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Relationship between expression of *serendipity* α and cellularisation of the *Drosophila* embryo as revealed by interspecific transformation

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SUMMARY

A dramatic reorganization of the cytoskeleton underlies the cellularisation of the syncytial *Drosophila* embryo. Formation of a regular network of acto-myosin filaments, providing a structural framework, and possibly a contractile force as well, appears essential for the synchronous invagination of the plasma membrane between adjacent nuclei. The *serendipity alpha* (*sry* α) gene is required for this complete reorganization of the microfilaments at the onset of membrane invagination. We compare here the structure and expression of *sry* α between *D. pseudoobscura*, *D. subobscura* and *D. melanogaster*. Interspersion of evolutionarily highly conserved and divergent regions is observed in the protein. One such highly conserved region shows sequence similarities to a motif found in proteins of the ezrin-radixin-moesin (ERM) family. Four 7-13 bp motifs are conserved in the 5′ promoter region; two of these are also found, and at the same position relative to the TATA box, in *nullo*, another zygotic gene recently shown to be involved in cellularisation. The compared patterns of expression of *D. melanogaster sry* α and *nullo*, and *D. pseudoobscura sry* α reveal a complex regulation of the spatiotemporal accumulation of their transcripts. The *D. pseudoobscura sry* α gene is able to rescue the cellularisation defects associated with a complete loss of *sry* α function in *D. melanogaster* embryos, even though species-specific aspects of its expression are maintained. Despite their functional homologies, the *D. melanogaster* and *D. pseudoobscura sry* α RNAs have different subcellular localisations, suggesting that this specific localization has no conserved role in targeting the *sry* α protein to the apical membranes.

Key words: *serendipity* α, *Drosophila* embryo, cellularisation, intracellular RNA localisation

INTRODUCTION

Most or all pterygote insects and at least some apterygote insects undergo a cleavage of the fertilised egg of the intralecithal kind, i.e., the yolk mass remains undivided while the zygote nucleus and its daughters divide and spread with accompanying divisions of their cytoplasmic haloes (Anderson, 1972). Formation of cells occurs after nuclei have migrated to the yolk-free periphery of the embryo to form a monolayer at the cortex. Cellularisation has been most extensively studied in the dipterans, especially *D. melanogaster*, where it occurs synchronously across the whole surface of the embryo at the end of mitosis 13 and during the interphase of cycle 14 (between 120 and 170 minutes after fertilisation; Foe and Alberts, 1983).

During cellularisation, the plasma membrane invaginates between adjacent nuclei, forming a hexagonal array of cleavage furrows around each nucleus, and subdivides the cortex of the embryo into individual cells. A dramatic redistribution of the cytoskeletal components occurs during cellularisation (Fullilove and Jacobson, 1971; Warn and Robert-Nicoud, 1990; Young et al., 1990). At the start of cellularisation, an actin-myosin hexagonal network forms, which provides a structural frame, and possibly the contractile force, for the synchronous invagination of membranes. During cellularisation, this network evolves into individual ring-like structures composed of filaments of actin and myosin II, at the base of the cleavage furrows. At the end of cellularisation, contraction of these rings results in the formation of the basal membrane of epithelial-like blastoderm cells (review by Warn et al., 1990).

Lack of the *sry* α zygotic gene activity results in erratic disruptions of the cytoskeleton early at mitotic cycle 14, culminating in the formation of abnormal, multinucleate cells. The *sry* α gene therefore appears specifically required for the integrity of the actin-myosin network (Merrill et al., 1988; Schweisguth et al., 1990). It encodes a transiently expressed protein which associates with membranes at the onset of cellularisation and accumulates at the base of the cleavage furrows during membrane invagination (Schweisguth et al., 1990, 1991 and unpublished). The temporal restriction of *sry* α accumulation is achieved both through a tight blastoderm-specific transcriptional control and by instability of both the *sry* α mRNA and protein products (Schweisguth et al., 1989, 1990).

No point mutations in the *sry* α gene have yet been iden-

tified despite several EMS screens of the 99D4-8 chromosome interval (Crozatier et al., 1992). In order to gain further insight into the specific role of *sry* α in cellularisation, and the control of its expression, we used an interspecific comparison to identify putative functional domains within the *sry* α protein and mRNA, as well as transcriptional *cis*-regulatory elements. We report here the sequence of the *sry* α genes from *D. pseudoobscura* and *D. subobscura*. Further, we examine the expression and functional properties of the *D. pseudoobscura* gene introduced into *D. melanogaster*. Together with a comparison of the expression of *sry* α and the other recently described cellularisation gene *nullo*, (Simpson-Rose and Wieschaus, 1992), this evolutionary comparison reveals a complex temporal and spatial regulation of the expression of cellularisation genes operating at the levels of transcription and possibly of RNA stability.

MATERIAL AND METHODS

Drosophila stocks

The Oregon R stock of wild-type flies was used for control in situ hybridizations. The *Df(3R)X3F* (referred to as *DfX3F*) stock was obtained from Dr J. Merriam, and the ry^{506} strain used in the transformation experiments from Dr W. Bender. The *LIMDF* deficiency strain uncovering the *nullo* gene was provided by Dr E. Wieschaus.

Molecular characterization of the *D. pseudoobscura* and *D. subobscura sry* α clones

All molecular methods described in this and other sections were carried out using standard techniques described in Sambrook et al. (1989). M. Aguadé and C. Segarra kindly provided us with DNA from the 91C region of *Drosophila subobscura* and the 62 region of *D. Pseudoobscura* in the form of overlapping clones (EMBL4 and EMBL3 phage vectors, respectively) containing the ribosomal protein 49 gene (Aguadé, 1988; Segarra and Aguadé, 1993). Crude maps of the *serendipity* gene cluster organization were obtained by Southern hybridization to phage DNA cut with *Bam*HI, *Eco*RI, and *Hin*dIII restriction endonucleases, using DNA probes made from the *D. melanogaster rp49* and *sry* β, α and δ genes. Relevant phage DNA fragments were subcloned into Bluescript (Stragagene) and sequenced in both orientations using the exonuclease directional deletion technique of Henikoff (1987).

Northern blot analysis and in situ hybridization

Embryos were collected from either fly cage populations (*D. melanogaster*) or flies raised in bottles (*D. pseudoobscura*). 30 minutes collections of synchronously developing embryos were obtained after two precollections of 1 hour on agar plates supplemented with baker's yeast and grape juice. The developmental stage of embryos from every collection was verified by optical inspection under the microscope following dechorionation. Development and times AEL (at 22°C) were checked by counting nuclei of embryos stained with DAPI using numbers of Zalokar and Erk (1976) (cycles 10-13) and by observing at the depth of membrane invagination during cycle 14. We found developmental times comparable to those given by Edgar et al. (1986, 1987). Hybridization probes were prepared from cloned fragments (a to d in Fig. 1) for the *D. melanogaster* and *D. pseudoobscura sry* α and rp49 genes, and the *Eco*RI-*Hin*dIII fragment of the *nullo* M1 cDNA, (a gift of L. Simpson-Rose). Isolated inserts were random primed for incorporation of either [^{32}P]dCTP for northern analysis, or digoxigenin-conjugated dUTP for in situ hybridization (Tautz and Pfeifle, 1989). Staining of digoxigenin-tagged DNA-RNA hybrids was performed with alkaline phosphatase-conjugated anti-digoxigenin antibodies (Boehringer-Mannheim) for usually 4 to 6 hours at room temperature. Nuclear dots seen in cycle 11 embryos were better stained when embryos had been fixed for twice the usual time. Views were taken using a Zeiss Axiophot photomicroscope equipped with epifluorescence and Nomarski optics. The domains of *sry* α transcript accumulation, given in percentage of egg length, were measured on five individual embryos for each stage.

P-element transformation and rescue assay

The p[*sry* α *pse*, ry^+] element was obtained by subcloning a 3.56 kb DNA segment, which contains the *D. pseudoobscura sry* α gene including 0.72 kb DNA upstream and 1.2 kb DNA downstream of the protein coding region, respectively, into the pDm23 P-element vector (Mismer and Rubin, 1987). P-element-mediated transformation was carried out following classical procedures (Rubin and Spradling, 1982). Appropriate crosses of p[*sry* α *pse*, ry^+] transformed flies with balancer and the *DfX3F* stocks were used to establish the strains Pa4 : p[*sry* α *pse*, ry^+] /p[*sry* α *pse*, ry^+], Y; *DfX3F/TM3, Sb* and Pa9 : p[*sry* α *pse*, ry^+] / *CyO*, *DfX3F/TM3, Sb.*

The progeny of these stocks were examined for cellularisation defects : cellular blastoderm embryos were devitellinized by hand-peeling and analyzed by staining F-actin and nuclei, and their phenotypes scored under the microscope (Schweisguth et al., 1990).

RESULTS

The *sry* α gene in *D. pseudoobscura* and *D. subobscura*

The *D. melanogaster serendipity* gene cluster is located immediately adjacent to the ribosomal protein rp49 gene on the third chromosome (99D4-8). Isolation and sequencing of the rp49 gene and its immediately dowstream region from *D. pseudoobscura* (Segarra and Aguadé, 1993) and *D. subobscura* (Aguadé, 1988) revealed that the relative locations of *sry* δ and rp49 had been conserved in these two *Drosophila* species. Starting from genomic phages containing rp49 (a gift of M. Aguadé), we determined the organization of the *sry* gene cluster (*sry* β−α−δ in the 5′ to 3′ direction of transcription) in *D. pseudoobscura* and *D. subobscura* and sequenced it in its entirety (Ferrer et al., 1993 and Fig. 1). Only the *sry* α gene region is of interest in this study and its nucleotide sequence is given in Fig. 2. The use of a dot matrix DNA sequence comparison illustrates the extent of sequence conservation between the *D. melanogaster* and *D. pseudoobscura sry* α genes (Fig. 3A). Highly conserved regions are observed interspersed with divergent regions both in the protein coding and immediately upstream sequences, while no significant homology could be detected 3′ to the translation stop codon.

Conservation of the *sry* α protein

The *D. melanogaster* and *D. pseudoobscura sry* α proteins share 65% amino acid identity and this sequence homology increases to 75% when conservative amino acid substitutions are considered as well. The *D. melanogaster* and *D. subobscura* proteins share 64.5% (75%) and *D. pseudoobscura* and *D. subobscura* 78% (84%) identity (similarity), respectively. In spite of a significant sequence divergence, the three proteins have nearly identical overall amino acid compositions (with around 36% hydrophobic residues), isoelectric points around 5.25 and similar hydropathy profiles (not shown). In all three proteins, the initiator methionine is

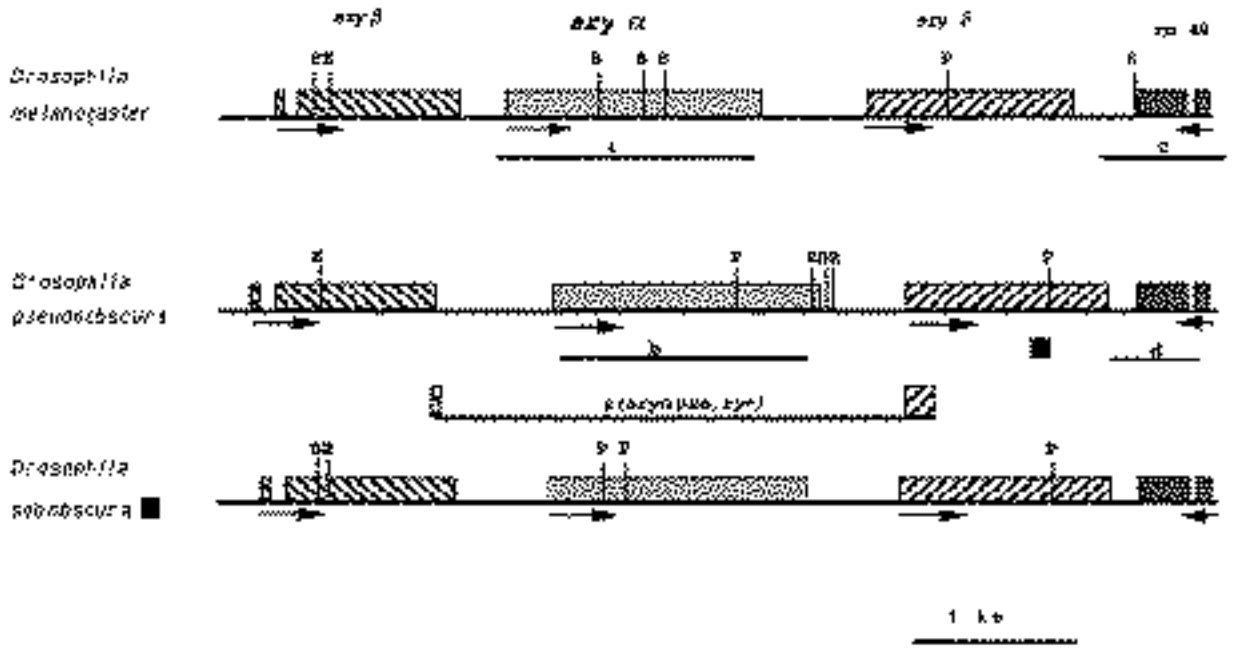


Fig. 1. Compared genomic organization of the *serendipity*/rp49 gene cluster in *D. melanogaster*, *D. pseudoobscura* and *D. subobscura* (Vincent et al., 1985; Ferrer et al., 1993). Position of *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H) and *Pst*I (P) restriction endonuclease sites is indicated. Open reading frames are boxed; arrows indicate the direction of transcription. Fragments a to d indicated by horizontal bars were used as separate probes for hybridization to RNA. The position of the *D. pseudoobscura* genomic fragment included in the P(*sry* α *pse*, *ry*$^{+}$) construct is indicated. Nucleotide sequences of the *D. pseudoobscura* and *D. subobscura* rp49 genes were taken from Segarra and Aguadé, 1993 and Aguadé, 1988, respectively.

immediately followed by a glutamic acid residue, which would predict a short average half-life (30 minutes) provided that the N-end rule applies in *Drosophila* (Varshavsky, 1992). One potential transmembrane segment is predicted at identical positions in the three *sry* α proteins (position 56-72 in *D. melanogaster*, see Fig. 2). There are 5 potential casein kinase II phosphorylation sites that are conserved (out of a total of 12, 8 and 9, in *D. melanogaster*, *D. pseudoobscura* and *D. subobscura*, respectively). Three of these sites are almost identical, two of them being included in a novel 14 a.a. conserved motif found twice in each protein (Fig. 3B). The extent of conservation is not constant across the length of the reading frame, and the *D. pseudoobscura* protein is 19 a.a. longer than the *D. melanogaster* protein and 6 a.a. longer than the *D. subobscura* protein. These differences in sizes are due to small insertion-deletions which generally separate highly conserved blocks. Each of these blocks, ranging in size from 15 to 46 a.a. residues (Fig. 3C), was used for searching of the Genbank and EMBL data bases. A region of limited homology was found between sry α and proteins of the ezrin-radixin-moezin (ERM) family (Fig. 3D). The ERM proteins appear to specifically localize to actin filament/ plasma membrane association sites (Sato et al., 1992). Databank searches did not reveal any other extended homology to known proteins.

sry α transcription in *D. melanogaster* and *D. pseudoobscura* embryos

The *sry* α transcription start in *D. pseudoobscura* and *D. subobscura* was deduced from sequence comparison with the *D. melanogaster* gene (Fig. 2). The predicted size of the *D. pseudoobscura sry* α mRNA was verified by northern blot analysis of RNA from 0- to 12-hour-old embryos. The major species detected is 2 kb long. It is only present in early embryos and at no other developmental stage tested. However, a second, minor 3.4 kb long transcript, complementary to *sry* α DNA, was also detected in embryos, as well as at other developmental stages (see below, Fig. 5A, and data not shown). A transcript of the same size was also detected when *sry* β DNA was used as a probe (Ferrer et al., unpublished data). This observation suggests that specific *sry* β-*sry* α read-through transcription occurs in *D. pseudoobscura*, a phenomenon first observed in *D. melanogaster* (Vincent et al., 1985, 1986).

The pattern of *sry* α transcript accumulation was compared in *D. melanogaster* and *D. pseudoobscura embryos* using whole-mount in situ hybridization (Fig. 4). In both species, *sry* α transcripts are first detected at mitotic cycle 11, as two dots in all somatic nuclei (Fig. 4A and A′ and data not shown). Such dots probably represent clusters of nascent transcripts at the site of the actively transcribed gene as postulated by Shermoen and O'Farrell (1991). The first hybridization signal detectable in the cytoplasm is in cycle 12 embryos. The level of *sry* α RNA increases as embryos progress through the 13th nuclear cycle (Fig. 4B,E) to peak in early cycle 14 embryos during the initial phase of cellularisation. This is the point when the *sry* α protein accumulates close to the folds and microprojections of the plasma membrane. At this stage, both transcript and protein appear uniformly distributed over the entire surface of the

embryo (Schweisguth et al., 1990). However, while *sry* α RNAs remain localized between the peripheral nuclei and the periplasmic membrane during cellularisation in *D. melanogaster* embryos (Fig. 4H; see also Schweisguth et al., 1989), this is clearly not the case in *D. pseudoobscura* embryos, where *sry* α mRNAs concentrate basally, just below the cortical nuclei (Fig. 4I).

A surprising aspect of the expression pattern observed in whole embryos, which previously passed unnoticed on embryo sections (Schweisguth et al., 1989), is that, while RNA is indeed present throughout the cortex of the embryo, with the exception of pole cells, the distribution of *sry* α RNA does not remain uniform after cellularisation has begun. In *D. melanogaster*, two broad bands along the anteroposterior axis, between 0 to 22% and 45% to 80% of the egg length (with 0% and 100% corresponding to the anterior and posterior ends, respectively), accumulate more RNA than the rest of the embryo (Fig. 4C). This banded pattern becomes more apparent as cellularisation progresses, when the level of *sry* α RNA decreases (Fig. 4D). By the time of ventral furrow formation, *D. melanogaster* embryos have virtually no more detectable *sry* α RNA (not shown).

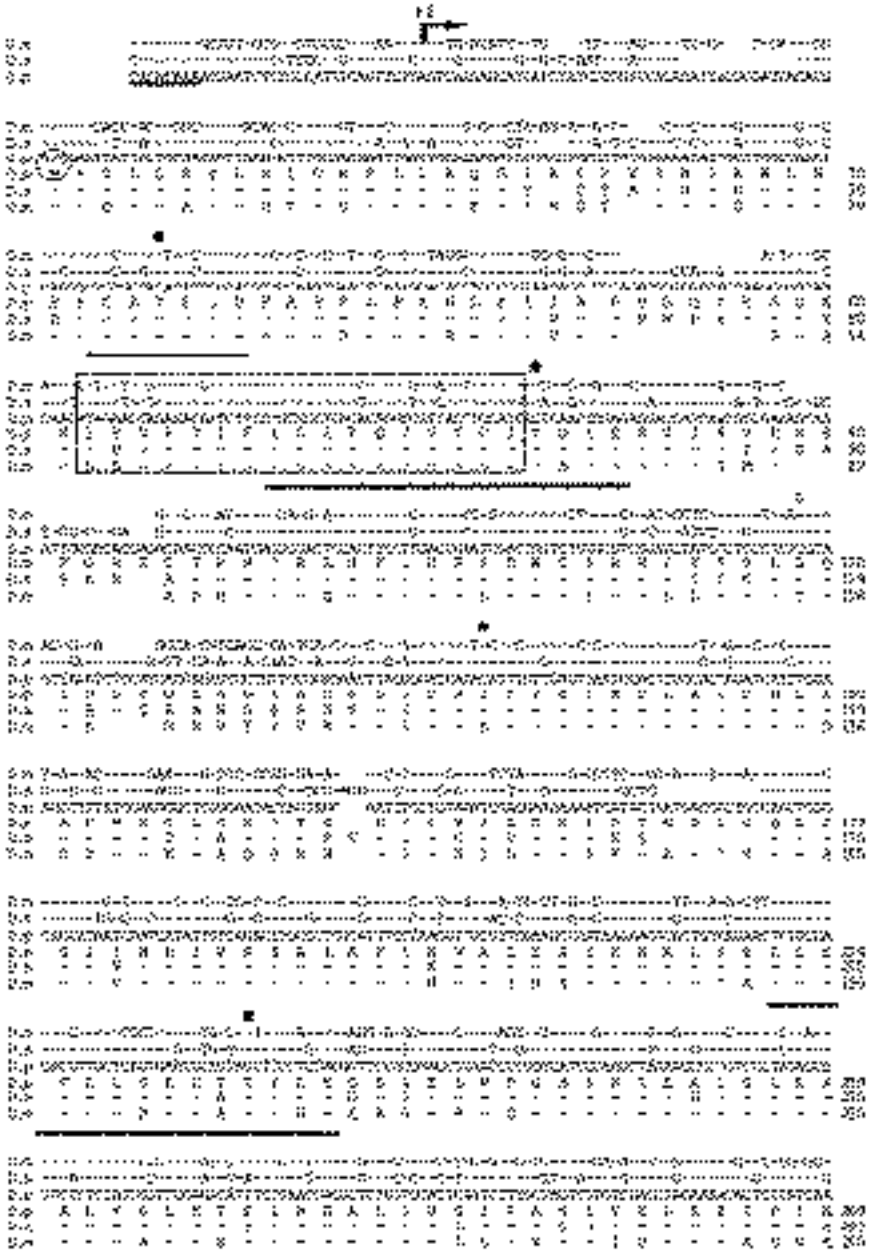
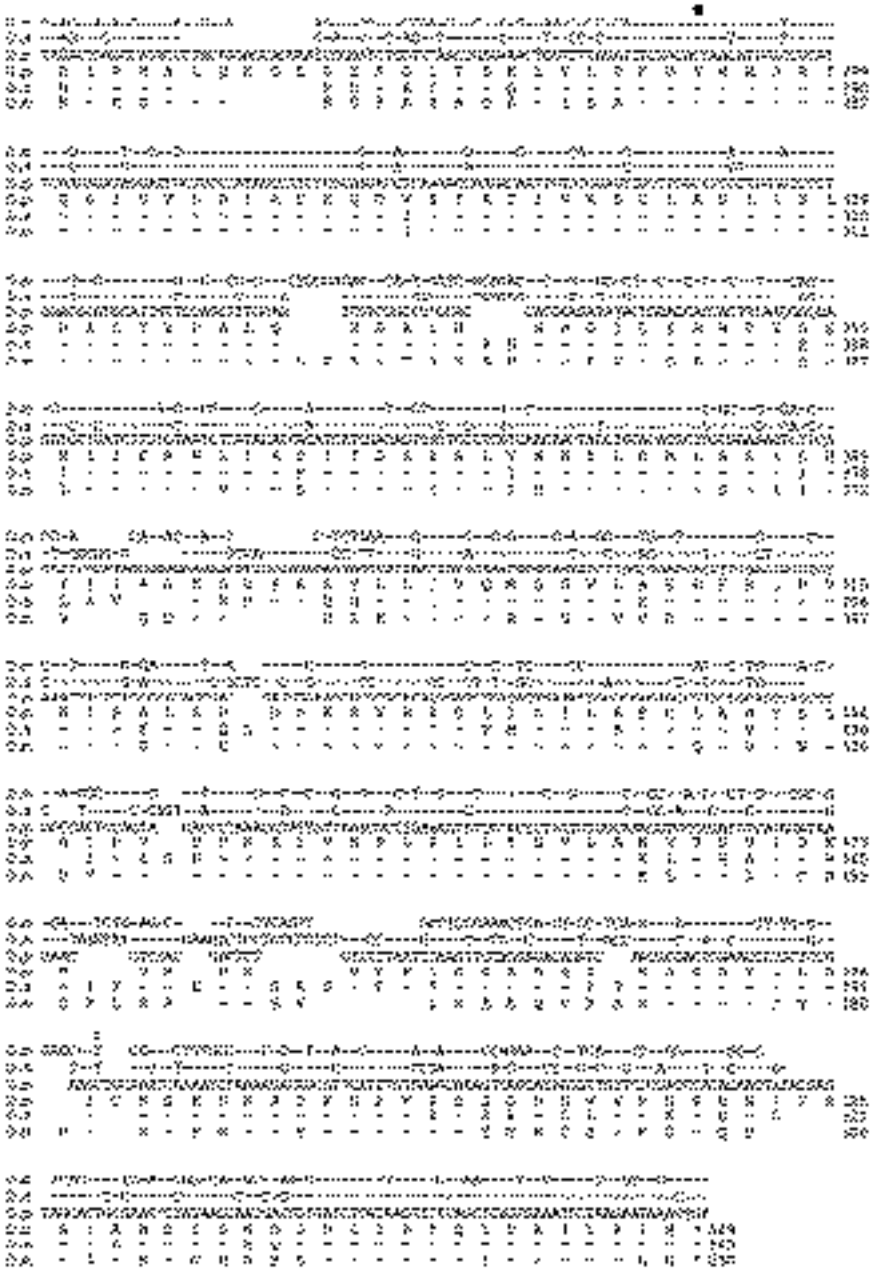


Fig. 2. Comparison of the nucleotide (top lines) and deduced amino acid sequences (bottom lines) of the *D. pseudoobscura* (D.p), *D. subobscura* (D.s) and *D. melanogaster* (D.m) *sry* α protein coding regions. The 5′ to 3′ sequences of the mRNA-like strand are presented, starting at the putative TATA box (underlined) and ending at the protein stop codon. The complete sequence is given for the *D. pseudoobscura* gene. Only bases or amino acids that differ between *D. pseudoobscura* and *D. subobscura* or *D. melanogaster* are shown; dashes indicate identical positions. The A nucleotide corresponding to the *D. melanogaster* transcription start is designated by a bent

The *D. pseudoobscura sry* α pattern during cycle 14 appears different from, and more complex than that of *D. melanogaster*. Early during the interphase, *D. pseudoobscura sry* α transcripts become more concentrated at both the anterior and posterior ends, and to a lesser extent at the center of the embryo, in domains covering roughly 0 to 22%, 80% to 100% and 35% to 65% of the total egg length, respectively (Fig. 4F). This pattern evolves during cellularisation into a prominent expression in the posterior region, the appearance of a clear dorsoventral asymmetry and the resolution of the central domain of expression into two separate bands (Fig. 4G). The *D. pseudoobscura sry* α transcripts persist longer than those in *D. melanogaster*, as they are still observed in specific groups of cells during invagination of the cephalic and posterior midgut furrows (not shown).

Expression of the *D. pseudoobscura sry* α gene in *D. melanogaster* transformant lines

Divergence of the *sry* α gene structure and pattern of RNA accumulation between *D. pseudoobscura* and *D. melanogaster* led us to examine the expression of the *D.*



arrow (Vincent et al., 1985) and referred to as nucleotide 1. Amino acid 1 corresponds to the 1st methionine in the open reading frame (circled). The coding region has been aligned on the basis of maximizing the amino acid homologies. Numbering on the right refers to the a.a. sequences. The potential transmembrane segment and casein kinase II phosphorylation sites found at identical positions in the three proteins are boxed, and indicated by asterisks, respectively. A novel duplicated 14 amino acid long motif, as well as one copy of its truncated version (see Fig. 3C), is underlined.

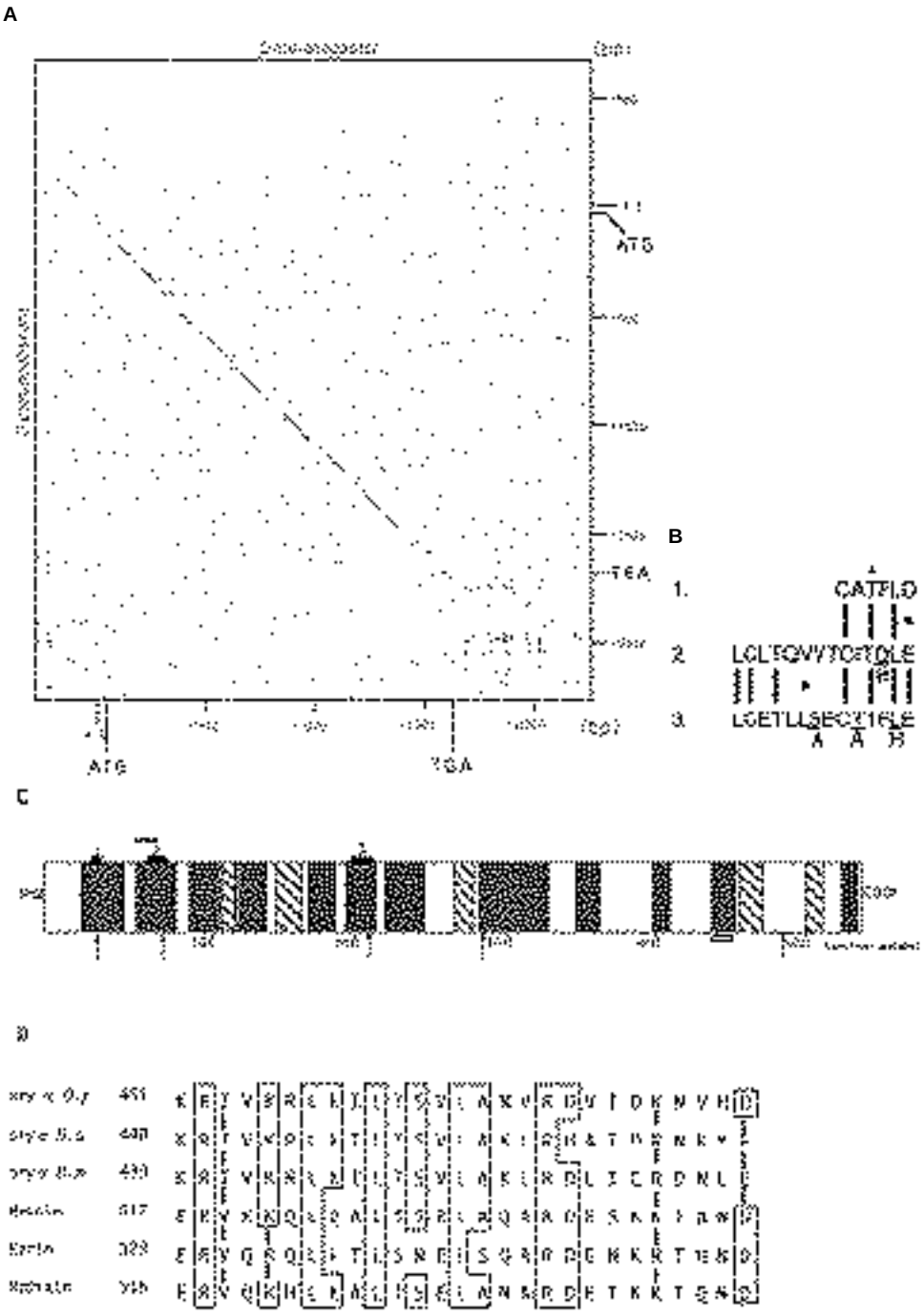


Fig. 3. (A) Dot matrix homology comparison of the *sry* α genomic region from *D. melanogaster* and *D. pseudoobscura*. The dot matrix homology comparison was generated using the COMPARE and DOTPLOT programs of the UWGCG DNA sequence analysis package. Eight matches or more over a 10 bp window (80%) are required to make a dot. Numbering refers to position +1 as the start of transcription. Positions of the start and stop codons of the *sry* α open reading frame are indicated. (B) Amino acid sequence of the closely related evolutionarily conserved motifs encompassing a casein kinase II phosphorylation site (*); numbering refers to the position in the *sry* α protein sequence, as indicated by black bars in panel C. (C) Highly conserved regions (8 of 10 identical residues at identical positions) between the *D. pseudoobscura, D. subobscura* and *D. melanogaster sry* α predicted proteins are indicated by shaded areas. Regions of high divergence between each of these species are indicated by striped areas. Positions of the potential transmembrane segment and of the region of homology to proteins of the ERM family (D) are indicated by the upper black and an open bar, respectively. Arrows indicate the positions of the conserved casein kinase II phosphorylation sites. (D) Comparisons of the sry α sequence to sequences available in the data bases revealed a region of weak homology to protein of the ezrin-radixin-moesin family (Sato et al., 1992). The homologous regions are aligned.

pseudoobscura sry α gene in *D. melanogaster*. A *D. pseudoobscura sry* α DNA fragment including the entire protein coding region bordered on the 5′ side by 0.72 kbp and 3′ side by 1.2 kbp of DNA, (p[*sry* α *pse, ry*$^{+}$]) was introduced into the *D. melanogaster* genome by germ line transformation (see Material and Methods). The inserted *D. pseudoobscura* fragment was expected to include all of the necessary *cis*-acting regulatory sequences, based on the structure of the gene cluster (Fig. 1), and the position of conserved 5′ sequence motifs (see below, Fig. 9). Two transformant lines, one on the X and one on the second chromosome, and designated below as Pa4 and Pa9 respectively, were established.

We first compared in detail the respective patterns of expression of the *D. pseudoobscura* and *D. melanogaster sry* α transcripts by northern blot analysis. The *D. pseudoobscura sry* α probe employed (see Fig. 1) allows discrimination between *D. pseudoobscura* and *D. melanogaster sry* α transcripts (Fig. 5A). To get an accurate comparison of the period of accumulation of the resident and transgenic *sry* α mRNA, developmental northern blots were made, using RNA from embryos collected every 30

minutes after egg laying (AEL), at 22°C (Fig. 5B). Accumulation of the *D. melanogaster* and transgenic *D. pseudobscura sry* α transcripts is concomitant (2.5 hours AEL at 22°C) and reaches a maximum between 3 and 3.5 hours AEL. However, the sharp decrease of *D. melanogaster* transcripts that follows is not paralleled by a

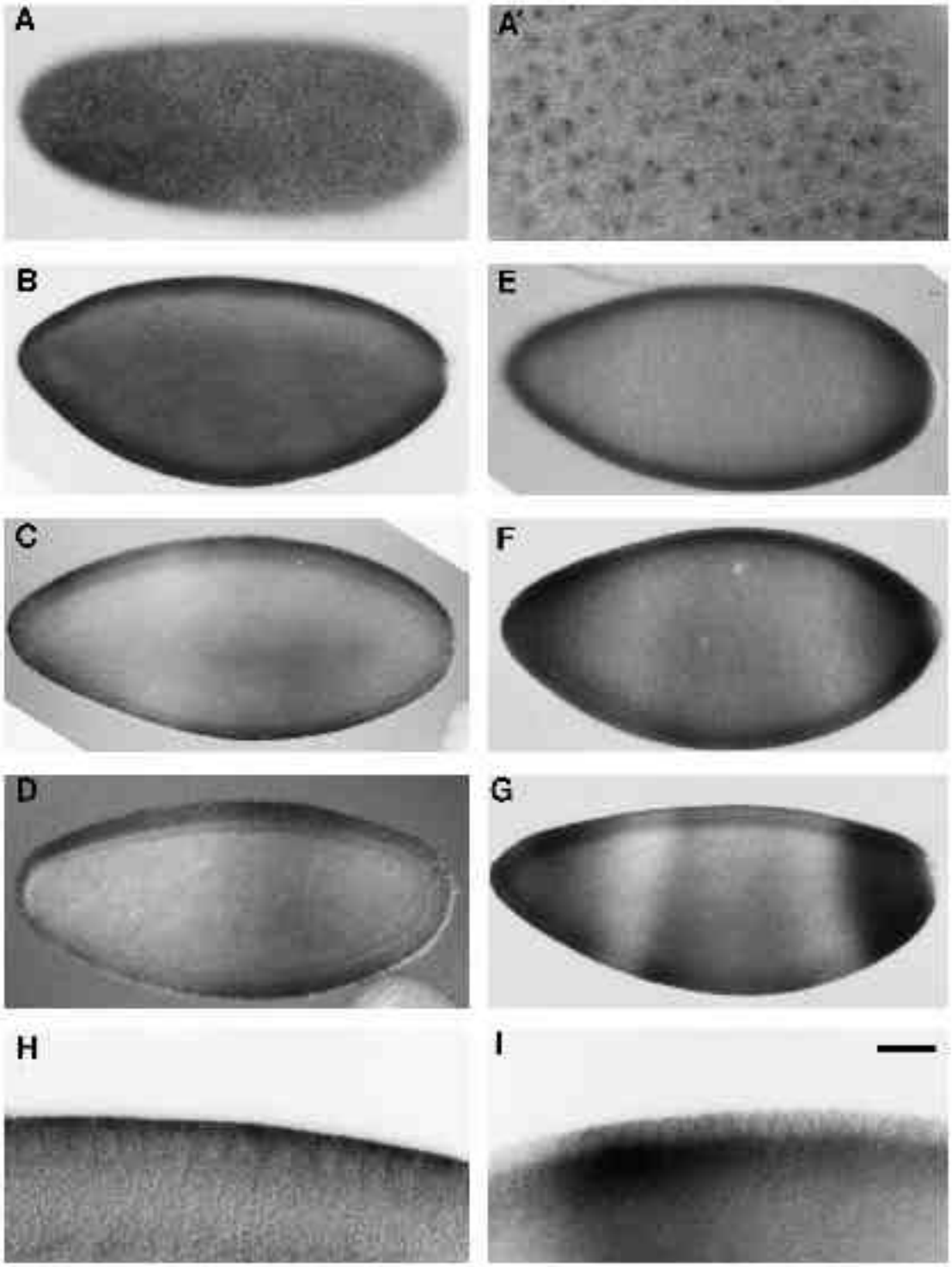


Fig. 4. In situ hybridization to *sry* α transcripts, in *D. melanogaster* (A-D, and H) or *D. pseudoobscura* (E-G and I) wild-type embryos. All embryos are oriented with anterior to the left and dorsal up, except G. 0% and 100% of egg length refer to the anterior and posterior ends, respectively. (A,A′) Embryo at nuclear cycle 11, showing one or two hybridization dots in each nucleus; (B,E) embryos at cycle 13; (C,F) embryos at early interphase of cycle 14, before nuclear elongation; (D,G) embryos at mid-cellularisation, with membranes invaginated to bases of nuclei; (H,I) details of embryos at mid-cellularisation, showing the apical and basal location, of *D. melanogaster* and *D. pseudoobscura sry* α transcripts, respectively. Scale bar corresponds to 60 mm (A-G) and ≈ 20 μm (A,H and I).

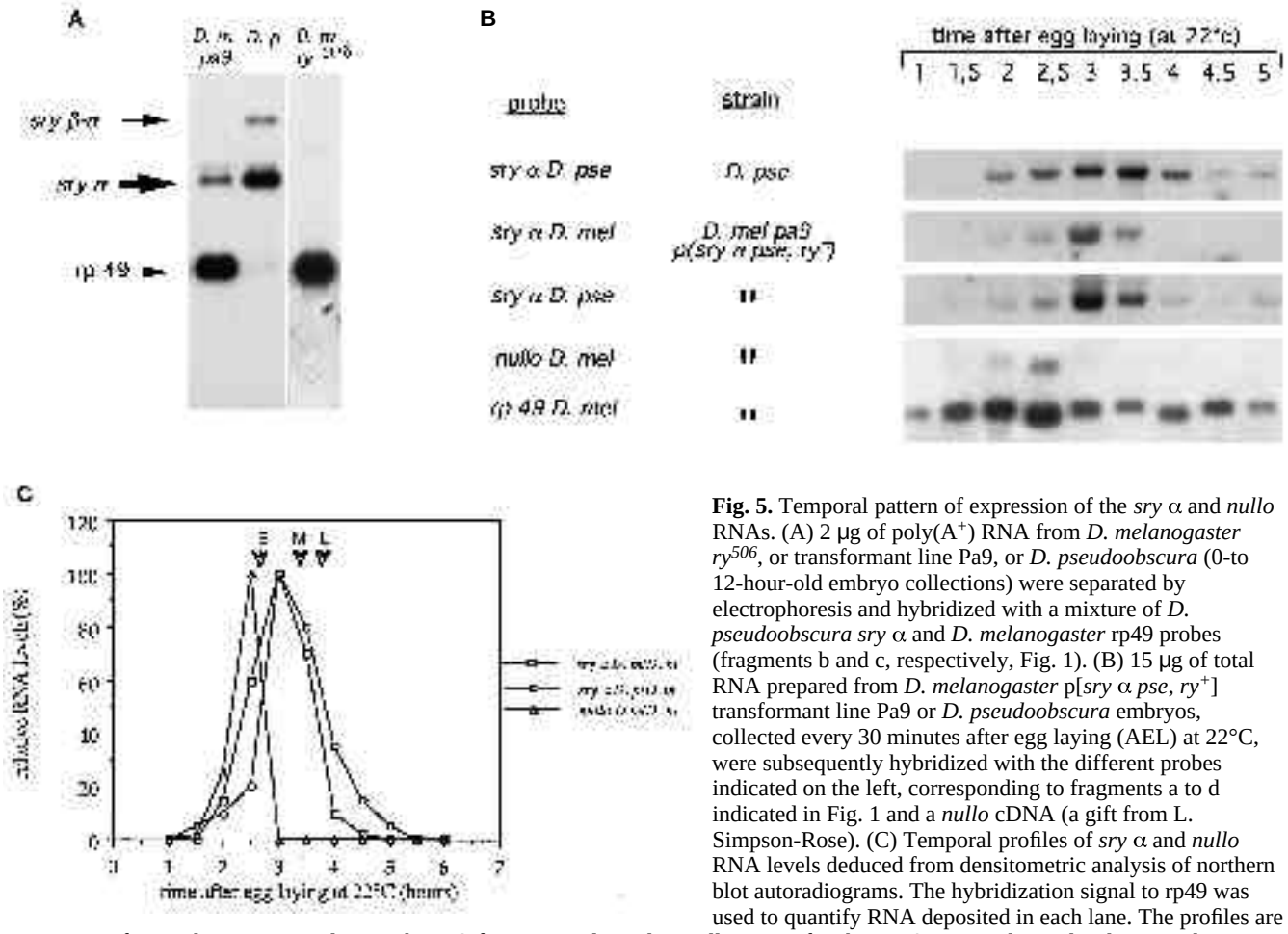


Fig. 5. Temporal pattern of expression of the *sry* α and *nullo* RNAs. (A) 2 μg of poly(A$^+$) RNA from *D. melanogaster* *ry*^{506}, or transformant line Pa9, or *D. pseudoobscura* (0-to 12-hour-old embryo collections) were separated by electrophoresis and hybridized with a mixture of *D. pseudoobscura sry* α and *D. melanogaster* rp49 probes (fragments b and c, respectively, Fig. 1). (B) 15 μg of total RNA prepared from *D. melanogaster* p[*sry* α *pse*, *ry*$^+$] transformant line Pa9 or *D. pseudoobscura* embryos, collected every 30 minutes after egg laying (AEL) at 22°C, were subsequently hybridized with the different probes indicated on the left, corresponding to fragments a to d indicated in Fig. 1 and a *nullo* cDNA (a gift from L. Simpson-Rose). (C) Temporal profiles of *sry* α and *nullo* RNA levels deduced from densitometric analysis of northern blot autoradiograms. The hybridization signal to rp49 was used to quantify RNA deposited in each lane. The profiles are composite of several experiments done with RNA from two independent collections of embryos. Arrows indicate developmental times for *D. melanogaster* embryos. E, early interphase 14 before nuclear elongation (≈160 minutes AEL); M, mid-cellularised embryos when the membrane reaches the bottom of nuclei (210 minutes AEL); L, cellularised embryos (240 minutes AEL).

similar decrease of the *D. pseudoobscura sry* α (*Pa9)* RNAs. Whereas by 4 hours AEL the *D. melanogaster* transcripts have become virtually undetectable (Fig. 5B,C), the transgenic *Pa9* transcripts persist, as they also do in the wild-type *D. pseudoobscura*.

Expression of the *D. pseudoobscura sry* α gene transformed into *D. melanogaster* was also examined by in situ hybridization on whole embryos of transformant lines Pa4 and Pa9 (Fig. 6). Expression of the transgene starts at the same time as that of the endogenous *sry* α gene (cycle 11) and similarly remains uniform during cycles 12 and 13 (not shown). However, starting early during cycle 14, the pattern of accumulation of the transgenic *D. pseudoobscura sry* α RNA differs from that of the resident (*D. melanogaster*) *sry* α transcripts and resembles the *sry* α pattern in *D. pseudoobscura* embryos (compare Figs 6A and 4F). Thereafter, the transgenic and wild-type patterns of *D. pseudoobscura sry* α RNA appear virtually identical (compare Figs 6B,C and 4G). Furthermore, transgenic *Pa4* or *Pa9 sry* α RNA do not localize to the apical region of newly forming cells, as do the endogenous *D. melanogaster sry* α transcripts but, like in *D. pseudoobscura*, they accumulate basal to nuclei (Fig. 6D).

The *D. pseudoobscura sry* α gene is functional in *D. melanogaster*

We have previously shown that a single copy of the *D. melanogaster sry* α gene is able to rescue the zygotic cellularisation defects associated with the *DfX3F* deficiency (Schweisguth et al., 1990). Whether the differences in *sry* α structure and/or expression described above are critical for *sry* α function in cellularisation may be tested by replacing the *D. melanogaster* gene by its homolog in *D. pseudoobscura*. The locally abnormal distribution of F-actin arrays in the *Df3XF* mutant embryos is illustrated in Figs 7 and 8. Transformed lines Pa4 and Pa9 were tested for their ability to rescue these cellularisation defects. The cellularisation phenotype was scored at the interphase of cycle 14, using phalloidin staining of embryos after devitellinisation (see Figs 7 and 8). The results given in Table 1 indicate that the *DfX3F* cellularisation phenotype is fully rescued (i.e. all nuclei are individually cellularised correctly) in line Pa9 and partly rescued in line Pa4. Partial rescue means here that not only the number of embryos showing cellularisation defects but also the average size of multinucleate cells in these embryos are consistently smaller in Pa4 embryos than in *DfX3F* homozygotes. The rescue experiments therefore

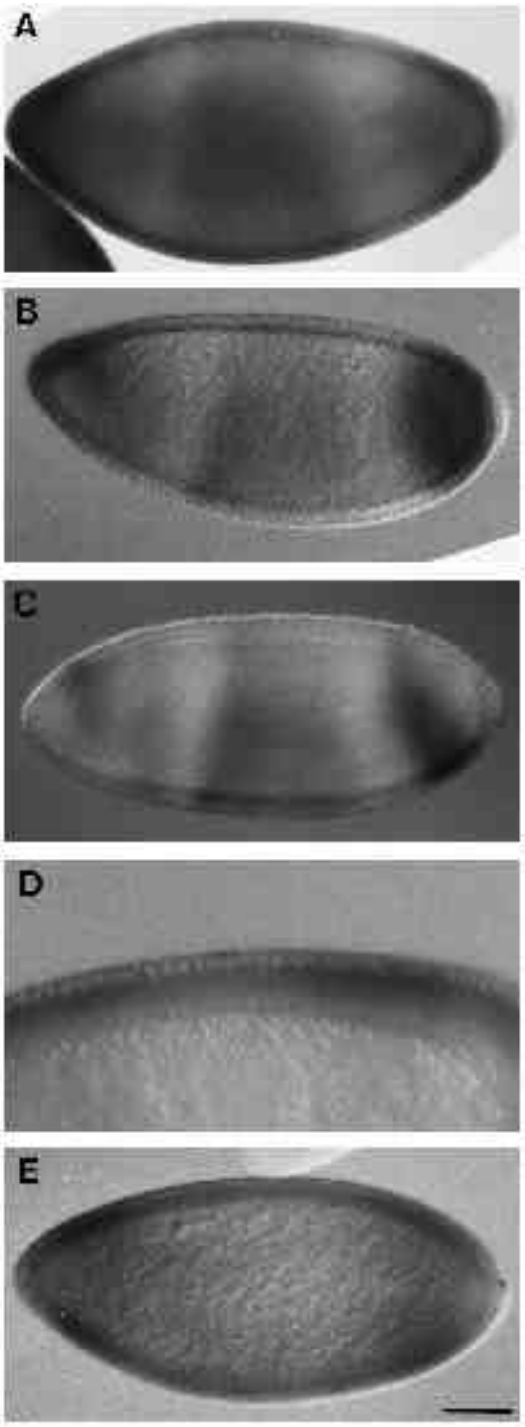


Fig. 6. Whole-mount in situ hybridization to *D. pseudoobscura*, *sry* α transcripts in embryos of the p[*sry* α *pse*, *ry*$^{+}$] transformant line Pa9. The *D. pseudoobscurasry* α probe employed (see Fig. 1) does not give a detectable hybridization signal on *D. melanogaster* wild-type (*ry*^{506}) embryos. (A) Embryos at early interphase of cycle 14 before nuclear elongation. (B) Embryo at mid-cellularisation with membranes invaginated to bases of nuclei. (C) Embryos after cellularisation is completed, when somatic cells have incorporated the cortical plasma. (D) Detail of embryo at mid cellularisation, showing the basal location of *D. pseudoobscura sry* α transcripts in *D. melanogaster* embryos. (E) Hybridization to *nullo* RNA in a wild-type embryo at the early interphase of cycle 14. Bar≈60 μm except in panel D (20 μm).

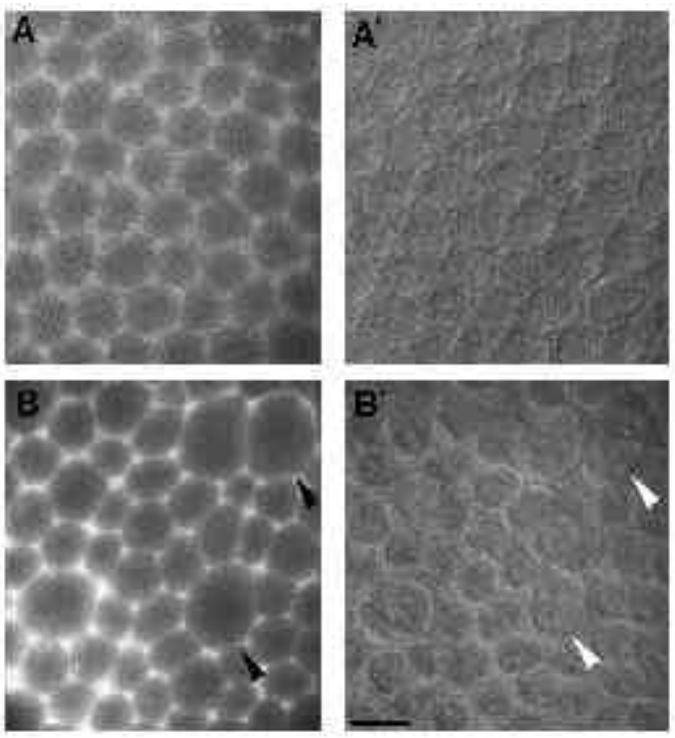


Fig. 7. (A) Surface view of (A) wild-type and (B) *DfX3F* embryos at the early interphase 14, stained with rhodamine-phalloidin to reveal actin-filaments just beneath the periplasmic membrane. Note the erratic disruptions of the F-actin network in *DfX3F* embryos; (A′,B′) same embryos observed under Nomarski optics to reveal the hexagonal array of cleavage furrows formed by the invaginating membranes. Note the exact superposition of disruptions of the F-actin network and defects in membrane invaginations, leading to the formation of multinucleate cells in *DfX3F* embryos. Bar≈15 μm.

show that the *D. pseudoobscura sry* α gene can, at least grossly, substitute for the *D. melanogaster* gene in cellularisation.

Conserved motifs in the *sry* α and *nullo* promoters

Previous promoter deletion analysis has shown that all *D. melanogaster sry* α *cis*-regulatory elements are contained within 311 bp 5′ upstream of its transcription start site, a fragment that includes the entire *sry* β-*sry* α intergenic region, (Schweisguth et al., 1989 and unpublished). Sequence comparison of the *sry* β-*sry* α intergenic region between *D. melanogaster*, *D. pseudoobscura* and *D. subob - scura* revealed several conserved nucleotide motifs (see Fig. 9A). A 50 bp region displays 32 identical nucleotides at identical positions that can be clustered into 3 motifs (designated I, II and III). These motifs are 12 bp, 8 bp and 13 bp long, respectively, and located at the same relative position in all three *Drosophila* species, although at a variable distance upstream of the putative TATA box. A fourth conserved motif (motif IV, 7bp long) close to the TATA box was identified. A search for sequence similarity between the 5′ upstream regions of *sry* α and *nullo*, the two *D. melanogaster* zygotic cellularisation genes for which

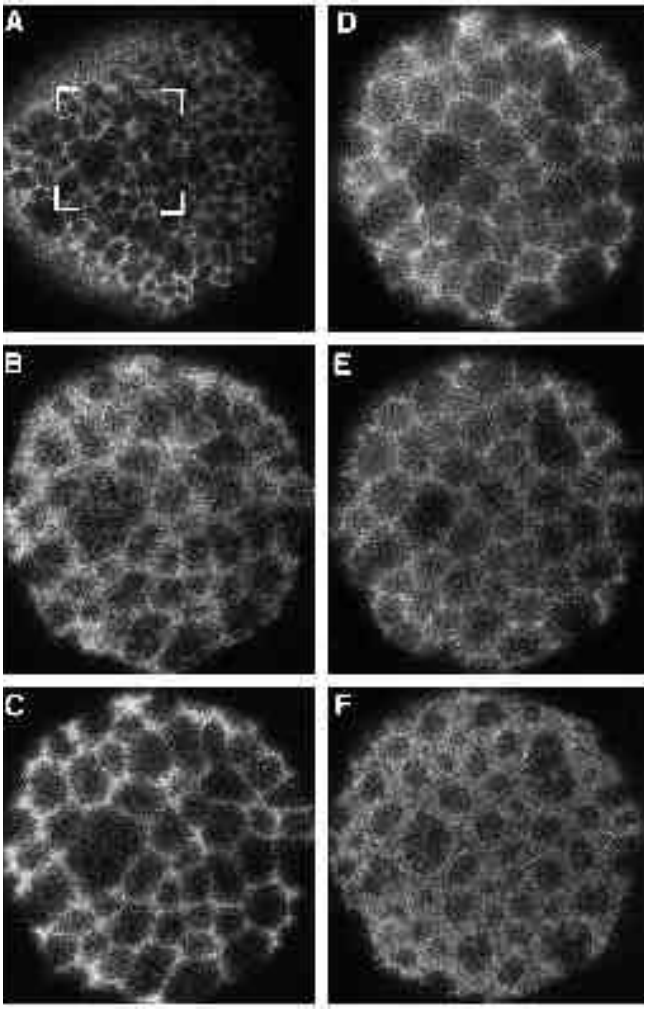


Fig. 8. Optical sections of a *DfX3F* embryo stained with rhodamine-phalloidin during the second phase of cellularisation, going from B (the apex) to F (the base of the forming cells). (A) Lower magnification view of the section C (anterior end of the embryo). Note the rounded shape of larger rings corresponding to multinucleate cells (F), indicating that the tensions equilibrate during ring contraction, in spite of initial irregular cell shape. Bar≈15 μm in all panels except A.

sequences are available (personal communication from L. Simpson-Rose), revealed a 33 bp region with a high degree of similarity, with 24 conserved nucleotide positions. This conserved region is located at the same position relative to the TATA box in the two genes and includes the *sry* α motifs I and II. It also includes a 8 bp GC-rich motif which is not as strongly conserved in *sry* α between different *Drosophila* species (Fig. 9B).

Subtle differences in expression and phenotype between the *sry* α and *nullo* cellularisation genes

sry α and *nullo* are the two already identified zygotic genes whose absence results in an abnormal formation of the actin-myosin network during cellularisation. Both genes are only expressed in embryos at the blastoderm stage (Vincent et al., 1985, Simpson-Rose et al., 1992). The possibility that they share some *cis*-acting regulatory elements (Fig. 9) led us to compare in detail their respective patterns of expression. *nullo* RNA accumulation starts at the same time as that of *sry* α but peaks earlier (by about 30 minutes at 22°C), such that *nullo* RNA has virtually disappeared when *sry* α accumulation is maximal (Fig. 5C). In situ hybridization on whole embryos with a *nullo* cDNA probe shows uniform expression at cycle 13 but bands of heavier *nullo* RNA accumulation at early cycle 14 (Fig. 6E and Simpson-Rose and Wieschaus, 1992). The bands of accumulation of *nullo* RNAs (covering between 8% to 22% and 75% to 90% of egg length, respectively) overlap but do not precisely coincide with those of *sry* α. Subtle phenotypic differences between *sry* α and *nullo* deficient embryos might parallel the difference in the patterns of RNA accumulation. The early defects observed in *DfX3F* precisely mark not only the position where multinucleate cells will form, but also their final size. Contrary to what is observed in *nullo* embryos, (Simpson and Wieschaus 1990, and data not shown), disruptions of the actin-myosin network of *DfX3F* embryos do not seem to increase during cellularisation, as there is complete absence of detectable residual F-actin between adjacent nuclei enclosed within a single multinucleate cell (Fig. 8). Finally, in *DfX3F* embryos the greatest disruptions occur at both poles of the embryo (Fig. 8A and Schweisguth et al., 1990), something that is not observed in *nullo* embryos. Whether the subtle phenotypic differences observed between *sry* α and *nullo* deficient embryos reflect differences in the patterns of expression of the two genes remains to be investigated.

DISCUSSION

Conservation of the *sry* α protein structure and function

The cellularisation phenotype associated with a complete loss of *sry* α function, and the similar distribution of F-actin and *sry* α protein during the transition between mitotic cycles 13 and 14 and the first phase of cellularisation suggested a role of *sry* α in the positioning and/or stabilisation of the hexagonal actin-myosin network (Schweisguth et al., 1990, 1991). As a first step towards the identification of functional domains within the *sry* α protein, we looked for conserved sequences between three *Drosophila* species, *D. melanogaster*, *D. pseudoobscura*, and *D. subobscura*. Estimated times for divergence from their common ancestor are approximately 46 million years between *D. melanogaster* and *D. pseudoobscura* or *D. subobscura*, and 10 million years betwen *D. pseudoobscura* and *D. subobscura*, enough time to permit the divergence of DNA sequences unconstrained for function (Beverley and Wilson, 1984). This sequence comparison revealed several highly conserved unique regions in the *sry* α protein, including one (residues 434-451) showing limited homology to proteins of the ezrin-radixin-moesin (ERM) family (Sato et al., 1992 and ref. therein). The ERM proteins have been postulated to serve as structural links between the plasma membrane and the cytoskeleton. Moreover, radixin has been recently shown to concentrate at specific regions where actin filaments are densely associated with plasma membranes

Table 1. Rescue of the cellularisation defect associated with the *DfX3F* deficiency by a transposon carrying the *D. pseudoobscura sry* α gene

		Cellular blastoderm embryos				
			With cellularisation defects			
				Expected		
Line	Genotype	Total number	Observed	Complete rescue	No rescue	Rescue (%)
DfX3F	$\frac{+}{+}\ \frac{DfX3F}{TM3}$	74	17 (1/4)	–	–	–
Pa4	$\frac{p(sry\alpha\ pse,\ ry+)}{p(sry\alpha\ pse,\ ry+)/Y};\frac{DfX3F}{TM3}$	173	9*	0	43 (1/4)	80
Pa9	$\frac{p(sry\alpha\ pse,\ ry+)}{Cy};\ \frac{DfX3F}{TM3}$	156	11	10 (1/16)	39 (1/4)	100

The genotypes of lines listed in the left column are given in the central column. The *DfX3F* stock is indicated on the first line as a control. Phenotype refers to the local disruptions of the hexagonal F-actin array around each nucleus in cellular blastoderm embryos (interphase of cycle 14). In the pa4 inter se cross :

$$♀♀\ \frac{p[sry\ \alpha\ pse,\ ry^+]}{p[sry\ \alpha\ pse,\ ry^+]}\ \frac{DfX3F}{TM3,\ Sb} \times ♂♂\ \frac{p[sry\ \alpha\ pse,\ ry^+]}{Y}\ \frac{DfX3F}{TM3,\ Sb},$$

all embryos receive at least one copy of the *p*[*sry* α *pse*] transposon. Complete rescue by this transposon is therefore expected to yield no embryos showing cellularisation defects; no rescue would yield 1/4 (*DfX3F* homozygous), i.e. 43/173, of all laid embryos showing defects.

In the pa9 cross :

$$\frac{p[sry\ \alpha\ pse,\ ry^+]}{Cy}\quad\frac{DfX3F}{TM3,\ Sb}\quad ♀♀ \times ♂♂$$

half of *DfX3F* homozygous embryos (1/8 of total progeny) carries one copy of the transposon while a quarter (1/16 of total) carries two copies. Therefore, complete rescue of the *DfX3F* cellularisation phenotype by one copy of the transposon is expected to leave only 1/16, i.e. 10/156, of all laid embryos showing cellularisation defects. No rescue could yield 1/4, i.e. 39/156, of all embryos with defects. The number of embryos showing cellularisation defects is given with reference to the total number of embryos examined. Calculated numbers expected from complete, (left), or no, (right) rescue by the transposon are also given. * indicates embryos showing weak cellularisation defects (see text).

(Sato et al., 1992). Whether the observed structural homology between *sry* α and radixin has functional significance remains to be investigated. That the *D. pseudoobscura sry* α gene was able to substitute for its *D. melanogaster* ortholog in rescue of the *DfX3F* cellularisation phenotype shows that sequence divergence in some regions of the *sry* α protein between *D. pseudoobscura* and *D. melanogaster* has no major consequence for *sry* α function in cellularisation. These regions of high divergence could serve as hinges between conserved functional domains. It must be emphasized, however, that only the cellularisation phenotype associated with the *DfX3F* deficiency

Fig. 9. (A) Sequence alignment of the conserved elements in the *sry* α 5′ upstream region between *D. melanogaster*, *D. pseudoobscura* and *D. subobscura*. Conserved motifs are boxed and numbered from I to IV, and the TATA box underlined. The given nucleotide positions correspond to the *D. melanogaster* sequence with position 1 corresponding to the *sry* α transcription start (see Fig. 2 and Vincent et al., 1985). The consensus sequence is given below the sequence alignment, with letters in bold and thin representing nucleotides conserved in 3 out of 3, and 2 out of 3 *Drosophila* species, respectively. (B) Sequence alignment of a 32 bp region showing a high degree of similarity between the *D. melanogaster sry* α and *nullo* 5′ upstream regions. The nucleotide positions are given relative to the transcription start [for *nullo*, see Simpson-Rose and Wieschaus, 1992]. Asterisks denote identical nucleotide positions between the two genes, with bold letters indicating identity with *sry* α consensus positions (panel A). An 8 bp GC-rich motif is boxed.

is scored in these experiments, while embryonic lethality is contributed to by genes other than *sry* α, such as *sry* δ (Schweisguth et al., 1990; Crozatier et al., 1992). Whether the sole lack of *sry* α activity results in embryonic lethality or defects other than cellularisation is still an open question in the absence of a *sry* α null mutation.

Specific subcellular localisation of *sry* α gene transcripts

In situ hybridization indicates that, in *D. melanogaster*, the *sry* α transcripts accumulate in the cortical region between the nuclei and the plasma membrane (Schweisguth et al., 1989; this study), i.e., the precise site of accumulation of the *sry* α protein at the end of cycle 13 (Schweisguth et al., 1990, and unpublished). This apical localisation is not observed for *sry* α-*lacZ* fusion transcripts, suggesting a requirement for sequences located in the 3′ region of the gene (Schweisguth et al., 1989). Recent data from Davis and Ish Horowicz (1991) show three classes of transcript localisation in *Drosophila* blastoderm embryos, i.e, apical, basal or intermediate, all of which depend on 3′ sequences suggesting that cytoplasmic transcript localisation is an active process. The functional relevance of the localization of *sry* α mRNA is challenged by the observation that this specific localization differs between *D. melanogaster* and *D. pseudoobscura.* It is consistent with the absence of significant sequence similarity between the *D. melanogaster* and *D. pseudoobscura* transcripts in their 3′ non coding sequences that correlates with their different localisation in *D. melanogaster* embryos. Interspecific rescue of the *DfX3F* cellularisation defects suggests that, in case of *sry* α, which encodes a membrane-associated protein, transcript localisation might not play a major role in protein targeting.

Putative *cis*-regulatory elements shared by the cellularisation genes *sry* α and *nullo*

Comparison of the *sry* α upstream region between the three *Drosophila* species examined revealed an evolutionary conservation of 4 sequence motifs in the −130 to −30 promoter region. Together with data showing that a [−160 −30] fragment placed upstream of a TATA box/lacZ construct is sufficient to confer blastoderm-specific expression to a reporter gene (Ibnsouda et al., unpublished data), it suggests that these conserved motifs might serve as binding sites for positive or negative *trans*-regulators. Supporting this hypothesis, deletion of the *sry* α upstream sequences containing the promoter up to the position −118, which removes half of motif I, results in a strong decrease in *sry* α promoter activity at the blastoderm stage together with an ectopic expression in precursor cells of parts of the PNS (Schweisguth et al., 1989). That the ectopic expression is due to an alteration of motif I was confirmed using a specific deletion of this motif in an otherwise intact *sry* α promoter, (S. Ibnsouda, unpublished results). Motif I as well as motif II are also found upstream of the transcription start of *nullo*, and at the same position relative to the putative TATA box. It raises the possibility of a coordinate control of the transcription, of at least, these two cellularisation genes. A third motif, located between motifs I and II, that is shared by *sry* α and *nullo* is less well conserved in *sry* α between different species. It could perhaps correspond to a site of species-specific regulation, as documented in this study. Nevertheless, phenotypic rescue data indicate that all *trans*-acting factors required for the functional expression of the *D. pseudoobscura sry* α gene are present in *D. melanogaster* embryos.

Transcription of cellularisation genes; a complex chronological and spatial regulation

Transcription of *sry* α, like *nullo*, starts at cycle 11, possibly as part of the general transcriptional activation of the zygotic genome (Edgar and Schübiger, 1986; Simpson-Rose and Wieschaus, 1992). However, most *nullo* RNA has already disappeared at the peak of accumulation of *sry* α RNA (170 minutes AEL, Figs 5 and 6 and Simpson-Rose and Wieschaus, 1992). Such a precise chronology of transcription of the two genes may be required for proper cellularisation. The *sry* α protein is present and localised to the membrane cap overlying each nucleus when *sry* α RNA reaches its maximal level and is uniformly distributed over the entire surface of the embryo (Schweisguth et al., 1990 and Fig. 4). In contrast, *nullo* activity seems required for both initial positioning and stability of the acto-myosin network during the cellularisation process, at a stage when the level of its transcription has greatly diminished (Simpson and Wieschaus, 1990; Simpson-Rose and Wieschaus, 1992, and this report). The next step will be to compare the pattern of expression of the protein products of the two genes. During progression into cycle 14, both *sry* α and *nullo* transcripts show a dynamic pattern of accumulation, evolving from a uniform to a banded distribution, a situation reminiscent of that observed for some pair-rule segmentation genes (See Edgar et al., 1989 for ref.). Whether this dynamic pattern of expression of cellularisation genes has any functional implications or is a reflection of the underlying complex regulation remains to be assessed. Nevertheless, it suggests that transcription of genes required for cellularisation possibly involves specific combinations of non-uniformly distributed factors (or factors whose distribution becomes itself non-uniform during cycle 14). The establishment of uniform expression through multiple locally restricted activators is not unprecedented in the *Drosophila* blastoderm embryo. Expression of the *sex-lethal* (*sxl*) gene requires non-uniform inputs (from *runt* and possibly, *deadpan)* and is influenced by the maternal anterior and terminal systems (*bicoid* and *torso*) (Duffy and Gergen, 1991; Younger-Sheperd et al., 1992). A potential rationale for such an intricate regulation operating at the transcriptional and/or RNA degradation levels could be a temporal link between cellular and molecular events that are involved in cell determination at the blastoderm stage (Edgar et al., 1987). Multiple modes of temporal regulation of zygotic gene expression underlie the events of the blastoderm transition in *Drosophila*, including dependence upon the number of mitotic cycles (nucleocytoplasmic ratio) (Edgar and Schubiger, 1986), ‘developmental time’ (Yasuda et al., 1991), and transcript size (Shermoen and O’Farrell, 1991; Rothe et al., 1992). Detailed knowledge of the control of expression of cellularisation genes should help in understanding how concerted morphogenetic and molecular transitions, such as those occurring in the fruit fly blastoderm embryo are temporally regulated.

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Note added in proof

The EMBL Data Bank Library accession numbers for the *D. subobscura* and *D. pseudoobscura sry* α sequences are L 19535 and L 19536, respectively.

This paper is dedicated to the memory of Gérard de Billy.