

Expression domains of a zebrafish homologue of the *Drosophila* pair-rule gene *hairy* correspond to primordia of alternating somites

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SUMMARY

her1 is a zebrafish cDNA encoding a bHLH protein with all features characteristic of members of the *Drosophila* HAIRY-E(SPL) family. During late gastrulation stages, *her1* is expressed in the epibolic margin and in two distinct transverse bands of hypoblastic cells behind the epibolic front. After completion of epiboly, this pattern persists essentially unchanged through postgastrulation stages; the marginal domain is incorporated in the tail bud and, depending on the time point, either two or three paired bands of expressing cells are present within the paraxial presomitic mesoderm separated by regions devoid of transcripts. Labelling of cells within the *her1* expression

domains with fluorescein-dextran shows that the cells in the epibolic margin and the tail bud are not allocated to particular somites. However, allocation of cells to somites occurs between the marginal expression domain and the first expression band, anterior to it. Moreover, the *her1* bands, and the intervening non-expressing zones, each represents the primordium of a somite. This expression pattern is highly reminiscent of that of *Drosophila* pair-rule genes. A possible participation of *her1* in functions related to somite formation is discussed.

Key words: pair-rule gene, zebrafish, somitogenesis, *her1*

INTRODUCTION

hairy is one of the *Drosophila* segmentation genes of the primary pair-rule class, acting between the gap and the secondary pair-rule genes in the regulatory cascade that controls segmentation (Nüsslein-Volhard and Wieschaus, 1980; Pankratz and Jäckle, 1993). *hairy* is the only segmentation gene known to encode a bHLH protein (Rushlow et al., 1989). The HAIRY protein is structurally related to the proteins encoded by the *Drosophila* genes of the *Enhancer of split* complex [E(SPL)-C; Klämbt et al., 1989; Delidakis and Artavanis-Tsakonas, 1992; Knust et al., 1992] and *deadpan* (Bier et al., 1992), as well as to the rat proteins HES-1 and HES-3 (Sasai et al., 1992). Genetic evidence suggests that the HAIRY protein acts as a repressor of the *achaete-scute* genes during sensory organ development (Botas et al., 1982; Moscoso del Prado and Garcia-Bellido, 1984) and of *fushi tarazu* during segmentation of *Drosophila* (Howard and Ingham, 1986; Ish-Horowicz and Pinchin, 1987). In addition, there is also biochemical evidence to show that this protein functions as a transcriptional repressor (Ohsako et al., 1994).

Drosophila has a long-germ embryo and, during the blastoderm stage, the pair-rule genes, including *hairy*, are expressed in seven stripes alternating with non-expressing cells (Hafen et al., 1984; Ingham et al., 1985; Howard and Ingham, 1986). An orthologue of the *Drosophila hairy* gene has been described in the short-germ embryo of the flour beetle *Tribolium castaneum* (Sommer and Tautz, 1993). This gene is expressed in two to three stripes, which most probably correspond to the primordia

of alternating segments. Patel et al. (1994) have cloned homologues of another *Drosophila* pair-rule gene, *even-skipped*, in a number of both short- and long-germ insects, among them *Tribolium*. Expression of these *even-skipped* homologues also occurs in three stripes.

We describe here aspects of the pattern of transcription of *her1*, a zebrafish gene of the *hairy-Enhancer of split* family (v. Weizsäcker, 1994; accession no. X97623). *her1* was cloned by PCR using degenerated primers directed to regions conserved among the proteins encoded by the *Drosophila* E(SPL)-C (Knust et al., 1992). It encodes a bHLH protein with moderate sequence homology to the HAIRY protein: 48% similarity and 26% identity over the entire coding region, and 63% similarity and 46% identity restricted to the bHLH domain (v. Weizsäcker, 1994). The *her1* transcription pattern is similar to both that of *hairy* and, in particular, to that of its *Tribolium* orthologue, suggesting that *her1* may in fact be functionally related to *hairy*. During late gastrulation stages and somitogenesis, *her1* is transcribed in the advancing epibolic margin (later the tail bud) and in metameric stripes, or bands, of paraxial mesodermal cells, which alternate with transcriptionally silent zones of similar shape and size. We have used intracellular injections of fluorescein-dextran to follow the development of the cells within the expression domains. The results show that neighbouring mesodermal cells close to the epibolic margin and in the tail bud contribute to the formation of several somites. *her1*-expressing cells within more anteriorly located regions are already allocated to particular segments. Each one of the expression bands corresponds to the primordium of a

somite, whereby the cells of the first band will give rise to the 5th somite, those of the second to the 7th somite, and so on. We speculate on potential roles of *her1* during segmentation.

MATERIALS AND METHODS

Maintenance of fish and staging of embryos

Zebrafish were kept on a 10/14 hour dark/light cycle at 28.5°C. Embryos were collected from spontaneous spawnings. Up to the tail bud stage, embryos were classified according to Warga and Kimmel (1990) and Kimmel et al. (1995); for later staging, we used the number of somites. Somites are easily distinguishable with the dissecting microscope in living embryos. After fixation, the somites are not longer clearly distinguishable; thus it is occasionally difficult to count the number of somites in fixed material. Therefore, references to somite stages in the manuscript are to embryos before fixing.

Whole-mount in situ hybridization

RNA/RNA hybridizations on whole embryos were performed with

digoxigenin-labelled probes according to the procedure described in Bierkamp and Campos-Ortega (1993), with minor modifications. Embryos were either observed as whole mounts or embedded in Durcupan (Fluka) and cut by hand into sections of about 100 µm.

Fate mapping of expression domains

Intracellular dye injections and in situ hybridizations were combined to follow the development of the cells within the *her1* transcription domains in the hypoblast. The positions of the expression domains were first determined on whole-mount in situ hybridizations with respect to embryonic landmarks, using a graticule of 8×8 squares, 65×65 µm/square, placed in the eye piece. As landmarks, we used the notochord, the epibolic margin and the somite boundaries; the distance of the corresponding expression domains from these landmarks was determined as precisely as possible. Embryos of similar size at 80%, 100% epiboly (tail bud) and 3-somite stages (Kimmel et al., 1995) were dechorionated, embedded in 1% low melting agarose/10% Hanks medium or in 4% methylcellulose (Serva)/embryo water (Westerfield, 1989) and oriented with a hair with the dorsal side towards the experimenter. The same graticule as

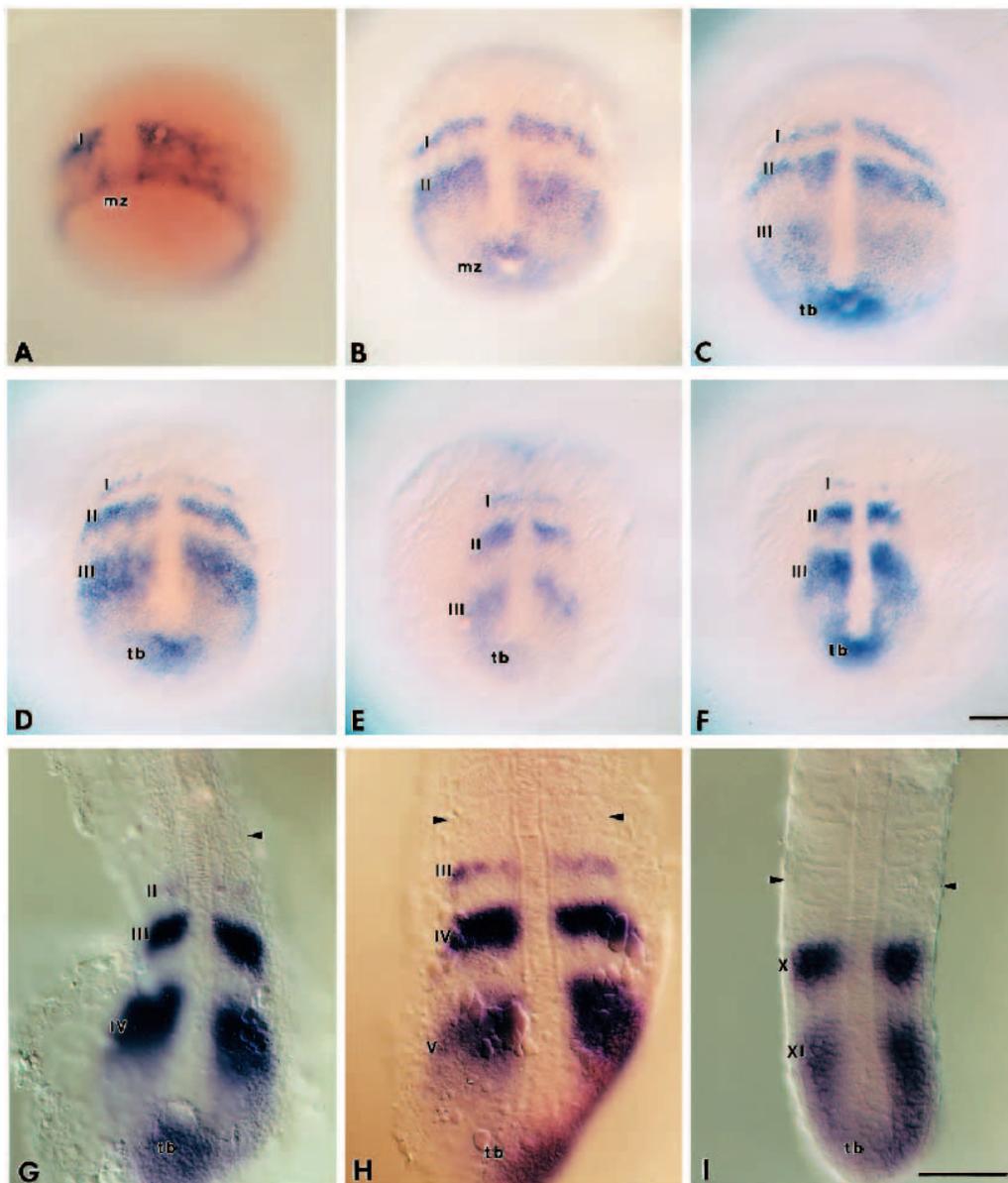


Fig. 1. Distribution of *her1* transcripts in late gastrulation and postgastrulation stages. (A-I) In situ hybridizations of embryos at 75% epiboly (A), 95% epiboly (B), 1- (C), 2- (D), 3- (E), 4- (F), 5- (G), 7- (H) and 21-somite (I) stages. During rostral displacement, the expression domains become narrower. As a consequence of convergence, the domain shapes change from stripes during gastrulation to compact blocks, in postgastrulation stages. Arrowheads in G, H and I point to the 5th, 7th and 21st somites, respectively. I-XI designate the corresponding expression domains. Anterior is to the top in all panels. mz, marginal expression zone; tb, tail bud. A-F and G-I, same magnification. Scale bars, 100 µm.

above was now used to determine the position of the embryonic cells to be labelled. Cells were labelled iontophoretically with fluorescein dextran amine (FDA, M_r 10×10^3 , 100 mg/ml in 0.2 M KCl, Molecular Probes, Eugene, Oregon; Gimlich and Braun, 1985) under a constant current of 5-10 nA for 15-20 seconds using an epifluorescence microscope with a fixed stage.

The accuracy of the labelling procedure was controlled in two ways. In the first series of controls, injections were made at the graticule position in which the expression domain was expected to lie, and the injected embryos were fixed immediately after injection and processed for in situ hybridization, followed by anti-fluorescein antibody staining. Due to the shrinkage of the embryos after in situ hybridization, direct extrapolation of the values determined from fixed material to the position of the expression domains in living embryos used for the intracellular injections gave rise to discrepancies. Therefore, empirical corrections of the position of the electrodes became necessary once the first control injections had been processed. To control for the accuracy of these corrections, the embryos injected each day were divided into two groups. Embryos of one group were stained immediately after injection. Those of the other group were allowed to develop and fixed and processed at a given age. The entire batch was discarded if any of the control injections turned out not to be within the targeted expression domain.

Injected embryos were fixed in 4% paraformaldehyde at 4°C, washed repeatedly in PBS and stored in cold 100% methanol for at least 30 minutes. Fluorescein dextran was detected by means of an anti-fluorescein antibody (Boehringer, Mannheim) and a peroxidase-conjugated goat anti-mouse secondary antibody. In situ hybridization was with digoxigenin-labelled RNA probes. Hybridization was for 16 to 24 hours. For simultaneous in situ hybridization and antibody staining, the anti-fluorescein antibody was mixed with the anti-digoxigenin-FAB-fragments (1:200).

RESULTS

her1 transcripts are expressed in a metameric pattern

RNA in situ hybridizations with digoxigenin-labelled probes

were performed on whole-mount staged zebrafish embryos. Some aspects of the pattern of transcription of *her1*, particularly its early features, have been previously described by v. Weizsäcker (1994) and here we extend these findings to later stages. *her1* transcription begins in the epibolic margin on the presumptive dorsal side of the embryo at the dome stage and, at about 30-40% epiboly, a ring of *her1* transcribing cells is visible at the epibolic margin. At the onset of gastrulation, transcription decreases in the prospective dorsal-most cells and, by 50-60% epiboly, *her1* transcripts are no longer detectable in the future axial mesoderm (not shown; v. Weizsäcker, 1994). As gastrulation proceeds, the marginal expression domain becomes fairly broad along the animal-vegetal axis, while the density of *her1* transcript containing cells declines toward the animal pole.

At about 70% epiboly (Fig. 1A), a pair of transverse stripes of *her1*-expressing cells (domain I) separates from the marginal domain. When it first appears, the density of *her1*-expressing cells within domain I is low. However, as the domain becomes displaced anteriorly, away from the epibolic margin, the cells pack closer together and thus the density of transcript-containing cells increases. At 80% epiboly, domain I is separated from the marginal domain by a band of non-expressing cells of approximately the same width. Optical sections reveal domain I to be restricted to the paraxial hypoblast (not shown). Before the end of gastrulation, at about 90% epiboly, a second pair of bands (domain II) of hypoblastic cells that express *her1* separates from the marginal domain (Fig. 1B). As a consequence of epiboly and convergence movements, domain I changes its position in relation to the marginal zone and thereby becomes considerably thinner. At the end of gastrulation, the marginal expression zone extends as a horseshoe shape around the tip of the tail bud. Another, broader pair of bands (domain III) of *her1*-expressing cells will emerge from this tail bud domain at the 1-somite stage. Hence, at the 1- to 2-somite stage, three pairs of *her1*-expressing cell

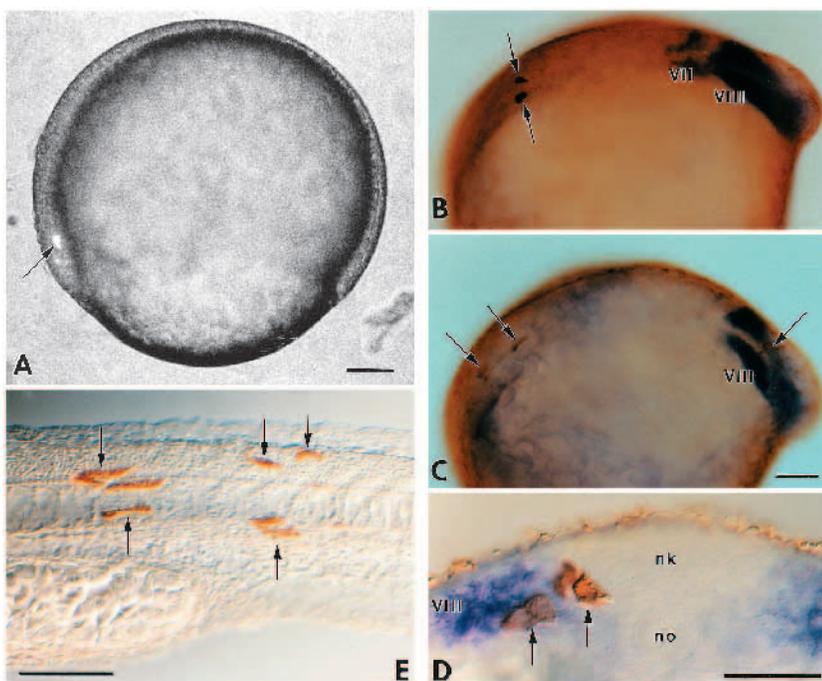


Fig. 2. Cells within the epibolic margin and the tail bud have not been allocated to somites. (A) Fluorescence micrograph of an injection in the hypoblast (arrow) at 80% epiboly. (B-D; 14-somite stage) show examples of the labelling resulting from injections as in A. These embryos were processed for in situ hybridization with digoxigenin-labelled RNA probes followed by anti-fluorescein antibody staining. (B) Fluorescein-labelled cells (brown, arrows) are located in the 9th somite; (D) fluorescein-labelled cells within expression domain VIII. (C) An embryo, in which hypoblastic and epiblastic cells were injected. The former have produced progeny distributed throughout somites 6-8 (two arrows), the latter contributed to the neural tube, in the presumptive territory of the 19th segment (one arrow). (E) Labelled cells (anti-fluorescein staining) in somites 15-19, following an injection in the tail bud at 100% epiboly. B and C, same magnification. Scale bars: 100 μ m.

blocks (domains I, II and III) and a single, unpaired domain posterior to the yolk plug can be distinguished (Fig. 1C-D). The mediolateral extent of these domains is modified as a consequence of convergence, their shape changing from a stripe during gastrulation to more of an oblong in postgastrulation stages.

As they become displaced anteriorly, the size and transcript density in the expression domains decreases. Two factors appear to contribute to this size reduction. On the one hand, expression domains become narrower associated with the ongoing convergence-extension morphogenetic movements; on the other hand, the size of the expression domains sharpens in the anteroposterior direction. In addition, transcript vanishes from expression domains in their anterior displacement. Thus, by the 4-somite stage, RNA is scarcely detectable in domain I, being restricted to its caudal end. In a few embryos at this stage (Fig. 1F), some cells may still contain *her1* transcripts in this domain I, now adjacent to the 4th somite. However, in most embryos, only two blocks of cells containing *her1* transcripts can be observed on each side of the presomitic mesoderm: one, corresponding to domain II, is centred some 10 cell diameters from the 4th somite, and the other, domain III, is further posterior, immediately adjacent to the expression domain at the tail bud.

At the 5-somite stage, three pairs of bands are visible again, the anteriormost probably corresponds to domain II, being separated from the 5th somite by 5-10 cell diameters, while the posteriormost is a newly emerged domain IV (Fig. 1G). This alternating pattern of two and three expression blocks persists during somitogenesis. Hence, the distribution of *her1* transcripts in the growing tail region (Fig. 1H,I) indicates that new expression domains originate posteriorly, advancing away from the tail bud region as growth proceeds; at the same time, new somites are added anteriorly. Sharpening of expression domains in their anterior displacement is particularly clear during tail development, when morphogenetic movements have finished. We have analysed the pattern up to the 21-somite stage, thus identifying up to 11 different expression domains, and it persists essentially unchanged during this time (Fig. 1I).

Cells in the marginal domain and the tail bud contribute to several somites

To study the behaviour of cells within the various *her1* expression domains, we injected fluorescein-dextran in cells at 80%, 95-100% epiboly, or 3 somites, and studied the distribution of the labelled cells and/or their progeny as embryogenesis progressed. In the first experiment, injections were made at the 80% epiboly stage in the dorsal paraxial mesoderm within the marginal zone, approximately 3-5 cell diameters anterior to the epibolic margin (Fig. 2A) and about 15-20 cell diameters lateral to the midline. A total of eight successfully injected embryos were allowed to develop until either the 10- or the 14-somite stage and processed for in situ hybridization and anti-fluorescein antibody staining. In these animals, fluorescein-labelled cells were found over a rather wide area. More precisely, labelled cells were located either in presomitic mesoderm, within (three cases) or in front of the expression stripes (one case; see Fig. 2D), or within somites (three cases; Fig. 2B,C), or in all three locations (one case), the most anterior labelled somite being the 6th and the most

posterior the 14th. This widespread distribution of label applied even when two or more neighbouring cells had been labelled in the same animal. That is to say, hypoblastic cells within the marginal zone at 80% epiboly, and/or their progeny, do not remain together after involution, but separate from each other, migrating over considerable distances. In some cases, injections were made accidentally both in the hypoblast and in the epiblast in the same embryo (Fig. 2C). In these cases, labelled cells were found both in the neural keel and in somites. Their positions along the anteroposterior axis, however, differed considerably; cells in the neural keel always being several segments further caudal than those in the somites. This again reflects the fact that marginal hypoblastic cells at 80% epiboly migrate rostrally, whereas the epiblastic cells do not change their anteroposterior position noticeably. These results indicate that cells of the marginal zone at the 80% epiboly stage have not been allocated to particular segments, and that, together with presomitic anlagen, they contribute mesoderm to the formation of at least the 6th-14th somite.

In order to establish a comparison between the marginal epibolic expression zone and the tail bud, injections were also made in the latter cells, a few cell diameters lateral and caudal to the blastopore, at 100% epiboly. The progeny of injected cells in nine different animals was analysed at the 28- to 30-somite stage. Also in this case, labelled cells were found widely distributed along the anteroposterior axis (Fig. 2F). However, all fluorescein-labelled cells were within somites. In four cases, labelled cells were restricted to a single somite; in the other five cases, labelled cells were distributed in several somites, up to five. The most anteriorly located labelled cells resided in the 13th somite, the most caudal ones in the 21st somite. Therefore, between the completion of gastrulation and the 28- to 30-somite stage, the tail bud contributes mesodermal cells to at least seven different somites, 15th to 21st.

Cells anterior to the marginal domain have been allocated to somites

The behavior of the various expression domains is suggestive of a relationship between *her1* expression and the process of segmentation in the mesoderm. The previous experiment showed that neighbouring cells have not been allocated to segments either within the marginal zone at 80% epiboly or, later on, in the tail bud. To test when the allocation of hypoblastic cells to somites occurs, we labelled cells at the same anteroposterior level on both sides of the embryo. Injections were made at 100% epiboly in different positions in front of the marginal expression domain. A total of nine bilateral injections were processed for this particular experiment (Fig. 3A). Five of these cases were injections in single cells in each side. In these five cases, labelled cells were located within the same somite on both sides of the embryo (Fig. 3C). In the remaining four animals, two or more neighbouring cells had been injected on one side. In these cases, progeny cells were found unilaterally in neighbouring somites; however, in each of these cases there were also labelled cells within the same somite on both sides (Fig. 3D). Thus, by 100% epiboly, cells in front of the marginal expression domain within the interband behind domain II and levels further anterior to it had already been allocated to particular somites.

***her1* expression domains correspond to somite anlagen**

The temporal evolution of the transcription pattern suggests that, as gastrulation proceeds and the tail region expands caudally, the cells within *her1* expression domains change their position with respect to the epibolic margin or tail bud. Moreover, it seems likely that the metameric expression domains could correspond to somitic primordia. In the tail region, where a comparison of the size of the bands to that of the adjacent somites is immediately possible (Fig. 1H, I), this appears indeed to be the case. However, during gastrulation stages, position and size of the somitic primordia are unknown. Hence, we were not able to tell whether or not *her1* expression domains represent segmental domains. To test these ideas, cells within domain II were injected at 100% epiboly, and the position of their progeny with respect to the expression domains was assessed at the 3-, 6- and 7-somite stages (Figs 4, 5). In situ hybridization of untreated embryos suggests that domain II remains distinguishable up to the 6 somite stage (Fig. 1A-F). Consequently, since involution is completed at the time of injection, any difference in the position of the labelled cells with respect to band II must have been caused by displacement of the expression domains and/or the labelled cells. A total of thirteen different control embryos were processed for in situ hybridization and anti-fluorescein antibody immediately after injection and, in all cases, injections had been made within expression domain II on one side of the embryo (Figs 4A, 6A). Three experimental animals were processed at the 3-somite stage (about 1 hour after injection); in all three, labelled cells were located in expression domain II (Figs 4B, 6B). Another six animals were allowed to survive until the 6-somite stage; labelled cells were in all six cases located in the still distinguishable domain II (Figs 4C, 6D). Finally, another six animals were processed at the 7-somite stage; labelled cells were located in the 7th somite in five cases (Fig. 4D), and immediately caudal to the 7th somite in the last case (Fig. 6E). These results confirm the assumption that expression domain II persists between 100% epiboly and the 6-somite stage. In addition, the results indicate (i) that cells labelled within domain II remain in this domain, moving with it toward more rostral levels; and (ii) that cells within domain II become incorporated into the 7th somite.

In another experiment, injections were made within domain I at the 3-somite stage (Figs 5, 6). The previous experiment had shown that expression domains I and II still contain *her1* transcripts at the 3-somite stage. Hence, we chose this stage because, due to the proximity of domain I to the 3rd somite, one can use this structure as a reliable landmark to inject cells within domain I. Six control embryos were processed immediately after injection and, in all cases, the injections had been made within domain I (Figs 5A, 6B). Another four animals were allowed to develop until the embryo had reached the 5-somite stage. In all four cases, labelled cells were found within somite 5 (Figs 5B,C, 6C). The results of this and the previous experiment indicate that the cells in *her1* expression domains II and I are incorporated into the 7th and the 5th somite, respectively. Therefore, the results can be taken as indicative that domains I and II define the primordia of somites 5 and 7, respectively.

DISCUSSION

Organization of the *her1* transcription domains

The *her1* expression domain at the marginal zone contains all the cells that will involute, or ingress, to form mesodermal derivatives (Kimmel et al., 1995). Accordingly, cells injected within this marginal expression zone at late stages of gastrulation distribute themselves over a considerable distance along the anteroposterior body axis. We had of course expected that hypoblastic cells close to the epibolic margin would move to the animal pole. However, it was somewhat surprising to see that neighbouring cells and their progeny did not remain together; although contiguous cells had been injected in the same embryo, their progenies were found in different somites. Similarly, cells of the tail bud were also found to separate from each other considerably, becoming distributed through at least eight somites. Therefore, the results of these two experiments indicate that mesodermal cells in the marginal zone and the tail bud have not been allocated to particular somites and that, initially, neighbouring cells can be separated by a considerable distance in the final distribution.

An interesting side-aspect of these two experiments is that their results permit us roughly to estimate how many somites develop from the mesodermal cells that have involuted by gastrulation. We have seen that, after hypoblast injections at 80% epiboly a few cell diameters in front of the marginal zone, the anteriormost somite containing labelled cells was the 6th, the posteriormost the 14th somite. Therefore, sufficient mesodermal cells to form six somite pairs must already have involuted by 80% epiboly; these mesodermal cells have, however, the potential to form at least 14 somites on either side of the embryo. This conclusion receives further support from the results of injections in the marginal zone at 100% epiboly: here, the anteriormost somite with labelled cells was the 13th. Consequently, we conclude that about 13 or 14 somite pairs may form from the mesoderm involuted, or ingressed, during gastrulation. The rest of the total of 30-34 somite pairs normally present (Kimmel et al., 1995) will form from the proliferation of the cells of the tail bud.

In addition to the expression in the marginal domain and the tail bud, *her1* transcripts are also detected in bilaterally disposed cell bands. The behaviour of the cells within these bands was different from that of the cells in the marginal domain and the tail bud, in that the former cells remained together whereas the latter became distributed into various somites. Bilateral injections at about the same anteroposterior level in the region between bands I, II and the marginal expression zone led to labelling cells in the same somites on both sides. These data thus suggest that the process of allocation of cells to particular segments occurs in the space between the marginal domain and the most caudally located expression band.

Expression stripes correspond to somitic primordia

The *her1* expression pattern is compatible with the idea that the appearance of a new expression band reflects the allocation to a particular somite of a new group of involuted cells; conversely, the cells in the intervening, non-expressing bands should give rise to the alternate somites. During tail development, observation of the size and location of the expression

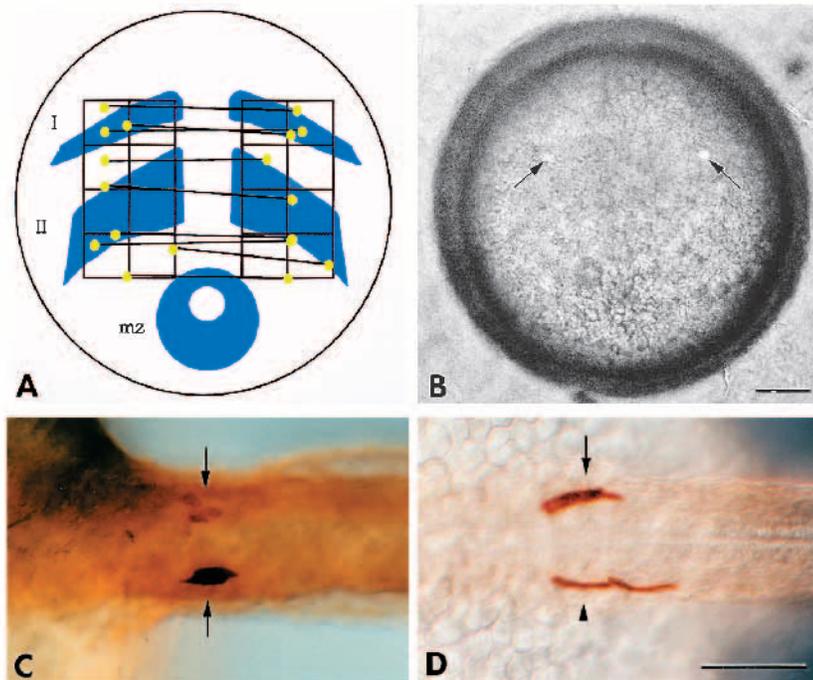


Fig. 3. Mesodermal cells in front of the marginal domain are already allocated to somites. (A) The diagram shows the position of cell pairs (yellow dots) injected at about the same anteroposterior level anterior to the marginal domain (mz) at 100% epiboly. Injections in a given embryo are linked. The graticule used for injections (see Materials and Methods) and the presumed location of the expression domains are projected onto the positions of the injected cells. (B) Fluorescence micrograph of a 100% epiboly embryo immediately after injection of hypoblast cells bilaterally at the same anteroposterior level (arrows). (C) In five cases, single cell injections were made on both sides; labelled cells (arrows) were restricted to the same somite on both sides. (D) In the remaining four embryos, two or more cells were injected on at least one side. In one embryo, labelling was restricted to the same somite on both sides; in the other three, progeny cells were found unilaterally in neighbouring somites; however, in each of these cases labelled cells were also within the same somite on both sides. C and D, same magnification. Scale bars, 100 μ m.

blocks immediately suggests that they mark the primordia of individual somites. During gastrulation, however, this correlation is not obvious, and the postulate that each of the bands delineates a somitomere requires experimental support. Indeed, the results of injecting cells within the domains II and I strongly support this possibility. Thus, injections in domain I and II show that their cells become incorporated into the 5th and 7th somite, respectively. Although direct experimental support is lacking, we assume that those expression domains that appear subsequently to I and II behave similarly.

On this basis, one may consider *her1* to be transcribed in a pair-rule-like fashion: zones of expression with the size of a

somitomere alternate with zones of non-expressing cells of the same size. The transcription pattern in the tail strongly suggests such an arrangement as well. It should be noticed that, as a given expression domain approaches the last differentiated somite and *her1* transcription is close to extinction, the extent of the domain appears smaller than one somitomere. However, this merely reflects the fact that transcripts vanish from expression domains in an anterior-to-posterior direction. The question arises as to whether the cells that will form somites 1 and 3 do not express *her1* when the somitomeres are constituted and, thus, behave in a different manner to those of the other somites. Domain I is the most anterior expression stripe

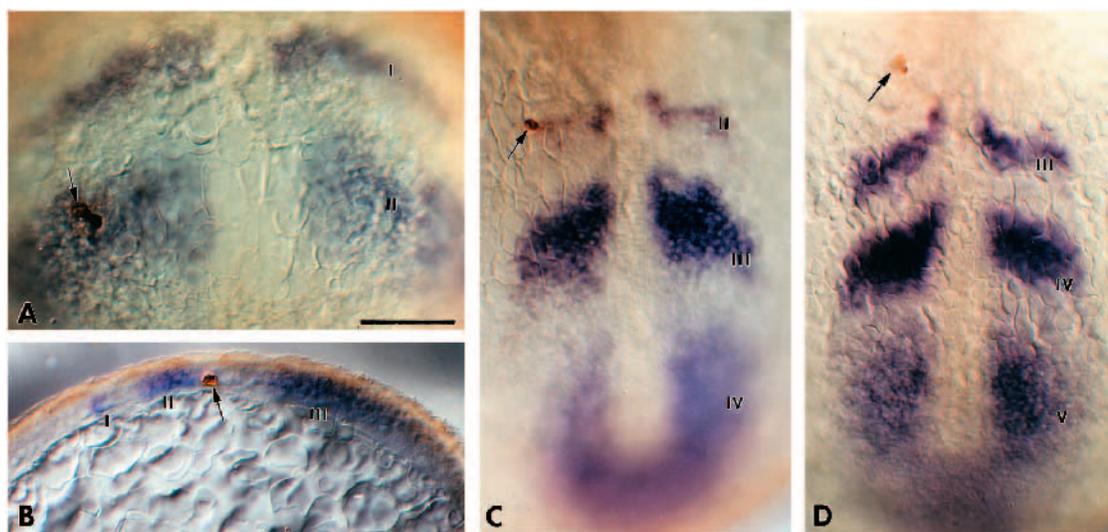


Fig. 4. Expression domains correspond to somitic primordia. (A) An embryo injected (three brown cells at the arrow) in expression domain II at 100% epiboly. This embryo is one of the control cases and was processed for in situ hybridization and anti-fluorescein antibody staining immediately after injection. (B) A parasagittal section ($\sim 100 \mu$ m) of a 3-somite stage embryo that had been injected in domain II at 100%

epiboly. The arrow points to a labelled cell caudally in domain II. (C) A double-stained 6-somite embryo (whole mount) following an injection in domain II at 100% epiboly. Labelled cells are located within domain II, immediately posterior to the 6th somite (no clearly visible). (D) A double-stained 7-somite stage (whole mount) following an injection in domain II at 100% epiboly. Labelled cells are located within the 7th somite. I-V, expression domains. Scale bar, 100 μ m.

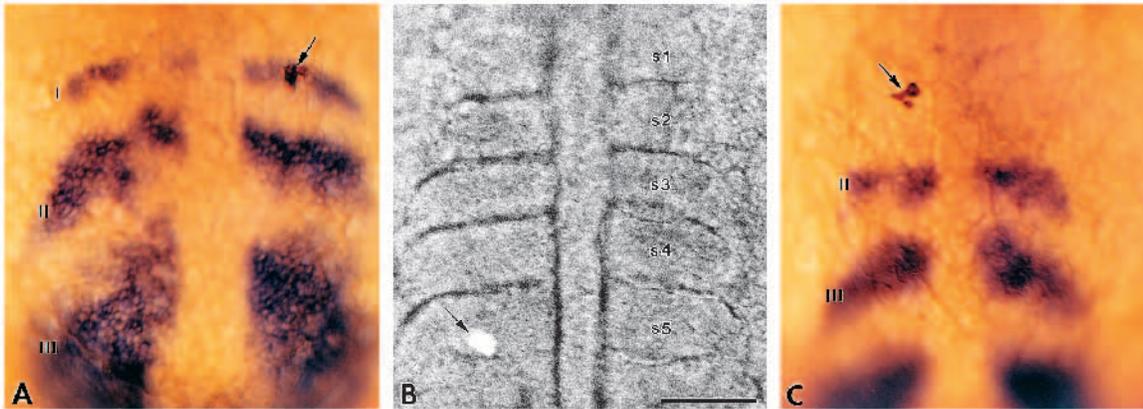


Fig. 5. Expression domains delineate somitic primordia. (A) An injection in expression domain I (three brown cells at the arrow) in an embryo at the 3-somite stage. This embryo is one of the control cases, and was processed for in situ hybridization and anti-fluorescein

antibody staining immediately after injection. (B) A fluorescence micrograph of an embryo in 5-somite stage that had been injected in domain I at the 3-somite stage. Labelling (arrow) is clearly visible within the 5th somite. (C) The same embryo after in situ hybridization and anti-fluorescein staining. I-III, expression domains. Scale bar, 100 μ m.

that we have found to be clearly separated from the marginal expression zone during gastrulation; this domain gives rise to the 5th somite. This means, either that *her1* activity does not define the primordia of somites 1 and 3, or this activity is not organized in well-developed stripes, or it is below the level detectable by our in situ hybridization technique.

***her1* expression is evolutionarily conserved**

We would like to emphasize two aspects of the expression pattern of *Drosophila* pair-rule genes that show a great deal of similarity to the *her1* pattern. First, like other pair-rule genes, *even skipped*, *fushi tarazu* and *hairy* are each expressed in seven stripes during the blastoderm stage; but these stripes are initially contiguous (Macdonald et al., 1986; Howard, 1988). In all three cases, the seven stripes develop by splitting of fewer, larger blocks of transcribing cells (Macdonald et al.,

1986; Howard, 1988; Pankratz et al., 1990). These observations are reminiscent of the splitting of *her1* expression stripes and blocks from the marginal zone and the growing tail. Second, during cellularization, the anteroposterior extent of the expression domains of *even skipped*, *fushi tarazu* and *hairy* sharpens from 4-5 cell diameters in stage 5 to only 2-3 in stage 7 (Macdonald et al., 1986; Howard, 1988; Lawrence and Johnston, 1989). These modifications of the expression domains of the *Drosophila* pair-rule genes are reminiscent of the sharpening of *her1* stripes that one observes both in the late gastrula and, particularly, during tail development. Indeed, as the *her1*-expressing cells separate from the tail bud, the domains they define are much broader than later, when they have progressed towards more anterior levels. Further anterior, transcription finally vanishes, but this should be distinguished from the sharpening referred to here.

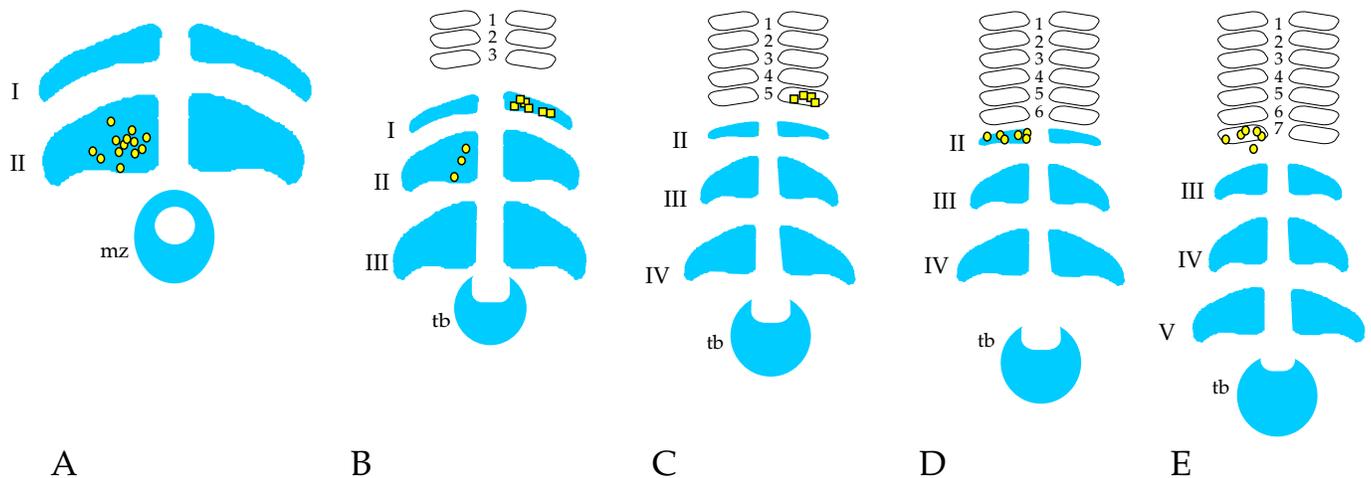


Fig. 6. Expression domains delineate somitic primordia. Schematic representation of the results of the previous two experiments, to illustrate the relationship of domains I and II to the 5th (right-hand side, squares) and 7th somite (left-hand side, yellow circles), respectively. (A) The positions of cells injected within domain II in control animals at 100% epiboly, processed immediately after injection. Each dot represents one case. Compare to Fig. 4A. The drawings at B, D and E indicate the positions of the labelled cells in animals that were allowed to develop to the 3-, 6- and 7-somite stages, respectively, after injection in domain II at 100% epiboly (yellow circles). (B) The positions of cells injected within domain I (yellow squares) in control animals at 3-somite stage, processed immediately after injection. (C) The positions of the labelled cells (yellow squares) in animals that were allowed to develop to the 5-somite stage after injection in domain I at 3-somite. I to IV refer to the corresponding expression domains. Anterior is to the top in all panels. mz, marginal expression zone; tb, tail bud.

The flour beetle *hairy* orthologue (Sommer and Tautz, 1993) exhibits a very similar transcription pattern. In the blastoderm stage, the gene is transcribed in two stripes, which become displaced anteriorly as the embryo grows. An additional domain forms later on at the posterior end of the embryo, similar to that in the tail bud of *her1*. Then, expression vanishes within the two anterior stripes, while the posterior domain splits into two stripes. All these features are strikingly reminiscent of the *her1* transcription pattern, as well as of that of the *Drosophila* pair-rule genes, and suggest similar regulatory mechanisms.

Is *her1* a segmentation gene?

The observations discussed above certainly do not prove that *her1* is a segmentation gene in zebrafish. However, they constitute a strong argument for the proposal that, in its expression, *her1* behaves like a pair-rule gene. As to the function of *her1* during somitogenesis, only speculations are possible at this time. The distribution of *her1* transcripts argues of course for a role of *her1* in formation and/or differentiation of somites. One possibility is that *her1* is involved in gene regulation processes related to the subdivision of hypoblastic and tail bud cells into portions of the size of one somite each. Consequently, a corollary of this assumption is that other genes exist in the zebrafish genome with complementary patterns of expression. To what extent these possibilities hold true must of course await the analysis of mutations in *her1* and other similarly expressed genes.

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REFERENCES

- Bier, E., Vässin, H., Younger-Shepherd, S., Jan, L. Y. and Jan, Y. N. (1992). *deadpan*, an essential pan-neural gene in *Drosophila*, encodes a helix-loop-helix protein similar to the *hairy* gene product. *Genes Dev.* **6**, 2137-2151.
- Bierkamp, C. and Campos-Ortega, J. A. (1993). A zebrafish homologue of the *Drosophila* neurogenic gene *Notch* and its pattern of transcription during early embryogenesis. *Mech. Dev.* **43**, 87-100.
- Botas, J., Moscoso del Prado, J. and Garcia-Bellido, A. (1982). Gene-dose titration analysis in the search of trans-regulatory genes in *Drosophila*. *EMBO J.* **1**, 307-310.
- Delidakis, C. and Artavanis-Tsakonas, S. (1992). The *Enhancer of split* [*E(spl)*] locus of *Drosophila* encodes seven independent helix-loop-helix proteins. *Proc. Natl. Acad. Sci. USA* **89**, 8731-8735.
- Gimlich, R. L. and Braun, J. (1985). Improved fluorescent compounds for tracing cell lineage. *Dev. Biol.* **109**, 509-514.
- Hafen, E., Kuroiwa, M. and Gehring, W. J. (1984). Spatial distribution of transcripts from the segmentation gene *fushi tarazu* during *Drosophila* embryonic development. *Cell* **37**, 833-841.
- Howard, K. (1988). The generation of periodic pattern during early *Drosophila* embryogenesis. *Development* **104** Supplement, 35-50.
- Howard, K. and Ingham, P. (1986). Regulatory interactions between the segmentation genes *fushi tarazu*, *hairy* and *engrailed* in the *Drosophila* blastoderm. *Cell* **44**, 949-957.
- Ingham, P., Howard, K. and Ish-Horowitz, D. (1985). Transcription pattern of the *Drosophila* segmentation gene *hairy*. *Nature* **318**, 439-445.
- Ish-Horowitz, D. and Pinchin, S. M. (1987). Pattern abnormalities induced by ectopic expression of the *Drosophila* gene *hairy* are associated with repression of *ftz* transcription. *Cell* **51**(405-415).
- Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. and Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev. Dynamics*, **203**, 253-310.
- Klämbt, C., Knust, E., Tietze, K. and Campos-Ortega, J. A. (1989). Closely related transcripts encoded by the neurogenic gene complex *Enhancer of split* of *Drosophila melanogaster*. *EMBO J.* **8**, 203-210.
- Knust, E., Schrons, H., Grawe, F. and Campos-Ortega, J. A. (1992). Seven genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop-helix proteins. *Genetics* **132**, 505-518.
- Lawrence, P. A. and Johnston, P. (1989). Pattern formation in the *Drosophila* embryo: allocation of cells to parasegments by *even skipped* and *fushi tarazu*. *Development* **105**, 761-768.
- Macdonald, P.M., Ingham, P. and Struhl, G. (1986). Isolation, structure and expression of *even skipped*: a second pair-rule gene of *Drosophila* containing a homeo box. *Cell* **47**, 721-734.
- Moscoso del Prado, J. and Garcia-Bellido, A. (1984). Genetic regulation of the *achaete-scute* complex of *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**, 242-245.
- Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795-801.
- Ohsako, S., Hyer, J., Panganiban, G., Oliver, L. and Caudy, M. (1994). *hairy* functions as a DNA-binding helix-loop-helix repressor of *Drosophila* sensory organ formation. *Genes Dev.* **8**, 2743-2755.
- Pankratz, M. J. and Jäckle, H. (1993). Blastoderm segmentation. In: *Development of Drosophila*. Cold Spring Harbor Publ. (ed. C. M. Bate and A. Martinez-Arias). pp 467-516.
- Pankratz, M. J., Seifert, E., Gerwin, N., Billi, B., Nauber, U. and Jäckle, H. (1990). Gradients of *Krüppel* and *knirps* gene products direct pair-rule gene stripe patterning in the posterior region of the *Drosophila* embryo. *Cell* **61**, 309-317.
- Patel, N.H., Condrón, B. and Zinn, K. (1994). Pair-rule expression patterns of *even-skipped* are found in both short- and long-germ beetles. *Nature* **367**, 429-434.
- Rushlow, A. C., Hogan, A., Pinchin, S. M., Howe, K. M., Lardelli, M. and Ish-Horowitz, D. (1989). The *Drosophila* hairy protein acts in both segmentation and bristle patterning and shows homology to N-myc. *EMBO J.* **8**, 3095-3103.
- Sasai, Y., Kageyama, R., Tagawa, Y., Shigemoto, R. and Nakanishi, S. (1992). Two mammalian helix-loop-helix factors structurally related to *Drosophila hairy* and *Enhancer of split*. *Genes Dev.* **6**, 2620-2634.
- Sommer, R. J. and Tautz, D. (1993). Involvement of an orthologue of the *Drosophila* pair-rule gene *hairy* in segment formation of the short germ-band embryo of *Tribolium* (Coleoptera). *Nature* **361**, 448-450.
- v. Weizsäcker, E. (1994). Molekulargenetische Untersuchungen an sechs Zebrafisch-Genen mit Homologie zur *Enhancer of split* Gen-Familie von *Drosophila*. Inaugural Dissertation. pp 114. Universität zu Köln.
- Warga, R. M. and Kimmel, C. B. (1990). Cell movements during epiboly and gastrulation in zebrafish. *Development* **108**, 569-580.
- Westerfield, M. F. (1989). *The Zebrafish Book*. Oregon Press

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