

## COMMENTARY

## POPing the fire into the pyrin?

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Initiation of an inflammatory response requires the co-ordinated participation of proteins within a scaffold in the cytosol of responding cells. The scaffold proteins contain members of a newly discovered family of pyrin-domain adaptor proteins that regulate complex assembly for initiation of nuclear factor  $\kappa$ B and interleukin-1 $\beta$  signalling. A paper in this issue of the *Biochemical Journal* by Stehlik et al. identifies a new member of the pyrin

family that may control signalling by sequestering pro-inflammatory components, shedding light on the origin of human inflammatory disorders.

**Key words:** cryopyrin, inhibitory  $\kappa$ B kinase (IKK), innate immunity, interleukin-1 $\beta$  (IL-1 $\beta$ ), pyrin.

Pyrin domains (PyD; also known as PAAD and DAPIN) are protein–protein interaction modules found at the N-termini of proteins thought to be involved in inflammatory signalling pathways [1,2]. For example, an increased incidence of hereditary inflammatory syndrome familial Mediterranean fever has been associated with mutations in the PyD-containing protein pyrin, whereas mutations within cryopyrin (PYPAF1, NALP3) have been linked to familial cold auto-inflammatory syndrome, Muckle–Wells syndrome and chronic infantile neurological cutaneous and articular syndrome [3]. The molecular mechanism by which these PyD-containing molecules regulate inflammatory responses is under intense investigation.

Studies suggest that both pyrin and cryopyrin utilize another PyD-containing adapter protein, ASC/TMS1/Pycard (containing a caspase recruitment domain, CARD), to activate (i) nuclear factor  $\kappa$ B (NF- $\kappa$ B), a transcription factor involved in pro-inflammatory responses, and (ii) pro-caspase 1, a cysteine protease required for processing and release of interleukin 1 $\beta$  (IL-1 $\beta$ ) [4,5]. Having both a PyD and a CARD, ASC (apoptosis-associated speck-like protein containing a CARD) serves as a molecular bridge between PyD- and CARD-containing signalling molecules that constitute the multiprotein assemblies proposed to induce pro-caspase 1 activation leading to IL-1 $\beta$  maturation [6,7]. The role of ASC as a potent pro-inflammatory mediator was proposed in a recent study describing the targeted disruption of pyrin in mice [8]. When stimulated with lipopolysaccharide, macrophages from these hypomorphic pyrin mutant mice showed increased caspase 1 activation and elevated levels of mature IL-1 $\beta$ . The authors proposed a model whereby proinflammatory cytokines induce ASC, which in turn activates pro-caspase 1, resulting in IL-1 $\beta$  maturation and fever induction. Conversely, an anti-inflammatory milieu induces pyrin, which sequesters ASC and blocks its interaction with caspase 1.

These studies underscore the importance of PyD-containing molecules in the modulation of inflammatory disorders. Using bioinformatics strategies, a novel PyD-encoding gene was identified in a study reported in this issue of the *Biochemical Journal* by Stehlik et al. [9]. This protein is unique in that it contains just a PyD, and hence was termed ‘POP1’ (PAAD-only protein-1) [9]. Given the relatedness of the POP1 PyD

to that of ASC and the lack of a CARD in POP1, it could be envisaged that POP1 might bind readily to ASC and spoil the aforementioned ASC-mediated signalling complexes. Consistent with this notion, POP1 suppressed NF- $\kappa$ B activation induced by co-expression of ASC and pyrin or cryopyrin [9]. This model would imply an inhibitory role for POP1 in inflammation and a potential therapeutic target in the treatment of inflammatory diseases involving ASC and other PyD proteins.

However, it is worth bearing in mind that the role of ASC in a physiological setting has yet to be established, and there are conflicting reports as to whether ASC may inhibit, or even promote, NF- $\kappa$ B and caspase 1 activation [5,7,10]. Possibly this conflict may depend largely on the experimental system utilized. ASC and POP1 presumably exert their biological effects by acting as adapter molecules to aggregate signalling complexes. In this context, small variations in adapter concentration would affect the stoichiometry of these intramolecular complexes, leading to diverse biological outcomes.

In humans, the gene encoding POP1 is situated on chromosome 16p12.1, approx. 14 kbp away from the ASC locus. Given the close phylogenetic similarity of these two loci, it is possible that POP1 and ASC arose by gene duplication. No apparent POP1 orthologue is found in the equivalent region of the mouse genome or, indeed anywhere in the mouse genome, decreasing the likelihood of determining its role by experimental gene ablation. However, POP1 may be akin to ICEBERG, a CARD-only protein that has no orthologues in mice. The presence of an additional regulator in humans may allow for more fine-tuning and precision in modulation of signalling, but the physiological relevance of POP1 still remains an enigma. For instance, whether POP1 expression is regulated or whether POP1, like ASC, is subject to methylation-induced silencing in human cancers [11] will provide critical information about POP1 biology. These new insights will potentially set the stage for the development of novel therapeutic strategies for treating hereditary inflammatory disorders and other human maladies.

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## REFERENCES

- 1 Fairbrother, W. J., Gordon, N. C., Humke, E. W., O'Rourke, K. M., Starovasnik, M. A., Yin, J. P. and Dixit, V. M. (2001) The PYRIN domain: a member of the death domain-fold superfamily. *Protein Sci.* **10**, 1911–1918
- 2 Martinon, F., Hofmann, K. and Tschopp, J. (2001) The pyrin domain: a possible member of the death domain-fold family implicated in apoptosis and inflammation. *Curr. Biol.* **11**, R118–R120
- 3 Gumucio, D. L., Diaz, A., Schaner, P., Richards, N., Babcock, C., Schaller, M. and Cesena, T. (2002) Fire and ICE: the role of pyrin domain-containing proteins in inflammation and apoptosis. *Clin. Exp. Rheumatol.* **20**, S45–S53
- 4 Srinivasula, S. M., Poyet, J. L., Razmara, M., Datta, P., Zhang, Z. and Alnemri, E. S. (2002) The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. *J. Biol. Chem.* **277**, 21119–21122
- 5 Stehlik, C., Fiorentino, L., Dorfleutner, A., Bruey, J. M., Ariza, E. M., Sagara, J. and Reed, J. C. (2002) The PAAD/PYRIN-family protein ASC is a dual regulator of a conserved step in nuclear factor  $\kappa$ B activation pathways. *J. Exp. Med.* **196**, 1605–1615
- 6 Tschopp, J., Martinon, F. and Burns, K. (2003) NALPs: a novel protein family involved in inflammation. *Nat. Rev. Mol. Cell Biol.* **4**, 95–104
- 7 Martinon, F., Burns, K. and Tschopp, J. (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- $\beta$ . *Mol. Cell* **10**, 417–426
- 8 Chae, J. J., Komarow, H. D., Cheng, J., Wood, G., Raben, N., Liu, P. P. and Kastner, D. L. (2003) Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. *Mol. Cell.* **11**, 591–604
- 9 Stehlik, C., Krajewska, M., Welsh, K., Krajewski, S., Godzik, A. and Reed, J. C. (2003) The PAAD/PYRIN-only protein POP1/ASC2 is a modulator of ASC-mediated nuclear-factor- $\kappa$ B and pro-caspase-1 regulation. *Biochem. J.* **373**, 101–113
- 10 Masumoto, J., Taniguchi, S., Ayukawa, K., Sarvotham, H., Kishino, T., Niikawa, N., Hidaka, E., Katsuyama, T., Higuchi, T. and Sagara, J. (1999) ASC, a novel 22-kDa protein, aggregates during apoptosis of human promyelocytic leukemia HC-60 cells. *J. Biol. Chem.* **274**, 33835–33838
- 11 Conway, K. E., McConnell, B. B., Bowring, C. E., Donald, C. D., Warren, S. T. and Vertino, P. M. (2000) TMS1, a novel proapoptotic caspase recruitment domain protein, is a target of methylation-induced gene silencing in human breast cancers. *Cancer Res* **60**, 6236–6242

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