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The 412 retrotransposon and the development of gonadal mesoderm in *Drosophila*

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Summary

We have shown that the expression of the 412 retrotransposon provides a useful early marker for the development of the gonadal mesoderm in *Drosophila* embryos. 412 is initially expressed in a set of parasegmentally repeated stripes from parasegments (PS) 2-14 in the mesoderm at the extended germ band stage. During germ band retraction the bulk of 412 expression declines except in dorsolateral clusters of cells in PS10, 11 and 12, where high levels of 412 expression remain. These mesodermal cell clusters are associated with germ cells and subsequently they coalesce, rounding up to form the gonads. The gonadal mesoderm thus appears to originate specifically from three abdominal parasegments, PS10, 11 and 12. We show that the maintenance of high levels of 412 expression in gonadal mesoderm is not induced by contact with germ cells, but rather depends on genetic control by the homeotic genes *abdominal-A* and *Abdominal-B*.

Key words: *Drosophila*, development, retrotransposon, homeotic genes, mesoderm, gonad.

Introduction

In *Drosophila*, the mesoderm arises during gastrulation when cells in the ventral region of the embryo invaginate to form an inner layer of cells beneath the ectoderm. This mesodermal primordium then undergoes considerable further differentiation and morphogenesis to give rise to a wide variety of specific tissues (Fullilove and Jacobson, 1978; Poulson, 1950). The separation of the mesoderm into two layers, the outer somatic and inner visceral mesoderm, is followed by subdivision of the somatic mesoderm into larger more ventrolateral and smaller more dorsolateral portions in each segment. The ventrolateral region forms the body musculature while the dorsolateral region gives rise to gonadal mesoderm, fat bodies and the cardioblasts of the dorsal vessel. The visceral mesoderm forms the muscles surrounding the gut.

Although the cellular and molecular mechanisms underlying mesodermal differentiation are still little understood, a small number of genes involved in these processes have now been identified. The zygotic genes, *twist* and *snail*, which respond to the maternal positional information specifying dorsoventral polarity, are expressed in the mesoderm anlage at the cellular blastoderm stage prior to ventral furrow formation (Simpson, 1983; Nusslein-Volhard et al., 1984; Boulay et al., 1987; Thisse et al., 1988). Both these genes are required for mesoderm formation. Since the *twist* and *snail* protein products belong to the helix-loop-helix and zinc finger protein families, respectively (Boulay et al., 1987; Thisse et al., 1988), they are likely to act as transcriptional regulators with roles in the specification of the mesodermal anlage and in subsequent mesodermal development through the activation of downstream mesodermal genes (Leptin, 1991).

Such downstream genes include the homeobox gene, *msh-2* (Bodmer et al., 1990), which is activated in the mesodermal anlage shortly after *twist* expression and is dependent on *twist* function. Although it is initially expressed in all mesodermal cells, *msh-2* is interesting in the context of mesodermal subdivision, as it subsequently becomes restricted to the dorsal mesoderm, which includes the primordia for the visceral mesoderm and the heart. At about the same time as *msh-2* expression is becoming confined to the dorsal mesoderm, another homeobox gene, H2.0, is activated in the visceral mesoderm (Barad et al., 1988). H2.0 expression is likely to depend on *msh-2* as it is abolished in embryos homozygous for the deletion of 93C-F, the chromosomal region that contains *msh-2* (Bodmer et al., 1990). Other genes identifying mesodermal subsets also encode putative transcription factors. The homeobox gene S59 is expressed in the somatic mesoderm specifically in muscle founder cells (Dohrmann et al., 1990). The *Drosophila* homologue of MyoD, *nautilus*, is expressed in segmentally repeated clusters of cells within the somatic mesoderm just prior to the emergence of fused muscle precursors and later in a subset of mature somatic muscles (Michelson et al., 1990). Also, the paired-domain gene, *pox meso*, identifies a segmentally repeated mesodermal subset in parasegments (PS) 3-14 in late extended germ band embryos (Bopp et al., 1989).

In this paper, we present a molecular marker that allows the study of a distinct mesodermal subset, the gonadal mesoderm. The gonads in *Drosophila* are composed of two very different cell types, the germ cells, derived from the pole cells formed at the posterior tip of the blastoderm, and the gonadal mesoderm, derived from the dorsolateral somatic mesoderm. The larval gonads each contain 10-12 germ cells intermingled with mesodermal 'interstitial cells' and surrounded by a mesodermal sheath (Fullilove and Jacobson, 1978; Poulson, 1950). In females, the mesodermal cells will give rise to the follicle cells and supporting structures of the mature ovary (King, 1970) and, in males, to a variety of support and nutritive cells in the testis (Cooper, 1950). The formation of the gonads presents some interesting developmental problems. The pole cells are swept inside the posterior midgut invagination during germ band extension and then must move through the gut wall to contact the gonadal mesoderm. How the gonadal mesoderm is specified within the dorsolateral mesoderm of a restricted number of abdominal segments and how the pole cells associate with this specific mesoderm, which then coalesces to form the gonads, is not known (Campos-Ortega and Hartenstein, 1985; Lawrence and Johnston, 1986).

Here we show that the expression of the 412 retrotransposon provides a useful marker for gonadal mesoderm, allowing a description of its formation, its association with pole cells and an analysis of the role of genes of the bithorax complex in its specification.

Materials and methods

Drosophila *stocks*

The wild-type strain used was Oregon R. All flies were maintained at 25°C unless stated otherwise. Flies carrying the deficiency covering the region 67C, Df(3L)AC1, *roe* p^p / TM3, Ser were obtained from the Bloomington Drosophila stock centre. Homozygous mutant embryos were collected from balanced heterozygous stocks: BX-C deficient embryos were collected from Dp(3:3)P5/Df(3R)P9 fly stocks (Lewis, 1978); *abd-A* mutant embryos from *abd-*A^{m1}/TM3 flies (Sanchez-Herrero et al., 1985) and *Abd-B* mutant embryos from *Abd-*B^{m1}/TM3 (m^-r^- mutant) or *Abd-*B^{m5}/TM3 (m^-r^+ mutant) (Casanova et al., 1986). Homozygous *osk*^{301} mutant embryos were collected at 20°C from *osk*^{301} homozygous progeny from a balanced heterozygous stock *th osk*$^{301}p^p$ / TM3 (Lehmann and Nüsslein-Volhard, 1986). The *esc*^{6} homozygous embryos derived from *esc*^{6} homozygous mothers were generated as described previously (Gould et al., 1990b).

Library screens for genomic and cDNA clones

An EMBL3 genomic library constructed by John Tamkun from an iso-1 strain (isogenic for chromosomes I and III) was a gift from Paul Lasko. The cloned immunopurified fragment IP72 (Gould et al., 1990a) was used as probe to screen the library at high stringency. Two genomic clones designated 72A and 72B were isolated.

A λgt10 3-12 hour embryonic cDNA library (Poole et al., 1985), provided by Nick Gay, was used to screen for cDNAs with the subcloned 10 kb *Sal*I restriction fragment from the 72B genomic clone as probe. Two independent cDNAs were isolated, designated 72.9 and 72.3.

DNA sequencing and analysis

DNA sequencing was carried out by the dideoxynucleotide chain termination method (Sanger et al., 1977) on double-stranded DNA using Sequenase (United States Biochemicals). The complete sequence of one strand of the 2.5 kb *Eco*RI fragment from the 72.9 cDNA was determined. About 300-400 bp of the ends of the 1.4 kb, 0.6 kb, 0.4 kb *Eco*RI fragments from the 72.9 cDNA, the 1.2 kb insert from the 72.3 cDNA and the 10 kb and 6 kb *Sal*I genomic fragments were also sequenced. DNA database searches scanned the EMBL database.

In situ hybridisation

Whole-mount embryo in situ hybridisations were carried out on 0-16 hour Oregon R embryos (Tautz and Pfeifle, 1989). DNA probes were either the 10 kb and 6 kb *Sal*I genomic fragments or *Eco*RI cDNA fragments that either had been gel purified or subcloned into Bluescript vectors (Stratagene) then labelled with digoxigenin (Boehringer). Photographs were taken on a Zeiss Axiophot microscope using Ilford PanF film.

Antibody labelling

Embryos that had been labelled by whole-mount in situ hybridisation were then labelled with an anti-vasa antibody (Lasko and Ashburner, 1990) as previously described (Gould et al., 1990b).

Results

The 412 retrotransposon at 67C

We encountered the expression pattern of the 412 retrotransposon whilst screening genomic clones from the 67C region for transcripts expressed in the embryo. Following a chromatin immunopurification strategy to isolate in vivo binding sites for the *Ultrabithorax* homeotic gene product (Gould et al., 1990a), we mapped a potential *Ultrabithorax* target sequence to 67C. The immunopurified fragment IP72 was used to probe a genomic library and *Sal*I fragments from the resulting two λ clones 72A and 72B (Fig. 1) were used to search for embryonic transcription units by whole-mount in situ hybridization. Transcripts were detected only from the 72A 6.0 kb and the overlapping 72B 10.0 kb fragments and these gave identical expression patterns. Using the 10 kb *Sal*I fragment as a probe, two independent non-overlapping cDNA clones, designated 72.3 and 72.9, were isolated (Fig. 1). Sequencing of the 2.5 kb *Eco*RI fragment from the 72.9 cDNA revealed perfect sequence homology to the 412 retrotransposon (Will et al., 1981; Yuki et al., 1986). Sequencing of the ends of the two cDNA clones revealed that they are entirely contained within the 412 sequence. Both cDNA clones gave the same in situ hybridization pattern as had been seen with the genomic clones and this, therefore, appears to be the expression pattern of the 412 retrotransposon.

Expression pattern of the 412 retrotransposon

The 412 retrotransposon shows a highly regulated pattern of expression during embryonic development. The first expression is seen after the completion of germ band extension (stage 10; staged according to Campos-Ortega and Hartenstein, 1985) in a stripe of cells in each parasegment of the mesoderm from PS 2-14. The level of transcript increases during stage 11 to give broad intense stripes of expression (Fig. 2A,B). The labelling then decreases close to the midline. This may result from cell migration or a

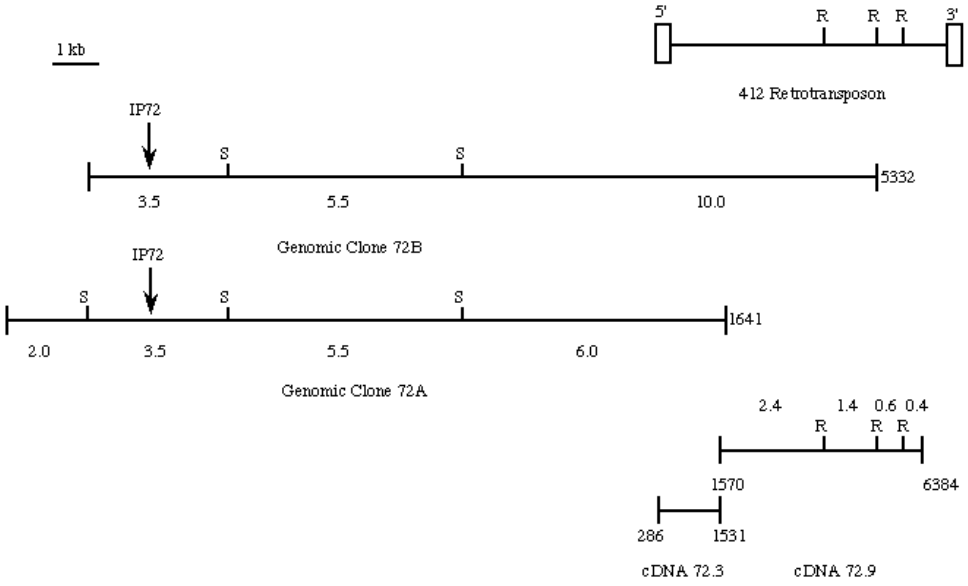


Fig. 1. Map of the genomic region at 67C containing the 412 insertion. A partial restriction map of the genomic clones 72A and 72B and alignment of the two cDNA clones, 72.9 and 72.3 with the 412 retrotransposon is shown. The approximate location of the IP72 immunopurified fragment (Gould et al., 1990a) within the 3.5 kb *Sal*I genomic fragment is indicated by an arrow. The nucleotide sequence of the 412 retrotransposon, as determined by Will et al. (1981) and Yukiet al. (1986), is shown as numbered positions along the cDNAs. The 412 nucleotide position at the ends of the two genomic clones are also indicated. S, *Sal*I; R, *Eco*RI.

local decrease in expression or a combination of the two. Thus, by mid stage 11, two large lateral clusters of 412-expressing cells have formed in each parasegment (Fig. 2C). In PS10-13, the labelled cell clusters are associated with large unlabelled cells. These large cells have been identified as germ cells by staining for the pole-cell-specific protein vasa (not shown). Fig. 2D shows the parasegmental registration of the 412 stripes. They are centred on the tracheal pits which lie roughly in the middle of each parasegment. During stage 12, at the onset of germ band retraction, segment-specific differences within the 412 pattern emerge. Levels of 412 expression generally decline, except in dorsolateral clusters in PS10, 11 and 12, where the labelling is often seen to be surrounding germ cells (Fig. 2E,F). The dorsolateral clusters fuse together to form elongated gonads containing the 412-expressing cells of gonadal mesoderm intermingled with germ cells (Fig. 2G). The gonads then round up and by stage 14 lie within the A5 segment (Fig. 2H). The pattern of 412 expression indicates therefore that gonadal mesoderm derives from PS10, 11 and 12.

Elsewhere in the embryo, 412 expression has decreased to a band of weakly staining cells extending posteriorly from T2 at the same dorsolateral level as the gonads. The gonads are later found to be embedded in the fat body, so this may represent labelling in the dorsal fat body (Fig. 2G). There is also some weak labelling in ventrolateral somatic musculature and in the midline of the central nervous system.

Mesodermal-germ cell interactions

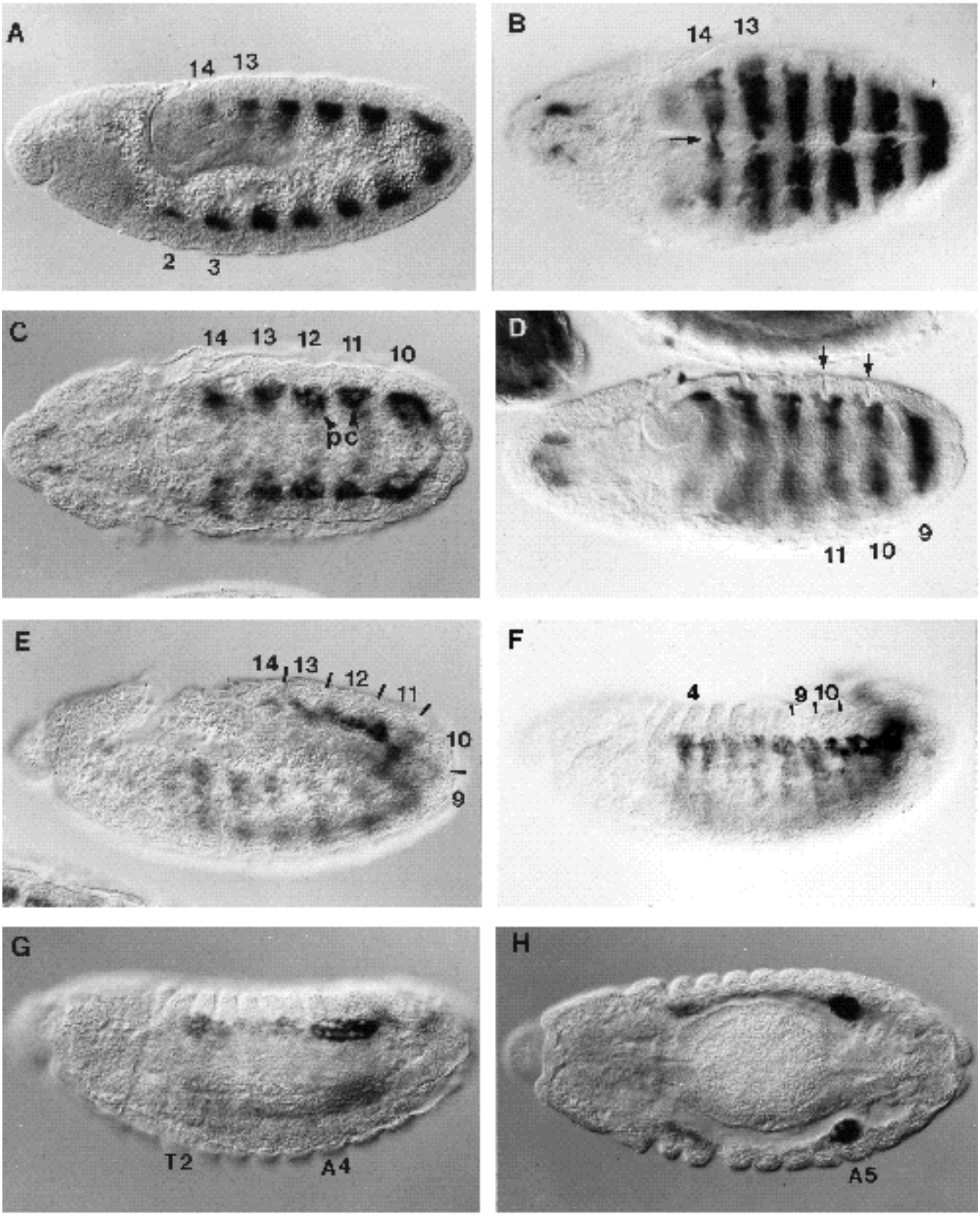
The description of the expression pattern of 412 indicates that pole cells come into contact with 412-expressing mesodermal cells soon after their internalisation through the wall of the hind gut at stage 11. Interactions between the mesoderm and the germ cells are presumably required for the lateral movement of the germ cells and their parcelling into gonads. It is striking that high level expression of 412 is maintained only in those clusters of dorsolateral mesoderm that are associated with germ cells. This led us to ask whether maintenance of high level 412 expression in mesodermal cells is dependent on their interaction with germ cells. Early observations on the development of the gonads (Aboim, 1945; Sonnenblick, 1941) suggested that, in the absence of pole cells, the mesodermal components differentiated otherwise normally into agametic gonads. 412 expression now provides a molecular marker to examine more specifically the development of gonadal mesoderm in embryos lacking pole cells. Embryos lacking maternal product from the *oskar* gene have neither abdominal segments nor pole cells. However, with the temperature-sensitive allele, osk^{301}, abdominal development is normal but pole cells are absent (Lehmann and Nüsslein-Volhard, 1986). We therefore examined 412 expression in embryos derived from homozygous osk^{301} mothers at the restrictive temperature. Antibody staining for the pole-cell-specific vasa protein (Hay and Jan, 1988; Lasko and Ashburner, 1990) confirmed the complete absence of pole cells. However, there was a normal pattern of 412 expression in the extended germ band stage (not shown) and, significantly, the normal high level of 412 expression was seen at later stages in gonadal mesoderm (Fig. 3A). Hence the high level of 412 expression characteristic of the differentiation of gonadal mesoderm is not dependent on the presence of pole cells.

The bithorax complex and gonadal mesoderm

If high levels of 412 expression in the dorsolateral mesoderm of parasegments 10-12 are not induced by contact with germ cells then how are they specified? The restriction of 412 expression to specific parasegments is likely to be dependent on the homeotic genes of the bithorax complex. Mutations in the bithorax complex are known to affect gonadogenesis and, specifically, *iab-4* mutants are viable but sterile, lacking gonads (Lewis, 1978; Karch et al., 1985).

In homozygous DfP9 mutant embryos, deficient for the entire bithorax complex, there is severe disruption of 412 expression. Initially, the pattern is normal but at germ band

shortening (stage 12) the normally high level of 412 expression in cells contributing to gonadal mesoderm is absent. Thus, no gonadal mesoderm forms in these mutant embryos. Interestingly, many of the germ cells are found close to the ventral midline in embryos undergoing germ band retraction (Fig. 3B). They appear to be stranded close



to their site of internalisation. Thus, absence of the correct abdominal segmental specification leads to a failure of germ cell movement and this might point to a role for abdominal mesoderm in the lateral transport of the germ cells.

Since gonadal mesoderm appears to be specified from parasegments 10-12, we looked more specifically at 412 expression in *abdominal-A* (*abd-A)* and *Abdominal-B* (*Abd-B)* mutant embryos. The reported domain of expression in the mesoderm of *abd-A* is PS8-12 (Karch et al., 1990; Macias et al., 1990) and of *Abd-B* is PS11-14 (DeLorenzi and Bienz, 1990), and both are expressed in gonadal mesoderm. In *abd-A* mutant embryos, we see little evidence of gonadal differentiation. There is a band of loosely scattered 412 expression in the dorsolateral mesoderm but no condensation of gonadal mesoderm and pole cells are scattered (Fig. 3C). Occasionally, we observed lone germ cells surrounded by a few mesodermal cells expressing high levels of 412. In *Abd-B* embryos after germ band shortening, fewer cells than normal exhibit strong 412 expression in dorsolateral mesoderm of parasegments 10-12 (Fig. 3D, c.f. Fig. 2F). There are many scattered pole cells and the resulting gonads are smaller, often fragmented and abnormally located across A5-A6. Thus although some gonadal mesoderm forms in *Abd-B* mutants, it is much reduced. These results indicate that both *abd-A* and *Abd-B* are required for normal 412 expression and gonadal mesoderm formation, with *abd-A* playing the major role.

The analysis above suggests that the restriction of gonadal mesoderm formation to parasegments 10-12 is due to the specific combination of *abd-A* and *Abd-B* expression in this region. This is supported by the observation that other regions of the embryo can be shown to be capable of forming gonadal mesoderm in the presence of ectopic *abd-A* and *Abd-B* expression. In *esc* homozygous mutant embryos derived from *esc* homozygous mothers, the normal domain boundaries restricting homeotic gene expression are relaxed and *abd-A* and *Abd-B* are coexpressed throughout the embryo (Simon et al., 1992; Struhl, 1981). This results in the differentiation of gonadal mesoderm along much of the length of the embryo, as revealed by a chain of clusters of cells expressing high levels of 412 (Fig. 3E). Most clusters lack germ cells, supporting the earlier observation that gonadal mesoderm forms independently of contact with pole cells. In addition, in *esc* mutants, the germ cells are positioned relatively normally along the anterior-posterior axis, being located in A5/A6 (Fig. 3F). This suggests that the germ cells may be guided to their specific segmental locations by their initial contact with mesoderm after they exit from the posterior midgut invagination.

412 expression in Df(3L)AC1 embryos

There are reported to be some 40 copies of the 412 retrotransposon within the *Drosophila* genome (Potter et al., 1979). The 412 insertion that we encountered was at cytological location 67C. As we came to this region through a candidate in vivo homeoprotein binding site and as the 412 expression apppears to be regulated by homeotic genes, it was conceivable that this 'binding site' might play a role in regulating 412 expression. As the 'binding site' lies outside the retrotransposon sequence (Fig. 1), the putative homeotic regulation would act only on the copy of 412 at 67C. We therefore investigated the possibility that the mesodermal expression pattern that we observe is due specifically to this copy of 412.

We have looked at 412 expression in embryos from a deletion strain Df(3L)AC1 covering the region 67A-D and observed exactly the same 412 pattern as in the wild type. Thus the expression pattern of the 412 retrotransposon is independent of the copy located at 67C and is likely to depend on intrinsic regulatory elements.

Fig. 2. Expression pattern of the 412 transcript in wild-type whole-mount embryos. Embryos are shown with anterior to the left and staged according to Campos-Ortega and Hartenstein (1985). The digoxigenin probes used for the in situ hybridisations were as described in Materials and methods. The numbers refer to parasegments. (A) Stage 11 extended germ band embryo showing 412 expression in a large subset of cells in each parasegment of the mesoderm from PS2-14. Occasionally a patch of weak labelling can be seen in PS1. (B) Dorsal view of a stage 11 embryo showing broad stripes of 412-expressing cells. Labelling is decreasing at the midline (arrow). Some labelling is also detected in the procephalon which will contribute to the pharyngeal musculature. (C) Dorsal view of a stage 11 embryo. 412-expressing cells form two large lateral clusters in each parasegment. Labelling surrounds large unlabelled cells, the pole cells (pc), in PS11 and 12 (arrows). (D) Dorsal view of a stage 11 embryo showing the position of the stripe of 412 expression with respect to the tracheal pits (arrowed in PS 11 and 12) which indicates that the 412 stripe is expressed in the middle of the parasegment. (E) A stage 12 embryo undergoing germ band retraction. Segment-specific differences in the 412 pattern emerge as levels of 412 generally decline. High levels of expression are maintained posteriorly in PS10, 11 and 12. Some embryos show high levels also in PS13, but this may be temporary. 412 expression has decreased in PS14 and also anteriorly to PS10. In this embryo, germ cells can be seen in contact with 412-expressing cells in PS10, 11 and 12. (F) A later stage 12 embryo showing the high levels of 412 expression in gonadal mesoderm at PS10. Lower levels are present in PS 4-9. Labelling is located in a dorsolateral region within each parasegment with weak expression extending laterally. (G) A stage 13 shortened germ band embryo showing condensation of 412-expressing cells with germ cells to form elongated gonads across A4-A7. A low level of 412 expression is maintained dorsally in anterior segments, possibly in the fat bodies, with a somewhat higher level in T2. (H) Stage 14 embryo. Tight clusters of 412-expressing cells with germ cells form the gonads located within A5.

Discussion

In this paper, we have described the origin and development of the gonadal mesoderm and have analysed its genetic specification. The origin of the gonadal mesoderm has been the subject of some debate. Until recently, the prevailing view was that gonadal mesoderm originates from a number of abdominal segmental primordia, although precisely which ones was not known (Gehring et al., 1976; Lawrence and Johnston, 1986). This has now been challenged by a detailed gynandromorph study which concludes that the progenitor cells for gonadal mesoderm are located within a single segment (Szabad and Nothiger, 1992). However, our work permits the progenitor cells of the gonadal

mesodermal to be visualised early in their development and strongly supports their multisegmental origin. The mesodermal cells that will later cluster together to enfold the germ cells and form gonads are clearly marked by their high levels of 412 expression in stage 12 embryos. These cells are seen as separate clusters in parasegments 10, 11 and 12 before they coalesce. The clusters come together to form a band of cells spanning several segments before

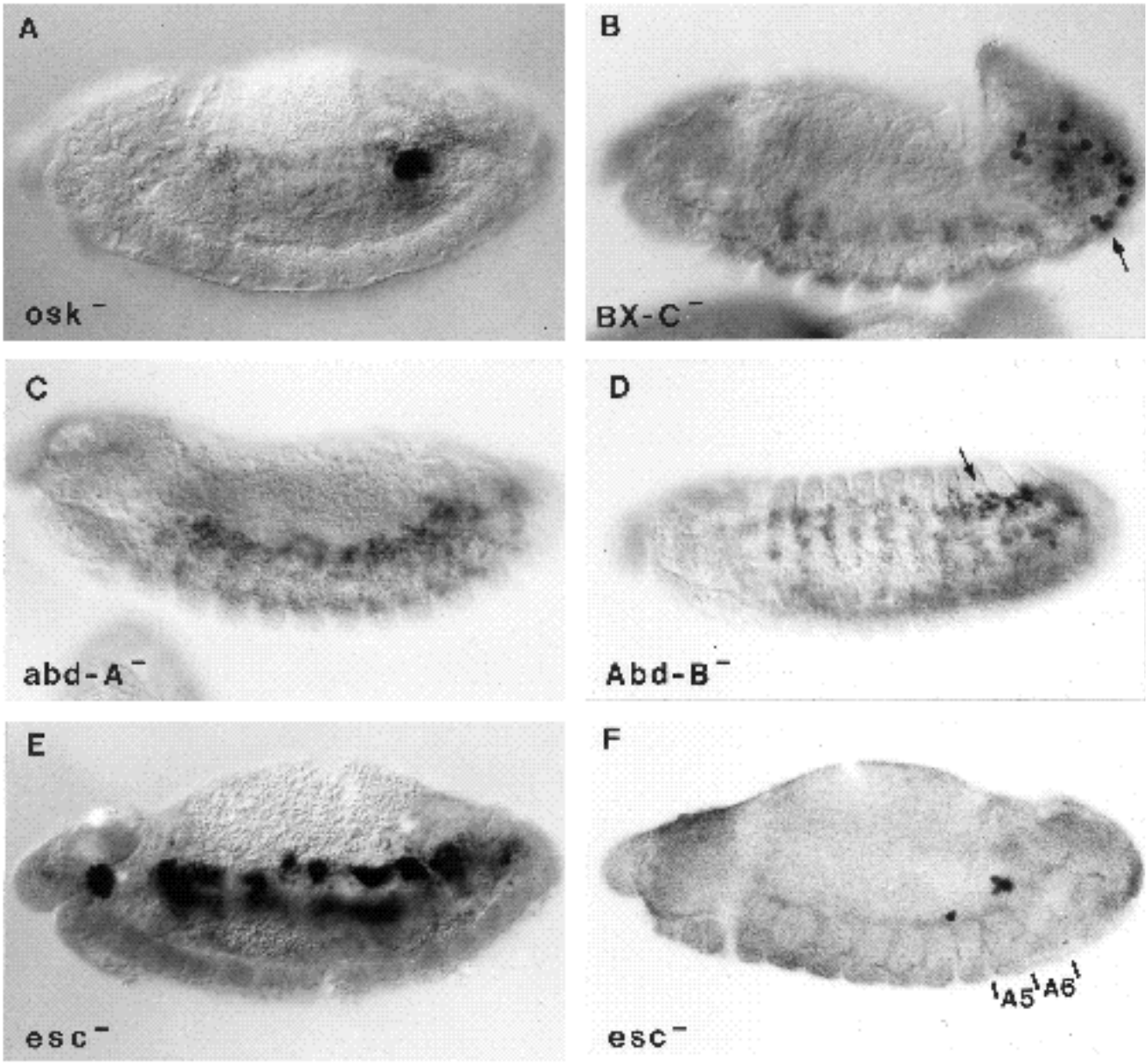


Fig. 3. 412 expression pattern in mutants. (A) *osk*^{301} homozygous mutant embryo. There is normal expression of 412 in the gonadal mesoderm of gonads which lack pole cells. These are normally located in A5. The absence of pole cells was confirmed by staining with an antibody against the pole-cell-specific protein vasa. (B) DfP9 homozygous mutant embryo deficient in the Bithorax (BX-C) complex. Germ band retraction is not complete. There is almost no 412 expression in posterior parasegments. No gonadal mesoderm forms. Pole cells remain scattered close to the ventral midline. Pole cells (arrowed) were labelled with an anti-vasa antibody. (C) *abd-A* homozygous mutant embryo. 412 expression in a stage 13 embryo remains scattered across the dorsolateral mesoderm. No gonadal mesoderm forms and pole cells are scattered. The level of 412 labelling is about the same as that normally seen in T2 (see Fig. 2G) and the dorsolateral stripe of labelled cells may be fat body. (D) *Abd-B* homozygous mutant embryo. 412 expression in stage 13 embryo shows a small amount of gonadal mesoderm forms across segments A4-A6. Many pole cells remain scattered and the gonads that do form are small and fragmented (arrowed, A5). Both the *Abd-B*^{m1} and *Abd-B*^{m5} mutants showed the same 412 pattern. (E) *esc* homozygous mutant embryo derived from a homozygous mutant mother. The level of 412 transcript in a stage 13 embryo is derepressed in anterior parasegments such that clusters of gonadal mesoderm form anterior to PS 10. The position of the pole cells and gonads are relatively normal. (F) *esc* mutant embryos as in E stained with an anti-vasa antibody. Pole cells cluster relatively normally in A5/A6. This embryo shows two ectopic pole cells more anteriorly. We note that these *esc* mutant embryos show a reduced number of pole cells.

rounding up within the A5 segment at stage 14. From this analysis of the 412 pattern, we conclude that the mesodermal cells of the gonads are derived from three parasegments, PS10, 11 and 12.

The gynandromorph analysis of Szabad and Nothiger (1992) gives a frequency of mosaicism of 10.5% for the gonads. On the assumption that the gonadal primordium is circular, this gives a diameter for the primordium that is similar to the distance across a single abdominal segment. It is possible that this estimate of diameter is misleading either because the frequency of mosaicism is an underestimate, due to the failure of small patches of ovary or testis to survive or be detected, or perhaps because the primordium is not circular. To reconcile the multisegmental view of gonadal mesoderm origin, depicted by the 412 expression pattern, with a single segmental origin of the progenitors would require a very early specification of these progenitors followed by migration into separate parasegmental 412-positive clusters but this seems, to us, unlikely.

We have shown that the normal specification of the gonadal mesoderm requires the activities of the homeotic genes *abd-A* and *Abd-B.* This accords with the expression of both of these genes in the gonadal mesoderm (DeLorenzi and Bienz, 1990; Karch et al., 1990). Previous analysis of viable mutations in the bithorax complex have implicated *abd-A* in gonadal formation as *iab-4* mutants, which affect the regulation of the *abd-A* gene, are sterile through a loss of adult gonads (Karch et al., 1985; Lewis, 1978). Recently Cumberledge et al., (1992) have shown that *iab-4* function is required autonomously in the mesodermal cells of the gonadal primordia. A role for *Abd-B* in the gonadal mesoderm has not been seen previously, but this has only been analysed in the viable mutations *iab-5* through *iab-8* (Karch et al., 1985).

Northern analysis has shown that the transcription of *Drosophila* transposable elements varies at different stages of development and that elements can be grouped according to their profiles of expression (Parkhurst and Corces, 1987). However, we were surprised by the degree of tissue specificity exhibited by the expression pattern of the 412 retrotransposon. Why should 412 expression be so highly restricted to the gonadal mesoderm? Transposable elements might be expected to be expressed specifically in the germline in order to ensure inheritance of transposition events and also to show restricted expression in the somatic tissues in order to minimise deleterious effects on the host. The P elements, which are members of the non-retrovirus-like class II group of elements, employ this type of strategy albeit at the post-transcriptional level. Thus P-element transposition is restricted to the germ-line through control of transposase production by a germ-line-specific splicing event (Laski et al., 1986). We do not know whether the retrovirus-like element, 412, is ever expressed in the germline. Its highly specific expression in the somatic cells of the embryonic gonad suggests the intriguing possibility that it may gain access to the germ cells through the production of an infectious retrovirus-like particle. However, it should be pointed out that although the 412 sequence contains an open reading frame with homology to the *pol* gene of Moloney murine leukaemia virus (Yuki et al., 1986), there is no corresponding open-reading frame with sequence homology to the *env* gene.

We have shown that 412 is an early molecular marker for gonadal mesoderm specification. Its pattern of expression raises the question of whether 412 is regulated by any of the genes known to be involved in mesoderm specification. Like 412, the *pox meso* gene is expressed in segmentally repeated stripes in the mesoderm of the extended germ band embryo (Bopp et al., 1989). However, the *pox meso* stripes span the parasegment boundary and are therefore out of phase with the 412 stripes. *pox meso* is thus a candidate for a negative regulator of the early 412 expression.

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