Dual Functions of the Heartless Fibroblast Growth Factor Receptor in Development of the Drosophila Embryonic Mesoderm

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ABSTRACT The Drosophila embryonic mesoderm forms by invagination of the ventral-most blastoderm cells. Subsequent development of this germ layer involves the dorsolateral migration of the internalized cells and expansion by cell division, followed by the specification of particular cell fates through the coordinate actions of both intrinsic and extrinsic regulatory mechanisms. The latter include several intercellular signals that function across germ layers. These processes combine to generate a diversity of mesodermal subtypes, including the cardiac and pericardial cells of the heart or dorsal vessel, a complete set of somatic muscle founders each with its unique identity, a population of cells that form the visceral musculature, and other cells that develop into hemocytes and the fat body. Here, we review recent evidence for the involvement of a fibroblast growth factor receptor (FGFR) encoded by the heartless (htl) gene in early directional migration of the Drosophila mesoderm. In addition, we provide new data that 1) demonstrate a second role for Htl in promoting the specification of the precursors to certain cardiac and somatic muscle cells in the Drosophila embryo, independent of its cell migration function, 2) suggest that Ras and at least one other signal transduction pathway act downstream of Htl, and 3) establish a functional relationship between the Ras pathway and Tinman (Tin), a homeodomain factor that is essential for specifying some of the same dorsal mesodermal cells that are dependent on Htl. Finally, parallels between requirements for FGFR signaling in Drosophila mesoderm development and other species, and then present new data that expand our current understanding of Htl function.

Overview of Drosophila Embryonic Mesoderm Development

The ventral region of the Drosophila blastoderm embryo is fated to adopt a mesodermal fate through the action of a series of maternal genes of the so-called dorsal group [Morisato and Anderson, 1995; Belvin and Anderson, 1996]. These genes encode the components of a conserved signal transduction pathway that generate a nuclear concentration gradient of the Dorsal (Dl) transcription factor. DI in turn activates patterns of zygotic gene expression that define particular fates along the dorsal-ventral axis of the embryo. For example, the highest nuclear levels of DI, which are found

INTRODUCTION

Fibroblast growth factors (FGFs), their receptors, and associated signal transduction pathways have been implicated in a wide range of developmental processes in a variety of organisms. A combination of gain- and loss-of-function experiments in Drosophila, Xenopus, nematode, and mouse, as well as the analysis of FGFR mutations in a number of human genetic diseases, have revealed diverse roles for FGF signaling in mesoderm induction, maintenance, and patterning [Deng et al., 1994; Yamaguchi et al., 1994; Gotoh and Nishida, 1996; Kroll and Amaya, 1996; Pownall et al., 1996], cell migration [Devore et al., 1995; Beiman et al., 1996; Gisselbrecht et al., 1996; Itoh et al., 1996; Ciruna et al., 1997; Deng et al., 1997; Shishido et al., 1997], limb morphogenesis [Tabin, 1995; Niswander, 1996], skeletal development [Muenke and Schell, 1995; Deng et al., 1996], angiogenesis [Friesel and Maciag, 1995], and the formation of a number of specific organs [Werner et al., 1993; Peters et al., 1994; Mima et al., 1995; Robinson et al., 1995]. In this article, we first review recently published findings on the involvement of the Heartless (Htl) FGFR in mesoderm development in Drosophila, highlight similarities between the mesodermal roles of FGFRs in Drosophila and other species, and then present new data that expand our current understanding of Htl function.

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Received for publication 29 August 1997; accepted 14 November 1997.
in the ventral-most blastoderm cells, induce the expression of twist (twi) and snail (sna) which in turn control mesoderm formation and suppress the development of more dorsolateral fates [Jiang et al., 1991; Kosman et al., 1991; Leptin, 1991; Ip et al., 1992; Thisse and Thisse, 1992]. The expression of Twi in the presumptive mesoderm (and also endoderm which is present at the anterior and posterior termini of the blastoderm embryo) is shown in Figure 1A and B.

The Twi-expressing mesodermal cells undergo a series of morphological changes at gastrulation which causes them to invaginate through the ventral furrow and initially to form an epithelial tube [Fig. 1C,D; Reuter and Casal, 1994; Leptin, 1995]. The internalized cells subsequently lose their epithelial characteristics, dissociate from each other, resume mitosis, and begin to spread beneath the ectoderm in a dorsolateral direction (Fig. 1E,F). By early stage 10, the mesoderm has formed a monolayer that spans the entire dorsoventral axis on either side of the extended germ band (Fig. 1G,H). The mesoderm subsequently is partitioned into visceral, somatic, and cardiac subdomains from which a variety of internal organs are derived [Bate, 1993]. This subdivision is accompanied by a modulation of the pattern of Twi expression which is functionally important for the specification of individual mesodermal cell types [Dunin Borkowski et al., 1995; Baylies and Bate, 1996] and is generated by segmentation genes acting within the mesoderm itself [Azpiazu et al., 1996; Reichmann et al., 1997]. In addition to these intrinsic regulatory mechanisms, several extrinsic factors affect mesodermal pattern formation. These include the secreted molecules encoded by the wingless (wg) and decapentaplegic (dpp) genes which are produced in the ectoderm but exhibit an inductive influence on the underlying mesoderm [Staehling-Hampton et al., 1994; Baylies et al., 1995; Frasch, 1995; Lawrence et al., 1995; Wu et al., 1995; Bate and Baylies, 1996; Ranganayakulu et al., 1996]. Dpp is responsible for inducing dorsal mesodermal fates including cardiac and visceral derivatives, while Wg is necessary for promoting the segregation of specific somatic muscle and cardiac founder cells. For these diffusible signals to function properly, it is essential that mesodermal cells acquire precise positions relative to the inducing ectoderm. The dorsolateral migration that the mesoderm undergoes following gastrulation is what assures that these cells come into correct contact with their inducers.

**Htl Function in Drosophila Mesoderm Migration**

Until recently, relatively little was known about the genetic control of mesoderm migration in the Drosophila embryo. However, it is now clear that this process is dependent in large part on the function of a mesodermally expressed FGFR encoded by the htl gene [Shishido et al., 1993, 1997; Beiman et al., 1996; Gisselbrecht et al., 1996]. The Drosophila genome contains two genes, breathless (btl) and htl, that encode receptor tyrosine kinases (RTKs) of the FGFR subfamily [Klambt et al., 1992; Shishido et al., 1993]. Btl is expressed in midline cells of the central nervous system (CNS) and in tracheal precursors. In contrast, expression of both htl RNA and protein in the early embryo is confined to the presumptive mesoderm [Fig. 2A,B; Shishido et al., 1993, 1997; data not shown]. By the end of germ band extension, the Htl pattern becomes modulated into alternating domains of high and low expression similar to that of Twi [Fig. 2C; Shishido et al., 1997]. Shortly thereafter but prior to the appearance of the tracheal pits, a discrete cluster of Htl-positive cells develops at the peak of the dorsal mesodermal crest in each hemisegment (Fig. 2D,E). This is reminiscent of the earliest Lethal of scute (L'sc)-expressing cell cluster that forms in this region [Carmena et al., 1995], and, as is the case with L'sc, a single progenitor is singled out from the Htl cluster (Fig. 2G). Moreover, both the dorsal Htl cluster and the associated progenitor express Even-skipped (Eve; Fig. 2F,H), establishing a further similarity to the dynamic mesodermal pattern of L'sc expression [A. Carmena, F. Jimenez, and AMM, unpublished observations]. In later stage embryos, Htl expression is lost from the Eve-positive cells [Shishido et al., 1997] but is maintained in cardiac, visceral mesoderm, and somatic muscle precursors (Fig. 2I). In addition, Htl is present in a subset of CNS glia [Shishido et al., 1997]. This pattern is consistent with the possible involvement of Htl in the specification of Eve progenitors, although not in their subsequent differentiation (see below).

**Loss-of-function mutations in htl cause profound defects in the formation of multiple mesodermal derivatives** [Fig. 3; Beiman et al., 1996; Gisselbrecht et al., 1996; Shishido et al., 1997]. Embryos homozygous for a null allele of htl lack the entire dorsal vessel, including both cardiac (Fig. 3A,B) and pericardial (Fig. 3D,E) cells, most dorsal somatic muscles (Figs. 3A,B and 7A,C) and many myofibers of the lateral and ventral groups (Fig. 3G,H). The residual muscles that do form in the absence of htl function have relatively normal morphologies, and, from their unique sizes, shapes, positions, and points of attachment, can in most cases be assigned particular wild type identities. However, occasional aberrant muscles are apparent in htl mutant embryos [data not shown; Beiman et al., 1996]. Abnormal morphogenesis of the gut musculature also occurs in such embryos [Beiman et al., 1996; Gisselbrecht et al., 1996; Shishido et al., 1997]. All of these defects can be traced to earlier stages of development where specific precursors of each mesodermal structure are absent or severely reduced in number. This is illustrated in Figure 3] -L for the precursors of the two pericardial cells and single dorsal somatic muscle that normally express Eve in every hemisegment.

The primary problem associated with loss of htl function became apparent when Twi expression was
analyzed in early mutant embryos. Gastrulation proceeds normally and the invaginated population of mutant cells proliferates as in wild type. However, newly internalized htl-deficient mesodermal cells do not appear to dissociate properly from each other, do not migrate in a dorsolateral direction and, as a conse-

Fig. 1. Twi expression during early stages of mesoderm development in wild-type embryos. Embryos of the indicated stages were immunostained with an antibody directed against the Twi protein, a marker for early mesodermal cells. Whole mount embryos are shown in A, C, E, and G. Anterior is to the left in each of these panels. A, E and G are lateral views and C is a ventral view. Transverse sections of similarly staged embryos are depicted in B, D, F, and H. 

A,B: Twi is expressed in the nuclei of the ventral-most cells which correspond to the presumptive mesoderm at the blastoderm stage. C,D: Twi-positive cells invaginate through the ventral furrow during gastrulation. E,F: As the germ band extends, the mesodermal cell mass begins to flatten as the internalized cells lose their epithelial appearance and dissociate from each other. G,H: By early stage 10, mesodermal cells have migrated to form a uniform monolayer that spans the entire dorsoventral axis on either side of the fully extended germ band.
sequence, fail to reach the dorsal edge of the ectoderm [Fig. 4A,B; Beiman et al., 1996; Gisselbrecht et al., 1996; Shishido et al., 1997]. Some degrees of cell spreading along the ectoderm does occur even in null mutants, but serial sections of the same embryo reveal that this migration is irregular and apparently undirected [Gisselbrecht et al., 1996]. Hypomorphic mutants show a similar, albeit less severe, defect in mesodermal spreading (Fig. 4C) which correlates with a milder phenotype in later embryos [Fig. 3L; Gisselbrecht et al., 1996].

The heart, dorsal somatic muscles, and visceral mesoderm are all known to be dependent on induction by Dpp which is produced by the dorsal ectoderm [Staehling-Hampton et al., 1994; Frasch, 1995]. Thus, the loss of dorsal mesodermal structures in htl mutants can be explained by failure of the corresponding precursors to migrate into the domain of Dpp expression. However, Dpp is not involved in the development of the ventrolateral muscles that are missing from htl mutant embryos. These defects might be accounted for by subtle abnormalities in cell positions resulting from the primary migration abnormality which could prevent Wg or perhaps as yet uncharacterized inductive signals from functioning properly. Indeed, mispositioning of mesodermal cells in ventral regions along the anteroposterior axis does occur in the absence of htl function [Gisselbrecht et al., 1996]. A second explanation for the missing ventral and lateral muscles is the possibility that Htl itself is involved directly in the specification of the corresponding muscle founders. Indirect evidence for the existence of an inductive signal for the ventral somatic mesoderm has been reported [Baker and Schubiger, 1995], and Htl could potentially fulfill this role. New results described herein strongly support the hypothesis that Htl is required for the specification of

Fig. 2. Wildtype expression of htl. Wild type embryos were stained with an antibody directed against the extracellular domain of Htl (A–E,G,I) or double-stained with anti-Htl (black) plus anti-Eve (reddish brown) antibodies (F,H). Anterior is to the left in all panels. A,B: Lateral (A) and ventral (B) views of cellular blastoderm embryos. Htl is found in the entire mesodermal primordium at this stage. C: Ventrolateral view of a stage 9/10 embryo illustrating modulation of the Htl pattern into alternating high (arrow) and low expression domains in every segment. D–H: By mid-stage 10, discrete clusters of Htl-expressing cells have formed in the dorsal-most region of the mesoderm. High magnification views of the regions corresponding to the area enclosed by the rectangle in D are shown in E–H. Htl-positive cell clusters (Cl in E and F) that are found at the peaks of the dorsal mesodermal crests also express Eve. Slightly later, single Eve-positive progenitors appear in this location (Pr in G and H). I: Lateral view of a stage 13 embryo. Htl is found in the developing cardiac cells (CC) of the dorsal vessel and in somatic muscle precursors (SM). The visceral mesoderm also expresses Htl (out of the plane of focus), as, Amnioticosa.
muscle and cardiac cell fates independent of its role in mesoderm migration (see below).

Although Htl function is essential for the movement of mesodermal cells into the Dpp-expressing region, activity of this FGFR is not required for competence to respond to the inductive effects of Dpp. Thus, dorsal mesodermal cell fates are recovered when Dpp expression is targeted to the mesoderm of htl mutant embryos [Beiman et al., 1996; Gisselbrecht et al., 1996]. In this respect, Htl plays an indirect role in determining particular mesodermal lineages by facilitating movement of the responding cells into a region where a positional signal (Dpp) can induce the proper fate. However, a more direct role for Htl in cell fate specification is not excluded by these experiments.

Htl clearly is required for the proper migration of early mesodermal cells, but how it influences this process remains unknown. It is possible that Htl is activated by a chemoattractant signal, perhaps in the form of a ligand gradient emanating from the adjacent ectoderm. This would account for the directional nature of the migration defect seen in htl mutant embryos, and is consistent with the finding that other RTKs influence cell movement by such a chemoattractant mechanism [Blume-Jensen et al., 1991; Kundra et al., 1994; Bladt et al., 1995]. Alternatively, Htl may affect mesoderm migration by regulating adhesion of the motile cells, either to each other or to the substratum which they traverse [Huttenlocher et al., 1995]. Distinguishing among these mechanisms requires more information.
about how Htl is activated, how it signals, and what are its downstream targets.

Insight into the mechanism of Htl signaling recently has been obtained from an analysis of the expression pattern of activated MAPK in the Drosophila embryo [Gabay et al., 1997a]. RTKs transduce their signals through a protein kinase cascade that includes MAPK [Marshall, 1995]. MAPK is activated by phosphorylation of neighboring threonine and tyrosine residues in its sequence, and an antibody that uniquely identifies this dually phosphorylated form in fixed embryo preparations has been generated [Gabay et al., 1997b]. Activated MAPK is expressed most strongly in the early mesoderm in the several rows of cells located at the leading edge of the initial migrating cell population. As mesodermal spreading proceeds, staining becomes restricted to the dorsal-most cells and is entirely dependent on Htl activity [Gabay et al., 1997a]. These findings suggest that Htl activation is dynamic and highly localized during mesoderm migration, and is consistent

Fig. 4. Mesoderm migration phenotypes associated with loss of htl function. Twi-stained transverse sections of early stage 10 embryos of the following genotypes are shown. A: Wildtype (WT), B: htl null (htl(AB42)), C: hypomorphic htl (htl(Y262)), and D: two copies of a UAS-dominant negative htl transgene expressed under the control of twi-Gal4 and Dmef2-Gal4 in an otherwise wild type genetic background (UAS-DN-htl). B: Complete loss of htl function results in a failure of mesodermal cells to undergo their normal dorsolateral migration. The mutant cells remain aggregated instead of forming a monolayer that is closely adherent to the overlying ectoderm along the entire dorsoventral axis (compare with A). C: Partial loss of htl function is associated with a weaker phenotype in which mesodermal cells migrate somewhat and appear less aggregated, but fail to reach the dorsal-most epidermal cells in most segments. D: Under the conditions employed here for expression of the dominant negative htl construct, early mesoderm migration is entirely normal. This reflects the slight delay inherent in the onset of Gal4-mediated activation of the UAS target gene relative to the endogenous promoters used to drive Gal4 expression [Greig and Akam, 1993; Ranganayakulu et al., 1996]. The resulting lag in accumulation of dominant negative Htl allows the endogenous wild type receptor to function in mesoderm migration before its action is blocked by the truncated form.
with the possibility that Htl plays an instructive role in this process.

**FGFRs in Vertebrate Mesoderm Development**

FGFs are effective inducers of mesoderm formation when added to Xenopus animal cap explant cultures [Kimelman and Kirschner, 1987; Slack et al., 1987; Gotoh and Nishida, 1996], and blocking FGF signaling by overexpression of a dominant negative FGFR in Xenopus embryos inhibits this process [Amaya et al., 1991]. In addition, later roles for FGF signaling in the maintenance of mesodermal gene expression and in the assignment of positional values along the anteroposterior axis of the embryo have been described [Kroll and Amaya, 1996; Pownall et al., 1996].

Functional FGFRs also are critical for mesodermal patterning in the mouse embryo. Targeted mutation of the murine fgfr1 gene produces early embryonic lethality accompanied by a thickening of the primitive streak, a reduction of the paraxial mesoderm, and an associated expansion of the axial mesoderm [Deng et al., 1994; Yamaguchi et al., 1994; Rossant et al., 1997]. This phenotype raised the possibility that FGFR1 is involved in cell migration through the primitive streak, an hypothesis that has gained recent support from the analysis of mosaics containing fgfr1 mutant embryonic stem cells [Ciruna et al., 1997; Deng et al., 1997; Rossant et al., 1997]. The latter studies revealed that cells lacking FGFR1 activity accumulate within the primitive streak and are deficient in their ability to contribute to endoderm and anterior mesodermal structures. Thus, like Htl, murine FGFR1 appears to regulate cell movements that are essential for the later development of specific lineages. Further conservation of this function is seen in C. elegans where a FGFR is required for the migration of a particular muscle cell type [Devore et al., 1995] and in chicken where FGF signaling is critical for myoblast migration from the somites to the limb buds [Itoh et al., 1996]. Results from the analysis of mosaic mouse embryos and of mice containing hypomorphic and gain-of-function FGFR1 alleles indicate additional functions of this receptor in limb bud formation and in the anteroposterior patterning of somite derivatives [Ciruna et al., 1997; Deng et al., 1997; Rossant et al., 1997]. This is consistent with related studies demonstrating similar functions for FGFs in limb development in chicken [Tabin, 1995; Niswander, 1996] and in anteroposterior pattern formation in Xenopus embryos [Pownall et al., 1996].

Investigations of cultured muscle cell lines have established that FGFs are potent regulators of myogenic cell proliferation and development [Olson, 1992]. FGFs and other growth factors promote the division and inhibit the differentiation of myoblasts in vitro. The latter function is due to the ability of FGFs to stimulate the protein kinase C-dependent phosphorylation of myogenic basic-helix-loop-helix transcription factors, thereby blocking their ability to bind to the regulatory regions of muscle-specific genes [Li et al., 1992]. Given these in vitro effects, FGF signaling could potentially promote skeletal myoblast proliferation and influence the timing of muscle differentiation during embryonic development [Marcelle et al., 1995; Cossu et al., 1996]. Indeed, some in vivo evidence to support these possibilities recently has been obtained [Itoh et al., 1996].

Another in vivo function of FGFR activity is the promotion of cardiac myocyte growth and/or survival at early stages of heart development in the chicken embryo [Mima et al., 1995]. FGFs, in combination with bone morphogenetic proteins (BMPs), also induce cardiac differentiation during avian embryogenesis [Lough et al., 1996]. Moreover, basic FGF functions as a neural tube-derived signal promoting skeletal myogenesis in paraxial mesoderm [Stern et al., 1997]. These somewhat disparate myogenic effects of FGFs in vertebrate embryogenesis are likely to reflect differences in developmental context, including the intrinsic properties of the responding cells as well as the range of environmental cues that must be integrated. The combinatorial effects seen with FGFs together with other growth factors are consistent with this hypothesis [Lough et al., 1996; Stern et al., 1997].

**Additional Developmental Functions of Htl**

Htl is additionally expressed in a small number of CNS glial cells in the Drosophila embryo [Shishido et al., 1997]. In htl mutant embryos, these glial cells do not migrate properly and fail to ensheathe longitudinal axon tracts as they do in wild type [Shishido et al., 1997]. Thus, in addition to its essential role in the mesoderm, htl functions in the embryonic CNS. Interestingly, the Drosophila Btl FGFR also is required for the migration of two different embryonic cell types, one of which corresponds to a separate subset of CNS glia from that affected by Htl [Klambt et al., 1992].

Htl expression is activated again in a later stage of the Drosophila life cycle. In wing and leg imaginal discs, Htl transcripts are found in adult muscle precursors [Emori and Saigo, 1993], raising the possibility that Htl is involved in the migration or differentiation of these cells. In eye discs, Htl is expressed within and posterior to the morphogenetic furrow where photoreceptor development occurs [Emori and Saigo, 1993]. A subregion of the larval brain also contains Htl transcripts [Emori and Saigo, 1993]. However, no studies have yet been reported to establish what functions, if any, Htl has during the development of adult structures.

The presence of occasional abnormal myofibers in embryos lacking Htl activity has led to the suggestion that Htl may play a late role in muscle morphogenesis, perhaps by regulating myotube growth cone migration or interactions between growing muscles and their ectodermal attachment sites [Beiman et al., 1996]. Since most myofibers that develop in htl mutant embryos appear to form a relatively normal residual
framework (see above), this aspect of Htl function may apply to only a small subset of muscles. Moreover, since Htl participates in muscle founder cell determination (see below), the development of aberrant myofibers in the absence of Htl function also could be attributable to the occasional misspecification of muscle identity.

Fig. 5. Differential rescue of the htl mutant phenotype by activated Ras1 and wildtype Htl. A–F: Stage 11 embryos of the indicated genotypes were stained for expression of Eve and are shown in lateral view with anterior to the left. G–K: Transverse sections of Twi-stained early stage 10 embryos of the indicated genotypes. WT, Wildtype; htl\textsuperscript{AB42}, a null allele of htl; UAS-Ras1\textsuperscript{Act}, ectopic expression of an activated form of Ras1 under the control of twi-Gal4 in an otherwise wildtype genetic background; UAS-Ras1\textsuperscript{Act}, htl\textsuperscript{AB42}, twi-Gal4-mediated ectopic expression of activated Ras1 in a null htl background; UAS-htl, ectopic expression of a full length form of Htl under the control of twi-Gal4 in an otherwise wildtype genetic background; UAS-htl, htl\textsuperscript{AB42}, twi-Gal4-mediated ectopic expression of full length Htl in a null htl background. A: Eve normally is expressed in small, segmentally repeated clusters of dorsal mesodermal cells that represent the founders of two pericardial cells and a single somatic muscle in each hemisegment (see Fig. 3J). B: Eve expression is completely missing from the dorsal mesoderm of an embryo lacking htl function. C,G: Activated Ras1 causes a marked increase in the number of Eve-expressing mesodermal founder cells, but has no effect on mesoderm migration in a wildtype embryo. D,H,I: Activated Ras1 induces a partial recovery of Eve-positive mesodermal cells when expressed in a null htl embryo. This partial rescue is also reflected by an improvement in mesoderm migration in some (I) but not all (H) regions of mutant embryos. E,J: Additional mesodermal expression of full length Htl has no effect either on the number of Eve-positive cells or on mesoderm migration in wildtype embryos. F,K: Full length Htl completely rescues both Eve founder cell formation and mesoderm migration in htl mutant embryos.
MATERIALS AND METHODS

Drosophila Strains and Genetics

The genetic and molecular characterization of the htl mutant stocks used in the present study have been described previously [Gisselbrecht et al., 1996]. htlAB42 is a null allele by both genetic and molecular criteria, and htlYY262 is associated with a hypomorphic phenotype. The null tin346 allele was provided by M. Frasch [Azpiazu and Frasch, 1993]. Targeted ectopic expression of transgenes was accomplished using the Gal4/UAS system [Brand and Perrimon, 1993]. The mesoderm-specific Gal4 lines employed included twi-Gal4 [Greig and Akam, 1993] and Dme2-Gal4 [Ranganayakulu et al., 1996]. Homozygous viable insertions of the former on the second chromosome and of the latter on the third chromosome were combined in a single strain using standard genetic crosses. Similarly, strains were constructed that contained Gal4 or UAS transgenes in htl or tin genetic backgrounds. UAS-Ras1Ac was provided by X. Lu and N. Perrimon, and has been described [Gisselbrecht et al., 1996]. Balance chromosomes marked with ftz-LacZ transgenic insertions were used for the identification of mutant embryos by double labeling for the expression of specific markers and that of β-galactosidase.

Cloning of a Dominant Negative Htl Construct

A dominant negative form of Htl was generated by truncating the intracellular portion of the receptor at Glu395 which corresponds to the last amino acid before the start of the tyrosine kinase domain [Shishido et al., 1993]. This was accomplished by PCR amplification using an intronless wild type genomic clone as template [Gisselbrecht et al., 1996] and the following primers: 5'-GGGATCCATATACCAAAAAATGGCTGCCGCCTG-3' and 5'-ACGTTCGAGTTATTTCCAGTCTTGGGATAC-3' which correspond to nucleotides 54–78 and 1294–1268 of the published htl sequence, respectively [Shishido et al., 1993]. The resulting PCR product was digested with BamHI and EcoRI, cloned into the corresponding sites of the pRSET-A bacterial expression vector (Invitrogen; Carlsbad, CA), and sequenced on both strands to insure that it encoded wild type Htl. A histidine-tagged Htl fusion protein was produced in Escherichia coli and subsequently purified by nickel-agarose affinity chromatography followed by polyacrylamide gel electrophoresis. This protein was used to immunize rabbits and to affinity purify the resulting antibody. Specificity of the antibody was demonstrated by lack of staining of embryos homozygous for a deficiency that uncovers htl [Shishido et al., 1993].

Antibody Production

A rabbit polyclonal antiserum directed against the extracellular portion of Htl was obtained, as follows. The region corresponding to amino acids 22 to 281 was PCR-amplified from a genomic htl template using the following primers: 5'-GGGATCCCTTGAGGACGCAC-GCCAATG-3' and 5'-GGGATCCCTAAGACCTGAG-TTGCGAGACAC-3'; the underlined nucleotides in the two primers indicate BamHI and EcoRI restriction sites, respectively. The resulting PCR product was digested with BamHI and EcoRI, and cloned into the corresponding sites in the pRSET-A bacterial expression vector (Invitrogen; Carlsbad, CA), and sequenced on both strands to insure that it encoded wild type Htl. A histidine-tagged Htl fusion protein was produced in Escherichia coli and subsequently purified by nickel-agarose affinity chromatography followed by polyacrylamide gel electrophoresis. This protein was used to immunize rabbits and to affinity purify the resulting antibody. Specificity of the antibody was demonstrated by lack of staining of embryos homozygous for a deficiency that uncovers htl [Shishido et al., 1993].

Antibody Staining, In Situ Hybridization, and Sectioning of Embryos

All methods for the fixation and staining of embryos using antibody or in situ hybridization probes have been reported previously [Tautz and Pfeifle, 1989; O'Neill and Bier, 1994; Gisselbrecht et al., 1996]. In the case of anti-Htl antibody, staining signal was enhanced by Tyramide Signal Amplification reagents (New England Nuclear; Boston, MA). Embryos were embedded in Spurr resin (Polysciences, Inc.; Warrington, PA) and sectioned, as described [Gisselbrecht et al., 1996]. Whole mounts and sections of embryos were examined under Nomarski optics using a Zeiss Axiophot microscope, and photographs were taken using Kodak EKTAPRO 160 film. Slides were scanned and the resulting digital images were assembled into figures using Adobe Photoshop and Adobe Illustrator software.

Quantitation of Eve Expression in the Dorsal Mesoderm

The numbers of hemisegments containing at least one Eve-expressing dorsal mesodermal cell were counted for stage 11 wild type and experimental embryos (see Fig. 6). Eighteen hemisegments (T2-3 and A1-7 on both sides of each embryo) in greater than 100 embryos of each genotype were examined. Data are expressed as the percentage of all scored hemisegments that contain one or more Eve-positive cells.
RESULTS AND DISCUSSION

Ectopic Expression of a Dominant Negative Htl FGFR Reveals a Second Function of Htl in Mesodermal Cell Fate Specification

As described above, the failure of mesodermal cells to migrate in a dorsolateral direction potentially can account for all of the defects in the dorsal mesoderm of htl mutant embryos. However, the lack of ventral and lateral somatic muscles, another prominent feature of the htl phenotype (Fig. 3H), raises the question of whether additional functions exist for the Htl signaling pathway in the embryonic mesoderm. One possibility is that Htl is necessary for specifying the fates of particular somatic muscle founder cells, as has been shown for Wg [Baylies et al., 1995; Lawrence et al., 1995; Wu et al., 1995; Ranganayakulu et al., 1996]. This potential function would be consistent with the early expression of Htl in mesodermal progenitors and the clusters from which these cells are derived (Fig. 2D–H). However, it is difficult to argue strongly for such a later function of Htl because of the extent to which the organization of early mesodermal cells is deranged in the available htl

Fig. 6. Quantitation of Eve expression in htl mutant and rescued embryos. The percentage of hemisegments containing at least one Eve-positive cell was determined for a large number of embryos of each of the indicated genotypes (see Materials and Methods for details). WT, Wildtype; htlY262, a hypomorphic allele of htl; htlAB42, a null allele of htl; UAS-Ras1Ac, htlAB42, twi-Gal4-mediated ectopic expression of activated Ras1 in a null htl background; UAS-htl, htlAB42, twi-Gal4-mediated ectopic expression of full length Htl in a null htl background. Whereas, no Eve-positive hemisegments are seen in the complete absence of htl function, a htl hypomorph is associated with some Eve expression. A significant but only partial recovery of Eve expression is seen when activated Ras1 is expressed in a null htl background. However, complete rescue of Eve expression is induced by full length Htl in this same genetic background. Note that these data were obtained by scoring a hemisegment as positive for Eve expression if it contained at least one such cell; no attempt was made to count and compare the number of Eve-expressing cells per hemisegment between the different genotypes.
mutants [Fig. 4B; Beiman et al., 1996; Gisselbrecht et al., 1996; Shishido et al., 1997]. Therefore, we tested the hypothesis that Htl is required for mesodermal founder cell fate specification by reducing its activity under conditions where earlier cell migration is not compromised.

The latter was accomplished by ectopic expression of a dominant negative form of the Htl FGFR using the Gal4/UAS targeting system [Brand and Perrimon, 1993]. Dominant negative Htl was constructed by truncating the intracellular domain of the receptor, as has been described for other RTKs [Amaya et al., 1991]. A combination of two mesoderm-specific Gal4 lines, twi-Gal4 [Greig and Akam, 1993] and Dme2-Gal4 [Ranganayakulu et al., 1996] was used to activate expression of the inhibitory receptor. We reasoned that it might be possible to inhibit a later function of Htl without affecting its role in mesoderm migration since 1) there is a slight lag in the onset of Gal4 expression relative to the endogenous gene promoters used to drive its expression [Greig and Akam, 1993; Ranganayakulu et al., 1996] and 2) it is necessary to reach a threshold level of dominant negative Htl in order to titrate out the wildtype receptor. Indeed, under these ectopic expression conditions, mesoderm migration proceeded normally with Twi-positive cells reaching the dorsal-most margin of the ectoderm (Fig. 4D). Nevertheless, dominant negative Htl induced numerous defects in mesodermal structures at multiple positions along the dorsoventral axis and at different stages of development.

In late stage embryos, somatic muscles were missing from ventral, lateral, and dorsal groups (Figs. 3I and 7B), and gaps occurred in the rows of cardial (Figs. 3C and 7B) and pericardial (Fig. 3F) cells. These defects could be traced to an earlier stage where the corresponding precursor cells were found to be lacking. For example, dominant negative Htl prevented the formation of progenitors of the Eve-expressing pericardial and somatic muscle cells (Fig. 3M, and data not shown). Small gaps in the normally continuous rows of visceral mesodermal precursors also were observed (data not shown). The penetrance of these phenotypes is partial, presumably due to an inability to completely block the activity of the endogenous wild type receptor. Consistent with this possibility, we found that the phenotypic severity associated with the ectopic dominant negative receptor is dosage-sensitive (data not shown). Of interest, the severity of the dominant negative Htl phenotype is remarkably similar to that of a hypomorphic htl allele (Fig. 3L,M) but without the perturbation of mesoderm migration characteristic of the latter (compare Fig. 4A,C,D). Although similar results with heat shock-induced expression of dominant negative Htl previously have been reported [Beiman et al., 1996], the present work with targeted expression of a related construct allow us to conclude that Htl must act autonomously within the mesoderm.

In summary, ectopic dominant negative Htl phenocopies the effects of weak htl loss-of-function mutations under conditions where mesoderm migration is not affected. Furthermore, the effects of dominant negative Htl are first manifest at the stage when mesodermal cells normally acquire particular fates. From these results, we conclude that the Htl signaling pathway is required not only for early mesodermal spreading, but also is essential for subsequent cell fate specification within this germ layer. The latter function is consistent with the expression of Htl in mesodermal progenitors and the clusters from which these cells are singled out (Fig. 2D–H). An inducer of ventral somatic muscle identity has been postulated to exist [Baker and Schubiger, 1995], and the present findings suggest that Htl may fulfill such a role. However, the migration-independent requirement of Htl for development of dorsal and lateral mesodermal derivatives (Fig. 3C,F,I,M) indicates that this RTK acts at all levels along the dorsoventral axis to specify mesodermal cell types. This establishes an additional parallel with murine FGFR1 which is involved not only in the migration of mesodermal cells through the primitive streak, but also in later pattern forming processes [Ciruna et al., 1997; Deng et al., 1997; Rossant et al., 1997].

**Differential Rescue of the htl Mutant Phenotype by Wildtype Htl and Activated Ras1—Implications for the Htl Signal Transduction Pathway**

A complete understanding of the mechanism by which Htl controls mesodermal migration necessitates knowledge of its intracellular signal transduction pathway. Ras is one of the well-established factors that act downstream of RTKs, including FGFRs from numerous species [van der Geer and Hunter, 1994]. We have found that Drosophila Ras1 also functions in the Htl signaling pathway since ectopic mesodermal expression of a constitutively active form of Ras1 is capable of partially rescuing a strong hypomorphic htl mutant [Gisselbrecht et al., 1996].

Partial rescue of a null htl mutation by activated Ras1 also occurs (Fig. 5A–D and G–I). In these experiments, twi-Gal4 was used to drive the expression of activated Ras1 under UAS control, and the degree of mesodermal migration was assessed from transverse sections of Twi-stained embryos. Activated Ras1 has no effect on migration when expressed in a wild type background (Fig. 5G). However, in some regions of htl mutant embryos, activated Ras1 causes a significant flattening and dorsolateral spreading of the mesodermal cell mass (Fig. 5I), whereas in others there is little or no effect (Fig. 5H).

Incomplete rescue of the htl phenotype by activated Ras1 also is manifest in the expression of Eve in the dorsal mesoderm. No Eve-positive cells are found in the complete absence of htl function, whereas a hypomor-
phic mutant contains a markedly reduced number of Eve-expressing segments (Figs. 3K,L, 5B, and 6). An increased number of Eve pericardial and muscle progenitors is induced in each segment by activated Ras1 in a wildtype background (Fig. 5C), an effect that is not due to a stimulation of cell division by Ras1 (data not shown). Rather, this reflects the involvement of Ras1 in the cell fate specification functions of Htl (see above and data not shown) and a second RTK that is essential for the development of Eve muscle founders, the Drosophila epidermal growth factor receptor, DER (E.M.B. and A.M.M., unpublished results). A variable recovery of Eve expression occurs under the influence of activated Ras1 in htl mutant embryos, and all of these Eve-positive cells are confined to the dorsal mesoderm in their usual segmental pattern (Figs. 5D and 6). This result is consistent with the involvement of Ras1 in both the migration and cell fate specification functions of Htl.

There are several possible explanations for the incomplete rescue of the htl phenotype by activated Ras1. The Gal4/UAS system employed may not accurately recapitulate the requisite temporal, spatial or quantitative aspects of signaling by the endogenous receptor. Alternatively, Ras1 may be only one of several components that transduce the signal mediated by Htl activation. Results from an experiment in which a wildtype form of Htl replaces Ras1 in the Gal4 UAS rescue construct argue in favor of the latter possibility. In marked contrast to activated Ras1, wild type Htl completely rescues the null htl phenotype as manifest by both Twi (Fig. 5J,K) and Eve (Fig. 5E,F) expression patterns. In

**Fig. 7.** Functional relationship between htl and tin. Stages 16 (A–D; dorsal views with anterior to the left), 10 (E; transverse section), and 11 (F,G; lateral views, anterior to the left) embryos of the indicated genotypes stained with antibodies against myosin heavy chain, Twist and Eve, respectively. WT, Wildtype; UAS-DN-htl, otherwise wildtype embryo expressing two copies of a dominant negative htl transgene under the control of twi-Gal4 and Dme2-Gal4; htlAB42, null allele of htl; tin346, null allele of tin; UAS-Ras1Act; tin346, twi-Gal4-mediated ectopic expression of activated Ras1 in a null tin background. A: Myosin is expressed in a stereotyped array of dorsal muscles and in the cardial cells of the heart. B: Ectopic mesodermal expression of a dominant negative form of Htl under conditions where mesoderm migration is not perturbed (Fig. 4D) induces a partial loss of dorsal somatic muscles and cardial cells (arrow). C: Complete loss of htl function is associated with a more severe loss of dorsal mesodermal derivatives than that seen with dominant negative Htl. D: tin loss-of-function causes a very similar loss of dorsal mesodermal phenotype to that of htl (compare with C). E: Mesoderm migration is normal in tin mutant embryos. F: No Eve-expressing cells are found in the dorsal mesoderm in a tin mutant. G: A null mutation in tin completely blocks the ability of activated Ras1 to induce the overproduction of Eve mesodermal cells (compare with Fig. 5C).
addition, there is a complete quantitative recovery of Eve expression when assayed in a large population of experimental embryos (Fig. 6). Whereas activated Ras1 causes only 24% of htl mutant segments to acquire at least one Eve progenitor, 100% of such segments contain Eve-positive mesodermal cells under the influence of ectopically expressed full length Htl. This implies that twi-Gal4 is capable of mimicking the correct qualitative and quantitative features of endogenous Htl expression. Although we cannot completely exclude the possibility that the failure of activated Ras1 to produce the same degree of rescue as wildtype Htl reflects variations in the efficiencies of transgene expression, we did obtain similar results with independent insertions of the activated Ras1 transgene in hypomorphic and null htl mutant backgrounds [Fig. 5; Gisselbrecht et al., 1996]. These findings lead us to suggest that components other than the Ras1 cascade are likely to act downstream of this RTK to transduce its signal. Among the candidates for such factors are Corkscrew [Perkins et al., 1992], Src [Takahashi et al., 1996], phospholipase Cg [Emori et al., 1994], phosphoinositide 3-kinase [Leevers et al., 1996], and the JAK-STAT pathway [Hou and Perrimon, 1997].

Epistatic Relationship Between Ras1 and tin Genes

The above results indicate that htl functions in the development of Eve-expressing dorsal mesodermal cells by two mechanisms. One is indirect and involves Htl in the movement of cells into a domain where they are exposed to Dpp, a position-specific inducer of dorsal mesodermal fate. The other is a more direct influence of Htl signaling on the actual specification of an Eve-positive identity. Interestingly, loss-of-function mutations in tinman (tin) and htl have identical effects on the development of Eve pericardial and somatic muscle cells [Figs. 5B and 7F; Azpiazu and Frasch, 1993; Bodmer, 1993]. Similarities are also seen between the cardial and dorsal somatic muscle phenotypes of these two genes (Fig. 7A–D). However, tin differs significantly from htl in its mechanism of action since mesoderm migration is completely normal in tin mutant embryos (Fig. 7E). This implies that tin is involved in only one of the processes affected by htl, namely the determination of dorsal mesodermal cell identities.

Given that htl and tin have similar phenotypes and appear to be involved in at least some of the same developmental processes, we were interested in determining if there is a functional relationship between these two regulatory genes. Since Ras1 functions in the Htl signaling pathway and activation of this signal transducer has the opposite effect on Eve progenitor development to tin loss-of-function, we were able to perform the following epistasis experiment. Expression of activated Ras1 in a tin mutant background results in an Eve expression phenotype corresponding to that of the latter (Fig. 7G). That is, tin loss-of-function completely blocks the ability of activated Ras1 to promote the formation of Eve pericardial cell and somatic muscle progenitors.

A Model for Pattern Formation in the Dorsal Mesoderm of the Drosophila Embryo

The result of the above epistasis experiment indicates either that tin and Ras1 lie on parallel pathways, both of which are necessary for Eve cell fate specification, or that tin acts downstream of Ras1. We believe that the available data more strongly support the former possibility. Whereas activated Ras1 generates a marked increase in the number of Eve-expressing mesodermal progenitors, the early cardiac/somatic mesoderm domain of tin expression is not similarly affected (data not shown). Furthermore, this Dpp-dependent expression of tin does not appear to be regulated by Htl after mesoderm migration is complete (data not shown), consistent with our prior evidence that Htl is not required for competence to respond to Dpp [Gisselbrecht et al., 1996]. Thus, although we cannot completely exclude the possibility that Tin acts downstream of Htl/Ras1, we favor the alternative hypothesis that these two regulatory pathways function in parallel to determine an Eve-positive mesodermal fate.

One model that is consistent with this proposal is illustrated in Figure 8A. In wildtype embryos, Htl initially is required for proper dorsolateral migration of the invaginated mesoderm. When mesodermal cells reach the dorsal-most region of the ectoderm, they are induced by Dpp to express Tin, thereby acquiring the competence to differentiate into visceral, cardiac, or dorsal somatic muscle derivatives [Fraser, 1995]. Superimposed on this process is the activation of the Ras1 pathway in a small subset of dorsal mesodermal cells. Ras1 activation is mediated by Htl in those cells destined to form the Eve pericardial progenitors, whereas both Htl and DER function together to generate a Ras1 signal specific to Eve-positive somatic muscle fate (Fig. 3M, and data not shown). In this sense, Htl/DER/Ras1 signaling serves to distinguish a fate characterized by Eve expression from additional dorsal mesodermal fates that are also dependent on tin. A further distinction between Eve pericardial and Eve muscle identities may depend on the different RTK signals that are necessary for each—the former requires only Htl but the latter depends on both Htl and DER (data not shown). A qualitative and/or quantitative difference in RTK signaling may generate the requisite outputs for these separate Eve-positive fates [Marshall, 1995]. In our model, it should be noted that Tin and Ras1 regulation are not required to function in any particular temporal order; one may precede the other or they may act simultaneously. The essential point is that both are absolutely required for the specification of Eve cardiac and somatic muscle fates in the dorsal mesoderm. In addition, because some ventral
A model for the dual function of Htl in embryonic mesoderm development. A: In wildtype (WT) embryos, Htl is first required for the proper dorsolateral migration of mesodermal cells. The dorsal ectoderm produces Dpp which induces the most dorsally migrating mesodermal cells to express Tin [Frasch, 1995]. Superimposed on this process is the activity of the Ras1 pathway (mediated by Htl and DER, the Drosophila epidermal growth factor receptor; data not shown) which activates Eve expression in a small subset of cells within the dorsal Tin domain. Thus, Htl participates in a second aspect of mesodermal patterning, the determination of specific cell fates. Given the additional ventral and lateral defects seen in embryos lacking htl function, we suggest that Htl has a similar cell fate specification function in other regions of the mesoderm. Note that Tin and Ras1 need not act in any particular temporal order in this model. B: In htl mutant embryos, cells do not migrate dorsally to reach the Dpp-expressing ectodermal cells. As a result, expression of neither Tin nor Eve is fully activated. C: In tin mutant embryos, the mesoderm migrates normally but dorsal cell fates are not specified because of the essential regulatory role of Tin in this process [Azpiazu and Frasch, 1993; Bodmer, 1993]. D: Ectopic expression of dominant negative Htl under the conditions used in the present study does not affect mesoderm migration nor dorsal Tin expression (data not shown) but does inhibit the later cell fate determination function of Htl. This is manifest by a reduction in the number of Eve-positive cells that are specified. E: Constitutive activation of Ras1 causes an overproduction of Eve-expressing dorsal mesodermal progenitors. Because Tin is required in addition to Ras1 for the specification of Eve cell fates, no Eve is expressed in tin mutant embryos having constitutive activation of the Ras1 pathway (Fig. 7G).
and lateral muscles also are dependent on Htl function (Fig. 3H,1), we suggest that this receptor plays a role in founder cell fate determination in other regions of the mesoderm.

All of our data are entirely consistent with the above model. With a null mutation in the htl gene, mesodermal cells are unable to migrate dorsally, Tin expression cannot be induced by Dpp and, as a consequence, Eve cell fates are not specified (Fig. 8B). In the absence of tin function, mesoderm migration is normal, cells are exposed to Dpp but competence to respond to Ras1 signaling is lost and Eve cells cannot be determined (Fig. 8C). With delayed loss of Htl activity, as obtained in our ectopic dominant negative Htl experiments, mesoderm migration proceeds normally, Dpp induces Tin but Eve expression does not occur because of a secondary requirement for Htl/Ras signaling for the specification of these cells (Fig. 8D). Constitutive activation of the Ras1 pathway throughout the mesoderm causes an overproduction of Eve progenitors which are confined to the competence domain demarcated by normal Dpp/Tin expression (Fig. 8E). Finally, because tin function is necessary for the response to Ras1 signaling, constitutive Ras1 should have no effect in the absence of tin, precisely the result of our epistasis experiment (Fig. 7G).

Our model highlights a striking similarity between Drosophila and vertebrate embryos with respect to FGFR requirements for mesoderm development. Drosophila Htl and mouse FGFR1 are both necessary for the proper migration of early mesodermal cells, and this has an indirect but profound impact on subsequent patterning events [Deng et al., 1994; Yamaguchi et al., 1996; Beiman et al., 1996; Gisselbrecht et al., 1996; Shishido et al., 1997]. Furthermore, independent of a role in cell movement, FGFR signaling is essential for induction of cardiac cell fates in both Drosophila and chicken embryos [present study; Lough et al., 1996]. This last similarity is even more remarkable when one considers how the conserved Dpp/BMP growth factors [Frasch, 1995; Lough et al., 1996; Schultheiss et al., 1997] and Tin/NKx transcription factors [Harvey, 1996] are integrated with FGFR signaling during heart development in divergent species. In both Drosophila [AMM, SG, A, Carmena and F. Jimenez, unpublished observations] and vertebrates [Lough et al., 1996], Dpp/BMP signals combine with the FGFR/Ras pathway to induce specific markers of cardiac differentiation, and Tin/NKx function is integral to this process. Thus, it appears that not just individual components but actually an entire cardiogenic program has been conserved throughout evolution.

CONCLUSIONS

In the past several years, the independent studies of numerous groups have converged to provide new insight into the regulation of pattern formation in the Drosophila embryonic mesoderm. These advances have been facilitated in large part by the ability to undertake genetic screens for new loci involved in these processes, or to test the effects of previously characterized genes whose expression domains or other properties suggest a possible involvement in mesoderm development. These studies also have revealed unsuspected evolutionary conservation among the components regulating muscle and heart development in diverse species. As summarized in the present article, the mesodermal functions of FGFR signaling underscore each of these points.

The approaches that already have proven productive for the study of mesoderm development in Drosophila are likely to yield answers to many new questions arising from what is currently known about the role of Htl signaling. One of the central issues for future work is identification of the ligand that activates this receptor. The ligand for Btl, the other Drosophila FGFR, is encoded by branchless (bll) [Sutherland et al., 1996], but both the expression pattern of this gene and its mutant phenotype make it unlikely that Bnl is also the Htl ligand. Molecular screens for Bnl homologs and genetic screens for new mutations associated with defects in mesoderm migration should be useful in the identification of a Htl ligand and the analysis of its function. Furthermore, such approaches should clarify whether there is a single Htl ligand or if different molecules stimulate its mesoderm migration and cell fate specification functions. Consistent with the latter possibility, a large number of molecules capable of activating vertebrate FGFRs exist, and these have quite diverse structures [Green et al., 1996]. In this context, genetic analysis in Drosophila could be useful for evaluating the in vivo functional significance of the hypothesis that neural cell adhesion molecules signal through FGFRs [Green et al., 1996].

Once identified, the expression of the Htl ligand(s) may reveal features of how this RTK functions to facilitate mesoderm migration. For example, is Htl permissive or instructive in this process? Bnl confers an instructive function on Btl in tracheal cell migration [Lee et al., 1996], and this is reflected in the intricate and dynamic way in which Bnl is expressed in the embryo [Sutherland et al., 1996]. The pattern of activated MAPK expression in the mesoderm suggests that Htl also may be activated by a highly localized ligand, thereby facilitating an instructive role for this RTK in mesoderm migration (Gabay et al., 1997a). Constitutive activation of the Htl pathway either by ubiquitous expression of an activated receptor or its cognate ligand could provide a direct test of whether graded activity of Htl is critical to its function in cell migration. Characterization of the Htl ligand(s) and further genetic studies may also reveal the existence and function of possible coreceptors for this FGFR [Schlessinger et al., 1995].

The targets of the intracellular signal transduction pathway functioning downstream of Htl should provide considerable insight into its mechanism of action. We
already have established that Ras1 is involved in this process, but the greater degree of phenotypic rescue obtained with the wild type receptor than with activated Ras1 suggests that additional signal transducers exist. Genetic screens or tests of known candidates should help to uncover these components, as has been the case for other Drosophila RTKs [Zilversmit and Rubin, 1994]. The same applies to the transcription factors that mediate the nuclear responses to Htl signaling. Yan, an ETS domain repressor, may correspond to one of these [Shishido et al., 1997; A.M.M., unpublished results]. However, since this factor also is involved in other RTK pathways [Rebay and Rubin, unpublished results], specificity may be conferred by unique transcriptional activators and repressors or combinations thereof.

In addition to its function in the embryonic mesoderm, it is necessary to evaluate potential roles of Htl during metamorphosis. The availability of htl alleles for use in generating mutant clones later in development, and of dominant negative receptor lines for ectopic expression at these stages, should facilitate such analyses.

We have proposed a model in which Ras1 signaling combines with Tin, a determinant of dorsal mesodermal fate, to specify a subset of pericardial and somatic muscle cells that express Eve. Additional factors controlling the development of these cells include Dpp [Frasch, 1995] as well as the WNT family member, Wg [Lawrence et al., 1995; Wu et al., 1995]. Future work employing both molecular and genetic approaches will be required to understand how all of these pathways are integrated to insure the proper patterning of embryonic mesodermal cells.

ACKNOWLEDGMENTS

We are grateful to N. Perrimon, X. Lu, J. Skeath, M. Akam, E. Olson, M. Frasch, R. Bodmer, D. Kiehart, S. Roth, M. Leptin, B. Patterson, T. Volk, and the Bloomington Drosophila Stock Center for fly lines and antibodies. A.M.M. is an Assistant Investigator of the Howard Hughes Medical Institute.

REFERENCES


