widespread downstream axonal projections from TRAPed LH neurons to other neuronal populations throughout the brain, many of which showed an increase in activity (as measured using the TRAP technique) during nest building. Future studies could test the necessity and sufficiency of these circuits to identify their specific roles in nest building and sleep regulation.

A final broad, speculative question is whether the role of the LH in regulating pre-sleep behaviors is generalizable not only across different strains of mice, but even across different species. The LH is conserved across many vertebrate organisms, with homologous populations of neurons (such as Hcrt and MCH neurons) identified in primates, rodents, birds, and fish[18,19]. It would be fascinating if the LH regulates pre-sleep behaviors in a variety of organisms that each exhibit different pre-sleep rituals. Do we have homologous LH neurons that are active as we switch off the lights and curl up under the covers? The formative findings of Sotelo *et al.*[8] lay the groundwork for comparative ethological studies of pre-sleep behaviors in other model organisms and provide behavioral scientists with many interesting future directions to sleep on.

DECLARATION OF INTERESTS

The author declares no competing interests.

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Tissue architecture: Two kinesins collaborate in building basement membrane

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Basement membranes are essential for tissue architecture and development. A new study reveals that two microtubule motors, kinesin-3 and kinesin-1, work collaboratively to direct basement membrane protein secretion in the *Drosophila* follicular epithelium for correct tissue movement.

The basement membrane is a self-organized extracellular sheet composed of extracellular matrix proteins that surrounds animal tissues and organs. Basement membranes mediate attachment of cells to maintain/change tissue shape, serve as barriers between tissues, and function as signaling platforms that are essential for tissue

polarization and development[1]. Despite the known biological significance of basement membranes in animal development, the regulation of the secretion and deposition of basement membrane proteins remains less well understood, due to the complexity of tissue organization and the difficulty of *in vivo* imaging. Now, a new study by Zajac and Horne-Badovinac published in this issue of *Current Biology* indicates that two microtubule motors are required for basement membrane protein transport in the *Drosophila* follicular epithelium[2].

In recent years, the fruit fly *Drosophila melanogaster* has emerged as an excellent model organism for studying basement membrane dynamics and development[3]. In each *Drosophila* egg follicle (also known as an egg chamber), a monolayer of somatic follicular epithelium composed of follicle cells encapsulates germline cells; this epithelium produces and secretes basement membrane proteins at the basal side to form a basement membrane on the outer surface of the egg chamber[4] (Figure 1A). The stereotypical geometry of the egg chamber, its accessibility for high-resolution *ex vivo* imaging, and the power of *Drosophila* genetics make it a perfect system to study a single-layer basement membrane produced by epithelial cells.

Previous work from the Horne-Badovinac group has shown that the basement membrane surrounding the follicle cell monolayer is essential for the collective follicle cell migration that applies mechanical constriction on the growing egg chamber and in turn elongates the egg along the anterior–posterior axis for its proper oval shape[5]. In their new study[2], Zajac and Horne-Badovinac use a combination of quantitative live imaging and genetic mutations to dissect the basement membrane secretion pathway of *Drosophila* follicles in detail. They demonstrated that the main basement membrane component, collagen IV, is packed and transported in Rab10-positive vesicles and secreted at the trailing edge of the basal surface of the moving follicle cells (Figure 1A,B). The authors examined the two previously described microtubule arrays in follicle cells: the vertical microtubule arrays that grow from the apical to the basal surface, as in other types of epithelial cells, and the horizontal basal microtubule arrays that grow from the leading edge to the trailing edge. They found that these two sets of microtubule arrays in fact form an interconnected network, whereby microtubules running from the apical side are bent when they reach the basal surface and then go along the basal surface toward the training edge (Figure 1B). More interestingly, the authors revealed that two plus-end-directed microtubule motors, conventional kinesin (a founding member of the kinesin-1 subfamily) and Khc-73 (a member of the kinesin-3 subfamily), are both involved in the transport of Rab10-positive secretory vesicles bearing basement membrane proteins to the basal trailing edge for proper secretion. Knockdown of both of these kinesins led to drastic mislocalization of basement membrane proteins to the apical side where they formed an aberrant basement membrane network that disrupted follicle cell movement and the structure of the cell monolayer.

Engagement of multiple motors for the transport of a single cargo is not unique to *Drosophila* follicle cells. Previously, kinesin-1 and kinesin-3 have been shown to synergize in the anterograde transport of neuropeptide[6] and TrkB receptor[7] in neurons, and in the centrifugal transport of lysosomes[8] and

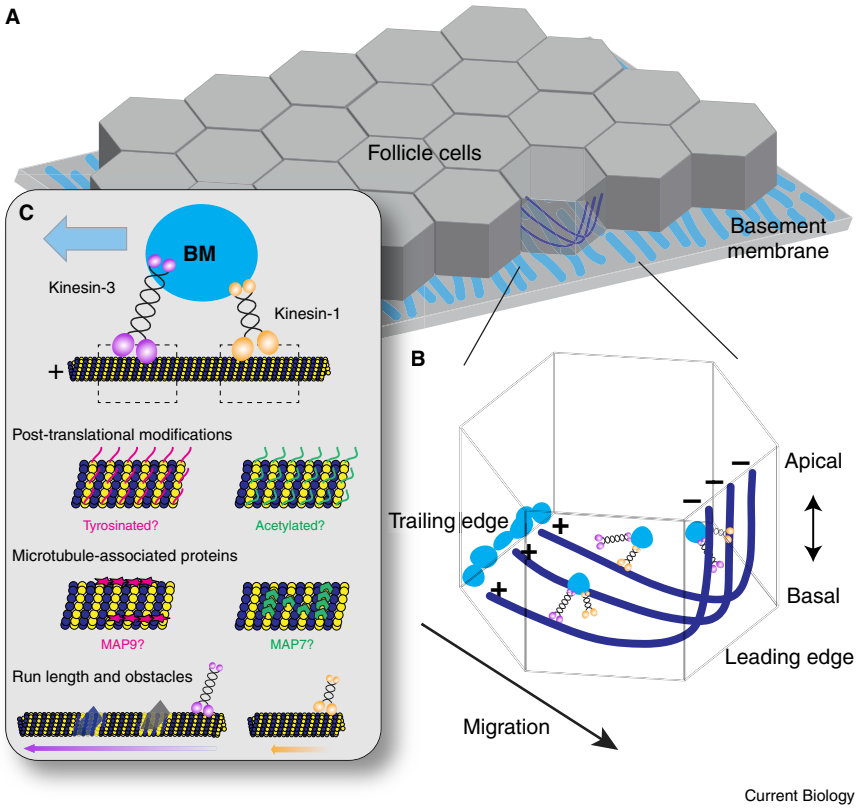

Figure 1. Kinesin-1 and kinesin-3 work together to transport basement membrane proteins in follicle cells.
(A) A monolayer of epithelial follicle cells forms a basement membrane on the outer surface of its basal side. (B) A single follicle cell with interconnected microtubules extending from the apical leading edge to the basal trailing edge. Both kinesin-1 (orange) and kinesin-3 (purple) motors are involved in basement membrane vesicle transport. (C) Different scenarios that require kinesin-1-dominant and kinesin-3-dominant transport of basement membrane vesicles: different post-translational modifications of microtubules would favor either kinesin-3-dependent or kinesin-1-dependent transport; different microtubule-associated proteins would allow the activation of either kinesin-3 or kinesin-1; different motor properties mean that kinesin-3 is a flexible motor that can overcome obstacles during long-range transport and that kinesin-1 is a stronger motor that can walk under higher load for short-distance travel.

Rab6-positive secretory vesicles[9] in HeLa cells. Teamwork between multiple motors also occurs within the same kinesin subfamily: two different kinesin-2 motors — a faster homodimeric KIF17 and a slower heterotrimeric KIF3A–KIF3B — collaborate in anterograde intraflagellar transport towards the ciliary tip[10]. Furthermore, an actin motor can collaborate with a microtubule motor to navigate microtubule tracks and the F-actin network to ensure the delivery of cargo to the correct position. For example, the actin motor myosin V teams up with either the plus-end-directed microtubule motor kinesin-1 or the minus-end-directed microtubule motor dynein for transporting the posterior polarity determinant *osk* mRNA in the *Drosophila* oocyte and the apical polarity determinant Crumbs in *Drosophila* follicle cells, respectively[11,12].

It is unclear why follicle cells employ two plus-end-directed microtubule motors, kinesin-1 and kinesin-3 (Khc-73), to transport basement membrane proteins along a continuous microtubule network. The authors report that knockdown of kinesin-1 alone does not phenocopy the Khc-73 loss-of-function mutations, implying that these two motors are not simply redundant but have distinct molecular functions. So why do two motors work better than one?

One plausible explanation is that the microtubule network in the follicular epithelium is not uniform, analogous to a situation where you need two different vehicles to drive on different roads. For example, kinesin-1 and Khc-73 may prefer different post-translational modifications on the tubulin that forms the follicular microtubule system (Figure 1C). In particular, it is known that kinesin-1 prefers to interact with acetylated microtubules, whereas kinesin-3 mainly 'walks' on tyrosinated microtubules in neurons and in HeLa cells[8,13]. In most interphase animal cells, microtubule-organizing centers (centrosomes and/or Golgi apparatus) are located at the center of the cell near the nucleus, with microtubule plus ends polymerizing outwards to the cell periphery[14]. As microtubule acetylation levels usually correlate with microtubule age, this microtubule organization creates a pattern of more acetylated microtubules in the perinuclear region and more tyrosinated microtubules at the periphery, allowing for more perinuclear transport by kinesin-1 and more peripheral transport by kinesin-3[8]. Considering the microtubule organization in follicle cells, the 'older' vertical apical–basal microtubules could have a higher acetylation level than the 'younger' horizontal microtubules running from the leading edge to the trailing edge. This difference in microtubule post-translational modifications along this microtubule network would require both kinesin-1 and kinesin-3 for the efficient transport of the basement membrane vesicles to their final destination (Figure 1C).

Alternatively, different sets of microtubule-associated proteins (MAPs) could be bound to the surface of the microtubules in this network. For instance, MAP7/ensconsin recruits kinesin-1 onto microtubules and activates kinesin-1-dependent transport[15,16], whereas it inhibits kinesin-3 activity[16]. In contrast, several other MAPs, including Tau, MAP2, doublecortin (DCX)/doublecortin-like kinase-1 (DCLK1), and MAP9, inhibit kinesin-1 activity; among these proteins, MAP9 specifically promotes kinesin-3 activity[17]. Furthermore, microtubules bend sharply at the basal leading edge to connect the apical–basal microtubules with the microtubules that run from the leading edge to the trailing edge (Figure 1B). Microtubules are rigid polymers and, to make such a sharp turn, certain MAPs, such as MAP65/PRC1/Feo, may be required to increase the flexibility of microtubules[18]. Altogether, it is very tempting to propose that kinesin-1 and kinesin-3 work together to travel through patches of different MAPs decorating microtubules from the apical leading edge to the basal trailing edge (Figure 1C).

Lastly, in addition to the differences underlying microtubule modifications and MAPs, kinesin-1 and kinesin-3 have quite distinct motor properties. Kinesin-3 is highly processive with an impressive average run length of $\sim$10 μm, almost ten times the run length of kinesin-1[19]. Although kinesin-3 has a higher velocity than kinesin-1, kinesin-3 detaches from and reattaches to microtubules more readily than kinesin-1 under load[20]. Therefore, with the higher processivity and higher rates of detachment and reattachment, kinesin-3 can more easily overcome large obstacles along microtubule tracks, making it a perfect motor for long-range transport (Figure 1C). On the other hand, kinesin-1 could serve as a stronger motor (that is, it could walk under higher load) for consistent short-distance travel (Figure 1C).

The present study by Zajac and Horne-Badovinac[2] not only sheds new light on how multiple microtubule motors work as a team to build a coherent layer of basement membrane, but also raises many interesting questions that await future studies. For example, how do kinesin-1 and kinesin-3 attach to the Rab10-positive basement membrane vesicles? Given that kinesin-1 light chain knockdown (*Klc-RNAi*) has a similar effect as the kinesin-1 heavy chain knockdown (*Khc-RNAi*) on basement membrane protein secretion, it is very likely that KLC functions to link Rab10 vesicles to the kinesin-1 motor. It will also be important to study whether the interaction between human kinesin-3 and Rab10-containing vesicles is conserved in the *Drosophila* system. Furthermore, both kinesin-1 and kinesin-3 are regulated by autoinhibition; it would therefore be crucial to investigate how the activities of kinesin-1 and kinesin-3 on the same vesicle are regulated and coordinated. It would also be intriguing to examine in follicle cells whether there are indeed differences in post-translational modifications and MAP localization on the microtubule network that impact potential motor-binding and -activation preferences. Finally, is the microtubule minus-end-directed motor, cytoplasmic dynein, involved in basement membrane vesicle transport? Knockdown of both kinesin-1 and Khc-73 causes ectopic accumulation of basement membrane proteins at the apical side, where microtubule minus ends reside. Additionally, roughly a third of transport events of Rab10-positive vesicles are directed towards the minus ends at the leading edge. Both observations imply the involvement of dynein in basement membrane vesicle transport. Additional

investigations are needed to address these questions and further elucidate the molecular machinery that follicle cells use for polarized basement membrane vesicle transport.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Physiology: Neutral buoyancy by an insect

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Larval phantom midges are remarkably adept at maintaining neutral buoyancy in water. A new study reveals that they do so using a previously unknown mechanism — modifying the volumes of internal air sacs using pH-driven changes in a protein embedded in the air-sac walls.

As scuba divers know, achieving neutral buoyancy underwater is difficult. It's just not something that we terrestrial primates do easily. The key is hours (or, in some cases, an entire lifetime) of practice with gear that allows us to actively counteract buoyancy-altering shifts in depth. Midwater organisms — of which the biodiversity and total biomass on the planet are stupefyingly large — of course also face the neutral buoyancy problem, though without the gear. They don't always *have* to be neutrally buoyant, but neutrality can save energy that would otherwise be spent swimming up or down. Moreover, open-water species often move vertically to forage or migrate, meaning that the buoyancy problem is dynamic and ongoing. So, how can these organisms achieve neutral buoyancy without dive gear and a cylinder of compressed air? For such a common problem, evolution has generated a