answers to these sorts of questions and, in so doing, identify the neural pathways that link sensory perception to behaviour.

References
1. Lettvin, J.Y., Maturana, H.R., W.S., M., and Pitts, W.H. (1959). What the frog's eye tells the frog's brain. Proc. IRE 47, 233–258.
2. Munch, T.A., da Silveira, R.A., Siegert, S., Viney, T.J., Awatramani, G.B., and Roska, B. (2009). Approach sensitivity in the retina processed by a multifunctional neural circuit. Nat. Neurosci. 12, 1308–1316.
3. Oyster, C.W., and Barlow, H.B. (1967). Direction-selective units in rabbit retina: distribution of preferred directions. Science 155, 841–842.
4. Preuss, S.J., Trivedi, C.A., vom Berg-Maurer, C.M., Ryu, S., and Bollmann, J.H. (2014). Classification of object size in retinotectal microcircuits. Curr. Biol. 24, 2376–2385.
5. Bianco, I.H., Kampff, A.R., and Engert, F. (2011). Prey capture behavior evoked by simple visual stimuli in larval zebrafish. Front. Sys. Neurosci. 5, 101.
6. Trivedi, C.A., and Bollmann, J.H. (2013). Visually driven chaining of elementary swim patterns into a goal-directed motor sequence: a virtual reality study of zebrafish prey capture. Front. Neural Circ. 7, 86.
7. Dean, P., Redgrave, P., and Westby, G.W. (1989). Event or emergency? Two response systems in the mammalian superior colliculus. Trends Neurosci. 12, 137–147.
8. Nevin, L.M., Robles, E., Baier, H., and Scott, E.K. (2010). Focusing on optic tectum circuitry through the lens of genetics. BMC Biol. 8, 126.
9. Gabriel, J.P., Trivedi, C.A., Maurer, C.M., Ryu, S., and Bollmann, J.H. (2012). Layer-specific targeting of direction-selective neurons in the zebrafish optic tectum. Neuron 76, 1147–1160.
10. Lowe, A.S., Nikolaou, N., Hunter, P.R., Thompson, I.D., and Meyer, M.P. (2013). A systems-based dissection of retinal inputs to the zebrafish tectum reveals different rules for different functional classes during development. J. Neurosci. 33, 13946–13956.
11. Nikolaou, N., Lowe, A.S., Walker, A.S., Abbas, F., Hunter, P.R., Thompson, I.D., and Meyer, M.P. (2012). Parametric functional maps of visual inputs to the tectum. Neuron 76, 317–324.
12. Sato, T., Hamaoka, T., Aizawa, H., Hosoya, T., and Okamoto, H. (2007). Genetic single-cell mosaic analysis implicates ephrinB2 reverse signaling in projections from the posterior tectum to the hindbrain in zebrafish. J. Neurosci. 27, 5271–5279.
13. Mysore, S.P., and Knudsen, E.I. (2011). The role of a midbrain network in competitive stimulus selection. Curr. Opin. Neurobiol. 21, 653–660.
14. Hunter, P.R., Lowe, A.S., Thompson, I.D., and Meyer, M.P. (2013). Emergent properties of the optic tectum revealed by population analysis of direction and orientation selectivity. J. Neurosci. 33, 13940–13945.
15. Huberman, A.D., Manu, M., Koch, S.M., Susman, M.W., Lutz, A.B., Ullian, E.M., Baccus, S.A., and Barres, B.A. (2008). Architecture and activity-mediated refinement of axonal projections from a mosaic of genetically identified retinal ganglion cells. Neuron 59, 425–438.
16. Huberman, A.D., Wei, W., Elstrott, J., Stafford, B.K., Feller, M.B., and Barres, B.A. (2009). Genetic identification of an On-Off direction-selective retinal ganglion cell subtype reveals a layer-specific subcortical map of posterior motion. Neuron 62, 327–334.
17. Tamamaki, N., Uhlrich, D.J., and Sherman, S.M. (1995). Morphology of physiologically identified retinal X and Y axons in the cat's thalamus and midbrain as revealed by intraaxonal injection of biocytin. J. Comp. Neurol. 354, 583–607.
18. Del Bene, F., Wyart, C., Robles, E., Tran, A., Looger, L., Scott, E.K., Isacoff, E.Y., and Baier, H. (2010). Filtering of visual information in the tectum by an identified neural circuit. Science 330, 669–673.
19. Baier, H. (2013). Synaptic laminae in the visual system: molecular mechanisms forming layers of perception. Annu. Rev. Cell. Dev. Biol. 29, 385–416.
20. Dhande, O.S., and Huberman, A.D. (2014). Retinal ganglion cell maps in the brain: implications for visual processing. Curr. Opin. Neurobiol. 24, 133–142.

MRC Centre for Developmental Neurobiology, King's College London, Guy's Hospital Campus, London SE1 1UL, UK.
E-mail: fatima.f.abbas@kcl.ac.uk, martin.meyer@kcl.ac.uk

Cell Polarity: Netrin Calms an Excitable System

In a tractable model for cell invasion, the *Caenorhabditis elegans* anchor cell migrates through basement membranes towards a polarity cue provided by netrin. A new study reveals that the anchor cell polarity network can break symmetry and oscillate in the absence of netrin, suggesting the presence of interlinked positive and negative feedback loops, which are common in polarity pathways.

Allison W. McClure
and Daniel J. Lew*

Establishment of cell polarity and guidance of cell growth or movement are critical processes for proper development of a multicellular organism. However, because of the difficulty in detecting and manipulating such processes *in vivo*, most studies on polarity mechanisms have focused on single-cell systems. A new study by Wang *et al.* [1] published in the *Journal of Cell Biology* exploits the transparent nematode worm *Caenorhabditis elegans* to demonstrate that polarity control principles recently discovered in single-cell systems also act in a multicellular context.

In order to initiate uterine–vulval attachment, the *C. elegans* anchor cell polarizes toward a netrin spatial cue and invades through the basement membranes separating the uterine and vulval tissues (Figure 1Ai) [2]. Netrin, a laminin-related protein, is secreted by the ventral nerve cord and is sensed by the anchor cell through the netrin receptor UNC-40 (the ortholog of the vertebrate deleted in colorectal cancer receptor, DCC). In addition to anchor cell invasion, netrin directs several other polarization events in *C. elegans*, including dendritic and axon outgrowth [3,4], synaptogenesis [5], and distal tip cell migration [6].

Cells that orient towards specific cues generally develop a stable polarity axis with a clear 'front'. But what happens when the spatial cue is removed? Wang *et al.* [1] found that, in the absence of netrin, UNC-40 and its effectors cluster at a random site in the anchor cell. Using high-resolution time-lapse microscopy, the authors found that these polarity clusters were dynamic: they disassembled and then reassembled at a new site in an oscillatory manner (Figure 1Aii). Clustering at random sites is suggestive of the existence of positive feedback, such that small asymmetries in polarity protein concentration are amplified to promote clustering. Subsequent cluster disassembly is suggestive of delayed negative feedback, which counteracts positive feedback.

The presence of interlinked positive and negative feedback loops has been inferred from similar oscillatory or excitable behavior in other systems. In appropriate environments, cells of the social amoeba *Dictyostelium discoideum* aggregate to form fruiting bodies. The cells migrate towards each other following gradients of the chemoattractant cyclic AMP (cAMP) and such cells stably concentrate the polarity regulators Ras-GTP and

phosphatidylinositol-3,4,5-triphosphate (PIP_3) at the front (Figure 1Bi). But when *D. discoideum* cells are deprived of a spatial cue, Ras-GTP and PIP_3 spontaneously cluster at random locations. The polarity clusters then disperse and reform at other sites (Figure 1Bii) [7]. In another example, cells of the budding yeast *Saccharomyces cerevisiae* stably polarize Cdc42 and associated polarity factors at preselected bud sites demarcated by a system of inherited bud-site-selection cues (Figure 1Ci). But in mutants lacking such cues, polarity factors spontaneously cluster at a random location on the cell cortex. These polarity clusters also disperse and reform, often, though not always, at the same site (Figure 1Cii) [8]. Similar oscillatory behaviors have also been noted for Cdc42 and related GTPases in T cells [9], fission yeast [10,11], and pollen tubes [12]. Although these various examples utilize different molecules and occur on different timescales (Figure 1Aii,Bii,Cii), they appear to reflect a common underlying architecture of interlinked positive and negative feedback loops [13].

Studies on biological oscillators, like those controlling circadian rhythms and the cell cycle, have established that interlinked positive feedback and delayed negative feedback loops constitute an excellent way to construct a robust oscillator [14,15]. But there is no obvious need for a polarity system to oscillate. Indeed, oscillatory or excitable behaviors of polarity regulators were only revealed when experimenters deprived polarizing cells of their normal spatial cues (Figure 1Aii,Bii,Cii). So, what benefits would interlinked positive and negative feedback provide for polarity networks?

One hypothesis on the potential role of feedback derives from the observation that, in their physiological contexts, polarizing cells are often called upon to track very shallow chemical gradients in a noisy environment. Positive feedback provides an effective way to amplify a shallow gradient signal into a strongly polarized response that can orient cell motility or growth. However, the resulting hair-trigger polarization may lead cells to polarize in the wrong direction, and, once polarized, positive feedback would make it hard to change direction. Theoretical studies showed that addition of a slower negative feedback loop could counteract these
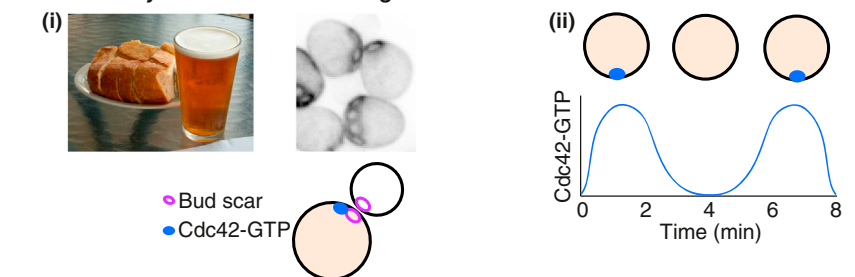
A *Caenorhabditis elegans* **anchor cell invasion**
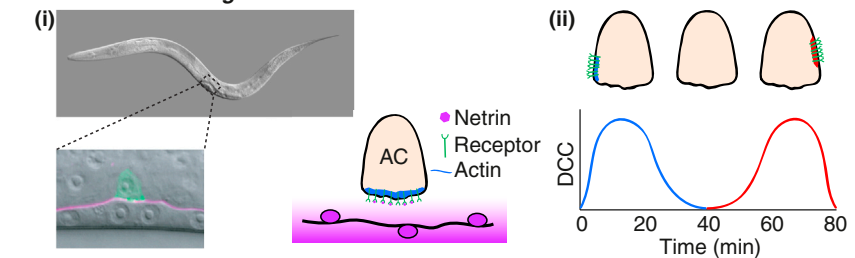
B *Dictyostelium discoideum* **chemotaxis**
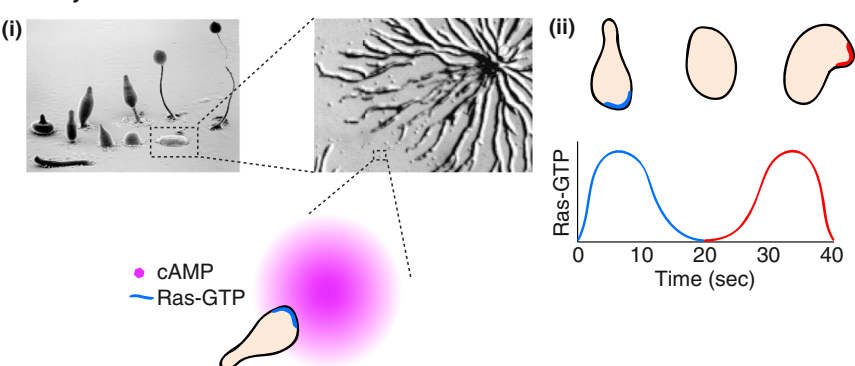
C *Saccharomyces cerevisiae* **budding**

Current Biology

Figure 1. Removal of spatial cues reveals a potential for oscillatory behavior in several polarity systems.

(Ai) The *C. elegans* anchor cell (AC) invades through the underlying basement membrane to initiate uterine–vulval attachment (pictures courtesy of Judith Kimble, University of Wisconsin-Madison, and [17]). The anchor cell polarizes towards the spatial cue, netrin, which is released by the ventral nerve cord. (Aii) In animals without netrin, the anchor cell netrin receptors cluster at a random location, disperse, and then repolarize at a different site. The graph represents the concentration of netrin receptor (UNC-40/DCC) at the blue and red sites in the picture [1]. (Bi) *D. discoideum* cells migrate up the cAMP gradient released by other cells as they aggregate (pictures courtesy of M.J. Grimson and R.L. Blanton, Texas Tech University, and [18]). (Bii) In uniform cAMP, the cells polarize transiently, then depolarize and repolarize in a random direction. The graph represents the local concentration of the polarity regulator Ras-GTP [7]. Note the faster time scale of clustering and disassembly. (Ci) *S. cerevisiae* cells polarize towards an internal spatial cue at the bud scar. (Cii) In mutants lacking the spatial cue, cells cluster Cdc42 at a random location in an oscillatory manner. The graph represents the local concentration of Cdc42-GTP in the cluster [8].

problems, allowing error correction and effective gradient tracking [16]. Thus, the circuit design revealed by oscillatory behavior in the absence of spatial information may have been optimized to allow effective gradient tracking when spatial cues are present. Alternatively, it has been speculated that negative feedback may serve to restrain the spreading of polarity clusters by positive feedback, making polarity systems more robust [8].

Testing whether these concepts hold in the anchor cell will require elucidation of the mechanisms of positive and negative feedback, so that they can be surgically removed. Wang *et al.* [1] show that UNC-40 and

its effectors localize to the polarity clusters, and that the UNC-40 receptor itself is required for polarity cluster oscillations. This suggests that the receptor is active even in the absence of netrin, and may itself participate in the posited feedback mechanisms. Given the polarizing roles of netrin/DCC in other cell types, it will be very interesting to see whether similar feedback loops are common in other developmental contexts. More generally, an understanding of the feedback mechanisms in different polarity systems will allow us to appreciate whether they are indeed employed to hone gradient tracking or whether they provide additional benefits.

References
1. Wang, Z., Linden, L.M., Naegeli, K.M., Ziel, J.W., Chi, Q., Hagedorn, E.J., Savage, N.S., and Sherwood, D.R. (2014). UNC-6 (netrin) stabilizes oscillatory clustering of the UNC-40 (DCC) receptor to orient polarity. J. Cell Biol. *206*, 619–633.
2. Ziel, J.W., Hagedorn, E.J., Audhya, A., and Sherwood, D.R. (2009). UNC-6 (netrin) orients the invasive membrane of the anchor cell in C. elegans. Nat. Cell Biol. *11*, 183–189.
3. Teichmann, H.M., and Shen, K. (2011). UNC-6 and UNC-40 promote dendritic growth through PAR-4 in Caenorhabditis elegans neurons. Nat. Neurosci. *14*, 165–172.
4. Adler, C.E., Fetter, R.D., and Bargmann, C.I. (2006). UNC-6/Netrin induces neuronal asymmetry and defines the site of axon formation. Nat. Neurosci. *9*, 511–518.
5. Colon-Ramos, D.A., Margeta, M.A., and Shen, K. (2007). Glia promote local synaptogenesis through UNC-6 (netrin) signaling in C. elegans. Science *318*, 103–106.
6. Hedgecock, E.M., Culotti, J.G., and Hall, D.H. (1990). The unc-5, unc-6, and unc-40 genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in C. elegans. Neuron *4*, 61–85.
7. Huang, C.H., Tang, M., Shi, C., Iglesias, P.A., and Devreotes, P.N. (2013). An excitable signal integrator couples to an idling cytoskeletal oscillator to drive cell migration. Nat. Cell Biol. *15*, 1307–1316.
8. Howell, A.S., Jin, M., Wu, C.F., Zyla, T.R., Elston, T.C., and Lew, D.J. (2012). Negative feedback enhances robustness in the yeast polarity establishment circuit. Cell *149*, 322–333.
9. Sinai, P., Nguyen, C., Schatzle, J.D., and Wulfing, C. (2010). Transience in polarization of cytolytic effectors is required for efficient killing and controlled by Cdc42. Proc. Natl. Acad. Sci. USA *107*, 11912–11917.
10. Das, M., Drake, T., Wiley, D.J., Buchwald, P., Vavylonis, D., and Verde, F. (2012). Oscillatory dynamics of Cdc42 GTPase in the control of polarized growth. Science *337*, 239–243.
11. Bendezu, F.O., and Martin, S.G. (2013). Cdc42 explores the cell periphery for mate selection in fission yeast. Curr. Biol. *23*, 42–47.
12. Hwang, J.U., Gu, Y., Lee, Y.J., and Yang, Z. (2005). Oscillatory ROP GTPase activation leads the oscillatory polarized growth of pollen tubes. Mol. Biol. Cell *16*, 5385–5399.
13. Wu, C.F., and Lew, D.J. (2013). Beyond symmetry-breaking: competition and negative feedback in GTPase regulation. Trends Cell Biol. *23*, 476–483.
14. Tsai, T.Y., Choi, Y.S., Ma, W., Pomerening, J.R., Tang, C., and Ferrell, J.E., Jr. (2008). Robust, tunable biological oscillations from interlinked positive and negative feedback loops. Science *321*, 126–129.
15. Novak, B., and Tyson, J.J. (2008). Design principles of biochemical oscillators. Nat. Rev. Mol. Cell Biol. *9*, 981–991.
16. Meinhardt, H. (1999). Orientation of chemotactic cells and growth cones: models and mechanisms. J. Cell Sci. *112*, 2867–2874.
17. Hagedorn, E.J., and Sherwood, D.R. (2011). Cell invasion through basement membrane: the anchor cell breaches the barrier. Curr. Opin. Cell Biol. *23*, 589–596.
18. Jang, W., and Gomer, R.H. (2008). Combining experiments and modelling to understand size regulation in Dictyostelium discoideum. J. Roy. Soc. Interface *5* (Suppl 1), S49–S58.

Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA.
*E-mail: Daniel.lew@duke.edu

Evolution: A Rapid Flight towards Birds

Remarkable feathered dinosaur fossils have blurred the lines between early birds and their non-avian dinosaur relatives. Rapid skeletal evolution and decreasing body size along one particular lineage of theropod dinosaurs paved the way for the spectacular radiation of birds.

Daniel T. Ksepka

With approximately 10,000 living species, ranging from tiny nectivorous hummingbirds to soaring raptors to secondarily aquatic penguins, birds represent one of the most remarkable vertebrate radiations. How did birds achieve such astonishing diversity? Birds split from their closest living relatives, the crocodilians, approximately 250 million years ago. This deep split places questions about the earliest phases of avian evolution beyond the reach of methods sampling only extant taxa. Thus, evolutionary biologists must turn to the fossil record. An ever-growing trove of fossil theropods — the clade of feathered bipedal dinosaurs that includes such well-known taxa as *Velociraptor* and

Tyrannosaurus — provides the raw data needed to reconstruct the crucial steps preceding the appearance of primitive birds roughly 150 million years ago. In a recent issue of *Current Biology*, Stephen Brusatte and colleagues [1] harness the fossil record of theropods to provide insight into rates of evolution near the transition between non-avian dinosaurs and birds.

Using an expansive morphological character dataset and methods for estimating rates of evolution, Brusatte and colleagues [1] identify a faster rate of skeletal character change along the theropod lineage leading to birds, as well as a faster rate within birds (as a clade) compared to other theropod clades. Conversely, the study detects no great leap between advanced theropods and basal birds

in morphospace (a multidimensional representation of the possible form of an organism, with each axis representing variation of a specific feature). This drives home the message that, although birds represent the endpoint of an exceptional fast-evolving lineage in the theropod evolutionary tree, there is no wholesale morphological transformation on the particular branch leading directly to birds (Figure 1). Rather, the earliest birds were extremely similar to their non-avian theropod contemporaries, and it was after the basic bird 'body plan' was acquired that they began a runaway diversification.

Early Birds
Investigating the evolution of birds requires several pre-requisite steps, including identifying the earliest bird. This is no longer an easy task. For much of the 20th century, the iconic Jurassic fossil bird *Archaeopteryx* seemed to be clearly separated from non-avian dinosaurs. Now, however, the former '*Urvogel*' (original bird) occupies an increasingly crowded and controversial region of the theropod tree. Indeed, several recent