Every cell has a built-in molecular network of 150-200 proteins, which once activated by the appropriate input signals, leads to cell death. By performing large scale anti-sense RNA screens in mammalian cells, we identified several new components of the cell death network. These genes, the DAPs (Death Associated Proteins), were further characterized at the structural and functional levels. Although the DAP genes are each rate limiting in global network performance, they differ substantially in their biochemical properties and intracellular localization. Each can therefore be thought of as defining a unique functional module within the death signaling network. Ultimately, a complete understanding of their individual functions will also require elucidation of the mode of their connectivity within the network topology.

DAP-kinase (DAPk), a Ca\(^{2+}\)/calmodulin (CaM) regulated, Ser/Thr kinase, is one of the most extensively studied proteins in our laboratory. One critical goal was to determine how DAPk remains silent in healthy cells yet becomes activated by death signals. By combining biochemical assays with the assessment of the cellular function of various mutants, we identified two mechanisms which simultaneously turn on its catalytic activity. These include binding of CaM to DAPk's CaM regulatory domain upon increased cytosolic calcium levels, and activation of a phosphatase which removes an inhibitory auto-phosphorylation at position 308 within the CaM binding domain. In a recent collaboration with M. Wilmanns at the EMBL, the 3D structure of the catalytic domain was determined, further confirming the regulatory predictions made by the experimental work. In parallel, we are investigating the signaling pathways activated downstream of DAPk that lead to cell death. To this end, we focused on identifying DAP-kinase substrates and interacting proteins. This extensive work showed that multiple signaling pathways emerge from DAPk, each coupled to a different cellular phenotype and containing different sets of substrates and interacting proteins. Two DAPk linked phenotypes were mainly studied: membrane blebbing and autophagic cell death. The first DAPk substrate identified was the myosin II regulatory light chain, phosphorylation of which mediates the process of membrane blebbing. In another approach, a novel small isoform of p19ARF, named smARF, was identified as a DAPk substrate. This isoform, which is produced by internal initiation of translation, localizes to the mitochondria, and disrupts mitochondrial membrane potential, ultimately leading to caspase-independent autophagic cell death. In addition, several previously unknown substrates were identified through unbiased, high throughput proteomics-based screens for DAPk interacting proteins and in vitro substrates, each potentially defining new functional arms of DAPk.

DAPk has two closely related kinases, ZIPk and DRP-1. They all share several common features, including a highly ordered basic loop located at the surface of the upper lobe of the catalytic domain, named the 'fingerprint' of the DAPk family. This basic loop mediates heterodimerization between DAPk and ZIPk, which results in trans-phosphorylation and subsequent functional activation of ZIPk. In addition, the basic loop enables the homodimerization of DRP-1, which is also critical for activation. The unique contact sites that occur between the catalytic domains and the interactions that are characteristic of this kinase family are elegantly revealed in the crystal structure of the DRP-1 dimers generated in collaboration with M. Wilmanns.

In addition to DAPk and its family members, our laboratory studies several other DAP genes, including DAP5, a translation initiation factor which directs IRES-dependent translation under stress conditions when cap-dependent translation is compromised. Several novel DAP5 mRNA targets were recently identified in our laboratory by screening cDNA arrays with labeled mRNAs that interact with the DAP5 protein. Analysis of the profile of DAP5 targets indicates that by changing the relative steady state levels of critical nodes in the network, the DAP5 protein can either stimulate or inhibit the process of cell death. Thus DAP5 lies at a critical junction of inter-module connectivity within the cell death network. Other rescued genes include the lysosomal protease cathepsin D, which may participate in autophagic cell death, DAP1, a small resident of the ER, and DAP3, a mitochondrial GTP-binding protein that impairs mitochondrial morphology by increasing the fission process.
One of the current challenges is to move from static diagrams of interconnections between proteins towards an understanding of principles which determine the output dynamics in cell death networks. To this end, we have initiated a system level computational approach to analyzing the functional weight of each protein within the network by specific perturbations. This is achieved by using RNAi technology to knock-down various components of the network singly or in combination. The general goal is to measure the impact of these perturbations on: a. the total network’s performance (expressed in numeric data which assess cell death probabilities); b. the inter-modular and intra-modular connections between proteins. We expect that the high throughput collection of data generated by the combinatorial knock-down experiments will reveal novel principles in the network’s function and will serve as a basis for its modeling.

Selected publications


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