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PERSPECTIVES

OPINION

SMOCs: supramolecular organizing centres that control innate immunity

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Abstract | The diverse receptor families of the innate immune system activate signal transduction pathways that are important for host defence, but common themes to explain the operation of these pathways remain undefined. In this Opinion article, we propose — on the basis of recent structural and cell biological studies — the concept of supramolecular organizing centres (SMOCs) as location-specific higher-order signalling complexes in which increased local concentrations of signalling components promote the intrinsically weak allosteric interactions that are required for enzyme activation. We suggest that SMOCs are assembled on various membrane-bound organelles or other intracellular sites, which may assist signal amplification to reach a response threshold and potentially define the specificity of cellular responses that are induced in response to infectious and non-infectious insults.

Perhaps no area of immunology has benefitted more from the sequencing of the human (and mouse) genome than that of innate immunity. Modern studies of innate immunity received widespread attention with the discovery in the late 1990s that Toll-like receptors (TLRs) link microbial detection with the induction of adaptive immunity[1]. Because TLRs and their associated families of signalling proteins have sequence homology, surveying the human and mouse genomes for uncharacterized orthologous proteins became a common approach to study these biological processes. Thus, within a few years of the discovery of cell-surface and endosomal TLRs[1–3], more than 100 genes had been identified that regulate the signalling pathways induced by these receptors[4–8], as well as the functionally related, cytosolic NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and others[9] (FIG. 1; TABLE 1). Individual members of these pattern recognition receptor (PRR) families detect conserved pathogen-associated molecular patterns (PAMPs) that are present on bacteria, viruses and fungi, or recognize intrinsic damage-associated molecular patterns (DAMPs) that are elicited by cellular injury. Upon ligand binding, these receptors activate numerous cellular responses to fight infection and restore homeostasis.

The success of using bioinformatics and reverse genetics to study innate immune signalling pathways came at the expense of alternative strategies to address these areas. As such, studies of the biochemistry, cell biology and dynamics of these signalling pathways have been much less common. In fact, most early studies of TLRs and their associated signalling proteins did not include any analysis of the subcellular localization of the newly identified protein(s). Thus, although we know the identity of many genes that are involved in innate immunity, the functional mechanisms of the proteins encoded by these genes, and how they interact in space and time, are poorly understood.

The lack of knowledge on the specific activities of the proteins that control innate immunity has given rise to biological models that do not address many aspects of the signalling process, such as the subcellular site where a given signalling event occurs, or the dynamics of putative protein–protein interactions. Current models of TLR, NLR or RLR signalling rather depict a series of arrows connecting receptors with downstream signalling proteins, yet we have little understanding of what these arrows actually represent. Do they represent direct protein–protein interactions? If so, are these interactions constitutive or are they induced upon microbial encounter? How are these interactions regulated and where in the cell do they occur? As described below, recent biochemical and cell biological studies have provided important insight into these questions. These new studies indicate that numerous protein regulators of innate immunity are organized into higher-order signalling complexes that define the subcellular sites and specificity of innate immune signal transduction.

In this Opinion article, we propose that these higher-order signalling complexes function as 'supramolecular organizing centres' (SMOCs) that control cellular responses induced by specific families of upstream receptors. We discuss how SMOCs can operate from various locations within the cell, and describe how they consist of proteins that either sense the activation of upstream receptors or elicit specific downstream effector responses. We further propose that these complexes include context-dependent components, which may be cell type-specific or organelle-specific regulators, such that a given SMOC can elicit diverse cellular responses depending on the stimulus. A benefit of coordinating innate immune signalling pathways around a set of organizing centres may be the modularity of the system, whereby numerous upstream stimuli can be directed into a common downstream module. Indeed, this is the case when considering the operation of other non-membranous organizing centres in mammalian cells, such as the microtubule organizing centre (MTOC) and the proteasome. In these examples, a large protein complex coordinates an entire biological process that may be needed to address diverse cellular needs.

SMOCs for PRRs

The classical view of signal transduction — involving a serial reaction in which ligands induce conformational changes in receptors followed by the activation of enzymes and the generation of second messengers — required

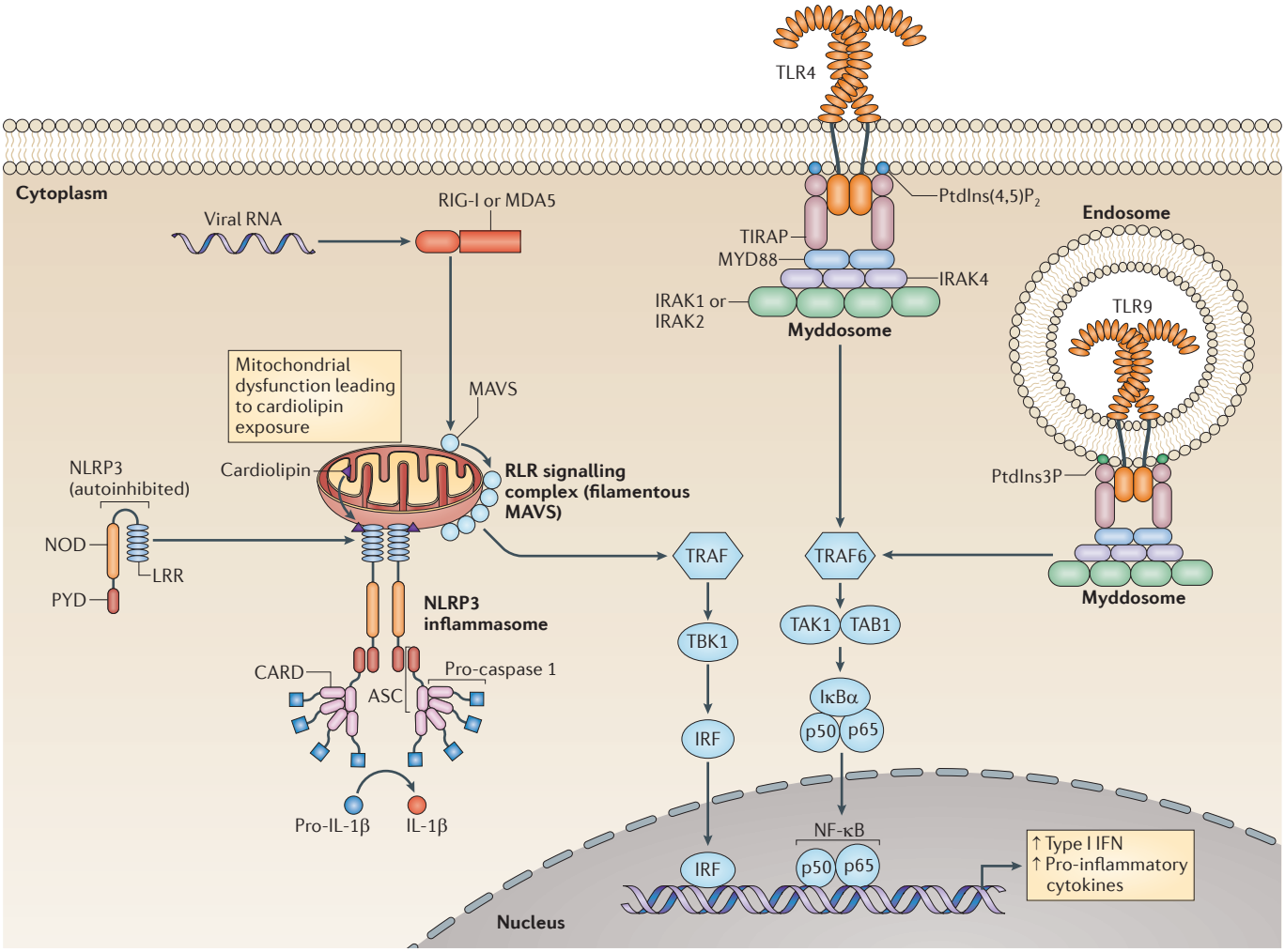

Figure 1 | **SMOC formation for TLRs, RLRs and NLRs.** Depicted are the best-studied supramolecular organizing centres (SMOCs), including the ligands and regulatory proteins that promote their assembly, and the downstream biological activities induced by these protein complexes. The figure does not show the exact stoichiometry of the protein components in each signalling complex. Binding of lipopolysaccharide (not shown) activates Toll-like receptor 4 (TLR4), leading to assembly of a Myddosome on the plasma membrane. By contrast, unmethylated CpG-containing DNA oligonucleotides (not shown) promote TLR9 to assemble an endosomal Myddosome. The TLR-specific sorting adaptor Toll/IL-1R domain-containing adaptor protein (TIRAP) facilitates Myddosome assembly. TIRAP has an amino-terminal lipid-binding domain that interacts promiscuously with acidic phosphoinositides and phosphatidylserine. For example, TIRAP is depicted as binding phosphatidylinositol-3-phosphate (PtdIns3P) and phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2) on the endosomal membrane and the plasma membrane, respectively. Binding of cardiolipin, which translocates to the outer mitochondrial membrane upon mitochondrial dysfunction, relieves the autoinhibited state of NOD-, LRR- and pyrin domain-containing 3 (NLRP3). This in turn may promote NLRP3 inflammasome assembly through downstream pyrin domain (PYD)–PYD and caspase activation and recruitment domain (CARD)–CARD interactions. Activation of RIG-I-like receptors — retinoic acid-inducible gene I (RIG-I) or melanoma differentiation-associated protein 5 (MDA5) — leads to the formation of higher-order oligomers of mitochondrial antiviral signalling protein (MAVS). IFN, interferon; IκBα, NF-κB inhibitor-α; IL, interleukin; IRAK, IL-1 receptor-associated kinase; IRF, IFN-regulatory factor; LRR, leucine-rich repeat; MYD88, myeloid differentiation primary response protein 88; NOD, nucleotide-binding oligomerization domain; NF-κB, nuclear factor-κB; TAB1, TAK1-binding protein 1; TAK1, TGFβ-associated kinase 1; TRAF, tumour necrosis factor receptor-associated factor.

serious modifications in the case of PRRs. The emerging concept for PRRs supports the formation of higher-order signalling complexes as the mode of signal transduction and amplification. Indeed, with the exception of the signalling pathway involving cyclic GMP–AMP synthase (cGAS) and stimulator of interferon genes protein (STING) described below, there is little or no role of second messengers in the earliest events associated with PRR signal transduction.

Early studies. Cellular studies using light microscopy imaging provided the first evidence for the existence of higher-order signalling complexes in innate immune pathways. The tumour necrosis factor (TNF) receptor superfamily comprises some of the earliest discovered members of the innate immune system, such as TNF receptor 1 (TNFR1) and FAS (also known as CD95 and TNFRSF6). These receptors do not have intrinsic enzymatic activity, but aggregate upon stimulation by trimeric ligands of the corresponding TNF superfamily. As an example, FAS — as part of the death-inducing signalling complex (DISC) — forms aggregated clusters or puncta of hundreds of nanometres to micrometres in diameter[10,11], which are much larger than ligand–receptor trimers. The formation of sizable clusters that are visible by light microscopy has become a recurrent observation for activated

innate immune receptors, including TLRs[12–14], RLRs[15,16], and NLR or absent in melanoma 2 (AIM2) inflammasomes[17,18] (FIG. 1; TABLE 1).

Biochemical and structural studies of innate immune signalling complexes indicated that such protein clusters are not random aggregates but instead have a defined molecular basis of assembly. An almost ubiquitous feature of innate immune pathways is the participation of signal transduction proteins with death domains, or the related death effector domains (DEDs), caspase activation and recruitment domains (CARDs) and pyrin domains (PYDs)[19]. Crystal structures of oligomeric death domain complexes revealed an ordered helical assembly mechanism that underlies the oligomerization of several proteins[19] including the 5:7 complex of p53-inducible protein with a death domain (PIDD) and RIP-associated ICH1/CED3-homologous protein with a death domain (RAIDD; also known as CRADD) in the core of the PIDDosome for caspase 2 activation[20]; the 5:5 complex of FAS and FAS-associated death domain protein (FADD) in the DISC for caspase 8 activation[21]; and the 6:4:4 complex of myeloid differentiation primary response protein 88 (MYD88), interleukin-1 receptor (IL-1R)-associated kinase 4 (IRAK4) and IRAK2 in the Myddosome for kinase activation in the TLR pathway[22] (TABLE 1). The common helical symmetry indicates that death domains and their related domains might be able to form large helical filaments and even larger filamentous signalling complexes to generate microscopically visible SMOCs.

Recent advances. Exciting new studies have now confirmed the structural predictions, as well as further extended our mechanistic understanding of innate immune signalling. The RLR signalling adaptor mitochondrial antiviral signalling protein (MAVS), which contains an amino-terminal CARD, forms helical filaments that activate the interferon pathway[16,23,24] (FIG. 2). The CARD-containing adaptor B cell lymphoma 10 (BCL-10) — which activates mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) for nuclear factor-κB (NF-κB) signalling downstream of several innate and adaptive immune receptors — assembles into similar helical filaments[25]. Recent studies of cytosolic inflammasomes, which activate caspase 1 to induce interleukin-1β (IL-1β) maturation and pyroptosis, show that filaments of the adaptor protein ASC (which contains both a PYD and a CARD) and caspase 1 (which contains a CARD) form star-shaped structures that mediate signal transduction and proximity-induced caspase 1 activation[26–28] (FIG. 2). Electron microscopy-based structural studies showed that these filaments are assembled through three conserved types of interactions that were initially observed in the crystal structures of death domain complexes[24,25,27].

These new advances indicate that nucleated polymerization may be a general principle in promoting the assembly of filamentous complexes upon stimulation to mediate innate immune signal transduction (FIG. 2). A sensor protein (receptor) in a pathway first becomes activated in the presence of ligands by overcoming autoinhibition and subsequently oligomerizes. The oligomerized sensor then provides the platform to nucleate a downstream protein to polymerize into filaments. For example, recognition of cytosolic double-stranded DNA (dsDNA) by the AIM2 inflammasome or the response to various infectious and danger signals by NLR inflammasomes disrupts intramolecular domain interactions[29] to enable oligomerization of the PYDs of inflammasome sensor proteins[26,27]. These oligomerized PYDs then function as a platform to nucleate the polymerization of the PYDs in the adaptor protein ASC. The ASC^{PYD} filaments bring the CARDs of ASC into proximity to nucleate the polymerization of the CARDs of caspase 1, which in turn promotes caspase 1 dimerization and activation. Similarly, the polyubiquitin-stabilized double CARD of retinoic acid-inducible gene I (RIG-I) forms a helical tetramer to imprint the polymerization of MAVS through its CARDs to promote downstream signalling[24,30–32].

Oligomerization mechanisms that do not involve the death domain superfamily have also been discovered in innate immunity[14,33]. A common molecular mechanism of signal transduction seems to be the tight coupling between oligomerization — regardless of the type — and allostery. On the one hand, ligand-induced allosteric changes in the respective receptors promote oligomerization. On the other hand, oligomerization of effector enzymes, such as kinases and caspases, enhances the allosteric changes that are presumed to be required for enzyme activation. This oligomerization-facilitated allostery is illustrated by the recently reported crystal structure of the unphosphorylated IRAK4 kinase domain asymmetric dimer captured in the conformation of the Myddosome-induced IRAK4 *trans*-autophosphorylation reaction[34]. In the crystal structure, one IRAK4 monomer exists in the active kinase conformation despite a lack of phosphorylation and it precisely binds the activation loop phosphosite of the other IRAK4 monomer for phosphotransfer. In solution, unphosphorylated IRAK4 forms weak dimers. By markedly increasing the local concentration of IRAK4, the Myddosome promotes dimerization to drive allosteric autoactivation of IRAK4. Oligomerization-driven allosteric changes probably have crucial roles in the higher-order signalling complexes of other innate immune receptors; it is the challenge of structural biologists to characterize these often weak and transient conformations.

Table 1 | **SMOCs of the innate immune response**

SMOC	Triggers or ligands	Receptors or sensors	Adaptors	Effectors	Functions
FAS DISC	FAS ligand	FAS	FADD	Caspase 8	Apoptosis
PIDDosome	DNA damage	PIDD	RAIDD	Caspase 2	Apoptosis
Myddosome	PAMPs	TLRs	TIRAP and MYD88	IRAKs	NF-κB activation
RLR complex	Viral RNAs	RIG-I and MDA5	MAVS	TRAFs	NF-κB activation and interferon response
Inflammasome	PAMPs and DAMPs	NLRs and ALRs	ASC	Caspase 1	Pyroptosis and IL-1β maturation

ALR, AIM2-like receptor; DAMP, damage-associated molecular pattern; DISC, death-inducing signalling complex; FADD, FAS-associated death domain protein; IL-1β, interleukin-1β; IRAK, IL-1 receptor-associated kinase; MAVS, mitochondrial antiviral signalling protein; MDA5, melanoma differentiation-associated protein 5; MYD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor-κB; NLR, NOD-like receptor; PAMP, pathogen-associated molecular pattern; PIDD, p53-inducible protein with a death domain; RAIDD, RIP-associated ICH1/CED3-homologous protein with a death domain; RIG-I, retinoic acid-inducible gene I; RLR, RIG-I-like receptor; SMOC, supramolecular organizing centre; TIRAP, Toll/IL-1R domain-containing adaptor protein; TLR, Toll-like receptor; TRAF, tumour necrosis factor receptor-associated factor.

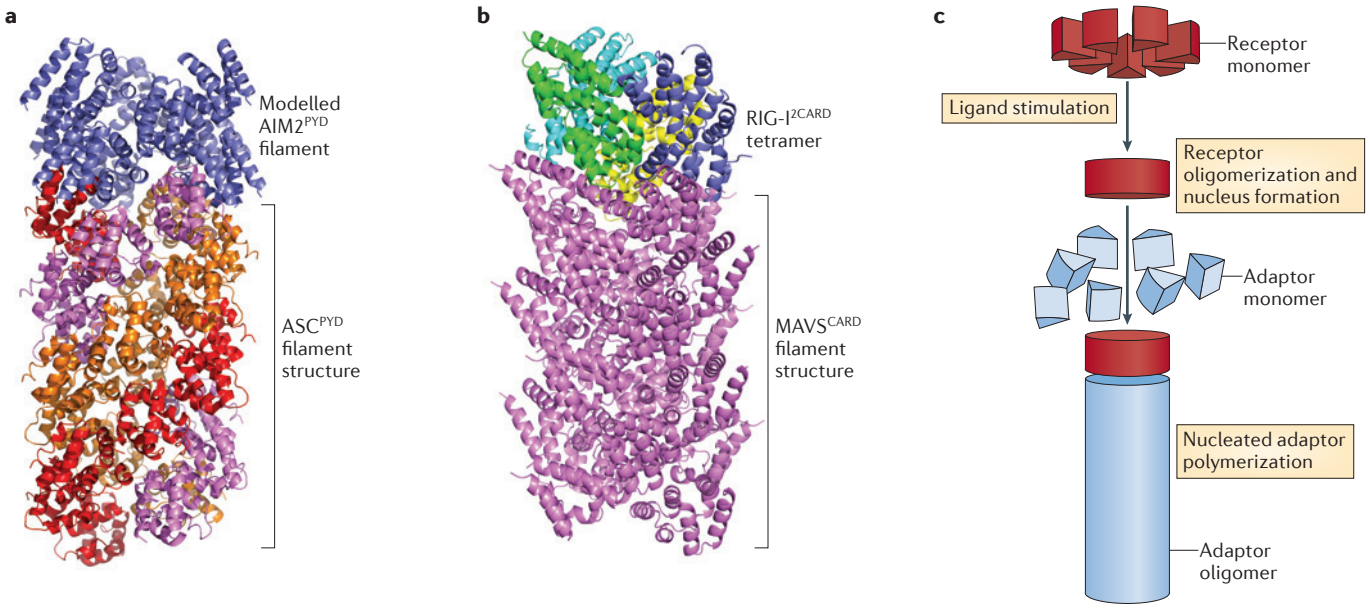

Figure 2 | **Structures of SMOCs that are formed by the mechanism of nucleated polymerization.** **a** | A ribbon diagram of the electron cryomicroscopic structure of polymerized ASC pyrin domain (ASC^{PYD}) filaments in inflammasomes (shown in red, purple and orange for each of the three helical strands), in complex with polymerized absent in melanoma 2 PYD ($AIM2^{PYD}$) filaments (shown in blue) formed upon double-stranded DNA (dsDNA) stimulation. **b** | A ribbon diagram of the electron cryomicroscopic structure of polymerized mitochondrial antiviral signalling protein caspase activation and recruitment domain ($MAVS^{CARD}$) in the RIG-I-like receptor (RLR) pathway (shown in purple), in complex with a retinoic acid-inducible gene I (RIG-I) double CARD ($RIG\text{-}I^{2CARD}$) tetramer (shown in blue, cyan, green and yellow) upon viral RNA stimulation. **c** | Proposed mechanism of nucleated polymerization for the formation of ASC^{PYD} and $MAVS^{CARD}$ SMOCs. Receptors (for example, AIM2 and RIG-I) are shown in red wedges for monomers and in red disks for oligomerized forms. Adaptors (for example, ASC and MAVS) are shown in blue wedges for monomers and in blue cylinders for filaments.

Mechanistic implications. The assembly of higher-order signalling complexes not only provides a phenomenological explanation for the observation of punctate structures in cells, but also implicates an elegant mechanism of signal amplification, preceding enzyme activation, that enables a response threshold to be reached. Nucleated polymerization ensures that a small number of sensor proteins can activate many downstream signalling proteins. For example, in the AIM2 inflammasome, a substoichiometric amount of AIM2 can polymerize many more ASC molecules[27]. In turn, activated ASC further oligomerizes many more caspase 1 molecules to amplify signal transduction. Within a single cell, it is likely that once the signalling cascade is successfully initiated, almost all caspase 1 molecules are recruited to inflammasomes so that a maximal response is generated. Signal amplification and the cooperativity in the assembly of higher-order signalling complexes may both contribute to the threshold, all-or-none host defence programmes that have been observed at the single-cell level for many innate immune pathways[35,36].

In addition, signal amplification may explain the sensitivity of mammalian cells to incredibly small numbers of bacteria. For example, it has been estimated that a single *Escherichia coli* bacterium can activate 1,000 macrophages through TLR4 (REF. 37), suggesting that very few receptors on any individual cell are engaged during infection. We suggest that SMOC formation around the cytosolic tail of TLR4 may explain the all-or-none response that macrophages often exhibit to a wide range of lipopolysaccharide (LPS) concentrations[37]. In addition, the role of SMOCs in signal amplification may be fundamentally analogous to that of second messengers, such as cAMP in signal transduction by G protein-coupled receptors. However, we believe that cAMP-mediated responses may be more graded than those mediated by SMOCs, with the strength of the response being determined by the amount, half-life and deactivation kinetics of the second messengers.

If signal amplification to reach a response threshold is the mechanism by which innate immune pathways are turned on, then once formed, how are SMOCs turned off to terminate signalling? In the case of BCL-10-containing signalosomes, they are recruited to autophagosomes through an interaction between ubiquitylated BCL-10 and the autophagy adaptor p62 (also known as SQSTM1), leading to degradation[38]. Deficiency of ATG16L1 — a protein that is required for autophagosome formation — has been shown to enhance endotoxin-induced IL-1β and IL-18 production, probably through a TLR-mediated pathway[39]. Given that SMOCs may be too large to be degraded efficiently by proteasomes, autophagosome-mediated degradation seems to make sense. It is interesting to note that the size distribution of puncta can be quite different for different SMOCs. For example, TLRs form relatively small clusters, which may account for the smaller size of the Myddosome compared with inflammasome filaments. Some inflammasomes, as well as STING (which mediates the interferon response upon stimulation of cGAS by cytosolic dsDNA), have gigantic perinuclear puncta[40,41]. Whether the distinct size of puncta is reflective of the threshold of activation and/or degradation mechanisms remains to be addressed.

Consistent with the ability of many death domain superfamily members to polymerize, several of these domains — including the CARD of MAVS and the PYD of ASC — have prion-like activities in yeast[16,27,28]. In mammalian cells, it seems that ASC-containing specks of inflammasomes are not degraded rapidly but are released upon pyroptosis into the extracellular space, where they promote further

IL-1β processing and can be engulfed by macrophages to induce inflammasome activation in the recipient cells[42,43]. Therefore, understanding the biophysical principles of SMOC assembly and degradation not only provides insights into unique signalling properties within each cell, but also implicates mechanisms of unexpected signal transduction between cells.

The subcellular localization of SMOCs

Cell biological and biochemical analyses of individual components of SMOCs have shown that these complexes are usually assembled on the cytosolic surface of membranous organelles of mammalian cells. This trend of SMOC assembly on membranes can be observed for TLRs, as well as for RLRs and NLRs. Because the RLRs and NLRs are not transmembrane proteins, their need to induce SMOC assembly on organelles is intriguing, and it indicates that membrane-based SMOC assembly is not simply a consequence of signal transduction being initiated by a transmembrane receptor such as a TLR.

Toll-like receptors. Although the Myddosome has been defined structurally[22], there is an incomplete understanding of the composition, dynamics and regulation of the endogenous TLR-induced Myddosome in mammalian cells. Recent work has provided insight into the behaviour of the Myddosome in macrophages, through the demonstration that it can form either at the plasma membrane or on endosomes[44]. For example, LPS treatment of macrophages activates TLR4 to assemble a Myddosome at the cell surface, whereas unmethylated CpG-containing DNA oligonucleotides activate TLR9 to assemble an endosomal Myddosome. In both cases, an activated receptor is not sufficient to induce Myddosome assembly; rather, the TLR-specific Toll/IL-1R (TIR) domain-containing adaptor protein (TIRAP; also known as MAL) is required for Myddosome assembly[44]. Thus, TIRAP is the first natural regulator of TLR-induced Myddosome formation to be identified. TIRAP is a peripheral membrane protein that contains an N-terminal lipid-binding domain that interacts promiscuously with acidic phosphoinositides and phosphatidylserine[45]. This promiscuity of lipid binding enables TIRAP to survey several plasma membrane and endosomal subdomains for the presence of an activated (ligand-bound) TLR. When TIRAP detects a ligand-bound TLR through its carboxy-terminal TIR domain[46,47], it recruits the core component of the Myddosome, MYD88, which seeds the formation of this SMOC through interactions with IRAKs[22,44,48]. Altering the lipid specificity of TIRAP such that it can only interact with either plasma membrane-localized or endosome-localized lipids restricts the ability of the cell to assemble Myddosomes from the location in which TIRAP resides[44]. Thus, although TIRAP contains a promiscuous lipid-binding domain, the individual targets of this domain enable location-specific assembly of a SMOC. TIRAP was originally thought to be a regulator that defines the specificity of signalling pathway activation induced by plasma membrane-localized TLRs, as compared with endosomal TLRs[8]. The observation that TIRAP regulates Myddosome formation from both subcellular locations reignites the question of how organelle-specific innate immune responses are achieved. We suggest that whereas TIRAP and the other components of the Myddosome participate in TLR signalling from multiple locations within the cell, organelle-specific regulators may exist to determine the specificity of signal transduction. Identifying these putative regulators may provide tools to understand how the composition and function of SMOCs can be modulated naturally, and perhaps therapeutically.

RIG-I-like receptors. The RLR system assembles a SMOC on membranes, despite the fact that the upstream receptors are cytosolic proteins. The core of the RLR-induced SMOC is MAVS, which is localized to membranes though a C-terminal transmembrane domain[15,16]. Although the localization domain of MAVS is not structurally similar to that of TIRAP, these domains are similar in that they can be targeted to multiple organelles. In the case of MAVS, its transmembrane domain enables it to localize to mitochondria, peroxisomes and the mitochondria-associated membranes (MAMs) of the endoplasmic reticulum[15,49,50]. Also, similar to TIRAP, MAVS can induce signalling responses from each of these organelles[15,49,50], although biochemical evidence for the formation of higher-order oligomers is only available for mitochondria-localized MAVS[16]. It remains to be determined whether similar MAVS-containing SMOCs are assembled on peroxisomes and MAMs during viral infections. Recent studies have shown that functional TIRAP or MAVS proteins can be generated by replacing the localization domains of either of these proteins with other domains that have similar membrane-targeting activities[15,44,50]. These data provide strong evidence that the sole function of these domains is to direct TIRAP or MAVS to the membranes that are necessary for PRR-induced SMOC assembly. Although it is clear that MAVS must localize to specific organelles to initiate SMOC assembly and signalling, it remains unclear why mitochondria, peroxisomes and MAMs have evolved as the preferred sites of RLR signal transduction. One possibility is that the metabolic functions of these organelles control MAVS activation, a theory that is supported by recent studies indicating that dysfunctional mitochondria cannot promote efficient RLR-dependent innate immune responses[51,52].

NOD-like receptors. The NLR system provides yet another example of SMOC assembly on membranes, at least in the case of NOD-, LRR- and PYD-containing 3 (NLRP3). Although NLRP3 is generally thought to be a cytosolic protein, it translocates to mitochondria when cells are stimulated with several types of inflammasome activators[53,54]. The translocation of NLRP3 to mitochondria has been reported to occur through interactions with the lipid cardiolipin — which is only displayed on the outer membrane of damaged mitochondria[55] — or with MAVS[56]. In this regard, NLRP3 is similar to TIRAP in that its ability to promote SMOC assembly is linked to its ability to interact with both membrane-bound lipids (phosphoinositides) and proteins (TLRs) in a specific region of the cell. It is unclear whether other NLR family members also interact with cardiolipin or any other lipid to promote SMOC assembly, but these studies provide a strong mandate to consider this possibility.

Conclusions and future perspectives

Examples now exist for the three major families of PRRs — TLRs, RLRs and NLRs — that SMOC assembly occurs on membranes, even when the upstream receptors are cytosolic proteins. In each documented example, a membrane protein seeds the formation of a higher-order signalling complex that activates specific innate immune responses. A biophysical explanation for the apparent membrane localization of SMOCs may be the marked energetic enhancement of protein–protein interactions on a two-dimensional membrane surface compared with those in a three-dimensional cellular milieu[57]. For this reason, we propose that future studies of the biochemical mechanisms of SMOC assembly and function would benefit from

a greater consideration of the subcellular sites where this assembly occurs. This analysis may also help to address the question of why SMOCs have evolved to operate from specific organelles. Additional studies of the relationship between organelle function and SMOC assembly should address the possibility that organelles are not solely needed as a scaffold for SMOC assembly, but rather that a metabolic or biochemical activity of the organelle may contribute as well. This cell biological analysis may reveal important means of controlling and manipulating SMOC assembly and subsequent inflammatory responses.

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Competing interests statement
The authors declare no competing interests.

Correction

In Figure 1 of the original version of this article, the Toll-like receptor within the endosome was incorrectly labelled as TLR4. This should have been labelled as TLR9 and has now been corrected online. *Nature Reviews Immunology* apologizes for this error.