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# Molecular networks controlling programmed cell death: DAP genes and beyond

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Function-based genetic selections were harnessed in our laboratory to identify rate limiting genes that control the complex molecular network of apoptosis. Half a dozen novel pro-apoptotic genes, named DAP genes, were discovered.

DAP-kinase (DAPk) was identified as a multi-domain calcium/calmodulin regulated serine/threonine kinase (Fig. 1A). Localization studies using a GFP fused protein confirmed its association with actin microfilaments (Fig.1B). This was consistent with the finding that myosin light chain is phosphorylated by DAPk both *in vitro* and *in vivo* on Ser19, a site known to promote acto-myosin contraction required for membrane blebbing. DAPk knock out mice were generated in our laboratory to assess its role in various normal and pathological conditions. Of special interest is the link to cancer, in light of the finding that DAPk activates p53 in a p19ARF-dependent manner, as part of a safeguard mechanism which eliminates pre-malignant cells.

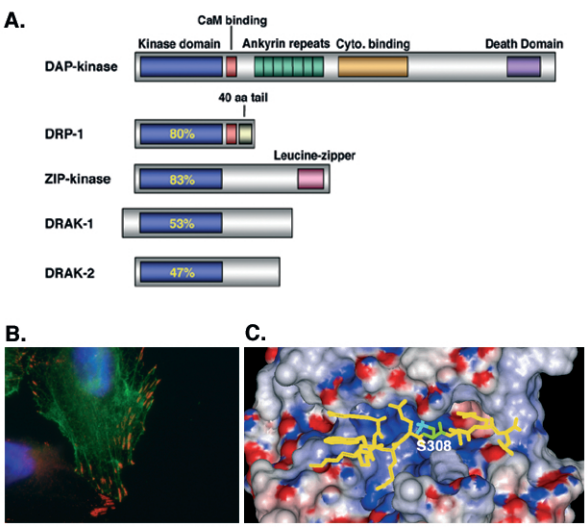


***Fig. 1*** *(A) DAPk family of proteins. (B) GFP-DAPk localizes to actin microfilaments which terminate at focal adhesions (red). (C) A model showing the position Ser308 phosphorylation within the catalytic cleft of DAPk.*

DAPk is the prototype of a novel family of death proteins. The five family members display homology in their catalytic domains while differing in their extra-catalytic segments (Fig.1A). The three closest members, DAPk, DRP-1 and ZIP-kinase cross interact in a novel kinase-kinase cascade. In addition, DAPk and DRP-1 share a unique auto-inhibitory mechanism that restrains their apoptotic activity. It involves autophosphorylation of Ser308 within the calmodulin-regulatory domain (Fig. 1C). In DRP-1, this phosphorylation also prevents homodimerization, required for the death promoting activity. Strikingly, electron microscopy analysis revealed that both DAPk and DRP-1 induce the formation of autophagic vesicles characteristic of type II cell death, suggesting that these kinases regulate several precise subcellular events.

The isolation of DAP5/p97 by our genetic selection uncovered the importance of translation control in apoptosis. This protein is a new member of the eIF4G family of translation initiation factors, which lacks the eIF4E-binding domain necessary for cap-dependent translation. We found that it is activated during cell death by a single caspase-mediated cleavage which removes its C-terminus. The cleaved DAP5/p86 promotes IRES-mediated translation of several death proteins including DAP5 itself, Apaf-1, XIAP and c-myc (Fig. 2A). This mechanism is responsible for the sustained translation of these important proteins during cell death, when cap-dependent translation is switched off.

A novel concept was recently raised once we found that DAP3, a GTP-binding protein, is localized to mitochondria (Fig. 2B). In contrast to the well characterized mechanisms which involve release of mitochondrial proteins to the cytosol, DAP3 remains inside the mitochondria following various apoptotic signals and thus operates within the organelle. The high conservation of DAP3 in evolution prompted us (in collaboration with B. Shilo) to knock out the gene in the Drosophila system in order to gain insight into its role in development. Finally, our genetic screen highlights the importance of an additional organelle, the lysosome, to apoptosis. It emerged from the isolation of cathepsin D, a lysosomal protease whose death-associated substrates are currently being isolated by a

high throughput approach.

In conclusion, the detailed structure/function studies of the DAP genes, the identification of substrates and interacting proteins and analysis of their connection to precise subcellular events, highlight critical parts of the apoptotic network on which, until now, very little was known.

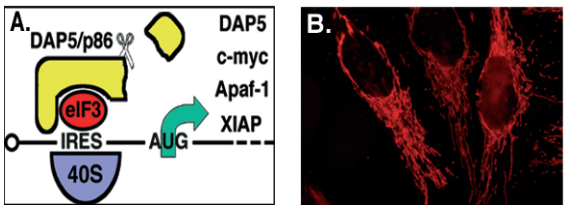


***Fig. 2** (A) DAP5 promotes protein translation from "death IRESs". (B)DAP 3 localizes to mitochondria.*

### *Selected Publications*


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*Equal contribution

### *Acknowledgements*


A.K. is the incumbent of the Helena Rubinstein Chair of Cancer Research. Supported by the Israel's Ministry of Science, DIP, Israel Academy for Science and Humanities, and the Feinberg Foundation.


### *For additional information see:*

www.weizmann.ac.il/molgen/scientists/kimchi.html