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Cilia born out of shock and stress

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Primary cilia are cell surface sensory organelles, whose dysfunction underlies various human genetic diseases collectively termed ciliopathies. A new study in *The EMBO Journal* by Villumsen *et al* now reveals how stress–response pathways converge to stimulate ciliogenesis by modulating protein composition of centriolar satellites. Better understanding of these mechanisms should bring us closer to identifying the cellular defects that underlie ciliopathies caused by mutations in centriolar satellite proteins.

Centrioles are barrel-shaped structures with two distinct identities. In proliferating cells centrioles provide structural support for the centrosome, a key microtubule-organizing centre, whereas in quiescent cells centrioles are converted into basal bodies and promote the assembly of primary cilia. In centrosomes, centrioles are embedded in pericentriolar material (PCM), a dynamic structure responsible for microtubule nucleation. PCM proteins exhibit cell cycle-dependent localisation, achieved at least in part by the regulation of their transport. Centriolar satellites, dense fibrous granules frequently clustered around the interphase centrosome, have been implicated in microtubule-dependent protein transport to centrosomes (Kubo *et al*, 1999). In particular, PCM-1, the core constituent of centriolar satellites, is required for centrosomal accumulation of several PCM components (Dammermann and Merdes, 2002). Although the proteomic composition of satellites is still elusive, the growing list of satellite proteins includes CEP131/AZI1 (Staples *et al*, 2012), CEP290 (Stowe *et al*, 2012), Bardet-Biedl syndrome protein 4 (BBS4) and Oral facial digital syndrome protein (OFD1; Lopes *et al*, 2011). Mutations in OFD1, CEP290 and BBS4 cause ciliopathies (Kim *et al*, 2008), underscoring a functional link between satellites and ciliogenesis. So far, two roles have been proposed for satellites in cilia formation: First, in cycling cells they may serve to sequester essential ciliary proteins (Stowe *et al*, 2012). Second, upon initiation of the ciliogenesis programme, centriolar satellite components seem to promote the recruitment of specific ciliary proteins to basal bodies (Ferrante *et al*, 2006; Lopes *et al*, 2011; Stowe *et al*, 2012).

In a new study in *The EMBO Journal*, Villumsen *et al* (2013) now describe how stress–response pathways conspire to control ciliogenesis. The authors observed that specific environmental stresses, such as ultraviolet light radiation (UV) or heat shock, but not ionizing radiation (IR), trigger rapid displacement of PCM-1, AZI1 and CEP290 from centriolar satellites. However, OFD1 remained associated with satellites, indicating that centriolar satellites persist despite UV-induced removal of PCM-1. This might come as some surprise, since PCM-1 depletion by RNA interference (RNAi) is thought to disrupt satellite integrity (Kim *et al*, 2008; Lopes *et al*, 2011); however, satellite loss upon PCM-1 RNAi may be a consequence of prolonged depletion of PCM-1, while acute PCM-1 displacement by stress might only 'remodel' centriolar satellites. It is also possible that not all satellites are created equal, and they do vary in protein composition (Kim *et al*, 2008; Staples *et al*, 2012). If so, UV-induced PCM-1 removal may disrupt some, but not all satellites.

A good candidate regulator of centriolar satellite remodelling was the stress-activated MAP kinase p38, and indeed, Villumsen *et al* (2013) found p38 MAPK activity to be stimulated by both UV and heat shock but not IR in U2OS cells, mirroring those very stress pathways that also cause displacement of AZI1 and PCM-1 from satellites. Furthermore, p38 MAPK was essential for UV-induced dispersal of PCM-1 and AZI1. The authors then tested the hypothesis that stress-induced centriolar satellite remodelling could involve changes in the interactome of AZI1, and—consistent with an earlier proteomics study (Akimov *et al*, 2011)—identified PCM-1, CEP290 and the mindbomb E3 ubiquitin protein ligase 1 (MIB1) as the main AZI1 binding partners. GFP-MIB1 localized to centriolar satellites and mono-ubiquitylated AZI1, PCM-1 and CEP290 in cycling cells. In response to UV, both ubiquitylation of these proteins and MIB1 activity were reduced; notably, UV-induced MIB1 inactivation was independent of p38 MAPK activity, indicating that these two enzymes may act via distinct pathways (Figure 1A).

What could be the purpose of MIB1-dependent ubiquitylation of these satellite proteins? It certainly does not seem to regulate subcellular targeting, as in MIB1-depleted cells, AZI1 and PCM-1 both localised normally to centriolar satellites and could still be displaced by UV. Instead, ubiquitylation seems to suppress the interaction between AZI1 and PCM-1, consistent with the observation that UV, a condition that also reduces their ubiquitylation, enhances the binding of AZI1 to PCM-1.

PCM-1, CEP290 and AZI1 all participate in ciliogenesis (Kim *et al*, 2008; Wilkinson *et al*, 2009; Stowe *et al*, 2012), raising the possibility that MIB1 might also affect this

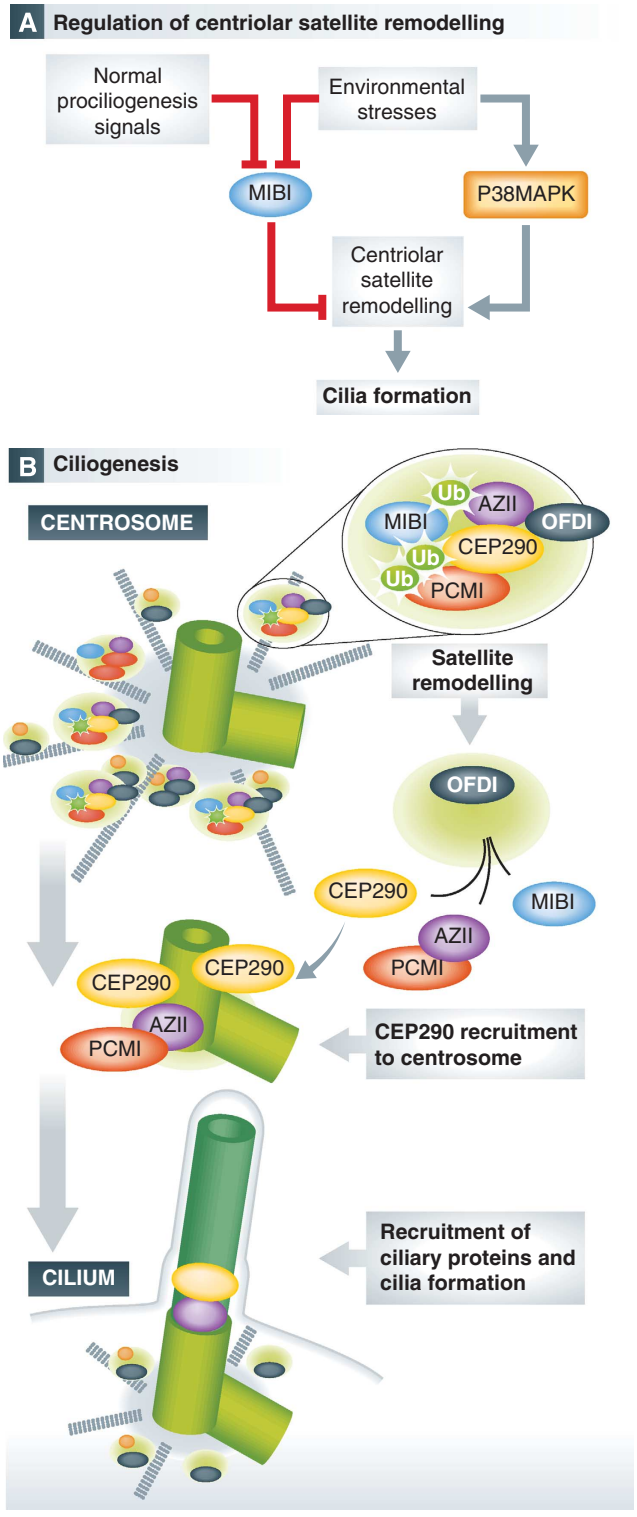

Figure 1 (**A**) Regulation of centriolar satellite remodelling. (**B**) Schematic summary of how centriolar satellite remodelling might facilitate ciliogenesis. See text for details.

process. Indeed, serum starvation, which is known to promote cilia formation, attenuated MIB1 activity. Furthermore, MIB1 overexpression reduced the ciliogenesis observed in serum-starved cells, while MIB1 depletion in proliferating cells triggered a marked increase in the proportion of cells that formed cilia; this seems to reflect a direct effect of MIB1 on ciliogenesis, since neither MIB1 depletion nor overexpression altered cell cycle progression. Taken together, downregulation of MIB1 enzymatic activity appears to be a pre-requisite for efficient ciliogenesis, regardless of whether it is triggered by physiological ciliogenesis-promoting signals or by environmental stresses, making MIB1 a novel negative regulator of cilia formation.

The recent discovery of ciliopathy-associated mutations in constituents of the DNA damage response signalling pathway pointed to a connection between DNA damage and ciliogenesis (Chaki *et al*, 2012). With the new link between UV and centriolar satellites, the authors next asked if UV radiation might affect ciliogenesis. Remarkably, UV and heat shock both triggered cilia assembly in RPE-1 cells in a p38 MAPK-dependent manner. MIB1 depletion further enhanced ciliogenesis after UV radiation, again implying an additive effect of p38 MAPK signalling and MIB1 suppression (Figure 1A).

While finer details on the precise role of centriolar satellite components in cilia formation are still lacking, a more coherent picture is finally starting to emerge. In cycling cells, ubiquitination by MIB1 could serve to limit the interaction between AZI1 and PCM-1 on centriolar satellites (Figure 1B). Under these conditions PCM-1 may bind and sequester CEP290, an essential ciliogenic protein, thereby precluding untimely cilia formation (Stowe *et al*, 2012). Both during normal and stress-induced ciliogenesis programs, remodelling of centriolar satellites creates a permissive environment for cilia formation, and a key step in this process is downregulation of MIB1 activity. While it remains to be established how the latter is achieved, it is clear that MIB1 inactivation causes loss of ubiquitylation and increased binding between AZI1 and PCM-1. Preferential interaction of PCM-1 with AZI1 could in turn facilitate release of CEP290 from centriolar satellites and its subsequent accumulation at the centrosome. Once CEP290 reaches the optimum concentration at the centriole/basal body, it could serve to tether AZI1–PCM-1 complexes. PCM-1 could then concentrate Rab8 GTPase near centrosomes, allowing CEP290 to recruit Rab8 into the cilium, where it acts to extend the ciliary membrane (Kim *et al*, 2008).

Collectively, the findings reported here provide strong experimental support to the notion that centriolar satellites are negative regulators of ciliogenesis in proliferating cells. Their role is central to limit untimely formation of cilia in cells. Environmental strains elicit stress–response pathways that converge to relieve the ciliogenesis block imposed by satellites. It is tempting to speculate that stress-induced cilia might serve as signalling platforms and contribute to checkpoint activation or perhaps initiation of repair mechanisms, but more work is needed to establish the true purpose of ciliogenesis in this context. It is of considerable interest that a recent study reports that autophagy, another stress-induced pathway, selectively removes OFD1 from satellites to promote ciliogenesis (Tang *et al*, 2013). Therefore stress-mediated centriolar satellite remodelling seems to be an evolving theme in the control of ciliogenesis.

Conflict of interest

The authors declare they have no conflict of interest.

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