such as the spatial organization of cortical areas or the arrangement of neuronal cell bodies in an entire worm. Analysis of this principle at finer resolution could not be carried out before now because we lacked enough detailed structural information. In recent years, however, a number of laboratories have dreamed of developing a complete wiring diagram of the brain — or at least of a small brain region. Chklovskii and his colleagues have shared this dream and have produced a complete structure of parts of the fruit fly brain. With this detailed information available, they have now been able to test the validity of the wire minimization principle for very small brain structures [3].

Information about the visual world is sensed by the fly's retina, and this information is first passed by photoreceptor cell axons to monopolar cells in a structure just behind the eye called the *lamina*. These lamina monopolar cells send their axons to the next visual processing stage — the medulla — and provide the fly with almost everything it needs to know about its visual world. 'Almost everything' because each unit of the fly's eye contains eight photoreceptor cells, six of which relay information to lamina cells and two of which provide visual information directly to the medulla.

Cell bodies of the lamina monopolar cells and of other lamina cells involved in the information processing (amacrine, glia, and some other cell types) are collected in the lamina cortex (a region just behind the retina) and the communications between photoreceptor axons and lamina cells occur in a region of neuropile subadjacent to the lamina cortex. This neuropil is complex, but very orderly. It is divided up into about 800 repeated units called *optic cartridges*, one for each pixel in the fly's image of the world. These cartridges are identical, are arranged in a hexagonal lattice, and each has something over 400 synapses, about 1 per μm^3, a synaptic density the same as that typically found in mammalian cortical neuropil.

The fact that the lamina neuropil has such an orderly structure suggests that the cartridges may conform to a minimum wire volume arrangement. To test this idea, Rivera-Alba *et al*. [3] used several approaches to determine if the placement of components indeed does minimize wire volume. In general, it is an extremely difficult problem to search through all of the possible component arrangements to find the one with minimum wire volume and then to compare this result to the actual arrangement. To make the problem manageable, Rivera-Alba *et al*. [3] exploited a symmetry in the cartridge structure: the arrangement of the largest components is nearly the same at each cross-section through the cartridge over its length. The authors kept constant the positions of these main components that are interconnected by side branches and found that the volume of the actual structure is less than that of a thousand structures whose average connectivity is the same as the real cartridge but where the actual interconnections have been replaced by random interconnections; the chances of this happening are less than about one in 10 million.

Rivera-Alba *et al*. [3] also used two other tests that permitted the main components to be moved around and again found that the actual arrangement had the minimum volume; this result is highly statistically significant (occurs by chance about one time in a hundred thousand). Furthermore, the authors examined other approaches, such as perturbing component sizes and connectivities, and again found the actual structure to have the smallest volume.

In the tests described above, Rivera-Alba *et al*. [3] assumed that the cross-sectional structure is basically uniform along the length of the cartridge, but this is not quite true. To see if the structural non-uniformity along the long axis of the cartridge is important, the authors incorporated observed differences in three longitudinal portions of the cartridge and examined each portion separately for minimum wire volume. As for the simpler computations above, the authors again found that wire minimization accounts well for the positions of the actual components.

In summary, then, this complete reconstruction of fly neuropil has been tested for conformity to the wire minimization principle, and this principle is found to explain the actual component arrangement satisfactorily. Rivera-Alba *et al*. [3] have thus discovered that the wire minimization principle operates down to the sub-microscopic level, at least in this brain region. As the complete structure of more brain regions becomes available, it should be possible — though increasingly difficult for less orderly neuropil —to learn the range of validity for this principle and to understand its exceptions.

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Neurogenesis: Premature Mitotic Entry Lets Cleavage Planes Take Off!

Mutations in the gene *microcephalin/MCPH1* result in the neurodevelopmental disease microcephaly. A recent report provides evidence that MCPH1 controls neuroprogenitor entry into mitosis via the Chk1–Cdc25b centrosome maturation pathway.

Priyanka Singh and Clemens Cabernard

Human primary microcephaly (MCPH) is an autosomal recessive disorder resulting in small but structurally normal brains and mild-to-moderate mental retardation [1]. At least seven loci, corresponding to the genes *MCPH1–7*, have been linked to

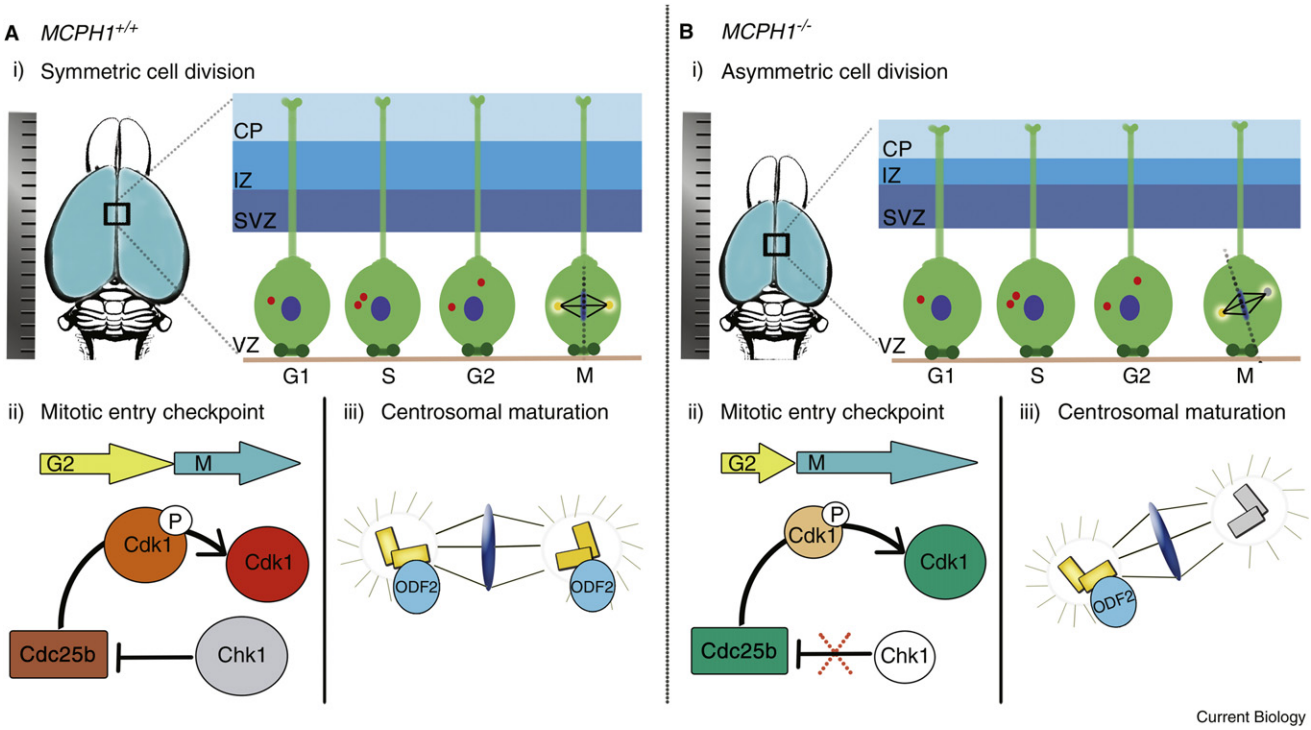

Figure 1. Lack of MCPH1 in mice recapitulates the microcephaly phenotype.

Schematics of phenotypes in (A) wild-type mouse brain and (B) *MCPH1*$^{-/-}$ mouse brain. (i) Mutant mouse brains have an overall reduction in size and reduced thickness of the cortical plate (CP) and the intermediate zone (IZ). The subventricular zone (SVZ) and ventricular zone (VZ) remain unchanged. In wild-type brains neuroprogenitors (green) residing in the ventricular zone (VZ) predominantly divide symmetrically early during neurogenesis, whereas *MCPH1*$^{-/-}$ mutant cells start dividing asymmetrically prematurely. (ii) The Chk1–Cdc25–Cdk1 pathway prevents wild-type neuroprogenitors from prematurely entering mitosis (M). MCPH1-deficient neuroprogenitors display reduced levels of Chk1, relieving Cdc25b inhibition too early. This results in dephosphorylation and activation of Cdk1 and a premature entry into M phase. (iii) Mature centrosomes, highlighted with symmetric ODF2 localization, properly orient the mitotic spindle in wild-type neuroprogenitors, whereas the immature centrosomes (asymmetric ODF2 localization) in *MCPH1*$^{-/-}$ neuroprogenitors fail to properly align the mitotic spindle.

microcephaly; MCPH1–7 proteins are ubiquitously expressed but localize, at least partially, to centrosomes during the cell cycle. Functional studies of the corresponding orthologues carried out in invertebrates and vertebrates suggested their involvement in centrosome maturation, spindle orientation and entry into mitosis [1].

MCPH1 has also been implicated in DNA-damage repair, chromosome condensation and the transcriptional regulation of DNA-damage genes [1]. However, how these functions are related to the formation of the severely decreased cerebral cortex in microcephalic brains has been unclear so far. In a recent paper, Gruber and colleagues [2] investigated the role of MCPH1 (also called microcephalin or BRIT1) during neurogenesis using a mouse model system. This new study provides evidence that MCPH1 regulates neuroprogenitor division by coupling the centrosomal cell cycle with mitotic entry (Figure 1).

To study the physiological function of MCPH1, Gruber *et al.* [2] first generated MCPH1-deficient mice. *MCPH1*$^{-/-}$ mice are viable, albeit sterile, and show smaller brains with a significant decrease in the thickness of the cortical plate and intermediate zone. These results are consistent with the described neuropathology of *MCPH1* patients. A reduction in brain size could be a consequence of increased cell death. Alternatively, it could also be attributed to a diminished self-renewal potential of neuroprogenitors. Indeed, labeling of apoptotic cells and *in vitro* monitoring of neuroprogenitor self-renewal revealed an increase in cell death but also a compromised ability to self-renew.

How could this *in vitro* finding be confirmed *in vivo*? Self-renewal versus differentiation of neuroprogenitors is regulated through the division mode, controlled in part by spindle orientation. Early during neurogenesis, neuroprogenitors undergo an amplification phase and divide symmetrically by positioning the cleavage plane perpendicular to the apical surface. Slight cleavage plane deviations will bisect the apical surface unequally, resulting in an asymmetric cell division. Gruber and colleagues [2] measured cleavage-plane orientation of MCPH1-deficient neuroprogenitors and found a significant number of dividing cells with unequal partitioning of the apical adherens junctions. Thus, the small brain phenotype associated with microcephaly could be a consequence of an increase in asymmetric cell divisions at the expense of neuroprogenitor self-renewal. Since MCPH proteins are involved in centrosome maturation (MCPH3), centrosome biogenesis (MCPH6), or directly in spindle positioning (MCPH5, MCPH7), loss

of MCPH1 could very likely affect neuroprogenitor self-renewal [1].

In order to test this hypothesis, Gruber *et al.* [2] analyzed the localization and phosphorylation profile of centrosomal kinases. Previously, it was shown that MCPH1 affects the centrosomal kinase Chk1 [3–5]. Gruber and colleagues [2] found that in neuroprogenitors Chk1 localized to centrosomes in interphase (G1/S/G2), disappeared at prophase and appeared again on both centrosomes in metaphase. However, quite surprisingly, MCPH1-deficient neuroprogenitors showed a significant decrease of Chk1 on centrosomes specifically in G2. Furthermore, phosphorylation of Cdk1, another centrosomal kinase, was reduced as well. What is the significance of this alteration in centrosomal kinase activity? In G2, Chk1 prevents the activation of Cdk1, avoiding a premature entry into mitosis by phosphorylating and therefore destabilizing Cdc25b. Relief of Cdc25b inhibition results in dephosphorylation of Cdk1 and entry into mitosis [6]. Thus, lower levels of MCPH1 should result in a premature entry into mitosis. Indeed, Gruber *et al.* [2] observed a shortened G2 phase but a prolonged M phase in *MCPH1* mutant neuroprogenitors, resulting in an increase in the overall cell cycle length.

How can this finding explain the increase in asymmetric cell divisions? To further address this question, the authors analyzed centrosome maturation upon mitotic entry in MCPH1-deficient cells. Usually, mitotic cells contain two mature centrosomes, revealed by symmetrical staining of the centriolar protein Cenexin/ODF2 in centrosomes. Neuroprogenitors depleted of MCPH1, however, display reduced ODF2 localization on one centrosome in almost all mitotic cells. This finding implies that the lack of MCPH1 impairs centrosome maturation and suggests that in *MCPH1*$^{-/-}$ neuroprogenitors centrosome maturation lags behind cell-cycle progression.

Immature centrosomes can be responsible for abnormal spindle formation, chromosome segregation and spindle alignment problems [7]. In agreement with this notion is Gruber *et al.*'s finding that a significant number of MCPH1-deficient neuroprogenitors and mouse embryonic fibroblasts (MEFs) contain abnormal spindles and misaligned chromosomes. Knockdown of Cdc25b expression specifically inhibited the formation of abnormal bipolar spindles and also significantly rescued the spindle orientation phenotype in MCPH1-deficient MEFs.

So far, the work by Gruber *et al.* [2] suggests that MCPH1 controls entry into mitosis via regulation of the Chk1–Cdc25b pathway but that MCPH1 also seems to be involved in centrosome maturation. But how important is the Chk1–Cdc25b pathway for neuroprogenitor cell fate determination *in vivo*? Gruber and colleagues [2] addressed this question by performing an *in utero* shRNA electroporation experiment, knocking down Chk1 and Cdc25b expression *in vivo*. In line with the previous findings, Chk1 shRNA treatment shifted the division plane and subsequently also increased the production of post-mitotic cells. This phenotype could be rescued by co-depletion of Cdc25b. Furthermore, depletion of Cdc25b rescued the *MCPH1* mutant phenotype and repressed the accumulation of post-mitotic cells in MCPH1-deficient neuroprogenitors.

In putting all of these data together, a picture starts to emerge: lack of MCPH1 could cause the small brain phenotype because it alters the cleavage-plane orientation in neuroprogenitors as a consequence of defective centrosome maturation. Incomplete centrosome maturation itself could be due to premature entry into mitosis, which is controlled by the Chk1–Cdc25b pathway. Immature centrosomes are unable to correctly position the mitotic spindle, resulting in premature asymmetric cell divisions (Figure 1). These results are in line with a previous report showing that in neuroprogenitors lacking the microcephaly gene *aspm/MCPH5* the cleavage plane is less frequently oriented perpendicular to the ventricular surface of the neuroepithelium [8].

Centrosome maturation seems to be a driving force in the correct establishment of spindle orientation as previously revealed by studies in *Drosophila melanogaster* [9,10]. Microcephaly proteins are also functionally conserved in invertebrates: the lack of Centrosomin (Cnn; orthologous to Cdk5Rap2), Sas-4 (Mcph6/CenpJ) and Anastral spindle 2 (Ana2; MCPH7/Stil) leads to spindle orientation defects in *Drosophila* neuroblasts, the precursors of the fly's central nervous system [11–13]. However, in contrast to microcephaly, uncontrolled spindle orientation in fly neuroblasts results in a shift from asymmetric towards symmetric divisions and subsequently an increase in the neuroblast pool [14].

Taken together, using a MCPH1-deficient mouse model, Gruber and colleagues [2] recapitulate the neuronal defects of microcephaly patients. This is thus a very informative study, providing molecular insight into the gene *MCPH1* in particular and microcephaly in general. In the future it will be interesting to learn whether the observed centrosome maturation defects in MCPH1-deficient neuroprogenitors are a consequence of the premature entry into metaphase or whether centrosome maturation and cell cycle control could act independently.

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