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# Familial Mediterranean Fever in the Post-Genomic Era: How an Ancient Disease is Providing New Insights into Inflammatory Pathways

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Abstract: Familial Mediterranean fever (FMF, MIM24900), described as a clinical entity only slightly over a half-century ago, has ancient roots among populations surrounding the Mediterranean basin. It is the most prevalent of the hereditary periodic fever syndromes, a group of disorders characterized by episodic attacks of fever and inflammation. Seven years ago, it was discovered that FMF is caused by mutations in *MEFV*, a gene that encodes a protein variously called pyrin or marenostrin. As exciting as that discovery was, physicians and patients alike were disappointed that the protein sequence of pyrin/marenostrin did not immediately suggest clues as to the molecular etiology of FMF. Though we are still far from a complete understanding of the function of pyrin/marenostrin at the cellular level, continued study of this intriguing protein is revealing new molecular details about inflammatory processes; the emerging information is relevant not only to FMF, but to innate immunity in general. Data from several laboratories demonstrate that pyrin/marenostrin is intimately connected to three important cellular pathways: apoptosis, cytoskeletal signaling and cytokine secretion. These connections occur, at least in part, through the direct interaction of the pyrin/marenostrin protein with two cytosolic protein adaptors: ASC (also called PyCARD or Tms1) and PSTPIP (also called CD2BP1). Here, we review the more recent literature regarding the molecular and cellular biology of pyrin/marenostrin and pinpoint open questions for future study.

**Keywords**: Pyrin, inflammation, apoptosis, cytoskeleton, periodic fever, cytokine secretion.

## CLUES FROM THE CLINIC

We begin with a brief overview of some of the clinical aspects of FMF that may relate directly to the specific function of pyrin/marenostrin (hereafter referred to as "pyrin" for simplicity). These aspects include the characteristics of typical attacks, possible triggers for these episodes, cytokine profiles in affected individuals during and between attacks, and the emerging relationships between FMF and other inflammatory disorders. This clinical information provides a framework within which emerging research data can be analyzed.

### General Features of FMF

FMF is characterized by recurrent attacks of fever and localized, painful inflammation (reviewed in [42]). Attacks usually resolve within 72 hours and attack-free intervals range from days to months. Inflammation typically involves the peritoneum, pleura, joints or skin. In recent years, vasculitic symptoms have been increasingly reported [70,99] and myalgias may also be a component [47]. Rarer manifestations include orchitis, which may be the initial presentation in young boys [54], and pericarditis [44]. All of these clinical features are highly variable in presentation and fever can at times be the only clinical manifestation of an attack.

Daily prophylaxis with colchicine results in suppression of FMF symptoms in the majority of patients. At present, the molecular mechanism underlying colchicine's effect is not known, but the realization that pyrin co-localizes with cellular microtubules [56] provides an interesting direction for further study. A severe complication of FMF, seen more commonly before the advent of colchicine prophylaxis, is multiorgan deposition of amyloid, of the SAA type. The kidney seems to be most sensitive to amyloid deposition, since significant amyloid in glomeruli causes nephrotic syndrome and chronic renal insufficiency, which may be fatal. In addition to limiting attack severity and frequency, colchicine prevents amyloidosis [77,114].

### Attack Triggers and Cellular Response

The body's immune system must be wired for balance; it must respond vigorously and systemically to a meaningful insult (e.g., a bacterial infection), but be willing to ignore minor violations (e.g., vigorous excercise). It has been proposed that FMF attacks are the result of a failure of that balance, so that minor inflammatory triggers stimulate robust responses. But what are the triggers for FMF patients? Anecdotally, some individuals have reported a correlation between physical or emotional stress and the onset of attacks [42]. Strenuous exercise, consumption of a fatty meal and menstruation have also been flagged as possible triggers. Attacks commonly decrease during pregnancy, leading to the speculation that the *MEFV* gene is regulated by estrogen.

It is interesting that many of the potential attack triggers identified by patients are associated with low levels of inflammation: strenuous exercise causes tissue injury and inflammation, including a detectable surge in inflammatory cytokines [87]; the onset of menstruation is accompanied by the appearance of large numbers of lymphoid and myeloid cells in the endometrium [30]; hormones released during stress can induce an acute phase response similar to that seen upon trauma or bacterial infection [14]. Thus, we can speculate that the induction of these mild inflammatory states might figure in the initial trigger mechanism. Pyrin may be a part of the cellular machinery that is necessary to quickly down-regulate this inflammation; its induction by a variety of pro-inflammatory cytokines could set in motion (or contribute substantially to) an anti-inflammatory response. But what does that response consist of on a cellular level, and what elements of that response are poorly (or differently) performed by mutant forms of pyrin?

An important aspect of the physiological response downstream of the trigger in FMF is a massive influx of polymorphonuclear lymphocytes (PMNs) into a relatively localized site. Sterile effusions in the pleural, peritoneal and synovial cavities of individuals experiencing attacks contain enormous numbers of these cells [77,42] and the exudates resolve concurrently with patients' symptoms. Similarly, histological evaluation of skin lesions reveals an extensive dermal infiltrate consisting primarily of PMNs [42]. Thus, PMNs seem to abnormally home to certain anatomical compartments and are involved in the establishment of localized and systemic inflammatory symptoms. However, mononuclear cells are also present in these tissue exudates [37] and evidence is emerging that some aspects of monocyte function are dependent upon pyrin [19]. Given the important role played by monocytes/macrophages in sensing and transducing inflammatory signals, these cells could figure prominently in the precipitation of an attack. At this time, it is not clear whether the pathophysiology of FMF involves an overly enthusiastic response to inflammatory triggers by resident macrophages (either a heightened sensing of these triggers or increased signal transduction as a result of such stimuli) or an abnormally energetic response to chemotactic signals on the part of neutrophils, or perhaps both. It is also possible that structural cells (i.e., local tissue fibroblasts) could be important in the generation of attacks, since some of these cells do express pyrin. But the mechanism of this is far from clear.

### Cytokine Profiles

Levels of blood cytokines and acute phase reactants have been measured in FMF patients and the results provide additional enticing clues. Typical laboratory findings during an attack include leukocytosis, an elevated erythrocyte sedimentation rate and increased acute phase reactants (e.g. serum amyloid A, fibrinogen, C-reactive protein) [91,9,31]. Several studies have now shown that these components are also elevated between attacks in FMF patients [9,48,76,26]. This tells us that though their overt attacks are self-limited in nature, FMF patients operate well above baseline with respect to their inflammatory state. Thus, patients seem

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poised to react to an otherwise innocuous inflammatory trigger with a vigorous response.

A striking finding in FMF patients is the apparent absence of a C5a/interleukin-8 (IL-8) inhibitor activity in the serosal fluids of FMF patients [65,66]. The activity of this inhibitor is also defective in primary fibroblast cultures derived from the serosa of FMF patients [64]. The anaphylotoxin C5a is a powerful chemoattractant for many leukocytes (e.g. neutrophils, monocytes) and acts as a pro-inflammatory mediator in a host of responses to infection (e.g. it stimulates increased vascular permeability and monocyte/granulocyte oxidative burst responses) [46]. The chemokine IL-8 is also a potent chemoattractant, primarily for neutrophils, although it is also implicated in monocyte adhesion [69]. The C5a/IL-8 inhibitor identified by Matzner and colleagues is a serine protease that inactivates both C5a and IL-8 *via* direct proteolysis. The hypothesis in regards to FMF, therefore, is that even with a normally insignificant inflammatory insult (e.g. minor trauma secondary to running) the absence of the C5a/IL-8 inhibitor allows IL-8 and C5a to accumulate, inducing a massive neutrophil chemotaxis that results in an inflammatory crisis. Although this hypothesis is intriguing, the connection between pyrin's function (see below) and the C5a/IL-8 protease is still unclear.

Less dramatic, but potentially relevant changes in the levels of other cytokines have also been observed. FMF patients exhibit increased levels of serum IL-6, IL-8, soluble ICAM-1 and soluble TNF receptors p55 and p75 relative to controls [45,9]. However, the findings seem to vary depending on the timing of cytokine measurement. It has been suggested that later stages of the attack may be characterized by depleted stores of TNF-$\alpha$ due to a previous massive release of this cytokine from monocytes at the onset of the attacks [85,84]. Gang *et al.* (1999) measured IL-1$\beta$ and IL-1 receptor antagonist levels and suggested that these components are unaltered during attacks [31]. But in another study, mRNA levels for TNF-$\alpha$, IL-1$\beta$, IL-6 and IL-8 were all increased relative to controls in circulating leukocytes of attack-free FMF patients [67]. Finally, Aypar *et al.* (2003) reported high levels of INF$\gamma$ production in FMF patients. Interestingly, the percentage of INF$\gamma$ positive T cells was also increased in FMF patients both during and between attacks [5]. Since the percentage of IL-4 positive T cells (Th2 cells) was not increased, these authors concluded that inflammation in FMF shows a Th1 polarization.

Collectively, these data indicate that individuals with FMF have sub-clinical inflammation when asymptomatic. Surprisingly, even some heterozygotes display increased inflammatory parameters, detectable in the laboratory [106,71]. Indeed, there is growing evidence that in some cases, heterozygotes can experience typical FMF symptoms [12]. Though FMF has generally been classified as a recessive disorder, the disease might be more accurately described as dominant, with reduced penetrance. Such a reclassification would also force the consideration of additional ideas regarding the function of wild type and mutant pyrin. Currently, the hypothesis most commonly embraced is that wild type pyrin acts as a suppressor of inflammation. The obvious corollary is that mutant forms of pyrin are non-functional (or less functional) and therefore ineffective inhibitors of inflammation. A "less functional" protein (rather than a totally inactive protein) actually fits better with the observed spectrum of mutations, which does not include gene deletions or frameshift mutations that lead to severe truncations of the protein. It would be more difficult to understand why missense mutations are more prominent than these "null" mutations if the disease phenotype actually reflects a complete loss of pyrin function.

Taken together, the data thus far do not strictly rule out the idea that pyrin mutations could be gain of function (e.g., mutant pyrin could actively promote the pro-inflammatory pathway or suppress the anti-inflammatory arm). The observation that heterozygotes display a laboratory phenotype of inflammation fits nicely with the gain of function scenario. Yet, another aspect of pyrin's biology could be important in this context: all of the current evidence suggests that pyrin functions in the context of large protein complexes. In such complexes, the inclusion of molecules with reduced function could potentially compromise complex function. This would provide a means whereby a molecule with limited function could exacerbate a phenotype in heterozygotes. Thus, on the basis of present evidence, it is not possible to definitively state whether the mutations in pyrin result in reduction or augmentation of its function.

### FMF and Other Inflammatory Disorders

Perhaps as a result of their heightened inflammatory state, FMF patients and even heterozygotes seem to be predisposed to other types of inflammatory syndromes. Thus, an increased incidence of Crohn's disease [29], juvenile idiopathic arthritis [71], and Bechet's disease [10,86,53] have all been described in *MEFV* carriers and/or patients. It is also possible that some of these disease associations reflect the function of *MEFV* mutations as modifiers of a common inflammatory pathway. Thus, new information concerning the molecular function of pyrin is being closely watched for its likely value in providing new insights into the regulation of general inflammatory networks.

## AFTER THE CLONING: THE MOLECULAR BIOLOGY OF PYRIN

Five years elapsed between the initial association of FMF with a region on human chromosome 16p and the nearly simultaneous identification of the *MEFV* locus by two Consortia [101,100]. Now, seven years after the cloning, we are just beginning to get a glimpse of some aspects of pyrin function at a molelcular level. Below, we summarize recent data concerning the *MEFV* gene, its expression and evolution, and we begin to connect its protein product, pyrin, with distinct cellular inflammatory pathways.

### The Heart of it All: the MEFV Gene

*MEFV* is composed of ten exons and its 3.7 kb transcript encodes a 781 amino acid protein (Fig. **1**). Exon 1 of pyrin encodes a death domain-related structure now known as a "pyrin domain". This turns out to be a particularly interesting portion of the molecule, but at the time of *MEFV* cloning, it was not recognized as such; we discuss this domain at length below. The region spanned by exons 2-10 of pyrin also harbors several known motifs: (a) a bZIP transcription factor basic domain located in exon 2; (b) a B-box zinc finger within exon 3; (c) two putative nuclear localization signals in exons 3-4, one of which is a Robbins/Dingwall consensus [80], (d) an alpha helical region, which may adopt a coiled coil configuration, spanning exons 3-8; and (e) a C-terminal domain variously termed the B30.2/SPRY/rfp domain encompassed within exon 10 where the majority of *MEFV* mutations are found. Although none of these domains has been assigned a definitive function in pyrin, each has relevance in directing investigations of pyrin.

When it was first cloned, pyrin was predicted to encode a transcription factor since it contained apparent nuclear localization signals and a bZIP domain, and exhibited a general similarity to nuclear factors of the B-box/coiled coil/rfp domain class (e.g., Xnf7, Ro52). However, thus far, the bulk of the extant evidence suggests that pyrin functions in the cytosol. In localization studies (done primarily by overexpression of epitope tagged versions of pyrin in transfected cells), pyrin is consistently cytosolic [79,74,56]. Mansfield *et al*. (2001) demonstrated that pyrin associates with actin in membrane lamellopodia and microspikes, and that the N-terminal portion of the molecule co-localizes with microtubules, a possible clue as to colchicine's effectiveness in treating FMF [56]. The two pyrin-interacting proteins thus far characterized, ASC and PSTPIP, are both primarily cytosolic as well [79,56] and PSTPIP in particular is an actin-associated protein [92]. The emerging picture is one in which pyrin functions in close proximity to the cytoskeletal network, a position that would permit modulation of both "inside out" and "outside in" signaling pathways [23].

Recently, a nuclear form of pyrin was identified: a splice isoform of pyrin that encodes a protein lacking exon 2 localizes to the nucleus in transfected cells [74]. This alternatively spliced isoform seems to represent only a minor fraction of the message population and no nuclear function has been ascribed to it as yet. Nevertheless, based on current information, it seems likely that pyrin can occupy both nuclear and cytoplasmic compartments, and our preliminary studies suggest that this can vary in a cell-type specific way. The further clarification of pyrin's sub-cellular location is clearly an important open avenue for further study, but will require the generation of antibodies that function in immunohistochemical applications.

The B-box zinc finger, coiled-coil domain and rfp domain of pyrin comprise a tripartite motif that is found in a large number of other proteins [38,104,78]. The majority of these proteins seem to form large protein complexes either in the nucleus or in the cytoplasm [16]. The B-box and coiled-coil regions of these proteins function cooperatively or individually to form specific homo- or hetero-dimers with other proteins [104,90]. Indeed, initial indications are that pyrin also functions in the context of large molecular aggregates (discussed further below).

The C-terminal rfp/B30.2/SPRY domain of pyrin is particularly interesting because it harbors the majority of FMF mutations. Yet, no function has been thus far ascribed to this domain, which is found on a surprisingly wide variety of proteins (reviewed in Henry *et al*. 1998, [38]). It has been speculated that the rfp domain is involved in signal transduction

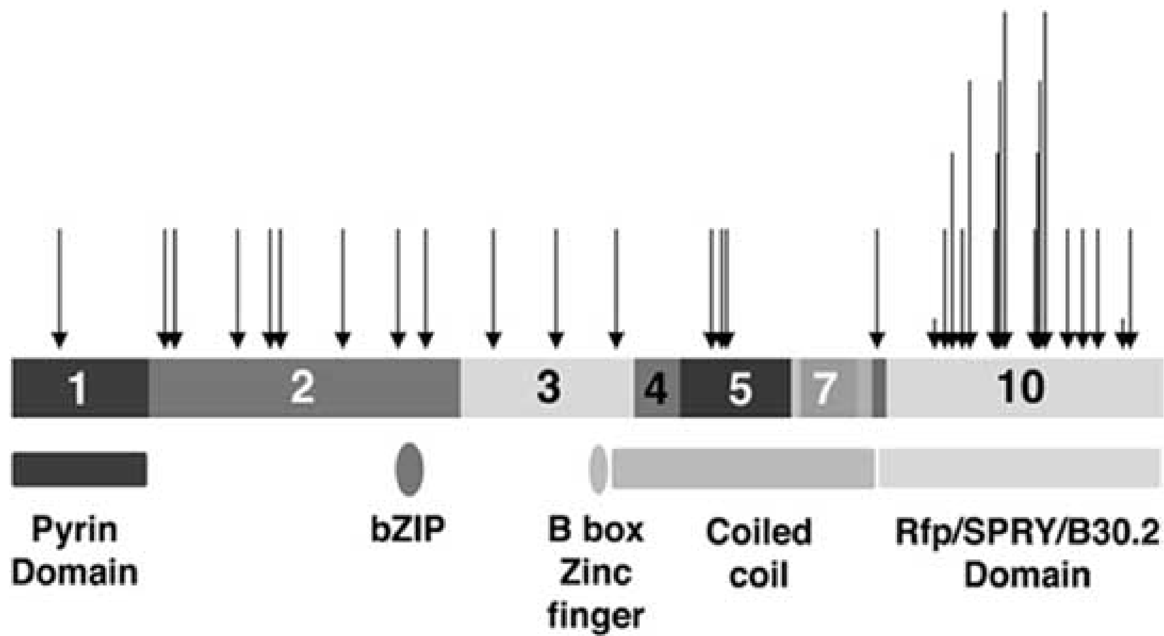


**Fig. (1).** *Schematic view of the pyrin protein.* Boxes represent exons, drawn to approximate size scale. Specific domains are annotated below. Mutations that are known to cause disease are indicated by arrows (information taken from the INFEVERS web site).

or ligand binding and the fact that many of the mutations seem to cluster within this domain suggests the location of an important functional interface or ligand binding site. However, direct evidence for this is lacking.

Curiously, analysis of the mouse and rat pyrin protein reveals that the rodent rfp domain is missing entirely; this is due to a frameshift in the genomic sequence in a region immediately 5' to this domain [18]. Perhaps the rfp domain performs a regulatory function (e.g., modifying a basal function of pyrin) that is dispensible or undesirable in rodents. Another region of striking divergence between human and mouse pyrin occurs in exon 2; though retained in both species, the amino acid sequences are less than 50% homologous. This exon is the site of eight additional FMF mutations. The occurrence of multiple mutations in *non-conserved* regions of the pyrin molecule runs contrary to the more common paradigm in which mutations cause disease precisely because they occur in functionally conserved amino acids. We will return to this unusual pattern of mutations when we discuss evolution of the rfp domain (see below).

**MEFV Expression and Regulation**

*MEFV* gene expression is activated during myelopoiesis near the time of lineage commitment and transcripts are strongly evident in mature neutrophils, eosinophils and monocytes [17]. Although one group reported low levels of expression in both T and B cells [103], others have not observed this [101,17]. It has been reported that *MEFV* is expressed in dendritic cells [49], and we have confirmed this at both the protein and mRNA level (unpublished data).

*MEFV* expression has also been demonstrated in primary lines of serosal and skin fibroblasts [64] as well as synovial fibroblasts ([100], our unpublished data). Thus, pyrin is expressed in a number of structural cells at sites where inflammation is commonly observed during FMF attacks. Whether this is important in the attack trigger or response has yet to be determined.

In many of these expressing cells, *MEFV* basal expression is low and can be inducibly upregulated, although there seem to be cell-specific differences in the *MEFV* response and there may also be species differences. In human monocytes, *MEFV* is upregulated when incubated for 24 hours with LPS, TNF-α, and IFN-γ while it is downregulated in response to anti-inflammatory cytokines such as IL-4, IL-10, and TGF-ß [17]. Induction of *MEFV* in response to LPS and IFN-γ occurs within 30 minutes, and is not inhibited by cycloheximide, suggesting that these molecules directly signal transcriptional upregulation. We have consistently detected pyrin induction in response to LPS and TNF-α, and have observed up-regulation in response to IL-4 and IL-10 as well. Inductive responses in general seem to be highly variable in human monocytes. In neutrophils, IFN-γ upregulates *MEFV* but LPS, TNF-α, IL-10 and IL-4 have no effect on *MEFV* expression in these cells [17]. Lastly, *MEFV* can be upregulated in primary peritoneal fibroblast cell lines in response to both IL-1β and PMA [1]. The apparent cell-type specificity in induction pattern may point to fundamental differences in pyrin's role within these various cells; uncovering the functional ramifications of this complex regulation is an important future goal.

In inbred mice, where genetic variation is minimized, a more well-defined picture of pyrin regulation emerges and pathways can be explored by controlled variation of the genetic background. In C57BL/6 mice, resident peritoneal macrophages respond to LPS, TNF-α and IL-1β with upregulation of pyrin protein within 24 hours. In addition, the anti-inflammatory cytokines IL-4 and IL-10, when given alone or in combination with LPS, are extremely potent stimulators of *MEFV* expression in mouse peritoneal macrophages [19]. Indeed, macrophages from IL-4 deficient mice exhibit reduced pyrin protein, indicating that this anti-inflammatory cytokine is necessary for basal levels of murine *MEFV* expression [19]. On the other hand, macrophages from IFN-γ null mice constitutively express pyrin protein, suggesting that IFN-γ normally suppresses expression of *MEFV*. The apparently different response of *MEFV* from mouse and human macrophages to IFN-γ stimulation could reflect species differences in gene regulation, or could be due to the fact that the human studies were done on CD34+ bone marrow cells or peripheral blood monocytes [17], while resident peritoneal macrophages were used in the mouse [19].

As predicted from the cell-specificity and inductive response of the *MEFV* gene, Centola *et al.* (2000) found *in silico* evidence of promoter binding sites for myeloid-specific factors (PU.1, AML, c-Myb, TAL-1) as well as sites for factors important in the mediation of inflammatory responses (NF-κB, AP-1, Interferon response elements). The important functional contributions of an NF-κB site at –163 and a C/EBPβ site at -55 were recently outlined by Papin *et al*. [73]. These authors found that the C/EBPβ site is required for TNF-α responsiveness, while the NF-κB site synergistically increases this response. Binding sites for both factors are also seen in the proximal promoter of the mouse *MEFV* gene. It will be interesting to see if this synergistic induction represents a key control step in FMF flares, a question that can be addressed in a suitable mouse model.

**Mutations**

Over 40 different *MEFV* mutations have now been identified (Fig. **1**). Current listings of all mutations and polymorphisms can be easily derived by visiting INFEVERS, a mutational database found at:

http://fmf.igh.cnrs.fr/infevers/ [82]. All except one of the documented mutations thus far demonstrated to cause disease are either missense mutations or in-frame deletions. The single exception is Y668X, a frameshift that encodes a C-terminal truncation and produces a protein lacking half of the rfp domain [68]. As discussed above, the complete lack of null or early truncation frameshift mutations in FMF patients is curious. Future experiments in engineered mouse models might reveal whether such mutations would produce different kinds of consequences, not recognized as the typical FMF profile.

Though concentrated in exon 10 (the rfp domain), *MEFV* mutations can be found in nearly every exon, and there are striking differences among mutations with respect to their severity and penetrance. For example, a number of studies have attested to the more severe nature of the M694V mutation, while the E148Q mutation seems to produce very mild disease or in some cases, no symptoms at all. In fact, there is still argument as to whether the E148Q change is truly a mutation or simply a polymorphism [3,72,2,98]. Booth *et al.* (2001) documented the over-representation of E148Q in a number of patients with other inflammatory disorders and proposed that this variant may act to generally augment the inflammatory state, conferring greater susceptibility to a variety of inflammatory diseases [15]. Though still to be proven, this notion that variations in the pyrin sequence could act in non-specific way to modulate the inflammatory response fits well with evolutionary findings discussed next.

**Epidemiology and Evolution**

FMF classically affects non-Ashkenazi (Sephardic) Jews of North African or Middle-Eastern descent, Armenians, Middle-Eastern Arabs and Turks [81,11,105], but clinical cases in individuals of non-Mediterranean ancestry, once thought to be rare, are increasingly reported now that genetic diagnosis is available. A fascinating finding still to be completely explained is the remarkable frequency with which mutant alleles are observed in many of the Mediterranean populations. Carrier frequencies of 1:3.5 to 1:4.7 have been reported for four mutations (M680I, M694V, V726A and E148Q) in Ashkenazi and Iraqi Jews, Moroccans and Muslim Arabs [32]. Aksentijevich *et al.* (1999) determined the frequency of known FMF mutations in 200 American Ashkenazi Jewish individuals undergoing genetic screening for other diseases and found a mutant allele frequency of 1:5 [3]. The carrier rate in Turkey has also been estimated at 1:5 [113].

These impressive carrier rates have led many authors to speculate that heterozygotes carrying mutant FMF alleles have a selective survival advantage, perhaps because their heightened inflammatory state helps them clear as yet unidentified endemic Mediterranean pathogen(s). Single nucleotide polymorphism (SNP) haplotype analyses also support this idea. SNP data indicate that several of the most common mutations (M694V, V726A M680I, and E148Q) are likely to be ancient mutations [3,101]. That is, the same founder haplotype is found in ethnic groups that have been geographically and socially isolated for thousands of years. The fact that these ancient mutations are still present at such high frequencies in multiple populations supports the notion that selective pressures have kept the mutant alleles prevalent [101,3].

Some additional support for the presence of a selective advantage comes from an analysis of pyrin's evolution in primates. Schaner *et al.* (2001) sequenced exon 10 of *MEFV* (the rfp domain is contained within this single exon) in 20 primates and 2 non-primate mammals and then applied maximum likelihood tests to examine the $d_N/d_S$ ratio, a measure of positive selection [83]. The $d_N/d_S$ ratio compares the relative rate of non-synonymous change ($d_N$, the mutation changes the amino acid sequence) to the relative rate of synonymous change ($d_S$, the mutation does not alter the amino acid state). Strong evidence of positive selection is present when the $d_N/d_S$ ratio is greater than 1.0. For comparison's sake, the $d_N/d_S$ ratio for most proteins ranges from 0.3 to 0.03 [51]. Schaner *et al.* found high $d_N/d_S$ ratios on several major evolutionary branch points. For example, the separation of platyrrhines (New World Monkeys) from catarrhines (Old World Monkeys, apes and human) is marked by a $d_N/d_S$ ratio of 1.5 on the branch leading to the New World Monkeys [83].

Perhaps even more striking, this study showed that at seven of the 10 mutant positions studied, amino acid residues that are considered "mutant" in humans were found as wild type in primates [83]. In fact, in several cases, the human mutations appear to recapitulate ancient sequence states during primate evolution. For example, at position 761, the wild type amino acid is arginine while the mutant amino acid is histidine. Examination of the primate sequences shows that only humans and apes carry arginine at this position. All non-primate mammals, all pro-simian primates, and all New World Monkeys carry histidine instead. Thus, the change from arginine to histidine was a relatively recent event that occurred after the separation of Old World Monkeys from the apes. Yet, a change back to arginine causes FMF in humans.

Together, these data suggest that pyrin is functionally evolving in response to environmental factors; the fact that changes in pyrin sequence often correlate with major cladistic branch points suggests that as species encounter new environments, new selective pressures may force a change in pyrin sequence. Like the absence of null mutations discussed above, this evolutionary pattern is consistent with the idea that the missense mutations somehow change the *character* of pyrin and that this change results in a phenotype that can be selected for. So, is FMF actually a dominant disease of reduced penetrance? Are the mutations gain of function mutations or do they create a molecule with reduced function? What are the environmental factors that pressure pyrin evolution? It will be fascinating to learn the nature of such selective pressures; it is possible that these triggers for pyrin change are also related to the triggers that exacerbate attacks.

**Mouse Models of FMF: Engineering the Pyrin Deficient Mouse**

The lack of human null mutations in *MEFV* tempts the speculation that such a mutation would either be lethal in consequence, or would have a very different phenotype. The worrisome extension of this speculation is that the pyrin null mouse would therefore provide a poor model for the human disease phenotype. In a clever variation on the knock-out scheme, Chae *et al*. (2003) engineered a mouse pyrin hypomorph [19]. The truncated pyrin protein produced from this targeted murine allele lacks the C-terminal half of the molecule (B box, coiled coil), but like the human pyrin protein, retains the N-terminal half, including the important "pyrin domain" (see below) encoded by exon 1.

This mouse model provides the best argument thus far that an FMF-like phenotype can result from reduced function of the pyrin molecule. Mice homozygous for this truncated form of pyrin exhibit heightened sensitivity to endotoxin challenge and show elevated production and secretion of IL-1β. Thus, the mice display an increased innate immune response, a phenotype that mirrors human FMF. Despite the fact that the myeloid cells of this mouse still express a portion of the pyrin molecule (and this portion has important functional roles, see below), heterozygote mice have a phenotype similar to wild type, suggesting that the truncated protein does not have a dominant negative function. Interestingly, the mice also display an apoptotic defect (macrophages exhibit reduced apoptosis in response to IL-4 and LPS) and this effect is not simply a result of increase in IL-1β secretion. This link to apoptosis is further discussed below.

While this hypomorphic mouse model will help to unravel some of the still enigmatic aspects of pyrin's function, there are important questions that most likely cannot be addressed in this system. The most central of these is: how do mutant forms of human pyrin cause disease? Since the mouse pyrin protein lacks the C-terminal rfp domain, the site of the majority of human mutations, it seems likely that a full appreciation of the *in vivo* function will require a model in which the mouse pyrin protein is replaced with its human counterpart. Such a model is indeed in the works. For this model to be useful, it will have to be demonstrated that the human pyrin protein retains all of its normal interactions in the mouse milieu.

## PYRIN INTERACTING PROTEINS – BROADENING THE PLAYING FIELD

When the *MEFV* gene was first cloned, its sequence did not suggest a function, and investigators were therefore left without an assay system to facilitate further investigations. To probe pyrin's intersection with known cellular pathways, yeast two hybrid screens were carried out in the Kastner laboratory as well as our own. Interestingly, despite the fact that the two labs used different cellular cDNA sources (monocytes and neutrophils, respectively), the two screens resulted in the isolation of the same two proteins: ASC/PyCARD/Tms1 and PSTPIP/CD2BP1 (called ASC and PSTPIP1 hereafter). The further investigation of these two proteins has led the field in interesting new directions.

**ASC, a Direct Connection to Apoptosis and Inflammation**

ASC (the acronym stands for "Apoptosis-associated Speck-like Protein with CARD Domain") was first cloned in late 1999 as a protein that forms large cytosolic "specks" in HL-60 cells undergoing apoptosis [60]. The authors noted that ASC's first exon was homologous to pyrin's first exon and that the domain encoded by this exon had some similarity to a CARD domain (a protein:protein interaction domain found on proteins

that participate in the apoptotic cascade). It is now known that pyrin and ASC are not the only two proteins to share this new variant of a CARD-like domain. It has been found in over 30 proteins in the human database [33], and has been the target of considerable excitement over the past three years, since proteins carrying this domain function in apoptosis and inflammation [40,13,4,58,75]. Of course, each laboratory has claimed the right to name the domain, and it has been variously referred to as a pyrin domain (PyD) [13,58,28], DAPIN domain [94], or PAAD domain [75], with PyD (or variations thereof) and PAAD being the more popular terms. Throughout the rest of this review, we will refer to this domain as the PyD (admitting some emotional link to this choice).

Nearly 20 members of the PyD family are structurally related. These proteins contain an N-terminal PyD, a central nucleotide binding domain (NBD) and a C-terminal leucine rich repeat (LRR) [36]. One of these is cryopyrin (also called PYPAF1, NALP3 or Caterpiller 1.1). Like pyrin, cryopyrin is mutated in a hereditary periodic inflammatory disease. Cryopyrin, its role in inflammation and its mutation in Muckle-Wells syndrome, familial cold urticaria as well as NOMID/CINCA are discussed more extensively in previous reviews [35,43].

**ASC Expression and Induction**

Structurally, ASC is a 195 amino acid protein composed of an N-terminal PyD and a C-terminal CARD, separated by a small linker exon [60,21]. Both of these domains are well-conserved between human and mouse [62]. Like FADD with its death domain and death effector domain [97], ASC functions as an adaptor, using its two protein:protein interaction domains to connect CARD-containing proteins to PyD-containing ones.

ASC is enriched in a number of myeloid cell lines, including HL-60, U937, K562 and THP-1 [60,62,21]. *In vivo,* ASC is expressed in a number of differentiated human epithelia, such as the squamous epithelium of the skin and cells of the upper colonic mucosa, suggesting that it is associated with cell differentiation [61]. Interestingly, these are also tissues that undergo a constant turnover of cells; new cells are constantly generated from stem cells as older cells are lost by apoptosis. It is possible that ASC plays a role in this apoptotic homeostasis.

ASC is also strongly present in CD14+ monocytes, moderately present in PMNs and CD3+ T lymphocytes, and absent in CD-20+ B lymphocytes [61]. In human neutrophils, ASC protein is upregulated in response to pro-inflammatory stimuli (i.e. IL-1α IL-1β, IFN-γ, IFN-α, TNF-α, and LPS), with expression peaking at two hours and returning to basal levels by four hours [88]. Pro-apoptotic signals also up-regulate ASC: PMNs treated with Fas ligand display increased ASC protein that peaks at four hours and continues at that level until at least eight hours [88]. These data suggest that ASC plays a part in both neutrophil inflammation and apoptosis, and that sustained, high levels of ASC expression may be a hallmark of neutrophil apoptosis.

Further investigation of the functional properties of ASC's PyD and CARD domains demonstrated that both of these domains have the ability to self and cross oligomerize [63,79]. Thus, like many proteins that contain DDs, CARDs and DEDs, ASC is able to self-associate; this capacity is essential for the function of ASC, as its major role seems to be to promote signal transduction cascades *via* proximity induced activation of binding partners.

**The ASC Speck**

At the cellular level, ASC can be seen in the nucleus as well as the cytosol both *in vivo* and *in transfecto* [61,79]. A hallmark of its ability to self-associate is the "speck", a large cytosolic aggregate of ASC that is still not molecularly well-defined. Specks have been seen *in vivo* as well as after transfection of ASC into a variety of cell types. At high magnification, the speck is a tangle of fiber-like processes likely caused by self-association of ASC in combination with other proteins [79]. The process of speck formation is dramatic in that the specks seem to corral virtually all of the cell's complement of ASC. It is likely that nucleation of the speck is the rate limiting step, and that once specks are present, cellular ASC is rapidly added to the ends of the growing fibrillar tangle. The isolation and further characterization of specks and their associated proteins is an important next step in their functional characterization. It is still not clear if the formation of the speck is necessary for apoptosis or if the speck represents a late-stage structure that forms as a consequence of the death process.

**ASC Interactions: a Myriad of Possibilities**

By virtue of its interaction with ASC, pyrin is brought to the hub of the cell's response to inflammatory triggers. ASC seems to be an important adaptor in several processes, including caspase-1 activation and IL-1β secretion, NF-κB activation and apoptosis. Fig. (**2**) summarizes some of the major ASC interactions that impact these pathways.

Proteins that can interact with the CARD of ASC appear to constitute key effectors of ASC-mediated signaling; the binding of CARD-containing factors to ASC's CARD can result in the activation of caspase-1 or NF-κB. For example, the CARD of ASC interacts directly with the CARD domain of caspase-1 [93,108,57]. Caspase-1, a long pro-domain caspase, is important in processing the cytokines IL-1β and IL-18 from their inactive zymogens to highly active pro-inflammatory molecules [24]. In addition, ASC's CARD interacts directly with the CARD of RICK/CARDIAK/RIP2 [96], a serine threonine kinase activator of the IKK complex (which activates NF-κB through phosphorylation and subsequent degradation of the cytosolic IκB inhibitor).

Finally, a third protein recruited by the ASC CARD is Ipaf/CLAN/CARD12. Downstream of its CARD domain, Ipaf resembles cryopyrin, in that it contains a nucleotide binding domain (NBD, also called a Nod or NACHT domain) and a series of leucine rich repeats (LRR). Interestingly, Ipaf's CARD is also capable of binding directly to the CARD of caspase-1, leading to caspase-1 activation [59]. Thus, both ASC and Ipaf alone can bind and activate caspase-1 *via* CARD:CARD

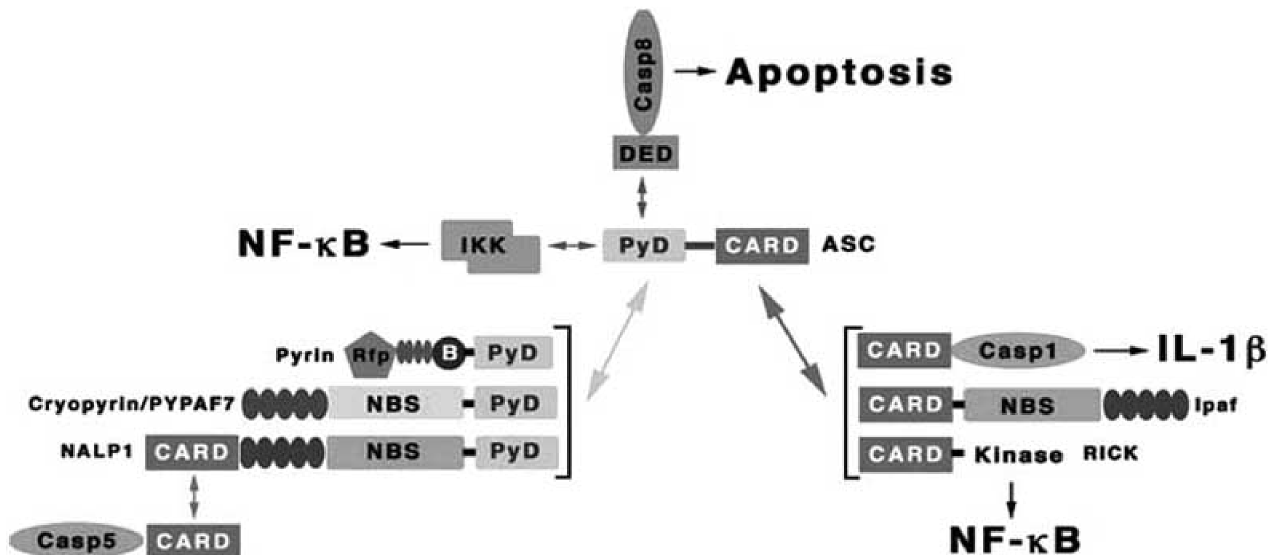


**Fig. (2).** *ASC and its interacting proteins*. Proteins known to interact with the ASC PyD are shown on the left, while those known to interact with the ASC CARD are shown on the right below the ASC molecule. Two more unconventional interactions are shown with smaller arrows directly at the ASC pyrin domain (IKK and caspase-8). The consequences of these interactions are discussed in the text.

interactions. But, ASC and Ipaf can also interact with each other *via* these CARD domains; the outcome of this interaction is activation of NF-kB and apoptosis [59].

Proteins that can bind to ASC's PyD include pyrin, cryopyrin, PYPAF5, PYPAF7 and DEFCAP/NAC/CARD7/NALP1. These interactions seem to impart regulation, either *via* oligomerization, or by other means. For example, though ASC can bind to RICK, transfection of cells with ASC alone activates NF-κB only weakly, and cryopyrin (or PYPAF5 or 7) alone does not stimulate NF-κB activity at all [108,55,34]. However, the co-expression of cryopyrin or PYPAF5/7 with ASC results in a robust activation of NF-κB. This effect requires the PyD and is mediated by the NBD-dependent oligomerization of the PyD-NBD-LRR (PYPAF) protein; oligomerization results in the proximity-induced activation of an effector such as RICK.

It is interesting that proteins containing the NBD/LRR motifs can interact either from the PyD side or the CARD side of ASC, depending upon whether they themselves contain a PyD (PYPAFs, pyrin) or a CARD (Ipaf) at the N-terminus. There is also a protein that contains the NBS-LRR motif and is surrounded by a PyD at one end and a CARD at the other. This protein, called DEFCAP/NAC/CARD7/NALP1, can bridge to ASC through its PyD and to caspase-5 through its CARD. Because ASC can bind to caspase-1, this brings both caspase-1 and 5 into proximity, an effect that may be important for the cell's inflammatory response. This structure (DEFCAP/NAC/CARD7/NALP1, ASC, caspase-5 and caspase-1) is known as the inflammasome [57]; it may have an important effector role in the macrophage response to inflammatory signals.

At least two other interactions are known to occur at ASC's PyD, though both involve unusual or poorly characterized binding by non-PyD containing proteins (Fig. **2**). First, caspase-8, an important initiator caspase, appears to bind to ASC *via* an unusual heterotypic interaction between its death effector domain (DED) and the PyD of ASC [59]. This seems to be a rather low affinity interaction that therefore sets up an interesting situation, since other PyD containing proteins could displace caspase-8 from ASC, with potentially important consequences. Indeed, this has been documented and is discussed further below. Second, at least at high concentrations, the PyD of ASC seems also to interact directly with the IKK complex [55], inhibiting its kinase activity, an action that results in down-regulation of NF-κB [59,95]. This interaction is also subject to competition (see below).

**Context, Competition and ASC Function**

As the myriad of interacting proteins might predict, the function of ASC seems to be dictated to a great degree by the cellular context. Not only is cell type at issue, but the cytokine milieu in which the cell finds itself is likely to influence ASC function. At low concentrations, ASC appears to be a weak NF-κB activator, and in the presence of cryopyrin, PYPAF5 or PYPAF7, NF-κB is synergistically activated [108]. But, in cells stimulated with TNF-α, ASC profoundly inhibits rather than activates NF-κB activation [95]. A similar bi-phasic effect of ASC on IL-1β secretion has also been demonstrated [96]. Though much more work is required to sort out these responses, the existing data point to a great deal of pathway crosstalk, with ASC at the hub of the conversation.

In the zebrafish, two proteins have been identified that interact with zASC: caspy1 and caspy2, encoding the zebrafish homologues of human caspase-1 and caspase-5. It is interesting that the most ancient forms of the PyD to be identified are found on zASC, caspase-1 and caspase-5, suggesting that the vertebrate inflammatory pathway had an early dependence on ASC-mediated signals. Evidence that these proteins further evolved in concert is perhaps provided by the finding that mammals seem to have evolved a specific multi-protein structure to keep all three of these proteins together in one complex, the inflammasome. In this complex, formed by the association of ASC, DEFCAP/NAC/CARD7/NALP1, caspase-1 and caspase-5, the processing of IL-1β is enhanced over that seen in the presence of activated caspase-1 alone [57].

The fact that so many proteins can interact with ASC sets the stage for competitive regulatory interactions, some with potential disease implications. Recently, Dowds *et al*. (2003) presented evidence that co-expression of cryopyrin and ASC results in activation of NF-κB as well as induction of apoptosis [25]. Since enforced oligomerization of the PyD of cryopyrin or oligomerization of ASC itself also had this effect, the authors concluded that triggered oligomerization of ASC by cryopyrin results in apoptosis and NF-κB activation *via* an induced proximity mechanism [25]. Strikingly, these pro-inflammatory ASC:cryopyrin interactions could be competitively disrupted by co-expression of wild type pyrin, a scenario that could account for the predicted anti-inflammatory activity of wild type pyrin. Since these studies were done in transfected HEK293 cells, it will be interesting to investigate whether the same competitive interactions occur in endogenous cells. Also, determing the effect of pyrin mutations on these interactions will be important.

In a similar way, Ipaf and ASC act together to induce NF-κB activation and apoptosis, effects that are mirrored by enforced oligomerization of the Ipaf CARD domain [59]. Both the CARD and the PyD of ASC are required for these activities; the CARD promotes the ASC:Ipaf interaction while the PyD interacts directly with caspase-8 or IKK [59]. Interestingly, co-expression of pyrin has an inhibitory effect on both NF-κB activation and apoptosis. The apoptosis seems to be mediated by a caspase-8 dependent pathway, since it is not seen in caspase-8 null fibroblasts [59]. The inhibitory effect of wild type pyrin seems to arise from its ability to displace caspase-8 from its association with ASC [59], perhaps inhibiting its proximity induced activation. We have confirmed the one-way competition between caspase-8 and pyrin using immunofluorescence (Fig. **3**). Caspase-8 binds to ASC specks *via* its DED, and pyrin displaces caspase-8 from specks in a manner that depends on its PyD. The interesting proposition that mutant forms of pyrin might not be as effective as wild type pyrin in competing caspase-8 from specks has not been borne out in our studies to date.

It is intriguing that several of the proteins of structure CARD/NBD/LRR function in some capacity as molecular recognition receptors that become activated in response to a stress related event [41]. They bind bacterial products *via* the LRR, oligomerize *via* the NBD and then carry out an effector function mediated by connection to another protein through the CARD. The proteins of the PyD/NBD/LRR structure may behave in a similar fashion, but the PyD would connect to ASC's PyD, and ASC, through its CARD, would then link to the effector. In this way, these PyD proteins could potentially act as molecular switches controlled ultimately by the binding of some ligand to their LRR (though none of the putative ligands are yet known).

Pyrin lacks the NBD and LRR, but the presence of its PyD might mean that it can modulate the functions carried out by members of the PyD/NBD/LRR group. Indeed, there is already evidence that pyrin's interaction with ASC modulates ASC's multiple functions, including speck formation and apoptosis [79], NF-κB activation [59] as well as caspase-1 activation and IL-1β secretion [19]. Thus, pyrin too has the potential to toggle the functional switches for inflammation and cell death pathways.

**Solving the PyD Structure: New Revelations**

Recently, the structure of cryopyrin's PyD was examined by nuclear magnetic resonance [39]. As is the case for other members of the death domain superfamily, the PyD consists of a bundle of six alpha helices. The remarkable aspect of the structure is that helix 3, a site important for intermolecular bridging in other death domain structures, appears to be locally unfolded. It has been proposed that this locally unfolded region could act like a conformational switch; regulated folding and unfolding of this helix could dictate downstream events. Other PyDs, including pyrin, also seem to have an amino acid composition in this region that suggests a low intrinsic helical character.

Interestingly, an FMF mutation, R42W, maps to the helix 3 region of pyrin's PyD and the data of Hiller *et al.* (2003) suggest that this mutation is located within the region that might be important for intermolecular contacts. Eliezer (2003) further examined the intrinsic helical propensity of wild type and mutant pyrin in this region and speculated that the R42W mutation might force higher helical character into the third helix, perhaps destroying its switching capabilities [27]. Unregulated (constitutive) binding of this well-folded PyD to some other effector molecule in the inflammatory cascade could thus result in uncontrolled inflammation. This predicts that "activated" pyrin functions as a pro-inflammatory molecule and that this particular disease mutation produces a constitutively active form of the protein. Of course, this scenario would not explain the periodic nature of attacks, nor would it fit with a loss of function model. The available data are also compatable with other interpretations that seem to fit these aspects better. For instance, given the predicted role of helix 3 in homotypic interactions with other PyDs, it is possible that the R42W mutation interferes with such interactions, rendering the molecule functionally ineffective.

Is it possible that under some conditions, the PyD or CARD of ASC interacts with more than one protein at once? Indeed, it has been proposed, on the basis of the Apaf-1:caspase-9 and the Drosophila Tube:Pelle structures, that these death domains actually possess two

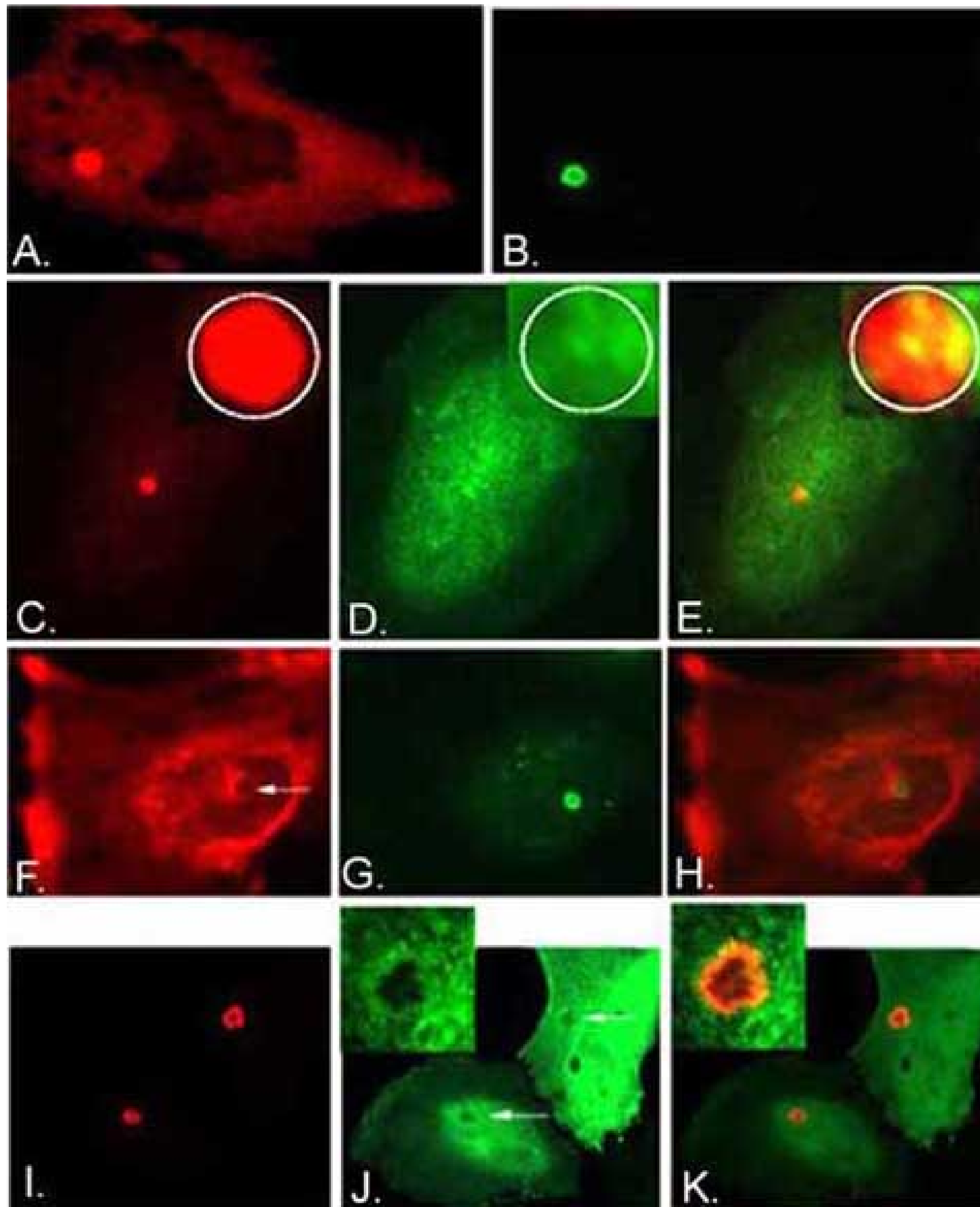


**Fig. (3).** *Caspase-8 binds to ASC specks; pyrin competes caspase-8 from ASC specks.* A,B) HeLa cells were transfected with 300 ng of myc-ASC and 400 ng of caspase-8m-HA (mutant form that does not cause apoptosis). A) Caspase-8 (red) is localized to an ASC speck in these doubly transfected cells. B) All of the ASC in the cell is collected in the speck (same cell as shown in A.). In the absence of ASC, caspase-8 is never found in specks and caspase-8 does not co-localize with pyrin in any visible structure (not shown). C-E) HeLa cells were transfected with 50 ng of myc-pyrin, 300 ng of myc-ASC and 400 ng of caspase8m-HA. C) Pyrin (red) is concentrated in the speck. D) Caspase-8 is highly expressed, but is not found in the speck. E) Overlay of the image of pyrin (red) and caspase-8 (green). Note that the speck is essentially red. Insets provide magnification of the speck. F-H) A pyrin molecule lacking exon 1 (the PyD) is unable to compete caspase-8 from specks. HeLa cells were transfected with 1300 ng myc-pyrin exon 2-10, 300 ng ASC and 400 ng caspase-8m-HA. F) Pyrin exon 2-10 (red) is not recruited to the speck. G) Caspase-8 (green) is localized in the speck. H. Overlay of pyrin (red) and caspase-8 (green) showing that the speck is essentially green. I-J) Caspase-9 is not recruited to specks. HeLa cells were transfected with 400 ng caspase-9m-HA and 300 ng myc-ASC. I) ASC (red) is collected in specks. J) Caspase-9 (green) is not present in specks. K) Overlay of ASC (red) and caspase-9 (green) showing that the specks are essentially red. Insets in J and K show specks at higher magnification. Thus, ASC binds specifically to caspase-8 (and not caspase-9) and recruits it to specks. Pyrin competes for caspase-8 binding to specks in a PyD-dependent manner.

interaction surfaces that are not mutually exclusive [109]. The fact that expression of ASC's PyD alone or its CARD alone results in the formation of long filaments (rather than dimers) in transfected cells that lack endogenous ASC is evidence that these domains each contain more than one interaction face. But at this point, it is not clear if two different molecules could interact at one time with a given domain of ASC. If this is the case, then the spectrum of possible interactions is considerably more complex than initially imagined.

### PSTPIP1, Connections to the Cytoskeleton

Besides ASC, the only other pyrin-binding protein identified to date is a second adapator protein called PSTPIP (or CD2BP1). Using full length pyrin as bait, both the Kastner laboratory and our laboratory isolated PSTPIP in a yeast two hybrid assay, and confirmed that interaction *via* immunoprecipitation and immunofluorescence ([89], unpublished data). We refer to this protein as PSTPIP1 in this review. Pyrin's interaction with PSTPIP1 points to some intriguing connections between the cytoskeleton, inflammation, and disease.

PSTPIP1 is a member of the pombe Cdc15 homology (PCH) family of proteins, and contains an N-terminal FCH (Fer, CIP4 homology) domain, a central coiled-coil domain and a C-terminal SH3 domain [50,92,52]. PSTPIP1 is present in both neutrophils and monocytes, where pyrin is found, though PSTPIP1 is also found in pyrin-negative tissues such as lung, spleen, thymus and small intestine [92,50].

As with other members of the PCH family, PSTPIP1 has been strongly implicated in the regulation of actin based structures. Overexpression of

PSTPIP1 induces formation of filapodia, suggesting that it functions in cytoskeletal reorganization [92]. Although a direct interaction between PSTPIP1 and actin has yet to be demonstrated, endogenous PSTPIP1 is associated with the cortical actin cytoskeleton, lamellipodia, and the cytokinetic cleavage furrow [92]. Indeed, it may be that PSTPIP1's interaction with the cytoskeleton is indirect, as it interacts (*via* it SH3 domain) with the proline-rich regions of two proteins that bind to the actin-based machinery: WASp and c-Abl [7,20].

WASp, the protein mutated in Wiskott-Aldrich Syndrome [112], is a complex modular protein important in integrating and modulating diverse cellular signals that regulate the actin cytoskeleton. The C-terminus of WASp directly binds the Arp2/3 complex and nucleates the growth of actin filaments [102]. Interestingly, pyrin also co-localizes with a subset of microfilaments [56], although it is not clear as yet if this is a direct or indirect association.

Another means by which PSTPIP1 connects to the cytoskeleton is through c-Abl, as this kinase directly binds to filamentous actin and phosphorylates proteins involved in the regulation of actin dynamics (e.g. paxillin, Crk, p130Cas) [111,107]. As is the case with WASp, PSTPIP1 binds to c-Abl *via* its C-terminal SH-3 domain. Infact, c-Abl phosphorylates PSTPIP on tyrosine 344, and this event increases the binding of PSTPIP to wild type pyrin [89].

Whereas c-Abl phosphorylates PSTPIP1, this action is reversed by the PEST phosphatase family of proteins (PTP-PEP, PTP-PEST, PTP-HSCF), all of which interact with the coiled coil of PSTPIP [50,92]. But the link between PSTPIP1 and the PEST phosphatases is not solely for the purpose of dephosphorylating PSTPIP1. PSTPIP1 serves as a molecular bridge that brings other substrates to these phosphatases; two of these substrates are WASp and c-Abl [22,20]. In the case of c-Abl, dephosphorylation *via* PEST phosphatases (requiring PSPTIP1) alters kinase activity [20].

While the functional significance of WASp dephosphorylation by PTP-PEST is unclear, PSTPIP1 is clearly necessary for certain WASp mediated activities, such as actin polymerization at the immunological synapse in T-cells [6]. In this case, PSTPIP1 serves to link the transmembrane receptor CD2 to WASp, allowing the reorganization of the actin cytoskeleton that accompanies synapse formation [6]. Furthermore, the ability of PSTPIP1 to interact with CD2 is enhanced by the dephosphorylation of PSTPIP1 by PTP-PEST. Thus, in a manner reminiscent to that outlined for ASC, PSTPIP1 serves as a regulated intermolecular bridge that controls the activity of actin-associated signal cascades.

Through its interaction with PSTPIP1, pyrin is placed in a perfect position to sense signals transduced by the cytoskeleton and/or to modulate cytoskeletal signal transduction. On the pyrin side, the pyrin-PSTPIP1 interaction requires pyrin's B-box and coiled-coil region; this region contains a number of FMF mutations, but it remains to be seen whether these mutations interfere with the interaction between these two proteins. Mutations in pyrin's rfp domain do not seem to affect pyrin-PSTPIP1 binding [89], leaving the molecular mechanism of mutant pyrin function still unclear. On the PSTPIP1 side, both the coiled-coil domain and SH3 domain of PSTPIP1 are necessary for the interaction with pyrin, but neither alone is sufficient.

### PSTPIP1 and Inflammatory Disease

A fascinating new disease connection further solidifies the importance of the functional interaction between pyrin and PSTPIP1 in the context of inflammation. Mutations in PSTPIP1 have recently been shown to result in pyogenic sterile arthritis, pyoderma gangrenosum, and acne (PAPA) syndrome (OMIM #604416) [110]. PAPA syndrome is an autosomal dominant autoinflammatory disorder in which individuals are beset by destructive, recurrent inflammation localized to skin, joints, and muscle. Histologically, lesions are typified by a massive infiltrate of neutrophils. Although there are significant symptomatic differences between PAPA syndrome and FMF, there are similar core components comprised of misregulated PMN infiltrates and recurrent arthritis and skin inflammation. This suggests the possibility that the interaction between PSTPIP and pyrin is related to the pathophysiology of PAPA syndrome and indeed there is now some evidence that supports this possibility.

Two mutations, E250Q and A230T, have been identified exclusively in affected PAPA syndrome kindreds. Both of these mutations diminish PSTPIP1's ability to bind to PTP-PEST and as a result, the mutant forms of PSTPIP are hyperphosphorylated. As discussed above, hyperphosphorylated forms of PSTPIP bind with higher affinity to pyrin [89]. But how does this enhanced pyrin/PSTPIP1 interaction lead to inflammation? A possible scenario was recently suggested [89]. Shoham *et al*. (2003) observed that monocytes from patients with PAPA syndrome display enhanced IL-1β secretion compared to wild type monocytes [89]. Furthermore, IL-6 and IL-12p70, targets induced by IL-1β, are markedly increased in PAPA patients. FMF patients too may have increased IL-1β levels and work in cultured cells (as well as in a pyrin hypomorphic mouse model) suggests that this is secondary to the failure of mutant pyrin to inhibit ASC-mediated activation of caspase-1 [19]. Putting these two observations together, a competitive model was put forward to account for etiology of PAPA syndrome [89]: enhanced binding of pyrin to mutant (hyperphosphorylated) forms of PSTPIP1 might draw pyrin away from ASC, so that it can no longer inhibit caspase-1 activation. The resulting excessive IL-1β activation would lead to an increasingly inflammatory state.

Though still to be confirmed experimentally, this is an interesting unifying model that places both diseases downstream of a reduced interaction between pyrin and ASC. Such an etiologic commonality could account for the clinical similarities between the two diseases, but we are still left to explain the clinical differences. At least some of these could result from the fact that the binding of PTP-PEST to PSTPIP1 is reduced, and this likely interferes with the ability of PSTPIP1 to perform its normal adaptor function, connecting PTP-PEST to its substrates (c-Abl, WASp, etc.).

And what about he MEFV mutations? Their effects on PSTPIP1-mediated pathways is not yet obvious as no change in the PSTPIP1/pyrin interaction was detectable when mutant forms of pyrin were tested [89]. Nevertheless, the clear link to the cytoskeleton provided by the PSTPIP1/pyrin interaction provides an exciting avenue for further exploration.

## CONCLUSION AND PERSPECTIVE

Since the cloning of *MEFV*, the study of the molecular biology of pyrin (as well as that of other periodic disease-associated proteins) has opened a whole new vista for molecular immunology. The evidence is convincing that pyrin, ASC and PSTPIP1 are interacting players in a signaling network that connects the cytoskeleton, inflammation, and apoptosis. A particularly exciting thread is the growing number of inflammatory syndromes that seem to be impacted by malfunctions in this signaling network. However, as confirmed by the number of open questions highlighted in this review, our understanding of these complex pathways is only in its infancy. Each new fact learned seems to point to many additional missing answers; and we are acutely aware that some of those missing answers are to questions that we do not yet know enough to ask. Impetus to continue the search for new connections comes directly from the 60% of individuals with periodic inflammatory disease for whom the genetic basis of their disease remains to be determined [89]. Based on past experience, it is likely that deciphering the cause of disease in these undiagnosed patients will provide an important source of new questions that will keep our international community of investigators engaged for years to come.

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