128:3381–3393; Developmental Biology 255:290–302; BBRC 325:439–444). We found that in fission yeast Schizosaccharomyces pombe, the distribution of TH to the cell cortex was dependent on Shk1, the yeast homolog of PAK1. Here, we report that TH and Xenopus PAK1 (X-PAK1) are functionally related during Schizosaccharomyces pombe 325:439–444). We found that in fission yeast Schizosaccharomyces pombe, the distribution of TH to the cell cortex was dependent on Shk1, the yeast homolog of PAK1. Here, we report that TH and Xenopus PAK1 (X-PAK1) are functionally related during Xenopus neurulation. (1) TH distribution to the cytoplasmic membrane of neural cells is under the positive control of X-PAK1. (2) Loss of X-PAK1 activity impedes neural plate cells’ ability to form the neural fold, to proliferate, and to differentiate. These phenotypes are identical to the neural defects induced by the loss of TH. (3) The targeting of TH to the neural cell cortex by expressing CAAX-tailed TH enhances TH function and rescues the neural fold defect induced by the loss of X-PAK1 activity. (4) TH is an in vitro substrate for X-PAK1. Our results were obtained through the analyses of neural plate cells that gained or lost X-PAK1 kinase activity by expressing either constitutively active X-PAK1/DE kinase or X-PAK1/KR inactive (dead) kinase. Our results suggest that TH and X-PAK1 work in the same pathway during neurulation. This study was supported by grants from NSF (L.D.E.).


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In vivo time-lapse analysis of cell divisions during neural tube closure
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We have used 4D confocal microscopy to study cell divisions in the spinal cord of intact Xenopus embryos during neural tube closure. Using a histone-GFP, we find that the divisions of secondary neurons in the spinal cord are highly polarized, dividing predominantly at a 50–60° angle with respect to the midline. To study mitotic spindles dynamics a Tau-GFP was utilized. This has revealed that mitotic spindles in these cells initially set up in a random orientation and then rotate rapidly into this polarity. By use of a Clip170-GFP growing and shrinking microtubules were examined. We find that astral microtubules probe the cell cortex during spindle rotations, indicating that some polarization cue may be present at the cortex to guide spindle orientation. In contrast to similar polarized cell divisions in the fish, disruption of previously studied planar cell polarity genes, such as Dishevelled and Frizzled, had no effect on this polarity. However, we do find evidence of a requirement of Cdc42 in polarizing these cell divisions. Work to examine how this protein is involved in spindle orientation and rotation is underway.

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Expression of Panza, an α2-macroglobulin, in a restricted dorsal domain of the primitive gut in Xenopus laevis
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α2-Macroglobulin is a major serum protein with diverse functions, including inhibition of protease activity and binding of growth factors, cytokines, and disease factors such as β-amyloid. We have cloned and characterized Panza, a new Xenopus laevis α2-macroglobulin. Panza has 56–60% amino acid similarity with previously identified Xenopus, mouse, rat and human α2-macroglobulins, indicating that Panza is a new member of the α2-macroglobulin family. Panza mRNA is first detected at the beginning of neurulation in the dorsal endoderm lining the primitive gut (archenteron roof). At the completion of neurulation and continuing through the late tadpole stage, Panza is restricted to a dorsal domain of the gut endoderm adjacent to the notochord and extending along the entire anterior–posterior axis. With outgrowth of the tailbud, Panza expression persists in the chordaneural hinge at the posterior end of the differentiating notochord and extends into the floor plate of the posterior neural tube. As gut coiling commences, Panza expression is initiated in the liver and the dorsal domain of Panza expression becomes limited to the midgut and hindgut. With further gut coiling, strong Panza expression persists in the liver but is lost from other regions of the gut. The expression of Panza in endodermal cells adjacent to the notochord points to a potential role in signal modulation and/or morphogenesis of the primitive gut.

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Xenopus tropicalis genetics identifies chromatoblast stem cell mutant
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We are developing frog genetics in Xenopus tropicalis and have identified eighteen confirmed mutations so far. We show that multigenerational mutant analysis is feasible and demonstrate that mutations can be identified, propagated, and readily characterized using hybrid, dihybrid, and even trihybrid crosses. Here, we document our progress in developing Xenopus as a genetic model organism. Several of the mutants are described including a recessive mutation leading to a defect in chromatoblast stem cell differentiation. The mutant shows normal melanocyte development but lacks the two other types of skin pigment cells: iridophores.

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