performance. An absence of entrainment in such species would raise the question of what else is required for entrainment.

One possibility is the propensity to engage in joint social action. A recent study of entrainment in human children [11] showed that young children find it difficult to entrain to a purely auditory stimulus (a disembodied metronomic beat), or to a visible drumbeating robot. They nonetheless entrain with a human adult in a socially-engaged game-playing context. Perhaps a similar propensity for social engagement underlies the apparent capacity for parrots, but not other birds, to entrain to a beat?

Parrots are long-lived, group-living birds, and their open-ended learning abilities are sometimes employed to develop vocal 'badges' of group membership [12]. Although the adaptive functions of parrot vocalizations remain poorly understood, they appear to be more group-oriented than the mostly individual territorial and courtship displays that typify songbirds, and this may be one factor explaining the complete dominance of parrot species in the YouTube sample. This hypothesis also suggests, given the capacity of dolphins to engage in imitation and joint action [13], that the potential for entrainment in this species deserves a closer experimental look.

What are the implications of these animal findings for research on human music and its evolution? The first is that we now have animal models to further explore the neural and genetic basis for entrainment. The second illustrates the fundamental point that evolutionary convergence or 'analogy' allows us to test evolutionary hypotheses, such as the vocal mimicry hypothesis [14]. Homologous traits represent a single evolutionary event, and count statistically as a single datapoint. In contrast, when different clades evolve the same trait convergently, these constitute statistically independent events [15], allowing us to test hypotheses about the evolution of human music or language that might otherwise remain 'just-so-stories'. At both mechanistic and functional levels, then, the discovery of parrot entrainment provides a rich foundation for further advances in understanding the biology and evolution of human music.

References

1. Clayton, N.S., Bussey, T.J., and Dickinson, A. (2003). Can animals recall the past and plan for the future? Nat. Rev. Neurosci. *4*, 685–691.
2. Kuhl, P.K., and Miller, J.D. (1978). Speech perception by the chinchilla: Identification functions for synthetic VOT stimuli. J. Acoustic. Soc. Am. *63*, 905–917.
3. Wallin, N.L., Merker, B., and Brown, S. (2000). The Origins of Music (Cambridge, Mass: The MIT Press).
4. Merker, B. (2000). Synchronous chorusing and human origins. In The Origins of Music, N.L. Wallin, B. Merker, and S. Brown, eds. (Cambridge, Mass.: The MIT Press), pp. 315–327.
5. Patel, A.D., Iversen, J.R., Bregman, M.R., and Schulz, I. (2009). Experimental evidence for synchronization to a musical beat in a nonhuman animal. Curr. Biol. *19*, 827–830.
6. Schachner, A., Brady, T.F., Pepperberg, I.M., and Hauser, M.D. (2009). Entrainment to music requires vocal mimicry: Evidence from non-human animals. Curr. Biol. *19*, 831–836.
7. Fitch, W.T. (2006). The biology and evolution of music: A comparative perspective. Cognition *100*, 173–215.
8. Patel, A.D. (2006). Musical rhythm, linguistic rhythm, and human evolution. Music Perception *24*, 99–104.
9. Patel, A.D. (2008). Music, Language, and the Brain (New York: Oxford University Press).
10. Janik, V.M., and Slater, P.B. (1997). Vocal learning in mammals. Adv. Stud. Behav. *26*, 59–99.
11. Kirschner, S., and Tomasello, M. (2009). Joint drumming: Social context facilitates synchronization in preschool children. J. Exp. Child Psychol. *102*, 299–314.
12. Farabaugh, S.M., Linzenbold, A., and Dooling, R.J. (1994). Vocal plasticity in budgerigars (*Melopsittacus undulatus*): evidence for social factors in the learning of contact calls. J. Comp. Psychol. *108*, 81–92.
13. Rendell, L., and Whitehead, H. (2001). Culture in whales and dolphins. Behav. Brain Sci. *24*, 309–324.
14. Fitch, W.T. (2006). On the biology and evolution of music. Music Perception *24*, 85–88.
15. Harvey, P.H., and Pagel, M.D. (1991). The Comparative Method in Evolutionary Biology (Oxford: Oxford University Press).

School of Psychology, University of St. Andrews, St. Andrews, Scotland, KY6 9JP, UK.
E-mail: wtsf@st-andrews.ac.uk

DOI: 10.1016/j.cub.2009.04.004

Chromosome Segregation: Ndc80 Can Carry the Load

Dynamic attachments between kinetochores and spindle microtubules are required for chromosome bi-orientation in mitosis. A new study provides biophysical insight into how the Ndc80 complex may contribute to the formation of these attachments.

Ajit P. Joglekar[1] and Jennifer G. DeLuca[2,*]

How cells generate the kinetochore–microtubule attachments required to drive chromosome movements in mitosis has long puzzled cell biologists. This problem is an interesting one, as attachments must be strong to generate forces for chromosome movements, yet flexible to allow for the constant gain and loss of tubulin subunits at the microtubule plus-ends. The search for molecular components that serve as load-bearing couplers between kinetochores and microtubules is active. Much effort has focused on the Ndc80 complex — composed of Ndc80 (Hec1 in humans), Nuf2, Spc24, and Spc25 — since it is essential for efficient kinetochore–microtubule attachment in cells and it can directly bind microtubules *in vitro* (reviewed in [1]). In budding yeast, the Ndc80 complex is thought to partner with the Dam1 complex to form kinetochore–microtubule attachments, perhaps through direct interaction [2]. Dam1 complexes are able to form rings and non-ring oligomers on microtubules *in vitro* [3,4], and both forms of assemblages can generate load-bearing attachments to growing and shortening microtubule plus-ends [5,6]. Although much evidence suggests that Dam1 complexes may serve as kinetochore–microtubule couplers, no convincing Dam1 homologs have surfaced in higher eukaryotes.

This raises the question: can the Ndc80 complex alone form load-bearing attachments to dynamic

microtubules? A recent report by Powers and colleagues [7] suggests that it can, and they argue it does so using a biased-diffusion mechanism. Hill originally proposed biased diffusion as a mechanism to explain chromosome movement coupled with microtubule depolymerization [8]. This mechanism relies on multiple weak interactions of binding proteins with the plus-end of a microtubule to produce depolymerization-dependent force. Hill modeled the microtubule-binding site at the kinetochore as a sleeve with regularly spaced weak interaction sites covering its inner surface based on the electron microscopy structure of the kinetochore available at the time. Thermal diffusion of this sleeve (facilitated by weak individual interactions) maintains the most favorable position of the sleeve in relation to the plus-end — one that maximizes the number of interactions between the sleeve and the microtubule lattice, minimizing the free energy of the system. Depolymerization at the microtubule plus-end biases the diffusion of the sleeve, and the sleeve then follows the receding plus-end to attain minimum free energy. This arrangement can generate a significant force (approximately 12–13 pN) in a microtubule-depolymerization-dependent fashion. Although the specific geometry of the binding site assumed by Hill may not be present in cells, biased diffusion of the kinetochore, facilitated by multiple weak interactions with the microtubule lattice, remains a compelling force generation mechanism. Experiments carried out by Powers *et al.* [7] demonstrate that the Ndc80 complex meets the requirements of the biased diffusion mechanism and suggest that it can serve as a kinetochore–microtubule coupler in cells.

Powers *et al.* [7] simulated interactions between microtubules and kinetochores by incubating polymerized microtubules with polystyrene microbeads coated with purified budding yeast or human Ndc80 complexes (Figure 1). When such beads were captured with a laser trap and held near the microtubules, they readily bound and rapidly diffused along the microtubule lattice, unbiased in either direction, at a rate of 2.3 ± 0.4 μm^2/sec. Beads bound to microtubules that underwent

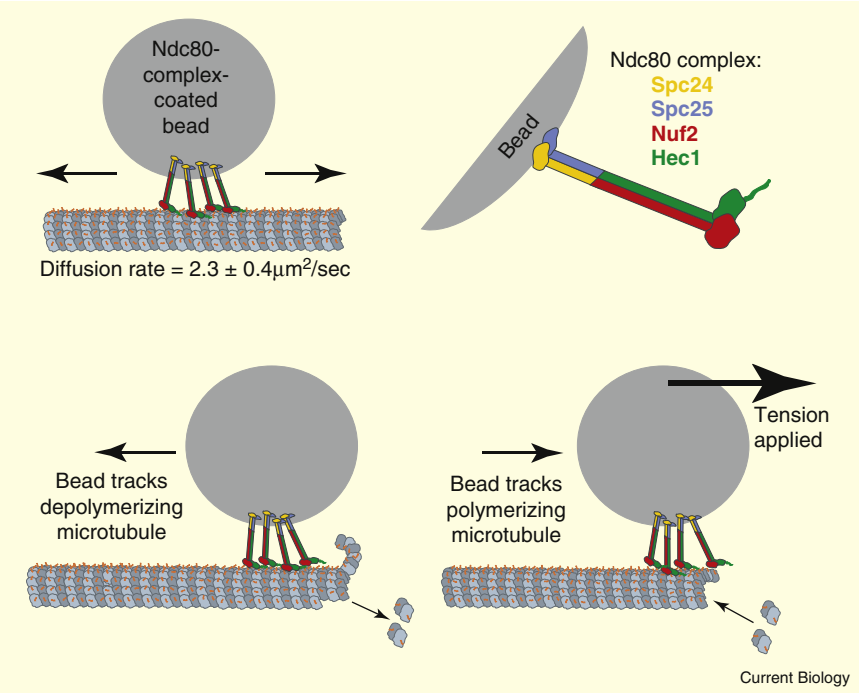

Figure 1. The Ndc80 complex can form load-bearing attachments to microtubules using a biased-diffusion-based mechanism.

Powers *et al.* [7] demonstrate that beads coated with Ndc80 complexes (top right) bind and diffuse rapidly along microtubules (top left). Ndc80-complex-coated beads can track depolymerizing microtubule plus-ends (bottom left) and, in the presence of tension, can track polymerizing microtubule plus-ends (bottom right). In the experiments described by Powers *et al.* [7], beads were coated evenly with Ndc80 complexes; however, only a subset is shown in the figure for clarity.

a catastrophe were able to persistently track depolymerizing plus-ends over long (and physiologically relevant) distances (average of 4.8 μm). These findings are consistent with a recent study by McIntosh *et al.* [9] in which beads coated with purified *Caenorhabditis elegans* Ndc80 complex bound microtubules *in vitro*, and a subset were able to track depolymerizing microtubule ends. In the Powers *et al.* [7] experiments, when force was applied to the beads to simulate a load, the beads also tracked polymerizing microtubule plus-ends without dissociating. This finding is significant, as during chromosome bi-orientation in cells, kinetochores must remain bound to both depolymerizing and polymerizing microtubules. Furthermore, Ndc80-complex-coated beads remained bound to microtubules under a load of up to 2.5 pN, which the authors estimate to be near the force generated at kinetochores during chromosome bi-orientation (0.4–8.0 pN) in cells [10,11]. The authors argue from these studies that the Ndc80 complex is competent, on its own, to serve as a biased-diffusion-based microtubule coupler at the kinetochore.

How does the Ndc80 complex interface the microtubule lattice to facilitate dynamic coupling? X-ray crystallographic data indicate that both Hec1 and Nuf2 contain calponin homology (CH) domains, which have been implicated in direct microtubule binding [12]. The CH domains in Hec1 and Nuf2 contain prominent positive ridges that have been proposed to interface the negatively charged carboxy-terminal tails of tubulin that extend from the microtubule lattice [12]. Supporting this notion, single point mutations in Hec1 or Nuf2 that reduce the positive charge in the ridge domain result in a 30–40-fold decrease in Ndc80 complex microtubule-binding activity *in vitro* [12]. However, the CH domains of Hec1 and Nuf2 alone are not sufficient to bind microtubules. Deletion of the positively charged amino-terminal 80 amino-acid tail domain of Hec1, which is not involved in CH domain folding, significantly impairs kinetochore–microtubule

attachment in cells [13,14] and reduces the affinity of the Ndc80 complex for microtubules by approximately 100-fold *in vitro* [12]. Modifying the charge of the Hec1 tail domain also prevents stable kinetochore–microtubule attachment in cells [13], presumably by altering electrostatic interactions between the oppositely charged tail domains of Hec1 and tubulin. Binding mediated through such interactions fits well into the model of diffusion-based coupling, which relies on multiple weak interactions to function [8]. In support of the role of electrostatic interactions, Powers *et al.* [7] demonstrate that increasing the ionic strength of their assay buffer increased the dissociation rate of Ndc80 complexes from microtubules. Rather than relying on a single binding site, it is likely that multiple contact points exist between the Ndc80 complex and microtubules, as suggested in a recent study by Wilson-Kubalek and colleagues [15]. They combined cryo-electron microscopy of Ndc80-complex-decorated microtubules with published Ndc80 complex crystallographic data to model the interaction. Fitting the crystal structure of the globular amino termini of Hec1 and Nuf2 into their electron density maps of Ndc80-complex-decorated microtubules led to a model in which the CH domain and the 80 amino-acid tail domain of Hec1 contact the microtubule directly. Interestingly, their data did not fit a model in which Nuf2 directly binds the microtubule lattice, suggesting the Ndc80 complex diffusion observed by Powers *et al.* [7] is primarily mediated through Hec1.

As expected for a Hill-type biased-diffusion-based coupler, Powers *et al.* [7] demonstrate that multiple Ndc80 complexes per microtubule are required for generating load-bearing attachments. When at least 360 Ndc80 complexes were bound per bead, attachment to microtubules persisted during cycles of growth and shortening, while decreasing the amount of Ndc80 complexes per bead significantly increased the detachment rate. The authors estimate, based on bead surface geometry, that 360 complexes per bead correspond to 14 ± 5 complexes interacting with a microtubule. Interestingly, this is close to previous estimates of Ndc80 complexes per kinetochore–microtubule *in vivo*: a range of 6–8 Ndc80 complexes per kinetochore–microtubule has been reported for various yeasts [16], and approximately 30 complexes have been reported per kinetochore–microtubule in *Xenopus* egg extracts [17]. Using soluble (non-bead-associated) Ndc80 complexes at very low concentrations, Powers *et al.* [7] demonstrated that complexes bound to microtubules singly or in pairs but did not exhibit tip-tracking behavior. Addition of antibodies to induce Ndc80 complex oligomerization restored the ability of the complexes to track the tips of depolymerizing microtubules. This observation indicates that multiple Ndc80 complex molecules are necessary for stable kinetochore–microtubule attachment. The need for aggregation is also important to consider when interpreting data from experiments using Ndc80 mutants that prevent kinetochore–microtubule attachment in cells or direct microtubule binding *in vitro*. Removal or mutation of certain domains may not disrupt the Ndc80–lattice interface but, instead, may prevent proper oligomerization of the complexes. For example, the Nuf2 CH domain may not be required for microtubule binding, but for oligomerization of the complexes, explaining the low affinity of a CH domain Nuf2 mutant for microtubules *in vitro* [12] and accounting for the inability to model Nuf2 as a direct binder of the microtubule lattice via cryo-electron microscopy [15].

Powers and colleagues [7] demonstrate that the Ndc80 complex alone can serve as a load-bearing coupler between microtubules and kinetochores. But does the Ndc80 complex really go it alone in cells? A recent study by Welburn *et al.* [18] suggests it may not, and kinetochores may also rely on the Ska1 complex to form load-bearing attachments to microtubules. The authors demonstrate that the Ska1 complex (containing Ska1, Ska2, and the newly identified Rama1 protein) can form oligomeric assemblages on microtubules and can couple bead movement along depolymerizing microtubules *in vitro*. The Ska1 components bind kinetochores in human cultured cells [18,19] and, similar to the Dam1 complex in budding yeast, kinetochore binding is dependent on both microtubules and the Ndc80 complex. A testable hypothesis is that the Ska1 complex increases the fidelity of Ndc80-mediated kinetochore–microtubule attachment. In support of this, Powers *et al.* [7] demonstrated that soluble Ndc80 complexes rarely exhibited microtubule binding in buffers of physiological ionic strength (lowering the ionic strength was required to measure diffusion rates), suggesting the presence of cellular factors that increase the ability of the Ndc80 complex to bind and diffuse along microtubules. Future studies probing the relationship between the two complexes will be key in understanding how cells generate the dynamic interface between kinetochores and microtubules.

References

1. Cheeseman, I.M., and Desai, A. (2008). Molecular architecture of the kinetochore-microtubule interface. Nat. Rev. Mol. Cell Biol. *9*, 33–46.
2. Shang, C., Hazbun, T.R., Cheeseman, I.M., Aranda, J., Fields, S., Drubin, D.G., and Barnes, G. (2003). Kinetochore protein interactions and their regulation by the Aurora kinase Ipl1p. Mol. Biol. Cell *14*, 3342–3355.
3. Miranda, J.J., De Wulf, P., Sorger, P.K., and Harrison, S.C. (2005). The yeast DASH complex forms closed rings on microtubules. Nat. Struct. Mol. Biol. *12*, 138–143.
4. Westermann, S., Avila-Sakar, A., Wang, H.W., Niederstrasser, H., Wong, J., Drubin, D.G., Nogales, E., and Barnes, G. (2005). Formation of a dynamic kinetochore-microtubule interface through assembly of the Dam1 ring complex. Mol. Cell *17*, 277–290.
5. Grishchuk, E.L., Spiridonov, I.S., Volkov, V.A., Efremov, A., Westermann, S., Drubin, D., Barnes, G., Ataullakhanov, F.I., and McIntosh, J.R. (2008). Different assemblies of the DAM1 complex follow shortening microtubules by distinct mechanisms. Proc. Natl. Acad. Sci. USA *105*, 6918–6923.
6. Gestaut, D.R., Graczyk, B., Cooper, J., Widlund, P.O., Zelter, A., Wordeman, L., Asbury, C.L., and Davis, T.N. (2008). Phosphoregulation and depolymerization-driven movement of the Dam1 complex do not require ring formation. Nat. Cell Biol. *10*, 407–414.
7. Powers, A.F., Franck, A.D., Gestaut, D.R., Cooper, J., Gracyzk, B., Wei, R.R., Wordeman, L., Davis, T.N., and Asbury, C.L. (2009). The Ndc80 kinetochore complex forms load-bearing attachments to dynamic microtubule tips via biased diffusion. Cell *136*, 865–875.
8. Hill, T.L. (1985). Theoretical problems related to the attachment of microtubules to kinetochores. Proc. Natl. Acad. Sci. USA *82*, 4404–4408.
9. McIntosh, J.R., Grishchuk, E.L., Morphew, M.K., Efremov, A.K., Zhudenkov, K., Volkov, V.A., Cheeseman, I.M., Desai, A., Mastronarde, D.N., and Ataullakhanov, F.I. (2008). Fibrils connect microtubule tips with kinetochores: A mechanism to couple tubulin dynamics to chromosome motion. Cell *135*, 322–333.
10. Nicklas, R.B. (1988). The forces that move chromosomes in mitosis. Annu. Rev. Biophys. Biophys. Chem. *17*, 431–449.
11. Pearson, C.G., Maddox, P.S., Salmon, E.D., and Bloom, K. (2001). Budding yeast chromosome structure and dynamics during mitosis. J. Cell Biol. *152*, 1255–1266.
12. Ciferri, C., Pasqualato, S., Screpanti, E., Varetti, G., Santaguida, S., Dos Reis, G.,

Maiolica, A., Polka, J., DeLuca, J.G., De Wulf, P., *et al.* (2008). Implications for kinetochore-microtubule attachment from the structure of an engineered Ndc80 complex. Cell *133*, 427–439.
13. Guimaraes, G.J., Dong, Y., McEwen, B.F., and Deluca, J.G. (2008). Kinetochore-microtubule attachment relies on the disordered N-terminal tail domain of hec1. Curr. Biol. *18*, 1778–1784.
14. Miller, S.A., Johnson, M.L., and Stukenberg, P.T. (2008). Kinetochore attachments require an interaction between unstructured tails on microtubules and Ndc80(Hec1). Curr. Biol. *18*, 1785–1791.
15. Wilson-Kubalek, E.M., Cheeseman, I.M., Yoshioka, C., Desai, A., and Milligan, R.A. (2008). Orientation and structure of the Ndc80 complex on the microtubule lattice. J. Cell Biol. *182*, 1055–1061.
16. Joglekar, A.P., Bouck, D., Finley, K., Liu, X., Wan, Y., Berman, J., He, X., Salmon, E.D., and Bloom, K.S. (2008). Molecular architecture of the kinetochore-microtubule attachment site is conserved between point and regional centromeres. J. Cell Biol. *181*, 587–594.
17. Emanuele, M.J., McCleland, M.L., Satinover, D.L., and Stukenberg, P.T. (2005). Measuring the stoichiometry and physical interactions between components elucidates the architecture of the vertebrate kinetochore. Mol. Biol. Cell *16*, 4882–4892.
18. Welburn, J.P., Grishchuk, E.L., Backer, C.B., Wilson-Kubalek, E.M., Yates, J.R., 3rd, and Cheeseman, I.M. (2009). The human kinetochore Ska1 complex facilitates microtubule depolymerization-coupled motility. Dev. Cell *16*, 374–385.
19. Hanisch, A., Sillje, H.H., and Nigg, E.A. (2006). Timely anaphase onset requires a novel spindle and kinetochore complex comprising Ska1 and Ska2. EMBO J. *25*, 5504–5515.

[1]Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA.
[2]Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO 80523, USA.
*E-mail: Jennifer.Deluca@ColoState.edu

DOI: 10.1016/j.cub.2009.04.014

Oxygen Sensation: Into Thick Air

Two recent studies show that a neural globin tunes oxygen responses in the nematode *Caenorhabditis elegans*. Analysis of wild *C. elegans* strains suggests that the commonly used Bristol strain may have adapted to life in the laboratory.

Mark J. Alkema

Each spring bar-headed geese leave their closely-related greylag geese behind at their winter feeding grounds in the lowlands of India to fly to their nesting grounds in Tibetan highlands. Bar-headed geese are the world's highest-flying migrants. They have been spotted flying over the top of Mount Everest where the oxygen concentration is one third of that at sea level. The hemoglobin of the bar-headed goose has a much higher affinity for oxygen than that of their lowland relatives allowing the bar-headed geese to cope with life and flight at high altitudes in the Himalayas [1]. Two papers recently published in *Neuron* [2] and *Nature* [3] show that globin polymorphisms also shape behavioral responses to oxygen in the nematode *Caenorhabditis elegans*.

The favorite breeding grounds of *C. elegans* are found in the soil, compost and rotting fruit, where oxygen concentrations can vary from near hypoxia to atmospheric levels; the worm avoids both hypoxia and hyperoxia and seems to prefer oxygen concentrations ranging from 5 to 12% [4]. Because *C. elegans* lacks a specialized respiratory system or circulatory organs, it must rely on diffusion for gaseous exchange. Previous work showed that wild *C. elegans* strains collected from different locations around the world have markedly different responses to oxygen. The *C. elegans* laboratory strain N2, originally isolated from a compost heap in Bristol England, is relatively indifferent to high oxygen concentrations as long as food is plentiful. The Hawaiian strain CB4856, which was isolated from a pineapple field, avoids high oxygen concentrations in the presence of food, and tends to aggregate and forage at the border of bacterial lawns. Polymorphisms in the neuropeptide receptor, *npr-1*, are largely responsible for differences in the behavioral responses between Bristol and Hawaiian strains [5]. The Bristol strain has a high activity *npr-1*(215V) allele resulting in a weak response to oxygen and aversion to carbon dioxide, whereas the Hawaiian strain has a low activity *npr-1*(215F) allele resulting in an aversion to high oxygen levels and indifference to carbon dioxide.

Changing oxygen concentrations alters locomotion rate and turning behavior of the worm, both of which are likely to allow the animal to find its preferred concentration on an oxygen gradient. McGrath *et al.* [2] and Persson *et al.* [3] found that the Hawaiian strain increased their locomotion and turning rate in response to small increases in the oxygen concentration (19/20% to 21%), while the Bristol strain were relatively unresponsive to small shifts in oxygen concentration. Using recombinant inbred strains between the Hawaiian and Bristol relatives, the two groups identified the *glb-5* locus as an additional factor required for the different behavioral responses. The *glb-5* gene encodes a protein with a globin domain and is a member of a large superfamily (including hemoglobin and myoglobin) of heme-binding proteins that play roles in oxygen storage, transport and sensation. In strains carrying both the Hawaiian *npr-1*(215F) and *glb-5* alleles, oxygen downshifts led to a marked increase in turning rate and a reduction of locomotion rate. Strains carrying the Hawaiian *npr-1*(215F) allele and the Bristol *glb-5* allele showed only minimal changes in turning and locomotion rate.

The Bristol *glb-5* locus contains an exon duplication that gives rise to splice variants encoding truncated versions of GLB-5. The Bristol *glb-5* allele is recessive to the Hawaiian *glb-5* allele, suggesting that the duplication reduces gene function. Absorption spectra of recombinant GLB-5 protein indicate that it can reversibly bind oxygen, like the human neuroglobins. The *glb-5* gene is expressed in the URX, AQR/PQR and the BAG sensory neurons, which have been identified as the oxygen and carbon dioxide sensing neurons of the worm. A GFP-tagged version of GLB-5 is highly enriched to the sensory ending of these neurons, suggesting a direct role in sensory transduction. To analyze how *glb-5* affects URX responses, both groups [2,3] used genetically encoded calcium indicators to measure intracellular calcium in the URX neurons. These analyses showed that changes in oxygen produced larger responses in the URX neurons of animals carrying the Hawaiian *glb-5* allele than in those carrying Bristol *glb-5*. High GLB-5