Motor Proteins

Mitra, Aniruddha; Peterman, Erwin J.G.

published in
Current Biology
2020

DOI (link to publisher)
10.1016/j.cub.2020.02.005

document version
Publisher's PDF, also known as Version of record

hydrocarbon perception with a wealth of respective olfactory receptors [19,20]. If, however, a queen naturally expresses other chemicals more prominently than cuticular hydrocarbons, they might be more likely to become a queen pheromone. In the case of the study of Steitz and Ayasse [2], the bee queen needs the lactones for the preparation of the underground brood cells and thus already produces these compounds in high amounts, which made them a likely candidate for a queen pheromone.

The study of Steitz and Ayasse fills an important gap in our knowledge about the evolution of queen pheromones, and additional studies on such small societies are needed for a more comprehensive understanding of the origin of royal scents in the eusocial insects.

REFERENCES

1. Hölldobler, B., and Wilson, E.O. (1990). The Ants (Cambridge, Massachusetts: Belknap Press).
2. Steitz, I., and Ayasse, M. (2020). Macrocyclic lactones act as a queen pheromone in a primitively eusocial sweat bee. Curr. Biol. *30*, 1136–1141.
3. Seeley, T.D. (1985). Honeybee Ecology: A Study of Adaptation in Social Life (Princeton, New Jersey: Princeton University Press).
4. Keller, L., and Nonacs, P. (1993). The role of queen pheromones in social insects: queen control or queen signal? Anim. Behav. *45*, 787–794.
5. Smith, A.A., and Liebig, J. (2017). The evolution of cuticular fertility signals in eusocial insects. Curr. Opin. Insect Sci. *22*, 79–84.
6. Ayasse, M., Engels, W., Lubke, G., Taghizadeh, T., and Francke, W. (1999). Mating expenditures reduced via female sex pheromone modulation in the primitively eusocial halictine bee, *Lasioglossum (Evylaeus) malachurum* (Hymenoptera : Halictidae). Behav. Ecol. Sociobiol. *45*, 95–106.
7. Endler, A., Liebig, J., Schmitt, T., Parker, J.E., Jones, G.R., Schreier, P., and Hölldobler, B. (2004). Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. P. Natl. Acad. Sci. USA *101*, 2945–2950.
8. Smith, A., Hölldobler, B., and Liebig, J. (2009). Cuticular hydrocarbons reliably identify cheaters and allow enforcement of altruism in a social insect. Curr. Biol. *19*, 78–81.
9. Holman, L., Jorgensen, C.G., Nielsen, J., and d'Ettorre, P. (2010). Identification of an ant queen pheromone regulating worker sterility. Proc. Biol. Sci. *277*, 3793–3800.
10. Van Oystaeyen, A., Oliveira, R.C., Holman, L., van Zweden, J.S., Romero, C., Oi, C.A., d'Ettorre, P., Khalesi, M., Billen, J., Wackers, F., *et al*. (2014). Conserved class of queen pheromones stops social insect workers from reproducing. Science *343*, 287–290.
11. Smith, A.A., Millar, J.G., and Suarez, A.V. (2015). A social insect fertility signal is dependent on chemical context. Biol. Lett. *11*, 20140947.
12. Hoover, S.E.R., Keeling, C.I., Winston, M.L., and Slessor, K.N. (2003). The effect of queen pheromones on worker honey bee ovary development. Naturwissenschaften *90*, 477–480.
13. Butler, C.G. (1957). The control of ovary development in worker honeybees (*Apis mellifera*). Experientia *13*, 256–257.
14. Vargo, E.L. (1997). Poison gland of queen fire ants (*Solenopsis invicta*) is the source of a primer pheromon. Naturwissenschaften *84*, 507–510.
15. Smith, A.A., Millar, J.G., and Suarez, A.V. (2016). Comparative analysis of fertility signals and sex-specific cuticular chemical profiles of *Odontomachus* trap-jaw ants. J. Exp. Biol. *219*, 419–430.
16. Matsuura, K., Himuro, C., Yokoi, T., Yamamoto, Y., Vargo, E.L., and Keller, L. (2010). Identification of a pheromone regulating caste differentiation in termites. Proc. Natl. Acad. Sci. USA *107*, 12963–12968.
17. Bradbury, J.W., and Vehrencamp, S.L. (2011). Principles of Animal Communication, 2 Edition (Sunderland, MA: Sinauer Associates Inc.).
18. Leonhardt, S.D., Menzel, F., Nehring, V., and Schmitt, T. (2016). Ecology and evolution of communication in social insects. Cell *164*, 1277–1287.
19. Zhou, X., Rokas, A., Berger, S.L., Liebig, J., Ray, A., and Zwiebel, L.J. (2015). Chemoreceptor evolution in Hymenoptera and its implications for the evolution of eusociality. Genome Biol. Evol. *7*, 2407–2416.
20. Pask, G.M., Slone, J.D., Millar, J.G., Das, P., Moreira, J.A., Zhou, X.F., Bello, J., Berger, S.L., Bonasio, R., Desplan, C., *et al*. (2017). Specialized odorant receptors in social insects that detect cuticular hydrocarbon cues and candidate pheromones. Nat. Comm. *8*, 297.

Motor Proteins: It Runs in the Family, but at Different Speeds

Aniruddha Mitra and Erwin J.G. Peterman*
Department of Physics and Astronomy, and LaserLaB Amsterdam, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
*Correspondence: e.j.g.peterman@vu.nl
https://doi.org/10.1016/j.cub.2020.02.005

The velocity of intraflagellar transport among evolutionarily distant organisms differs substantially, while the transport machinery is well conserved. A new *in vitro* study finds that the velocity difference is encoded in the motor proteins driving transport.

Although eukaryotic organisms vary widely in size and shape, they share many fundamental biological mechanisms and functions. An example is active, intracellular transport driven by motor proteins that walk along the cytoskeleton to distribute cellular components around the cell. Although the machineries of intracellular transport in distinct eukaryotes are largely conserved and evolutionarily related, substantial differences occur in transport parameters and mechanisms. In a new study in this issue of *Current Biology* [1], Sonar and coauthors zoom in on the distinct properties of a heterotrimeric kinesin-2 motor protein driving intraflagellar transport (IFT) in *Chlamydomonas reinhardtii*, in which the transport is substantially faster than in other organisms.

Eukaryotic cells critically depend on active, ATP-driven transport of cargoes such as vesicles, organelles, RNA and protein complexes along the cytoskeleton. Three distinct superfamilies

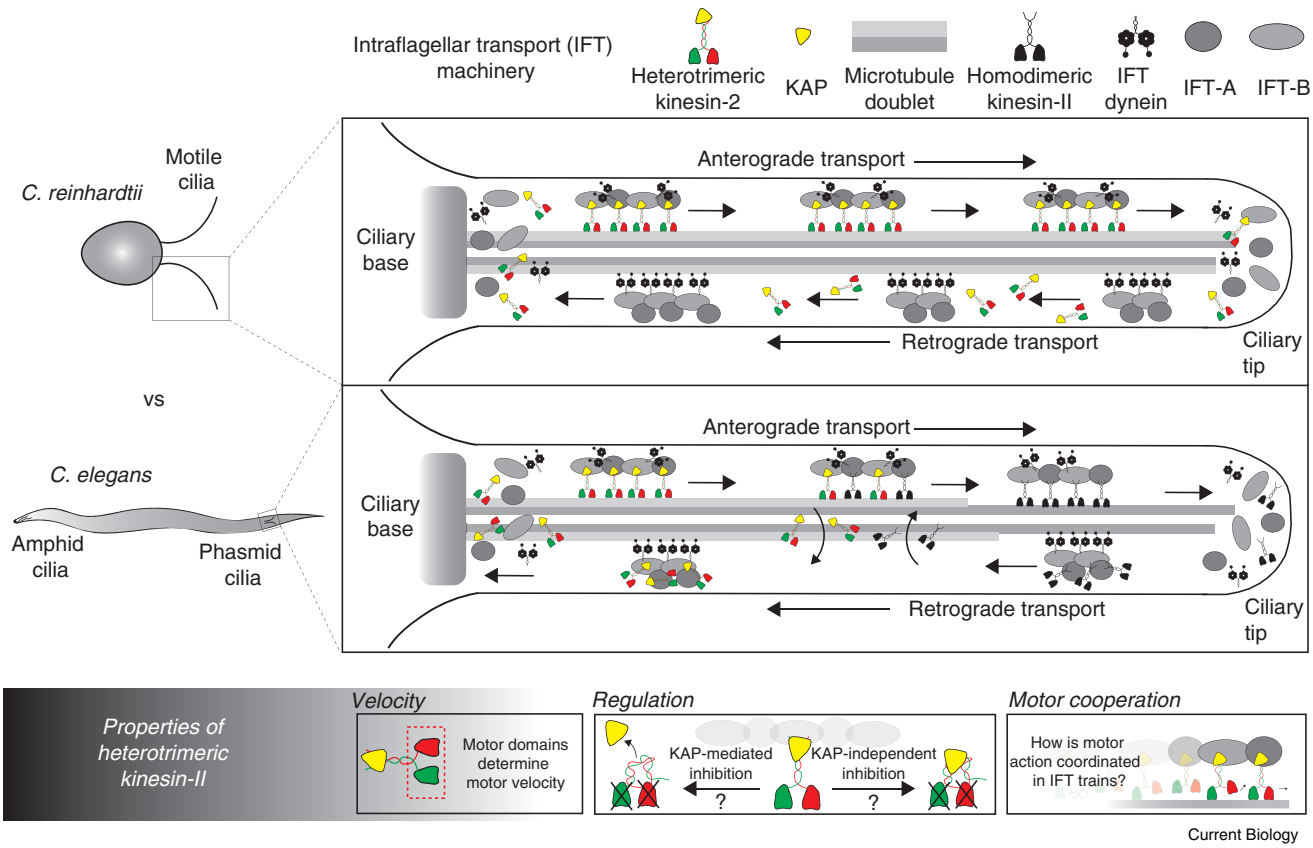

Figure 1. IFT and role of heterotrimeric kinesin-2 in *C. reinhardtii* and *C. elegans*.
Schematic comparison of IFT dynamics in *C. elegans* chemosensory cilia and *C. reinhardtii* motile cilia, with focus on heterotrimeric kinesin-2 function, motor velocity, regulation and collective action.

of motor proteins driving this transport have been identified: myosins (which use actin as track), kinesins and dyneins (which both use microtubules as tracks). Organisms contain many different varieties of motor proteins, with specialized function, structure and localization. For example, mammalian genomes encode 45 kinesin superfamily members, which can be classified into 15 families [2].

Intraflagellar transport (IFT) is a specialized transport mechanism that takes place in the cilia of many eukaryotic cells [3]. Cilia are microtubule-based, membrane-enveloped organelles that protrude from the cell surface. Cilia can be classified into motile cilia (also called flagella), which drive cell propulsion or fluid flow, and non-motile cilia (also called primary or sensory cilia), which are important sensory hubs, receiving and transmitting extracellular signals. For assembly and maintenance, cilia critically depend upon IFT along the axoneme (a bundle of microtubules). Anterograde IFT (from ciliary base towards ciliary tip) is driven by kinesin-2 family motors, while transport in the opposite, retrograde direction relies on IFT dynein. IFT is organized in trains, coupled protein complexes, called IFT particles, to which multiple motors and cargo dock. Cargo includes ciliary building blocks like axonemal components and proteins of the signal-transduction machinery.

Although the IFT machinery is well conserved and individual proteins share substantial homology, important differences in IFT properties and mechanism exist between cilia from different organisms. The well-studied motile cilia of *C. reinhardtii* and chemosensory cilia of *Caenorhabditis elegans* are striking in this respect (Figure 1). In *C. reinhardtii*, anterograde transport is driven by a heterotrimeric kinesin-2, the FLA8–10–KAP complex, resulting in a velocity of over 2 μm/s [4]. In *C. elegans*, on the other hand, anterograde transport is driven by an intricate interplay between a heterotrimeric kinesin-2, KLP11–20–KAP1, also called kinesin-II, and a homodimeric kinesin-2, OSM-3. KLP11–20–KAP1 is responsible for the assembly and import of IFT trains into the cilium with a velocity of ~0.5 μm/s. A few μm into the cilium, the faster motor OSM-3 gradually takes over, resulting in an acceleration to ~1.5 μm/s [5]. The velocities measured *in vivo* correspond to those of purified individual motors *in vitro* [6].

In their new study, Sonar *et al.* [1] use an *in vitro* approach to study the properties and mechanism of FLA8–10–KAP, the heterotrimeric kinesin-2 driving IFT in *C. reinhardtii*. They recombinantly express and purify the protein, and probe it using single-molecule fluorescence motility assays. They show that FLA8–10–KAP velocity *in vitro* is ~2 μm/s, consistent with the velocity of IFT observed in *C. reinhardtii*. Chimeric motor

constructs with *C. reinhardtii* and *C. elegans* domains swapped show that the catalytic motor domains and not the coiled-coil tails are the determinants of motor velocity. Further studies involving chimeric motor constructs with short sequence swaps, bioinformatics or structure determination will still be needed to understand what amino acids or structural elements in the conserved motor domains determine the velocity of these kinesins. A more fundamental question is why different species have evolved IFT with different velocities. In this respect, it is remarkable that also dynein-driven, retrograde IFT in *C. reinhardtii* is faster than in *C. elegans* (~3 μm/s versus ~1.7 μm/s [3]). Could it be that overall velocity has some relationship to ciliary length or plays a part in diversification of ciliary structure and complexity [7]? Velocity is, however, not the only relevant parameter, given the substantially different anterograde IFT mechanisms in *C. reinhardtii*, where only one kinesin-2 is deployed, and in *C. elegans*, where two distinct kinesin-2 types cooperate [8].

Tight regulation of motor activity is crucial for efficient IFT, where trains cycle back and forth from base to tip. Anterograde trains, driven by kinesin-2, also transport IFT dynein as inactive cargo [9]. In *C. elegans*, retrograde, IFT-dynein-driven trains have inactive kinesin-2 as cargo, while in *C. reinhardtii* inactive heterotrimeric kinesin-2 diffuses back to the base [10]. Sonar *et al.* [1] show that heterodimeric FLA8–10 constructs move at two distinct velocities, ~2 and ~1 μm/s, which the authors interpret to represent active and inhibited motor conformations, respectively. The inhibited conformation is absent in heterotrimeric FLA8–10–KAP constructs. Distinct conformations with different activity have been identified before in homodimeric kinesin-1 and kinesin-2 motor constructs, where cargo binding has been inferred to relieve motor auto-inhibition [11,12]. Mutation of a single amino acid has been shown to suppress the inhibited conformation by preventing the folding of the tail domains on the motor domains [11]. Sonar *et al.* [1] show that an equivalent mutation has the same effect in FLA8–10. Although the authors provide strong evidence for active and inactive states of *C. reinhardtii* kinesin-2, it remains unclear what regulates or drives transitions between the states *in vivo*. It is generally assumed that the motors form stable heterotrimers *in vivo*. As a consequence, binding and unbinding of KAP most likely is not the trigger, and a more likely trigger could be the binding and unbinding of IFT particles to the heterotrimer. In addition, phosphorylation has been shown to regulate heterotrimeric kinesin-2 activity [13], but it is unclear whether or how this connects to the different conformations observed in the current study.

In anterograde IFT, tens of kinesin-2 motors are mechanically coupled in compact IFT trains. How do these motors cooperate efficiently? Sonar *et al.* [1] addressed this question by studying the *in vitro* motility parameters of pairs of kinesin-2 motors coupled via a short DNA strand. They observed that the motor pairs move with velocity and run length very similar to those of single motors, which they attribute to only one of the two motors associating with the microtubule most of the time. *C. reinhardtii* and *C. elegans* motor constructs behaved very similarly to each other, suggesting that motor cooperativity is a conserved property. These observations for kinesin-2 are surprisingly different from previous *in vitro* multi-motor assays (on kinesin-1, kinesin-14 and dynein) that showed that run length increases substantially with motor number, while velocity is largely unaffected [14–16]. Those previous studies appear more in line with the *in vivo* situation, where the velocity of IFT trains, containing tens of motors, is similar to *in vitro* single-motor velocities (as demonstrated for *C. reinhardtii* in the current study), while the effective run length of trains (moving in one go from ciliary base to tip) appears to be substantially larger than that of single motors *in vitro*. What could be the cause of this apparent discrepancy between run of the tens of motors coupled *in vivo* compared with the pair of motors coupled *in vitro*? First of all, it could be that two coupled motors are too few to extrapolate to the *in vivo* situation. Furthermore, the densely packed ciliary environment and the attachment of trains to the ciliary membrane could enhance the rebinding efficiency of unbound motors. Finally, there might be other factors in the *in vitro* assays that fail to mimic the *in vivo* context — for example, ionic strength, the mechanical properties of the connection between the motors and the spacing between the motors.

In summary, the new results demonstrate that the high anterograde IFT velocity in *C. reinhardtii* is caused by the intrinsically higher velocity of heterotrimeric kinesin-2. Other motility parameters, including cooperativity and inactivation, are conserved with the homologous motor from *C. elegans.* Future studies by IFT and kinesin researchers will be needed to reveal the exact molecular basis for the difference in speed of related motors, the regulatory mechanism employed by IFT motors, and the reason for IFT *in vivo* making use of tens of mechanically coupled motors.

REFERENCES

1. Sonar, P., Youyen, W., Cleetus, A., Wisanpitayakorn, P., Mousavi, I.S., Stepp, W.L., Hancock, W.O., Tüzel, E., and Ökten, Z. (2020). Kinesin-2 from C. reinhardtii is an atypically fast and auto-inhibited motor that is activated by heterotrimerization for intraflagellar transport. Curr. Biol. *30*, 1160–1166.

2. Hirokawa, N., Noda, Y., Tanaka, Y., and Niwa, S. (2009). Kinesin superfamily motor proteins and intracellular transport. Nat. Rev. Mol. Cell Biol. *10*, 682–696.

3. Prevo, B., Scholey, J.M., and Peterman, E.J.G. (2017). Intraflagellar transport: mechanisms of motor action, cooperation, and cargo delivery. FEBS J. *284*, 2905–2934.

4. Cole, D.G., Diener, D.R., Himelblau, A.L., Beech, P.L., Fuster, J.C., and Rosenbaum, J.L. (1998). Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in Caenorhabditis elegans sensory neurons. J. Cell Biol. *18*, 993–1008.

5. Prevo, B., Mangeol, P., Oswald, F., Scholey, J.M., and Peterman, E.J.G. (2015). Functional differentiation of cooperating kinesin-2 motors orchestrates cargo import and transport in C. elegans cilia. Nat. Cell Biol. *17*, 1536–1545.

6. Mohamed, M.A.A., Stepp, W.L., and Ökten, Z. (2018). Reconstitution reveals motor activation for intraflagellar transport. Nature *557*, 387–391.

7. Soares, H., Carmona, B., Nolasco, S., Viseu Melo, L., and Gonçalves, J. (2019). Cilia distal domain: Diversity in evolutionarily conserved structures. Cells *14*, 8.

8. Scholey, J.M. (2013). Kinesin-2: a family of heterotrimeric and homodimeric motors with diverse intracellular transport functions. Annu. Rev. Cell Dev. Biol. *29*, 443–469.

9. Jordan, M.A., Diener, D.R., Stepanek, L., and Pigino, G. (2018). The cryo-EM structure of intraflagellar transport trains reveals how dynein is inactivated to ensure unidirectional anterograde movement in cilia. Nat. Cell Biol. *20*, 1250–1255.

10. Chien, A., Shih, S.M., Bower, R., Tritschler, D., Porter, M.E., and Yildiz, A. (2017). Dynamics of the IFT machinery at the ciliary tip. Elife, e28606.

11. Imanishi, M., Endres, N.F., Gennerich, A., and Vale, R.D. (2006). Autoinhibition regulates the motility of the C. elegans intraflagellar transport motor OSM-3. J. Cell Biol. *174*, 931–937.

12. Wedaman, K.P., Meyer, D.W., Rashid, D.J., Cole, D.G., and Scholey, J.M. (1996). Sequence and submolecular localization of the 115-kD accessory subunit of the heterotrimeric kinesin-II (KRP85/95) complex. J. Cell Biol. *132*, 371–380.

13. Liang, Y., Pang, Y., Wu, Q., Hu, Z., Han, X., Xu, Y., Deng, H., and Pan, J. (2014). FLA8/KIF3B phosphorylation regulates kinesin-II interaction with IFT-B to control IFT entry and turnaround. Dev. Cell. *30*, 585–597.

14. Furuta, K., Furuta, A., Toyoshima, Y.Y., Amino, M., Oiwa, K., and Kojima, H. (2013). Measuring collective transport by defined numbers of processive and nonprocessive kinesin motors. Proc. Natl. Acad. Sci. USA *110*, 501–506.

15. Derr, N.D., Goodman, B.S., Jungmann, R., Leschziner, A.E., Shih, W.M., and Reck-Peterson, S.L. (2012). Tug-of-war in motor protein ensembles revealed with a programmable DNA origami scaffold. Science *338*, 662–665.

16. Driver, J.W., Jamison, D.K., Uppulury, K., Rogers, A.R., Kolomeisky, A.B., and Diehl, M.R. (2011). Productive cooperation among processive motors depends inversely on their mechanochemical efficiency. Biophys. J. *101*, 386–395.