Temporal and molecular separation of the kit receptor tyrosine kinase’s roles in zebrafish melanocyte migration and survival

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Abstract

The Kit receptor tyrosine kinase (Kit) is required for vertebrate melanocytes for their migration and survival. The relationship between these developmental roles of Kit, however, remains poorly understood. Here, we use two genetic approaches to demonstrate that Kit’s roles in the migration and survival of embryonic melanocytes in the zebrafish (Danio rerio) are temporally and functionally independent. We use a temperature-sensitive kit mutation to show that kit promotes melanocyte migration and survival during distinct stages of development. These experiments additionally reveal that melanocyte migration is neither necessary nor sufficient for subsequent survival. We also identify kit alleles that molecularly separate kit’s roles in migration and survival. These results suggest that the melanocyte changes its response to Kit receptor signaling and function during development, first to promote migration, then to promote survival through distinct Kit-dependent mechanisms.

Keywords: Kit; RTK; Melanocyte; Melanophore; Neural crest; Migration; Survival; Conditional; Temperature-sensitive

Introduction

The Kit receptor tyrosine kinase (Kit) is required for the normal development of a variety of mammalian cell types, including melanocytes, hematopoietic progenitors, primordial germ cells, and interstitial cells of Cajal (Russell, 1979; Silvers, 1979; Chabot et al., 1988; Geissler et al., 1988; Nocka et al., 1990; Tan et al., 1990; Besmer et al., 1993; Huizinga et al., 1995). Additionally, activating mutations in the Kit receptor have been associated with a number of human cancers (see Boissan et al., 2000; Heinrich et al., 2002), further compelling a thorough characterization of Kit function during normal development. Within these multiple cell types, Kit promotes a range of developmental processes, including cell differentiation, migration, and survival. How the Kit receptor functions to specifically promote the appropriate process at the appropriate stage in development of these lineages is unclear.

The developmental roles of Kit are best characterized in melanocytes, because they are inherently labeled and not directly required for viability. Mutations in mammalian Kit or its ligand, Steel factor (Sf), cause embryonic melanocyte precursors, or melanoblasts, to fail to migrate away from the neural crest and subsequently disappear from the embryo (Silvers, 1979; Geissler et al., 1988; Nocka et al., 1990; Tan et al., 1990; Motro et al., 1991; Morrison-Graham and Weston 1993; Cable et al., 1995; Wehrle-Haller and Weston, 1995; Bernex et al., 1995; Yoshida et al., 1996; Jordon and Jackson, 2000; Wehrle-Haller et al., 2001). In contrast to mammalian Kit mutants, zebrafish embryos homozygous for null alleles of kit (sparse) develop pigmented embryonic melanocytes, but like mammalian Kit mutants, these cells largely fail to migrate and subsequently undergo programmed cell death and coincident extrusion from the animal (Parichy et al., 1999). Despite the long-standing availability of kit mutations, the relationship between kit’s roles in embryonic melanocyte migration and survival re-
mains poorly understood. For example, it is possible that melanocytes in kit mutants fail to migrate because they are undergoing cell death, or that melanocytes in kit mutants die because they are unable to migrate to their target sites (where they may receive additional trophic factor support), or that kit promotes melanocyte migration and survival independently. Furthermore, it remains unclear when during embryonic and larval stages kit is required to promote these different aspects of melanocyte development.

Kit belongs to the type III receptor tyrosine kinase subfamily, characterized by an extracellular domain consisting of five immunoglobulin like (Ig) repeats, a single transmembrane domain, and a cytoplasmic tail containing a split tyrosine kinase domain (Yarden et al., 1987). Biochemical and cell culture studies of Kit receptor function have led to the identification of several functional domains of the receptor, including those required for ligand binding, receptor dimerization, and interaction with other proteins (reviewed by Linnekin, 1999; Taylor and Metcalfe, 2000). Although several studies have identified the domains and residues required for specific developmental roles of several other receptor tyrosine kinases (e.g., Aroian et al., 1994; Lesa et al., 1997; Klinghoffer et al., 2001), the mechanistic bases of their roles remain unclear. Likewise, very little is known of how distinct functional domains of the Kit receptor and the different signaling pathways downstream from Kit relate to the receptor’s numerous developmental roles. Here, we present evidence that zebrafish kit promotes melanocyte migration and survival during distinct developmental stages through separable domains of the receptor.

Materials and methods

Stocks and temperature-shift treatments

Zebrafish were reared according to standard protocols at 28.5°C, unless otherwise noted. Temperature-shift experiments were conducted by using the permissive temperature of 25°C and the restrictive temperature of 33°C (Johnson and Weston, 1995; Rawls and Johnson, 2001). All developmental staging in days postfertilization (dpf) corresponds to staging at the standard temperature of 28.5°C. Staging at restrictive and permissive temperatures is translated to 28.5°C staging according to the following equation (Kimmel et al., 1995): (dpf@25°C)(0.805) = (dpf@28.5°C) = (dpf@33°C)(1.245). Alleles kit<sup>ky85</sup> (Parichy et al., 1999), kit<sup>j1e249</sup> (Rawls and Johnson, 2001), and kit<sup>j1e249</sup> (Rawls et al., 2003) have been previously described. All other kit alleles used in this study were generated in a previously described noncomplementation screen for new ENU-induced alleles of kit (Rawls and Johnson, 2001). Because kit<sup>j1e249</sup> is temperature-sensitive (Rawls et al., 2003), all experiments involving this allele were performed at 33°C.

All references to mutant alleles correspond to homozygotes, unless otherwise noted.

Melanocyte counts

To facilitate the counting of melanocytes, animals were immersed in 10 mg/mL epinephrine for 5 min to contract melanosomes (Johnson et al., 1995; Rawls and Johnson, 2000). Larvae were then fixed in 4% paraformaldehyde in PBS and their melanocytes were counted under a dissecting microscope. To assess the migration of melanocytes away from their places of origin along the dorsal aspect of the neural tube, melanocytes located on the dorsum of the larvae (dorsal melanocytes) were counted separately from melanocytes in the C4 null mutant phenotype, in which melanocytes largely fail to migrate (Parichy et al., 1999).

All animals shifted from the restrictive to the permissive temperature, as well as any unshifted controls, were thereafter treated with phenylthiourea (PTU) to prevent pigmentation of new melanocytes, thereby facilitating exclusive observation of melanocytes present at the time of downshift. To counteract pharmacological lag, PTU was added to the media approximately 1 h prior to downshift in all PTU treatments. PTU treatment consisted of constant immersion in 0.2 mM PTU with daily feedings and media changes (Milos and Dingle, 1978; Rawls and Johnson, 2000). Because the efficacy of PTU treatment is limited to approximately 7 days at 28.5°C, all PTU treatments in this study were restricted to 6 days. All assessments of melanocyte number and position are shown as the mean of data from at least six individual animals, with error estimates shown as 95% confidence intervals. Statistical analyses were performed by using Microsoft Excel.

Sequence analysis

To identify lesions in new kit alleles, overlapping fragments of kit cDNA were amplified from first-strand cDNA, and then sequenced by using nested primers and BigDye dye terminator chemistry (Perkin Elmer). Sequencing reactions were resolved on an ABI-377 automated sequencer, and sequence traces from each animal were compiled by using the Phred-Phrap-Consed package (Ewing and Green, 1998). The resulting full-length consensus cDNA sequence from each allele was compared with the reference wild-type sequence to identify any lesions. Multiple individuals of each allele were sequenced in this manner.
Results and discussion

**kit**\textsuperscript{1le99} facilitates the selective removal or restoration of kit function

To assess the temporal requirements for *kit* in promoting melanocyte migration and survival, we utilized a temperature-sensitive allele of *kit* (kit\textsuperscript{1le99}, Rawls and Johnson, 2001). Melanocytes in wild-type zebrafish, or kit\textsuperscript{1le99} mutants reared at the permissive temperature (25°C), initiate migration away from the dorsal neural tube, begin to synthesize melanin, and become visible approximately 1 dpf. By 2 dpf, most melanocytes have differentiated and reached their target positions. This pattern is largely maintained through the onset of the larval-adult pigment pattern metamorphosis that occurs at approximately 14 dpf (Table 1; Milos and Dingle, 1978; Johnson et al., 1995; Parichy et al., 1999; see Rawls et al., 2001). In contrast, melanocytes in kit\textsuperscript{1le99} embryos reared continuously at the restrictive temperature (33°C) develop similarly to those in kit\textsuperscript{b5} null mutants (Parichy et al., 1999), largely failing to migrate and undergoing apoptosis beginning at approximately 4 dpf (Fig. 1B, D, and F). In addition, the total number of melanocytes in these animals and in kit\textsuperscript{b5} null mutants at 3 dpf is approximately 70% of the wild-type complement (not shown; Parichy et al., 1999). To determine when during larval development *kit* is required to promote melanocyte migration and survival, we subjected kit\textsuperscript{1le99} mutants to temperature-shift regimens discussed below (Fig. 2).

**kit** is required for melanocyte migration prior to 2 dpf

We first sought to determine when during larval development *kit* is required to promote melanocyte migration. We allowed kit\textsuperscript{1le99} embryos to develop at the restrictive temperature through stages equivalent to 2 dpf (see Materials and methods), at which time melanocytes have differentiated along the dorsal aspect of the neural tube, with few having migrated into peripheral locations. Embryos were then shifted to the permissive temperature and assessed for effects of restoring *kit* function to melanocytes. Since restoration of *kit* function during larval zebrafish development induces the formation of de novo melanocytes from unpigmented precursors (typically at or after 8 dpf; J.F.R., C. Yang, and S.L.J., unpublished results), we inhibited the melanization of de novo melanocytes for the ensuing 6 days following downshift by treating larvae with phenylthiourea (PTU, a tyrosinase inhibitor; Milos and Dingle, 1978; Rawls and Johnson, 2000). This allowed us to use the preexisting melanin as a lineage marker to follow the development of melanocytes present at the time of downshift and ignore the contribution of any new melanocytes that differentiated de novo following downshift. By comparing the number of dispersed melanocytes in larvae at the time of downshift to the number of dispersed melanocytes 6 days after temperature shift, migration of preexisting melanocytes could be quantified (see Materials and methods). We found little or no additional migration of melanocytes following downshift to the permissive temperature at 2 dpf (Fig. 2A). Specifically, 29 ± 2% of all melanocytes are located in the periphery in 2 dpf animals.
reared at the restrictive temperature, and downshifted embryos at 8 dpf have a similar number of peripheral melanocytes (25 ± 2%). In contrast, 8 dpf kit j1e99 mutants reared continuously at the permissive temperature have 55 ± 2% peripheral melanocytes (Fig. 2A), indicating that kit is required for melanocyte migration prior to 2 dpf. While downshifts as early as 1 dpf resulted in only minor deficits in overall melanocyte migration, some melanocytes (for instance, those that originate behind the otic vesicle) clearly fail to migrate following downshift at 1 dpf (not shown). This indicates that the migration-promoting role of kit may be complete as soon as 24 h postfertilization in some melanocytes. Since kit transcript is first detected in neural crest cells at approximately 18 h postfertilization (Parichy et al., 1999), melanocytes or melanoblasts may have a very narrow developmental window for kit-mediated migration. Taken together, our results show that kit transiently promotes migration of embryonic melanocytes prior to 2 dpf.

The finding that kit-promoted migration response is transient is consistent with several possible interpretations. First, the migration machinery or signal transduction pathway by which kit normally promotes melanocyte migration may no longer be available by 2 dpf in the absence of kit function. Alternatively, the extracellular matrix composition along melanocyte migration pathways (Morrison-Graham and Weston, 1989), or other anatomical features of the migration pathway (Erickson and Weston, 1983), may change by 2 dpf and render melanocyte migration impossible, despite restoration of kit’s potential to transduce migration cues.

Interestingly, the restoration of kit function at 2 dpf was sufficient to promote the division of pigmented melanocytes (Fig. 2A). Specifically, the total number of melanocytes per embryo after downshift (384 ± 20) equals or exceeds the number prior to downshift at 2 dpf (313 ± 18). Because these embryos were immersed in PTU at downshift, the excess melanocytes at 8 dpf result from division of differentiated pigmented melanocytes. Division of differentiated melanocytes is not observed at these developmental stages in kit j1e99 mutants maintained at either the permissive or restrictive temperatures (Fig. 2A and B). The division of differentiated melanocytes observed here may therefore reveal a role for a terminal kit-dependent melanoblast division that normally occurs at 2 dpf.

Table 1
Novel kit alleles specifically disrupt either melanocyte migration or survival

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Migration</th>
<th>Survival</th>
<th>Nucleotide</th>
<th>Protein</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>Quantitative*</td>
<td>Qualitative*</td>
<td>Quantitative*</td>
</tr>
<tr>
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<td>50 ± 1</td>
<td>+</td>
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</tr>
<tr>
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<td>52 ± 3</td>
<td>+</td>
<td>−2 ± 6</td>
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<td>25 ± 2</td>
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<tr>
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<td>−</td>
<td>33 ± 1</td>
<td>+</td>
<td>+11 ± 4</td>
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<td>−</td>
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<td>+</td>
<td>+0 ± 5</td>
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<td>19 ± 1</td>
<td>+</td>
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<tr>
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<tr>
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<td>46 ± 2</td>
<td>+/−</td>
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<td>−1 ± 7</td>
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<td>+</td>
<td>42 ± 4</td>
<td>−</td>
<td>−73 ± 5</td>
</tr>
</tbody>
</table>

* Qualitative assessment of melanocyte migration into periphery at 3 dpf for each genotype.
* Quantitative assessment of melanocyte migration into periphery at 3 dpf, shown as average percent of total melanocytes found in peripheral positions at 3 dpf for each genotype.
* Qualitative assessment of melanocyte survival through 12 dpf for each genotype.
* Quantitative assessment of melanocyte survival through 12 dpf, shown as average percent change in total melanocyte number between 3 and 12 dpf for each genotype. Since PTU was not used in these experiments, changes in melanocyte number also include appearance of newly differentiated melanocytes. Therefore, significant negative change in melanocyte number is indicative of melanocyte death and is accompanied by observation of melanocytes extruding through the skin (not shown).
* Nucleotide mutation in kit open reading frame.

\(^d\) Parichy et al., 1999.

We next sought to determine when during larval development kit is required for melanocyte survival. Since apoptotic melanocytes in teleosts become TUNEL-positive...
as they are extruded through the epidermis and lost from the animal (Parichy et al., 1999; Sugimoto et al., 2000), we assessed melanocyte cell death by quantifying the loss of melanocytes. We first considered whether kitj1e99 melanocytes that were differentiated and held at the restrictive temperature survived following downshift and restoration of kit function. When 2 dpf kitj1e99 embryos were shifted to the permissive temperature, we found no evidence for subsequent melanocyte cell death. First, observations on downshifted embryos failed to reveal melanocytes extruding through the skin (not shown), a hallmark of melanocyte death in zebrafish (Parichy et al., 1999). Additionally, the number of melanocytes persisting through the 6 days following downshift remains high (Fig. 2B, but see above). In contrast, when kitj1e99 embryos were downshifted to the permissive temperature at 4 dpf only 59% of all melanocytes survive (Fig. 2B). This loss of cells is accompanied by the appearance of contracted melanocytes extruding through the skin (not shown). These results indicate that kit is not required prior to 2 dpf for subsequent melanocyte survival. The finding that melanocytes that fail to migrate into the periphery survive in normal numbers when kit function is...
restored at 2 dpf (Fig. 2A) indicates that migration to the periphery, for instance to receive additional trophic factor support is not a necessary condition for melanocyte survival.

**Survival-dependence on kit is transient**

To determine how long melanocytes require kit to promote their survival, we reared embryos at the permissive temperature through 2, 4, or 8 days postfertilization, shifted them to the restrictive temperature, and counted the melanocytes that persisted for 10 days following upshift. By comparing the total number of melanocytes in animals at the time of upshift with the number of total melanocytes 10 days after upshift, we could assess the effect of removing kit function on melanocyte survival. We found that removal of kit function at 2 dpf was sufficient to cause melanocyte death indistinguishable from individuals held continuously at the restrictive temperature (Fig. 2C). These results indicate that availability of kit function through 2 dpf is not sufficient to promote subsequent melanocyte survival. In contrast, removal of kit function beginning at 4 or 8 dpf resulted in the survival of a large fraction of the melanocytes present at the time of upshift. Our observation that the fraction of kit-independent melanocytes does not significantly increase between 4 and 8 dpf raises the possibility that a small fraction of all melanocytes never acquire kit-independence. Nevertheless, we interpret these results to indicate that melanocytes in the presence of kit activity begin to acquire kit-independence sometime between 2 and 4 dpf.

Acquisition of kit-independence by melanocytes has been previously demonstrated in a number of experimental systems (Morrison-Graham and Weston, 1993; Nishikawa et al., 1991; Yoshida et al., 1996; Rawls and Johnson, 2001). Our temperature shift experiments reveal that acquisition of kit-independence in zebrafish embryonic melanocytes initially occurs between 2 and 4 dpf, the same temporal window during which kit is initially required to promote melanocyte survival. This suggests that individual melanocytes might require kit activity for survival during a relatively brief period in their development, rather than requiring kit continuously. We suggest that individual melanocytes use the presence of kit activity between 2 and 4 dpf as surrogate evidence for successful completion of kit-mediated developmental processes (e.g., migration, division, or differentiation) at earlier morphogenetic stages. In this model, the presence or absence of kit activity between 2 and 4 dpf would determine whether that cell acquires kit-independence or undergoes cell death, respectively.

These upshift experiments also further uncover the relationship between melanocyte migration and survival. Although our downshift experiments showed that migration to the periphery was not necessary for survival it remained possible that melanocyte migration to peripheral positions could provide melanocytes with additional local trophic support that would allow them to survive independently of kit function. However we found that removal of kit function at 2 or 4 dpf in larvae reared initially at the permissive temperature resulted in similar fractions of dying melanocytes in both dispersed (17 and 56%, respectively) and dorsal (23 and 55%, respectively) melanocyte populations over the subsequent 10 days of development. These observations indicate that migration of melanocytes to positions in the periphery is not sufficient for their subsequent survival in the absence of kit function.

Taken together, the reciprocal temperature shift experiments reported here indicate that kit promotes melanocyte migration prior to 2 dpf, and promotes melanocyte survival after 2 dpf (see Fig. 4A). Furthermore, these studies show that melanocyte migration is neither necessary nor sufficient for their subsequent survival. We interpret these results to suggest that kit promotes melanocyte migration and survival independently at different stages of development. Interest-

![Fig. 3. New kit alleles separate kit's developmental roles in melanocyte migration and survival. Melanocytes in kit\textsuperscript{1/e60} mutants largely fail to migrate to peripheral locations through 2.5 dpf (A), yet persist through 12 dpf (B) along the dorsal aspect of the neural tube (black arrowheads). In contrast, melanocytes in kit\textsuperscript{1/e78} mutants at 2.5 dpf (C) successfully migrate to peripheral locations (black arrowheads), but subsequently disappear from the animal by 12 dpf (D). Scale bars: 1 mm (A, C); 1 mm (B, D).](image-url)
ingly, our finding that zebrafish kit promotes melanocyte migration before it promotes survival is consistent with previous mouse neural crest explant studies that suggest kit does not promote melanoblast survival until after the onset of melanoblast migration (Morrison-Graham and Weston, 1993).

New kit alleles selectively disrupt distinct developmental roles in melanocyte migration and survival

Our finding that kit independently promotes embryonic melanocyte migration and survival led us to hypothesize that different mutations in the kit gene might disrupt one of these developmental roles while leaving the other intact. We therefore screened through 249 ENU-induced kit non-complementing mutations (Rawls and Johnson, 2001; Rawls et al., 2003) for those with larval melanocyte phenotypes in which only migration or survival was disrupted. We identified 18 mutations in which embryonic melanocytes largely fail to migrate yet persist through subsequent stages of development. Of these 18, 11 kit alleles were recovered and 4 selected for further analysis in this study (j1e60, j1e89, j1e177, j1e249; Fig. 3A and B). Quantitative analysis confirmed the specific effects of these mutations on melanocyte migration rather than survival (Table 1). To determine the molecular basis of these phenotypes, we sequenced kit cDNAs for each allele (see Materials and methods). We found that all four migration-defective alleles encode unique single nucleotide mutations resulting in single amino acid substitutions in the extracellular domain of the Kit receptor (Table 1; Fig. 4B). Specifically, allele j1e249 encodes a substitution in the third Ig repeat, j1e177 encodes a substitution in the fifth Ig repeat, and allele j1e60 encodes a substitution of a conserved cysteine to arginine within the second Ig repeat. Studies of mammalian Kit orthologs have previously indicated molecular roles for Kit’s extracellular domain. For example, Ig repeats 1–3 have been implicated in promoting ligand binding and specificity (Blechman et al., 1993; Lev et al., 1993), while the fifth Ig repeat is required for proteolytic cleavage of Kit from the cell surface (Broudy et al., 2001).

The migration-specific effects of our mutations in the Ig domains of the Kit receptor suggest that these or other functions of the extracellular domain may promote migration independent of the signals involved in promoting...
survival. For example, Kit might promote melanocyte migration through migration-specific signal transduction cascades. Alternatively, Kit might promote melanocyte migration more directly by serving as a cell adhesion factor. If so, the ligand affinity of the extracellular domain mutations described here might be sufficiently reduced to disrupt their migration-promoting adhesive properties, yet still allow enough transient receptor activation to promote survival. The previous finding that soluble Slf in mouse promotes melanoblast migration while membrane bound Slf promotes subsequent melanoblast survival (Wehrle-Haller and Weston, 1995) raises the possibility that multiple Kit ligands may also exist in zebrafish, each promoting different developmental processes through their respective interactions with the Kit receptor. If so, then the phenotypes of the migration defective kit alleles described here could be due to either failure of these mutant receptors to interact with a migration-specific ligand, or preferential interaction between these mutant receptors and a survival-specific ligand.

We identified a single kit allele (jle78) in which embryonic melanocytes migrate normally but then die during subsequent developmental stages (Fig. 3C and D; Table 1). Sequencing of cDNAs from this allele revealed an amino acid substitution of a conserved alanine to threonine in the catalytic loop of the tyrosine kinase domain (Table 1; Fig. 4B; Hanks and Hunter, 1995). Although biochemical roles for this specific residue in other vertebrate species have not been described, substitutions at other residues in or adjacent to the catalytic loop of Kit have been associated with coat color spotting in mouse (Tan et al., 1990) and piebaldism in human (Spritz and Beighton, 1998; Syrris et al., 2002). One intriguing possibility is that kit\(^{jle78}\) mutant receptors bind ligand normally to promote migration, yet fail to promote survival due to defective ligand-mediated receptor dimerization or signaling. While the downstream signaling pathways affected in these mutant animals remain unknown, at least two possibilities exist. First, Kit has been shown to promote survival through a PI3 kinase-Akt-Bad pathway in cultured cells (Blume-Jensen et al., 1998). Second, in vivo analysis in mouse suggests that Kit normally regulates embryonic melanoblast survival through a pathway involving RAS, the Nf1 RAS GTPase-activating protein, and MEK1 (Ingram et al., 2000; Wehrle-Haller et al., 2001). Interestingly, these two pathways have been shown to interact downstream of other receptor tyrosine kinases (Klinghoffer et al., 1996; Yart et al., 2001), providing a possible link between these two signaling mechanisms in the context of Kit function. Our survival-defective kit allele should provide new opportunities to resolve the kit-dependent signaling pathways that promote cell survival.

An alternative to the notion that Kit’s migration-specific role is mediated by the extracellular domain, as suggested by our mutations, is the possibility that the signal transduction mechanisms promoting migration are more sensitive to slight deficits in kit function than are those that promote survival. This possibility was compelled by the high observed frequency of migration-defective kit alleles (18/249) compared with the frequency of survival-defective alleles (1/249). To test this possibility, we reared temperature-sensitive kit\(^{jle60}\) mutants at different temperatures to produce kit hypomorphic animals with varying degrees of kit function. We found that migration and survival are similarly affected by titration of kit function, or possibly that survival is slightly more sensitive (Fig. 2D). This adds further support to the notion that our migration-defective alleles specifically disrupt migration-specific signaling pathways or mechanisms mediated by the extracellular domain of Kit.

Since the kit mutations that disrupt migration are located in regions of the Kit receptor distinct from that of the mutation that disrupts survival, we asked whether these two classes of mutations can complement each other in trans. We found that the phenotype of transheterozygotes depended on the individual migration-defective allele used (Table 1). For example, kit\(^{jle78}\)/kit\(^{jle249}\) larvae revealed significant complementation, with melanocytes surviving normally and only slight deficits in melanocyte migration. In contrast, kit\(^{jle78}\)/kit\(^{jle60}\) transheterozygotes show a phenotype similar to kit\(^{jle78}\)/kit\(^{jle78}\) homozygous animals, with larval melanocytes migrating normally but subsequently dying. Importantly, kit\(^{jle78}\+) heterozygous larvae display a wild-type phenotype (not shown), indicating that the dominance of kit\(^{jle78}\) in some transheterozygote combinations (e.g., jle78/jle60) results from interaction with the migration defective allele rather than from a general dominant effect. Interallelic noncomplementation can be difficult to interpret, especially in multimeric proteins such as the Kit receptor. However, our finding that the most severe migration-defective kit allele (jle249) complements the survival-defective kit allele (jle78) provides further evidence that kit’s roles in migration and survival are separable molecularly as well as temporally.

Summary

Several lines of evidence presented here support the model that kit independently promotes the processes of larval melanocyte migration and survival. First, temperature shift experiments revealed that kit transiently promotes melanocyte migration and survival during distinct developmental stages (Fig. 4A). Second, temperature-shift experiments also showed that melanocyte migration is neither necessary nor sufficient for subsequent survival. Finally, mutational analysis demonstrates that kit’s roles in melanocyte migration and survival are independently mutable, and are dependent on distinct domains of the Kit receptor (Fig. 4B). Our results are consistent with the model that tyrosine kinase activity of the Kit receptor promotes survival, while other signaling pathways or mechanisms promoted by Kit may independently mediate migration at distinct developmental stages.
Acknowledgments

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