experiments on the hindmost and middle legs of the stick insect *Pseudoproscopia scabra*. In the stick insect's hind legs, they found the same thing as in the locust — joint capsule forces which could flex the joint. Looking at the middle legs of the stick insect, they found passive forces within the joint capsule, but these were, unlike the locust, much more symmetric; the capsule forces in the middle leg were able to generate both flexions and extensions. Lastly, data from the rearmost legs of another stick insect, *Carausius morosus*, showed joint capsule forces that were the inverse of those found in the locust; the *Carausius* joint capsule forces generated extensions and not flexions [4,5].

Ache and Matheson [5] had thus found joint capsule forces in all three possible permutations: aiding flexion, aiding extension, and aiding both symmetrically. They had found them in locusts and stick insects. Lastly, they had found them in legs used primarily for jumping and in legs used primarily for walking. The joint capsule forces were thus important and ubiquitous, yet they lacked neural correlates, and had thus far had been 'invisible' to previous neurophysiological investigations [8,9]. On the most conservative level, they had discovered a new kind of passive force, a 'silent partner' that must be considered when analysing the control of motion. In a larger sense, they had demonstrated, by elegant example, that combining biomechanics with neurobiology can yield research dividends [10].

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Development: Scaling to Size by Protease Inhibition

The dorsal half of bisected *Xenopus laevis* embryos can regenerate a well-proportioned organism on a smaller scale. A new study indicates that the removal of ventral tissue generates a steeper Chordin gradient by reducing Sizzled, a secreted inhibitor of Tolloid chordinases.

Edward M. De Robertis and Gabriele Colozza

How is a perfectly patterned embryo formed time after time? Development is a very robust process and animal embryos can self-regulate, adjusting to changes in environmental temperature and variations in egg size. Pattern self-regulation after bisection or transplantation of a dorsal Spemann organizer is an intriguing property of the dorsal-ventral morphogenetic field of *Xenopus laevis* gastrula stage embryos. The dorsal side secretes a cocktail of growth factor antagonists, most prominent of which are the BMP antagonists Noggin and Chordin [1,2]. On the ventral side, high BMP signaling promotes Sizzled production [3]. Sizzled regulates BMP signaling indirectly, by stabilizing Chordin through the competitive inhibition of Tolloid proteases that degrade Chordin (Figure 1A) [4]. Previous work has uncovered an extracellular network of interacting proteins that regulate the dorsal-ventral BMP gradient [5]. This includes other components such as ADMP (Anti-dorsalizing Morphogenetic Protein), BMP2/4/7, ONT1, Crossveinless-2 and Crescent (Figure 1B). This patterning system is self-regulating because dorsal components are transcribed at low BMP levels and ventral genes are expressed at high BMP levels [6,7]. In a new study [8], Hidehiko Inomata and colleagues present a simplified model in which scaling to size is explained mainly by the long-range regulation of Chordin stability caused by the removal of Sizzled-producing ventral tissue after bisection (Figure 1C).

Evidence in the literature had already suggested that the extracellular BMP antagonist Chordin and its regulator Sizzled were key players in dorsal-ventral patterning. In zebrafish, the only ventralizing (high BMP) gastrulation mutations found in extensive genetic screens corresponded to *chordin* and *sizzled* [9,10]. In *Xenopus*, Chordin and Sizzled are very abundantly secreted in the gastrula and, if uniformly distributed, would reach concentrations of about 30 nM each in the extracellular space [4]. Depletion of Chordin with antisense morpholino results in loss of all embryonic inducing activity by transplanted Spemann organizer [11]. Furthermore, knock-down of Chordin or Sizzled with morpholinos results in identical high-BMP

phenotypes in *Xenopus* (Figure 2) even though these genes are expressed at opposite poles of the embryo [4]. Taken together, these data indicated that proteolytic control of Chordin and its regulation by Sizzled played a central role in the harmonious development of the embryo.

To study the formation of the Chordin gradient, Inomata and colleagues [8] used a clever experimental trick in which endogenous dorsal-ventral pattern was erased by depleting β-Catenin required for dorsal organizer gene expression, such that BMP signaling was uniformly high across the entire embryo. In this system, a single dorsal injection of *chordin* mRNA at the 8-cell stage was sufficient to regenerate dorsal-ventral patterning, while mRNA for another BMP antagonist, *noggin,* caused a more uniform dorsalization. This raised the possibility that the difference could be due to Chordin diffusing more slowly than Noggin in the embryo [8]. To test this idea, the authors microinjected mRNA encoding Noggin or Chordin fused to Green Fluorescent Protein into animal blastomeres. The proteins diffused in the extracellular space between uninjected neighboring cells in ectodermal explants. Beautiful time-lapse movies of fluorescence recovery after photobleaching (FRAP) demonstrated that, contrary to expectations, Chordin diffused quickly into the bleached area. The diffusion coefficients were measured and Noggin was found to diffuse more slowly than Chordin (0.7 vs 1.9 $\mu m^2/\mu s$). These new diffusion constants — combined with the previously determined biochemical dissociation constants of most protein–protein interactions [4,12,13] (Figure 1B) — will provide invaluable parameters for a future quantitative understanding of embryonic patterning.

Since Chordin is able to diffuse, why is it able to pattern the embryo more efficiently than Noggin? To address this, the stability of these organizer proteins was investigated by injecting recombinant proteins into the blastula cavity. Chordin was rapidly degraded with a half-life of 30 minutes, which was prolonged by *sizzled* mRNA injection, as Sizzled inhibits Tolloid.

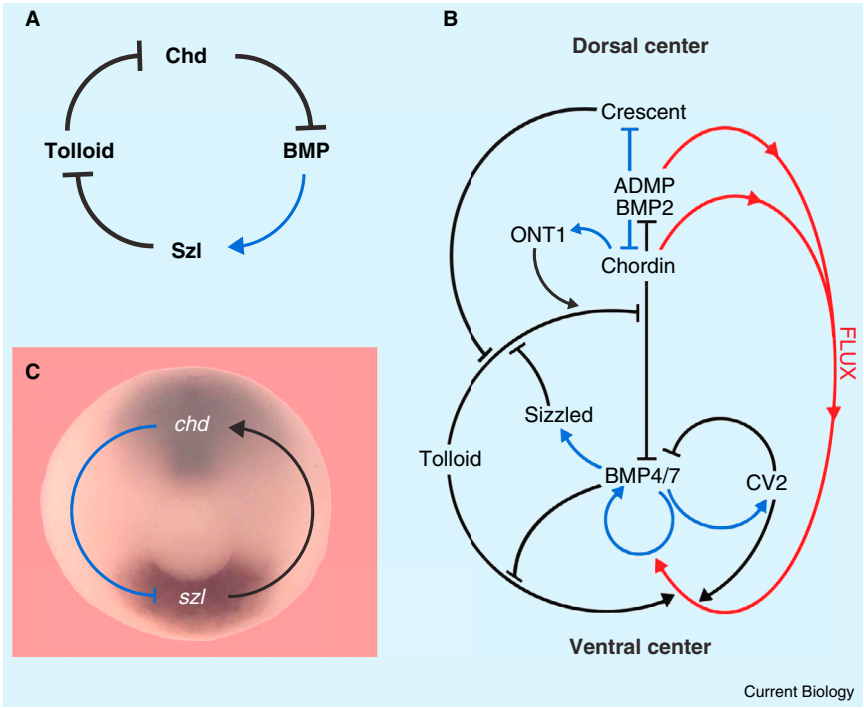

Figure 1. Extracellular protein interactions in *Xenopus* dorsal-ventral patterning.
(A) Chordin inhibits BMPs, which activate expression of Sizzled, an inhibitor of Tolloid proteases that degrades Chordin. (B) The dorsal-ventral network consists of many components under opposite transcriptional control by BMPs [7]. Crescent is a Sizzled homolog expressed in the Spemann Organizer [13]. (C) A simplified pathway in which Chordin and Sizzled regulate each other [8]. The *in situ* hybridization in the background illustrates that *chd* and *szl* mRNAs are expressed at opposite poles of the mid-gastrula [4]. The arrows indicate direct protein–protein interactions in black, transcriptional regulation by BMP signaling in blue, and diffusion (flux) of Chordin/BMP in red.

In contrast, Noggin and Sizzled proteins were considerably more stable [8]. Moreover, endogenous Sizzled protein levels accumulated during gastrulation. These observations lead to a model in which the extracellular accumulation of Sizzled causes the gradual stabilization of Chordin, which in turn inhibits dorsal BMP signaling, such that the transcription of Sizzled itself becomes restricted to the ventral-most part of the embryo. Thus, the long-range interaction between the dorsal and ventral sides of the embryo is mediated by the diffusion and accumulation of Sizzled protein [8].

But how does this explain scaling, and how is normal patterning restored in the dorsal half after bisection? The authors present a mathematical model that tracks the evolution of dorsal-ventral patterning proteins during gastrulation and explains how the BMP gradient is able to scale to reductions in embryonic size. Scaling occurs because removal of ventral tissue decreases the Sizzled-producing area, leading to higher Tolloid activity and a shorter (and steeper) Chordin gradient. Bisection experiments confirmed that microinjected epitope-tagged Chordin was indeed degraded more rapidly in the dorsal half when the ventral side was removed [8]. In conclusion, the new work uncovered a key role for the accumulation of Sizzled protein during gastrulation, which stabilizes Chordin at the opposite pole of the embryo.

As with all good papers, new questions are raised. The great insight was taking a minimalistic approach [8], reducing the problem of scaling of embryonic size to Chordin and Sizzled protein accumulation (Figure 1C). However, the key role of the diffusion of dorsally secreted BMPs (ADMP and BMP2) in scaling [14] was not fully highlighted. Previous work has shown that ADMP depletion results in the loss of self-regulation by

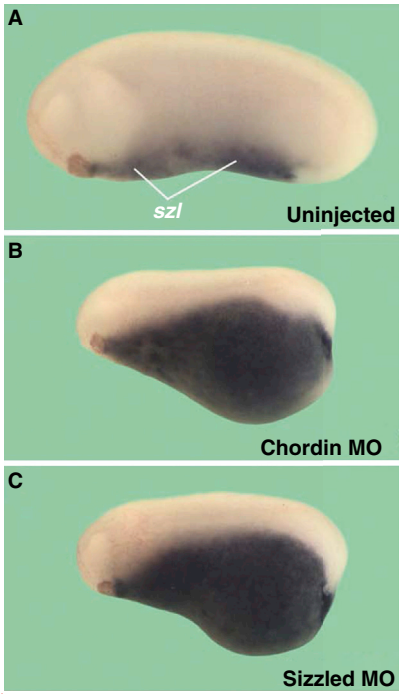

Figure 2. Chordin and Sizzled are key regulators of dorsal-ventral patterning.

Depletion of Chordin or Sizzled with morpholinos (MOs) results in identical high-BMP phenotypes marked by an expanded ventral domain of *sizzled* mRNA expression. [4].

dorsal half-embryos [6]. The new study [8] notes that epitope-tagged ADMP can diffuse to the ventral side (presumably shuttling together with Chordin). This observation is important, because the mathematics of diffusion-reaction equations are such that, in order to obtain a stable pattern it is necessary for an activator (which increases its own synthesis) and a long-range inhibitor to be produced from the same cellular source [15]. At the dorsal side (low-BMP), Chordin activates its own synthesis, while the more diffusible ADMP/BMP2 would correspond to the inhibitor [16]. On the ventral side, BMP4/7 activate their own transcription, while Sizzled (through inhibition of Tolloid) would act as the long-range inhibitor [4]. In the embryo, these two local circuits become interlocked because of the opposite transcriptional regulation of the dorsal and ventral centers by BMP signaling [6].

The Chordin/BMP/Tolloid pathway has an ancient evolutionary origin, regulating dorsal-ventral patterning in organisms as diverse as vertebrates, amphioxus, hemichordates, spiders and fruit flies [17]. However, the *Drosophila melanogaster* genome does not contain Sizzled (or any other secreted Frizzled-related protein). The homologue of Chordin, Short gastrulation (Sog), is a main regulator of dorsal-ventral patterning in flies. Self-regulation of pattern does occur in insects such as crickets [5]. Perhaps other extracellular regulators of BMP signaling — such as Crossveinless-2, Twisted gastrulation, or the non-competitive inhibition of Tolloid by BMP [12] — may take the role of the inhibitor in the high-BMP side, replacing Sizzled in fruit flies.

Among vertebrates, zebrafish, *Xenopus*, and chick embryos show a very robust expression of Sizzled in regions of high BMP signaling during gastrulation. However, the Sizzled gene was lost in the mammalian lineage. This correlates with the loss of vitellogenin (and therefore yolk in the egg) as well as other genes of the *Xenopus* dorsal-ventral pathway (ADMP, ONT1 and Crescent) [18]. It is possible that the Sizzled patterning system evolved to self-adjust the BMP gradient during epiboly movements in yolky eggs, in which the size of the blastopore is constantly changing in circumference while the BMP gradient remains constant.

Self-regulation in *Xenopus* is mediated by the diffusion of Chordin, BMPs and Sizzled over long distances. In *Drosophila*, Sog forms a gradient that shuttles BMPs away from the source of Sog in a very defined extracellular region, the perivitelline space [19,20]. In *Xenopus*, development is normal after removal of the vitelline membrane, implying that the dorsal-ventral gradient travels through the interior of the embryo. The next challenge will be to identify the region of the embryo in which the endogenous Chordin/Sizzled gradient is formed.

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