Enriching the human apoptosis pathway by predicting the structures of protein–protein complexes

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Abstract

Apoptosis is a matter of life and death for cells and both inhibited and enhanced apoptosis may be involved in the pathogenesis of human diseases. The structures of protein–protein complexes in the apoptosis signaling pathway are important as the structural pathway helps in understanding the mechanism of the regulation and information transfer, and in identifying targets for drug design. Here, we aim to predict the structures toward a more informative pathway than currently available. Based on the 3D structures of complexes in the target pathway and a protein–protein interaction modeling tool which allows accurate and proteome-scale applications, we modeled the structures of 29 interactions, 21 of which were previously unknown. Next, 27 interactions which were not listed in the KEGG apoptosis pathway were predicted and subsequently validated by the experimental data in the literature. Additional interactions are also predicted. The multi-partner hub proteins are analyzed and interactions that can and cannot co-exist are identified. Overall, our results enrich the understanding of the pathway with interactions and provide structural details for the human apoptosis pathway. They also illustrate that computational modeling of protein–protein interactions on a large scale can help validate experimental data and provide accurate, structural atom-level detail of signaling pathways in the human cell.

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1. Introduction

Apoptosis was first defined as a general mechanism of controlled cell death which regulates animal cell populations. The term (apoptosis is a Greek word for “falling or dropping off” like leaves from trees) was used to underscore the aspect of kinetics (Kerr et al., 1972). The recent definition of apoptosis is programmed cell death in multicellular organisms; that is, cells committing suicide by activating an intracellular death program; getting engulfed and digested by macrophages without harming their neighbors. Apoptosis helps in regulation of cell number and size, such as the differentiation of fingers in a developing embryo by the programmed death of cells between them; or removal of infected or damaged cells. Apoptotic processes are regulated by extrinsic (also called as extracellular, cytoplasmic or death receptor-induced) and intrinsic (also called intracellular, mitochondrial or B-cell lymphoma 2 (Bcl-2) controlled) pathways (Ghobrial et al., 2005; Sprick and Walczak, 2004; Strasser et al., 2000).

Central players in signal transduction in both the extrinsic and intrinsic pathways are the caspsases (cysteine-dependent aspartate-directed proteases). Caspases are members of the protease family, which are synthesized as inactive precursors or procaspases. Procaspases are activated by proteolytic cleavage by other members of their family in response to inducing signals. Once they are activated and become caspases, they can activate other procaspases by cleaving them. In this manner, initiator caspases, such as procaspase-8 and -10, become activated and cleave the inactive effector caspases, such as procaspase-3. The extrinsic pathway is mediated by the death receptors. These include the Tumor Necrosis Factor receptor (TNF-R; also known as DR1), Fas (TNF receptor superfamily, member 6; also known as CD95, DR2, APO-1) and...
TNF-related apoptosis-inducing ligand receptors (TRAIL-R1; also known as DR4, APO-2 and TRAIL-R2; also known as DR5) (Portt et al., 2011). Following the activation of the death receptors by death ligands, initiator caspases and death domain (DD)-containing adaptor molecules such as FADD (Fas-associated via death domain) are recruited to the DD of the death receptors. This recruited complex (composed of TRAIL-R, FADD, procaspase-8 and FLIP) is called DISC (death-inducing signaling complex) (Kischkel et al., 1995) and it activates a signaling cascade. Effector caspases are activated by this complex for cleavage of death substrates, e.g. DNA fragmentation factor 45 (DFF45), causing events such as DNA or nuclear fragmentation which trigger apoptosis (Portt et al., 2011).

On the other hand, the intrinsic pathway is initiated by stress signals, such as UV-irradiation, γ-irradiation, DNA damage, and genotoxic stress, causing cytochrome C (CytC) release from the mitochondria. The pro-apoptotic members of the Bcl-2 protein family (Bcl-2-associated X protein (Bax), Bcl-2-associated death promoter (Bad), Bcl-2 homologous antagonist/killer (Bak)) are required for making the mitochondrial membrane permeable for the release of CytC, whereas the anti-apoptotic members (Bcl-extra large (Bcl-XL) and Bcl-2) inhibit CytC release by blocking pro-apoptotic members (Portt et al., 2011). Released CytC binds to apoptotic protease activating factor 1 (Apaf-1) to form the apoposome and activate initiator caspase, e.g. procaspase-9, which activates the executioner caspases caspase-3, -6 or -7 (Portt et al., 2011). There is another intrinsic pathway, in which caspases do not function, and it is mediated by a pro-apoptotic protein called apoptosis inducing factor (AIF), which causes DNA fragmentation upon its release from the mitochondria (Susin et al., 1999). The extrinsic and intrinsic pathways of apoptosis are observed to cross-talk via caspase-8, which leads to the initiation of the intrinsic pathway by activating the pro-apoptotic Bcl-2 member Bid (Brunelle and Letai, 2009) and the release of CytC from the mitochondria, in addition to its role of activating caspase-3 and triggering apoptosis in the extrinsic pathway.

Proteins interact with each other specifically and selectively to achieve their biological functions. Consequently, it is crucial to know which proteins interact and how, especially in signaling pathways. Structural studies of the PPIs in the apoptosis network focused on the death receptor signaling (especially the structure of DISC) (Ashkenazi and Dixit, 1998; Bodmer et al., 2000; Carrington et al., 2006; Chinnaiyan et al., 1995; Johnstone et al., 2008; Krueger et al., 2001; Lavrik et al., 2005; Peter and Kramer, 2003; Wajant, 2002), on the mediators (inducing or inhibitory) of the pathway (Chen and Goeddel, 2002; Mihara et al., 2003; Riedl et al., 2001a,b; Yu et al., 2009) and on the general mechanisms of apoptosis regulation (Yan and Shi, 2005).

Considerable information is still missing in the human signaling networks. Large scale analysis by experimental methods such as the yeast two-hybrid system, mass spectrometry, tandem affinity purification, DNA and protein microarrays and phage display have limitations; as such they are complemented by computational methods (Shoemaker and Panchenko, 2007). Computational PPI prediction methods mainly handle the task as a classification problem and solve it via a statistical or machine learning approach. Classification methods determine whether a pair of proteins interact or not, but do not give information about how they interact. Structural knowledge of proteins helps: docking methods can predict how proteins interact, if it is known that they do. However, docking methods are computationally expensive (Hue et al., 2010) on a large scale and usually need additional biochemical data.

PPIs take place through an interface region formed by the complex of two interacting proteins. The interface region is more conserved than the overall structures of proteins (Caffrey et al., 2004) and structurally and functionally different protein pairs can associate through similar interface motifs (Keskin et al., 2004). Based on these considerations, we proposed a high performance PPI prediction method called PRISM (PProtein Interactions by Structural Matching) (Aytuna et al., 2005; Ogmen et al., 2005; Tuncbag et al., 2011a), which uses interfaces of known protein complexes to predict new potential interactions that can use similar interfaces.

Apoptosis is involved in the pathogenesis of many diseases. If the cells fail to undergo apoptosis, an uncontrolled proliferation rate can cause diseases such as cancer, autoimmune diseases and viral infections (Vaux et al., 1994). On the other hand, accelerated rates of apoptosis may cause diseases that are related to cell loss, such as AIDS (acquired immunodeficiency syndrome), neurodegenerative diseases, ischemic injury and toxin-induced liver disease (Thompson, 1995). It is crucial to know the details of the apoptosis signaling pathway, especially structural details of protein–protein interactions, in order to identify targets and design drugs. So far, not many studies have concentrated on the structures of interacting proteins in signaling pathways, such as apoptosis. In this paper, we aim to predict the structures of the complexes formed by interacting protein–protein pairs in the apoptosis pathway of humans, by using the PRISM algorithm and to figure out the implications of the newly obtained structural network.

2. Materials and methods

PPIs in the human apoptosis signaling pathway, which was adopted from KEGG (Kanehisa and Goto, 2000), are analyzed and new interactions are predicted by using the PRISM algorithm. Below, we describe the PRISM algorithm and the template and the target sets that are used for prediction.

2.1. The PRISM algorithm

PRISM requires two input sets: the template and the target. The template set consists of interfaces extracted from protein pairs that are known to interact (PDB complexes) and the target set comprises of protein chains (PDB chains), the interactions of which we want to predict (Fig. 1). The two sides of a template interface are compared with the surfaces of two target monomers. If regions on the target surfaces are similar to the complementary sides of the template interface, then these two targets are predicted to interact with each other through the template interface architecture.

The prediction algorithm consists of four steps, which are depicted in Fig. 1. In the first step, interacting surface residues of target chains are extracted by using the Naccess (Hubbard and Thornton, 1993) program which calculates the accessible surface area of residues (a residue is accepted as a surface residue if its relative surface accessibility is greater than 5%). In the second step the complementary chains of template interfaces are separated and structurally compared with each of the target surfaces by using the MultiProt structural alignment tool (Shatsky et al., 2004). In the third step the structural alignment results are filtered according to some threshold values. For example, if the template chain has less than or equal to 50 residues, then 50% of the template residues should match the target surface residues; if larger than 50, a 30% match of template to target residues is required. In addition, at least one ‘hotspot’ residue on the template interface should match one of the hotspots on the target surface. The resulting set of target surfaces are transformed onto the corresponding template interfaces to form a complex. PRISM then checks if following transformation the residues of the target chains collide with those of the complementary target partners. Finally, in the last step, the FiberDock (Mashiach et al., 2009, 2010) algorithm is used to refine the
interactions allowing some flexibility, resolve steric clashes of side chains, compute the global energy of the complex and rank the solutions according to these energies.

2.2. Template set

A template set contains structurally nonredundant (nonhomologous) unique interfaces. The template sets are constructed by using the interface dataset described by Tuncbag et al. (2008). The dataset was generated by hierarchical clustering of 49,512 two-chain interfaces (extracted from all PDB complexes available in the version of February 2006) into 8205 clusters. Representative interfaces are chosen from each cluster. This large set was classified into groups, obligate and nonobligate (by using the NOXclass PPI type prediction tool) (Zhu et al., 2006); and homodimer and heterodimer interfaces. In this work, the nonobligate (157) (Table S1) and heterodimer (1037) (Table S2) interface sets are combined in the template set (48 elements of these two sets are in common). These sets were selected because the target set is a signaling pathway, and proteins in the signaling pathways typically interact with each other nonobligately by forming heterodimer complexes.

2.3. Target set

PRISM is a prediction algorithm based on three-dimensional protein structures and therefore it can only be applied to proteins with known (experimental or high quality modeled) structures. The target set may contain a minimum of two proteins and the number of proteins in the set can increase up to any desired number, that is, all the proteins in a given pathway. This is the case in our study, which focuses on the apoptosis pathway. A total of 503 proteins with structures (PDB IDs) are obtained from the human apoptosis pathway in KEGG (available at: http://www.genome.jp/dbget-bin/get_pathway?org_name=hsa&mapno=04210, Figure S1) (Kanehisa and Goto, 2000) by following the links on the genes that are available on the map. However, this set includes multiple structures, most of which have more than 90% sequence similarity, for the same target protein. When these are eliminated by using representative structures (with the highest resolution) for each similar group, the number of targets decreases to 124 (Table S3, see Table S4 for the annotated target set). These final target structures are used to predict the potential binary interactions.

2.3.1. Validation of the method

The good performance of PRISM algorithm is validated on 88 benchmark complexes by finding 87 correct binding regions (with high quality protein complex models) for the target set (Tuncbag et al., 2011b).

2.3.2. The drawbacks of the method

Similar to many interaction prediction algorithms, PRISM may predict some false positive (over prediction) and false negative (under prediction) interactions. For example, over predictions may include predictions between intra and extra cellular proteins. This can be minimized by using template sets that are special to the target set, rather than the default template sets. However, this adds another restriction such that the performance of the prediction algorithm depends on the quality of the template set. Additionally, the targeted proteins should have 3D structures in order to apply PRISM algorithm.

3. Prediction results

The prediction results of PRISM are separated into two groups: predicted interactions that were already present in the target human apoptosis pathway in KEGG, and those absent from KEGG. We note that proteins whose structures are not available could not be used in the structural pathway prediction. After eliminating those (15) KEGG interactions between proteins without experimental structures or homology models with low sequence identity
(Table S5), the remaining (57) binary interactions between the targets are extracted from the KEGG map (Table S6). By using two different template sets for prediction, PRISM predicted 242 interactions (Table S8). 29 of the predicted interactions were already shown to interact with each other in the target pathway. However, the structures of 21 of these interacting complexes are newly predicted (Table S6). On the other hand, although 27 of the remaining 213 predicted interactions could be validated by experimental data in the literature via the STRING (a search tool for recurring instances of neighboring genes) web-server (Snel et al., 2000) they have not been shown to interact with each other in the apoptosis pathway in KEGG (Tables S7 and S8). These two interaction sets which were modeled by PRISM are shown in Figs. 2 and 3, respectively, where for clarity and usefulness, they are marked directly on the apoptosis pathway map. No direct experimental data was used for the prediction; only the knowledge that these proteins function in the apoptosis pathway. The remaining 186 predicted interactions might be used as targets for experimental validation. These results are summarized in Fig. 4. See also Table S9 for the interactions between targets that are shown in STRING but could not be predicted by PRISM.

4. Analysis of the predicted complexes

After enriching the human apoptosis pathway with the predicted protein complex structures, we focus on their analysis. Among these, there are two cases in which each of the two complexes (FLIP–Procaspase-8 and TRAIL–TRAIL-R) are composed of targets that are complementary chains of a PDB structure (3h11 and 1du3) (Cha et al., 2000; Yu et al., 2009) and hence the predicted structures are comparable to the experimental ones. These two complexes are structurally aligned with the corresponding experimental structures in the PDB (Figs. 5 and 6) and the predicted structures are verified based on the low RMSD values (below 1 Å) of the alignments calculated by the MultiProt server (Shatsky et al., 2004). The predicted FLIP–Procaspase-8 complex structure is especially important because it was not in the KEGG human apoptosis pathway although it is an important complex in the formation of DISC (death-inducing signaling complex).

Next, we analyzed the predicted complex structures that are already in the KEGG human apoptosis pathway. We searched the literature for any structural data for those complexes in order to validate them. The results are summarized in Table S6. Two case studies are presented below, for which we validated two predicted complex structures that do not have PDB structures, via experimental data in the literature (Figs. 7 and 8). In the first case study, the TNFα–TNF-R1 complex structure is predicted with a binding energy of −26.35 kcal/mol (Fig. 7). The tumor necrosis factor (TNF) is a transmembrane cytokine which is crucial for human body defense against infectious diseases and carcinogenesis (Aggarwal, 2003). However, like many molecules functioning in the host defense, excess TNF can cause autoimmune diseases such as rheumatoid arthritis, Crohn’s disease, and ulcerative colitis (Feldmann and Maini, 2003). TNF functions as the ligand for two
membrane receptors TNF-R1 and TNF-R2, and TNF-R1 is the key mediator of TNF signaling (Wajant et al., 2003). Although the TNFα–TNF-R1 complex does not have a structure in the PDB, there

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Fig. 3. Experimentally validated interactions that were predicted by PRISM and are not in the KEGG human apoptosis pathway. The interactions represented with pink lines are undirected PRISM predictions; that is, the algorithm predicts the complex structures of interacting proteins without suggesting a direction for the interaction. Some species such as FLIP and IAP are moved to different map location for clarity.

Fig. 4. Venn diagram of PRISM predictions. The numbers in the circles are those obtained by PRISM predictions. PRISM predicted a total of 242 interactions. Out of these, three in the KEGG pathway are already noted in the KEGG apoptosis pathway; however, no crystal structures of the complexes are available. Eight of them have structures in the PDB and additional experimental information in the literature, as compiled by STRING. Eighteen have experimental data in the literature, but no structures of the complexes are available. Twenty-five interactions are not in KEGG, however, they are validated by experimental data. Overall, 56 predictions are validated by some experimental data.

Fig. 5. Verification of the predicted FLIP (CFLAR)–Procaspase-8 complex by aligning with the corresponding PDB complex 3h11. PDB complex: FLIP (blue, 3h11A)–Procaspase-8 (red, 3h11B); predicted complex (with −91.78 kcal/mol energy): FLIP (green, 3h11A)–Procaspase-8 (orange, 3h11B); template interface: 1pyo8D (Caspase 2, p12 subunit dimer).
are experimental studies which provide information about key binding residues. According to Mukai et al., Arginine 31 and 32 (R31 and R32) on TNFα and Histidine 69 (H69) and Serine 72 (S72) on TNF-R1 are critical in binding (Mukai et al., 2009). When the interface of the predicted complex is analyzed, these four residues are observed (Fig. 7). However, the TNFα chain (1a8mB) is a mutant structure, with Aspartic Acid as the 31st residue in the interface instead of Arginine (R31D).

In the second case study, the Caspase-8–Procaspase-3 complex structure is predicted with a binding energy of $-66.04$ kcal/mol (Fig. 8). Caspases play important roles in apoptosis and cytokine processing. Caspase-8 is an initiator caspase whereas procaspase-3 is an effector caspase, which needs to be activated by an initiator caspase. Procaspase-3 (activated form of procaspase-3) is assumed to be the executioner of apoptosis (Cohen, 1997) due to its role of destructing important proteins and causing cell death. One of the substrates of caspase-8 is procaspase-3 (Rank et al., 2001) and this interaction is important for the activation and functioning of caspase-3. Similar to the previous case study, the predicted complex does not have a structure in the PDB but the important residues in binding are obtained from the literature. According to Rank et al., isoleucine 172, glutamic acid 173 and threonine 174 (I172, E173 and T174) residues on the activation site of procaspase-3 are critical for the specificity of caspase-8 binding (Rank et al., 2001). When the interface of the predicted complex is analyzed, these three residues are observed (Fig. 8). However, since procaspase-3 chain (2j32A) is a mutant, we observe Alanine as the 173rd residue in the interface instead of glutamic acid (E173A). The shared binding sites of hub proteins in the network, such as caspases and IAP, are also analyzed. If a hub protein interacts with different partners through the same interface, then these interactions are not expected to take place simultaneously. However, if the hub protein binds to different partners through different interfaces then these interactions can occur simultaneously, as long as there are no structural overlaps elsewhere or conformational changes that make these interactions impossible. Some case studies for multi-partner proteins with shared and different interfaces are analyzed in this section (Figs. 9 and 10).

A multi-partner protein example, which interacts with its partners via the same and/or different interfaces, is the XIAP (BIRC4) protein. The X-linked inhibitor of apoptosis protein (XIAP) functions in the regulation of apoptosis by inhibiting caspases (Suzuki et al., 2001). According to the predicted structures of the complexes, the XIAP inhibitor binds to caspase-7 and procaspase-3...
through the same region (BIR2 domain), so these two interactions cannot take place simultaneously (Fig. 9A), whereas it binds to caspase-9 through the BIR3 domain (Fig. 9B) allowing for the simultaneous interaction of XIAP–Caspase-9 and XIAP–Caspase-7 (or procaspase-3).

Another multi-partner protein example is Caspase-8, which interacts with its partners BID and procaspase-3 via different interfaces. Procaspase-3 is activated by the initiator caspase-8 and this interaction causes the signal propagation for apoptosis to take place via the extrinsic pathway. BID, on the other hand, is a pro-apoptotic protein containing a BH3 interacting domain and is a member of the Bcl-2 family. BID is a specific proximal substrate of caspase-8 (Li et al., 1998) and once it is activated by caspase-8, the intrinsic apoptosis pathway is initiated (Brunelle and Letai, 2009) and CytC is released from the mitochondria. When we align two different predicted complexes of caspase-8 with BID and procaspase-3, it is observed that caspase-8 uses different interfaces to interact with these two partners (Fig. 10) so that it can simultaneously interact with these and activate the extrinsic and intrinsic apoptosis pathways, respectively.

As a validation, the accuracy of the results is calculated by comparing the predicted interactions with the yeast two-hybrid interaction data set, called CCSB-HI1, of Rual et al. (2005). Thirty-two of 57 targets (Table S4) were found in this data set and when their interactions are examined a total of 32 interactions were obtained. Thirteen out of 32 interactions were also predicted by PRISM (true positives) and the remaining 19 are accepted as false negatives. All possible binary interactions between the target set proteins constitute 496 interactions (\(\frac{32!}{2!}\)). There are 103 pairs that PRISM predicted as interacting and 13 of them are true but the remaining predictions are not found in the experimental data set, so the number of false positives is 90. Lastly, the number of true negatives is calculated as 374. Based on these numbers the accuracy of the predictions is 0.78, sensitivity is 0.41 and specificity is 0.81 (see Table S10). Similarly, PRISM was used in another study about ubiquitins and the accuracy of the results was found as 0.76 (Kar et al., 2011).

5. Concluding remarks

The application of the structural interaction prediction algorithm – PRISM – to the human apoptosis pathway yielded many interactions: for those already in the apoptosis pathway in KEGG it provided predictions for the structures of the complexes; in addition it predicted interactions that were not in the KEGG apoptosis pathway, but later confirmed via a literature search. For those where crystal structures were available the agreement between the prediction and the crystal structures is excellent (considering that for one of these two cases the template, target and the validation structures were the same). In addition, it predicted others, for which currently no experimental data is available. The complex structures that could not be validated due to lack of previous structural information can be used as models for future studies. Thus, these results enriched the available apoptosis pathway. In addition,
the availability of atomic-level information can help in the understanding of the regulation and in drug design. The aim of this work was to enrich the human apoptosis pathway with the 3D structures of protein–protein complexes by predicting new complex structures that are in accord with information in the literature and by suggesting new complex structures which can be used as starting points for future studies. By structurally complementing the information on how complexes interact with each other, we aimed to model the apoptosis structural pathway. In addition we were able to account for multi-partnered hubs. Shared binding sites imply that the ‘host’ protein cannot simultaneously interact with those partners that bind through the same site, and these partners compete with each other. Which one binds is likely the outcome of prior binding, or post-translational modification events, which allosterically lead to some conformational change. The availability of the structural pathway of a fundamental functional route is important for the understanding of the key steps in regulation; how the signaling takes place; and for elucidating drug targets with low toxicity. Using a computational tool for supplementing pathways is advantageous: it provides predictions which can be checked by experiment, which would be more straightforward than searching for unknown interactions. Here the predictions from the PRISM tool are also validated where data is available. Overall, it illustrates that computations can help experiment for large scale pathways and network construction, and as such, insight into regulation.

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Appendix A. Supplementary data


References
