Charting the NF-κB pathway interactome map

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1 Nuclear Factor Kappa-B: a prominent mediator of inflammation

→ One of the phenomena observed in human aging is the **progressive increase of a systemic inflammatory state**, a condition referred to as "**inflammaging**" [Franceschi 2000], negatively correlated with longevity.

→ Prominent mediators of inflammation are the five components of the **Nuclear Factor kappa B (NF-κB)** family, that acts as key transcriptional regulator of many genes coding for pro-inflammatory cytokines.

→ Several different signaling pathways activated by very diverse stimuli converge on NF-κB, resulting in a regulatory system characterized by **high complexity** (fig. 1) [Gilmore 2006, Perkins 2007].

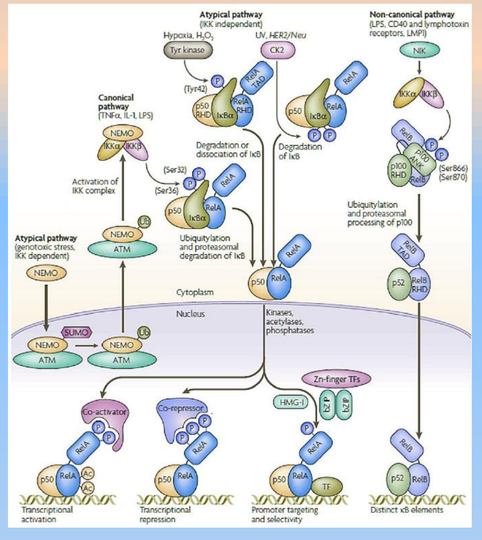

Fig. 1: Distinct NF-κB activation pathways: canonical, atypical IKK dependent and IKK independent, and non canonical pathways. Legend: Ac, acetylation; bZIP, leucinezipper-containing transcription factor; HMG-I, high-mobility-group protein-I; IκB, inhibitor of κB; IKK, IκB kinase; LMP1, latent membrane protein-1; LPS, lipopolysaccharide; 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.

2 From the NF-κB pathways to the NF-κB pathway interactome network

→ It is increasingly recognized that the number of components that impinges upon phenotypic outcomes of signal transduction pathways may be **higher than those taken into consideration from canonical pathway representations**.

→ Recent screens and interpretation of new findings suggest the participation of **hundreds of components** (the cell functionality may be based on a single "mega-network with limited isolation"), instead of the tens classically involved in canonical signaling pathway representations [Fraser 2009].

→ In this scenario, limiting the analyses to **canonical NF-κB pathway elements may reveal inadequate** in unveiling crucial mechanisms in the regulation of this system.

→ Aim of this study is to frame the structure of the NF-κB-activating pathways in a **wider, systemic picture, the NF-κB pathway interactome,** that may give further insights on components that would have been neglected following a more narrowed approach.

3 NF-κB pathway interactome reconstruction

→ We retrieved and integrated • binary protein-protein interaction (PPI) data, • protein annotation data, and • literature data. We also collected • NF-κB downstream genes data. Data retrieval, interactome reconstruction and analysis have been mainly carried out using the Cytoscape platform , and APID, UniProt and KEGG databases.

→ **DI: Direct NF-κB interactome from PPI data** (fig. 2). The direct NF-κB interactome, DI, is composed by all the proteins that show experimental evidence of interaction with at least one of the five NF-κB members. Using this method, we identified a total of **377 proteins** (including the five NF-κB members) accounting for **4119 non-directional interactions** (including self-interactions).

→ **UI: Uniprot-annotated NF-κB interactome** (fig. 3). Searching the UniProt Knowledge Base with the parameters: *«annotation:(type:non-positional "nf kappa b") and organism:"Homo sapiens (Human) [9606]"»* we retrieved a list of 235 proteins, that have been manually screened and checked to obtain a final, validated list of 229 proteins with evidence of implications in the NF-κB functioning. **210** out of 229 proteins are present in the APID database, from where their interaction data are downloaded. 150 out of 210 proteins form a main cluster, accounting for **550 interactions**, while other 60 are isolated from the main cluster.

→ **MCI: Manually curated NF-κB interactome** (fig 4). We selected and manually screened a set of 37 top quality, highly cited literature papers focused on NF-κB to identify proteins that take part with different roles and functions to the signaling cascade leading to NF-κB activation. The criteria for protein selection and inclusion in the MCI set are based on its presence and role described in each paper, either as directly involved in the cascade dynamics, or collaterally participating with a well recognized and described role. **140 proteins** have been identified, and PPI data have been added to build the first version of the MCI by using the APID database, that accounts for **829 non-directional interactions** (including self-interactions).

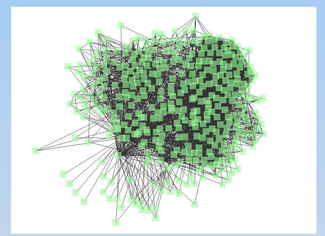

Fig. 2: Direct Interactome (DI)
377 proteins, 4119 interactions
Proteins that show experimental evidence of interaction with at least one member of the NF-κB family.

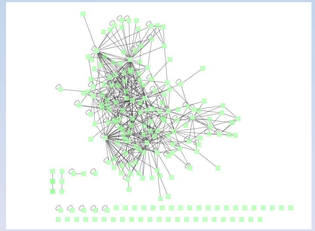

Fig. 3: Uniprot Interactome (UI)
210 proteins, 568 interactions
Selection based on protein annotation in the UniProt database, PPI data from the APID database. 60 out of 210 proteins result isolated from the main connected core.

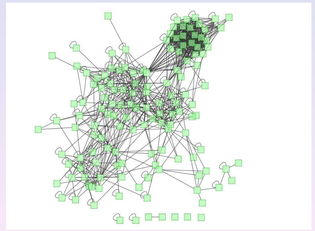

Fig. 4: Manually Curated Interactome (MCI)
140 proteins, 829 interactions
Protein set manually selected starting from literature data, interaction data retrieved from the APID database.

4 Results and discussion

→ The three interactomes are quite differentiated in their dimensions as well as in the protein composition. We also considered the **Union** set (DI ∪ UI ∪ MCI), that accounts for 622 proteins and 5071 interactions, and the intersection of all three (DI ∩ UI ∩ MCI), that accounts for only 15 proteins and 79 interactions.

→ There is a limited grade of **overlap** among the three interactomes (Fig. 5): **DI and UI** share only the **9.1%** of their elements, **DI and MCI** the **6.2%**, while **UI and MCI** share the **13.3%** of the total number of their proteins. These numbers partly reflect the differences and **dishomogeneity in data types and databases** that have been queried.

→ The evident discrepancies in the composition of the interactomes cannot be totally explained taking into account the differences in data types, and thus pave the way to new questions about the **adequacy of the classical pathway descriptions and their completeness**.

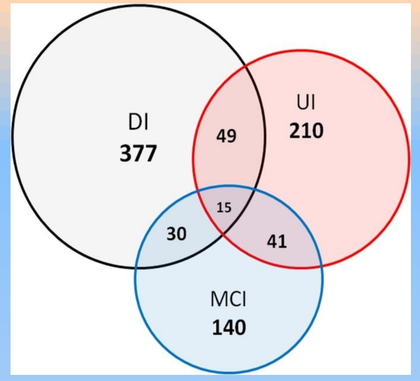

Fig. 5: Union and intersections of the interactomes .
The union set (DI ∪ UI ∪ MCI) acconts for 622 proteins and 5071 interactions, while the intersection of all three (DI ∩ UI ∩ MCI) accounts for only 15 proteins and 79 interactions.

→ The network analysis highlights that among most central proteins (table 1) we find the components of the NF-κB family and their direct regulators, inhibitors and inhibitors kinases (NEMO, IKB and IKK families), but also some general purpose kinases (SRC, BTK), receptor mediator proteins (TRAF family) and Ubiquitin.

→ **Table 1: top 15 proteins for betweenness centrality, Union set**

ID	Betw Centr	Description	NCBI gene	UP ID
TF65	0.24167207	Transcription factor p65	RELA	Q04206
NFKB2	0.09973567	Nuclear factor NF-kappa-B p100 subunit	NFKB2	Q00653
NFKB1	0.09248578	Nuclear factor NF-kappa-B p105 subunit	NFKB1	P19838
UBIQ	0.07499786	Ubiquitin	RPS27A	P62988
NEMO	0.07404715	NF-kappa-B essential modulator	IKBKG	Q9Y6K9
TRAF6	0.06950459	TNF receptor-associated factor 6	TRAF6	Q9Y4K3
IKKE	0.06168222	Inhibitor of nuclear factor kappa-B kinase subunit epsilon	IKBKE	Q14164
IKKA	0.05233459	Inhibitor of nuclear factor kappa-B kinase subunit alpha	CHUK	O15111
TRAF2	0.0478487	TNF receptor-associated factor 2	TRAF2	Q12933
RELB	0.04614179	Transcription factor RelB	RELB	Q01201
IKKB	0.04600286	Inhibitor of nuclear factor kappa-B kinase subunit beta	IKBKB	O14920
IKBA	0.04019644	NF-kappa-B inhibitor alpha	NFKBIA	P25963
M3K14	0.0342034	Mitogen-activated protein kinase kinase kinase 14	MAP3K14	Q99558
SRC	0.02986319	Proto-oncogene tyrosine-protein kinase Src	SRC	P12931
BTK	0.02586853	Tyrosine-protein kinase BTK	BTK	Q06187

→ Overrepresentation of specific pathways (table 2) confirms the well-known involvement of NF-κB in cancerogenesis, in Chagas disease and particularly in inflammation.

→ **Table 2: top 9 overrepresented pathways in Union set proteins**

KEGG ID – Description	*% on total (protein count)*
hsa05200 - Pathways in cancer	**8.7%** (54)
hsa04010 - MAPK signaling pathway	**7.7%** (48)
hsa04620 - Toll-like receptor signaling pathway	**6.3%** (39)
hsa03050 - Proteasome	**5.0%** (31)
hsa04722 - Neurotrophin signaling pathway	**5.0%** (31)
hsa04210 - Apoptosis	**4.7%** (29)
hsa04060 - Cytokine-cytokine receptor interaction	**4.7%** (29)
hsa04062 - Chemokine signaling pathway	**4.7%** (29)
hsa05142 - Chagas disease	**4.5%** (28)

→ **NF-κB downstream genes and feedback loops**. Data extracted from a manually curated list of NF-κB-downstream genes (www.nf-kb.org) and from the Transcriptional Regulatory Elements Database (TRED) have been elaborated to constitute a list of 444 gene names that result to be up- or down-regulated in response to the activation of at least one of the NF-κB family members. 441 valid ENSEMBL gene identifiers mapping to 422 gene product unique identifiers have been obtained. 384 out of 422 protein identifiers have been found in the APID database to check for PPIs. Of these 384 NF-κB-regulated proteins, 49 are present in the Union set (Fig. 6). This means **13% (49 out of 384) of NF-kB downstream genes express proteins that belong to the Union set**, and that **8% (49 out of 622) of the NF-kB Union interactome is regulated by NF-κB itself**.

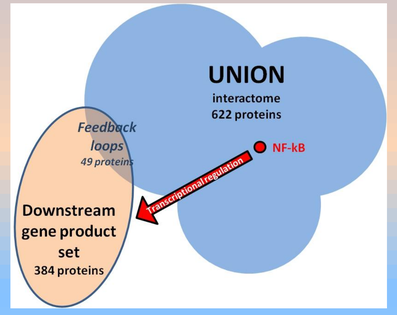

Fig. 6: NF-κB Union interactome and downstream gene product set.
13% (49 out of 384) of NF-κB downstream genes express proteins that belong to the Union set;
8% (49 out of 622) of the NF-κB Union interactome is regulated by NF-κB itself.

5 Conclusions

→ The reconstruction of a comprehensive NF-κB interactome map from the integration of existing, multiple-source data highlights that **the number of elements impinging upon pathway outcomes are higher than those usually taken under consideration in canonical representations**.

→ Substantial **divergences** in interactomes' composition open questions about the adequacy and **comprehensiveness of classical pathway descriptions and representations**, suggesting the participation of **hundreds** –and not tens- of proteins, and turning the idea of pathway **from an isolated entity into a open and unbound one**.

→ The observation of a number of **feedback loops** (proteins interacting/involved in NF-κB activation and which genes are regulated by NF-κB itself) deserves a deeper investigation, since these interactions might be potential critical points for the NF-κB system regulation.

→ This reconstruction confirms that the structure of the NF-κB system may resemble a **bow tie architecture** [Tieri 2010], with a fan in (Union interactome), a core (the NF-κB family), a fan out (downstream genes) and feedback loops.

→ The integrative approach shown here leads to a wider, systemic picture that may help in shedding light on the hidden role and dynamics of proteins that are classically not taken into consideration for what concerns NF-κB activation.

6 Essential bibliography

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