Simultaneous cleavage of both sites of proBMP4 leads to loss of activity in mice, perhaps due to disrupted interactions with the ECM. Zwaan and Hendrix (1974) hypothesized that simultaneous cleavage of both sites of proBMP4 leads to loss of activity because of the ECM. BMPs are generated as latent precursors that are proteolytically activated by proprotein convertases (PCs). ProBMP4 is initially cleaved at a site adjacent to the mature ligand domain (S1), and then at an upstream site within the prodomain (S2). Sequential cleavage of proBMP4 is driven in part by the presence of optimal (RXKR) and minimal (RXXR) PC consensus cleavage motifs at the S1 and S2 sites, respectively. When the S2 site of proBMP4 is mutated to an optimal consensus motif, the two sites are cleaved rapidly and stochastically rather than sequentially. Ectopic expression of this precursor (BMP4S2K) in Xenopus embryos generates a ligand with enhanced activity, suggesting that ordered cleavage restricts ligand activity. To test this hypothesis, we generated mice carrying a knockin point mutation that introduces an optimal consensus cleavage motif at the S2 site. Rather than generating a gain of function allele as predicted, BMP4S2K is a severe hypomorphic allele. Most BMP4S2K homozygotes die by E12.5, and they display reduced BMP-reporter activity. Equivalent levels of proBMP4 and cleaved prodomain are present in wild type and mutant embryos, indicating that the point mutation does not interfere with expression or cleavage of proBMP4. Surprisingly, pulse chase analysis suggests that both sites of proBMP4 are cleaved extracellularly. Because the prodomain of BMP4 binds to the extracellular matrix (ECM) protein, fibrillin, and cleavage at the S2 site dissociates the mature ligand from the prodomain, we hypothesize that simultaneous cleavage of both sites in proBMP4S2K causes premature release, or prevents the mature ligand from being deposited into the ECM, thus leading to loss of local signaling activity.

doi:10.1016/j.ydbio.2011.05.042

Proximal–distal patterning of the vertebrate limb is initiated by altered exposure to secreted signals
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The developing vertebrate limb has long been a model for understanding the complex processes that organize the embryo. The proximal–distal (PD) axis in particular, running from the shoulder to the tips of the fingers, has been an important example of the progressive patterning of embryonic structures. However, the mechanisms by which this is achieved have remained elusive. Using a novel system combining in vitro and in vivo culture, we demonstrate that limb PD patterning is independent of the length of time the undifferentiated limb cells grow and rather depends on the signaling environment they encounter. The proximal limb in particular is established through continued exposure to flank-derived signals, with the developmental program determining the distal segments only being initiated in the limb in domains that have grown beyond their influence. This result allows us to differentiate between two broad classes of models that have been proposed to explain PD patterning and puts this paradigmatic patterning problem.

doi:10.1016/j.ydbio.2011.05.042