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 cellular stress responses for successful brain ageing [published online ahead of print January 18, 2012]. Nat Rev Neurosci. doi:10.1038/nrn3151.


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 signalling 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 heterogeneous 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<sup>rd16</sup> mice, which have a pheno-
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 Cep290rd16 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 Cep290rd16 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 Cep290rd16 and Mkks knockout alleles in mice. Mice homozygous mutant for Mkks (Mkks−/− mice) had a weaker retinal ciliopathy phenotype if they carried a Cep290rd16 allele. The converse was also true; the phenotype of Cep290rd16/+ 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...
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 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\textsuperscript{d1/D2},MKKS\textsuperscript{b5} 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 checkpoint short circuit (Figure 1A). 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.

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