Structure, Vol. 11, February, 2003, ©2003 Elsevier Science Ltd. All rights reserved. PII S0969-2126(03)00012-1

Switching Off the Switch

The solution structure of the catalytic domain of the dual-specificity phosphatase PAC-1 reveals new insight about the enzymes that deactivate mitogen-activated protein kinases (MAPK).

Protein phosphorylation is a key regulatory process that modulates protein activity. In eukaryotes, many extracellular signals are relayed via a hierarchical network of mitogen-activated protein kinases (MAPKs; see Figure) [1]. The downstream targets of these MAPK cascades are transcription factors that are involved in the regulation of cell cycle progression, signaling, metabolism, growth, and differentiation [2]. MAPKs are activated by tyrosine and threonine phosphorylation of a Thr-Xxx-Tyr signature by upstream kinases. The important cellular responses that are elicited by MAPKs have to be tightly controlled and require a fast response to changing environmental stimuli. This is achieved by MAP kinase phosphatases (MKP) that control the MAPK switches by removing the phosphate, and thus "switching off the switch."

MKPs belong to the family of dual-specificity phosphatases (DSPs) that are characterized by a common active site HCX_5R sequence motif [3]. Hydrolysis of phosphorylated substrates involves the formation of a cysteinyl phosphate intermediate. The transition state during formation and hydrolysis of this intermediate is stabilized by an active site arginine, and a conserved aspartic acid in the so-called "acid loop" functions as a general acid/base catalyst. Dual-specificity phosphatases can act on both phosphorylated serine/threonine and tyrosine residues. This enzymatic activity correlates with a shallow active site binding cleft as observed in three-dimensional structures of the catalytic domains of the vaccinia HI-related DSP (VHR) [4] and the mitogen-activated protein kinase phosphatase 3 (MKP-3/Pyst1) [5].

A unique feature of the MKP family—with the exception of VHR—is the presence of a less conserved N-terminal substrate binding domain (SBD) which, in most cases, is required for substrate-specific activation of the MKP. Intriguingly, recent data suggest that MKP-3 activity is allosterically controlled by substrate-induced MKP-3 activation. Zhou and coworkers have previously determined the structure of the N-terminal SBD of MKP-3 and demonstrated that this domain interacts with both the kinase substrate as well as with the phosphatase catalytic domain (CD) [6]. MKPs generally exhibit distinct substrate specificity toward three major classes of MAPKs: extracellular signal-regulated kinases (ERKs), c-*jun* N-terminal kinases/stress-activated protein kinases (JNKs), and p38 proteins. Whereas MKP-3/Pyst1 is specific for the p42/44 MAP kinase isoforms (ERK1/2), PAC-1 is highly expressed in hematopoietic cells and was originally discovered by virtue of its inactivation of ERKs in T cells.

In this issue of *Structure*, Zhou and coworkers describe the NMR structure of the PAC-1 phosphatase catalytic domain [7]. In addition, by using NMR titration experiments, they characterize its molecular interaction with substrates and the substrate binding domain, shedding new light on the molecular basis for substrate-induced MKP activation.

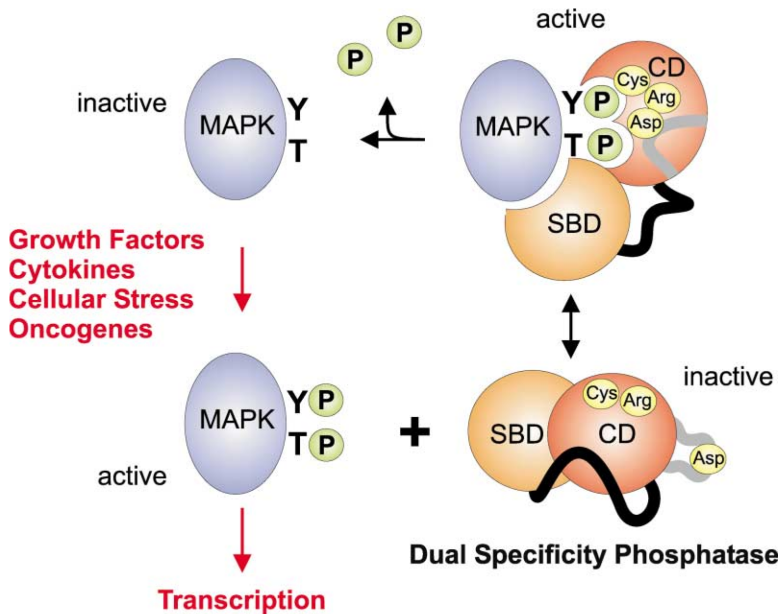
The authors find that the conserved aspartate in the acid loop is remote from the active site cysteine and arginine residues, similar to what has been observed for the MKP-3 catalytic domain [5]. This indicates that activation requires a conformational change of the acid loop, to bring the aspartate residue in close proximity to the active site (see Figure). Whereas the overall fold of the PAC-1 catalytic domain is very similar to MKP-3, Farooq et al. observe a different conformation for the segment comprising helices $\alpha 4$ and $\alpha 5$ near the active site (see Figure 2 in [7]). They also provide evidence that this region comprises a second phospho-amino acid binding pocket, in a similar location as in the VHR catalytic domain [8]. This suggests that structural variations in this second binding pocket could contribute to substrate-specific recognition of a phosphorylated MAPK by DSPs.

To their surprise, Farooq et al. did not observe a tight interaction of the catalytic domain of PAC-1 alone with a bis-phosphorylated peptide resembling the activation loop of the ERK2 kinase substrate, nor with the full-length ERK2 substrate. However, the authors confirm that ERK2 binding and substrate recognition requires the presence of the N-terminal substrate binding domain in PAC-1. Furthermore, in NMR titration experiments with the PAC-1 catalytic domain, the authors show that in the absence of substrate, both the SBD and the connecting linker region contact the C-terminal phosphatase domain. Intriguingly, this SBD/CD interaction does not affect the catalytically active site directly. Together with the finding that the SBD is required for binding the kinase substrate, this strongly supports the idea of an allosteric, ligand-induced activation mechanism for MKPs.

Finally, Zhou and coworkers make an interesting observation regarding the stability of the SBD and CD fragments alone and in complex with each other. Based on their NMR spectra, the SBD and the CD fragments alone require high- and low-salt conditions, respectively, in order to stabilize a unique conformation in solution. In contrast, the SBD/CD complex is stable at both high- and low-salt conditions. This may suggest that the intramolecular interaction between the SBD and the CD stabilizes the full-length PAC-1 protein and that this molecular interface is also implicated in the conformational change induced upon substrate binding.

In summary, the new results by the Zhou lab indicate that their structure of the catalytic domain of PAC-1 represents the general inactive state of DSPs and that a substrate-induced structural rearrangement, which is relayed by the SBD, alters the conformation of the acid loop in the CD toward an active state conformation.

While the findings by Farooq et al. provide further evidence for an allosteric activation mechanism of MKPs, the molecular details still remain elusive. Scientific research once again relates to the many-headed



Regulation of MAP Kinase Signaling by MAP Kinase Phosphates (MKPs)

Activated, phosphorylated MAP kinases are dephosphorylated by dual-specificity phosphates (DSPs), which are comprised of a substrate binding domain (SBD) and a catalytic domain (CD). In the inactive state, the catalytic aspartate in the acid loop is structurally disengaged from the active site. MAPK binding to the SBD results in a conformational rearrangement and produces the catalytically active geometry of the involved cysteine, arginine, and aspartate residues.

serpent of the Hydra. Answers obtained so far give rise to many new questions, such as: what are the structural details of MKP activation via substrate binding? How does binding of the MAP kinase substrate by the SBD alter the conformation of the catalytic domain? How is substrate specificity achieved? Structures of the full-length MKPs with and without substrate will be required to address these questions. Naturally, the Hercules's of our time have already set out for the answers.

Philipp Selenko and Michael Sattler
EMBL Heidelberg
Meyerhofstrasse 1
69117 Heidelberg
Germany

Selected Reading

1. Keyse, S.M. (2000). Curr. Opin. Cell Biol. *12*, 186–192.
2. Camps, M., Nichols, A., and Arkinstall, S. (2000). FASEB J. *14*, 6–16.
3. Denu, J.M., and Dixon, J.E. (1998). Curr. Opin. Chem. Biol. *2*, 633–641.
4. Yuvaniyama, J., Denu, J.M., Dixon, J.E., and Saper, M.A. (1996). Science *272*, 1328–1331.
5. Stewart, A.E., Dowd, S., Keyse, S.M., and McDonald, N.Q. (1999). Nat. Struct. Biol. *6*, 174–181.
6. Farooq, A., Chaturvedi, G., Mujtaba, S., Plotnikova, O., Zeng, L., Dhalluin, C., Ashton, R., and Zhou, M.-M. (2001). Mol. Cell *7*, 387–399.
7. Farooq, A., Plotnikova, O., Chaturvedi, G., Yan, S., Zeng, L., Zhang, Q., and Zhou, M.-M. (2003). Structure *11*, this issue, 155–164.
8. Schumacher, M.A., Todd, J.L., Rice, A.E., Tanner, K.G., and Denu, J.M. (2002). Biochemistry *41*, 3009–3017.