zygotic-genome dynamics in plants, including, perhaps, some aspects of heterosis, are established almost immediately after fertilization. ■

Christopher J. Hale *is in the Department of Molecular, Cell and Developmental Biology, and* **Steven E. Jacobsen** *is at the Howard Hughes Medical Institute, University of California, Los Angeles, Los Angeles, California 90095, USA.*
e-mails: cjhale@ucla.edu; jacobsen@ucla.edu

1. Tadros, W. & Lipshitz, H. D. *Development* **136,** 3033–3042 (2009).
2. Autran, D. *et al. Cell* **145,** 707–719 (2011).
3. Nodine, M. D. & Bartel, D. P. *Nature* **482,** 94–97 (2012).
4. Cao, J. *et al. Nature Genet.* **43,** 956–963 (2011).
5. Vielle-Calzada, J.-P., Baskar, R. & Grossniklaus, U. *Nature* **404,** 91–94 (2000).
6. Grosberg, R. K. & Strathmann, R. R. *Annu. Rev. Ecol. Evol. Syst.* **38,** 621–654 (2007).
7. Sundaresan, V. & Alandete-Saez, M. *Development* **137,** 179–189 (2010).
8. Twell, D. *Sex. Plant Reprod.* **24,** 149–160 (2011).
9. Feng, S., Jacobsen, S. E. & Reik, W. *Science* **330,** 622–627 (2010).
10. Walser, C. B. & Lipshitz, H. D. *Curr. Opin. Genet. Dev.* **21,** 431–443 (2011).
11. Meyer, S., Pospisil, H. & Scholten, S. *Plant Mol. Biol.* **63,** 381–391 (2007).

MOLECULAR MOTORS

A staggering giant

The protein dynein 'walks' along filaments to transport various cargoes within the cell. Two studies reveal that, unlike other motor proteins, dynein's steps are not strictly coordinated.

WILHELM J. WALTER & STEFAN DIEZ

When you are walking down the street your feet probably take alternating steps, with one foot passing the other each time. Alternatively, you might walk without letting your feet pass one other, in a limping motion. However, regardless of the stepping pattern, your walking will be highly coordinated. At the molecular scale, the motor protein dynein also 'walks' along filaments to carry vesicles and organelles to specific locations within a cell. But is coordination between dynein's two 'feet' required for walking? Two papers — one by DeWitt *et al.*[1] in *Science* and another by Qiu *et al.*[2] in *Nature Structural & Molecular Biology* — independently address this question and come to the surprising conclusion that dynein uses both random and coordinated walking.

Cytoplasmic dynein is a giant, multi-subunit protein that uses the chemical energy stored in the molecule ATP to transport cargoes[3]. It moves along microtubules — long polymeric tubes composed of dimers of the protein tubulin, typically arranged to form 13 parallel tracks. Dynein has two 'head' domains linked by their respective 'tails', whereby the heads act as 'feet' for walking along the microtubules. Each head contains four binding pockets for ATP and a microtubule-binding site. Of the four ATP-binding pockets, one catalyses ATP hydrolysis to generate energy for walking; the other three are thought to be important for the regulation of dynein activity[4].

Previous work[5,6] using single dynein motors bound to beads revealed that during processive motility on microtubules — the process by which a motor takes multiple steps without dissociating from the microtubule — dynein moves by 8-nanometre steps. This distance corresponds to the periodicity of the dimeric tubulin subunits along the microtubule tracks. However, little is known about the coordination of the two dynein heads during motion.

To make the dynein heads visible and study the stepping mechanism during processive motion, DeWitt *et al.*[1] and Qiu *et al.*[2] attached fluorescent markers to the dynein heads and used fluorescence microscopy. Moreover, to distinguish between the movement of the two individual heads, both groups used markers that emitted fluorescence of a different colour for each head, a method previously applied[7,8] to another motor protein, myosin V.

To facilitate the two-colour labelling, both groups generated dynein molecules in which the two subunits had been artificially linked to each other. DeWitt *et al.* attached different fluorescent quantum dots (semiconductor nanocrystals) to modified dynein heads that dimerized in the presence of the small molecule rapamycin. By contrast, Qiu *et al.* attached complementary DNA strands and different fluorescent organic molecules (less stable but smaller than quantum dots) to dynein heads, so that the DNA molecules self-associated to link both heads together. To observe the stepping mechanism, both groups[1,2] slowed dynein's movement by using an ATP concentration that was much lower than that normally found in the cell's cytoplasm. From computerized image analysis, they were able to track the positions of individual dynein heads in two dimensions with an accuracy of about 3 nm.

The primary finding of both studies is that dynein has a highly variable stepping pattern (Fig. 1). For most steps, the heads moved alternately, but they passed each other only infrequently. After each step, the head-to-head distance varied widely, from less than 5 nm to 50 nm, which is indicative of uncoordinated stepping. By contrast, other motor proteins such as kinesin-1 show strictly coordinated stepping. In kinesin-1, only the lagging head can bind ATP, because of a mechanism that relies on the strain between the two heads. This mechanical coupling forces the heads to step in a strictly alternating pattern, because the hydrolysis of the ATP molecule causes the lagging head to make the next step[9].

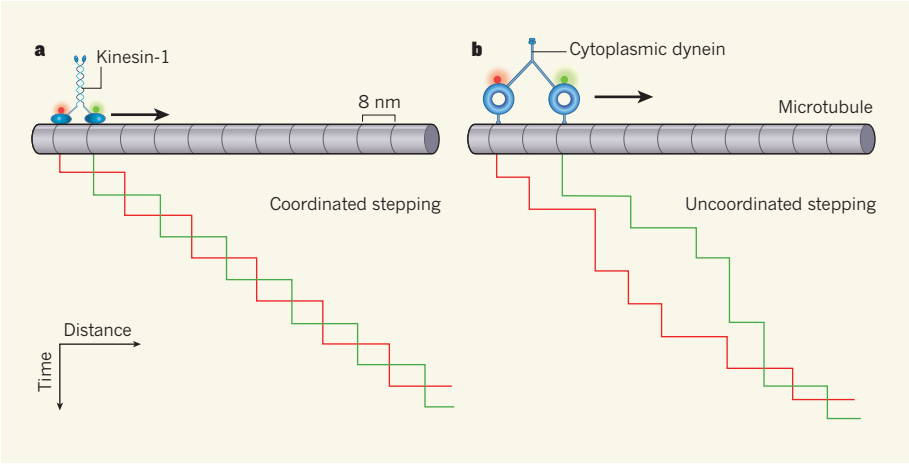

Figure 1 | Stepping patterns in motor proteins. **a**, Some motor proteins, such as kinesin-1, move in a highly coordinated way along microtubules (polymeric protein tubes; grey). The traces show the stepping of individual motor heads (each labelled with a different fluorescent marker, red or green). **b**, DeWitt *et al.*[1] and Qiu *et al.*[2] show that, by contrast, cytoplasmic dynein moves in a variable stepping pattern. Although the heads often move alternately, they only seldom pass each other, and the spacing between them varies strongly over time.

Interestingly, the current studies[1,2] show that the degree of coordination in dynein stepping depends on the head-to-head distance: the stepping pattern is random when the distance is small, but becomes coordinated when the distance is large. This implies a tension-based coordination mechanism similar to the mechanical coupling seen in kinesin-1, but less pronounced.

To further test for the necessity of coordination to dynein motility, DeWitt *et al.*[1] generated a dynein motor in which one of the two heads could not hydrolyse ATP. This head associated only weakly with the microtubule track, probably because transition to a strong binding state requires ATP hydrolysis. This motor moved reliably along microtubules, however, suggesting that coordination is not required for long-range transport.

The high accuracy with which both groups[1,2] managed to track dynein's motion on the surface of microtubules allowed them to make yet another discovery: the two heads exhibit an inherent left–right asymmetry, with the right head more likely to be in the lead. Thus, dynein can be described as staggering sideways along the microtubule.

In light of the two papers' unexpected findings, several exciting questions and ideas for follow-up experiments arise. First, the observed effect of the suggested tension-based mechanism for head coordination is rather small; however, because both groups used modified dyneins, the effect might be more pronounced in normal dynein. It is known[10] that slight modifications to the tail domains, which help to link dynein's two subunits, affect the protein's stepping pattern. Full coordination might thus rely on full-length tail domains and additional subunits, both of which are present in wild-type dynein but lacking in the artificially linked constructs analysed here.

Second, the regulatory ATP-binding sites should not be neglected. It is possible that the disordered stepping patterns observed by the authors are the result of limited ATP binding to the regulatory ATP-binding sites, because all experiments were performed at low concentrations of ATP.

And, third, the highly variable step sizes of the dynein heads — smaller than 16 nm on average — suggest that the centre of mass of the motor dimers tends to move less than 8 nm per step. But it is not clear how this can be reconciled with the aforementioned results of previous studies[5,6] of similar dynein constructs, which were found to take 8-nm steps.

Further development of optical technologies — towards even faster image-acquisition rates at tracking accuracies similar to the outstanding ones presented by DeWitt *et al.*[1] and Qiu *et al.*[2] — will, step by step, allow for fascinating insight into the functioning of dynein and other molecular motors under physiological conditions. Knowledge about the flexibility in the stepping behaviours of various motors might allow us to explain their astonishing ability to move along crowded microtubules in a dense cytoplasmic environment. ■

Wilhelm J. Walter and **Stefan Diez** *are at the B CUBE — Center for Molecular Bioengineering, Technische Universität Dresden and at the Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.*
e-mails: walter@bcube-dresden.de; diez@bcube-dresden.de

1. DeWitt, M. A., Chang, A. Y., Combs, P. A. & Yildiz, A. *Science* **335,** 221–225 (2012).
2. Qiu, W. *et al. Nature Struct. Mol. Biol.* http://dx.doi.org/10.1038/nsmb.2205 (2012).
3. Vallee, R. B., Williams, J. C., Varma, D. & Barnhart, L. E. *J. Neurobiol.* **58,** 189–200 (2004).
4. Sakakibara, H. & Oiwa, K. *FEBS J.* **278,** 2964–2979 (2011).
5. Toba, S., Watanabe T. M., Yamaguchi-Okimoto, L., Toyoshima, Y. Y. & Higuchi, H. *Proc. Natl Acad. Sci. USA* **103,** 5741–5745 (2006).
6. Gennerich, A., Carter, A. P., Reck-Peterson, S. L. & Vale, R. D. *Cell* **131,** 952–965 (2007).
7. Warshaw, D. M. *et al. Biophys. J.* **88,** L30–L32 (2005).
8. Churchman, L. S., Ökten, Z., Rock, R. S., Dawson, J. F. & Spudich, J. A. *Proc. Natl Acad. Sci. USA* **102,** 1419–1423 (2005).
9. Rosenfeld, S. S., Fordyce, P. M., Jefferson, G. M., King, P. H. & Block, S. M. *J. Biol. Chem.* **278,** 18550–18556 (2003).
10. Ori-McKenney, K. M., Xu, J., Gross, S. P. & Vallee, R. B. *Nature Cell Biol.* **12,** 1228–1234 (2010).

PRECISION MEASUREMENT

A comb in the extreme ultraviolet

A 'comb' of photons at evenly spaced frequencies in the extreme ultraviolet has been generated. It will allow a more precise search for variation in the fine-structure constant, which sets the strength of the electromagnetic force. SEE LETTER P.68

LINDA YOUNG

That improved measurement precision yields new physics is axiomatic. In the case of optical spectroscopy, the quest for ultimate precision was revolutionized around the turn of the century by the realization of the optical frequency comb[1–3]. This elegant invention, which was honoured by the 2005 Nobel Prize in Physics[4,5], provides a direct link between radio and optical frequencies, allowing one to count cycles of an electromagnetic field at near-petahertz frequencies (1 petahertz is 10^{15} Hz). On page 68 of this issue, Cingöz *et al.*[6] extend the frequency range of combs to the extreme-ultraviolet spectral regime.

An optical frequency comb is produced by a mode-locked ultra-fast laser in which a light pulse of sub-picosecond duration (1 picosecond is 10^{-12} s) circulates inside an optical cavity made up of a set of highly reflective mirrors. The laser emits an infinite train of pulses at a certain repetition rate (f_{rep}) that is determined by the time the pulses take to make a round trip of the cavity's mirrors[7,8]. This train of pulses has two distinguishing properties: the pulses are equally spaced in time, and the phase (where a wave's peaks and troughs lie) of the electromagnetic field in each pulse is precisely shifted from that in the subsequent pulse (the locked-phase relationship).The train produces a comb of sharp spectral lines uniformly spaced in frequency.

The frequencies of the comb teeth are defined by a simple formula: $f_n = nf_{rep} + f_o$, where f_{rep} and f_o are radio frequencies, f_n is optical frequency and n is an integer of the order of 10^6–10^7 (Fig. 1). With one comb tooth, f_n, referenced to an absolute standard such as the frequency of a caesium atomic clock, the absolute frequency of an unknown optical laser may be counted as a beat note relative to another comb tooth with an accuracy at the level of one part in 10^{14}. This greatly exceeds the accuracy achievable using conventional wavelength-based measurements, which are limited by wavefront distortion to an accuracy at the 10^{-10} level.

A prominent early example of the application of the frequency comb was the measurement[1] of a two-photon transition in hydrogen to an accuracy of more than 2×10^{-14}. This measurement, combined with one performed about four years later[9], placed a limit on the variability of the fine-structure constant — a fundamental constant that characterizes the strength of the electromagnetic interaction and whose variation in time informs the standard model of particle physics. Beyond precision spectroscopy, the optical frequency comb has fostered a multitude of applications, including a clock based on optical frequencies, massively parallel multi-wavelength spectroscopy of unknown chemicals, and long-distance transmission of ultra-stable clock signals, which are useful for next-generation telescope arrays or accelerator systems.