

genes), which is surprising because mitochondrial and plastid genomes often get pulled in similar evolutionary directions, particularly those from the same species [8].

Any plastome that is housed in a non-photosynthetic species faces the threat of outright loss.

The holoparasitic angiosperm *Rafflesia lagascae* is thought to have abandoned its ptDNA [10] as has the colourless green alga *Polytomella* [11]. Arguably, a key prerequisite for relinquishing a plastome (while still maintaining a plastid) is the genetic transfer of functionally crucial ptDNA-encoded genes to the nuclear genome and the successful targeting of their protein products back to the plastid [12]. Such an outcome seems unlikely for *Balanophora*: presumably, the massive AT-bias and novel genetic code of the ptDNA would prevent successful transfer of any plastid-encoded gene to the nucleus. In other words, the amazing plastomes of *Balanophora* are here to stay. Whether or not they have reached peak AT content or are inching their way back to a more moderate nucleotide composition only evolutionary time will tell.

REFERENCES

1. Su, H.J., Barkman, T.J., Hao, W., Jones, S.S., Naumann, J., Skippington, E., Wafula, E.K., Hu, J.-M., Palmer, J.D., and dePamphilis, C.W. (2019). Novel genetic code and record-setting AT-richness in the highly reduced plastid genome of the holoparasitic plant *Balanophora*. *Proc. Natl. Acad. Sci. USA* **116**, 934–943.
2. Lang-Unnasch, N., and Aiello, D.P. (1999). Sequence evidence for an altered genetic code in the *Neospora caninum* plastid. *Int. J. Parasitol.* **29**, 1557–1562.
3. Tang, K., Guo, Y., Zhang, L., Rowe, L.A., Roellig, D.M., Frace, M.A., Li, N., Liu, S., Feng, Y., and Xiao, L. (2015). Genetic similarities between *Cyclospora cayetanensis* and cecum-infecting avian *Eimeria* spp. in apicoplast and mitochondrial genomes. *Parasit. Vectors* **8**, 358.
4. Moore, R.B., Oborník, M., Janouškovec, J., Chrudimský, T., Vancová, M., Green, D.H., Wright, S.W., Davies, N.W., Bolch, C.J., Heimann, K., and Slapeta, J. (2008). A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* **451**, 959–963.
5. Bellot, S., and Renner, S.S. (2015). The plastomes of two species in the endoparasite genus *Pilostyles* (Apodanthaceae) each retain just five or six possibly functional genes. *Genome Biol. Evol.* **8**, 189–201.
6. Petersen, G., Zervas, A., Pedersen, H.A.E., and Seberg, O. (2018). Genome reports: contracted genes and dwarfed plastome in mycoheterotrophic *Sciaphila thaidanica*
- (Triuridaceae, Pandanales). *Genome Biol. Evol.* **10**, 976–981.
7. Alkatib, S., Scharff, L.B., Rogalski, M., Fleischmann, T.T., Matthes, A., Seeger, S., Schöttler, M.A., Ruf, S., and Bock, R. (2012). The contributions of wobbling and superwobbling to the reading of the genetic code. *PLoS Genet.* **8**, e1003076.
8. Smith, D.R., and Keeling, P.J. (2015). Mitochondrial and plastid genome architecture: Reoccurring themes, but significant differences at the extremes. *Proc. Natl. Acad. Sci. USA* **112**, 10177–10184.
9. Osawa, S., and Jukes, T.H. (1999). Codon reassignment (codon capture) in evolution. *J. Mol. Evol.* **28**, 271–278.
10. Molina, J., Hazzouri, K.M., Nickrent, D., Geisler, M., Meyer, R.S., Pentony, M.M., Flowers, J.M., Peiser, P., Barcelona, J., Inovejas, S.A., and Uy, I. (2014). Possible loss of the chloroplast genome in the parasitic flowering plant *Rafflesia lagascae* (Rafflesiaceae). *Mol. Biol. Evol.* **31**, 793–803.
11. Smith, D.R., and Lee, R.W. (2014). A plastid without a genome: evidence from the nonphotosynthetic green algal genus *Polytomella*. *Plant Physiol.* **164**, 1812–1819.
12. Janouškovec, J., Tikhonenkov, D.V., Burki, F., Howe, A.T., Kolísko, M., Mylnikov, A.P., and Keeling, P.J. (2015). Factors mediating plastid dependency and the origins of parasitism in apicomplexans and their close relatives. *Proc. Natl. Acad. Sci. USA* **112**, 10200–10207.

Developmental Biology: Go with the Flow to Keep the Body Straight

Daniel T. Grimes

Institute of Molecular Biology, Department of Biology, University of Oregon, Eugene, OR 97403, USA

Correspondence: dtgrimes@uoregon.edu

<https://doi.org/10.1016/j.cub.2018.12.011>

It has long been noticed that zebrafish defective in ciliary beating develop abnormal body curvatures. Recently, insights into how cilia keep the body straight have emerged, with implications for understanding human scoliosis.

A straight head-to-tail animal body axis allows for coordinated, directional movement. But how the body first attains and then maintains straightness during development, growth and adulthood is little understood. The need to comprehend how the body stays straight is underscored by the remarkable

prevalence of scoliotic spinal curves in human populations. With this goal, researchers have turned to the zebrafish model organism. Three recent studies [1–3] on zebrafish provide fresh mechanistic understanding of body straightening and provide a deeper appreciation of the causes of scoliosis.

The story starts with a simple but striking phenotype: curly tail down. For many years, researchers studying mutant zebrafish embryos had noticed this common defect, where the body axis is not straightened and the tail instead loops downwards in a ventral curl. Several curly tail down mutant lines were recovered



during large-scale mutagenesis screens in the mid-1990s, and many more have been generated since. Gradually, it was realized that most of these mutants harbored abnormalities in their motile cilia (for examples see [4,5]). Motile cilia resemble tiny hairs that protrude from the surface of specialized cells, and beat back and forth to generate fluid flows across epithelial sheets and along tubes [6]. Perhaps the best known example are those of the airways that are responsible for moving mucus out of the lungs. In zebrafish, though flow patterns are complex and not well understood, motile cilia in the brain ventricles and central canal of the spinal cord are thought to contribute to cerebrospinal fluid (CSF) flow [7,8]. So why do zebrafish embryos with defective ciliary beating, and thereby defective CSF flow, show curly tail down? What roles do cilia and flows play in the straightening of the body?

Cantaut-Belarif *et al.* [1] shed light on this puzzle. They demonstrated a link between motile cilia and an extracellular thread called the Reissner fiber, which runs through the brain ventricles and down the central canal. The Reissner fiber is predominantly composed of the glycoprotein SCO-spondin and, though it was first discovered in 1860, its function has remained enigmatic. By generating zebrafish mutants lacking *scospondin*, Cantaut-Belarif *et al.* [1] demonstrated that the Reissner fiber is needed to keep the body straight: mutant embryos exhibited classic curly tail down, mimicking cilia motility mutants. Consequently, the authors assessed motile cilia and CSF flow, but they found that both were normal in the absence of the Reissner fiber. Instead, they discovered that cilia mutants lacking CSF flow cannot properly assemble the fiber, suggesting that the Reissner fiber requires intact motile cilia to form. As the authors suggest, CSF flow forces may promote the aggregation of SCO-spondin monomers into the Reissner fiber.

In a second study, Zhang *et al.* [3] probed other mechanisms acting downstream of CSF flow in body straightening. They investigated gene expression differences between wild-type embryos and *zmynd10* mutants, which exhibit curly tail down owing to loss of cilia motility and CSF flow. While many

differences were found, Zhang *et al.* [3] focused on the strong reduction of *urp1* and *urp2* expression, two genes encoding urotensin II neuropeptides. Follow-up experiments showed that injecting synthetic Urp1 into the CSF partially rescued the abnormal curve of CSF flow-lacking mutants, while combined loss of *urp1* and *urp2* led to curly tail down. These findings invited new questions: how do motile cilia activate Urp expression and, critically, how do these neuropeptides then promote body straightness?

To address the first question, Zhang *et al.* [3] assessed a panel of CSF-residing signaling molecules for their ability to rescue *urp1* expression in the *zmynd10* motility mutant. Whole-embryo treatment with adrenaline was able to restore *urp1* expression and, indeed, also rescued the curly tail down of motility mutants. In wild-type zebrafish embryos, adrenergic neurons are mainly located in the brain ventricles while *urp1* and *urp2* are expressed distantly, in CSF-contacting neurons (CSF-cNs) within the spinal canal [3]. Thus, adrenaline is likely transported by CSF flow from the brain ventricles down the central canal to CSF-cNs, where it drives Urp neuropeptide expression. Intriguingly, the Reissner fiber is known to bind and transport adrenaline molecules [9], and so its formation downstream of CSF flow [1] might play a critical part in the delivery of adrenergic signals to CSF-cNs. It will now be interesting to test this model by asking whether *urp1/2* expression is affected in *scospondin* mutants that lack the Reissner fiber but maintain CSF flow, and whether adrenaline treatment can rescue the curly tail down of these mutants.

So, how do Urp1 and Urp2 neuropeptides secreted by CSF-cNs straighten the body? To address this, Zhang *et al.* [3] first searched for the Urp1 receptor. They found that Urp1 engages the protein Uts2ra, which is expressed by slow-twitch muscle cells specifically on the dorsal side of the embryo. Knock-down of *uts2ra* led to curly tail down without affecting motile cilia and, moreover, neither adrenaline nor Urp1 treatment was able to rescue *uts2ra* loss. Given these results, Zhang *et al.* [3] propose that Urp1 activates Uts2ra on dorsal muscles and causes them to contract. This dorsal contraction then acts to pull the tail upwards, straightening the

initial ventral curl of zebrafish embryos. In this model, CSF-cNs act as chemosensors of adrenergic signals and sources of Urp1. Indeed, CSF-cNs are ideally positioned to sense molecules within the CSF: their apical surface extends into the fluid and they harbor a single cilium, a well-known sensory organelle [10,11]. But evidence also suggests that CSF-cNs are mechanosensory [12,13], so they could also respond to the force of CSF flow.

Sternberg *et al.* [2] added weight to this possibility. While CSF-cNs in wild-type embryos exhibited spikes of Ca^{2+} activity, they found this was almost entirely lost in cilia mutants lacking CSF flow. Moreover, the polycystin family cation channel Pkd2l1, a marker of CSF-cNs across species, was essential for this Ca^{2+} activity. Other polycystin channels have been implicated in flow sensory responses in distinct contexts [14,15]. This supports a model in which CSF-cNs are mechanically activated by CSF flow, with Pkd2l1 playing a key role.

Together, these findings add much to our understanding of CSF sensation and the straightening of the body axis in zebrafish embryos. But what about the maintenance of this straight body throughout growth? In human populations, idiopathic scoliosis is prevalent: this disease, in which the spinal column aberrantly curves and rotates, typically onsets during the adolescent years [16]. Though idiopathic scoliosis affects around 4% of adolescents, we know little about its causes. Could cilia and CSF flow — and potentially the downstream mechanisms described here — also be controlling body straightness at later stages?

Some evidence supports this hypothesis. By using a temperature-sensitive mutation in the cilia motility gene *cfap298* (previously called *c21orf59*) [17], researchers inactivated ciliary beating and CSF flow in a temporally controlled way. By allowing these mutants to progress normally through developmental stages at permissive temperatures and only later shifting them to restrictive temperatures, they discovered that motile cilia function is needed during juvenile growth phases to keep the vertebral column straight [8]. Furthermore, motile

cilia and CSF flow were found to be disrupted at post-embryonic stages [8] in zebrafish *ptk7* mutants that were known to exhibit juvenile-onset spinal curves [18]. These late-onset spinal curve mutants showed many idiopathic scoliosis-like features, suggesting they are exceptional models of this complex disease [19]. Additionally, disruptions to CSF flow in human patients have been associated with the development of spinal curves.

Cilia and CSF flow, therefore, seem to be essential for keeping the spine and body straight, both in embryos and during juvenile growth. This leaves open the question of whether the Reissner fiber, urotensin neuropeptides and/or CSF-cN sensory function are also required during later stages. While some evidence hints that these mechanisms might be at play — *pkd2l1* mutant adults show mild spinal kinks akin to kyphosis [2], hunching of the back, while *uts2ra* mutants show juvenile-onset 3D curves reminiscent of idiopathic scoliosis [3] — a thorough understanding will require precise genetic perturbations in a spatially and temporally controlled manner. An exciting challenge remains: to understand how these mechanisms intersect in the context of the growing brain and spine. Zebrafish research is now well-poised to address these outstanding issues and to advance our understanding of body straightening and diseases like idiopathic scoliosis.

REFERENCES

1. Cantaut-Belarif, Y., Sternberg, J.R., Thouvenin, O., Wyart, C., and Bardet, P.L. (2018). The Reissner fiber in the cerebrospinal fluid controls morphogenesis of the body axis. *Curr. Biol.* 28, 2479–2486.
2. Sternberg, J.R., Prendergast, A.E., Brosse, L., Cantaut-Belarif, Y., Thouvenin, O., Orts-Del'Immagine, A., Castillo, L., Djenoune, L., Kurisu, S., McDermid, J.R., et al. (2018). *Pkd2l1* is required for mechanosception in cerebrospinal fluid-contacting neurons and maintenance of spine curvature. *Nat. Commun.* 9, 3804.
3. Zhang, X., Jia, S., Chen, Z., Chong, Y.L., Xie, H., Feng, D., Wu, X., Song, D.Z., Roy, S., and Zhao, C. (2018). Cilia-driven cerebrospinal fluid flow directs expression of urotensin neuropeptides to straighten the vertebrate body axis. *Nat. Genet.* 50, 1666–1673.
4. Hjeij, R., Onoufriadis, A., Watson, C.M., Slagle, C.E., Klena, N.T., Dougherty, G.W., Kurkwiak, M., Loges, N.T., Diggle, C.P., Morante, N.F., et al. (2014). *CCDC151* mutations cause primary ciliary dyskinesia by disruption of the outer dynein arm docking complex formation. *Am. J. Hum. Genet.* 95, 257–274.
5. Becker-Heck, A., Zohn, I.E., Okabe, N., Pollock, A., Lenhart, K.B., Sullivan-Brown, J., McSheene, J., Loges, N.T., Olbrich, H., Haefner, K., et al. (2011). The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation. *Nat. Genet.* 43, 79–84.
6. Spassky, N., and Meunier, A. (2017). The development and functions of multiciliated epithelia. *Nat. Rev. Mol. Cell Biol.* 18, 423–436.
7. Kramer-Zucker, A.G., Olale, F., Haycraft, C.J., Yoder, B.K., Schier, A.F., and Drummond, I.A. (2005). Cilia-driven fluid flow in the zebrafish pronephros, brain and Kupffer's vesicle is required for normal organogenesis. *Development* 132, 1907–1921.
8. Grimes, D.T., Boswell, C.W., Morante, N.F., Henkelman, R.M., Burdine, R.D., and Ciruna, B. (2016). Zebrafish models of idiopathic scoliosis link cerebrospinal fluid flow defects to spine curvature. *Science* 352, 1341–1344.
9. Caprile, T., Hein, S., Rodriguez, S., Montecinos, H., and Rodriguez, E. (2003). Reissner fiber binds and transports away monoamines present in the cerebrospinal fluid. *Brain Res. Mol. Brain Res.* 110, 177–192.
10. Malicki, J.J., and Johnson, C.A. (2017). The cilium: cellular antenna and central processing unit. *Trends Cell Biol.* 27, 126–140.
11. Orts-Del'Immagine, A., and Wyart, C. (2017). Cerebrospinal-fluid-contacting neurons. *Curr. Biol.* 27, R1198–R1200.
12. Bohm, U.L., Prendergast, A., Djenoune, L., Nunes Figueiredo, S., Gomez, J., Stokes, C., Kaiser, S., Suster, M., Kawakami, K., Charpentier, M., et al. (2016). CSF-contacting neurons regulate locomotion by relaying mechanical stimuli to spinal circuits. *Nat. Commun.* 7, 10866.
13. Jalalvand, E., Robertson, B., Wallen, P., and Grillner, S. (2016). Ciliated neurons lining the central canal sense both fluid movement and pH through ASIC3. *Nat. Commun.* 7, 10002.
14. Norris, D.P., and Grimes, D.T. (2012). Developmental biology. Cilia discern left from right. *Science* 338, 206–207.
15. Ma, M., Gallagher, A.R., and Somlo, S. (2017). Ciliary mechanisms of cyst formation in polycystic kidney disease. *Cold Spring Harb. Perspect. Biol.* 9 (11). pii: a028209. <https://doi.org/10.1101/cshperspect.a028209>.
16. Cheng, J.C., Castelein, R.M., Chu, W.C., Danielsson, A.J., Dobbs, M.B., Grivas, T.B., Gurnett, C.A., Luk, K.D., Moreau, A., Newton, P.O., et al. (2015). Adolescent idiopathic scoliosis. *Nat. Rev. Dis. Primers* 1, 15030.
17. Jaffe, K.M., Grimes, D.T., Schottenfeld-Roames, J., Werner, M.E., Ku, T.S., Kim, S.K., Pelliccia, J.L., Morante, N.F., Mitchell, B.J., and Burdine, R.D. (2016). *c21orf59/kurly* controls both cilia motility and polarization. *Cell Rep.* 14, 1841–1849.
18. Hayes, M., Gao, X., Yu, L.X., Paria, N., Henkelman, R.M., Wise, C.A., and Ciruna, B. (2014). *ptk7* mutant zebrafish models of congenital and idiopathic scoliosis implicate dysregulated Wnt signalling in disease. *Nat. Commun.* 5, 4777.
19. Boswell, C.W., and Ciruna, B. (2017). Understanding idiopathic scoliosis: a new zebrafish school of thought. *Trends Genet.* 33, 183–196.