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 and performed 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 of all the proteins that show experimental evidence of interaction with at least one of five NF-κB members. By using the method that was previously established [Castellani et al.], we identified a total of 377 proteins (including 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 (Homo (19860))" 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 collaboratively 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).

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 U UI U 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.

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) drives 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 (Unión interactomes), 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