Although considerable work remains in the continued quest to delineate the molecular and mechanistic basis of evolutionary change within and between species, the work of Chan *et al.* [8] provides some of the best evidence yet that parallel genetic changes in the response to a selective pressure may be an important component in the adaptive evolution of polygenic traits. In many ways, the more we learn about evolution the more it resembles François Jacob's famous analogy that evolution is a tinkerer [17], an imperfect process that makes do with the parts (genetic variation) that are available to it.

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Pronuclear Migration: No Attachment? No Union, but a Futile Cycle!

How do pronuclei migrate towards each other? The zebrafish *futile cycle* gene is shown to encode a maternally expressed membrane protein required for nuclear attachment and migration along the sperm aster.

Phuong A. Nguyen*, Keisuke Ishihara, Martin Wühr, and Timothy J. Mitchison

During fertilization, the male and female pronuclei migrate toward each other and congress to mix genetic materials from both parents. In many animal species, the sperm donates the sole pair of centrioles. The centrosome — essentially centrioles coated with microtubule-nucleating material — thus assembles near the male pronucleus and nucleates microtubules for the sperm aster [1]. Once contacted by the expanding sperm aster, the female pronucleus moves along the microtubules toward the centrosome, close to the male pronucleus (Figure 1A), in a dynein-dependent manner [2–4].

One approach to elucidate pronuclear migration mechanisms is forward genetics. This requires maternal effect mutations that alter the molecular composition of the egg while allowing the mother and her eggs to develop. The maternal-effect mutation *futile cycle* in the zebrafish zygote abolishes pronuclear congression and DNA segregation in subsequent mitoses. Early embryonic cell cycle progresses normally and cytokinesis still occurs, orchestrated by centrosomes and microtubule asters without DNA, thus resulting in enucleated cells [5]. In this issue of *Current Biology*, Lindeman and Pelegri trace the *futile cycle* mutation to a gene encoding a vertebrate-specific *lymphoid-restricted membrane protein* (Lrmp) [6]. Lrmp protein localizes mainly to the nuclear envelope. In the metaphase spindle, where nuclear envelope is absent, the Lrmp protein is found juxtaposed with the centrosomes at the spindle poles. In *futile cycle* mutant zygotes, centrosomal material is detached from both pronuclei, which stay far apart from each other, and no detectable Lrmp protein localization is observable. Using various localization approaches in fixed embryos and a novel method to genetically rescue the maternal effect mutation, the authors suggest that Lrmp provides a physical link between the nuclear envelope and microtubules [6].

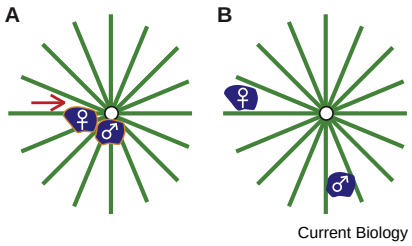

Figure 1. Lrmp protein is required for pronuclear migration in the zebrafish embryo.

(A) In wild-type embryos, the male pronucleus moves with the centrosome while the female pronucleus is captured by the microtubule aster and transported to the center, fusing with the male pronucleus. Lrmp protein (orange) localizes to the nuclear envelope. (B) In *futile cycle* (*fue*) embryos, where the encoded mutant Lrmp protein does not localize to the nuclear envelope, both male and female nuclei fail to interact faithfully with microtubules or centrosomes, and pronuclear fusion is abolished.

Lrmp homologues are present only in vertebrates [6], and were thought to be lymphocyte-specific. Previously characterized human and mouse Lrmp homologs localize to the endoplasmic reticulum (ER) in lymphocytes [7], where they are targeted post-translationally via a single transmembrane segment at the carboxyl terminus [8]. Their function has been unclear. Since the outer nuclear membrane is contiguous with the ER, how might zebrafish Lrmp target specifically to the nuclear envelope? One possibility is that Lrmp is a novel, vertebrate-specific component of a linker of the nucleoskeleton and cytoskeleton (LINC) protein pair, which has been best characterized in the invertebrates *Caenorhabditis elegans* and *Drosophila*. The general model posits that SUN (Sad1/UNC-84) proteins in the inner nuclear membrane and KASH (Klarsicht/Anc-1/Syne homology) proteins in the outer nuclear membrane create a nuclear envelope bridge via protein–protein interaction in the luminal space [9]. The carboxyl terminus of zebrafish Lrmp contains a predicted coiled coil, a transmembrane, and a luminal domain that partially aligns with the KASH consensus sequence [6]. To test whether this minimal carboxy-terminal fragment is sufficient for nuclear envelope targeting, as previously observed for a KASH protein in *C. elegans* [10,11], the authors expressed a GFP fusion by injecting mRNA into 1-cell embryos and detecting GFP fluorescence in embryos at the 8–12 cell cycle stage [6]. The carboxy-terminal fusion protein localized to both the nuclear envelope and the ER, partially recapitulating the localization of endogenous full-length Lrmp protein [6]. Further experiments will be required to test the hypothesis that zebrafish Lrmp might represent a novel KASH protein that localizes to the nuclear envelope by interacting with a yet to be identified SUN protein.

A fascinating aspect of zebrafish Lrmp is that its mRNA is localized to centrosomes and the mitotic spindle in wild-type embryos, the same place that the protein functions [6]. This suggests Lrmp may also be localized by local translation. The localization of the mRNA was completely abolished in *futile cycle* embryos [6], suggesting Lrmp protein may localize its own transcript, which would be a novel targeting mechanism in embryos. The mRNA was diffuse before fertilization [6], suggesting it is recruited to the sperm centrosome either during centrosome maturation, or as a consequence of microtubule nucleation. This raises the questions: how does Lrmp protein localize its own mRNA and what are the functional implications of the mRNA localization?

How mRNAs localize in embryos is a generally interesting question [12]. One possible mechanism for localization of the mRNA encoding Lrmp is that Lrmp protein itself might directly bind to its own mRNA. However, no predicted RNA-binding motif is present in Lrmp and preliminary RNA-immunoprecipitation experiments were negative [6]. A second possibility, not considered in the paper, is that Lrmp mRNA is transported to the centrosome by dynein, and this transport somehow depends on Lrmp protein. This could be tested by injecting the dynein inhibitor p150-CC1 into unfertilized embryos [13,14]. In the most parsimonious model, a dynein-dependent function of Lrmp could account for both localization of Lrmp mRNA to the centrosome as well as its requirement for pronuclear migration. The authors propose that co-translational localization of the mRNA and protein on the spindle near the reassembling nuclear envelope might be required for the specific membrane targeting of Lrmp. While this explanation might be attractive, especially in spatially large cells such as early zebrafish blastomeres, localized translation is not necessarily the only functional role of mRNA localization [15]. In any case, this finding may open new directions for the mRNA localization field: future studies should elucidate the role of the amino-terminal half of zebrafish Lrmp and its homologues, where

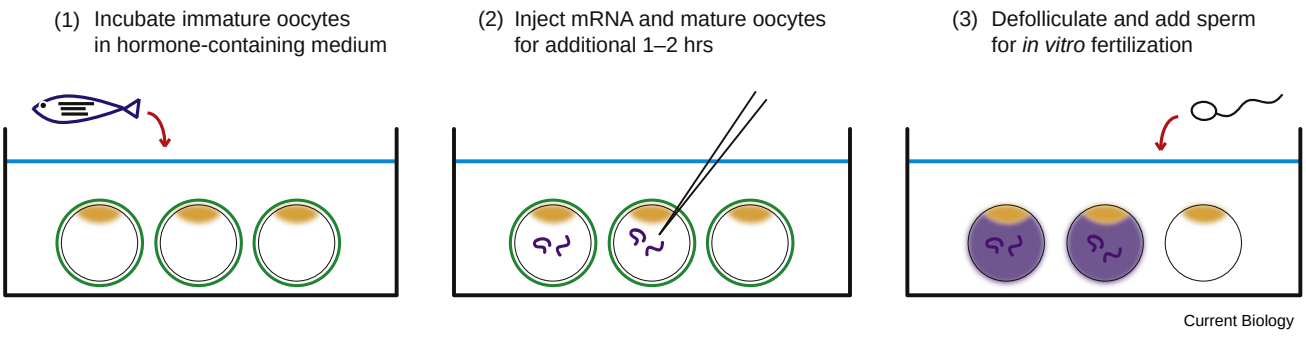

Figure 2. Genetic rescue of early embryogenesis in zebrafish.

As reported by Lindeman *et al.* [6], a new method that combines *in vitro* oocyte maturation, mRNA injection and *in vitro* fertilization allows rescue experiments for processes involved in early embryogenesis, such as pronuclear migration and fusion.

mutation in a single conserved residue results in abolishment of mRNA localization.

One exciting aspect of the paper is a new method for rescuing maternal effect mutations that could have wide applicability for research on early development in zebrafish. Early embryonic cell biology is governed by maternally inherited factors, as zygotic gene expression is not activated in zebrafish until the midblastula transition that initiates about 10 cell cycles into development [16]. Researchers therefore had to either raise genetically manipulated adults or inject protein shortly after fertilization to alter the protein content of cleavage-stage embryos [14]. In this study, the authors injected mRNA into immature oocytes. Once oocytes were matured and fertilized *in vitro* [17], the injected mRNA was fully expressed (Figure 2) [6]. By injecting antisense oligonucleotides into immature oocytes it might be possible to also knock down proteins of interest. While similar methods have been available for many years in *Xenopus laevis* [18], zebrafish embryos provide unique experimental advantages, especially for live imaging. The possibility to genetically manipulate and image zebrafish embryos during cleavage stages within a day rather than months should drastically increase the attractiveness of the zebrafish for early embryonic research.

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Cortical Circuits: Layer 6 Is a Gain Changer

Stimulation of excitatory cells in layer six of mouse visual cortex results in net inhibition of the spiking of neurons in upper cortical layers; this ascending intra-cortical drive provides a mechanism for gain modulation of sensory-evoked responses.

Mateo Vélez-Fort and Troy W. Margrie

Since the seminal work of the early neuroanatomists, and more recently of physiologists [1], the significance of the laminar organization of the neo-cortex has been a subject of intense investigation. Both *in vivo* and *in vitro* studies indicate not only a rich functional, genetic and morphological diversity of neurons within [2] and across layers [3] but also that intra- and inter-laminar connectivity is cell type and layer specific [4]. In work on rodents, much of the experimental focus has been on the whisker or barrel cortical system, where there is a somatotopic map of the vibrasse receptive field. This primary sensory area benefits from having been extensively mapped and its cytoarchitecture specifies distinct barrel columns — with known receptive fields — that can be repeatedly targeted in both the intact and sliced brain preparation [5]. From the wealth of electrophysiological and morphological data on this region, it is clear that there are stereotypical patterns in intra- and inter-layer connectivity and this has inspired the first attempts to model the function of a six-layered column of cortex [6]. But the precise function of specific layers and pathways within the cortex remains to be elucidated. In a recent study, Olsen *et al.* [7] have taken up this challenge and used an optogenetic approach to determine the effect of