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Requirements of DFR1/Heartless, a mesoderm-specific *Drosophila* FGF-receptor, for the formation of heart, visceral and somatic muscles, and ensheathing of longitudinal axon tracts in CNS

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SUMMARY

***DFR1* encodes a mesoderm-specific fibroblast growth factor receptor in *Drosophila*. Here, we identified and characterized a protein-null mutant of *DFR1* and examined DFR1 expression in embryos using anti-DFR1 antibody. Mutant phenotypes were completely rescued by a genomic fragment from the *DFR1* locus. After invagination, mesodermal cells expressing DFR1 undergo proliferation and spread out dorsally to form a monolayer beneath the ectoderm. In mutant embryos, however, the mesoderm is not capable of extending to the normal dorsal limit and consequently mesodermal cells fail to receive ectodermal signals and thus rendered incapable of differentiating into primordia for the heart, visceral and somatic muscles. *DFR1* is also required for normal development of the central nervous system. The absence of *DFR1* resulted in the failure of longitudinal glia to enwrap longitudinal axon tracts. *DFR1* mutant phenotypes were partially mimicked by the targeted expression of activated Yan, thus demonstrating the MAP kinase pathway to be involved in differentiation of mesoderm.**

Key words: *Drosophila*, FGF receptor, *yan*, longitudinal glia, mesoderm formation

INTRODUCTION

In *Drosophila*, the first apparent morphogenetic event, gastrulation, takes place 3 hours after egg laying (AEL) (Campos-Ortega and Hartenstein, 1985; Bate, 1993). During gastrulation, ventral cells of the blastoderm move into the interior of the embryo along the invaginating ventral furrow. The invaginated cells form the mesoderm from which are derived many internal organs and tissues including somatic and visceral muscles, fat body and heart. All these different cells first start differentiating at the time of germ-band retraction (7-8 hours AEL; Bate, 1993).

Events decisive for mesoderm fate are thought to occur between gastrulation and germ-band retraction (Bate, 1993). Immediately after gastrulation, mesodermal cells undergo proliferation and spread out to form a monolayer beneath the overlying ectoderm (Campos-Ortega and Hartenstein, 1985). Dorsoventral and anteroposterior subdivisions of each segment have been shown to be importantly involved in partitioning of mesoderm into progenitors of different derivatives (Frasch, 1995; Azpiazu et al., 1996). Three different signals are considered to be essential for mesodermal subdivision. Decapentaplegic (Dpp) signals from the dorsal-most region of the ectoderm determine which cells of the mesoderm become competent for developing into visceral mesoderm and heart (Frasch, 1995). The opposing roles of *hedgehog* (*hh*) and *wingless* (*wg*) appear essential for what becomes of mesoderm along the anterior-posterior axis (Azpiazu et al, 1996). In addition, a recent experiment showed that Twist, a helix-loop-helix transcription factor, regulates mesodermal differentiation and propels a specific subset of mesodermal cells into somatic myogenesis (Baylies and Bate, 1996).

Studies on *Xenopus* embryos indicate the importance of certain growth factors to mesoderm formation (reviewed in Jessell and Melton, 1992; Gurdon, 1992). Fibroblast growth factor (FGF) and transforming growth factor (TGF-β) have been shown to be associated with inducing activity of mesoderm. Exposure to FGF induces formation of mesodermal derivatives, such as muscle and mesenchyme, in the animal hemisphere isolated from *Xenopus* embryos (Kimmelman and Kirschner, 1987; Slack et al., 1987). On deleting an element of FGF signaling, it became evident that the FGF signaling pathway is essential for the formation of mesoderm (Amaya et al., 1991).

High-affinity receptors for FGFs (FGF-Rs) constitute a family of transmembrane proteins each possessing an intracellular tyrosine kinase domain (reviewed in Johnson and Williams, 1993). The extracellular region usually contains two to five immunoglobulin-like domains and a highly acidic region. Members of the FGF-R family have been shown

present in many different mammals, amphibia and insecta. *DFR1* encodes one of the two *Drosophila* FGF-R species (Shishido et al., 1993). In early embryos, the expression of *DFR1* mRNA is specific to mesoderm. Using a small deficient chromosome from which the *DFR 1* locus had been deleted, the possible involvement of *DFR1* was previously shown in the patterning of muscle precursor cells (Shishido et al., 1993). But this does not provide sufficient indication of the functions of *DFR1*. Using the above deficiency, not only *DFR1* but also several other genes with functions possibly indispensable for early mesodermal development are removed (Bellen et al., 1992) .

Here, we describe the isolation and phenotype characterization of a mutant lacking the ability to produce DFR1. Dynamical changes in DFR1 expression, visualized by anti-DFR1 antibody, are also described. Mutant phenotypes indicate that *DFR1* is essential not only for mesodermal cell differentiation but also for CNS formation. DFR1 mutant phenotypes are partially mimicked by targeted ectopic expression of activated Yan.

While this paper was in review, two papers (Beiman et al., 1996; Gisselbrecht et al., 1996) appeared which report requirements of *DFR1* for migration and differentiation of early mesodermal cells. Our results include considerable extensions and partial amendments of their observations.

MATERIALS AND METHODS

Fly strains and genetics

Df(3R)sr^{16} was described previously (Shishido et al., 1993). *wg*^{CX4} is a null allele of *wg*. A fly line with UAS-*yan*^{ACT} was obtained from G. Rubin (Rebay and Rubin, 1995). Four *DFR1* (*htl*) mutants, *htl*^{80}, *htl*^{108}, *htl*^{117} and *htl*$^{S1-28}$ were identified among 112 lethals previously shown to fall within the *Df(3R)P14* deficiency (Campbell et al., 1994). These *htl* chromosomes were lethal in *trans* to each other and over *Df(3R)sr*^{16}. Genotypes of embryos were determined using a suitable blue-balancer. P-mediated germ-line transformation was carried out as follows. After genomic fragments derived from the *DFR1* locus were cloned into a transformation vector pYCM (Fridell and Searles, 1991), resultant constructs were introduced into fly lines carrying *htl*^{80}. Several transformant lines recovered were crossed with *Df(3R)sr*^{16} flies and tested for survival of *htl*^{80}/*Df(3R)sr*^{16}. Two of five E12 transformants rescued *htl*^{80}/*Df(3R)sr*^{16}.

Antibody production

Anti-DFR1 (anti-Htl) antiserum was prepared as follows. A 768bp *Bal*I fragment of cFR4-6 (Shishido et al., 1993) was subcloned into pET3b (Rosenberg et al., 1987) to overproduce a T7-gene-10 protein fused with a part of the extracellular domain of DFR1 (from amino acid position 30 (N) to 285 (V)) in *Escherichia coli* cells. Partially purified fusion protein was used for immunization of rabbits and resultant antiserum was affinity-purified as described previously (Higashijima et al., 1992). The resultant antibody was specific to DFR1, there being no signals in embryos homozygous for *Df(3R)sr*^{16}.

Antibody staining and in situ hybridization

Antibody staining was carried out as described previously (Higashijima et al., 1992) with the exception of the usage of Cy3- or FITC-conjugated second antibody for confocal microscopic observation. Primary antibodies were: rabbit anti-DFR1 antibody used at 1:1000, rabbit anti-Eve antibody (Frasch et al., 1987) used at 1:50000, rabbit anti-MHC (myosin heavy chain) antibody (Kiehart and Feghali, 1986) used at 1:500, rabbit anti-Twist antibody (Thisse et al., 1988) used at 1:500, mouse anti-Fas III antibody (Snow et al., 1989) used at 1:10, mouse anti-Fas II antibody used at 1:5 (Lin et al., 1994) and mouse anti-Eve antibody used at 1:2 (Patel et al., 1992). In situ hybridization was performed as described previously (Shishido et al., 1993). *tin* cDNA was obtained from R. Bodmer. Araldite sections of embryos were prepared according to Azpiazu and Frasch (1993).

RESULTS

Expression of DFR1 FGF-R in mesodermal cells

The mesoderm-specific expression of *DFR1* RNA noted previously (Shishido et al., 1993) indicates *DFR1* to possibly be required for mesodermal cell development. DFR1 protein expression must be examined in detail for clarification of its place in embryogenesis. Antibody was thus initially raised against the extracellular domain of DFR1. The DFR1 protein expression occurred initially in all mesodermal cells prior to gastrulation (stage 5; Fig. 1A,B). In contrast to uniform cell-surface distribution at later stages, DFR1 at this stage was localized exclusively on the apical cell surface (Fig. 1A, inset). Internalized mesodermal cells, spreading out below the ectoderm, persistently showed DFR1 expression (stage 6-10; Fig. 1C-E). This early mesodermal expression of DFR1 (first phase expression) is positively regulated by *twist* and *snail* (Shishido et al., 1993). As shown in Fig. 1J, it is significant that DFR1 distribution at stage 8-10 was homogeneous along the dorsoventral axis.

During stage 9, the area expressing DFR1 was divided into two domains, cephalic and postcephalic mesoderms, and the pattern of DFR1 expression in the latter began to show signs of a metameric fluctuation along the anteroposterior axis (Fig. 1E). A similar segmental expression pattern has been reported in Twist (Dunin Borkowski et al., 1995). Using *ftz-lacZ* stripes as internal references, we compared Twist and DFR1 expression (Fig. 1G-I), and found that, as in the case of Twist, future parasegmental borders are situated in periodic patches exhibiting relatively high DFR1 signals. During stage 10, DFR1 expression showed steady decrease, the reduction being sharp at stage 11 with residual but strong expression remaining in a fraction of somatic muscle precursor cells (Fig. 1F,K). Little or no DFR1 signals were detected in heart precursors at stage 11 (Fig. 1K). We presume the second phase expression of DFR1 in most somatic muscle precursors to start during stage 11.

At stage 12, the second-phase DFR1 expression occurred in heart precursors (Fig. 1L,N). Staining for Even-skipped (Eve) and DFR1 in heart primordia (Fig. 1O,P) revealed only cardial cell precursors, negative to Eve (Azpiazu and Frasch, 1993), to express DFR1. The second round of DFR1 expression in visceral muscle precursors for midgut and hindgut became discernible at stage 14 (Fig. 1Q,R), when dorsal pharyngeal muscles and foregut visceral muscles, derivatives of the cephalic mesoderm, were clearly observable as DFR1-positive tissues (Fig. 1R). From stage 12 onward, DFR1 expression was evident in a fraction of CNS cells (see below). Most DFR1 signals in somatic muscles (Fig. 1M) had faded out by stage 16 (Fig. 1S) except for signals in a few muscles including muscle 25 (ventral transverse muscle 1) in abdominal segments (A2-A7) and ventral internal segmental muscle 2, 4 and 5 in the thorax.

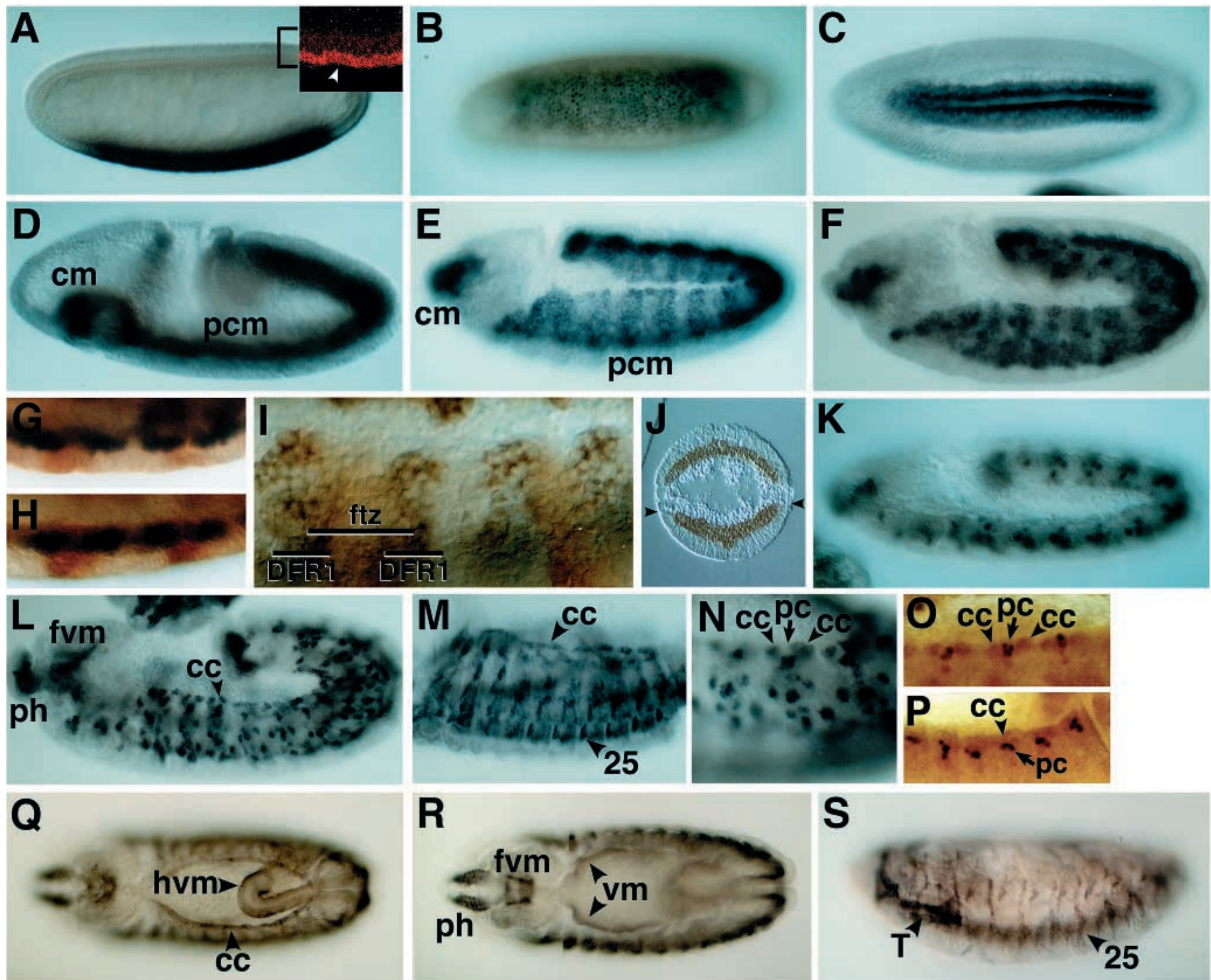

Fig. 1. DFR1 expression in wild-type embryos visualized with anti-DFR1 antibody. (A-I, K-S) Anterior is left and dorsal is up; (A,C-I,K-P,S) lateral views; (B) ventral view; (Q,R) dorsal views. (A,B) Stage 5; inset, a confocal image showing the apical localization of DFR1 signals. Arrowhead, apical cell surface; bracket, cell depth. (C) Stage 6; (D) stage 8; (E) stage 9; note a metameric fluctuation of DFR1 expression. (F) Stage 10/11; (G,H) stage-10 embryos stained for *ftz-lacZ* (brown) and Twist (black; G) or DFR1 (black; H). Note that metameric fluctuation of Htl is similar to that of Twist. (I) Enlargement of H; (J) transverse section (stage 10) showing DFR1 to distribute homogeneously. Arrowheads, ectodermal dorsal limit. (K) Stage 11; (L) stage 12; (M) stage 13; (N) enlargement of L. Arrow, pericardial cell (pc) precursor position. (O,P) Anti-DFR1 (brown)/anti-Eve (black) double staining at stages 12 (O) and 13 (P). Note that pericardial cells (pc) are Htl-negative. (Q,R) Stage 14; (S) stage 16. cc, cardial cells; cm, cephalic mesoderm; fvm, foregut visceral mesoderm; hvm, hindgut visceral mesoderm; pcm, postcephalic mesoderm; ph, pharyngeal muscle precursors; T, thoracic ventral internal segmental muscles (2,4 and 5); 25, muscle 25.

Isolation of DFR1 mutants with defective somatic muscle and heart formation

DFR1 has been assigned to the cytological position, 90D-E, uncovered by the deficiency $Df(3R)sr^{16}$ (Shishido et al., 1993). For clarification of the functions of *DFR1*, recessive lethal mutations were sought in the 90D-E region. Using anti-DFR1 antibody, study was made to determine whether embryos of 112 lethals, isolated by Campbell et al. (1994), are defective in DFR1 protein production. For htl^{80}, no protein stainable with anti-DFR1 antibody could be found throughout embryogenesis (Fig. 2D), indicating the absence of DFR1 protein production and this mutant thus to be a protein-null allele of *DFR1*. To further confirm this, examination was made of whether htl^{80} lethality is rescued by P-mediated transformation with wild-type genomic sequences. E12, a 12 kb fragment from the *DFR1* locus (Fig. 3A), was found not only to rescue the lethality of $htl^{80}/Df(3R)sr^{16}$ but to recover DFR1 expression as well (Fig. 2A,G). htl^{80} is thus concluded to be a protein-null allele of *DFR1* all of whose essential parts are present within the E12 region. Three other *DFR1* alleles, htl^{108}, htl^{117} and $htl^{S1\text{-}28}$, were identified as alleles incapable of complementing htl^{80}. In contrast to htl^{80}, these alleles exhibited DFR1 expression detectable with anti-DFR1 antibody, indicating that they are not protein-null alleles of *DFR1*.

Using $Df(3R)sr^{16}$, *DFR1* was previously shown to be involved in muscle development in embryos (Shishido et al.,

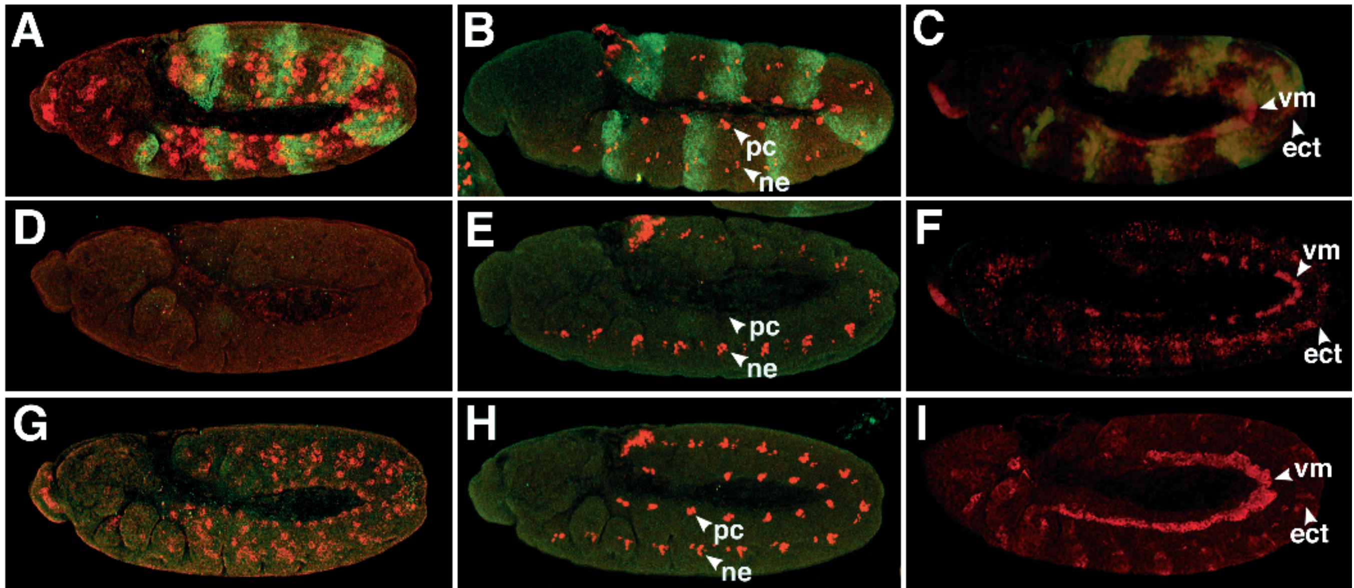

Fig. 2. E12-dependent rescue of *htl* mutant defects in the expression of Htl in somatic muscle primordia (A,D,G), Eve in heart primordia (B,E,H) and Fas III in visceral mesoderm (C,F,I) at stage 12. Red, Htl, Eve or FasIII signals. (A-C) *htl/TM3β* embryos expressing *ftz-lacZ* (green); (D-F) *htl* mutant embryos; (G-I) E12 transformants. ect, ectoderm; ne, neurons; pc, pericardial cells; vm, visceral mesoderm. Note the expression of *htl*, *eve* and *fas III* is restored in transformants.

Fig. 3. (A) Restriction map of the DFR1 locus (Shishido et al., 1993; Ito et al., 1994; this work), and (B,C) anti-MHC staining of wild type, (D-I) *htl* mutants and (J) a transgenic embryo at stage 16. (B-J) Anterior is left; (B,D,F-J) dorsal views; (C,E) lateral views. (B,C) Wild type; (D,E) $htl^{80}/Df(3R)sr^{16}$. The dorsal vessel (dv) and most of dorsal muscles are missing. (F) $htl^{108}/Df(3R)sr^{16}$; (G) $htl^{117}/Df(3R)sr^{16}$; (H) $htl^{S1\text{-}28}/Df(3R)sr^{16}$; (I) htl^{80} homozygotes; (J) a $htl^{80}/Df(3R)sr^{16}$ embryo having E12. Virtually all defects in mutant muscles are rescued. p, pleural transverse muscles; v, ventral longitudinal muscles.

1993). Mutant phenotype examination was thus made using anti-MHC antibody (Kiehart and Feghali, 1986). In *trans* to $Df(3R)sr^{16}$, all *DFR1* mutants showed loss of the heart, and severely defective somatic and visceral muscles as in the case

of *htl*^{80} homozygotes (Fig. 3B-I and data not shown). All defects in *DFR1* mutants were eliminated by the *DFR1* transgene expression (Fig. 3J). Thus, it was concluded that *DFR1* is essential for the formation of the heart, midgut visceral muscles and somatic muscles.

Since all *DFR1* mutants examined to date exhibit the heartless phenotype, and possible confusion in naming of *Drosophila* FGF-R genes (*DFR1/DFR2* (Shishido et al., 1993) vs. *DFGF-R2/DFGF-R1* (Klämbt et al., 1992)) should be avoided, *DFR1* is hereafter referred to as *heartless* (*htl*) in accordance with the nomenclature of other workers (Beiman et al., 1996; Gisselbrecht et al., 1996). Unless otherwise stated, *htl*^{80}/*Df(3R)sr*^{16} transheterozygotes are used as mutants lacking *htl* activity.

Requirements of *htl* for mesodermal cell migration and proper mesoderm subdivision

During stages 8 and 9, the mesoderm spreads with dorsolateral orientation along the underlying ectoderm to form a monolayer extending to the border between dorsal ectoderm and amnioserosa (see Fig. 1J). Heart, visceral and somatic muscles are produced through separate subdivisions of the extended mesoderm (Azpiazu and Frasch, 1993; Frasch 1995; Dunin Borkowski et al., 1995; Azpiazu et al., 1996). In these tissues or direct precursors, the second phase of Htl expression occurred from late stage 11 onward (see Fig. 1). Most, if not all, *htl* phenotypes observable from late stage 11 onward should thus be formed as a result of the absence of both the first and second phases of *htl* expression. Using *Df(3R)sr*^{16}, we previously indicated *htl* not to be required for invagination (Shishido et al., 1993). Thus, with *twist* and *tinman* (*tin*; Azpiazu and Frasch, 1993), molecular markers of stages 8-10, *htl* (first phase expression) was studied for its being essential for early mesodermal cell migration after gastrulation.

Twist is expressed in all early mesodermal cells (Thisse et al., 1988). In wild-type embryos, the dorsal edge of extended mesoderm reached the dorsal crest at stage 9 or 4 hours AEL (Fig. 4A-D), while that of mesoderm in the *htl* mutant embryos failed to reach the dorsal circumference presumably because of incomplete spreading; rather, it was situated several cells away from the wild-type dorsal tip (Fig. 4E-H). Since both wild-type and mutant embryos were noted to have virtually the same number of Twist-positive nuclei (data not shown), the proliferation of mesodermal cells should be normal in *htl* embryos, and loss of *htl* activity may limit the dorsal migration of mesodermal cells.

To obtain stronger evidence of the presence of defects in *htl* mutant mesodermal cells in early migration, search was made for changes in the *tin* expression of these cells. Precursors for larval heart are derivatives of dorsal-most subdivisions of mesoderm (Bate, 1993; Bodmer, 1993; Azpiazu and Frasch, 1993; Frasch, 1995). *tin* encodes a homeodomain protein (Bodmer et al., 1990) essential for the formation of the heart and visceral muscles (Azpiazu and Frasch, 1993; Bodmer, 1993). It is initially expressed in all mesodermal cells (Fig. 4I; Bodmer, 1993; Azpiazu and Frasch, 1993; Frasch, 1995) and is positively regulated by *twist* (Bodmer et al., 1990). Soon following completion of mesodermal cell migration, *twist*-dependent *tin* expression gradually fades out to be replaced by *dpp*-dependent strong *tin* expression directly below dorsal ectodermal cells expressing *dpp* (Frasch, 1995). At stage 10, mesodermal cells not below *dpp*-expressing ectodermal cells completely lose *tin* RNA (Frasch, 1995). As shown in Fig. 4J, each domain of *dpp*-dependent *tin* expression splits into heart and midgut visceral mesoderm regions at stage 11 (Azpiazu and Frasch, 1993). In *htl* embryos, *tin* mRNA is initially expressed in all mesodermal cells (Fig. 4L) and later becomes restricted to dorsal part of mesoderm as with wild type (Fig. 4M). However, intensity of *tin* expression decreased extensively and one of the two subdomains was lost (compare Fig. 4J and M). The *tin* subdomain in the *htl* mutant at stage 11 appeared positioned virtually the same as in the wild-type subdomain for visceral mesoderm and disappeared at stage 12 as also noted for the wild-type visceral subdomain (Azpiazu and Frasch, 1993; Fig. 4N). In the wild-type heart domain, *tin* expression persists at later stages (Fig. 4K; Azpiazu and

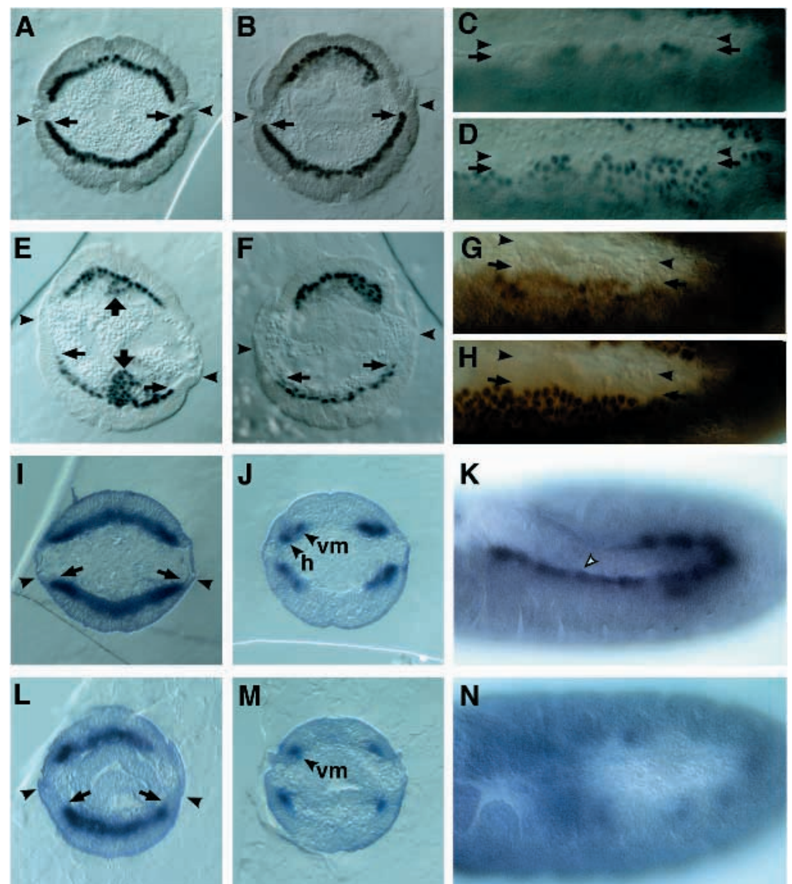

Fig. 4. Defects in early mesoderm migration visualized with anti-Twist antibody staining (A-H) and *tin* expression in the *htl* mutant (I-N). (A-D,I-K) Wild-type embryos; (E-H,L-N) *htl*^{80}/*Df(3R)sr*^{16} embryos. Focal planes in C and G, ectodermal cell layer. Focal planes in D and H, mesodermal cell layer. Arrowheads, ectoderm-amnioserosa borders; small arrows, dorsal limits of mesoderm; large arrow, abnormally protruded mesoderm; open arrowhead, cardial cell precursors; vm, visceral mesoderm; h, heart precursors.

Frasch, 1993). Dorsal *tin* expression itself would thus appear independent of *htl*, but reduction in the number of mesodermal cells capable of receiving *dpp* signals from the dorsal-most ectoderm (Frasch, 1995) causes changes in the dorsoventral subdivision of mesoderm, in particular, loss of the *tin* domain for heart progenitors in *htl* mutant embryos. Consistent with this, *htl* mutant mesodermal cells responded to ectopic Dpp signals, with consequent induction of the expression of *eve,* a gene specific to heart primordia (pericardial cell precursors; Beiman et al., 1996; Gisselbrecht et al., 1996).

htl essential for heart precursor formation

The larval heart is a pulsating vessel situated quite close to the ectoderm in the dorsal midline region (Bate, 1993; Zaffran et al., 1995) and comprises of two apposed layers of myoendothelial cells (cardial cells) surrounded by pericardial cells. Pericardial cell precursors are detectable as cells expressing a pair-rule gene, *eve* (Fig. 2B; Frasch et al., 1987). In *htl* embryos, no Eve-expressing cell clusters could be found in the region for prospective heart precursors (Fig. 2E). At the start of the germ-band retraction, *tin* expression is evident only in a continuous row of cells on the dorsal rim in wild-type embryos, which later form the heart (Fig. 4K; Bodmer et al., 1990; Bodmer, 1993): *tin* expression in the prospective midgut visceral mesoderm has faded away by the end of stage 11 (Azpiazu and Frasch, 1993). In *htl* embryos, *tin* expression could be no longer detected from stage 12 onward (Fig. 4N), indicating that heart precursors are not specified or formed in the *htl* mutant. Defects in the expression of *eve* and *tin* in the *htl* mutant was eliminated completely by the expression of the *htl* transgene (Fig. 2H and data not shown). *eve* expression was partially rescued by ectopic *dpp* expression driven by *twist*-Gal4 (Beiman et al., 1996; Gisselbrecht et al., 1996). Thus, it is concluded that, in *htl* embryos, presumptive heart primordia fail to come into contact with dorsal ectoderm from which Dpp signals are emitted and consequently never take on the status of heart primordia.

Interrupted Fasciclin III expression in *htl* mutant precursors for visceral midgut muscles

The larval gut is coated with a layer of visceral muscles as the source of power for peristaltic movement of digestion (Bate, 1993). Fasciclin III (Fas III) may serve as a marker for progenitors of midgut visceral mesoderm. At stage 11, Fas III is initially expressed in segmental patches. The expression becomes strong in cells situated along the ventral periphery of the patches and these cells form a band of visceral mesoderm in which Fas III expression continues up to stage 13 (Fig. 2C; Bate, 1993; Azpiazu and Frasch, 1995). In *htl* embryos, Fas III expression was frequently interrupted (Fig. 2F).

Connectin (Con) is a cell surface molecule, which marks a subset of visceral midgut muscle precursors (Nose et al., 1992; Bate, 1993). Our unpublished data showed Con expression cells to decrease considerably in mutant embryos. The defects in Fas III and Con expression in the mutant were completely rescued by the E12 transgene (Fig. 2I and data not shown).

The presence of Fas-III-positive and Con-positive cells indicates that specification of visceral muscle precursors takes place to some extent in *htl* mutant embryos, although Fas-III- or Con-positive cells are greatly reduced in number, consistent with the failure of spreading mesodermal cells in *htl* mutants.

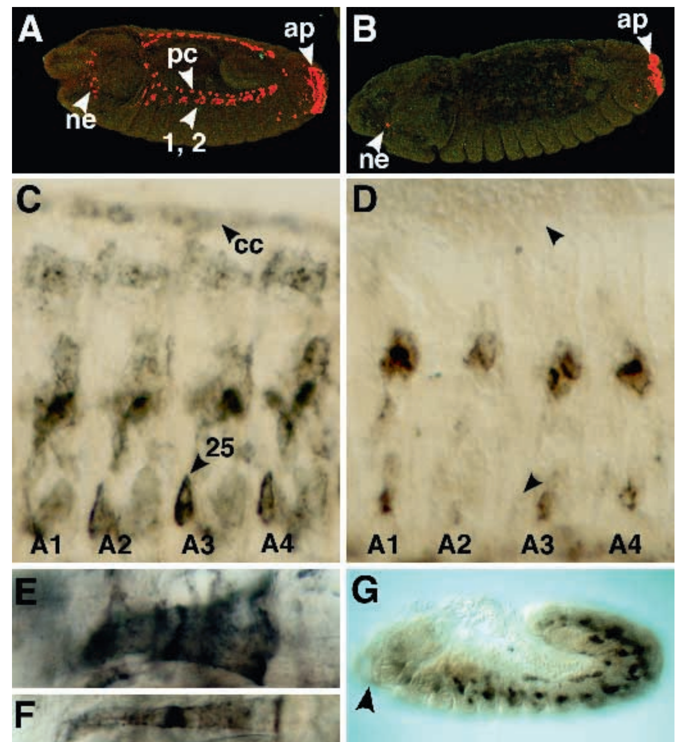

Fig. 5. Muscle defects in *htl* mutants. (A,B) Anti-Eve staining (confocal images) of wild-type (A) and $htl^{80}/Df(3R)sr^{16}$ (B) embryos at stage 14. In the mutant, no Eve expression occurred in pericardial cells (pc) and dorsal muscles, 1 and 2. ap, anal plate; ne, neurons; pc, pericardial cells. (C,D) Anti-Htl antibody staining of wild-type (C) and $htl^{S1\text{-}28}/Df(3R)sr^{16}$ (D) somatic muscles in abdominal segments (A1-A4). In mutants, Htl expression is absent from muscle 25 and cardial cells (cc). (E,F) Thoracic ventral internal segmental muscles (2,4 and 5) in wild-type (E) and $htl^{S1\text{-}28}/Df(3R)sr^{16}$ (F) embryos at stage 16. Note the reduction of Htl expression in F. (G) A $htl^{S1\text{-}28}/Df(3R)sr^{16}$ embryo. The arrowhead shows the absence of cephalic Htl signals.

Reduction in somatic muscles in *htl* embryos

Larval abdominal hemisegments (A2-A7) normally each contain a set of 30 syncytial muscle cells all of which can be distinguished in size, shape and point of attachment from late embryogenesis (Bate, 1993). Larval muscle formation is initiated with start of germ-band retraction (7.5 hour AEL). Somatic muscles in 111 hemisegments from late *htl* embryos were examined using anti-MHC antibody and dorsal oblique muscles were observed to have decreased to 26% of those of the wild-type and ventral longitudinal and oblique muscles, to 54%. Pleural and dorsal transverse muscles in the lateral body wall showed moderate decrease (86%) and, as expected, no Eve-positive dorsal-muscle cells corresponding to muscle 1 could be found at stage 14 in the *htl* mutant (Fig. 5A,B). Many unfused myoblasts were seen scattered in mutant embryos (Fig. 3D), possibly indication of fewer dorsal muscle precursor cells (putative muscle founder cells; Bate, 1993; Rushton et al., 1995) to which undifferentiated myoblasts become fused.

Anti-Htl antibody staining showed Htl expression in abdominal ventral transverse muscle 1 (muscle 25; Fig. 5C,D) and thoracic ventral internal segmental muscles (2, 4 and 5;

Fig. 5E,F), strongly expressing Htl from stages 13 and 15, respectively, in the wild-type, to be absent from or reduced in most hemisegments of *htl*$^{S1-28}$, a producer of an inactive form of Htl. Although Htl expression in cephalic mesoderm was evident at stage 10, Htl expression disappeared in the derivatives of the cephalic mesoderm, dorsal pharyngeal muscles and foregut visceral muscles, at later stages (Figs 1L, 5G).

It is quite feasible that late defects in somatic musculature are partly due to the absence of the second phase or somatic-muscle-specific expression of *htl*. Intricate somatic muscles are considered to form in accordance with positional information in the ectoderm (Bate, 1993; Staehling-Hampton et al., 1994). Formation of the mutant cuticle was followed but nothing abnormal was found. Muscle defects in mutant embryos thus appeared to be intrinsic to the mesoderm.

Requirements of *htl* for CNS differentiation

Our previous experiment (Shishido et al., 1993) showed *htl* to be expressed in CNS cells. To determine whether these cells are neuronal or glial, wild-type embryos at late stage 11/early stage 12 were subjected to double-staining with anti-Htl antibody and anti-Reverse potential (Repo), which specifically labels virtually all CNS glial cells (Xiong et al., 1994; Campbell et al., 1994; Halter et al., 1995). Most, if not all, Htl-positive CNS cells (several cells per hemisegment (see Fig. 6K)), occupying a position for future longitudinal tracts (Jacobs and Goodman, 1989), were found to express Repo (Fig. 6L), indicating Htl-positive CNS cells to be interface glia (IG), which consist of longitudinal glia (LG) and their immediate glial neighbours (Fig. 4 in Halter et al., 1995). Consistent with this, two rows of Htl-positive non-neuronal cells, running parallel with each other along the dorsal face of developing longitudinal connectives, were visualized by anti-Htl antibody at late stage 12, early stage 13 (Fig. 6G). At stage 16, nuclei of LG cells are arranged in two rows on each side of the midline (Campbell et al., 1994) and LG cells ensheath longitudinal axon tracts (Jacobs and Goodman, 1989). Anti-Htl antibody staining of the wild-type CNS showed Htl to be expressed in the extended cytoplasmic sheets growing out of the IG/LG cell bodies, which encircle the neuropil from lateral and medial sides (Fig. 6A,C).

It is possible to identify *htl* mutant IG/LG cells in *htl*$^{S1-28}$/*Df(3R)sr*^{16} embryos by staining with anti-Htl antibody. As shown in Fig. 6H, in mutant embryos at stage 12/13, IG/LG cells were not only spatially disorganized but also exhibited a significant reduction in migration rate (compare Fig. 6H with G). Unlike wild-type cells, mutant IG/LG cells were rounded and appeared to be incapable of increasing their surface area. In contrast to wild type, mutant IG/LG cells at stage 16 could not line up in two files and appeared to fail to enwrap longitudinal axon tracts (Fig. 6B,F). Fig. 6D,E shows the interval between bilateral rows of IG/LG cells in the mutant to be 2-3 times larger than that of the wild-type. Mutant longitudinal axon fascicles were virtually normal in appearance (Fig. 6I,J). Thus, we conclude that *htl* is essential for IG/LG cell migration and ensheathing longitudinal connectives.

Partial mimicking of the *htl* mutant phenotype by targeted mesodermal expression of activated Yan

The MAP-kinase pathway has been shown to be involved in signaling by Breathless, another *Drosophila* FGF-R (Reichman-Fried et al., 1994). Thus, reduction in MAP-kinase signaling activity in mesodermal cells may result in *htl* mutant phenotype. To test this possibility, flies with a construct that drives spatially restricted Gal4 expression under the control of *htl* regulatory elements (Ono and Saigo, unpublished data) and flies expressing an activated form of Yan, a downstream repressor of the MAP-kinase pathway, under the control of Gal4 upstream sequences (Rebay and Rubin, 1995) were crossed. Embryos derived from this cross express activated Yan in all mesodermal cells from stage 10 (data not shown). The effects of activated Yan on the expression of Eve, Fas III, and Htl in the wild-type background were studied.

The ectopic expression of activated Yan apparently caused reduction in Eve-expressing cells (Fig. 7A,B); a much less extensive effect on Fas III expression was detected (Fig. 7C,D).

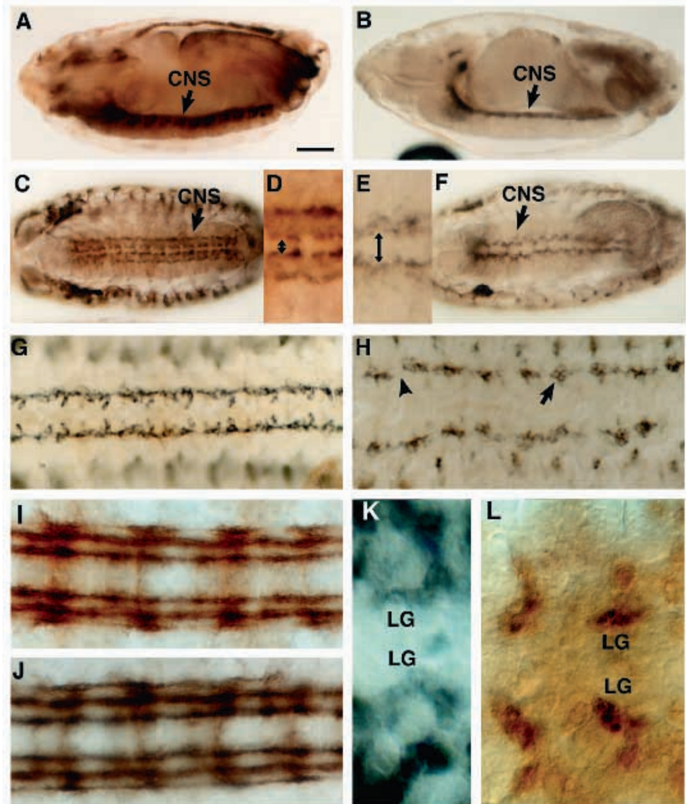

Fig. 6. Requirements of *htl* in CNS formation. (A,C,D,G,I,K,L) Wild-type embryos, (B,E,F,H,J) *htl*$^{S1-28}$/*Df(3R)sr*^{16} embryos. (A-F) Anti-Htl antibody staining (stage 16). (A,B) Lateral views; (C-F) ventral views; (D,E, respectively) enlargements of C and F. Arrows with double heads, bilateral medial IG/LG cells. Note that, in E, Htl-positive glial cells are disorganized and appear to fail to extend cell bodies properly. (G,H) Anti-Htl antibody staining of dissected, flattened sections at late stage 12/early stage 13. The arrow indicates round mutant IG/LG cells. Arrowhead, gapped region. Note that the wild-type CNS has neither round IG/LG cells nor gapped region. (I,J) Anti-Fas II antibody staining patterns of CNS at stage 16. No apparent difference can be seen between wild type and mutants. Anti-Htl antibody staining (K) and double staining with anti-Htl (brown)/anti-Repo (black) at early stage 12, when Repo is strongly expressed in LG (Halter et al., 1995). (L) LG indicates IG/LG cells. Scale bar in A, 50 μm for A-C and F; 25 μm for D,E,G and H; 12.5 μm for I-L.

Anti-MHC antibody staining indicated normal heart and somatic muscle formation in part not to occur (Fig. 7E,F), though that of midgut visceral muscles appeared to proceed normally (data not shown). These findings, and the fact that the *htl* mutant phenotype is partially rescued by the targeted mesodermal expression of an activated form of Ras1 (Gisselbrecht et al., 1996), may indicate that the MAP-kinase pathway acting downstream of Htl or parallel to its pathway or both is essential for the formation of heart and somatic muscles.

Wingless/Hedgehog dependency of Htl expression in putative somatic muscle precursors

The second phase of Htl expression extends from stage 11 onward in putative precursors for heart, somatic and visceral muscles. Heart formation was previously shown to require both *wg* and *hh*, with only *hh* being required for normal visceral mesoderm formation (Wu et al., 1995; Park et al., 1996; Baylies et al., 1995). Htl expression in somatic muscles is segmental (see Fig. 1E). Study was thus made to see whether Htl expression in somatic muscles requires Wg and/or Hh signals. Anti-Htl staining showed many muscle precursors in *hh* mutants to be capable of producing Htl (data not shown). In contrast, Htl expression in a large fraction of presumptive somatic muscle precursors was *wg*-dependent (Fig. 7G,H). *wg* is expressed in the mesoderm only for a short period after gastrulation (Lawrence et al., 1994); the results thus suggest that the second phase of Htl expression may be controlled by Wg signals from ectodermal cells.

DISCUSSION

Htl signaling and formation of mesodermal tissues

The mode of Htl expression suggest that signaling relevant to Htl occurs in at least three stages of mesodermal development:(1) an early stage for spreading of dorsolaterally orientated mesodermal cells, and (2) that for segregation of the mesoderm subdivided by inductive signals. The third stage of signaling is induced by the second phase of Htl expression in precursors of each mesodermal tissue or organ. This study, and those of Beiman et al. (1996) and Gisselbrecht et al. (1996), established the functions of Htl at the earliest stage of mesodermal development by characterizing mutant phenotypes.

The dorsal subdivision of the mesoderm has been shown elicited by signals derived from the ectoderm (Frasch, 1995; Staehling-Hampton et al., 1994). On preventing the mesoderm from interacting with the dorsal part of the ectoderm, induction of the formation of the heart and visceral muscles was not possible (Baker and Schubiger, 1995). Genetic analysis showed Dpp to serve as central signaling molecules from the dorsal ectoderm to underlying mesoderm and these Dpp-dependent signals to be essential for *tin* activation in dorsal subdivision of mesoderm (Frasch, 1995). Since *tin* is a homeobox gene required for the formation of the heart and visceral muscles (Bodmer et al., 1990), restriction of *tin* expression to dorsal mesodermal cells is absolutely required for early regional subdivision of the mesoderm (Frasch, 1995).

Maggert et al. (1995) noted that narrowing of the ventral furrow (prospective mesoderm) caused loss or reduction of heart and visceral muscles. In their mutants, there was no spreading of mesoderm to the dorsal region; therefore, fewer cells could come into contact with *dpp*-expressing ectodermal cells and, as a result, dorsal *tin*-expression region failed to be formed. These phenotypes appear to be the same as those observed here for *htl* mutants. In the absence of Htl, not only does properly directed migration of mesodermal cells fail to occur (see Fig. 4A-H), but there is also no normal formation of heart and visceral muscles (see Figs 2, 3). *tin* domains for heart precursors and visceral muscles, respectively, did not form and was reduced in size (see Fig. 4J,M). Since *htl* mutant mesodermal cells are capable of expressing *eve*, a homeobox gene specific to pericardial cells (Frasch et al., 1987), in response to exogenous *dpp* signaling (Beiman et al., 1996; Gisselbrecht et al., 1996), the first phase of Htl expression in primitive mesoderm may be essential for the dorsolateral

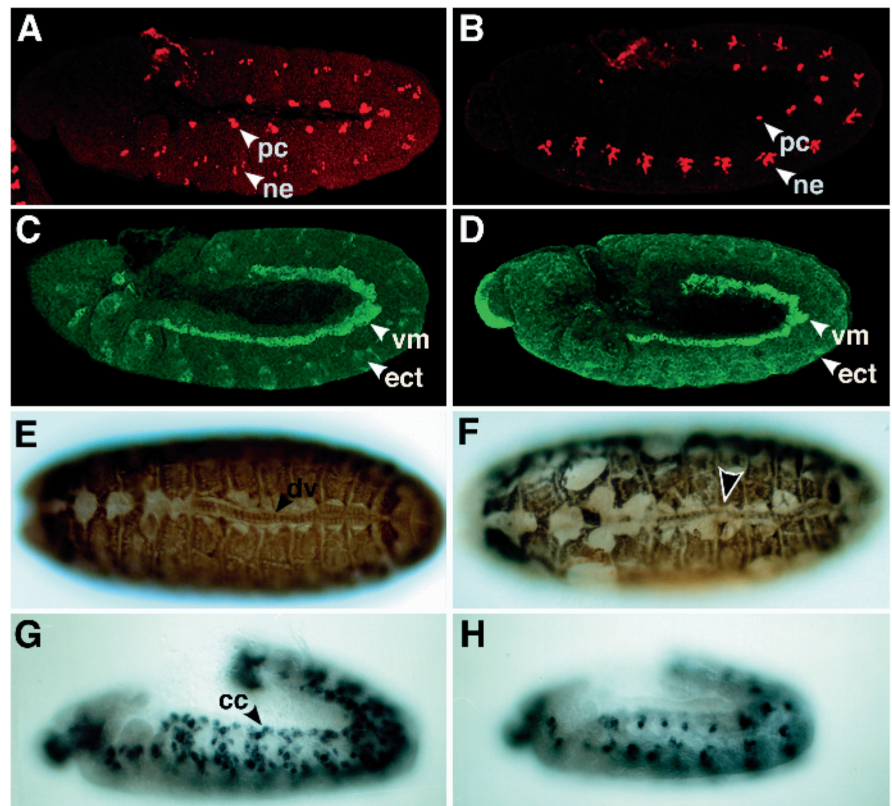

Fig. 7. Effects of the targeted expression of activated Yan (A-F) and the absence of *wg* (G,H). (A,C,E,G) Wild-type embryos; (B,D,F) embryos expressing activated Yan. Heart and skeletal muscle formation is deranged. (H) A wg^{CX4} embryo. Embryos were stained with anti-Eve (A,B), anti-Fas III (C,D), anti-MHC (E,F) and anti-Htl (G,H) antibodies. (A-D) Confocal images. cc, cardial cells; ne, neurons; pc, pericardial cells; vm, visceral mesoderm. The arrowhead in F shows a deranged dorsal vessel.

migration of mesodermal cells and the loss of heart precursors and reduction of visceral mesoderm in *htl* mutants may be due to the failure of mutant mesodermal cells to reach the dorsal-most region.

A recent model of trachea formation suggests that the pathway involving Branchless FGF and Breathless (Btl) FGF-R specifies the mode of trachea branching by guiding tracheal cell migration during primary branch formation (Sutherland et al., 1996). However, whether Htl FGF-R responds to putative chemotactic signals for primitive mesodermal cell migration is not known. Anti-Htl antibody staining of transverse sections of wild-type embryos could indicate no preferential distribution of Htl (see Fig. 1J). Htl appeared distributed evenly on the surface of all primitive mesodermal cells except for those at stage 5, a stage before gastrulation, in which Htl is localized in the apical cell surface (see the inset of Fig. 1A). It is possible that mesodermal cells are activated by putative Htl ligands possibly present in perivitelline fluid at stage 5 and, consequently, acquire the ability for chemotaxis at later stages.

Twist expression is known to be modulated in a metameric fashion at stage 9/10 of development (Dunin Borkowski et al., 1995). Baylies and Bate (1996) recently showed that levels of Twist expression are critical determinants of mesodermal differentiation in the early embryo. The absence of Twist causes defects in somatic muscle formation, while high levels of Twist switch cells into somatic myogenesis during subdivision of the mesoderm, resulting in various derangements in heart and visceral muscle formation. Thus, similarity in modulation of expression between Twist and Htl is an intriguing finding (see Fig. 1G-I). One might imagine that Htl serves as a downstream element of Twist to segregate the subdivided mesoderm.

The second phase of Htl expression is *wg*-dependent and most, if not all, positive signals emerged in the regions of each parasegment with relatively high levels of the first phase of Htl expression (see Fig. 1F,L), possibly suggesting that the metameric modulation of the first phase of Htl expression is also *wg*-dependent. Since gene expression and/or differentiation of a fraction of somatic muscles, pharyngeal muscles and foregut visceral muscles were affected at later stages by the absence of *htl* (see Fig. 5), it is quite feasible that *htl* is also implicated in late differentiation of mesodermal cells.

As with mesodermal cells, Htl is required for migration and morphogenesis of interface glia including LG cells (see Fig. 6). Klämbt et al.(1992) showed another *Drosophila* FGF-R, Btl, to play an important role in migration of midline glia. Together, these findings indicate the concerted expression of two FGF-Rs in glial cells to be essential for CNS formation.

Possible roles of FGF-R in vertebrate mesoderm development

In chicken embryos, cardiac myocytes are derived from antero-lateral mesoderm called the heart-forming region (HFR) (Mima et al., 1995; Lough et al., 1996). When cells in HFR become committed to a cardial lineage, FGF and FGF-R are expressed at high levels (Mima et al., 1995). Introducing dominant-negative FGF-R showed that FGF-R is required for cardiac myocyte proliferation in early heart development (Mima et al., 1995). FGF-R has been shown necessary for the proper cell proliferation of mesoderm in mice (Deng et al., 1994). Abnormal axial formation in FGF-R-deficient mice does not arise from intrinsic blocks in mesodermal differentiation since cells from FGF-R-deficient mice can produce many mesodermal cell types in vitro.

Lough et al. (1996) showed BMP-2 of the TGF-β growth factor family and FGF-4 to be involved in the early heart development in vertebrates. Although neither FGF-4 nor BMP-2 alone induces the formation of cardial cells, together, they induce cardiogenesis. BMP-2 mimics cardiogenic effects on precardiac mesoderm but cannot support the viability of mesoderm. FGF-4 does not induce cardiogenesis in non-precardiac mesoderm. In *Drosophila*, heart formation requires FGF-R (Htl) and TGF-β (Dpp). As with *Drosophila tin*, *Nkx2-5*, a mammalian *tin* homolog, is expressed in early mesodermal development (reviewed in Harvey, 1996). A functional relationship would thus appear to exist between *tin* and *Nkx2-5*. It is of interest that vertebrates and invertebrates (*Drosophila*) both require essentially the same molecules for establishing the cardial system during embryogenesis.

MAP kinase pathway involvement in heart formation

Examination of the targeted expression of activated Yan (see Fig. 7A-F) indicated the involvement of the MAP kinase pathway in the heart formation and somatic muscles. Since no activated Yan expression could be detected prior to stage 10 by antibody staining (Ono and Saigo, unpublished data) and no appreciable effect of activated Yan on Fas III expression was detected (Fig. 7D), it is quite feasible that, under the present conditions, activated Yan can affect only late mesodermal differentiation starting from stage 11/12. Gisselbrecht et al. (1996) showed that the targeted mesodermal expression of an activated form of Ras1 can partially rescue hypomorphic *htl* mutant phenotypes. They also showed that activated Ras1 is capable of partially eliminating defects from oriented migration of mesodermal cells. It may thus follow that the MAP kinase pathway is required for normal differentiation of the heart throughout development.

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REFERENCES

Amaya, E., Musci, T. J. and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the FGF receptor disrupts mesoderm formation in Xenopus embryo. *Cell* **66,** 257-270.

Azpiazu, N. and Frasch, M. (1993). *tinman* and *bagpipe*: two homeobox genes that determine cell fates in the dorsal mesoderm of *Drosophila*. *Genes Dev.* **7,** 1325-1340.

Azpiazu, N., Lawrence, P. A., Vincent, J.-P., and Frasch, M. (1996). Segmentation and specification of the *Drosophila* mesoderm. *Genes Dev.* **10,** 3183-3194.

Baker, R. and Schubiger, G. (1995). Ectoderm induces muscle-specific gene expression in *Drosophila* embryos. *Development* **121,** 1387-1398.

Bate, M. (1993). The mesoderm and its derivatives. In *The Development of* Drosophila melanogaster (ed. M. Bate and A. Martinez Arias). pp. 1013-1090. New York: Cold Spring Harbor Laboratory Press.

Baylies, M. K., Martines Arias, A. and Bate, M. (1995). *wingless* is required for the formation of a subset of muscle founder cells during *Drosophila* embryogenesis. *Development* ***121*,** 3829-3837.

Baylies, M. K. and Bate, M. (1996). *twist*: A myogenic switch in *Drosophila. Science* **272,** 1481-1483.

Beiman, M., Shilo, B.-Z. and Volk, T. (1996). Heartless, a *Drosophila* FGF receptor homolog, is essential for cell migration and establishment of several mesodermal lineages. *Genes Dev.* **10,** 2993-3002.

Bellen, H. J., Vaessin, H., Bier, E., Kolodkin, A., D'Evelyn, D., Kooyer, S. and Jan, Y. N. (1992). The *Drosophila couch potato* gene: An essential gene required for normal adult behavior. *Genetics* **131,** 365-375.

Bodmer, R. (1993). The gene *tinman* is required for specification of the heart and visceral muscle in *Drosophila. Development* **118,** 719-729.

Bodmer, R., Jan, L. Y. and Jan, Y. N. (1990). A new homeobox-containing gene, *msh-2*, is transiently expressed early during mesoderm formation in *Drosophila. Development* **110,** 661-669.

Campbell, G., Göring, H., Lin, T., Spana, E., Anderson, S., Doe, C. Q. and Tomlinson, A. (1994). RK2, a glial-specific homeodomain protein required for embryonic nerve cord condensation and viability in *Drosophila. Development* **120,** 2957-2966.

Campos-Ortega, J. A. and Hartenstein, V. (1985). The embryonic development of *Drosophila melanogaster*. Springer-Verlag, Berlin.

Deng, C., Wynshaw-Boris, A., Shen, M. M., Daugherty, C., Ornitz, D. M. and Leder, P. (1994). Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev.* **8,** 3045-3057.

Dunin Borkowski, O, M., Brown, N. H. and Bate, M. (1995). Anterior-posterior subdivision and the diversification of the mesoderm in *Drosophila. Development* **121,** 4183-4193.

Frasch, M., Hoey, T., Rushlow, C., Doyle, H. and Levine, M. (1987). Characterization and localization of the even-skipped protein of Drosophila. *EMBO J.* **6,** 749-759.

Frasch, M. (1995). Induction of visceral and cardiac mesoderm by ectodermal Dpp in the early *Drosophila* embryo. *Nature* **374,** 464-467.

Fridell, Y.-W. C. and Searles, L. L. (1991). *vermilion* as a small selectable marker gene for *Drosophila* transformation. *Nuc. Aci. Res.* **19**, 5082.

Gisselbrecht, S., Skeath, J. B., Doe, C. Q. and Michelson, A. M. (1996). *heartless* encodes a fibroblast growth factor receptor (DFR1/DFGF-R2) involved in the directional migration of early mesodermal cells in the *Drosophila* embryo. *Genes Dev.* **10,** 3003-3017.

Gurdon, J. B. (1992). The generation of diversity and pattern in animal development. *Cell* **68,** 185-199.

Halter, D. A., Urban, J., Rickert, C., Ner, S. S., Ito, K., Travers, A. A. and Technau, G. M. (1995). The homeobox gene *repo* is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. *Development* **121,** 317-332.

Harvey, R. P. (1996). NK-2 homeobox genes and heart development. *Dev. Biol.* **178,** 203-216.

Higashijima, S., Michiue, T., Emori, Y. and Saigo, K. (1992). Subtype determination of *Drosophila* embryonic sensory organs by redundant homeo box genes *BarH1* and *BarH2*. *Genes Dev.* **6,** 1005-1018.

Ito, M., Matsui, T., Taniguchi, T. and Chihara, K. (1994). Alternative splicing generates two distinct transcripts for the *Drosophila melanogster* fibroblast growth factor receptor homolog. *Gene* **139,** 215-218.

Jacobs, J. R. and Goodman, C. S. (1989). Embryonic development of axon pathways in the Drosophila CNS. I. A glial scaffold appears before the first growth cones. *J. Neurosci. 97,* 2402-2411.

Jessell, T. M. and Melton, D. A. (1992). Diffusible factors in vertebrate embryonic induction. *Cell* **68,** 257-270.

Johnson, D. E. and Williams, L. T. (1993). Structural and Functional diversity in the FGF receptor multigene family. *Adv. Cancer Res.* **60,** 1-41.

Kiehart, D. P. and Feghali, R. (1986). Cytoplasmic myosin from *Drosophila melanogaster*. *J. Cell Biol.* **103,** 1517-1525.

Kimmelman, D. and Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGF-β and the identification of an mRNA coding for FGF in the early Xenopus embryo. *Cell* **51,** 869-877.

Klämbt, C., Glazer, L. and Shilo, B-Z. (1992). *breathless*, a Drosophila FGF receptor homolog, is essential for migration of tracheal and specific midline glial cells. *Genes Dev.* **6,** 1668-1678.

Lawrence, P. A., Johnston, P. and Vincent, J.-P. (1994). *Wingless* can bring about a mesoderm-to-ectoderm induction in *Drosophila* embryos. *Development* **120,** 3355-3359.

Lin, D. M., Fetter, R. D., Kopczynski, C., Grenningloh, G. and Goodman, C. S. (1994). Genetic analysis of Fasciclin II in *Drosophila*: Defasciculation, refasciculation, and altered fasciculation. *Neuron* **13,** 1055-1069.

Lough, J., Barron, M., Brogley, M., Sugi, Y., Bolemder, D. L. and Zhu, X. (1996). Combined BMP-2 and FGF-4, but neither factor alone, induces cardiogenesis in non-precardiac embryonic mesoderm. *Dev. Biol.* **178,** 198-202.

Maggert, K., Levine, M. and Frasch, M. (1995). The somatic-visceral subdivision of the embryonic mesoderm is initiated by dorsal gradient thresholds in *Drosophila. Development* **121,** 2107-2116.

Mima, T., Ueno, H., Fishman, D. A., Williams, L. T. and Mikawa, T. (1995). Fibroblast growth factor receptor is required for in vivo cardiac myocyte proliferation at early embryonic stages of heart development. *Proc. Natl. Acad. Sci. USA* **92,** 467-471.

Nose, A., Mahajan, V. B. and Goodman, C. S. (1992). Connectin: A homophilic adhesion molecule expressed on subset of muscles and the motoneurons that innervate them in *Drosophila. Cell* **70,** 553-567.

Park, M., Wu, X., Golden, K., Axelrod, J. D. and Bodmer, R. (1996). The Wingless signaling pathway is directly involved in *Drosophila* heart development. *Dev. Biol.* **177,** 104-116.

Patel, N. H., Ball, E. E. and Goodman, C. S. (1992). Changing role of *even-skipped* during the evolution of insect pattern formation. *Nature* **357,** 339-342.

Rebay, I. and Rubin, G. M. (1995). Yan functions as a general inhibitor of differentiation and is negatively regulated by activation of the Ras1/MAPK pathway. *Cell* **81,** 857-866.

Reichman-Fried, M., Dickson, B., Hafen, E. and Shilo, B. Z. (1994). Elucidation of the role of *breathless*, a Drosophila FGF receptor homolog, in tracheal cell migration. *Genes Dev*. **8,** 428-439.

Rosenberg, A. I., Lade, B. N., Chui, D., Lin, S.-W., Dunn, J. J. and Studier, F. W. (1987). Vectors for selective expression of cloned DNAs by T7 RNA polymerase. *Gene* **56,** 125-135.

Rushton, E., Drysdale, R., Abmayr, S. M., Michelson, A. and Bate, M. (1995). Mutations in a novel gene, *myoblast city*, provide evidence in support of the founder cell hypothesis for *Drosophila* muscle development. *Development* **121,** 1979-1988.

Shishido, E., Higashijima, S., Emori, Y. and Saigo, K. (1993). Two FGF-receptor homologues of *Drosophila*: one is expressed in mesodermal primordium in early embryos. *Development* **117,** 751-761.

Slack, J. M. W., Darlington, B. G., Heath, J. K. and Godsave, S. F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature* **326,** 197-200.

Snow, P. W., Bieber, A. J. and Goodman, C. S. (1989). Fasciclin III: A novel homophilic adhesion molecule in *Drosophila. Cell* **59,** 313-323.

Staehling-Hampton, K., Hoffmann, F. M., Baylies, M. K., Rushton, E. and Bate, M. (1994). *dpp* induces mesodermal gene expression in Drosophila. *Nature* **372,** 783-786.

Sutherland, D., Samakovlis, C. and Krasnow, M. A. (1996). *branchless* encodes a Drosophila FGF homolog that controls tracheal cell migration and the pattern of branching. *Cell*, **87,** 1091-1101.

Thisse, B., Stoetzel, C., Gorostiza-Thisse, C. and Perrin-Schmitt, F. (1988). Sequence of the *twist* gene and nuclear localization of its protein in endomesodermal cells of early *Drosophila* embryos. *EMBO J.* **7,** 2175-2183.

Wu, X., Golden, K. and Bodmer, R. (1995). Heart development in *Drosophila* requires the segment polarity gene *wingless. Dev. Biol.* **169,** 619-628.

Xiong, W.-C., Okano, H., Patel, N. H., Blendy, J. A. and Montell, C. (1994). *repo* encodes a glial-specific homeo domain protein required in the *Drosophila* nervous system. *Genes Dev.* **8,** 981-994.

Zaffran, S., Astier, M., Gratecos, D., Guillen, A. and Semerivia, M. (1995). Cellular interactions during heart morphogenesis in the *Drosophila* embryo. *Biol. Cell.* **84,** 13-24.

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