VISUALIZING INTERNAL MESODERM MIGRATION DURING *XENOPUS LAEVIS* GASTRULATION USING DIl STAINING

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One of the most dramatic events during *Xenopus Laevis* gastrulation is the migration of mesoderm from its initial position in the blastocoel wall towards the interior of the embryo. This change in position is coupled with the change in cell sorting behavior which allows mesoderm to remain separated from the blastocoel roof cells as it advances towards the animal pole of the blastocoel (1). So far the details of mesoderm migration remained obscured by the lack of transparency associated with *Xenopus Laevis* embryos. Here we report a novel method for visualizing the advancement of mesoderm cells using point or linear DiI staining. Using DiI treated eyelash tips the stain is delivered to the inside of the embryo and depending on the depth of desired staining the resulting DiI trace will have a point or linear shape. Staining is performed at various stages of development after which the embryos are left to heal and to continue developing. Stained embryos are fixed at various time intervals, fractured and visualized under UV florescence. Acquired images are analyzed for various deformations of the DiI trace.

*Xenopus Laevis* embryos were obtained and staged according to Nieuwkoop and Faber (2). All of the experiments were repeated with at least 3 batches of embryos. Some of the embryos were not treated (wt, wild-type), (Fig 1.A), while the others received microinjections of either dnPak-1 (dominant negative construct of Pak-1, p-21 activated kinase) (Fig. 1 B, C), or a combination of dnPak1 and full length wtPak-1 constructs in equal aliquots (Fig.1 D). Microinjections were performed after the second cleavage into the marginal zone of two dorsal blastomeres.

The staining was done at stage 10 by locally applying small quantities of DiI cell tracking dye. This was accomplished by inserting the tip of a DiI treated eyelash into dorsal embryonic tissues. After a short time the eyelash was removed and the embryos were left to heal in a mixture of 2% Ficol and 1xMBS. The embryos were then left to develop until stage 10.5 in 1/10xMBS after which they were fixed in 4% formaldehyde, fractured along the line of staining and observed under UV light. Figure 1A shows the results observed in wild-type embryos. As expected, the mesoderm involutes from the marginal zone and then climbs along the blastocoel roof (BCR) towards the animal pole (1, 3). There is an obvious separation between the staining in the BCR wall and that of the stained mesoderm. In Fig.1 B the mesoderm failed to move along the BCR wall due to the effects of dnPak-1 injections. The effect was limited to the cells adjacent to the BCR as the cells slightly deeper to these managed to move past the affected cells (Fig 1C). Co-injections of full length wtPak-1 together with the dnPak-1 rescued the inhibitory effect of dominant negative construct as can be seen in Fig 1 D.
Figure 1. Dil staining of the Xenopus Laevis gastrula. All of the embryos were stained at stage 10 and visualized at 10.5. (A) As expected in wt embryos, the mesoderm has moved around the dorsal lip (white arrowheads) and up, past the point of initial staining in the BCR. (B) Dominant negative Pak-1 construct was injected dorsally and has caused mesoderm to remain attached to the BCR preventing it from moving upwards. (C) deeper cells remain unaffected by the dominant negative injection and so they move upwards partially by active migration and partially due to vegetal rotation. (D) rescue injections of wt Pak-1 and dnPak-1 and a recovery of normal mesoderm movement towards the animal pole of the blastocoel.

This approach allows us to study various molecules which potentially play a role in cell movements during gastrulation. By inhibiting or over expressing the activity of particular molecules of interest, it is possible to observe a block or a gain of function in the tissues of injected embryos by Dil staining and a subsequent analysis of the Dil trace. This simple staining procedure could be used wherever it is necessary to observe a relative movement between two or more layers of tissue.

Literature:

