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by the cell as a regulatory signal to control many different biological processes¹⁰. Given the versatile nature of this signal, the various functions now attributed to BRCA1 may well be mediated by the E3 ligase activity of the BRCA1-BARD1 heterodimer. Needed insights into the mechanism of BRCA1-mediated tumor suppression should emerge from further structural studies of the BRCA1-BARD1 complex, its associated proteins and the catalytic properties of its RING motifs.

Richard Baer is at the Institute of Cancer Genetics, College of Physicians & Surgeons,

Columbia University, New York, New York 10032, USA. email: rb670@columbia.edu

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A cell death-promoting kinase

Adi Kimchi

Death-associated protein kinase (DAP-kinase; DAPK) has been implicated in programmed cell death and tumor suppression. The recently solved crystal structure of the catalytic domain of human DAP-kinase reveals interesting 'fingerprint' regions that may be functionally important.

Death-associated protein kinase (DAPk or DAP-kinase) is a multidomain Ser/Thr kinase regulated by Ca^{2+} -calmodulin (CaM). It is localized to the cytoskeleton, specifically in association with the actin microfilaments^{1,2}. It was originally identified by applying a function-based genetic screen in mammalian cell cultures aimed at discovering novel genes that participate in programmed cell death³. Since its discovery in 1995, this large multidomain protein kinase has been implicated in a wide range of apoptotic systems and in tumor suppression^{4–7}. In parallel, loss of DAP-kinase expression was detected in a wide spectrum of human cancers, in some cases in association with more aggressive stages of disease^{8–14}. Thus, it is clear that molecular studies of DAP-kinase are critical to the advancement of several areas of biomedical research and that the protein is a likely drug target.

Both the pro-apoptotic and tumor suppressor functions of DAP-kinase depend on its kinase activity^{2,4,7}. This further implies that a better definition of the catalytic domain is necessary for deciphering important features concerning its regulation and mode of action. On page 899 of this issue of *Nature Structure Biology*, Tereshko *et al.*¹⁵ describe the crystal structure of the catalytic domain of human DAP-kinase. This structure highlights

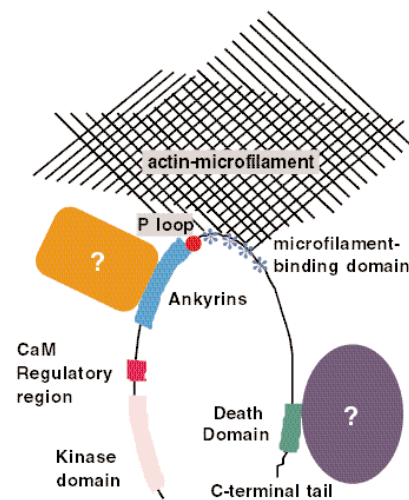


Fig. 1 Schematic representation of DAP-kinase. The various motifs and domains are shown. The crossed lines represent the actin microfilament network. Question marks indicate yet unidentified DAP-kinase interacting proteins.

unique features of DAP-kinase in comparison to other Ser/Thr kinases with known structures. One of these features is a highly basic and structured loop that may possess a critical function, based on its specific location in the three-dimensional structure. The importance of this basic loop is further supported by its conservation in the recently identified members of DAP-kinase family of proteins^{16,17}.

DAP-kinase

The region encompassing the catalytic domain of DAP-kinase, which was utilized

by Tereshko *et al.* for determining the crystal structure, occupies 285 out of the 1,431 amino acid residues of the full length protein. The catalytic domain resides at the N-terminus of the protein and precedes a segment comprising the calmodulin (CaM) binding and regulatory domains (Fig. 1). Ca^{2+} -calmodulin binding to this segment relieves its inhibitory effect on the catalytic site and is necessary for activation of the catalytic domain². In addition, the protein carries eight ankyrin repeats, two extra-catalytic nucleotide-binding P-loops, a cytoskeleton binding region and a conserved death domain. The death domain is followed by a short Ser-rich stretch of amino acids at the C-terminus of the protein (for a review, see ref. 18).

It has been shown experimentally that various domains of the protein contribute to the final cellular action of the protein^{2,4,7}. This includes modules that mediate protein-protein interactions, such as the death domain and the ankyrin repeats that may bind downstream or upstream effector proteins, possibly generating



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important for recognizing peptide substrates. This information was used as a starting point to construct a positional scanning substrate library and has resulted in the discovery of a tentative synthetic peptide substrate. The search for substrates and specific peptide inhibitors has important clinical implications especially in light of recent work that suggests DAP-kinase is involved in ischemia-induced neuronal cell death²¹.

Another critical question relates to the functional role of the above-mentioned basic loop present on the surface of the N-terminal domain. Interestingly, this unique basic loop, which is absent from all other kinases with known structures, represents a 'fingerprint' of the new members of the death-associated protein kinase family (Box 1). In principle, this basic loop could be involved in regulating the kinase or in recognizing substrates. In a recent work it was found that mutations that perturbed this loop in DAP-kinase had no significant effect on the K_m values of a synthetic peptide substrate²⁰. The lack of additional experimental data concerning other potential effects of these mutations leaves the present status of our knowledge on the functional role of the basic loop in a stage of mere speculation. The basic loop could be a site of interaction with another regulatory protein, or a site of interaction with another domain in DAP-kinase protein itself. In any case it should regulate an event that has a common basic role in all death kinases belonging to the DAP-kinase family. Thus, the report of Tereshko *et al.*¹⁵ provides important leads for designing future experiments that otherwise could not be conducted due to lack of critical data.

Finally, the finding that the DAP-kinase catalytic domain is found in its active closed conformation, combined with the flexibility of the peptide binding loop discussed above, is consistent with the lack of activating phosphorylation in this region¹⁵. This feature distinguishes members in the DAP-kinase family from many other kinases that are activated by phosphorylation. This further suggests the existence of additional tightly controlled mechanisms that would keep the active kinase in a silent harmless state in viable cells. One obvious mechanism is the auto-inhibition by the calmodulin regulatory segment (see above) that is specifically released once calcium-activated calmodulin binds to it². This mechanism may be further reinforced by additional layers of regulation that restrain the DAP-kinase function in growing cells to prevent its harmful death effects — as long as the cells are not exposed to external death signals. An example of such regulation was recently identified, involving autophosphorylation of the calmodulin regulatory segment that inhibits the activity of the protein. The theme of auto-inhibition extends to other functional domains of DAP-kinase, as the Ser-rich C-terminal tail also displays auto-inhibitory effects on the death-promoting functions of this protein¹⁹.

In conclusion, the structural information provided by Tereshko *et al.*¹⁵ has revealed important new features concerning the function of the catalytic domain in its isolated form. It has also paved the way for future structure-assisted discovery of new active site-directed inhibitors of DAP-kinase as therapeutics. To get a closer physiological insight in the mode of action of DAP-kinase, one must analyze the catalytic properties of the kinase

domain in the context of the full length protein and, perhaps, in the context of complexes with other interacting proteins. This study of Tereshko *et al.*¹⁵ is at the beginning of a long and complicated road.

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Adi Kimchi is in the Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel. email: adi.kimchi@weizmann.ac.il

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