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Attraction rules: germ cell migration in zebrafish

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The migration of zebrafish primordial germ cell towards the region where the gonad develops is guided by the chemokine SDF-1a. Recent studies show that soon after their specification, the cells undergo a series of morphological alterations before they become motile and are able to respond to attractive cues. As migratory cells, primordial germ cells move towards their target while correcting their path upon exiting a cyclic phase in which morphological cell polarity is lost. In the following stages, the cells gather at specific locations and move as cell clusters towards their final target. In all of these stages, zebrafish germ cells respond as individual cells to alterations in the shape of the *sdf-1a* expression domain, by directed migration towards their target — the position where the gonad develops.

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Introduction

In many different organisms, primordial germ cells (PGCs) are specified early in development in a position that is distinct from that of the gonad, the location where they differentiate into gametes [1–3]. Therefore, the cells have to reach the somatic part of the gonad in a process that, in addition to its crucial role in the propagation of the species, serves as a general model for long-range cell migration.

PGC migration has been studied in diverse vertebrate and invertebrate species. These investigations highlighted the important role of somatic cells that act along the migratory route, offering a permissive environment for cell migration and directing the cells by providing attractive or repulsive cues [2,3].

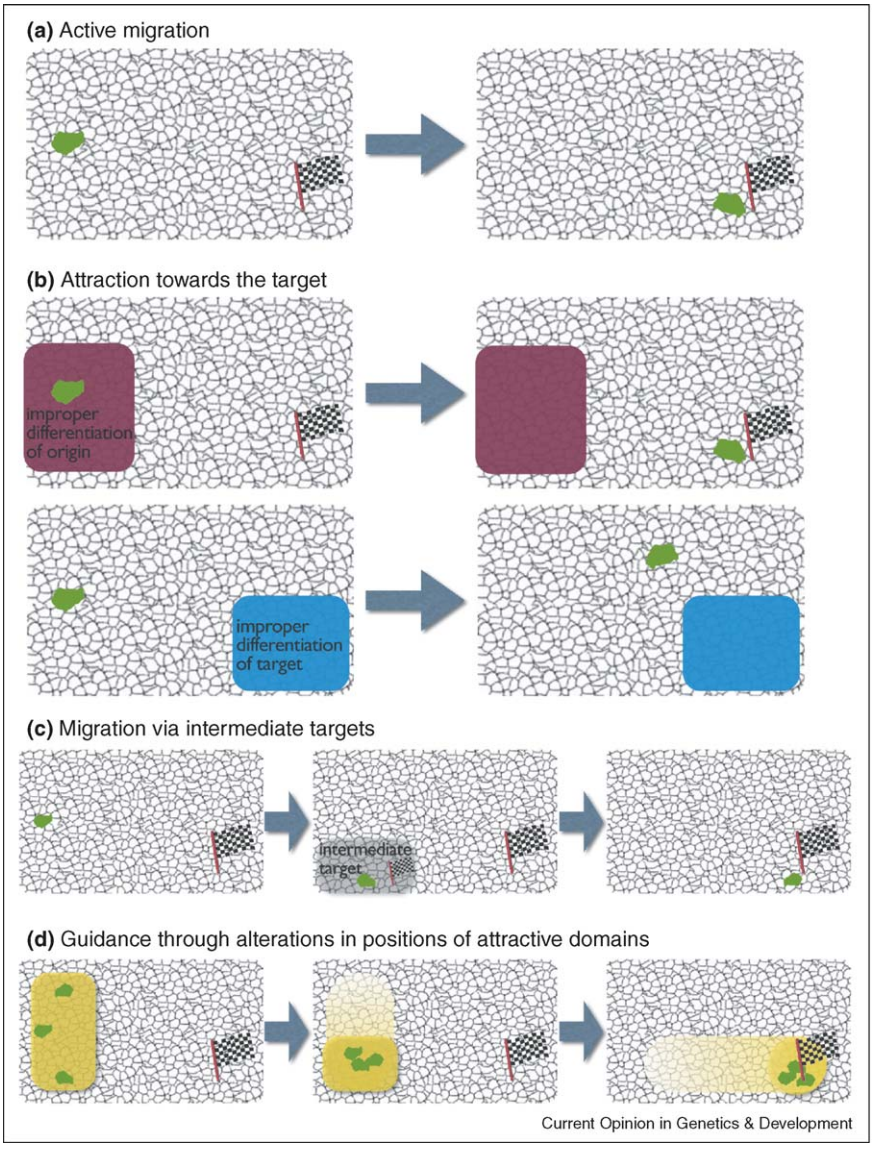
A relatively new model system in which PGC migration is being studied is the zebrafish [4]. A combination of forward and reverse genetic tools coupled with the extra-uterine development of the translucent embryo makes the zebrafish an excellent organism for investigating this process. In this review, we focus on recent insights into the mechanisms that enable zebrafish PGCs to arrive at their target with high reliability and precision.

Clues from embryological studies

PGC migration in zebrafish presents a unique and interesting developmental problem because the migrating cells are specified at four different locations that are randomly positioned with respect to the developmental axis of the embryo [5,6]. Despite this unusual starting point, practically all the PGCs arrive at the site where the gonad develops within the first day of development. The robustness of the process was clearly demonstrated by transplanting PGCs into ectopic positions within the early embryo, a manipulation that did not prevented proper migration of the transplanted cells towards their normal target [7].

The initial analysis of PGC migration in zebrafish embryos provided important clues regarding the developmental mechanisms responsible for the directed migration of the cells. First, throughout their migration, the cells exhibit active displacement owing to their intrinsic motility (Figure 1a) [8,9], rather than being passively pulled along by the movement of neighboring tissues or cells; this is in contrast with phases of passive movements of either *Drosophila* and avian PGCs, which move with the developing posterior midgut and translocate via the circulatory system, respectively [1,10]. Second, analyzing PGC migration in mutant embryos in which specific tissues do not develop properly supported the idea that attractive cues are responsible for directing the cells towards their targets [6,8]; consistent with cell guidance by way of attraction, abnormal development of the target tissues resulted in abnormal PGC migration, whereas faulty development of tissues vacated by PGCs proved to be of no consequence to the process (Figure 1b) [6,8]. Last, rather than aiming directly at their final target, the cells initially cluster at intermediate targets from which they move towards the region of the prospective gonad prospective gonad (Figure 1c) [8,9]. Although the actual molecules directing the PGCs were yet to be identified, these observations provided a principle explanation for the properties of PGC migration in wild type as well as in mutant embryos: namely, the findings were consistent with the idea that broad attractive domains attracting PGCs at the onset of migration alter their own shape to focus the cells into forming cell clusters. Moving the clustered cells to their final target could again be a result of shifting the attractive domain to another position. As

Figure 1



Zebrafish PGCs migrate actively in response to attractive cues and arrive at intermediate targets on the way to the final target. **(a)** A schematic representation of active PGC migration, during which the cells exhibit morphological shape changes and movement relative to their somatic neighbors. **(b)** Consistent with guidance by attractive cues, improper differentiation of the site of origin (purple) has no effect, whereas improper differentiation of the target site (blue), leads to migrational defects. **(c)** The migration towards the final target is indirect and involves arrival at an intermediate target (grey). **(d)** Alterations in the shape and position of attractive domains (yellow) are at the basis of cell clustering and arrival at the target.

the cells migrate from one position to another by active migration, the alterations in the shape and position of the attractive domains must result from changes in the relevant properties of the somatic environment, such as alterations in the transcription pattern of putative attractants (Figure 1d).

Molecular aspects of directional migration

The observations described above are in agreement with the notion that zebrafish PGCs are guided by attractive cues provided by somatic cells along their migratory route. The molecular identity of the receptor for these cues was revealed in two genetic screens. In these studies, a crucial role was demonstrated for CXCR4b, a chemokine receptor that is expressed in PGCs [11,12]. PGCs in which the activity of the receptor is reduced maintain their highly motile behavior but they do not show directed migration towards the target, and they therefore arrive at ectopic locations within the embryo. Of the chemokines SDF-1a (Stromal-derived factor-1alpha) [11] and SDF-1b [12], the two putative CXCR4 ligands that have been suggested to guide PGC migration, the RNA

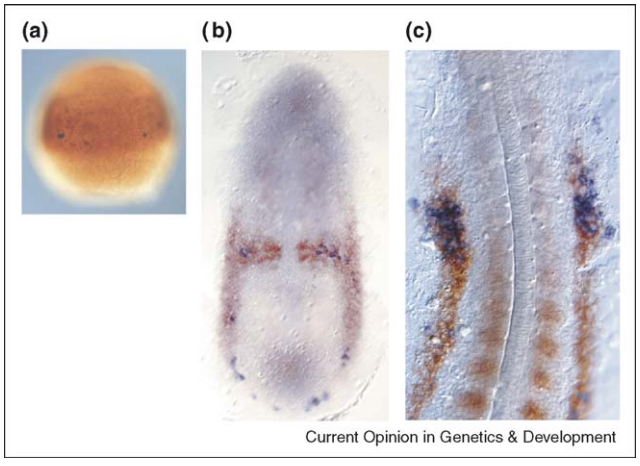
expression pattern of the former is more tightly correlated with the position of the migrating cells (Figure 2). Indeed, reducing SDF-1a activity causes more severe and more penetrant migrational defects than those inflicted by reduced SDF-1b. In keeping with the idea that the PGCs are attracted to sites of chemokine expression within the embryo, somatic cells engineered to express SDF-1a [11] or SDF-1b [12] in ectopic locations are able to attract PGCs there.

Following the identification of SDF-1a as the key cue for zebrafish PGC migration, it was pertinent to investigate the mechanisms controlling cell polarization and directed migration in response to changes in the chemokine expression pattern. The molecular machinery underlying the cellular response of zebrafish germ cells is unknown. It is known, however, that Gi proteins are likely to function downstream of the receptor, because expression of Pertussis toxin within the PGCs abrogates directional migration [13]. By contrast, phosphoinositide 3-kinase (PI3K) and its product phosphatidylinositol (Ptd-Ins) (3,4,5) P_3 (PIP_3), which are important for directing polarized pseudopod formation in neutrophils and *Dictyostelium discoideum* cells [14•], appear to play no such role in migrating PGCs in zebrafish [13]. PI3K is nevertheless important for attaining normal cellular morphology and cell motility; PGCs expressing a dominant negative form of PI3K show reduced cell polarity and stability of filopodia, as well as slower migration speed [13].

PGC behavior from specification to arrival at the target

A major advantage in studying PGC migration in zebrafish is the ease with which cell behavior can be examined *in vivo*. Labeling the migrating cells with fluorescent proteins and observing different steps during cell migration by means of time-lapse movies provides important insights into the cellular mechanisms facilitating cell motility and directed migration towards the target.

Figure 2



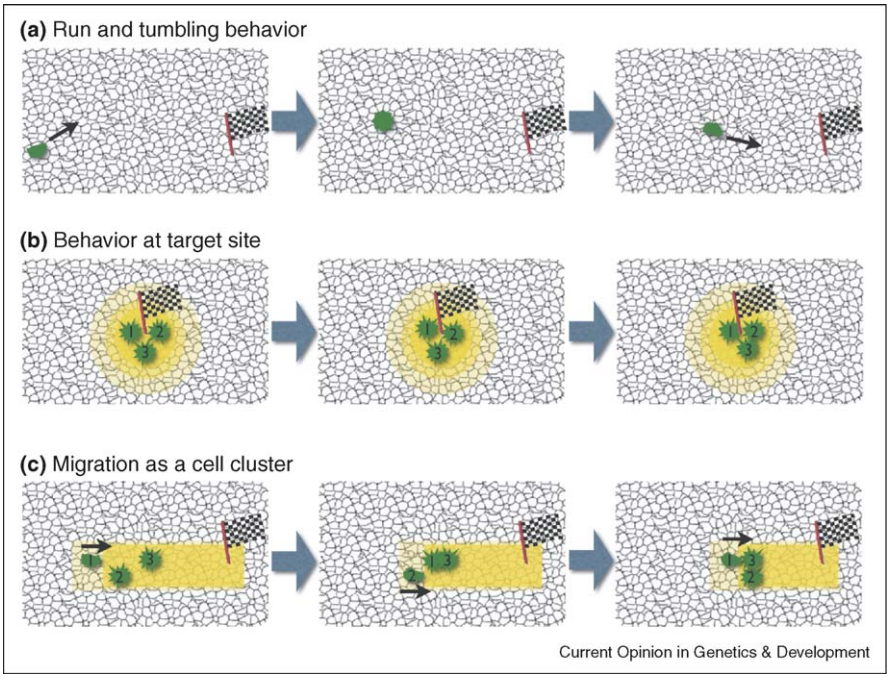
The RNA expression pattern of *sdf-1a* (brown) is tightly correlated with the position of the migrating PGCs (blue) in different stages of embryonic development. **(a)** Soon after PGCs mature to become migrating cells, they move as individual cells. **(b)** PGCs on the way to an intermediate target. **(c)** PGCs migrate toward the final target as single cells in a cluster. Reproduced with permission from Doitsidou *et al*. [11].

The generation of transgenic fish carrying a construct that includes a maternal promoter fused to the green fluorescent protein gene, as well as elements directing specific stabilization and translation of the RNA to the germ cells enabled monitoring of PGC behavior from the earliest stages of their development [15•]. Findings of particular interest yielded by this analysis were the cellular events following PGC specification, which culminate in the transformation into highly motile cells that respond effectively to chemotactic cues. This transition is observed in different processes in development and disease (e.g. in the development of neural crest cells in vertebrates [16], or border cells in *Drosophila* [17,18], and in metastasizing cancer cells [19]). In zebrafish PGC development, this process could be divided into three steps that together span the first 1.5 hours subsequent to PGC specification. During the initial 30 minutes, PGCs are morphologically indistinguishable from their somatic neighbors, showing a simple, round morphology and no active migration. In the next phase, the PGCs extend multiple protrusions in all directions, and yet do not actively migrate. It is only 1.5 hours following their specification that the PGCs appear polarized, extend broad protrusions in the direction of migration and move relative to somatic cells. These changes are PGC-autonomous, because cells that have matured to become migrating cells retain these properties when transplanted into early embryos. The significance of these early events for guided migration was demonstrated by challenging the cells with a gradient of SDF-1a; in contrast to mature cells that displayed directed long-range migration towards the source of the chemokine, cells that have not completed their early maturation showed no response to SDF-1a [15•].

Although the molecular or cytoskeletal basis for the early maturation steps of the PGCs are not known, one gene product whose function was shown to be essential for PGC motility is the putative RNA-binding protein Dead end [20]. Interestingly, PGCs depleted of Dead end perform normally during the first two phases of their development, demonstrating the passage from round to protruding cells [15•]. Yet although these cells cease the protrusion activity, they fail to proceed into the last phase, because they do not polarize or form broad pseudopodia and do not actively migrate [15•]. Identifying the RNA substrates of Dead end and determining their biochemical function would therefore provide meaningful insights into the molecular requirements for active cell migration.

Once PGCs acquire the ability to migrate, they move as individual cells until they form two cell clusters on either

Figure 3



Modes of behavior during PGC migration. **(a)** Individually migrating cells periodically stop moving, lose their polarity and correct their migration path. **(b)** After arrival at their target, the PGCs positioned at a local peak of SDF-1a (yellow) expression show no polarity and exhibit only limited movement relative to each other. **(c)** Migrating as a cluster, PGCs that are positioned where SDF-1a expression is reduced (light yellow) respond individually by migrating into sites of higher chemokine levels (strong yellow).

side of the body axis (Figure 2). Remarkably, throughout their migration the PGCs cycle between 'run' phases, during which they are polarized and actively migrate, and 'tumble' phases, in which they lose their polarity and remain on the spot (Figure 3a) [21•]. The loss of cell polarity during tumbling phases is followed by reacquisition of cellular polarity and resumption of the migration. Considering that the majority of the changes in the course of PGC migration occur after the periodic loss of cell polarity, it is conceivable that the function of tumbling is to allow for corrections in the route of migration and, thus, to facilitate precise arrival at the target.

Upon reaching the clustering site, where stable high-levels of *sdf-1a* RNA are detected, the cells maintain their position for several hours. During this stage, the cells extend small protrusions in all directions and barely move (Figure 3b) [21•]. This behavior, which is significant for cells arriving at their target where they participate in building tissues and organs, presumably results from a uniform peak activation of the receptor at the cell membrane.

Another pattern of zebrafish PGC behavior was identified when cells were examined as they migrate as a cell cluster toward the site of the developing gonad (Figure 2c) [21•]. The conclusion from this analysis was that despite the close proximity of the migrating cells to one another their migration is not coordinated. Rather, the movement of the cluster is the sum of the independent migration of individual cells in response to alterations in the distribution of the chemokine SDF-1a (Figure 3c).

Conclusions

Irrespective of the PGC site of origin, their migration route or the mechanism by which the cells are specified, an important aspect of PGC migration in different organisms lies in the functional conservation of the attractant, the chemokine SDF-1, and its receptor, the 7-transmembrane receptor CXCR4. In addition to controlling PGD migration in zebrafish, SDF-1 is involved in the same process in mouse and chick embryos [22,23]. Furthermore, this conservation appears to extend to *Drosophila*, in which a 7-transmembrane protein was reported to be involved in PGC migration [24]. However, important differences between the role of SDF-1a in zebrafish and that in other organisms should be highlighted.

In contrast to the idea that SDF-1a plays a central role in PGC migration in zebrafish, findings in other model organisms depict a much more complicated scenario in which passive displacement of the cells and guidance by a number of attractive and repulsive cues other than SDF-1 direct the cells towards their target [2,3]. Other than HMGCoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, the activity of which is crucial for the attraction of *Drosophila* PGCs [25] and which was shown to be

required for optimal PGC migration speed in zebrafish [26], no additional molecule was systematically examined for its role in zebrafish PGC migration. It would be particularly interesting to determine whether enzymes and signaling molecules such as Wunen molecules and Hedgehog in *Drosophila*, which generate a repulsive and attractive environment, respectively [27–30], and the interferon-induced transmembrane protein, which generates a repulsive environment for mouse PGCs [31], play an equivalent role in PGC migration in zebrafish. It is possible, however, that the special onset of PGC migration from random positions within the zebrafish embryo dictates a unique solution that is centered on a single guidance cue, SDF-1a.

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