editing, which was subsequently confirmed by amplification of the target regions of the TDO gene and DNA sequencing. If injections were done early enough (prior to pronuclear migration), the chromatophores were completely free of pigmentation. This is a particularly important result because it shows that both alleles of the TDO gene are being edited. If this result is the norm, it should be relatively straightforward to generate loss-of-function edits for other target genes in the squid. What genes might those be? Crawford *et al.* [4] make some suggestions. At the top of their list are genes involved in the wiring of the squid brain. This is an obvious choice. The cephalopod brain and the behaviors that it generates are so remarkable [9] that it has even been suggested that cephalopod evolution involved genes that originated in extraterrestrial viruses [10]. A close second on their list are the genes involved in the extraordinary amount of RNA editing that occurs in the squid nervous system [11,12]. And for developmental biologists, there are the many genes that are likely to be involved in constructing the fascinating squid body plan [13], which comes in forms ranging from pygmies to giants, and piglets to vampires.

Unfortunately, the squid species that Crawford *et al.* [4] used, *D. pealii*, cannot be cultured beyond the hatching stage. However, this does not preclude doing a lot of interesting science, since a lot of biology happens to produce a hatchling squid. But what one obviously wants sooner than later are adult squids that have CRISPR-edited genes. This isn't as wishful thinking as one might guess. The MBL has done amazing things with cephalopod culture in the last few years [14], and they now culture two species of squids with closed life cycles, *Eurprymna scolopes* and *Sepioloidea lineolata*. With the experience of doing CRISPR gene editing in *D. pealii*, it seems obvious that one or both of these species is next in line.

Finally, it is fitting that this work was done at the MBL. The squid is a special animal there. The work on the squid giant axon reported by J.Z. Young in his 1938 paper [1] was carried out at the MBL during the summer of 1936. You can buy squid hats at the MBL gift shop. And how many places have a job whose title is Manager of Cephalopod Operations? I think I can now predict with some degree of certainty that it won't be too long before we will see mutant squid swimming in the tanks at the MBL.

REFERENCES

1. Young, J.Z. (1938). The functioning of the giant nerve fibres of the squid. J. Exp. Biol. *15*, 170–185.
2. Hodgkin, A.L., and Huxley, A.F. (1939). Action potentials recorded from inside a nerve fibre. Nature *144*, 710–711.
3. Arnold, J.M. (1965). Normal embryonic stages of the squid, Loligo pealii (Lesueur). Biol. Bull. *128*, 24–32.
4. Crawford, K., Diaz Quiroz, J.F., Koenig, K.M., Ahuja, N., Albertin, C.B., and Rosenthal, J.J.C. (2020). Highly efficient knockout of a squid pigmentation gene. Curr. Biol. *30*, 3484–3490.
5. Blitz, I.L., Biesinger, J., Xie, X., and Cho, K.W. (2013). Biallelic genome modification in F(0) Xenopus tropicalis embryos using the CRISPR/Cas system. Genesis *51*, 827–834.
6. Jao, L.E., Wente, S.R., and Chen, W. (2013). Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system. Proc. Natl. Acad. Sci. USA *110*, 13904–13909.
7. Nakayama, T., Fish, M.B., Fisher, M., Oomen-Hajagos, J., Thomsen, G.H., and Grainger, R.M. (2013). Simple and efficient CRISPR/Cas9-mediated targeted mutagenesis in Xenopus tropicalis. Genesis *51*, 835–843.
8. Figon, F., and Casas, J. (2019). Ommochromes in invertebrates: biochemistry and cell biology. Biol. Rev. *94*, 156–183.
9. Hanlon, R.T., and Messenger, J.B. (2018). Cephalopod Behaviour (Cambridge University Press).
10. Steele, E.J., Al-Mufti, S., Augustyn, K.A., Chandrajith, R., Coghlan, J.P., Coulson, S.G., Ghosh, S., Gillman, M., Gorczynski, R.M., Klyce, B., *et al*. (2018). Cause of Cambrian Explosion - Terrestrial or cosmic? Prog. Biophys. Mol. Biol. *136*, 3–23.
11. Alon, S., Garrett, S.C., Levanon, E.Y., Olson, S., Graveley, B.R., Rosenthal, J.J., and Eisenberg, E. (2015). The majority of transcripts in the squid nervous system are extensively recoded by A-to-I RNA editing. eLife *4*, e05198.
12. Vallecillo-Viejo, I.C., Liscovitch-Brauer, N., Diaz Quiroz, J.F., Montiel-Gonzalez, M.F., Nemes, S.E., Rangan, K.J., Levinson, S.R., Eisenberg, E., and Rosenthal, J.J.C. (2020). Spatially regulated editing of genetic information within a neuron. Nucleic Acids Res. *48*, 3999–4012.
13. Callaerts, P., Lee, P.N., Hartmann, B., Farfan, C., Choy, D.W., Ikeo, K., Fischbach, K.F., Gehring, W.J., and de Couet, H.G. (2002). HOX genes in the sepiolid squid Euprymna scolopes: implications for the evolution of complex body plans. Proc. Natl. Acad. Sci. USA *99*, 2088–2093.
14. https://www.mbl.edu/cephalopod-mariculture/.

Molecular Motors: Kif14's Disordered Dongle

Yean Ming Chew and Robert A. Cross*
Centre for Mechanochemical Cell Biology, Warwick Medical School, Coventry CV4 7LA, UK
*Correspondence: r.a.cross@warwick.ac.uk
https://doi.org/10.1016/j.cub.2020.06.095

Modern life is replete with function-expanding dongles, and life at the molecular scale is, it turns out, no exception. Hanging out of the back of the Kif14 molecular motor is an intrinsically disordered domain that gives it superpowers.

A typical eukaryotic cell, if there is such a thing, might contain anywhere between 20 and 70 different types of kinesin molecular motors, whose biological functions are encoded in their primary structure in non-obvious ways. One way in which nature encodes function is to place sequence inserts within the kinesin tails that enable them to recognise cargo adaptors, or indeed bind directly to particular cargoes [1]. Another way is to place inserts within the motor heads, or at their margins [2]. Kinesin head domains bind and hydrolyse ATP and, as they

do so, they interact cyclically with microtubules to generate impulses of force and motion. Precisely how each of these inserts affects the mechanisms of each kinesin motor is, by and large, not yet clear. New work from Zhernov and colleagues [3], published in this issue of *Current Biology*, now poses this question for Kif14, a kinesin-3 motor required for building and stabilising the central spindle [4] and primary cilium [5]: via *in vitro* reconstitution analyses, these authors demonstrate how the amino terminus of this motor provides functional versatility.

Kif14 has a substantial amino-terminal subdomain of 350 residues — about the same size as the entire kinesin head. Remarkably, this subdomain is predicted to be partially or entirely unstructured. Intrinsic disorder appears to be widespread in the kinesin family [6]. Natively unstructured sequences can of course transiently gain some structure, often by interacting with binding partners [7], but, even with this in mind, exactly how Kif14's disordered amino-terminal extension might contribute to its function is difficult to imagine. Pursuing this question, Zhernov *et al.* [3] have dug deep into their repertoire of *in vitro* reconstitution techniques and been rewarded with a full basket of surprising insights.

The new work uses a range of truncated constructs up to and including the full-length Kif14 protein, carboxy-terminally tagged with GFP and expressed in insect cells. Constructs that include a section of the Kif14 carboxy-terminal tail equilibrate between monomer and dimer states, as visualised by monitoring the fluorescence intensity of individual GFP-tagged molecules attached to microtubules. Constructs that contain a larger portion of the carboxy-terminal tail have a greater tendency to dimerise (Figure 1).

Earlier work on Kif14 determined a high-resolution X-ray structure of the motor head in its nucleotide-free (apo) state in the absence of microtubules [8], using monomeric fusion constructs that lacked the amino-terminal extension. Binding of these constructs to microtubules stabilised them against cold-depolymerisation [8], and strongly activated their ATPase activity. But when coated on to a surface, these

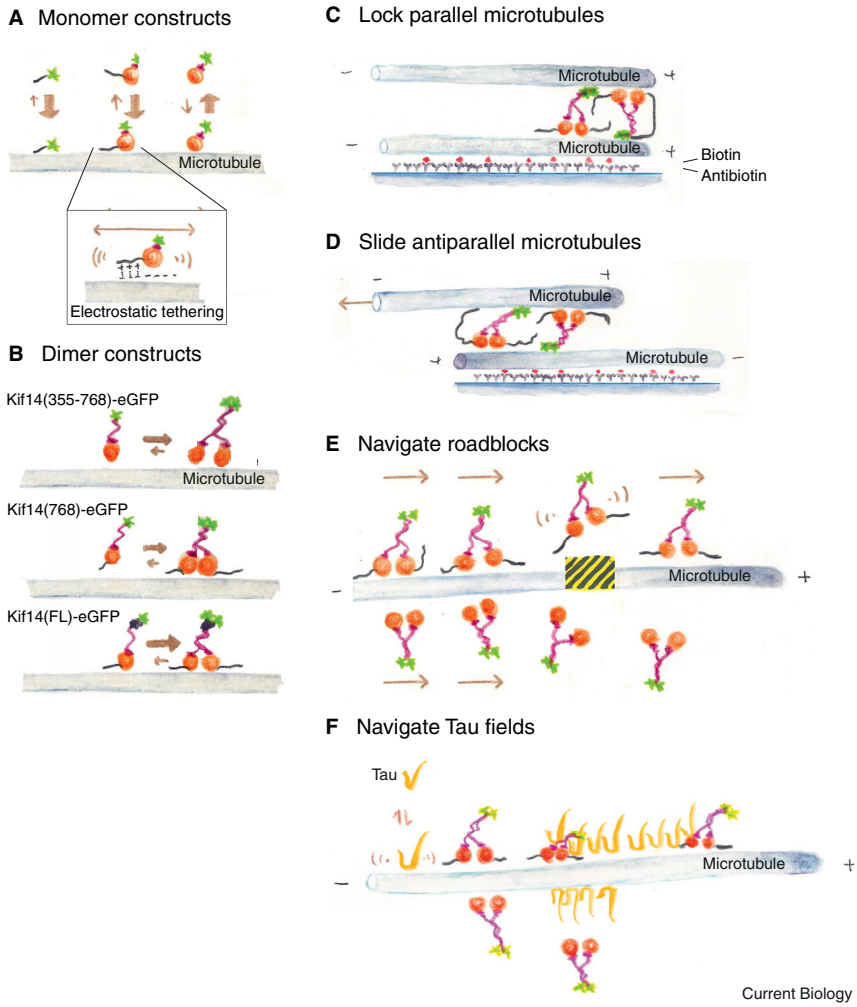

Figure 1. Actions of Kif14's intrinsically disordered amino-terminal subdomain.
(A) Monomer constructs that contain the intrinsically disordered amino-terminal subdomain are electrostatically tethered to the microtubule. The amino-terminal subdomain is depicted as a grey wavy line, the motor head group as orange circles, the carboxyl termini in pink, and the eGFP tag as a green star. (B) Monomers and dimers are in equilibrium on the microtubule surface, with full-length (FL) constructs dimerising most efficiently. (C) Full-length Kif14 crosslinks and locks parallel microtubules. The amino terminus is long enough to bridge the two microtubules and directly crosslink them, whilst also promoting head-mediated crosslinking. (D) Full-length Kif14 slides antiparallel microtubules, with the amino-terminal subdomain enhancing processivity. (E) The amino terminus helps Kif14 navigate roadblocks. (F) The amino terminus allows Kif14 to traverse tau fields.

constructs slid microtubules at only a few nanometres per second, consistent with full-length Kif14 motors acting as near-static clamps. The new work by Zhernov *et al.* [3] shows in apparent contrast that individual Kif14 molecules can actually move quickly as soloists — dimers can move processively for up to several microns at 200 nanometres per second. Deletion of the amino-terminal subdomain reduces this run length and accelerates progress by ~20%, establishing that the binding of the amino-terminal subdomain to the microtubule improves single-molecule processivity. The processivity of dimers with intact amino termini allows them to slow down and pile up at the plus ends of GMPCPP (i.e. stabilised) microtubules, whilst dimers lacking the amino terminus do not.

The amino terminus has a net positive charge and the interactions that bind it to the negatively charged microtubule appear to be largely electrostatic. These electrostatic interactions are clearly in rapid exchange, because GFP-tagged single-head Kif14 heads (and

GFP-tagged single amino termini) diffuse backwards and forwards along the lattice (Figure 1). Full-length dimers were seen on rare occasions to switch between diffusive and unidirectional motion. It seems possible that the long processive runs typical of full-length Kif14 dimers consist of a series of shorter runs, linked by imperceptibly brief bursts of diffusion mediated by the amino termini. If so, a team of Kif14s will be required to do sustained work within the jostling crowd of molecular players that assembles and stabilises the central spindle [4].

The need to make directional progress through crowds of microtubule binders poses any microtubule motor with the acute problem of how to deal with roadblocks. Kinesin-1, the prototypical long distance walker, is bad at this — it typically waits for a few hundred milliseconds for the problem to solve itself, and then gives up and lets go of the microtubule [9], although it can sometimes find its way past. Other kinesins are better at side-stepping obstacles [10]. Having a lengthy neck linker helps [11], as does connecting multiple motors to a single cargo, so that the cargo can roll around the obstacle [12].

But in the case of a microtubule region enriched in the microtubule-associated protein tau, none of these options will help. The Braun-Lansky lab have previously shown that tau molecules, which are themselves substantially unstructured, accrete by diffusing on the microtubule surface and can interact with one another to form a collective with some of the properties of a liquid drop [13]. Faced with a tau collective as an obstacle, Kif14 has a unique trick — it burrows (Figure 1). Zhernov *et al.* [3] find that intact Kif14 dimers, like kinesin-8 dimers [13], move through tau fields almost unhindered. Details are unclear, but it seems likely that the rapidly exchanging electrostatic contacts made by the unstructured Kif14 amino-terminal domain allow the motor to wait, tethered to the microtubule, until a gap opens in the tau field, and then occupy the gap.

What further powers does Kif14's amino terminus confer? Remarkably, a construct consisting of the amino-terminal domain alone, tagged with GFP, prefers GMPCPP (stable) microtubules to GDP (dynamic) microtubules, and binds preferentially to the plus ends of growing dynamic microtubules (Figure 1). Digging deeper, Zhernov *et al.* [3] show that, even in the absence of their amino-terminal domain, Kif14 heads prefer GMPCPP microtubules to GDP microtubules, and preferentially bind the tips of growing microtubules, without influencing their growth rate. So the amino-terminal subdomain serves to enhance a pre-existing preference in the motor core for microtubule plus ends. Interestingly, recent work from the Holzbaur lab showed that another processive kinesin-3, Kif1A, has exactly the opposite preference [14], allowing it to detach selectively at microtubule tips.

Like other crosslinking kinesins, Kif14 dimers can link and slide antiparallel-overlapped microtubules apart, whilst locking and stabilising parallel-overlapped microtubules (Figure 1). A further surprise is that just the amino-terminal domain on its own is sufficient to link microtubules (Figure 1). Here too then, the amino terminus seems to be functioning as an amplifier or enhancer of a basal property of the core motor domain. Notably, the amino terminus of Kif14 also interacts with PRC1, a microtubule crosslinker that stabilises antiparallel microtubule overlaps [15]. Although Zhernov *et al.* [3] did not in this latest work study PRC1 directly, it seems very likely that this interaction will direct Kif14 into antiparallel microtubule overlaps. Kif4, a kinesin-4 that also organises central spindles, interacts with PRC1 as well [16], as does kinesin-6, which binds via its partner CYK1 [17]. Clearly, a case could be made that it is not the motors that recruit the crosslinker, but the crosslinker that recruits the motors, one of which is Kif14. Alternatively, the zone of antiparallel microtubule overlap can be pictured as the machine that acquires both crosslinkers and motors [18]. Whichever way up you hold the map, the important point is that, in each perspective, the same, specific mechanochemical mechanisms are recruited. The power of *in vitro* reconstitution when done well is that it can dissect these mechanisms and open the way to interrogating their *in vivo* roles.

Zhernov *et al.* [3] speculate that, *in vivo* as *in vitro*, Kif14 can transition between monomeric and dimeric states, perhaps regulated by post-translational modifications that switch its processivity on and off. A further possibility, not discussed by the authors, is that the interaction with PRC1, an antiparallel dimer, might influence Kif14 dimerisation.

This new Kif14 work emphasises the great value of careful *in vitro* reconstitution and shows that Kif14's intrinsically unstructured amino-terminal dongle confers profound functional versatility. The challenge now is to understand where and how and to what extent each of the mechanisms identified plays its part in the teeming teams of molecules that shape the central spindle and the primary cilium. The suspicion must be that it is the rapid dynamics of KIf14's natively unstructured amino-terminal domain that allow it to interrupt and direct force generation by the folded motor core. Perhaps this is a more general principle [19]. Perhaps this new paper might even turn out to be the first in a new field — the unstructural biology [20] of molecular motors.

REFERENCES

1. Verhey, K.J., and Hammond, J.W. (2009). Traffic control: regulation of kinesin motors. Nat. Rev. Mol. Cell Biol. *10*, 765–777.

2. Shen, Z., Collatos, A.R., Bibeau, J.P., Furt, F., and Vidali, L. (2012). Phylogenetic analysis of the kinesin superfamily from Physcomitrella. Front. Plant Sci. *3*, 230.

3. Zhernov, I., Diez, S., Braun, M., and Lansky, Z. (2020). Intrinsically disordered domain of kinesin-3 Kif14 enables unique functional diversity. Curr. Biol. *30*, 3342–3351.

4. Lee, K.-Y., Davies, T., and Mishima, M. (2012). Cytokinesis microtubule organisers at a glance. J. Cell Sci. *125*, 3495–3500.

5. Pejskova, P., Reilly, M.L., Bino, L., Bernatik, O., Dolanska, L., Ganji, R.S., Zdrahal, Z., Benmerah, A., and Cajanek, L. (2020). KIF14 controls ciliogenesis via regulation of Aurora A and is important for Hedgehog signaling. J. Cell Biol. *219*, e201904107.

6. Seeger, M.A., Zhang, Y., and Rice, S.E. (2012). Kinesin tail domains are intrinsically disordered. Proteins *80*, 2437–2446.

7. Schneider, R., Jensen, M.R., and Blackledge, M. (2019). Experimental studies of binding of intrinsically disordered proteins to their partners. In Intrinsically Disordered Proteins, N. Salvi, ed. (Oxford: Elsevier), pp. 139–187.

8. Arora, K., Talje, L., Asenjo, A.B., Andersen, P., Atchia, K., Joshi, M., Sosa, H., Allingham, J.S., and Kwok, B.H. (2014). KIF14 binds tightly to microtubules and adopts a rigor-like conformation. J. Mol. Biol. *426*, 2997–3015.

9. Crevel, I.M.-T., Nyitrai, M., Alonso, M.C., Weiss, S., Geeves, M.A., and Cross, R.A. (2004). What kinesin does at roadblocks: the coordination mechanism for molecular walking. EMBO J. *23*, 23–32.

10. Mitra, A., Ruhnow, F., Girardo, S., and Diez, S. (2018). Directionally biased sidestepping of Kip3/kinesin-8 is regulated by ATP waiting time and motor–microtubule interaction strength. Proc. Natl. Acad. Sci. USA *115*, E7950–E7959.

11. Hoeprich, G.J., Thompson, A.R., McVicker, D.P., Hancock, W.O., and Berger, C.L. (2014). Kinesin's neck-linker determines its ability to navigate obstacles on the microtubule surface. Biophys. J. *106*, 1691–1700.

12. Ferro, L.S., Can, S., Turner, M.A., ElShenawy, M.M., and Yildiz, A. (2019). Kinesin and dynein use distinct mechanisms to bypass obstacles. eLife *8*, e48629.

13. Siahaan, V., Krattenmacher, J., Hyman, A.A., Diez, S., Hernández-Vega, A., Lansky, Z., and Braun, M. (2019). Kinetically distinct phases of tau on microtubules regulate kinesin motors and severing enzymes. Nat. Cell Biol. *21*, 1086–1092.

14. Guedes-Dias, P., Nirschl, J.J., Abreu, N., Tokito, M.K., Janke, C., Magiera, M.M., and Holzbaur, E.L.F. (2019). Kinesin-3 responds to local microtubule dynamics to target synaptic cargo delivery to the presynapse. Curr. Biol. *29*, 268–282.e8.

15. Gruneberg, U., Neef, R., Li, X., Chan, E.H.Y., Chalamalasetty, R.B., Nigg, E.A., and Barr, F.A. (2006). KIF14 and citron kinase act together to promote efficient cytokinesis. J. Cell Biol. *172*, 363–372.

16. Subramanian, R., Ti, S.-C., Tan, L., Darst, S.A., and Kapoor, T.M. (2013). Marking and measuring single microtubules by PRC1 and kinesin-4. Cell *154*, 377–390.

17. Lee, K.-Y., Esmaeili, B., Zealley, B., and Mishima, M. (2015). Direct interaction between centralspindlin and PRC1 reinforces mechanical resilience of the central spindle. Nat. Commun. *6*, 7290.

18. Wijeratne, S., and Subramanian, R. (2018). Geometry of antiparallel microtubule bundles regulates relative sliding and stalling by PRC1 and Kif4A. eLife *7*, e32595.

19. Seeger, M.A., and Rice, S.E. (2013). Intrinsic disorder in the kinesin superfamily. Biophys. Rev. *5*, 233–247.

20. Tompa, P. (2011). Unstructural biology coming of age. Curr. Opin. Struct. Biol. *21*, 419–425.

Spatial Ecology: Herbivores and Green Waves — To Surf or Hang Loose?

William F. Fagan* **and Eliezer Gurarie**
Department of Biology, University of Maryland, College Park, MD 20742, USA
*Correspondence: bfagan@umd.edu
https://doi.org/10.1016/j.cub.2020.06.088

A classic distinction in spatial ecology is between range-residency and migration. A new study — on dozens of ungulate populations worldwide — demonstrates how the dynamics of food resources help determine which movement pattern animals will exhibit.

Across the globe, many species of ungulate (hoofed animals, including members of the deer, horse, and cow families) migrate seasonally as they seek out food resources, shelter from predation, or suitable areas for reproduction. Some of the most recognizable terrestrial migrations occur in remote environments — think Arctic caribou or the great Serengeti wildebeest migrations — but ungulate migrations are also surprisingly common in temperate zones of North America and even densely populated western Europe. In fact, major new migratory routes are still being discovered [1]. Unlike caribou or wildebeest, where essentially all individuals in a particular herd migrate, temperate ungulates exhibit a wide variety of strategies: some populations within a generally migratory species may be range-resident (living year-round in a home range), some individuals within an otherwise cohesive population may not migrate, and individual animals may even choose to migrate or not in a given year [2]. Collectively, these phenomena are known as 'partial migration'. Understanding the costs, benefits, timing and mechanisms of terrestrial migration is a long-standing ecological challenge; understanding whether and why certain individuals within a partially migratory population migrate adds an additional layer of mystery.

A recent conceptual contribution to the study of migration is the 'green wave surfing' hypothesis [3,4]. This hypothesis posits that migrating ungulates follow a progressive spatial pulse of the earliest, most digestible and most nutritious plant growth in spring as that pulse moves across the landscape in a wave-like fashion — either poleward across latitudes or upward in elevation. Where present, these green waves follow a clearly discernible pattern that can be observed using time series of satellite imagery of NDVI (normalized difference vegetation index), a useful measure of vegetative greenness and productivity on large scales [3,5]. But spring green-up progresses across landscapes in very different ways in different places. In some landscapes, spring arrives in a 'surfable', wave-like fashion. In others, spring might creep up slowly, suddenly appearing over a large area. Or spring may arrive unpredictably and patchily, depending on highly localized characteristics of the dominant vegetation. A study in this issue of *Current Biology* by Ellen Aikens,