models to zebrafish, a diurnal animal relying on cone-driven visual inputs to a much higher degree than nocturnal mice. The authors conducted an unbiased screen among the members of the short-chain dehydrogenase/reductase enzyme family aiming to identify enzymes expressed in cones that are able to perform 11-*cis*-retinol oxidation. This screen pointed to a previously uncharacterized fish homolog of human RDH12, called ZCRDH. Cones from zebrafish with a loss-of-function mutation in the gene coding for this enzyme lost their ability to regenerate visual pigments after supplementation with exogenous 11-*cis*-retinol. This experiment was followed by a detailed side-by-side analysis of the enzymatic properties of the fish and human enzymes, which reinforced the notion that ZCRDH and RDH12 are functional homologs.

Of particular interest is the fact that RDH12 belongs to a group of proteins whose mutations cause a blinding condition in human patients known as Leber's congenital amaurosis (LCA)[15]. Mutations of the *Rdh12* gene account for ~10% of all LCA cases. Different from mutations in other LCA-associated genes, mutations in *Rdh12* affect primarily cones, with relative sparing of rods[16]. The study by Kaylor and colleagues suggests that this pathology may relate to the inability of cones from these patients to perform efficient pigment regeneration. Conversely, understanding that RDH12 is a part of the cone visual cycle highlights the vital role of this cycle in maintaining the healthy status of our retinas.

In summary, the demonstration that RDH12 is the cone-specific 11-*cis*-retinol oxidase concludes an over two decade-long quest to characterize the enzymatic pathway responsible for regenerating visual pigments in these photoreceptor cells. This pathway is uniquely designed to sustain the high demand for cone opsin regeneration in bright light.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Confined migration: Microtubules control the cell rear

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Cell migration through complex 3D environments relies on the interplay between actin and microtubules. A new study shows that, when cells pass through narrow constrictions, CLASP-dependent microtubule stabilisation at the cell rear controls actomyosin contractility to enable nuclear translocation and preserve cell integrity.

Cell migration is a challenging process that requires coordination of actin polymerisation, which drives cell extension at the leading edge, and

actomyosin contraction at the cell rear[1]. Poor coordination of these processes perturbs cell morphology and inhibits cell displacement but can also lead to more dramatic outcomes such as cell rupture[2]. The mutually exclusive activities of RhoA and Rac GTPases polarise the dynamics of the actin network[3]. An additional layer of regulation is provided by microtubules, which can also polarise along the front–rear axis[4]. Microtubules serve as tracks for the transport of components involved in cell adhesion and as a scaffold for signaling molecules controlling actin dynamics[5].

The function of microtubules becomes even more critical when cells invade soft and spatially constrained environments[6,7]. The exact role of microtubules depends on the cell migration mode. Mesenchymal cells, such as fibroblasts, endothelial cells or some cancer cells moving in 3D hydrogels, extend long exploratory protrusions that penetrate into the pores formed by extracellular matrix fibres. Local stabilisation of microtubules in these protrusions is key to their maintenance and necessary for efficient cell migration[8,9]. Furthermore, asymmetric distribution of microtubules between protrusions ensures overall cell asymmetry to allow persistent movement in 3D environments[10] (Figure 1, left). In contrast, in cells undergoing ameboid migration, including different types of immune cells, microtubules are less critical[6,7]. Still, in ameboid cells with long protrusions, such as dendritic cells, microtubules coordinate protrusion retraction and thus prevent cell entanglement and fragmentation[2]. An important player in this process is the RhoA GEF GEF-H1, a signalling protein that is inhibited by binding to microtubules[11]. Local microtubule disassembly in cell protrusions releases GEF-H1, which then triggers actomyosin contraction and protrusion retraction[2].

The balance between microtubule assembly and disassembly is controlled by numerous cellular factors[12], such as CLASPs[13] — represented in mammals by two homologs, CLASP1 and CLASP2. These proteins accumulate at microtubule plus ends to promote growth and prevent shrinkage; they also associate with the centrosome and Golgi membranes to stimulate microtubule nucleation and minus-end stability. Also, CLASP2, which is known to ensure plus-end growth, is rapidly recruited to damaged microtubule lattices and protects these sites from depolymerisation[14]. This CLASP function can be particularly relevant during cell movement in complex environments because mechanical forces generated by the actomyosin cytoskeleton can bend and damage microtubules[15] (Figure 1, left).

It is thus not surprising that CLASPs are important for cell migration. In motile cells, CLASPs are often enriched at the leading edge of the cell, and CLASP1 depletion impairs microtubule stabilisation in membrane protrusions and prevents cell migration in 3D[8]. Recently, it was shown that compressive mechanical forces that bend microtubules induce the relocalisation of plus-end tracking proteins, including EB1 and CLASP2, from growing tips to the damaged lattice, thereby stabilising microtubules and making them more resistant to nocodazole-induced disassembly[16]. This process contributes to the stabilisation of microtubules at the leading edge of cells migrating in a confined space. CLASP2 depletion abolished this mechanical stabilisation and significantly slowed the passage of cells through narrow constrictions[16] (Figure 1, left). However, it remained unclear how this stabilisation of microtubules by external mechanical forces, or by autonomous cell-contractile forces, in turn affects the cellular actomyosin contraction necessary for the acute deformation of cells passing through constrictions. In addition, the contribution of these local stabilisation events to the global integration of the motile machinery at the cellular level remained to be elucidated.

Now, a new study by Ju *et al.*[17] reports that melanoma cells with reduced CLASP1 expression rupture when migrating in 3D collagen gels. To further

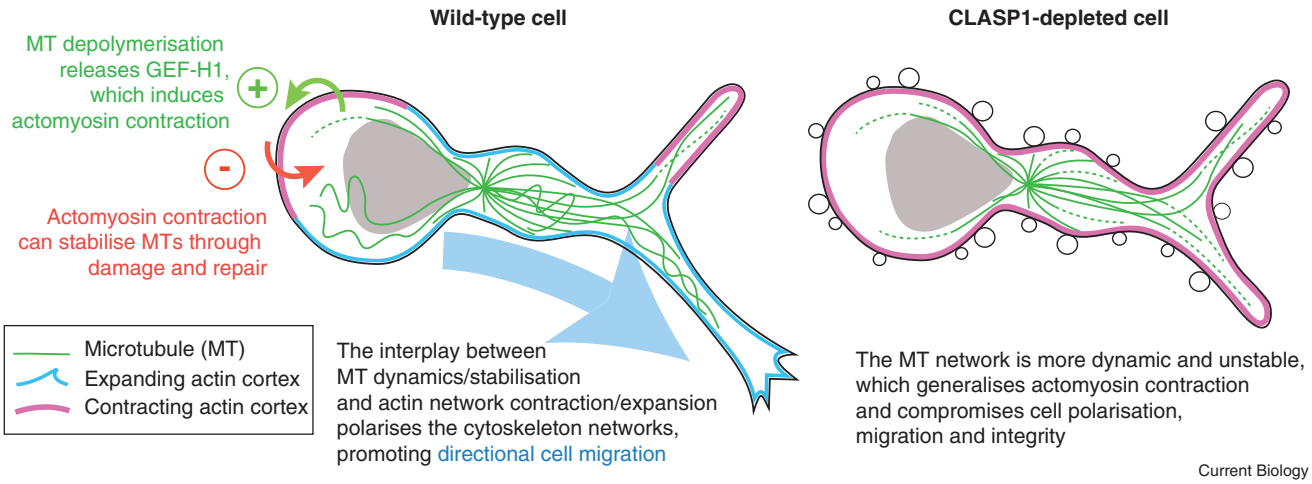

Figure 1. Regulation of cell polarisation and migration by the interplay between actin and microtubule networks.
(Left) 3D migration of a wild-type mesenchymal cell through a constriction that restricts the passage of the nucleus. Eventually, the polarisation of the cell and its contraction at the rear will push the nucleus through the constriction. (Right) In a CLASP1-depleted cell, the cell remains blocked at the constriction. These cells are unable to polarise, undergo blebbing at the plasma membrane, and remain stuck in the constriction for hours, where they often die.

investigate this sensitivity, the authors designed dense arrays of micropillars to force cells to pass through very narrow constrictions and performed high-resolution live-cell imaging. They found that, as the cells passed through the constrictions, their nucleus was obstructed. Cell contraction at the rear concentrated relatively stable, partly acetylated microtubules at the back of the cell, and these microtubules accumulated CLASPs on their lattice, a localisation consistent with microtubule damage by contractile forces and its subsequent repair. The network of microtubules present at the rear disassembled shortly before nuclear propulsion, and this correlated with an increase in the activation of GEF-H1 and RhoA and the accumulation of myosin. In contrast, in CLASP1-depleted cells, microtubules were rapidly lost after nuclear obstruction; such cells displayed numerous and evenly distributed sites of GEF-H1 and RhoA activation and actomyosin contraction. This lack of polarity prevented cells from passing through the constrictions; instead, the cells exhibited extensive blebbing all over their surface and died after several hours of random and ineffective contractions (Figure 1, right). The authors propose that the lack of repair and stabilisation of microtubules damaged by cell contraction in CLASP1-deficient cells affected the mechanical stabilisation of the cell rear and disrupted the polarised release of GEF-H1, thus forcing excessive cell blebbing and ultimately causing cell death.

These results confirm the critical importance of the fine regulation of microtubule dynamics and stability by CLASP1 for 3D cell migration[8] and the importance of mechanical microtubule stabilisation[16] for cell migration in complex 3D environments. Importantly, these results reveal that the lack of CLASP1 perturbs the positioning of contractile regions and the overall organisation of cell polarity in confined cells. It is still unclear why the coupling between microtubule dynamics and cell contractility becomes particularly critical in the presence of tight constrictions. Ju *et al.*[17] propose that this is due to the regulation of the hydrostatic pressure balance between the cell front and cell rear, which are isolated by the nuclear obstruction in the constriction, with microtubules acting as a 'cushion' at the back, preventing this compartment from being squeezed empty. However, it is also possible that the loss of microtubules from a significant part of a contractile cell is toxic for other reasons, for example, due to the abrogation of vesicle transport. Accelerated microtubule disassembly at the rear upon CLASP1 depletion is likely specific for constricted cells because microtubules already present at the rear are short-lived due to the absence of stabilisation of plus ends and the lattice, whereas new, dynamic microtubules emanating from the microtubule-organising centre, located in front of the nucleus in the studied cell type, cannot penetrate behind the nucleus. The decreased microtubule growth persistence at the obstacles, which would be expected following CLASP depletion based on *in vitro* work[18], likely contributes to this effect.

This work also raises many interesting questions, such as the dissection of different aspects of CLASP function. CLASPs are essential for cell division[13], and one might wonder whether cell division abnormalities in CLASP1-depleted cells prime them for death in migration assays. Cell migration defects are typically studied in conditions of partial CLASP loss, such as depletion of one of the two CLASP isoforms. Although the two CLASPs show redundancy, it would be interesting to know whether they have distinct activities in different processes, such as catastrophe suppression and lattice repair. Finally, since CLASPs affect the stability of both microtubule ends and lattices, separating the contribution of different microtubule turnover pathways to the observed cellular phenotypes remains challenging.

Another set of questions concerns the release of GEF-H1 from microtubules, as not only microtubule disassembly but also microtubule acetylation associated with stabilisation has been shown to release GEF-H1[19]. This further points to the need to clarify what is meant by microtubule 'stability': extended lifetime due to the absence of tubulin turnover at the ends, high rescue frequency at the dynamic ends, or the ability to resist or repair mechanical damage? Does accumulation of post-translational modifications, such as acetylation, play a role, or is it simply a correlate of microtubule longevity? Or is the structure of the microtubule lattice, such as its expanded or compacted state, which was recently shown to correlate with microtubule stability[20], the functionally relevant factor? Any or all of these could have a distinct and specific impact on GEF-H1 release, RhoA activation and local contractility.

Due to their stiffness, microtubules appear to be sensitive mechanical sensors in the cytoplasm. Combined with their ability to sequester or release biochemical signals, they can locally regulate specific pathways, acting not only as antennas but also as transmitters. It is crucial to elucidate how microtubule mechanical sensitivity and biochemical regulation are coupled to the excitability of the contractile actin network and how this interplay directs or prevents cell polarisation, thereby controlling not only cell migration but also cell integrity and survival.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Chromosome biology: Too big to fail

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Spindles are microtubule-based machines that segregate chromosomes during cell division. Spindle morphology and dynamics are malleable based on forces within the spindle, and a new study reveals the extreme plasticity of the *Saccharomyces cerevisiae* spindle to adapt and segregate engineered mega-chromosomes.

Cells must faithfully segregate their chromosomes during cell division. Failure to properly separate chromosomes can lead to negative outcomes, including aneuploidy[1] and cellular death[2], which are associated with human health conditions such as cancer progression[3] and congenital birth defects[4]. In mitosis, replicated chromosomes consist of two identical sister chromatids that are pulled apart by the spindle, a dynamic molecular machine. The spindle is a bipolar structure, composed of microtubules that attach to sister chromatids and generate sufficient force to retract them towards the poles during anaphase[5] (Figure 1). Microtubules in the spindles fall into three categories based on their role: first, kinetochore microtubules attach and pull chromosomes apart via the kinetochore, a protein structure that assembles on centromeric DNA; second, interpolar or core microtubules provide structural integrity for the spindle; and third, astral microtubules reach away from the main spindle to position it within the cell[6] (Figure 1A). While the spindle is a critical and highly conserved cellular machine, its morphology and dynamic capabilities are highly variable across species, and even within species. Previous studies have shown that various spindle features, including the length of the spindle and the number of microtubules, can vary based on the number of chromosomes[7,8] and attachments to the spindle[9]. However, we have lacked a clear understanding of how chromosome demands on the spindle ultimately shape its structure and function, and how dynamic the spindle can be in response to their chromosome loads. In a study published in this issue of *Current Biology*, Kunchala *et al.*[10] utilize the genetically engineered 'mega-chromosomes' in *Saccharomyces cerevisiae* to probe the plasticity of the spindle. Findings from this study suggest that the spindle is highly responsive, altering its morphology and segregation dynamics in response to the mega-chromosome karyotype. Ultimately, the spindle is able to adaptively accommodate to the load, revealing that, for cells, chromosome segregation is too big to fail (Figure 1B).

Spindles are tasked with the critical role of pulling chromosomes apart, but this function is highly complex. This singular task requires spindles to generate force,