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Regulation of primary cilia formation by the ubiquitin–proteasome system

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Primary cilia form at the surface of most vertebrate cell types, where they are essential signalling antennae for signal transduction pathways important for development and cancer, including Hedgehog. The importance of primary cilia in development is clearly demonstrated by numerous disorders (known as ciliopathies) associated with disrupted cilia formation (ciliogenesis). Recent advances describing functional regulators of the primary cilium highlight an emerging role for the ubiquitin–proteasome system (UPS) as a key regulator of ciliogenesis. Although there are well-documented examples of E3 ubiquitin ligases and deubiquitases in the regulation of cilia proteins, many putative components remain unvalidated. This review explores current understanding of how the UPS influences primary cilia formation, and also how recent screen data have identified more putative regulators of the UPS. Emerging research has identified many promising leads in the search for regulators of this important organelle and may identify potential novel therapeutic targets for intervention in cancer and other disease contexts.

Introduction

Cilia are small cellular protrusions that mediate a variety of cellular functions. Cilia are classified as motile or immotile, differentiated by the structural features of the core cilia microtubule axoneme. Primary (immotile) cilia form at the apical surface of most vertebrate cell types and differ from motile cilia in their structure, function and solitary nature [1]. Notably, primary cilia lack a central microtubule structure, and dyenin arms responsible for beating in motile cilia [1]. Primary cilia extend from distal end of the mother centriole in a cell cycle-dependent manner, mutually exclusive with mitosis [2]. The primary cilium is resorbed during prophase with the associated centriole re-localising to the cytoplasm to form part of the mitotic spindle [2]. Centriolar satellites are granular structures comprised of pericentriolar material 1 (PCM1) and other key proteins [3,4] surrounding the centrosome are important for centrosome maintenance and ciliogenesis [5].

Primary cilia persist through mammalian gestation in a lineage-dependent manner and are important regulators of cell signalling [6]. Effector components of the Hedgehog (Hh) signalling pathway have been localised to the primary cilium [7], and disruption of ciliogenesis has been shown to hinder effective Hh signal transduction [8]. Aberrant Hh signalling has been linked to cancers of the skin [9], brain [10], pancreas [11], breast [12], lung [13] and colon [14]. Primary cilia are also thought to be involved in sensing mechanical force via Ca^{2+}-permeable channels [15]. Disruption of cilia through mutation of genes encoding cilia proteins has been linked to multiple disorders — often referred to as ciliopathies — including Meckel–Gruber syndrome and Joubert syndrome [16]. It is likely that observed phenotypes of ciliopathies are the result of impaired signal transduction, as many mutations associated with the more common ciliopathies encode proteins localising to the cilium or basal body [16].

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Defining regulators of ciliogenesis and the cilia proteome

Recent studies have helped to define a discrete ciliary proteome and associated protein–protein interactions [17–19]. Many of the proteins identified have not previously been implicated in cilia biology, suggesting the existence of as yet undescribed regulatory processes controlling ciliogenesis. A better understanding of these protein interactions will provide mechanistic insight into developmental and disease processes and may form the basis for potential therapeutic intervention targeting sensitive signalling pathways, such as Hh. Similarly, novel functional regulators of ciliogenesis have been identified through RNAi screening [20], but definition of signalling networks through functional validation of many of these putative novel regulators is yet to be undertaken.

The ubiquitin–proteasome system

Post-translational modifications of cilia proteins (e.g. phosphorylation and acetylation) are key regulators of cilia formation [21–23]. More recently, functional and proteomics analyses have implicated the ubiquitin–proteasome system (UPS) as a key regulator of cilia formation and maintenance. Many UPS components have been identified in the cilia proteome [18] and via functional genomics (RNAi) screens [20] (Table 1). The attachment of the small protein modifier ubiquitin (Ub) to target proteins (ubiquitination) is mediated by a hierarchical enzymatic cascade and is a key regulator of numerous cellular processes [24,25]. Covalent attachment of Ub to lysine side chains in substrate proteins is ultimately catalysed by E3 Ub ligases, which largely determine substrate specificity (Figure 1A) [26]. Conversely, bound Ub tags can be removed from substrates by deubiquitase enzymes (DUBs). Ub modifications can take the form of either mono-Ub or poly-Ub chains via repeated rounds of Ub attachment [26], with the topology of poly-Ub chains driving various downstream outcomes, including protein degradation by the 26S proteasome [27] (Figure 1A).

Amongst UPS components localising to cilia (Table 1) [18] were vasolin-containing protein (VCP, also known as p97), a druggable [28] chaperone involved in Ub signalling quality control [29], Ub-activating enzymes, UBA1 [30] and UBA6 [31], and E3 Ub ligases, Neural precursor cell-expressed, developmentally down-regulated 4-like, E3 Ub protein ligase (NEDD4L) [32] and MYC-binding protein 2, E3 Ub protein ligase (MYCBP2) [33]. Disrupted UPS activity at the primary cilium has been linked to ciliopathy. For example, the ciliary-localising protein RPGRIP1L is mutated in Joubert syndrome [34] and is known to regulate proteasomal subunit PSMD2 at the base of the cilium [35]. Functional genomics screens [20] identified many UPS factors as putative regulators of ciliogenesis and cilium length (Table 1). Factors potentially affecting ciliogenesis included many proteins with putative or known E3 Ub ligase activity, based on the presence of RING (really interesting new gene) and HECT (homologous to E6-associated protein) domains associated with E3 Ub ligase activity [26]. Even though only a limited number of putative hits were subsequently validated, these data highlight the importance of upstream regulators of the UPS in ciliogenesis [20]. A more recent functional genomics screen [36] identified 164 genes necessary for normal cell cycle transition and ciliogenesis. Two gene enrichment clusters were identified linking the hits to mRNA processing and the proteasome. Depletion of various UPS components was shown to affect ciliogenesis and the maintenance of quiescence during the G_1 to S phase transition after serum withdrawal. Furthermore, components of the UPS were required for correct cilia construction and disassembly [36]. Further characterisation of the link between cilia and cell cycle progression may provide insight into molecular mechanisms of ciliopathies and related disorders.

Centriolar satellite organisation and ciliogenesis

Several components of the UPS have well characterised roles in organisation of centriolar satellites and primary cilia formation. E3 Ub ligases are the most numerous class of components in the UPS and largely determine the specificity of the Ub cascade [37]. As such they are likely to specifically regulate expression and/or activity of proteins important for the growing axoneme during ciliogenesis. E3 Ub ligases are known to regulate cilia assembly in a cell cycle-dependent context (Figure 1B). The E3 ligase Mindbomb1 (MIB1) has been shown to localise to centriolar satellites tethered by PCM1 [38]. PCM1 binds MIB1 through its N-terminal domain and this interaction is important for ciliogenesis [38]. The requirement of MIB1 tethering for ciliogenesis is probably mediated by the maintenance of centriolar satellite proteins otherwise targeted by MIB1 for degradation. MIB1 directly regulates levels of PLK4 — a kinase that activates PCM1 by S372 phosphorylation during G_1 phase [39] — stabilising centriolar satellites and in turn, cilia [23]. Depletion of PLK4 results in dispersed centriolar satellites and reduced ciliogenesis [23]. Localisation of PCM1 is strongly associated with cell cycle

Table 1 UPS-related regulators of primary cilia formation
Genes of interest were taken selectively from preliminary screen data [18,20]. Confirmed hits were identified in a subsequent confirmation screen or independent study.

Gene ID	Full identifier	UPS function	NCBI gene ID #	Uniprot ID	Validated
Localised to cilia [18]					
UBA1	Ub-like modifier-activating enzyme 1	E1	7317	P22314	No
UBA6	Ub-like modifier-activating enzyme 6	E1	55236	A0AVT1	No
NEDD4L	Neural precursor cell-expressed, developmentally down-regulated 4-like, E3 Ub protein ligase	E3	23327	Q96PU5	No
MYCBP2	MYC-binding protein 2, E3 Ub protein ligase	E3	23077	Q7TPH6	No
VCP	Valosin-containing protein	Chaperone	7415	P55072	No
Validated positive regulators [43,46,47]					
CUL3	Cullin 3	E3	8452	Q13618	Yes
VHL	von Hippel–Lindau tumour suppressor	E3	7428	P40337	Yes
USP21	Ub-specific peptidase 21	DUB	27005	Q9UK80	Yes
Putative positive regulators [20]					
UBA7	Ub-like modifier-activating enzyme 7	E1	7318	P41226	No
UBE2C	Ub-conjugating enzyme E2C	E2	11065	O00762	No
HERC2	HECT domain and RLD 2	E3	8924	O95714	Yes
RNF148	Ring finger protein 148	E3	378925	Q8N7C7	No
RNF26	Ring finger protein 26	E3 (putative, contains RING)	79102	B0BN75	Yes
RNF39	Ring finger protein 39	E3 (putative, contains RING)	80352	Q9H2S5	No
TRIM2	Tripartite motif-containing 2	E3	23321	Q9ESN6	No
TRIM3	Tripartite motif-containing 3	E3	10612	O75382	Yes
TRIM8	Tripartite motif-containing 8	E3	81603	Q9BZR9	No
TRIM38	Tripartite motif-containing 38	E3	10475	O00635	No
UBR5	Ub protein ligase E3 component recognin-5	E3	51366	O95071	No
OTUB1	OTU domain, Ub aldehyde-binding 1	DUB	55611	Q96FW1	No
USP36	Ub-specific protease 36	DUB	57602	Q9VRP5	Yes
Validated negative regulators [41]					
MIB1	Mindbomb E3 Ub protein ligase 1	E3	57534	Q86YT6	Yes
Putative negative regulators [20]					
ARIH1	Ariadne RBR E3 Ub protein ligase 1	E3	25820	Q9Y4X5	Yes
HECTD2	HECT domain-containing 2	E3	143279	Q5U5R9	No
HERC3	HECT domain and RLD 3	E3 (putative, contains HECT)	8916	Q15034	No
UBE4A	Ubiquitination factor E4A	E4	9354	Q14139	No

progression, as PCM1 dissociates from the centrosome as the cell enters mitosis [40]. MIB1 overexpression suppresses ciliogenesis [41], possibly due to ectopic localisation. MIB1 can also directly ubiquitinate PCM1 [38,41] and TALPID3 [38], which are both required for ciliogenesis. The details of the mechanism by which PCM1 ubiquitination by MIB1 regulates ciliogenesis remain to be defined. One study demonstrated that, in the absence of cellular stress (UV), PCM1 and other centriolar satellite proteins are mono-ubiquitinated by MIB1, inhibiting their function independent of proteasomal degradation [41]. However, another study demonstrated MIB1-dependent poly-ubiquitination of PCM1, resulting in PCM1 destabilisation [38].

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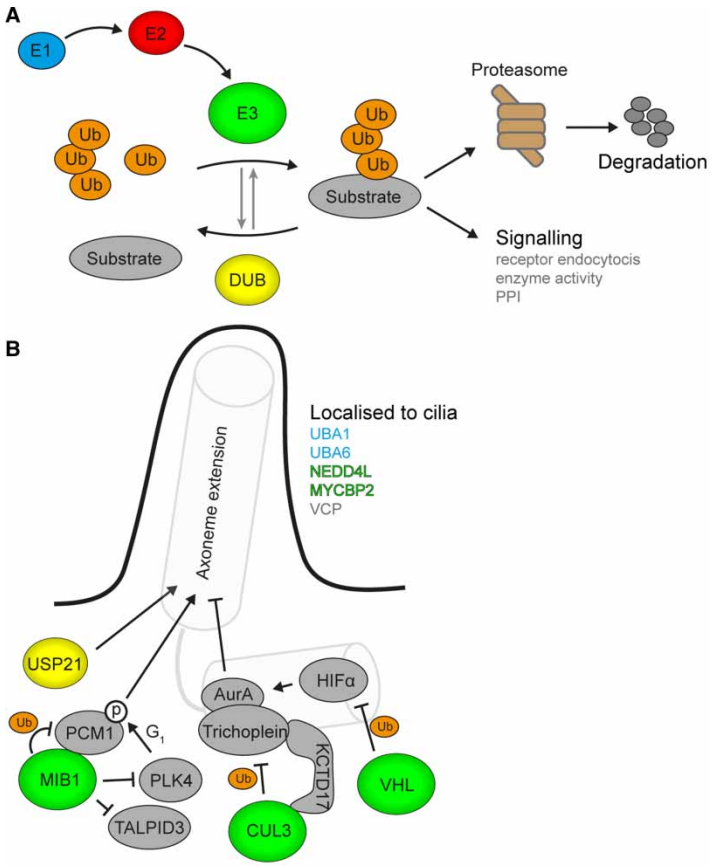

Figure 1. Dynamic regulation of cilia/centriolar components by the UPS in ciliogenesis.

(**A**) Ubiquitination of substrate proteins occurs in a cascade of three enzymes. E1 Ub-activating enzymes, E2 Ub-conjugating enzymes, and E3 Ub ligases [26]. There are 617 putative E3 Ub ligases in the human genome [37], providing the primary substrate specificity of the ubiquitination cascade. Substrate ubiquitination is maintained through a balance of the actions of E3 Ub ligases and DUBs, which can also act on each other to mediate co-ordinate regulation. (**B**) Currently known UPS components required for the regulation of cilium axoneme extension. Many UPS interactions occur with centrosomal proteins. E3 Ub ligases are shown in green and the DUB USP21 is shown in yellow.

Primary cilia are tightly linked to cell cycle progression, which is also tightly regulated by the UPS. Inhibitors of ciliogenesis are present during mitosis and destabilised by the UPS during G_1 to allow axoneme extension (Figure 1B). Ciliogenesis is inhibited by Aurora A (AurA), which is bound and activated by trichoplein at the centriole, preventing axoneme extension [42]. Degradation of trichoplein by a CUL3–KCTD17 E3 Ub ligase complex results in disassembly of the repression complex, promoting ciliogenesis [43]. The E3 Ub ligase von Hippel-Lindau tumour supressor (VHL) localises to primary cilia, where it is thought to promote the maintenance of cilia via GSK3β signalling that is independent of E3 Ub ligase activity [44]. VHL has also been shown to directly ubiquitinate HIF-α [45], a transcriptional regulator of AurA [46], and thus may have an indirect role in regulating ciliogenesis.

The clear role of ubiquitination in the maintenance of centriolar satellites and cilium disassembly probably requires the tightly co-ordinated action of E3 Ub ligases and DUBs to correctly regulate protein levels. A study of the subcellular localisation of 66 DUBs showed that Ub specific peptidase 21 (USP21) localises strongly to the centrosome and microtubules, and knockdown of USP21 greatly reduced cilia formation in human RPE cells [47]. An earlier study of DUB interactors [48] found relevant USP21-interacting partners; however, none were significantly more ubiquitinated with sufficient USP21 knockdown [47]. No specific DUBs were identified in the ciliary proteome by proximity labelling [18]; however, it is possible that others act remotely in the initiation of ciliogenesis. Functional screens indicated a possible role for the DUBs OTUB1 and USP36 in ciliogenesis in RPE cells (Table 1) [20].

Targeting the UPS in disease

Cilia are visible in murine embryos from E6.0 and are probably important for development [6], as evident in the strong phenotypes associated with more common ciliopathies. Potential future therapeutic intervention requires a more in-depth understanding of the regulatory mechanisms involved with ciliogenesis. The UPS is clearly required for both cilium formation and maintenance, as inhibiting the proteasome with MG-132 [36] disrupts both processes. The proteasome is an attractive drug target for destabilising cilia, as drugs targeting the proteasome, such as Bortezomib (Velcade®), are currently in use for treating myeloma and mantle cell lymphoma [49]. Bortezomib treatment is known to reduce Hh target gene transcription in ovarian cancer [50] and medulloblastoma cell lines [51], but it is unclear if this effect is related to cilia. Bortezomib is known to lead to Bcl-2 phosphorylation and corresponds with promoting apoptosis and G_2/M arrest [52], which is consistent with disrupting cilia disassembly. A confounding factor in the cancer context is that primary cilia are reduced or absent from certain cancers and the frequency of ciliated cancer cells is variable [53–56], yet cilia are probably important for signal transduction in Hh-driven cancers. Paracrine Hh signalling involving tumour stromal cells featuring intact cilia may also represent a potential therapeutic target [57,58].

Summary

Numerous lines of evidence implicate the UPS in the regulation of formation and maintenance of primary cilia. Although there are already clearly defined E3/DUB components regulating ciliogenesis, data from functional genomics and proteomics screens indicate that many more UPS components may play a role, but are yet to be fully validated [20,36]. Perhaps, more important is the identification of UPS factors in the maintenance of cilia/cell cycle dynamics [36]. The precise role of cilia in cancer is still unclear. Aberrant Hh signalling is common in many cancers and is thought to require an intact cilium for transduction. However, many cancers are ciliated with a very low frequency, confounding our understanding of co-regulation of cilia and cell cycle, and hence how targeting the UPS might influence cancer cells. Similarly, comprehensive analysis of cilia frequency in a large cancer cohort using standardised detection methods would be very informative in helping to identify tumour types that may be susceptible to therapies targeting disruption of cilia and related signalling pathways. Current literature describing the presence and frequency of cilia in cancers and surrounding tissues is fragmented and incomplete. Further validation of UPS components identified as putative regulators of ciliogenesis will not only provide a more comprehensive mechanistic understanding of the process, but may also in future provide the basis for therapeutic disruption of cilia formation or cell cycle progression in appropriate cancers.

Abbreviations

AurA, Aurora A; DUBs, deubiquitase enzymes; Hh, Hedgehog; Ub, ubiquitin; UPS, ubiquitin–proteasome system.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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