the selective transport of p75 in polarized cells involves selective cargo binding. Cargo binding depends on phosphorylation of the cargo-binding region of the motor as well as on scaffolding proteins that link the motor to its cargo (Lee and Hollenbeck, 1995; Blasius et al., 2007). It could be that efficient cargo binding depends on modifications or scaffolding proteins that are only present in polarized cells.

Finally, because only the final distribution of p75 is assessed in the presence of impaired KIF5B function, there is another possible mechanistic explanation for the data presented by Jaulin et al. (2007). Instead of mediating transport, KIF5B could mediate the budding of p75-containing post-Golgi carriers from the TGN in polarized cells. It was previously shown that, in unpolarized cells, the budding of tubules (though not of vesicles) from the TGN depends on kinesins (Kreitzer et al., 2000). The step at which KIF5B is acting could be determined by high-resolution, time-lapse imaging of individual p75-containing carriers in the presence of KIF5B inhibition.

In any case, Jaulin et al. (2007) show that, in the presence of KIF5B inhibition, there is a clear difference in the mechanisms used for redistribution of p75 in polarized versus unpolarized MDCK cells. This differential regulation of p75 transport suggests that the development of epithelial cell polarity is accompanied by a major reorganization of microtubule-based transport, which may include specificity in motor-cargo interactions along with the development of specialized microtubule "tracks" that lead these motor-cargo pairs to specific subcellular destinations. Further work is needed to fully understand the intricacies of this reorganization and how specific kinesins and microtubule modifications could contribute to protein targeting in polarized epithelial cells.

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miRNA in Embryonic Development: The Taming of Nodal Signaling

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The TGF-β family member Nodal is one of the most potent molecules known to an embryo. Studies on Nodal regulation thus far have focused on its transcription, maturation, and interaction with antagonists. Two recent studies on the role of microRNAs (miRNAs) in Nodal signaling now reveal that something important was missing from the picture.

During embryonic development Nodal activates a signaling pathway primarily known in vertebrates for being essential to the induction and patterning of the mesoderm and the establishment of the left-right axis (Shen, 2007). Nodal signals via a receptor complex that comprises type I and type II serine/threonine receptors and an EGF-CFC coreceptor—once activated, this complex phosphorylates the intracellular transducers Smad2 or Smad3, which in turn trigger the transcription of target genes. Among these is *Nodal* itself, thereby promoting its own expression, as well as *Lefty* and *Cerberus*, which code secreted antagonists of Nodal signaling. This highly conserved pairing of both a positive and a negative autoregulatory feedback loop in the regulation of *Nodal* expression, and the regulation of the processing of *Nodal*'s immature form, have so far provided the explanation for its dynamic pattern and the very delicate modulation of its activity.

Mature miRNAs are ~22-nucleotide-long regulatory RNAs (Bushati and Cohen, 2007). They are generated after cleavage of a longer precursor, called a pri-miRNA, a process involving the Dicer enzyme. A miRNA blocks translation and/or promotes the degradation of mRNAs harboring in their 3′UTR a sequence complementary to a specific stretch of seven to eight nucleotides present at the miRNA 5′ end (called the "seed" sequence). Scores of miRNAs have been found and computer predictions have identified hundreds of possible targets for each of them. Several studies, using loss-of-function or gain-of-function approaches, have confirmed their role in various processes. Such is the case for the zebrafish miR-430 family. Previous work in the Schier lab had shown that miR-430, abundantly expressed during early development, is necessary for proper brain morphogenesis and the clearance of maternal mRNAs (Giraldez et al., 2005, 2006). The latter requirement suggests that miR-430 facilitates a smooth transition from one developmental state to another.

A new study from the same lab (Choi et al., 2007), published in *Science*, now focuses on the impact of miR-430 on the modulation of the activity of the Nodal signaling pathway. A computer search of 3′-UTRs identified a *Nodal*-related gene called *squint* (*sqt*) and the *Lefty* genes *lft1* and *lft2* as potential targets of miR-430. The authors demonstrate that this prediction is correct and that the activity of *sqt*, *lft1*, and *lft2* is repressed by miR-430. To address the physiological role of these interactions, they design antisense morpholinos that overlap with the miRNA target site and yet are specific to one species of mRNA. They prove the effectiveness and the specificity of these morpholinos—called target protectors (TPs)—and go on to inject either sqt-TP$^{miR-430}$ or lft2- TP$^{miR-430}$ into embryos to assess the role of miR-430 repression on the activity of each of these genes. Predictably, the injected embryos display phenotypes consistent with having been subjected to a higher level of Nodal signaling in the first case or a lower level of Nodal signaling in the second. To find out whether it matters if the embryo simultaneously dampens both *sqt* and *lft2*, the two TPs are coinjected. Interestingly, this does not seem to affect the injected embryos much at the blastula stage, but a stronger phenotype appears during gastrulation, when a reduced number of endodermal and forerunner cells are produced, which is evidence that Nodal signaling was compromised. The authors therefore suggest that miR-430 is necessary to balance the counteracting inputs of *sqt* and *lft*. The fact that the embryo copes better with increased levels of miR-430-sensitive *sqt* or *lft* mRNA than with loss of miR-430-mediated regulation for each of these mRNAs also suggests that miR-430 contributes to the robustness of embryonic development. The mode of action of miRNAs and their broad implication in the determination of the size of progenitor fields, as shown in this study, designate them as potent agents in the evolution of phenotypic changes.

While the miR-430 target sites that were identified by the above study in zebrafish are also present in the *Nodal* and *Lefty* genes of amphibians and mammals, suggesting a possible conservation of this mode of regulation in these species, a second study, from the Piccolo lab and published in *Nature* the same week (Martello et al., 2007), concentrates on a miRNA-mRNA interaction that does not appear to be conserved in teleosts. Yet it plays a critical role in the regulation of the Nodal signaling pathway in *Xenopus*, and may explain how the initial asymmetry in Wnt/β-catenin, set up after fertilization, translates into a gradient of Nodal activity and leads to the dorsal localization of Spemann's organizer.

An in silico inspection of core components of the Nodal signaling pathway in *Xenopus* led the authors to focus on the interaction between the miRNAs miR-15 and miR-16 and the mRNA for Acvr2a, a type II receptor for Nodal and Activin ligands that is found to possess two conserved putative binding sites for miR-15 and miR-16 in its 3′UTR. They carefully demonstrate that the expression level of the miRNAs affects the amount of Acvr2a protein produced and the activity of the Nodal/TGF-β/Smad signaling pathway—more miR-15 and miR-16 means less Acvr2a protein and an attenuation of Nodal/Activin signaling. miRNA-injected embryos show a marked reduction in the strength and breadth of gene expression for organizer and mesoderm markers at the gastrula stage, and exhibit anterior defects typical of a deficit in organizer function later on. Coinjection of an Acvr2a mRNA desensitized to miR-15 and miR-16 action efficiently rescues the expression of organizer markers, demonstrating that it is through Acvr2a that the miRNAs exert their action. Loss-of-function experiments, making use of 2′-O-methyl antisense oligonucleotides (anti-miRNAs) or of morpholinos to inhibit miR-15 and miR-16, lead to increased responsiveness to Nodal signaling and an expansion of organizer tissue, confirming the critical role of this novel regulatory layer in determining the size of the Spemann's organizer.

The authors then demonstrate that while the expression of the precursor for miR-15 and miR-16 is uniform along the dorso-ventral axis of the embryo, it is more efficiently processed in the ventral region, thereby generating a ventral-to-dorsal concentration gradient of the mature miRNAs. As a result, the concentration of Acvr2a is highest on the dorsal side, where it generates a peak of phosphorylated Smad2, prompting the authors to suggest that this is a key step in the establishment of the Spemann's organizer. Finally, they find that the processing of the pri-miRNA is inhibited by Wnt/β-catenin signaling on the dorsal side, and that this takes place early on, before the midblastula transition that marks the onset of zygotic transcription. These results bring into focus the tight control exerted by the Wnt/β-catenin pathway over the induction of the Spemann's organizer. Not only is it contributing to the transcriptional activation of *Nodal*-related organizer genes, it also restricts responsiveness to their gene products to the dorsal side of the embryo.

How much of this applies to mammals? Possibly, quite a lot. Martello et al. (2007) show in cell cultures that the interaction between miR-15 and

Acvr2a is conserved in humans. In addition, while zebrafish embryos depleted for maternal and zygotic Dicer manage to establish an axis and even form somites, mouse embryos mutant for *Dicer* fail to establish a body plan, which demonstrates a greater and earlier reliance on miRNA-mediated regulation in mammals (Bernstein et al., 2003; Tang et al., 2007). As well as being necessary for the patterning of the early embryo, the Nodal signaling pathway has been implicated in the maintenance of stem cells' pluripotency and in tumorigenesis. These two studies open exciting perspectives for the study of these phenomena.

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Putting the Brakes on Cytokinesis with α-Actinin

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Although signal transduction pathways provide spatiotemporal control of cytokinesis, additional regulation likely occurs through complex cytoskeletal network interactions. In this issue of *Developmental Cell*, Mukhina et al. (2007) show that myosin-II modulates the cortical lifetime of the actin crosslinker α-actinin, which in turn tunes actin filament dynamics, thereby controlling furrow ingression.

Cytokinesis is the process of reshaping one cell into two daughter cells and is driven by actin, actin crosslinkers, and myosin-II. Classically, cytokinesis contractility is thought to occur through the constriction of a sarcomeric-like contractile ring (a purse-string) of actin and myosin-II filaments. However, contractile ring structure varies widely among different organisms, from a highly ordered ring in *S. pombe* to a more disordered actin network in some mammalian cell types (including the normal rat kidney [NRK] cells used in the study by Mukhina et al., [2007]). α-actinin is an actin crosslinker that localizes to the cleavage furrow region in a variety of cells, from yeast to mammals. Yet, until Mukhina et al. (2007), α-actinin's function in cytokinesis in a cell type with a more disorganized contractile network had not been studied. Mukhina et al. (2007) discovered that α-actinin modulates furrow ingression dynamics and actin turnover and that myosin-II activity modulated the lifetime of α-actinin at the equatorial cortex.

To determine how α-actinin contributes to cytokinesis, Mukhina et al. (2007) studied how increasing or decreasing α-actinin expression levels influences the actin cortex and cytokinesis fidelity. When they overexpressed α-actinin, furrow ingression slowed down or reversed, leading to failure of cytokinesis. Under these conditions, the authors observed increased equatorial F-actin levels and slower actin turnover, which may explain the effects on furrow formation. Increased concentration of F-actin at the furrow likely increases viscoelasticity, slowing the removal of cytoskeleton and cytoplasm during furrow ingression. Higher concentrations of α-actinin may also enhance the stability of the polymerized actin network. Indeed, one function of myosin-II at the cleavage furrow is to increase actin turnover, facilitating furrow ingression (Murthy and Wadsworth, 2005).

Conversely, when Mukhina et al. (2007) reduced α-actinin expression by RNAi, ectopic furrows formed and furrow ingression rates increased. Again, the impact of lowering α-actinin levels on cytokinesis shape changes may be through α-actinin's modulation of F-actin, since F-actin levels are reduced by α-actinin RNAi. These results suggest that inhibition of actin turnover by α-actinin may act as a brake to