C. elegans if cultivated at a new temperature with or without food. Mohri *et al.* [8] recently examined the time-course of the switch in preferred temperature following cultivation at a new temperature with or without food. In the experiments where worms were cultivated at temperature A plus food and then switched to temperature B plus food, it took about two to three hours to switch preference from temperature A to temperature B. If cultivated at temperature A with food and then switched to temperature A without food, the time to shift preference differed when the temperature was high compared to when it was low. If worms were cultivated at 25°C and then starved at 25°C, it took them about 10–20 minutes to begin to avoid 25°C; but when the cultivation temperature was 17°C and then worms were starved at 17°C, it took them more than 2 hours to begin to avoid 17°C.

Understanding the parametric features of this plasticity should facilitate investigation into the mechanisms underlying the behavioral changes. It will be very interesting to see whether the plasticity observed in the aerotactic response shows similar time-courses and characteristics as those reported for thermotaxis by Mohri *et. al.* [8].

Taken together, these studies emphasize the amazing abilities that *C. elegans* has to learn about, and to remember features of its environment that predict the presence of food, and to use those cues to move to areas where there is increased likelihood of finding food. Thus far, studies have shown that a single cue, such as a taste or smell, or the ambient temperature or oxygen level, can all be used as predictors of food. At this time experiments have only varied one of these cues at a time, in order to fully understand how the worm addresses the question 'Now, where was I?' we will need to understand whether, and if so how, these sensory inputs are integrated to form a memory that can guide behavior.

References

1. Wen, J. Y. M., Kumar, N., Morrison, G., Rambaldini, G., Runciman, S., Rousseau, J., and Van der Kooy, D. (1997). Mutations that prevent associative learning in *C. elegans*. Behav. Neurosci. *111*, 354-368.
2. Morrison, G. E., Wen, J. Y., Runciman, S., and van der Kooy, D. (1999). Olfactory associative learning in *Caenorhabditis elegans* is impaired in *lrn-1* and *lrn-2* mutants. Behav. Neurosci. *113*, 358–367.
3. Hedgecock, E. M., and Russell, R. L. (1975). Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *72*, 4061-4065.
4. Mori, I. (1999). Genetics of chemotaxis and thermotaxis in the nematode *Caenorhabditis elegans*. Annu. Rev. Genet. *33*, 399–422.
5. Cheung, H.H., Cohen, M., Rogers, C. Albayram, O. and de Bono, M. (2005). C. elegans integrates signals from food and ambient oxygen to regulate exploratory behavior and modify aerotaxis responses. Curr. Biol. *15*, this issue.
6. Cheung, H.H., Arellano-Carbajal, F., Rybicki, I., and de Bono, M (2004). Soluble guanylate cyclases act in neurons exposed to the body fluid to promote *C. elegans* aggregation behavior. Curr. Biol. *14*, 1105-1111.
7. Gray, J.M. , Karow, D.S.Lu, H., Chang. A.J., Chang, J.S., Ellis, R.E., Marietta, M.A. and Bargmann, C.I. (2004). Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. Nature *430*, 317-322.
8. Mohri, A., Kodama, E., Kimura, K. D., Koike, M., Mizuno, T., and Mori, I. (2005). Genetic control of temperature preference in the nematode *Caenorhabditis elegans*. Genetics *169*, 1437-1450.

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BMP Signalling: Synergy and Feedback Create a Step Gradient

More than a decade ago, genetic evidence predicted the existence of a Dpp gradient in the early *Drosophila* embryo. Two recent studies finally reveal Dpp distribution, providing further insights into the mechanism of BMP gradient formation.

Hilary L. Ashe

A central theme in the development of multicellular organisms is that fields of cells are patterned by gradients of signalling molecules in a concentration dependent manner. For example, gradients of bone morphogenetic proteins (BMPs), which are members of the TGF-β superfamily, pattern the dorsal–ventral axes in vertebrate and invertebrate embryos [1]. In the *Drosophila* embryo, this process requires two BMP signalling molecules, Decapentaplegic (Dpp) and Screw (Scw) [2]. Two groups have recently visualised the distribution of Dpp in wild-type and mutant embryos [3,4]. These studies have shed new light on BMP gradient formation by demonstrating that a Dpp–Scw heterodimer is a potent signalling molecule [3], and that a positive feedback mechanism reinforces signalling at peak levels of activity [4].

In the early *Drosophila* embryo, *dpp* is uniformly transcribed in the dorsal ectoderm, which encompasses the dorsal 40% of the embryonic circumference, whereas *scw* is ubiquitously expressed [2]. However, a wealth of experimental evidence has pointed to the existence of an extracellular Dpp and Scw protein gradient, which patterns the dorsal ectoderm. Peak signalling at the dorsal midline leads to formation of the extra-embryonic amnioserosa, whereas lower levels of signalling specify dorsal epidermis [5].

Dpp and Scw bind to Thickveins–Punt (Tkv–Put) and Saxophone–Punt (Sax–Put) receptor complexes, respectively. The signal is then transduced by the Smad transcription factors, Mad and Medea, which enter the nucleus following phosphorylation of Mad by the activated receptors [2]. Genetic evidence had suggested a continuous gradient of Dpp and Scw activity with the highest signalling activity at the dorsal midline, gradually decreasing towards the lateral regions [5]. However, visualisation of activated Smads revealed a step gradient instead. Active Smads are initially detected in a broad stripe in dorsal nuclei, which subsequently narrows to a tight stripe of nuclei in cells fated to become amnioserosa. Until recently, the

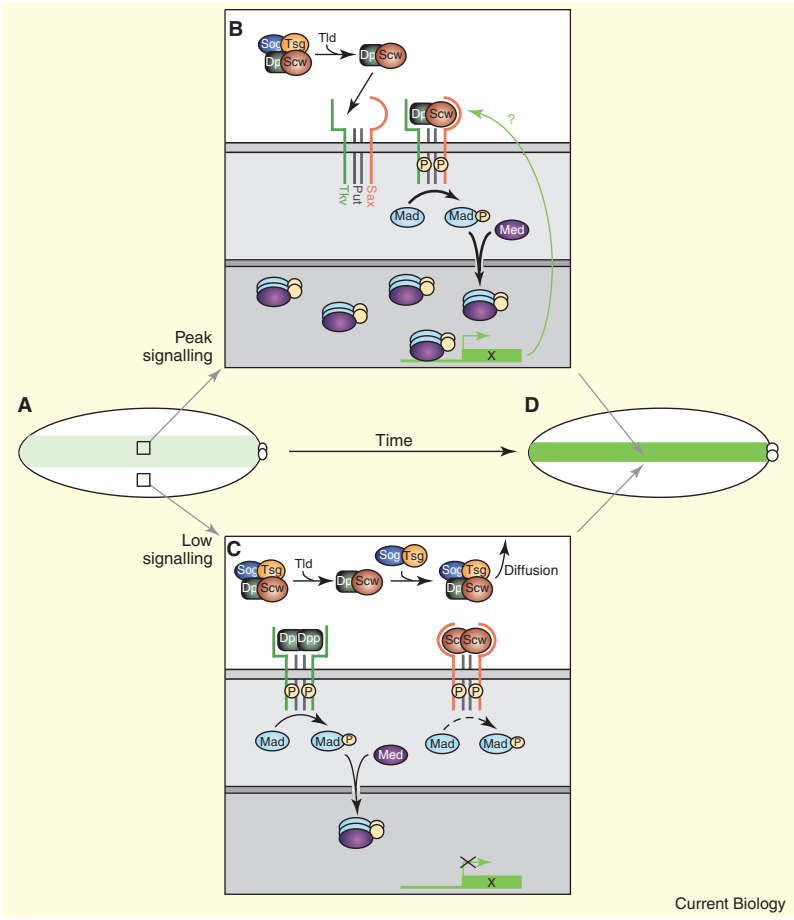

Figure 1. Formation of a BMP step gradient.

(A) Dorsal view of a late cellularisation stage *Drosophila* embryo showing the broad stripe of Dpp protein in green.(B) Dpp–Scw heterodimers are transported to the dorsal midline in a complex containing Sog and Tsg. Following cleavage by Tld, Dpp–Scw heterodimers signal through Tkv–Put–Sax complexes, leading to a high nuclear concentration of phosphorylated Mad and Medea. These transcription factors activate target genes including 'X' which encodes an unknown factor that promotes BMP–receptor interactions.(C) In more lateral regions, Tld cleavage of the inhibitory complex releases Dpp–Scw heterodimers which are rebound by Sog and Tsg, and transported dorsally. Homodimers can signal but lower levels of phosphorylated Mad and Medea accumulate in the nucleus, which are insufficient to activate 'X'.(D) Extracellular transport and positive feedback refine Dpp distribution, shown in green, to a narrow stripe at the dorsal midline in embryos prior to gastrulation.

basis for this unusual Smad distribution has been unclear [2].

Now, visualisation of tagged Dpp in embryos by the O'Connor [3] and Ferguson [4] groups revealed that Dpp protein is initially uniformly distributed, but subsequently accumulates in a broad domain centred around the midline at the late cellularisation stage. This domain is refined into a narrow stripe at the dorsal midline, as observed for nuclear Smads (Figure 1A,D). However, in *scw* mutant embryos the localised Dpp and Smad distribution is lost, suggesting the existence of a Dpp–Scw heterodimer [2–4]. Wang and Ferguson [4] interpreted results from Scw misexpression experiments as supporting a synergy between Dpp and Scw homodimers. In contrast, Shimmi *et al*. [3] provide evidence for Dpp–Scw heterodimers. In addition to biochemical evidence, exposure of cultured cells to Dpp–Scw heterodimers leads to accumulation of 10 or 100 times more phosphorylated Mad than exposure to Dpp or Scw homodimers, respectively. Genetic evidence also suggests that a heterodimer is a more potent ligand in the embryo [3].

Short Gastrulation (Sog) and Twisted Gastrulation (Tsg) are secreted proteins that bind to and facilitate Dpp or Scw diffusion [6,7]. Analysis of Sog and Tsg revealed that Dpp–Scw heterodimers bind to Sog and Tsg with higher affinity than either homodimer. Therefore, the major inhibitory complex with respect to BMP ligands in the embryo is a Dpp–Scw–Tsg–Sog complex, and this complex focuses Dpp–Scw signalling at the dorsal midline [3,4].

An additional level of regulation was identified by Wang and Ferguson [4] who, by measuring Smad activation, demonstrated that *dpp* mutant cells are less responsive to misexpressed Dpp than wild-type cells. Visualization of Dpp–receptor interactions revealed that these are disrupted in *medea* mutant embryos. The observations suggest that previous Dpp–Scw signalling is necessary to promote future Dpp–Scw–receptor interactions, consistent with an autonomous positive feedback mechanism. This feedback mechanism requires Medea and therefore appears to be mediated by an as yet unidentified Dpp target gene [4].

These findings can be integrated in a model for embryonic BMP gradient formation. In late cellularisation embryos, Sog and Tsg facilitate the extracellular transport of Dpp–Scw heterodimers, redistributing them into a broad domain at the dorsal midline (Figure 1A). This transport system redistributes Dpp–Scw to dorsal most regions in a similar manner as previously described for Dpp and Scw homodimers [6,7]. The Dpp–Scw–Sog–Tsg complex is cleaved by the Tolloid (Tld) protease, and in more lateral regions, where Sog is present [8], Dpp/Scw will be rebound by Sog and transported (Figure 1C). Cleavage of the complex in the dorsal most regions, which lack Sog [8], will render the Dpp/Scw heterodimer free to signal. As both Tkv and Sax are required for signalling by the heterodimer, this synergistic signalling results in high levels of Smad activation (Figure 1B). Dorsolateral cells fated

to become dorsal epidermis predominantly receive signals in the form of Dpp and Scw homodimers, which have a broader distribution due to their lower affinity for Sog and Tsg. However, homodimers activate Smads less efficiently than heterodimers, and thus a biphasic signalling profile is generated [3] (Figure 1C).

In addition to the transport system, a positive feedback mechanism is proposed to promote BMP–receptor interactions at the dorsal midline via an unidentified Dpp target gene (Figure 1B). This creates a biphasic profile of BMP–receptor interactions, with heterodimers at the dorsal midline having increased capacity for receptor binding, whereas homodimer–receptor interactions in dorsolateral regions are reduced. In this way, the peak of Dpp/Scw signalling is refined to a tight stripe in embryos at the onset of gastrulation [4] (Figure 1D).

This model leaves at least two outstanding questions. First, what is the molecular mechanism for the increased levels of active Mad by Tkv–Put–Sax versus Tkv–Put or Sax–Put receptor complexes? Perhaps Smads are recruited to the Tkv–Put–Sax receptor complex with greater efficiency or an increased stoichiometry. Alternatively, an inhibitor may exist which is more readily displaced from Tkv–Put–Sax complexes [9], or Tkv–Put–Sax complexes may be preferentially sorted into distinct endocytic vesicles which favour signalling [10]. Second, what is the Dpp target gene which promotes BMP-receptor interactions? High levels of BMP signalling may activate a co-receptor which increases the affinity of BMP–receptor interactions, or an inhibitor of post-transcriptional receptor downregulation thereby restricting downregulation to regions of low signalling [4]. It is possible that the target gene reinforces Dpp/Scw synergy, for example by stabilising the Sax receptor.

Mathematical modelling suggests that Dpp–Scw heterodimers are more robust to changes in gene dosage than homodimers [3]. As heterodimers are also more potent signalling molecules, it is likely that other BMPs function as heterodimers as well. There is some supporting evidence for this in *Drosophila* [11,12], as well as in vertebrates [13–16].

Formation of the Dpp–Scw gradient in the embryo is an unusual case in that *dpp* and *scw* transcripts are uniform in the dorsal ectoderm. In contrast, in the wing imaginal disk a gradient of Dpp forms from a localised source through diffusion which is restricted by heparan sulphate proteoglycans [17]. These distinct mechanisms for creating BMP gradients emphasise the resourcefulness of evolution in solving a biological problem in different developmental contexts.

References

1. Hogan, B.L. (1996). Bone morphogenetic proteins in development. Curr. Opin. Genet. Dev. *6*, 432–438.
2. Raftery, L.A., and Sutherland, D.J. (2003). Gradients and thresholds: BMP response gradients unveiled in Drosophila embryos. Trends Genet. *19*, 701–708.
3. Shimmi, O., and O'Connor, M.B. (2005). Facilitated transport of a Dpp/Scw heterodimer by Sog/Tsg leads to robust patterning of the *Drosophila* blastoderm embryo. Cell *120*, 873–886.
4. Wang, Y.C., and Ferguson, E.L. (2005). Spatial bistability of Dpp-receptor interactions during *Drosophila* dorsal-ventral patterning. Nature *434*, 229–234.
5. Podos, S.D., and Ferguson, E.L. (1999). Morphogen gradients: new insights from DPP. Trends Genet. *15*, 396–402.
6. Ashe, H.L. (2002). BMP signalling: visualisation of the Sog protein gradient. Curr. Biol. *12*, R273–R275.
7. Eldar, A., Dorfman, R., Weiss, D., Ashe, H., Shilo, B.Z., and Barkai, N. (2002). Robustness of the BMP morphogen gradient in *Drosophila* embryonic patterning. Nature *419*, 304–308.
8. Srinivasan, S., Rashka, K.E., and Bier, E. (2002). Creation of a Sog morphogen gradient in the *Drosophila* embryo. Dev. Cell *2*, 91–101.
9. Dorfman, R., and Shilo, B.Z. (2001). Biphasic activation of the BMP pathway patterns the *Drosophila* embryonic dorsal region. Development *128*, 965–972.
10. Miaczynska, M., Pelkmans, L., and Zerial, M. (2004). Not just a sink: endosomes in control of signal transduction. Curr. Opin. Cell Biol. *16*, 400–406.
11. Ray, R.P., and Wharton, K.A. (2001). Context-dependent relationships between the BMPs gbb and dpp during development of the *Drosophila* wing imaginal disk. Development *128*, 3913–3925.
12. Kawase, E., Wong, M.D., Ding, B.C., and Xie, T. (2004). Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the *Drosophila* testis. Development *131*, 1365–1375.
13. Aono, A., Hazama, M., Notoya, K., Taketomi, S., Yamasaki, H., Tsukuda, R., Sasaki, S., and Fujisawa, Y. (1995). Potent ectopic bone-inducing activity of bone morphogenetic protein-4/7 heterodimer. Biochem. Biophys. Res. Commun. *210*, 670–677.
14. Nishimatsu, S., and Thomsen, G.H. (1998). Ventral mesoderm induction and patterning by bone morphogenetic protein heterodimers in *Xenopus* embryos. Mech. Dev. *74*, 75–88.
15. Schmid, B., Furthauer, M., Connors, S.A., Trout, J., Thisse, B., Thisse, C., and Mullins, M.C. (2000). Equivalent genetic roles for bmp7/snailhouse and bmp2b/swirl in dorsoventral pattern formation. Development *127*, 957–967.
16. Butler, S.J., and Dodd, J. (2003). A role for BMP heterodimers in roof plate-mediated repulsion of commissural axons. Neuron *38*, 389–401.
17. Belenkaya, T.Y., Han, C., Yan, D., Opoka, R.J., Khodoun, M., Liu, H., and Lin, X. (2004). *Drosophila* Dpp morphogen movement is independent of dynamin-mediated endocytosis but regulated by the glypican members of heparan sulfate proteoglycans. Cell *119*, 231–244.

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DNA Dynamics: Bubble 'n' Flip for DNA Cyclisation?

A recent demonstration of the facile *in vitro* formation of DNA microcircles of fewer than 100 base pairs throws new light on the basis of DNA flexibility.

Andrew Travers

One of the most stringent experimental tests of DNA flexibility is the formation of small DNA circles. But how does DNA form a small circle in solution? A common view is that the stacking between adjacent base pairs can vary within rather small limits, thus enabling the narrowing or widening of the DNA grooves that is a concomitant of DNA bending and circularisation. These conformational fluctuations would also accommodate small correlated changes in DNA twist. As a circle must necessarily contain an integral number of