are sensitive to statistical regularity in syllable sequences, an observation that is remarkably consistent with existing findings in humans[8]. Further, the authors report that the canine auditory cortices, bilaterally, exhibit a sensitivity for structured sequences, manifest as repetition enhancement. Boros, Magyari *et al.*[2] suggest that this may reflect ongoing perceptual learning, but that the absence of an effect of familiarisation suggests that there is no evidence for the development of long-term representations of the 'words' embedded in the syllable sequences. In other words, the specific computational mechanisms that characterise statistical learning of speech segments and the locations where these are executed seem to be shared in dogs and humans, even if the evidence for recognition of the words is limited.

These results are striking for several reasons. First and foremost, when viewed in conjunction with previous work by this group[9] demonstrating a canine capacity to separately process lexical and prosodic information, the findings of Boros, Magyari *et al.*[2] further indicate that dogs are not simply passive recipients of human speech, but apply sophisticated computational tricks when segmenting and processing their owners' utterances. This is even more impressive when considering that these computations are applied here to not their own but an 'alien' communication system that differs markedly in its structural and semantic properties.

How could this have come about? How can a dog segment human speech in the same way as humans? Two potential options exist. Either dogs have convergently evolved the neural substrates necessary for speech segmentation over the short period of evolutionary time that they have interacted with humans (~10,000 years) or they apply phylogenetically conserved pattern recognition strategies that they employ in their own natural communication, or behaviour, to human speech. Parsimony would favour the latter and is supported by previous related findings that complex language-like associations (such as non-adjacent dependencies) in auditory input can be extracted by other non-human species, likely through the novel application of evolutionarily ancient mechanisms for computing relationships between objects, agents, or events[10]. One promising way to begin to disentangle these alternatives is to turn to the ancestor of dogs, wolves, and their speech segmentation capacities. But to make it a truly fair comparison, and control for exposure to human speech, ideally wolves who have been intensively socialised with humans. Such data in combination with a targeted investigation of the phylogenetic limits of this ability will be crucial to understanding if this is a case of new dogs, old tricks or, indeed, new dogs, new tricks.

REFERENCES

1. Saffran, J.R., Aslin, R.N., and Newport, E.L. (1996). Statistical learning by 8-month-old infants. Science *274*, 1926–1928.
2. Boros, M., Magyari, L., Török, D., Bozsik, A., Deme, A., and Andics, A. (2021). Neural processes underlying statistical learning for speech segmentation in dogs. Curr. Biol. *31*, 5512–5521.
3. Heimbauer, L.A., Beran, M.J., and Owren, M.J. (2011). A chimpanzee recognizes synthetic speech with significantly reduced acoustic cues to phonetic content. Curr. Biol. *21*, 1210–1214.
4. Santolin, C., and Saffran, J.R. (2018). Constraints on statistical learning across species. Trends Cogn. Sci. *22*, 52–63.
5. Wilson, B., Slater, H., Kikuchi, Y., Milne, A.E., Marslen-Wilson, W.D., Smith, K., and Petkov, C.I. (2013). Auditory artificial grammar learning in macaque and marmoset monkeys. J. Neurosci. *33*, 18825–18835.
6. Hauser, M.D., Newport, E.L., and Aslin, R.N. (2001). Segmentation of the speech stream in a non-human primate: Statistical learning in cotton-top tamarins. Cognition *78*, B53–B64.
7. Karuza, E.A., Emberson, L.L., and Aslin, R.N. (2014). Combining fMRI and behavioral measures to examine the process of human learning. Neurobiol. Learn. Mem. *109*, 193–206.
8. Kotz, S.A., Schwartze, M., and Schmidt-Kassow, M. (2009). Non-motor basal ganglia functions: A review and proposal for a model of sensory predictability in auditory language perception. Cortex *45*, 982–990.
9. Andics, A., Gabor, A., Gacsi, M., Farago, T., Szabo, D., and Miklosi, A. (2016). Neural mechanisms for lexical processing in dogs. Science *353*, 1030–1032.
10. Watson, S.K., Burkart, J.M., Schapiro, S.J., Lambeth, S.P., Mueller, J.L., and Townsend, S.W. (2020). Nonadjacent dependency processing in monkeys, apes, and humans. Sci. Adv. *6*, eabb0725.

Embryonic polarity: Focusing on Dishevelled

Charles A. Ettensohn
Department of Biological Sciences, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA
Correspondence: ettensohn@cmu.edu
https://doi.org/10.1016/j.cub.2021.10.060

The early polarity of animal embryos is typically established by molecules that are asymmetrically localized in the oocyte. A new study reveals that the Wnt signaling effector Dishevelled, an evolutionarily conserved, maternal regulator of embryonic polarity, is dynamically redistributed during oogenesis in a process regulated by the cell cycle and by the Wnt receptor Frizzled 1.

Developmental biologists have long been fascinated by the mechanisms that establish the polarity of the early embryo. In most metazoans, the primary (animal–vegetal, or anterior–posterior) axis of the embryo can be traced to the molecular asymmetry of the oocyte. The identity of the key maternal factors, the processes that control their localization, and the mechanisms by which they control gene expression and cell fates are therefore fundamental questions. A new study in

this issue of *Current Biology* by Swartz *et al.*[1] provides insights into these issues. These authors uncover mechanisms that polarize the embryo of the sea star *Patiria miniata*, a representative of the echinoderms. This new work reveals that the Wnt signaling effector Dishevelled, a key effector of Wnt signaling and a conserved regulator of embryonic polarity, is redistributed during oogenesis in a manner controlled by the cell cycle and by the Wnt receptor Frizzled 1 (Fzd1).

The localized accumulation of β-catenin at one pole of the early embryo is a highly conserved and critically important event in animal development. In echinoderms, including sea urchins and sea stars, β-catenin becomes enriched in the nuclei of blastomeres during cleavage in a graded manner, with the highest levels of nuclear β-catenin in the vegetal-most cells, where it acts in a concentration-dependent manner to activate endodermal and mesodermal gene regulatory networks[2–5]. This polarized nuclear accumulation of β-catenin along the primary, animal–vegetal (A–V) axis is associated with a gradient in β-catenin stability; the half-life of the protein is more than two orders of magnitude longer in vegetal blastomeres than in animal blastomeres[6].

The stability of β-catenin along the A–V axis is modulated by Dishevelled (Dvl), which acts as a negative regulator of the β-catenin destruction complex[7]. In Wnt-responsive cells, the binding of Wnt ligands to Frizzled receptors promotes an interaction between Dvl and Frizzled at the plasma membrane[8,9], where Dvl acts to inhibit the molecular machinery that degrades β-catenin. Both *in vitro* and *in vivo*, Dvl forms oligomers through its amino-terminal DIX (Dishevelled and Axin) domain. Binding of Wnt to Frizzled stimulates oligomerization of Dvl at the plasma membrane and this is thought to be essential for the disruption of the β-catenin destruction complex, although many aspects of the underlying mechanisms remain unclear[9,10]. Related to its capacity for oligomerization, Dvl has been observed to form cytoplasmic puncta in a wide variety of cells. These puncta were at one time thought to represent vesicles but are now widely believed to be membrane-free, Dvl-containing protein complexes[11]. One challenge in examining the organization of Dvl in cells is that overexpression of the protein at high levels can induce the formation of oligomeric complexes that are much larger than endogenous complexes and that may have limited physiological significance[10,12].

In the oocytes of diverse metazoans, including echinoderms, frogs and sea anemones, Dvl-containing puncta are found concentrated at the pole where β-catenin will accumulate in cell nuclei during early embryogenesis. These puncta have been detected with antibodies against the endogenous proteins and are therefore not an artifact of Dvl overexpression[13–15]. In echinoderms, *dvl* mRNA is distributed throughout the oocyte and early embryo, but Dvl protein is highly enriched near the vegetal pole, where it is found mostly in cortical puncta[6,15,16]. High-resolution imaging has revealed that these puncta consist of clusters of pedestal-shaped structures[17]. In frogs, where Dvl was first implicated in early embryonic axis specification, the role of Dvl has remained controversial[18]. In sea urchins, however, expression of a dominant-negative isoform of Dvl prevents the vegetal accumulation of nuclear β-catenin and dramatically disrupts endomesoderm formation, phenocopying the effects of disrupting β-catenin nuclear accumulation by several independent approaches[6]. These findings provide strong evidence that in sea urchins Dvl plays a critically important role in the stabilization of β-catenin in vegetal cells and in the activation of gene regulatory networks that drive endomesoderm formation.

In the new study, Swartz *et al.*[1] provide an exciting look into the mechanisms of Dvl localization in oocytes of the sea star *P. miniata*. They take advantage of two very valuable characteristics of this experimental model. First, sea star oocytes can be collected from adult females while arrested in prophase I and then induced to mature synchronously *in vitro* by the addition of the hormone 1-methyladenine. Second, sea star oocytes, like the eggs and embryos of many echinoderms, are highly transparent and ideally suited for *in vivo* imaging. Swartz *et al.*[1] capitalize on these virtues of sea star oocytes to examine the dynamics of Dvl localization during oogenesis in ways that were not previously possible.

To first determine whether Dvl is essential for axis specification in sea stars, as it is in sea urchins, Swartz *et al.*[1] knock down Dvl expression by microinjecting a translation-blocking morpholino into prophase I oocytes. They find that the specification of mesoderm and endoderm is perturbed. These effects are less pronounced than those previously reported in sea urchins when Dvl function is inhibited with dominant-negative Dvl[6]; one possible explanation for this is that the function of pre-existing, maternal Dvl, which cannot be affected by the morpholino, is inhibited by the dominant-negative protein.

By expressing a GFP-tagged form of Dvl in oocytes and in parallel experiments using an antibody against the endogenous protein, Swartz *et al.*[1] analyze the dynamics of Dvl localization during the oocyte-to-embryo transition. They find that in prophase I-arrested oocytes Dvl–GFP localizes in puncta uniformly throughout the cortex, although it should be noted that these puncta do not seem to be detected with the Dvl antibody. Hormonal stimulation of the arrested oocytes triggers their maturation, which is associated with germinal vesicle breakdown and the completion of meiosis I. During oocyte maturation, prophase I Dvl–GFP cortical puncta disappear very rapidly (that is, on a timescale of a few minutes), revealing a connection between the resumption of the cell cycle and dynamic changes in Dvl organization. Soon thereafter, Dvl puncta accumulate in the peripheral cytoplasm near the vegetal pole, as visualized using Dvl–GFP or the Dvl antibody. These Dvl puncta are inherited by vegetal blastomeres during embryonic cleavage and presumably support the stabilization of β-catenin in those cells. Accumulation of Dvl–GFP in vegetal puncta is unaffected when protein synthesis is blocked with emetine, indicating that the puncta are assembled from pre-existing pools of protein. In contrast, inhibition of cyclin-dependent kinase activity with flavopiridol prevents the formation of vegetal puncta (visualized with Dvl–GFP), supporting a link between meiotic progression and Dvl dynamics.

Several observations indicate that the formation of vegetal Dvl puncta occurs through a local assembly process. Measurements of the motion of

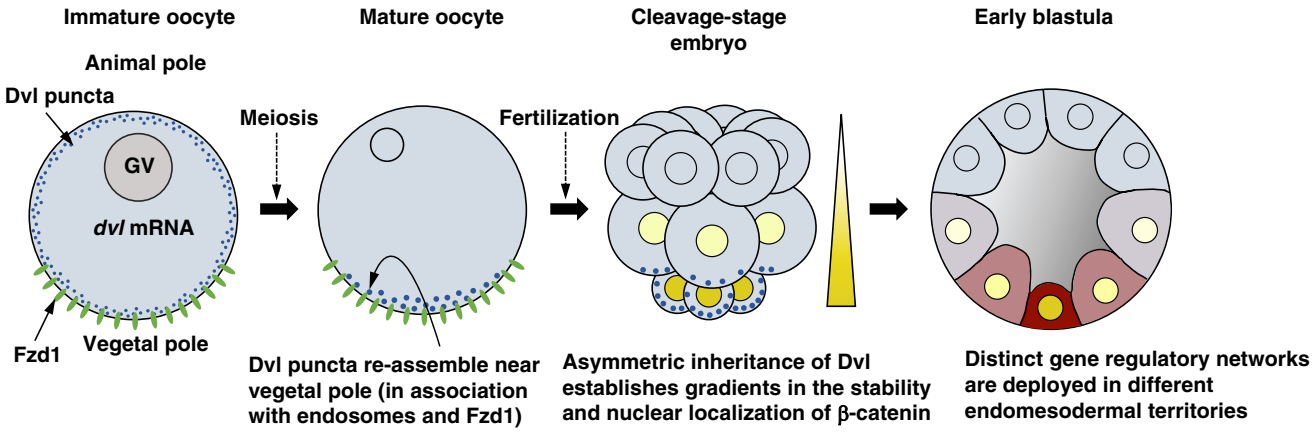

Figure 1. Current model of the establishment of embryonic polarity along the animal–vegetal axis in echinoderms.
The model is based on the study by Swartz *et al.*[1] and previous work[2–6,15]. *dvl* mRNA (pale blue) is uniformly distributed during oogenesis and early embryogenesis. Dvl protein is distributed in puncta (dark blue dots) throughout the cortex early in oogenesis, but progression through the meiotic cell cycle triggers the disassembly of these complexes and the local reformation of Dvl puncta in the vegetal cytoplasm in a Fzd1-dependent manner. In the mature oocyte, vegetal Dvl puncta are associated with both endosomes (not shown) and Fzd1. After fertilization, Dvl complexes are inherited by vegetal blastomeres and establish a gradient of β-catenin stability. This produces a gradient of nuclear β-catenin (shown in yellow) along the A–V axis, highest at the vegetal pole, which in turn leads to the regional activation of distinct gene regulatory networks along the embryonic axis, as illustrated by the red and pink cells in the early blastula stage embryo.

Dvl–GFP-containing puncta show that they do not translocate directionally toward the vegetal region, and the appearance of vegetal puncta is unaffected by chemical disruption of cytoskeletal networks that might be expected to support long-distance transport. Perhaps most elegantly, Swartz *et al.*[1] show that Dvl–GFP puncta assemble autonomously at the vegetal pole when surgically isolated, vegetal oocyte fragments are treated with 1-methyladenine. This finding, combined with the emetine studies, suggests that the vegetal domain contains both assembly cues and a localized pool of Dvl protein.

Swartz *et al.*[1] further leverage *in vivo* imaging to characterize several features of the vegetal Dvl–GFP puncta. They show that puncta located in the cell cortex are much less mobile than those found deeper in the cytoplasm. Fluorescence recovery after photobleaching analysis of the prophase I puncta and those that form vegetally after the resumption of meiosis reveals that Dvl protein exchanges readily between cytoplasmic pools and each type of complex with a half-time for recovery of around 2.5 minutes. A much lower fraction of Dvl protein within vegetal, cortical puncta is mobile, however, presumably reflecting different binding environments for Dvl in these two contexts. Swartz *et al.*[1] also reveal a striking colocalization of vegetal puncta with Lamp1^{+}–Rab7^{+} endosomes, as shown by both live imaging of Dvl–GFP and immunostaining. While very few Dvl puncta in prophase I-arrested oocytes are associated with endosomes, this association is dramatically enhanced upon maturation: almost 40% of vegetal Dvl puncta in mature oocytes colocalize with endosomes. Thus, at least in sea star oocytes, the answer to the long-standing question of whether Dvl puncta represent membrane-bound structures or protein complexes appears to be that both descriptions are partially correct.

These findings led the authors to suspect that a maternally localized, membrane-associated molecule might support the assembly of vegetal Dvl puncta. As noted above, Dvl interacts with Frizzled proteins during Wnt signal transduction. Fzd1 is maternally expressed in sea star oocytes, and Swartz *et al.*[1] find that Fzd1–GFP accumulates in the vegetal region of prophase I-arrested oocytes (that is, prior to maturation and the vegetal, cortical localization of Dvl), where it remains localized throughout early cleavage. Significantly, knockdown of maternal Fzd1 largely blocks the vegetal, cortical accumulation of Dvl, and this effect is fully rescued by overexpression of a full-length, morpholino-insensitive form of Fzd1. These new findings point to Fzd1 as an essential component of the machinery that localizes Dvl at the vegetal pole and establishes the polarity of the early embryo (Figure 1).

Of course, the study by Swartz *et al.*[1] raises many exciting new questions. Those interested in examining the establishment of embryonic polarity know that this endeavor can be a 'will-o-the-wisp': if maternally localized Fzd1 is important in restricting the distribution and/or activity of Dvl, then what mechanisms are responsible for the vegetal localization of Fzd1? The study by Swartz *et al.*[1] includes tantalizing images showing colocalization of Fzd1–GFP with endogenous, vegetal Dvl puncta; it will be important to examine the distribution of endogenous Fzd1 protein and determine the extent to which the two proteins colocalize as well as the timing of their association. The findings reported in this study will likely also prompt studies of the possible evolutionary conservation of Fzd1 function in the establishment of embryonic polarity. Localized Frizzled receptors have been implicated as regulators of axis determination in a cnidarian[19], hinting at possible evolutionary conservation. Another exciting prospect will be to explore the molecular connections between cyclin activity and the redistribution of Dvl during oogenesis. Lastly, what are we to make of the unexpected interaction between Dvl granules and endosomes? Several

studies have suggested that endocytosis of Frizzled–Dvl complexes plays an important role in Wnt signal transduction, although this is currently a controversial aspect of Wnt signaling[20]. Is there any connection between internalized Wnt signaling complexes and the Dvl–endosome complexes observed in oocytes? These questions sparked by the work of Swartz *et al.*[1] show that even a protein as intensively studied as Dvl has yet to reveal all of its secrets.

REFERENCES

1. Swartz, S.Z., Tan, T.H., Perillo, M., Fakhri, N., Wessel, G.M., Wikramanayake, A.H., and Cheeseman, I.M. (2021). Polarized Dishevelled dissolution and reassembly drives embryonic axis specification in sea star oocytes. Curr. Biol. *31*, 5633–5641.
2. Wikramanayake, A.H., Huang, L., and Klein, W.H. (1998). Beta-catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. Proc. Natl. Acad. Sci. USA *95*, 9343–9348.
3. Logan, C.Y., Miller, J.R., Ferkowicz, M.J., and McClay, D.R. (1999). Nuclear beta-catenin is required to specify vegetal cell fates in the sea urchin embryo. Development *126*, 345–357.
4. Miyawaki, K., Yamamoto, M., Saito, K., Saito, S., Kobayashi, N., and Matsuda, S. (2003). Nuclear localization of beta-catenin in vegetal pole cells during early embryogenesis of the starfish *Asterina pectinifera*. Dev. Growth Differ. *45*, 121–128.
5. McCauley, B.S., Akyar, E., Saad, H.R., and Hinman, V.F. (2015). Dose-dependent nuclear β-catenin response segregates endomesoderm along the sea star primary axis. Development *142*, 207–217.
6. Weitzel, H.E., Illies, M.R., Byrum, C.A., Xu, R., Wikramanayake, A.H., and Ettensohn, C.A. (2004). Differential stability of beta-catenin along the animal-vegetal axis of the sea urchin embryo mediated by dishevelled. Development *131*, 2947–2956.
7. Nusse, R., and Clevers, H. (2017). Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. Cell *169*, 985–999.
8. Wong, H.C., Bourdelas, A., Krauss, A., Lee, H.J., Shao, Y., Wu, D., Mlodzik, M., Shi, D.L., and Zheng, J. (2003). Direct binding of the PDZ domain of Dishevelled to a conserved internal sequence in the C-terminal region of Frizzled. Mol. Cell *12*, 1251–1260.
9. Gammons, M., and Bienz, M. (2018). Multiprotein complexes governing Wnt signal transduction. Curr. Opin. Cell Biol. *51*, 42–49.
10. Ma, W., Chen, M., Kang, H., Steinhart, Z., Angers, S., He, X., and Kirschner, M.W. (2020). Single-molecule dynamics of Dishevelled at the plasma membrane and Wnt pathway activation. Proc. Natl. Acad. Sci. USA *117*, 16690–16701.
11. Schwarz-Romond, T., Merrifield, C., Nichols, B.J., and Bienz, M. (2005). The Wnt signalling effector Dishevelled forms dynamic protein assemblies rather than stable associations with cytoplasmic vesicles. J. Cell Sci. *118*, 5269–5277.
12. Kan, W., Enos, M.D., Korkmazhan, E., Muennich, S., Chen, D.H., Gammons, M.V., Vasishtha, M., Bienz, M., Dunn, A.R., Skiniotis, G., and Weis, W.I. (2020). Limited Dishevelled/Axin oligomerization determines efficiency of Wnt/β-catenin signal transduction. eLife *16*, e55015.
13. Miller, J.R., Rowning, B.A., Larabell, C.A., Yang-Snyder, J.A., Bates, R.L., and Moon, R.T. (1999). Establishment of the dorsal-ventral axis in *Xenopus* embryos coincides with the dorsal enrichment of Dishevelled that is dependent on cortical rotation. J. Cell Biol. *146*, 427–437.
14. Lee, P.N., Kumburegama, S., Marlow, H.Q., Martindale, M.Q., and Wikramanayake, A.H. (2007). Asymmetric developmental potential along the animal-vegetal axis in the anthozoan cnidarian, *Nematostella vectensis*, is mediated by Dishevelled. Dev. Biol. *310*, 169–186.
15. Peng, C.J., and Wikramanayake, A.H. (2013). Differential regulation of Disheveled in a novel vegetal cortical domain in sea urchin eggs and embryos: implications for the localized activation of canonical Wnt signaling. PLoS One *8*, e80693.
16. Leonard, J.D., and Ettensohn, C.A. (2007). Analysis of Dishevelled localization and function in the early sea urchin embryo. Dev. Biol. *306*, 50–65.
17. Henson, J.H., Samasa, B., Shuster, C.B., and Wikramanayake, A.H. (2021). The nanoscale organization of the Wnt signaling integrator Dishevelled in the vegetal cortex domain of an egg and early embryo. PLoS One *16*, e0248197.
18. Tadjuidje, E., Cha, S.W., Louza, M., Wylie, C., and Heasman, J. (2011). The functions of maternal *Dishevelled 2* and *3* in the early *Xenopus* embryo. Dev. Dyn. *240*, 1727–1736.
19. Momose, T., and Houliston, E. (2007). Two oppositely localised Frizzled RNAs as axis determinants in a cnidarian embryo. PLoS Biol. *5*, e70.
20. Rim, E.Y., Kinney, L.K., and Nusse, R. (2020). β-catenin-mediated Wnt signal transduction proceeds through an endocytosis-independent mechanism. Mol. Biol. Cell *31*, 1425–1436.

Evolution: The oldest sex chromosomes

Deborah Charlesworth
Institute of Evolutionary Biology, University of Edinburgh, Edinburgh EH9 3FL, UK
Correspondence: Deborah.Charlesworth@ed.ac.uk
https://doi.org/10.1016/j.cub.2021.10.062

The first sex chromosomes in plants were described in bryophytes, and liverwort genome sequences reported in a new study are now starting to help us understand the similarities and differences in the evolution of haploid and diploid systems.

Sex chromosomes were first described in 1905 in the beetle *Tenebrio molitor*, with the discovery of its small male-specific chromosome[1], implying that this species has an XY system with a much smaller Y than X chromosome. In 1918, genetic degeneration leading to loss of Y-linked genes and shrinkage of Y chromosomes was understood to be a consequence of a lack of recombination[2]. In haploid plants, the spores germinate into free-living haploid gametophytes that in many species are either female, developing archegonia at sexual maturity, or male, bearing antheridia that produce motile, flagellated sperm. Gametophyte sex is often genetically determined, and male and female gametophytes