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What Is Left Behind—Quality Control in Germ Cell Migration

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During all stages of animal development and adult homeostasis, the processes of cell differentiation, cell migration, cell death, and cell proliferation are tightly controlled. Failure to regulate these processes can lead to tumor formation and metastasis. For example, defects in cell migration and the ensuing ectopic location of cells in the body can result in deregulated cell proliferation, invasive behavior, and cell spreading—features that characterize tumorigenic cells. A strategy to prevent tumor formation is the disposal of these aberrant cells by induction of cell death.

Primordial germ cells (PGCs) are an excellent model for studying guided cell migration in vivo. These cells are specified early in development and typically migrate from their site of origin toward the gonads, where they differentiate into sperm or eggs (*1*). Despite the accuracy with which PGCs have been observed to arrive at their targets, some PGCs do not reach the gonads and are left behind at ectopic locations. Ectopic PGCs are often found in midline structures, a location at which human pediatric germline tumors commonly arise (*2*). It is therefore thought that PGCs escaping elimination during embryogenesis give rise to such extragonadal germline tumors (*3*). Thus, in addition to defining the mechanisms involved in guiding PGC migration, it is important to understand the processes responsible for the elimination of ectopic cells.

After their formation, *Drosophila* PGCs are internalized and become associated with cells of the midgut. Guided by repulsive and attractive cues, the cells leave the midgut and migrate away from the midline in two groups to form two cell clusters at the region where the gonad develops. Cells that fail to vacate the midline region die in a process that depends on two functionally redundant gene products, Wunen and Wunen-2 (*4*) (Fig. 1). These two genes encode extracellular lipid phosphate phosphatases (LPP3) that dephosphorylate yet-to-be identified phospholipids. The *Wunen* genes were originally identified as directive cues on the basis of their role in generating a repulsive environment for migrating PGCs (*5*–*7*). The expression of Wunen in the central nervous system, which is located at the midline of the *Drosophila* embryo, is necessary and sufficient for directing migrating PGCs away from the midline and toward their target. It was later suggested that the Wunen substrate might also be a survival factor and that Wunen activity depletes this survival signal from the extracellular environment (*8*, *9*). Thus, *Drosophila* PGCs appear to migrate to locations with low Wunen activity and large amounts of survival-promoting phospholipids. Conversely, when positioned in structures with high Wunen activity (such as the midline), they die, presumably due to insufficient levels of the phospholipid.

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Although different molecules are involved, a similar course of events has been recently described by Runyan *et al*. for mouse PGCs (*10*) (Fig. 1). Responding to multiple guidance cues, mouse PGCs start their active migration from the posterior primitive streak region into the adjacent endoderm, which gives rise to the hindgut (*11*–*13*). In a process that is qualitatively similar to that in *Drosophila*, PGCs leave the hindgut and separate into two clusters on either side of the midline. Cells that migrate properly arrive at the two clusters, where they interact with somatic gonadal precursor cells in the genital ridges and eventually differentiate into gametes. In contrast, PGCs that fail to emigrate from the gut remain in the midline region and are eliminated (*14*).

A survival signal for PGCs present in the midline during early migration is Steel, the c-Kit ligand (*15*–*19*). The receptor tyrosine kinase c-Kit and its ligand were initially identified as genes important for a wide range of developmental processes such as pigmentation, hematopoiesis, and germ cell survival and migration (*20*, *21*). Careful analysis of hypomorphic mutations in the *Steel* locus encoding the ligand suggested that the c-Kit–Steel pathway functions in promoting PGC survival, proliferation, and migration depending on the stage of development (*22*). Recent work by Runyan and colleagues aimed at exploring the mechanisms controlling the elimination of the PGCs from the midline (*10*) supports this idea.

Steel-deficient mice show a progressive decrease in the number of PGCs that is mediated by the proapoptotic Bcl2 family member Bax (*23*, *24*). Runyan and colleagues used double-knockout mice lacking both Bax and Steel ($Steel^{-/-}$ $Bax^{-/-}$ mice) to elucidate the precise mechanism of PGC elimination in this context (*10*). In wild-type mice, apoptosis of PGCs that remained at the midline was enhanced compared to that of non-ectopic cells. Analysis of RNA levels in PGCs showed specific up-regulation of genes encoding proteins involved in the intrinsic apoptotic pathway (Bax, Bak, Bad, Bim, Caspase 3) and no expression at this stage of genes encoding proteins involved in the extrinsic apoptotic pathway (Fas, Caspase 8). Although c-Kit protein was found in the PGCs irrespective of their developmental stage or position, the abundance of the ligand Steel was reduced in the midline at the time of PGC elimination. This suggests that maintenance of PGCs depends on survival signals mediated through Steel–c-Kit signaling.

Further analysis of Steel-Bax mutant mice revealed a role for Steel in supporting cell survival at early stages of development and in promoting PGC proliferation at later stages when PGCs have migrated from the midline. Compared to wild-type mice, $Steel^{-/-}$ $Bax^{-/+}$ mice showed reduced numbers of PGCs at early stages of development, a phenotype that was rescued in $Steel^{-/-}$ $Bax^{-/-}$ mice. This supported the hypothesis that germ cells are normally protected from Bax-dependent apoptosis by Steel-Kit activity. At later stages of development, germ cell numbers in $Steel^{-/-}$ $Bax^{-/-}$ mice were similar to those found during earlier

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stages. Independent of Bax function, mice carrying an intact allele of *Steel* showed markedly increased germ cell numbers at later stages of development. Thus, beyond its role in germ cell survival, Steel is required for germ cell proliferation once cells leave the gut. Runyan *et al*. suggest that, in addition to its roles in controlling cell proliferation and survival, Steel is also important for PGC migration: In Steel$^{+/+}$ Bax$^{-/-}$ mice, PGC migration was normal, whereas in Steel$^{-/-}$ Bax$^{-/-}$ mice, most germ cells failed to emigrate from the hindgut. This is markedly similar to the proposed mechanism of PGC elimination in *Drosophila* (Fig. 1). In both organisms, removal of a survival factor (removal of phospholipids by Wunen activity or down-regulation of Steel expression in the mouse) seems to be responsible for guiding cells away from the midline and to eliminate those PGCs unable to follow.

In the case of Steel, however, it remains unclear whether the survival signal itself acts as a guidance cue or whether the failure of germ cells to leave the hindgut is due to other reasons. Mouse PGCs are guided toward the genital ridges by the chemokine CXCL12 (*25*). The receptor to CXCL12, CXCR4, is normally expressed in mouse PGCs, and the authors speculate that Steel absence could lead to a down-regulation of CXCR4 expression and thus prevent directional migration. Alternatively, the directional cue conferred by CXCL12 secretion from the genital ridges might be a short-range signal that is too far to elicit a chemotactic response in germ cells in the midline.

When studying guided cell migration, it is common to focus on the mechanisms that are responsible for guiding the cells toward their target. Nevertheless, the possible pathological consequences resulting from inaccuracies in the process argue that more attention should be given to the mechanisms responsible for eliminating cells that end up in the wrong environment along the migration route. The identification of the molecules involved in PGC elimination in the mouse and the fact that similar events were described in other organisms suggest that this process is crucial for proper development and survival. In addition, a close investigation of the involved processes reveals that a single signaling pathway can play different roles in the same cell population at different stages of development or cell migration. Integrating information from different model systems will provide a better understanding of this phenomenon by filling gaps in our knowledge of one system with information from another.

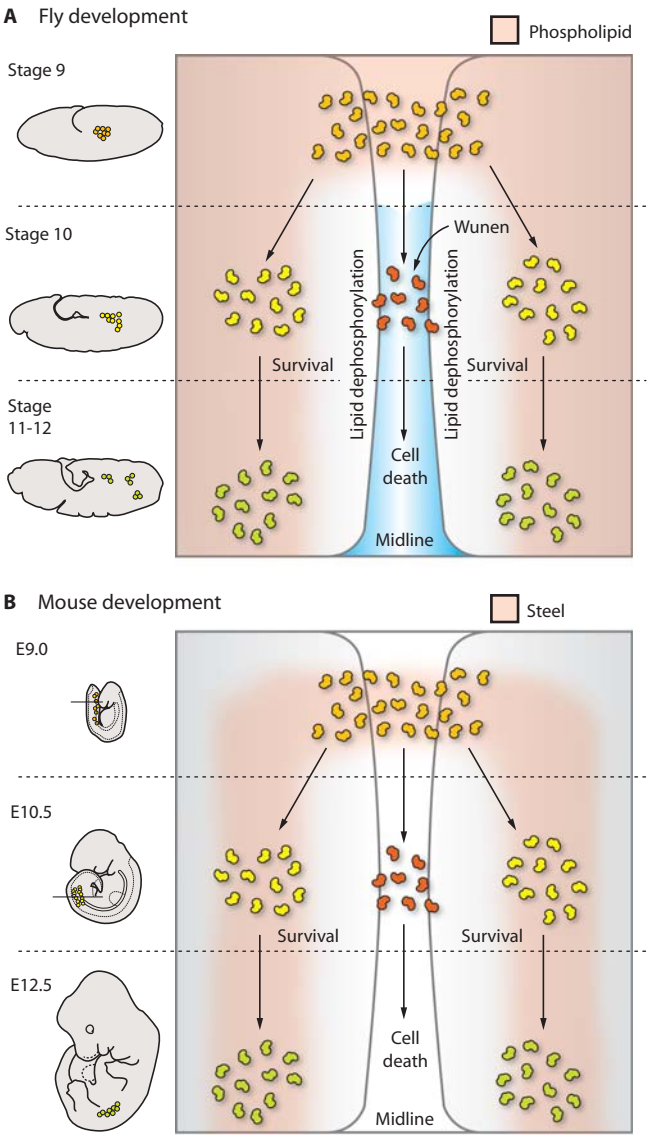

Fig. 1. Models of PGC elimination in midline structures in *Drosophila* and the mouse. Germ cells in *Drosophila* (**A**) and the mouse (**B**) are eliminated by qualitatively similar mechanisms. During early fly and mouse development, germ cells are found close to the midline [orange cells, stage 9 in *Drosophila* and embryonic day 9.0 (E9.0) in the mouse]. Germ cells then follow directional cues and leave the midline (yellow cells). Frequently, some PGCs are left behind in ectopic midline positions (red cells, stage 10 and E10.5). In *Drosophila*, Wunen activity (blue shadow) in midline structures causes PGC death through dephosphorylation of a yet-to-be-identified extracellular phospholipid. In the mouse, cells at this position are eliminated by withdrawal of the survival factor Steel. By stages 11 to 12 and E12.5, most of the ectopic PGCs have been eliminated from the midline. The cartoons for the fly embryos were adapted from FlyMove (http://flymove.uni-muenster.de); the cartoons for E9.0 and E10.5 mouse embryos were adapted from Kunwar *et al*. (*1*); and the cartoon for the E12.5 mouse embryo was adapted from the Edinburgh Mouse Atlas Project (http://genex.hgu.mrc.ac.uk/intro.html).

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