Proteasome inhibition: a new anti-inflammatory strategy. J Mol Med. 2003 Apr;81(4):235-45.
- Franzoso G, Bours V, Park S, Tomita-Yamaguchi M, Kelly K, Siebenlist U - The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-kappa B-mediated inhibition - Nature 359(6393):339-4 (1992)
- Gilmore T, Gapuzan ME, Kalaitzidis D, Starczynowski D - Rel/NF-kappa B/I kappa B signal transduction in the generation and treatment of human cancer - Cancer letters 181(1):1-9 (2002)
- Häcker H, Karin M - Regulation and function of IKK and IKK-related kinases - Science's STKE: signal transduction knowledge environment 2006(357):re13 (2006)
- Hayden MS, Ghosh S - Signaling to NF-kappaB - Genes & development 18(18):2195-224 (2004)
- Hinz M, Löser P, Mathas S, Krappmann D, Dörken B, Scheidereit C - Constitutive NF-kappaB maintains high expression of a characteristic gene network, including CD40, CD86, and a set of antiapoptotic genes in Hodgkin/Reed-Sternberg cells - Blood 97(9):2798-807 (2001)
- Ishinaga H, Jono H, Lim JH, Kweon SM, Xu H, Ha UH, Xu H, Koga T, Yan C, Feng XH, Chen LF, Li JD. TGF-beta induces p65 acetylation to enhance bacteria-induced NF-kappaB activation. EMBO J. 2007 Feb 21;26(4):1150-62.
- Krappmann D, Wegener E, Sunami Y, Esen M, Thiel A, Mordmuller B, Scheidereit C. The IkappaB kinase complex and NF-kappaB act as master regulators of lipopolysaccharide-induced

gene expression and control subordinate activation of AP-1. Mol Cell Biol. 2004 Jul;24(14):6488-500.

- Malek S, Chen Y, Huxford T, Ghosh G - IkappaBbeta, but not IkappaBalpha, functions as a classical cytoplasmic inhibitor of NF-kappaB dimers by masking both NF-kappaB nuclear localization sequences in resting cells - The Journal of biological chemistry 276(48):45225-35 (2001)
- Malek S, Huang DB, Huxford T, Ghosh S, Ghosh G - X-ray crystal structure of an IkappaBbeta x NF-kappaB p65 homodimer complex - The Journal of biological chemistry 278(25):23094-100 (2003)
- Moorthy AK, Savinova OV, Ho JQ, Wang VY, Vu D, Ghosh G. The 20S proteasome processes NF-kappaB1 p105 into p50 in a translation-independent manner. EMBO J. 2006 May 3;25(9):1945-56. Epub 2006 Apr 13.
- Pasparakis M Luedde T Schmidt-Supprian M. Dissection of the NF-kB signalling cascade in transgenic and knockout mice. Cell Death and Differentiation (2006) 13, 861–872
- Perkins ND - Integrating cell-signalling pathways with NF-kappaB and IKK function – Nature reviews. Molecular cell biology - 8(1):49-62 (2007)
- Pham LV, Tamayo AT, Yoshimura LC, Lin-Lee YC, Ford RJ - Constitutive NF-kappaB and NFAT activation in aggressive B-cell lymphomas synergistically activates the CD154 gene and maintains lymphoma cell survival - Blood 106(12):3940-7 (2005)
- Rodriguez MS, Thompson J, Hay RT, Dargemont C - Nuclear retention of IkappaBalpha protects it from signal-induced degradation and inhibits nuclear factor kappaB transcriptional activation – The Journal of biological chemistry 74(13):9108-15 (1999)
- Saccani S, Pantano S, Natoli G - Modulation of NF-kappaB activity by exchange of dimer - Molecular cell 11(6):1563-74 (2003)
- Salminen A, Huuskonen J, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T - Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging – Ageing research reviews 7(2):83-105 (2008)
- Schmitz ML, Baeuerle PA - The p65 subunit is responsible for the strong transcription activating potential of NF-kappa B - The EMBO journal 10(12):3805-17 (1991)
- Taylor IW and Wrana JL - SnapShot: The TGF Pathway Interactome - Cell 133:378-378.e1 (2008)
- Thompson JE, Phillips RJ, Erdjument-Bromage H, Tempst P, Ghosh S - I kappa B-beta regulates the persistent response in a biphasic activation of NF-kappa B - Cell 80(4):573-82 (1995)
- Ward KR, Zhang KX, Somasiri AM, Roskelley CD, Schrader JW. Expression of activated M-Ras in a murine mammary epithelial cell line induces epithelial-mesenchymal transition and tumorigenesis. Oncogene. 2004 Feb 12;23(6):1187-96. Erratum in: Oncogene. 2004 Nov 18;23(54):8858.
- Wegener E et al. - Essential role for IkappaB kinase beta in remodeling Carma1-Bcl10-Malt1 complexes upon T cell activation - Molecular and cellular biology 23(1):13-23 (2006)
- Weil R, Israel A. Deciphering the pathway from the TCR to NF-kB. Cell Death and Differentiation (2006) 13, 826–833
- Wertz IE et al. - De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling - Nature. 430(7000):694-9 (2004)
- Whiteside ST, Epinat JC, Rice NR, Israël A - kappa B epsilon, a novel member of the I kappa B family, controls RelA and cRel NF-kappa B activity - The EMBO journal 16(6):1413-26 (1997)
- Wulczyn FG, Naumann M, Scheidereit C - Candidate proto-oncogene bcl-3 encodes a subunitspecific inhibitor of transcription factor NF-kappaB - Nature 358(6387):597-9 (1992)

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References

- Adriaens M.E., Jaillard M., Waagmeester A., Coort S.L.M., Pico A.R., Evelo C.T.A. (2008) The public road to high-quality curated biological pathways. *Drug Discov Today* **13**, 856–862
- Albert R., Jeong H., Barabasi A.L. (2000) Error and attack tolerance of complex networks. *Nature*, **406,** 378-382.
- Alderson D.L., Li L., Willinger W., Doyle J.C. (2005) Understanding internet topology: principles, models, and validation. *IEEE/ACM Transactions on Networking,* **13**, 1205–1218.
- Alon U., Surette M.G., Barkai N., Leibler S. (1999) Robustness in bacterial chemotaxis. *Nature* **397**, 168-171.
- Assenov, Y., Ramírez, F., Schelhorn, S.E., Lengauer, T., Albrecht, M. (2008) Computing topological parameters of biological networks. *Bioinformatics*, **24**, 282-284
- Avila-Campillo I., Drew K., Lin J., Reiss D.J., Bonneau R. (2007) BioNetBuilder: automatic integration of biological networks. *Bioinformatics*. **23**, 392-3.
- Bader G.D., Cary M.P., Sander C. (2006) Pathguide: a pathway resource list. *Nucleic Acids Res*. **34**, D504-6
- Baitaluk M., Sedova M., Ray A., Gupta A. (2006) BiologicalNetworks: visualization and analysis tool for systems biology. *Nucleic Acids Res*. **34,** W466-71
- Barabasi A.L., Oltvai Z.N. (2004) Network biology: Understanding the cell's functional organization. *Nature Reviews Genetics*, **5**, 101-U15.

- Barabasi AL, Albert R, (2002) Statistical mechanics of complex networks, Rev Mod Phys,; **74**, 47-97
- Baranov VS, Baranova EV. (2007) Genetic aspects of aging. Adv Gerontol.; **20**(2): 26-34.
- Barbieri M, Ferrucci L, Ragno E, Corsi A, Bandinelli S, Bonafè M, et al. (2003) Chronic inflammation andthe effect of IGF-I on muscle strength and power in older persons. Am J Physiol Endocrinol Metab; **284**(3): E481-7.
- Batagelj, V., Mrvar A. (2003): Pajek - Analysis and Visualization of Large Networks. in Jünger, M., Mutzel, P., (Eds.) *Graph Drawing Software*. Springer, Berlin. 77-103
- Bauer-Mehren A., Furlong L.I., Sanz F. (2009) Pathway databases and tools for their exploitation: benefits, current limitations and challenges. *Mol Syst Biol*., **5**, 290
- Bhalla US, Iyengar R. (1999) Emergent properties of networks of biological signaling pathways. *Science*, **283**, 381-7
- Bhalla U.S. (2003) Understanding complex signaling networks through models and metaphors, *Progress in Biophysics and Molecular Biology* **81**, 45-65
- Bindea G., Mlecnik B., Hackl H., Charoentong P., Tosolini M., Kirilovsky A., Fridman W.H., Pagès F., Trajanoski Z., Galon J. (2009) ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics*. **25**, 1091-3.
- Boccaletti S., Latora V., Moreno Y., Chavez M., Hwang D.U., (2006) Complex networks: Structure and dynamics. *Physics Reports-Review Section of Physics Letters*, **424**, 175-308.
- Bollobàs B, (2002) Modern Graph Theory, Springer Verlag, Berlin-New York.

- Bray D. (1990) Intracellular signalling as a parallel distributed process. *J Theor Biol* **143**, 215-231.
- Bray D. (1995). Protein molecules as computational elements in living cells. *Nature* **376**, 307-312
- Butler RN, Miller RA, Perry D, Carnes BA, Williams TF, Cassel C et al. (2008) New model of health promotion and disease prevention for the 21st century. BMJ; **337**: a399.
- Capri M, Salvioli S, Cevenini E, Celani L, Sevini F, Bellavista E, Lanzarini C, Lukas S, Lescai F, Tieri P, Monti D, Franceschi C (2009) Aging and Longevity in Animal Models and Humans, in: Life-Span Extension, Aging Medicine, Sell C et al. eds., Springer/Humana Press, DOI: 10.1007/978-1-60327-507-1_11, 175-191
- Cedersund G, Roll J. (2009) Systems biology: model based evaluation and comparison of potential explanations for given biological data. FEBS J; **276**(4): 903-22.
- Ceol,A.,Chatr-Aryamontri,A.,Licata,L.,Cesareni,G., (2008) Linking entries in protein interaction database to structured text: The FEBS Letters experiment. *FEBS Letters* **582**, 1171-1177.
- Cevenini E, Invidia L, Lescai F, Salvioli S, Tieri P, Castellani G et al. (2008) Human models of aging and longevity. Expert Opin Biol Ther; **8**(9): 1393-405.
- Cevenini E, Bellavista E, Tieri P, Castellani G, Lescai F, Francesconi M, Mishto M, Santoro A, Valensin S, Salvioli S, Capri M, Zaikin A, Monti D, de Magalhães JP, Franceschi C (2009) Systems Biology and Longevity: an emerging approach to identify innovative anti-aging targets and strategies, Curr. Pharm. Des., in press.

- Chen K.C., Calzone L., Csikasz-Nagy A., Cross F.R., Novak B., Tyson J.J. (2004). Integrative analysis of cell cycle control in budding yeast. *Mol Biol Cell* **15**, 3841-3862.
- Chen K.C., Csikasz-Nagy A., Gyorffy B., Val J., Novak B., Tyson J.J. (2000) Kinetic analysis of a molecular model of the budding yeast cell cycle. *Mol Biol Cell* **11**, 369-391.
- Choi C., Krull M., Kel A., Kel-Margoulis O., Pistor S., Potapov A., Voss N., Wingender E. (2004) TRANSPATH-A High Quality Database Focused on Signal Transduction. *Comp Funct Genomics*, **2**, 163-8.
- Chondrogianni N, de C M Simoes D, Franceschi C, Gonos ES. (2004) Cloning of differentially expressed genes in skin fibroblasts from centenarians. Biogerontology; **5**(6): 401-9.
- Cloke B, Huhtinen K, Fusi L, Kajihara T, Yliheikkilä M, Ho KK, Teklenburg G, Lavery S, Jones MC, Trew G, Kim JJ, Lam EW, Cartwright JE, Poutanen M, Brosens JJ (2008) The androgen and progesterone receptors regulate distinct gene networks and cellular functions in decidualizing endometrium. Endocrinology. **149**(9):4462-74.
- Cui Q., Yu Z., Purisima E.O., Wang E. (2006) Principles of microRNA regulation of a human cellular signaling network. *Mol Syst Biol* **2**, 46.
- Davies L, Karthikeyan N, Lynch JT, Sial EA, Gkourtsa A, Demonacos C, Krstic-Demonacos M (2008) Cross talk of signaling pathways in the regulation of the glucocorticoid receptor function. Mol Endocrinol. **22**(6):1331-44.
- De Benedictis G, Carotenuto L, Carrieri G, De Luca M, Falcone E, Rose G et al. (1998) Age-related changes of the 3'APOB-VNTR genotype pool in ageing cohorts. Ann Hum Genet; **62**(Pt 2): 115-22.

- De Benedictis G, Franceschi C. (2006) The unusual genetics of human longevity. Sci Aging Knowledge Environ 2006; **10**: pe20.
- de la Fuente A., Fotia G., Maggio F., Mancosu G., Pieroni E. (2008) Insights into biological information processing: structural and dynamical analysis of a Human Protein Signalling Network. *Journal of Physics A*, **41**, 224013..
- Demetrius L. Aging in mouse and human systems: a comparative study. (2006) Ann N Y Acad Sci; **1067**: 66-82.
- Doyle FJ 3rd, Stelling J. (2006) Systems interface biology. J R Soc Interface; **3**(10): 603-16.
- Eden E, Roy Navon, Israel Steinfeld, Doron Lipson and Zohar Yakhini. (2009) GOrilla: A Tool For Discovery And Visualization of Enriched GO Terms in Ranked Gene Lists, BMC Bioinformatics, **10**:48.
- Erdős, P. Rényi, A. (1959). On Random Graphs. I. Publicationes Mathematicae **6**: 290–297.
- Ferrell J.E., Jr. (1996) Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. *Trends Biochem Sci* **21**, 460-466.
- Fraga MF, Ballestar E, Paz MF, Roper S, Setien F, Ballestar ML et al. (2005) Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A; **102**(30): 10604-9.
- Fraga MF, Esteller M. (2007) Epigenetics and aging: the targets and the marks. Trends Genet; **23**(8): 413-418.
- Franceschi C, Mondello C, Bonafè M, Valensin S, Sansoni P, Sorbi S. (1999) Long telomeres and well preserved proliferative vigor in cells from centenarians: a contribution to longevity? Aging; **11**(2): 69-72.

- Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci.;**908**:244-54.
- Franceschi C, Olivieri F, Marchegiani F, Cardelli M, Cavallone L, Capri M et al. (2005) Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. Mech Ageing Dev; **126**(2): 351-61.
- Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, Salvioli S. (2007a) Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev.;**128**(1):92-105.
- Franceschi C. (2007b) Inflammaging as a major characteristic of old people: can it be prevented or cured? Nutr Rev; **65**(12 Pt 2): S173-6.
- Franceschi C, Motta L, Motta M, Malaguarnera M, Capri M, Vasto S et al. (2008) IMUSCE. The extreme longevity: the state of the art in Italy. Exp Gerontol; **43**(2): 45-52.
- Funahashi, A., Tanimura, N., Morohashi, M., and Kitano, H. (2003) CellDesigner: a process diagram editor for gene-regulatory and biochemical networks, *BIOSILICO*, **1**, 159-162
- Gardy J.L., Lynn D.J., Brinkman F.S., Hancock R.E. (2009) Enabling a systems biology approach to immunology: focus on innate immunity. *Trends Immunol*. **30**, 249-62.
- Gems D, Partridge L. (2008) Stress-response hormesis and aging: "that which does not kill us makes us stronger". Cell Metab; 7(3): 200-3.

- Gerstein,M., Seringhaus,M., Fields,S., (2007) Structured digital abstract makes text mining easy. *Nature* **447**, 142.
- Gilmore T.D., (2010) Rel/NF-kB Transcription Factors website, http://www.nf-kb.org
- Goh K.I., Cusick M.E., Valle D., Childs B., Vidal M ., et al. (2007) The human disease network. *Proceedings of the National Academy of Sciences of the United States of America*, **104,** 8685-8690.
- Goldbeter A., Koshland D.E., Jr. (1981) An amplified sensitivity arising from covalent modification in biological systems. *Proc Natl Acad Sci U S A* **78**, 6840-6844.
- Kitano H, (2002) Systems Biology: a brief overview, Science, **295**:1662-1664,
- Hasegawa M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Núñez G, Inohara N. (2008) A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. EMBO J. **27**(2):373-83.
- Helikar T., Konvalina J., Heidel J., Rogers J.A. (2008) Emergent decision-making in biological signal transduction networks. *Proc Natl Acad Sci U S A* **105**, 1913-1918.
- Hernandez-Toro J., Prieto C., De las Rivas J. (2007) APID2NET: unified interactome graphic analyzer. *Bioinformatics*. **23**, 2495-7.
- Hotamisligil GS. (2006) Inflammation and metabolic disorders. Nature; **444** (7121): 860-7.
- Hunter PJ, Borg TK. (2003) Integration from proteins to organs: the Physiome Project. Nat Rev Mol Cell Biol; **4**(3): 237-43
- Invidia L, Salvioli S, Altilia S, Pierini M, Panourgia MP, Monti D et al. (2009) The frequency of Klotho KL-VS polymorphism in a large Italian population, from

young subjects to centenarians, suggests the presence of specific time windows for its effect. Biogerontology; in press.

- Ivakhno S, Armstrong JD. (2007) Non-linear dimensionality reduction of signaling networks. *BMC Syst Biol*. **8**, 1:27
- Jensen L.J., Saric J., Bork P. (2006) Literature mining for the biologist: from information retrieval to biological discovery. *Nat Rev Genet*. **7**, 119-29
- Jeong H., Mason S.P., Barabasi A.L., Oltvai Z.N. (2001) Lethality and centrality in protein networks. *Nature*,. **411,** 41-42.
- Jeong H., Tombor B., Albert R., Oltvai Z.N., Barabasi A.L. (2000) The large-scale organization of metabolic networks. *Nature*, **407**, 651-654.
- Jiang C., Xuan Z., Zhao F., Zhang M.Q. (2007) TRED: a transcriptional regulatory element database, new entries and other development. *Nucleic Acids Res*. **35**, D137-40
- Kanehisa, M., Goto, S. (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res*. **28**, 27-30
- Kanehisa, M., Goto, S., Furumichi, M., Tanabe, M., and Hirakawa, M. (2010) KEGG for representation and analysis of molecular networks involving diseases and drugs. *Nucleic Acids Res*., in press
- Kaput J, Dawson K. (2007) Complexity of type 2 diabetes mellitus data sets emerging from nutrigenomic research: a case for dimensionality reduction?. Mutat Res; **622**(1-2): 19-32.
- Keshava Prasad T.S., Goel R., Kandasamy K., Keerthikumar S., Kumar S., Mathivanan S., Telikicherla D., Raju R., Shafreen B., Venugopal A., Balakrishnan L., Marimuthu A., Banerjee S., Somanathan D.S., Sebastian A., Rani S., Ray S., Harrys Kishore C.J., Kanth S., Ahmed M., Kashyap M.K., Mohmood R.,

Ramachandra Y.L., Krishna V., Rahiman B.A., Mohan S., Ranganathan P., Ramabadran S., Chaerkady R., Pandey A. (2009) Human Protein Reference Database--2009 update. *Nucleic Acids Res*. **37,** D767-72.

- Kholodenko B.N. (2006) Cell-signalling dynamics in time and space. *Nat Rev Mol Cell Biol* **7**, 165-176.
- Kirkwood TB. (2008) A systematic look at an old problem. Nature; **451**(7179): 644-7.
- Kloetzel PM. Antigen processing by the proteasome. Nat Rev Mol Cell Biol 2001; **2**(3): 179-87.
- Larbi A, Franceschi C, Mazzatti D, Solana R, Wikby A, Pawelec G. (2008) Aging of the immune system as a prognostic factor for human longevity. Physiology (Bethesda); **23**: 64-74.
- Lee MP, Dunn BK. (2008) Influence of genetic inheritance on global epigenetic states and cancer risk prediction with DNA methylation signature: challenges in technology and data analysis. Nutr Rev; **66** Suppl 1: S69-72.
- Leitner, F., Valencia, A., (2008) A text-mining perspective on the requirements for electronically annotated abstracts. *FEBS Lett*., **582**, 1178-1181.
- Leroi AM, Bartke A, De Benedictis G, Franceschi C, Gartner A, Gonos ES, et al. (2005) What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mech Ageing Dev; **126**(3): 421-9.
- Levin M.D., Morton-Firth C.J., Abouhamad W.N., Bourret R.B., Bray D. (1998) Origins of individual swimming behavior in bacteria. *Biophys J* **74**, 175-181.
- Liang W, Kujawski M, Wu J, Lu J, Herrmann A, Loera S, Yen Y, Lee F, Yu H, Wen W, Jove R. (2010) Antitumor Activity of Targeting Src Kinases in

Endothelial and Myeloid Cell Compartments of the Tumor Microenvironment. Clin Cancer Res. In press.

- Licastro F, Grimaldi LM, Bonafè M, Martina C, Olivieri F, Cavallone L, et al. (2003) Interleukin-6 gene alleles affect the risk of Alzheimer's disease and levels of the cytokine in blood and brain. Neurobiol Aging; **24**(7): 921-6.
- Lin C.Y., Chin C.H., Wu H.H., Chen S.H., Ho C.W., Ko M.T. (2008) Hubba: hub objects analyzer--a framework of interactome hubs identification for network biology. *Nucleic Acids Res*. **36,** W438-43
- Liu W., Li D., Zhang J., Zhu Y., He F. (2006) SigFlux: a novel network feature to evaluate the importance of proteins in signal transduction networks. *BMC Bioinformatics* **7**, 515.
- Lutgens E, Lievens D, Beckers L, Wijnands E, Soehnlein O, Zernecke A, Seijkens T, Engel D, Cleutjens J, Keller AM, Naik SH, Boon L, Oufella HA, Mallat Z, Ahonen CL, Noelle RJ, de Winther MP, Daemen MJ, Biessen EA, Weber C. (2010) Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. J Exp Med. In press.
- Ma'ayan A., Jenkins S.L., Neves S., Hasseldine A., Grace E., Dubin-Thaler B., Eungdamrong N.J., Weng G., Ram P.T., Rice J.J., Kershenbaum A., Stolovitzky G.A., Blitzer R.D., Iyengar R. (2005) Formation of regulatory patterns during signal propagation in a Mammalian cellular network. *Science* **309**, 1078-1083
- Maere S., Heymans K., Kuiper M. (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics*. **21**, 3448-9.

- Matthews L., Gopinath G., Gillespie M., Caudy M., Croft D., de Bono B., Garapati P., Hemish J., Hermjakob H., Jassal B., Kanapin A., Lewis S., Mahajan S., May B., Schmidt E., Vastrik I., Wu G., Birney E., Stein L., D'Eustachio P. (2009) Reactome knowledgebase of human biological pathways and processes. *Nucleic Acids Res*. **37,** D619-22
- Matys V., Fricke E., Geffers R., Gössling E., Haubrock M., Hehl R., Hornischer K., Karas D., Kel A.E., Kel-Margoulis O.V., Kloos D.U., Land S., Lewicki-Potapov B., Michael H., Münch R., Reuter I., Rotert S., Saxel H., Scheer M., Thiele S., Wingender E. (2003) TRANSFAC: transcriptional regulation, from patterns to profiles. *Nucleic Acids Res*. **31**, 374-8.
- Mishto M, Bellavista E, Dyllick-Brenzinger M, Dahlmann B, Franceschi C. (2009) In: NOVA Publishers, The ubiquitin proteasome system in nervous system: from physiology to pathology. 2008 Update". New York, DiNapoli M and Wojcik C, in press.
- Mishto M, Bellavista E, Santoro A, Stolzing A, Ligorio C, Nacmias B et al. (2006) Immunoproteasome and LMP2 polymorphism in aged and Alzheimer's disease brains. Neurobiol Aging; **27**(1): 54-66.
- Mori T, Kai S. (2002) Noise-induced entrainment and stochastic resonance in human brain waves. Phys Rev Lett; **88**(21): 218101.
- Nelius T, Filleur S, Yemelyanov A, Budunova I, Shroff E, Mirochnik Y, Aurora A, Veliceasa D, Xiao W, Wang Z, Volpert OV (2007) Androgen receptor targets NFkappaB and TSP1 to suppress prostate tumor growth in vivo. Int J Cancer. Sep 1;**121**(5):999-1008.
- Newman M.E.J. (2000) Models of the small world. *Journal of Statistical Physics*, **101**, 819-841.

- Noorbakhsh F, Overall CM, Power C. (2009) Deciphering complex mechanisms in neurodegenerative diseases: the advent of systems biology. Trends Neurosci; **32**(2): 88-100.
- Ordovas JM. (2007) Gender, a significant factor in the cross talk between genes, environment, and health. Gend Med; **4** Suppl B: 111-22.
- Pahl HL. (1999) Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene. **18**(49):6853-66.
- Palvimo JJ, Reinikainen P, Ikonen T, Kallio PJ, Moilanen A, Jänne OA (1996) Mutual transcriptional interference between RelA and androgen receptor. J Biol Chem. **271**(39):24151-6.
- Paulsson J. (2004) Summing up the noise in gene networks. Nature; **427**: 415–18;
- Péant B, Diallo JS, Lessard L, Delvoye N, Le Page C, Saad F, Mes-Masson AM. (2007) Regulation of IkappaB kinase epsilon expression by the androgen receptor and the nuclear factor-kappaB transcription factor in prostate cancer. Mol Cancer Res. **5**(1):87-94.
- Perkins ND. (2007) Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol. **8**(1):49-62.
- Pico A.R., Kelder T., van Iersel M.P., Hanspers K., Conklin B.R., Evelo C. (2008) WikiPathways: pathway editing for the people. *PLoS Biol.* **6**, e184
- Pieroni, E., de la Fuente, S., van Bentem, Mancosu, G., Capobianco E., Hirt, H., de la Fuente A. (2008) Protein networking: insights into global functional organization of proteomes. *Proteomics* **8**, 799-816
- Platzer A., Perco P., Lukas A., Mayer B. (2007) Characterization of protein-interaction networks in tumors. *BMC Bioinformatics*. **8**, 224.

- Prieto C., De Las Rivas J. (2006) APID: Agile Protein Interaction DataAnalyzer. *Nucleic Acids Res*. **34**, W298-302
- Raser JM, O'Shea EK. (2005) Noise in gene expression: origins, consequences, and control. Science; **309**: 2010–3.
- Rattan SI. (2007) The science of healthy aging: genes, milieu, and chance. Ann N Y Acad Sci; **1114**: 1-10.
- Ravasz, E, Somera AL, Mongru DA, Oltvai ZN, Barabási AL. (2002) Hierarchical organization of modularity in metabolic networks. Science **297** 1551-1555
- Raza S, Robertson KA, Lacaze PA, Page D, Enright AJ, Ghazal P et al. (2008) A logic-based diagram of signalling pathways central to macrophage activation. BMC Syst Biol; **23**: 2-36.
- Reimand J., Tooming L., Peterson H., Adler P., Vilo J. (2008) GraphWeb: mining heterogeneous biological networks for gene modules with functional significance. *Nucl. Acids Res*. **36**, W452-W459
- Russell DF, Wilkens LA, Moss F. (1999) Use of behavioural stochastic resonance by paddle fish for feeding. Nature; **402**(6759): 291-4.
- Salminen A, Huuskonen J, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T. (2008) Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. Ageing Res Rev. **7**(2):83-105.
- Salvioli S, Olivieri F, Marchegiani F, Cardelli M, Santoro A, Bellavista E et al. (2006a) Genes, ageing and longevity in humans: problems, advantages and perspectives. Free Radic Res; **40**(12): 1303-23.
- Salvioli S, Capri M, Valensin S, Tieri P, Monti D, Ottaviani E, et al. (2006b) Inflamm-aging, cytokines and aging: state of the art, new hypotheses on the role

of mitochondria and new perspectives from systems biology. Curr Pharm Des; **12**(24): 3161-71.

- Salvioli S, Capri M, Tieri P, Loroni J, Barbi C, Invidia L et al. (2008a) Different types of cell death in organismal aging and longevity: state of the art and possible systems biology approach. Curr Pharm Des; **14**(3): 226-36.
- Sansoni P, Vescovini R, Fagnoni F, Biasini C, Zanni F, Zanlari L, Telera A, Lucchini G, Passeri G, Monti D, Franceschi C, Passeri M. (2008b) The immune system in extreme longevity. Exp Gerontol. **43**(2):61-5.
- Sauro H.M., Kholodenko B.N. (2004). Quantitative analysis of signaling networks. *Prog Biophys Mol Biol* **86**, 5-43.
- Schaefer C.F., Anthony K., Krupa S., Buchoff J., Day M., Hannay T., Buetow K.H. (2009) PID: the Pathway Interaction Database. *Nucleic Acids Res*. **37**, D674-9.
- Schumacher B, van der Pluijm I, Moorhouse MJ, Kosteas T, Robinson AR, Suh Y et al. (2008) Delayed and accelerated aging share common longevity assurance mechanisms. PLoS Genet; **4**(8): e1000161.
- Shannon P., Markiel A., Ozier O., Baliga N.S., Wang J.T., Ramage D., Amin N., Schwikowski B., Ideker T. (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. **13**, 2498-504
- Tieri P, Valensin S, Latora V, Castellani GC, Marchiori M, Remondini D, Franceschi C. (2005) Quantifying the relevance of different mediators in the human immune cell network. *Bioinformatics*. **21**, 1639-43
- Tieri, P., Termanini, A., Fariselli, P., and Franceschi, C. (2008) Embedding machine-readable proteins interactions data in scientific articles for easy access

and retrieval. Available from *Nature Precedings* <http://hdl.handle.net/10101/npre.2008.2337.1>

- Tieri P, de la Fuente A, Termanini A, Franceschi C (2010) Integrating omics data for signaling pathway and interactome reconstruction and functional analysis, in: Bioinformatics for Omics Data: Methods and Protocols, Mayer B Ed., Springer/ Humana Press, in press.
- Travers, J., Milgram, S. (1969) An Experimental Study of the Small World Problem. *Sociometry*, **32**, 425-443
- Tyson J.J., Chen K., Novak B. (2001) Network dynamics and cell physiology. *Nat Rev Mol Cell Biol* **2**, 908-916.
- Tyson J.J., Chen K.C., Novak B. (2003) Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell. *Curr Opin Cell Biol* **15**, 221-231.
- Usher M, Feingold M. (2000) Stochastic resonance in the speed of memory retrieval. Biol Cybern; **83**(6): L11-6.
- Vescovini R, Biasini C, Fagnoni FF, Telera AR, Zanlari L, Pedrazzoni M, et al. (2007) Massive load of functional effector CD4+ and CD8+ T cells against cytomegalovirus in very old subjects. J Immunol; **179**(6): 4283-91.
- Villoslada P, Steinman L, Baranzini SE. (2009) Systems biology and its application to the understanding of neurological diseases. Ann Neurol. **65**(2):124-39.
- Watts D.J., Strogatz S.H. (1998) Collective dynamics of 'small-world' networks. *Nature*, **393,** 440-442.

- West GB, Bergman A. (2009) Toward a systems biology framework for understanding aging and health span. J Gerontol A Biol Sci Med Sci; **64**(2): 205-8.
- Williams GC. (1957) Pleiotropy, natural selection, and the evolution of senescence. Evolution; **11**: 398–411.
- Yashin AI, De Benedictis G, Vaupel JW, Tan Q, Andreev KF, Iachine IA et al. (1999) Genes, demography, and life span: the contribution of demographic data in genetic studies on aging and longevity. Am J Hum Genet; **65**: 1178-93.
- Yi T.M., Huang Y., Simon M.I., Doyle J. (2000) Robust perfect adaptation in bacterial chemotaxis through integral feedback control. *Proc Natl Acad Sci U S A* **97**, 4649-4653.
- Zahn JM, Poosala S, Owen AB, Ingram DK, Lustig A, Carter A, Weeraratna AT et al. (2007) AGEMAP: a gene expression database for aging in mice. PLoSGenet.; **3**(11): e201.
- Zaikin A, Pöschel T. (2005) Peptide-size-dependent active transport in the proteasome. Europhys Lett; **69** (5): 725-31.
- Zhao F., Xuan Z., Liu L., Zhang M.Q. (2005) TRED: a Transcriptional Regulatory Element Database and a platform for in silico gene regulation studies. *Nucleic Acids Res*. **33**, D103-7