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A “so cilia” network: cilia proteins start “social” networking

Frédéric Saudou

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Commentary

Cilia are unique cellular organelles found in nearly all cell types. In recent years, the importance of these organelles has been highlighted by the discovery that mutations in genes encoding proteins related to cilia biogenesis and function cause a class of complex syndromes termed ciliopathies. Emerging evidence suggests interactions among the various ciliopathy-associated proteins, but the precise mechanisms by which these interactions generate functional networks have remained elusive. In this issue of the *JCI*, Rachel and colleagues have now clearly linked two ciliopathy-associated proteins (CEP290 and MKKS). Surprisingly, the effects of a hypomorphic disease-causing *Cep290* allele were rescued by loss of MKKS function, suggesting that it might be possible to treat some ciliopathies by fine-tuning interactions within the expanding ciliary network.

ing pathways involving the ER and the plasma membrane, thereby triggering the UPR and subsequent programmed cell death (Figure 1). A similar scenario may occur in familial PD caused by Parkin deficiency, because Parkin is thought to function to protect the ER and mitochondria. Indeed, it was recently reported that both mitochondrial and ER stress induce Parkin expression and that Parkin counteracts the stress in both organelles, thereby preventing neuronal death (17). The new evidence generated by Selvaraj et al. (6) indicating involvement of impaired SOCE in a mitochondrial toxin–based model of PD suggests potential new therapeutic approaches aimed at halting the neurodegenerative process in PD. These include activation or upregulation of TRPC1 channels, enhancement of ER Ca^{2+} uptake, and tweaking of the UPR.

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Cilia are unique cellular organelles found in nearly all cell types. In recent years, the importance of these organelles has been highlighted by the discovery that mutations in genes encoding proteins related to cilia biogenesis and function cause a class of complex syndromes termed ciliopathies. Emerging evidence suggests interactions among the various ciliopathy-associated proteins, but the precise mechanisms by which these interactions generate functional networks have remained elusive. In this issue of the *JCI*, Rachel and colleagues have now clearly linked two ciliopathy-associated proteins (CEP290 and MKKS). Surprisingly, the effects of a hypomorphic disease-causing *Cep290* allele were rescued by loss of MKKS function, suggesting that it might be possible to treat some ciliopathies by fine-tuning interactions within the expanding ciliary network.

Cilia are microtubule-based organelles surrounded by membranes that protrude from the cells. The functional importance of these organelles has only recently been recognized through the identification of genetic diseases associated with defects in ciliogenesis that are now termed ciliopathies (1). Ciliopathies affect diverse organ systems, with overlapping but distinct phenotypes observed in different syndromes. The complexity of this class of disorders is exemplified by Bardet-Biedl syndrome (BBS), one of the best-characterized ciliopathies, with mutations in at least 14 loci identified as causative (2). Moreover, different mutations in a given gene can lead to different syndromes with differing phenotypes. For example, the centrosomal protein 290 kDa (*CEP290*) gene is mutated in individuals with Senior-Løken syndrome, Joubert syndrome, Meckel-Gruber syndrome, and BBS. Similarly, the McKusick-Kaufman syndrome (*MKKS*, also known as *BBS6*) gene is found mutated in individuals with BBS and in those with MKKS. In this issue of the *JCI*, Rachel et al. report new complexity within the ciliopathies (3). Their data indicate that CEP290 and MKKS

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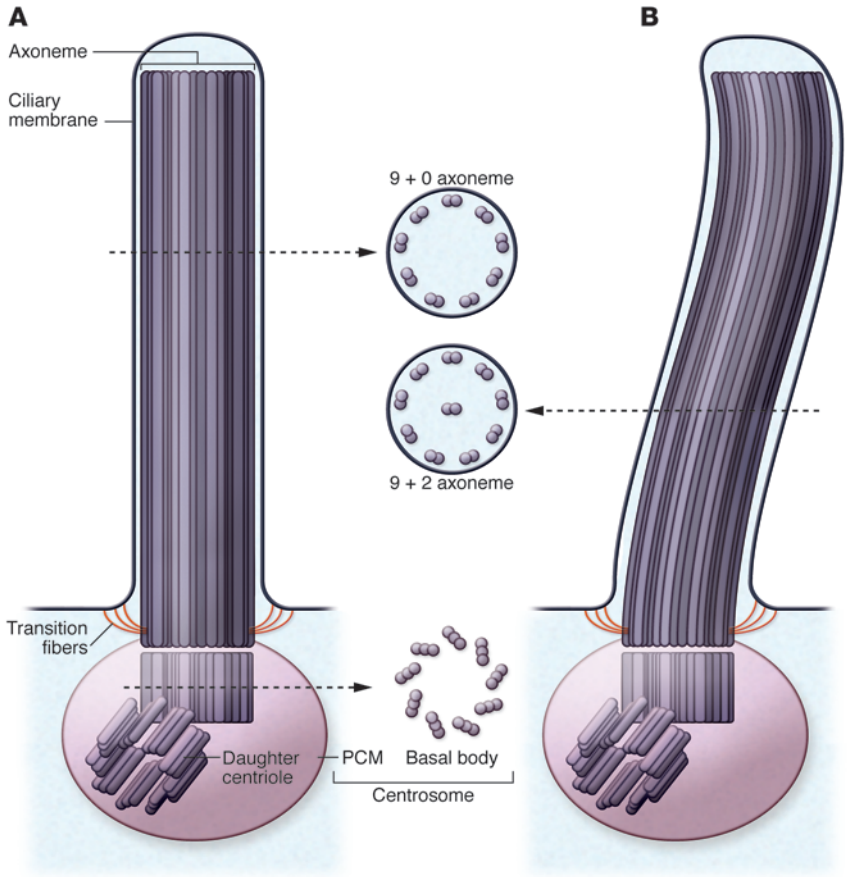

Figure 1
The cilia. (**A**) Primary cilia are nonmotile, as their axonemal microtubules have 9 microtubule pairs (9 + 0). (**B**) Motile cilia have an additional pair of central microtubules (9 + 2).

interact as part of an expanding network of ciliopathy-associated proteins, leading them to suggest that modulating this network may provide a novel approach for treating human ciliopathies.

Cilia — the basics

Over the past few decades, cell biological and signaling studies have established a crucial role for cilia in sensory functions, motility, and flow generation (4). Cilia originate from the mother centriole of the centrosome (5). As a result of intense trafficking of protein complexes to the pericentriolar material (PCM), a structural matrix that surrounds the centrioles, the mother centriole differentiates into a basal body, and the cilium assembles from this structure (Figure 1).

Cilia can be broadly classified as primary or motile based on the pattern of their axonemal microtubules; there are nine microtubule pairs in primary cilia (9 + 0), while motile cilia have an additional pair of central microtubules (9 + 2). Primary and motile cilia have different functions. Motile cilia serve to generate flow, such as the flow of mucus in the respiratory tract that is crucial for eliminating bacteria. Because they lack the central microtubule pair, primary cilia are immotile. They serve as antennae to sense environmental cues and link them to key signalling pathways, such as the Hedgehog signaling pathway.

Primary cilia, photoreceptors, and retinal dystrophies

Primary cilia are usually localized at the cell surface. However, in sensory cells, such as photoreceptors of the retina, nonmotile primary cilia are located within the cell, connecting the outer and inner segments (Figure 2). Interestingly, although different ciliopathies have highly variable clinical phenotypes, retinopathy is a recurrent phenotypic manifestation of many of the ciliopathies, suggesting that this system is particularly vulnerable.

Leber congenital amaurosis (LCA) is a rare early-onset childhood inherited retinopathy, characterized by severe visual impairments that occur shortly after birth. This clinically and genetically heterogenous disease is caused by mutations in at least 16 genes (Table 1), with mutations in the *CEP290* gene being the most common cause of LCA. Mutations in *CEP290* can also cause other ciliopathies, including Joubert syndrome, Meckel-Gruber syndrome, and BBS (6). While extending their previous work on LCA (7), Rachel et al. (3) found that almost 10% of individuals with LCA have a mutation in *MKKS*, a gene also found mutated in individuals with MKKS and BBS. This further reveals the extreme overlap and complexity among the ciliopathy-associated proteins and the ciliopathies.

CEP290 and MKKS act in concert

Given the substantial fraction of mutations in *CEP290* or *MKKS* genes in patients with LCA, Rachel et al. postulated that CEP290 and MKKS could be functionally linked (3). Importantly, some patients with LCA carrying mutations in *CEP290* had potentially pathogenic variant alleles of *MKKS*. The authors then demonstrated that MKKS interacts with the CEP290 domain termed the deleted in sensory dystrophy (DSD) domain. This domain is absent in the Cep290 protein expressed by *Cep290*^{rd16} mice, which have a pheno-

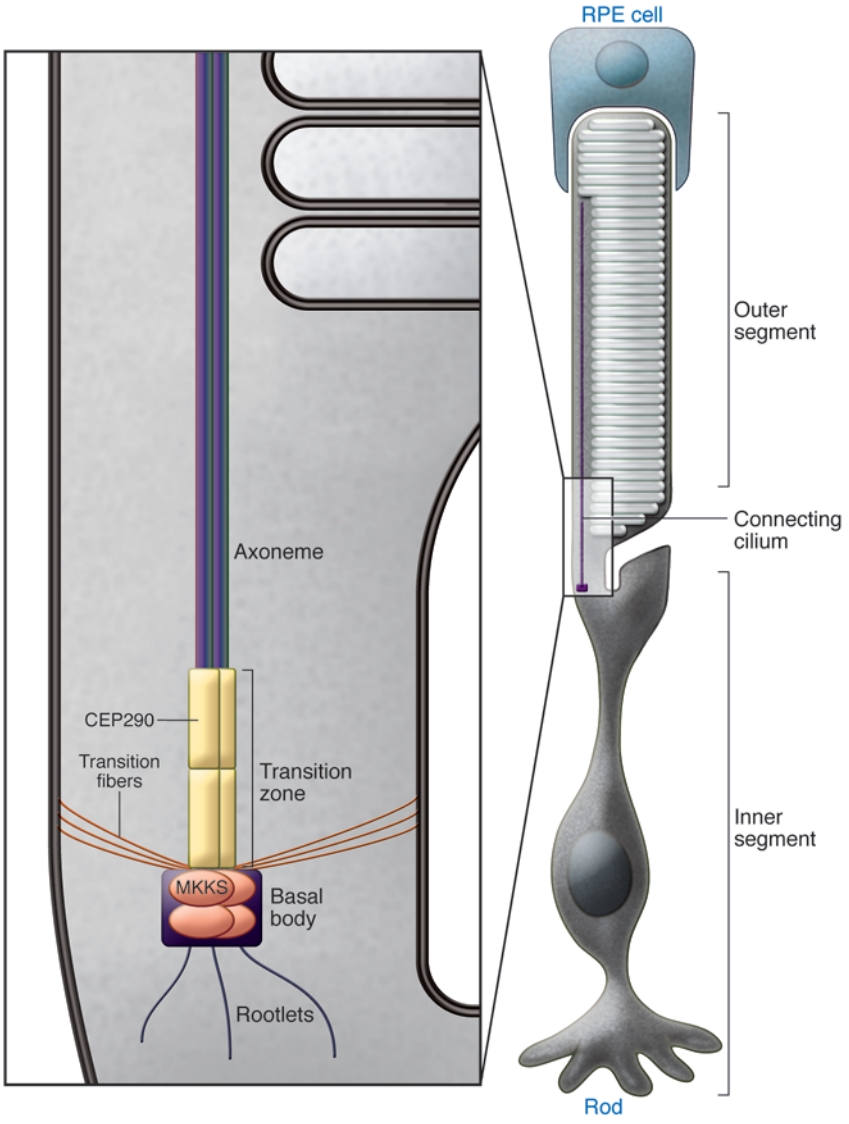

Figure 2
Scheme of the photoreceptor cell. The connecting cilium is a specialized organelle that regulates trafficking into and out of the outer segment of the photoreceptor cell. CEP290 is found at the transition zone adjacent to the basal body where MKKS is localized. Given their localization at this strategic transport zone between the inner and outer segments, these two proteins act together to regulate ciliogenesis, possibly by controlling the trafficking of various cargos and proteins, including BBS and IFT proteins. RPE, retinal pigment epithelium.

type that resembles LCA (8). The DSD domain was sufficient for CEP290-MKKS interaction. Mutant MKKS encoded by BBS-associated *MKKS* alleles failed to efficiently interact with CEP290, further supporting the functional interaction between CEP290 and MKKS. Importantly, coinjection of subminimal doses of *cep290* and *mkks* morpholinos in zebrafish larvae induced a synergistic effect on eye and ear morphogenesis, implying that these proteins, together, are critical for the proper development and maintenance of these sensory organs. Rachel et al. next analyzed two different mouse models of ciliopathies (3), the *Cep290*^{rd16} mouse model of LCA (8) and the *Mkks* knockout mouse model of BBS, which also exhibits retinal degeneration (9). Since the Mkks-binding DSD domain of Cep290 is absent in *Cep290*^{rd16} mice, the authors speculated that concomitant reduction of Cep290 and Mkks dosage should be synergistic, as in the zebrafish larvae (3). However, they observed a rescue of the ciliary defects and the resulting retinal phenotypes when they combined *Cep290*^{rd16} and *Mkks* knockout alleles in mice. Mice homozygous mutant for Mkks (*Mkks*$^{-/-}$ mice) had a weaker retinal ciliopathy phenotype if they carried a *Cep290*^{rd16} allele. The converse was also true; the phenotype of *Cep290*$^{rd16/+}$ mice was less severe if they were also heterozygous for the *Mkks* knockout allele. Restoration of normal phenotype was not restricted to photoreceptor cells, as Rachel et al. observed a marked rescue of both kinocilia and stereocilia of the inner ear and of sensory cilia of the olfactory epithelium.

In photoreceptor cells as well as sensory neurons in the ear and olfactory epithelium, MKKS was shown to localize to the basal body, while CEP290 was found adjacent to the basal body in a region known as the transition zone (3). These findings argue in support of CEP290 having a specific gatekeeper role at the transition zone (10) and of it working in coordination with the MKKS protein in the basal body (Figure 2).

An important aspect of the findings of Rachel et al. (3) relates to the heterogeneity of ciliopathies that is likely a consequence of the diversity of causative mutations within a given gene. As shown in mice, deletion of the DSD domain of CEP290 impairs the interaction between CEP290 and MKKS and alters ciliogenesis, but, strikingly, this mutation does not impair

Table 1
LCA phenotypes and associated genes

Phenotype	Location	Gene/locus	Function/localization
LCA1	17p13.1	***GUCY2D***	Retinal guanylate cyclase
LCA2	1p31	***RPE65***	Retinal pigment epithelium-specific protein 65 kDa
LCA3	14q31.3	*SPATA7*	Spermatogenesis-associated protein 7
LCA4	17p13.1	*AIPL1*	Aryl hydrocarbon receptor interacting protein-like 1
LCA5	6q14.1	*LEBERCILIN*	Ciliary protein
LCA6	14q11	*RPGRIP1*	Retinitis pigmentosa GTPase regulator interacting protein 1
LCA7	19q13.3	*CRX*	Cone-rod homeobox
LCA8	1q31-q32	***CRB1***	Crumbs homolog 1
LCA9	1p36	?	?
LCA10	12q21	***CEP290***	Centrosomal and ciliary protein
LCA11	7q31.3-q32	*IMPDH1*	Guanine synthesis
LCA12	1q32.3	*RD3*	Retinal membrane guanylyl cyclase inhibitor
LCA13	14q24.1	*RDH12*	Retinol dehydrogenase
LCA14	4q31	*LRAT*	Lecithin/retinol acyltransferase
LCA15	6p21.3	*TULP1*	Tubby-like protein 1
LCA16	2q37	*KCNJ13*	Potassium channel subunit Kir7.1

The symbols of the most frequently mutated genes in LCA are in bold typeface. *CEP290* is also known as *NPHP6*.

CEP290 cellular localization at the transition zone. One attractive hypothesis, given the potential gatekeeper role of CEP290 at this location, is that some mutations affect the transport of some but not other cargos into and out of the cilium. However, this is unlikely to be as clear-cut an explanation of the phenotypic heterogeneity as one might like, given the large and overlapping distribution of the various mutations within *CEP290* (11).

Cilia proteins, understanding the rules

What is needed now is a better understanding of the function of CEP290, a likely multifunctional protein. Assessment of its various functional domains and of the functional defects of individual mutant proteins could help dissect such functions. In addition to the transition zone, CEP290 is found on centriolar satellites, interacts with PCM1, and is essential for the proper localization of the small GTP-binding protein Rab8 (12). The Rab8-targeting function of CEP290 is inhibited when CEP290 binds to the centrosomal protein CP110, levels of which decrease in quiescent cells (13). Whether such interaction is altered in some patients remains to be investigated. Also, deletion of the DSD domain in CEP290 or other ciliopathy mutations could impact the capacity of CEP290 to transport Rab8 or other cargos. In *Chlamydomonas*, Cep290 functions to control the flagellar protein content (14). Protein checking at the transition zone could be under the control of signaling pathways, such as the Hedgehog pathway. Also, *CEP290* mutations could induce signaling defects in Raf-1 kinase pathways, as deletion of either *Cep290* or the CEP290 DSD induces accumulation of the Raf-1 kinase–inhibitory protein (15). Whether regulation of CEP290 function in response to signaling pathways is altered by mutations of potential posttranslational modification sites remains to be investigated.

Although the work of Rachel et al. points to a crucial partnership between CEP290 and MKKS (3), how the reduction of MKKS levels rescues CEP290 dysfunction induced by deletion of its DSD domain remains to be understood. MKKS is a cochaperone protein that interacts with BBS10 and BBS12 (16). Until now, MKKS was not identified within the BBSome, a core complex of highly conserved BBS proteins that are closely associated with BBS4 (17). However, MKKS may participate with BBS10 and BBS12 in the assembly of the BBSome, since the BBSome does not form in the absence of MKKS (16). Intriguingly, BBS4 and some intraflagellar transport (IFT) proteins accumulate in *Cep290* mutant *Chlamydomonas* flagella, suggesting an impairment in the sorting out of proteins from the flagella induced by the loss of Cep290. Proteins of the BBSome, such as BBS4 or IFTs, are likely candidates for proteins whose normal trafficking might be rescued in the double $Cep290^{rd16};MKKS^{ko}$ mutants analyzed by Rachel and colleagues.

Therapeutic perspectives

Recently, several groups reported successful therapy using adeno-associated virus vectors to rescue vision in patients with LCA carrying mutations in the retinal pigment epithelium-specific protein 65 kDa (*RPE65*) gene (18). Strikingly, adeno-associated virus–mediated therapies allowed the recovery of visual cortex activity, even after long sensory deprivation, making such therapies very effective, even in early-onset syndromes (19). Since then, other genetic mutations causing LCA have been targeted either clinically or preclinically, demonstrating the feasibility of such approaches (20). However, the work of Rachel et al., in this issue of the *JCI* (3), indicates that some mutations in *CEP290* are not simple loss-of-function mutations that could be restored by reexpression of CEP290. It does suggest additional approaches to restoring cilia function by intervening at the level of interaction among distinct ciliopathy-associated proteins that would be based on the nature of the disease-causing mutations harbored by the affected individual. Given the allelic heterogeneity of patients suffering from ciliopathies, this will require extensive study of the various allelic combinations, but this is probably the only way to propose efficient treatment for these patients.

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Chk'ing p53-deficient breast cancers

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Loss or functional impairment of p53 occurs in many human cancers, and its absence is often associated with a poor response to conventional chemotherapy. Hence, much effort is currently devoted to developing novel treatments for p53-deficient malignancies. One approach is to target pathways that are selectively required for the survival of p53-deficient cancer cells, thus exploiting a synthetic lethal interaction. Previous studies have demonstrated that inhibition of the ataxia telangiectasia and Rad3-related (ATR) and checkpoint kinase 1 (Chk1) pathway in p53-deficient cells can induce such a synthetic lethal outcome. In this issue of the *JCI*, Ma et al. take these findings a step closer to the clinic by demonstrating that highly specific inhibitors of Chk1 synergize with chemotherapy to stem progression of p53-deficient triple-negative breast cancers in a xenotransplant model of this disease. Together with other recent studies, this report highlights the promise of ATR and Chk1 inhibitors in targeted cancer treatment.

Breast cancers are a heterogeneous group of tumors that can be classified into several subtypes based on histological observations and molecular profiling. Each subtype can vary in epidemiology, response to treatment, and risk of progression and recurrence. Triple-negative breast cancer (TNBC) is defined by the loss of estrogen receptor and progesterone receptor expression as well as the lack of human epidermal growth factor receptor 2 (*HER2*) amplification (1). Management of patients with these cancers can represent a serious challenge, as TNBCs are generally very aggressive and unresponsive to the standard molecularly targeted therapy (HER2 interference and hormonal therapy). Hence, there is much interest, and recent preliminary success, in identifying and manipulating other targets for the treatment of this disease (2). Notably, the p53 pathway is often disrupted in TNBC. In this issue of the *JCI*, Ma et al. report data from a human-in-mouse model of TNBC that highlight the promise of checkpoint kinase 1 (Chk1) inhibitors as targeted therapy for p53-deficient TNBCs (3).

Targeting Chk1 in an advanced experimental model of TNBC

The ataxia telangiectasia and Rad3 related (ATR) and Chk1 kinases function in a linear pathway that serves as a "shock absorber" to perturbations to DNA replication. Specifically, activation of the ATR/Chk1 pathway during replication stress both prevents collapse of troubled replication forks into DNA double-strand breaks and inhibits cell-cycle progression into M phase. Previous culture-based studies have demonstrated that suppressing the G_2-M phase checkpoint through ATR and Chk1 inhibition is particularly toxic when combined with loss of G_1-S checkpoint function via p53 deficiency (4–9). This dual loss produces a checkpoint short circuit (Figure 1A). These observations, together with the fact that TNBCs frequently harbor mutations in *TP53*, led Ma and colleagues to hypothesize that p53-deficient TNBCs might be sensitive to selective inhibition of Chk1 (3).

To best model TNBC, Ma et al. grafted cancerous tissue obtained from patient

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