The *serendipity alpha* gene encodes a membrane-associated protein required for the cellularization of the *Drosophila* embryo

François Schweisguth,^{1,3} Jean-Antoine Lepasant,¹ and Alain Vincent²

¹Institut Jacques Monod, Centre National de la Recherche Scientifique et Université Paris 7, 75251 Paris Cedex 05, France;

²Centre de Recherches de Biochimie et Génétique Cellulaires du Centre National de la Recherche Scientifique, 31062 Toulouse Cedex, France

The *Drosophila serendipity alpha* (*sry α*) gene is specifically transcribed at the blastoderm stage, from nuclear cycle 11 to the onset of gastrulation, in all somatic nuclei. This pattern of transcription and a zygotic cellularization defect observed in embryos homozygous for *Df(3R)X3F*, a deficiency covering the *sry* locus, suggest that *sry α* plays a role in the cellularization of the syncytial blastoderm embryo. P-element rescue experiments show that one copy of the *sry α* gene rescues the defective cellularization phenotype associated with *Df(3R)X3F*. Lack of *sry α* activity results in erratic disruptions of the cytoskeleton at the beginning of the interphase of mitotic cycle 14. Multinucleate cells form during plasma membrane invagination. Immunodetection of the *sry α* protein using anti-*sry α* polyclonal antibodies indicates that the 58-kD *sry α* protein accumulates transiently at the blastoderm stage. The *sry α* protein is associated with the invaginating plasma membrane and colocalizes with F-actin. We propose that *sry α* is involved in the localization of membrane furrows within the syncytial blastoderm.

[Key Words: *serendipity*; cellularization; F-actin; blastoderm; *Drosophila*]

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The powerful molecular genetics of *Drosophila* make its embryo an attractive model for the identification of the various cytoskeletal components involved in cellular blastoderm formation. Despite increasing knowledge of the genetic control of *Drosophila* embryogenesis (Ingam 1988), little is known about the initiators and mechanistic effectors of the synchronous cellularization of the somatic nuclei at the blastoderm stage.

Following fertilization, the zygotic nuclei divide 13 times without cytokinesis to form the syncytial blastoderm embryo (Foe and Alberts 1983). Most of the nuclei, initially centrally located, migrate stepwise to the cortical cytoplasm during cycles 7–10. The first cells to be formed are the pole cells, precursor of the germ line cells. Polar buds form during cycle 9 and pinch off at the end of mitotic cycle 10 (1 hr and 20 min after fertilization). Three additional division cycles (nuclear cycles 11–13) take place at the cortex. All 6000 somatic nuclei, which form a uniform monolayer, are then synchronously cellularized during the interphase of cycle 14 (2 hr to 2 hr and 50 min). Cellularization is preceded by an increase in plasma membrane surface with the forma-

tion of multiple villous projections (Fullilove and Jacobson 1971). The cellularization process may be separated into two phases based on the cellularization rate: an initial "slow phase" (35 min) followed by a "fast phase" (20 min). These differences in the rate of membrane invagination presumably reflect different mechanisms of furrow formation (Merrill et al. 1988), such as the unwinding of the membrane accumulated in villous projections (Fullilove and Jacobson 1971), which results in the flattening of the egg surface, or as the lateral fusion of newly synthesized endomembranes (Sanders 1975; Turner and Mahowald 1976). The invagination of the plasma membrane between adjacent nuclei is accompanied by a reorganization of the cytoskeleton (Warn et al. 1980; Warn and Magrath 1983; Warn and Warn 1986; Pesacreta et al. 1989). During the slow phase, F-actin, which accumulates in the cortex, is associated with the newly formed furrows. Although the cortical actin meshwork progressively disappears during the fast phase, the actin filaments (F-actin) are found along the furrow membrane and associated at the base of the furrow with myosin (Warn and Warn 1986), forming a ring-like structure, or contractile ring, enveloping the furrow canal. The furrow canal is defined as the widening of the intercellular space between the apposed

³Corresponding author.

membranes that surround each nucleus at the level of the cellularization front (Fullilove and Jacobson 1971).

The genetic basis of cellularization is still poorly documented. Female sterile mutations have been isolated that block development of the embryo at cellularization (Rice and Garen 1975; Zalokar et al. 1975; Schüpbach and Wieschaus 1989), indicating that some gene products involved in this process are deposited in the egg during oogenesis. For example, cytoskeletal proteins such as actin, myosin, tubulin and spectrin, are already accumulated in the unfertilized egg (Karr and Alberts 1986; Warn 1986; Pesacreta et al. 1989). In addition to the maternal contribution, at least eight loci are zygotically required for the formation of the cellular blastoderm embryo (Merrill et al. 1988; Wieschaus and Sweeton 1988). Among these, one locus defined by the deficiency *Df(3R)X3F* (99D1–2; 99E1) (K. Kongsuwan, J. Merriam, and J.A. Lengyel, pers. comm.) is required for the regular spacing of the invaginating membrane around each nucleus (Merrill et al. 1988). The deletion of this chromosomal region leads to the formation of cells that may contain multiple nuclei.

The *Df(3R)X3F* deficiency encompasses the blastoderm-specific *serendipity alpha* (*sry* α) gene (99D 4-8) (Vincent et al. 1985). *sry* α transcription is first detected by in situ hybridization at the syncytial blastoderm stage (nuclear cycle 11), peaks during cellularization, and is no longer detectable during germ-band extension (Schweisguth et al. 1989). In spite of a detailed molecular characterization of the *sry* α gene (Vincent et al. 1985), the function of the *sry* α protein has remained elusive. We show here that the cellularization defects observed in *Df(3R)X3F* homozygous embryos are due to the lack of the *sry* α gene. Data are presented on the spatio-temporal distribution and subcellular localization of the *sry* α protein, which suggest that it is involved in the localization of the actin filaments just prior to and during plasma membrane invagination.

Results

Cellularization defects of Df(3R)X3F homozygous embryos

Here, we extend the original description given by Merrill et al. (1988) of *Df(3R)X3F* homozygous embryos at the blastoderm stage. Embryos were examined with DAPI, (4,6 diamidino-2-phenylindole), which reveals nuclei, and with fluorescently labeled phalloidin, which specifically interacts with F-actin (Warn and Magrath 1983). No zygotic defects could be observed during the first 13 cleavage divisions, except for occasional irregularities in nuclei migration and distribution. These were shown to be due to a dominant maternal effect (data not shown), possibly resulting from aneuploidy, as has been documented in other cases (Garcia-Bellido et al. 1983). During interphase of cycle 14, local disruptions in the hexagonal array of F-actin are detectable (Fig. 1B). Such

defects were observed in one-quarter of the progeny (see Table 1) as expected for a fully penetrant zygotic phenotype. The cellularization process is not arrested, but the plasma membrane invaginates irregularly around clustered nuclei (Fig. 1D), enclosing multiple nuclei, from 1 to ~25, into single cells. However, the initiation and rate of plasma membrane invagination, as well as nuclear elongation and nuclei position relative to the surface, appear normal. These irregularities in the cytoskeleton array are extended over the whole surface of the egg, the greatest disruptions predominantly occurring at both poles (Fig. 1B). Despite these defects, cellularization and F-actin distribution appear normal in regions where mononucleate cells are formed. In particular, the baso-apical distribution of F-actin at the cortex and along the furrows is essentially similar to that observed in wild-type embryos (Fig. 1E,F).

F-actin is not the only cytoskeleton component whose distribution is abnormal in embryos homozygous for *Df(3R)X3F*. Spectrin, a major component of the meshwork associated with the inner face of the plasma membrane (Pesacreta et al. 1989), is distributed in a manner strictly similar to F-actin both in wild-type and *Df(3R)X3F* blastoderm-stage embryos (Fig. 2). In these surface views, F-actin and spectrin appear concentrated around normal or multinucleate cells, because of the deep cleavage furrows. Anti- α -spectrin antibodies (a kind gift from Dr. Branton) thereby provide an alternative probe to phalloidin. As phalloidin binding is lost following the methanol fixation procedure commonly used in immunofluorescence, and as the methanol fixation procedure allows processing of a larger number of embryos compared to devitellinization through hand peeling, anti- α -spectrin antibodies were subsequently used in the double fluorescence-labeling experiments. Microtubule distribution was analyzed by using an anti- α -tubulin monoclonal antibody in embryos homozygous for *Df(3R)X3F*, identified as such by the disruptions in the spectrin pattern. At mid-cellularization, both axial and cortical microtubules are partly organized following cell limits corresponding to the multinucleate cells, although cortical microtubules are still detectable between clustered nuclei (data not shown). However, although an irregular pattern of spectrin may already be observed at the time of furrow positioning (Fig. 3C), the tubulin network still appears normal at that stage (Fig. 3D). Centrosome splitting is also normal (data not shown).

Phenotypic rescue of Df(3R)X3F homozygous embryos with an additional copy of sry α

The *Df(3R)X3F* deficiency spans the genomic region 99D1–2; 99E1 (K. Kongsuwan, J. Merriam, and J.A. Lengyel, pers. comm.). It uncovers several transcription units, among which the *rp49*, the *sry*, and *Janus* (*Jan*) genes have been extensively characterized (Kongsuwan et al. 1985; Vincent et al. 1985; Yanicostas et al. 1989). These transcription units contribute to the complex em-

Figure 1. Disruption of the F-actin array in cycle 14 embryos homozygous for *Df(3R)X3F*. The distribution of F-actin (A, B, E, and F) and nuclei (C and D) is visualized on whole embryos using fluorescently labeled phalloidin and DAPI. A wild-type embryo at the interphase of cycle 14 is shown as a control (A, C, and E). Note the irregular actin arrays (B and F) and abnormal clustering of nuclei (D) in the *Df(3R)X3F* embryo at the cellular blastoderm stage; the most extensive disruptions in the F-actin array are observed at both poles. Embryos were maintained at 22°C. Embryos, ~450 μ in length, are oriented with the anterior pole to *left*. Bar, 5 μ (in E and F).

bryonic lethal phenotype exhibited by *Df(3R)X3F* homozygous embryos. However, because the zygotic cellularization phenotype can be detected very early, at the onset of zygotic transcription, it very likely results from the nonexpression of a strictly zygotic gene at the blastoderm stage. We therefore hypothesized that the cellularization phenotype of *Df(3R)X3F* homozygous embryos could result from the lack of the blastoderm-specific *sry* α gene product. This hypothesis was tested using P-element-mediated germ line transformation with constructs (Table 1) containing either the *sry* α gene alone [P[*sry* α , *ry*⁺]], or the entire *Jan* and *sry* loci [P[*Jan/sry*, *ry*⁺]]. Chromosomes carrying a P[*sry* α , *ry*⁺] or a P[*Jan/sry*, *ry*⁺] were then crossed in the *Df(3R)X3F* line and tested for their ability to suppress the cellularization phenotype, as visualized at the interphase of cycle 14 by using DAPI and phalloidin. The results, shown in Table 1, indicate that the cellularization phenotype associated with *Df(3R)X3F* is fully rescued in both lines, that is, the hexagonal actin array is no longer disrupted and all nuclei are regularly spaced and individually cellularized. A single copy of the *sry* α gene is thus sufficient to rescue the cellularization defect seen in *Df(3R)X3F* embryos.

Immunodetection of the 58-kD *sry* α protein

Polyclonal antibodies specific to the *sry* α protein were prepared to follow the expression and localization of the *sry* α protein during cellularization. Sera were raised in rabbits against *sry* α polypeptides produced in *Escherichia coli*. Immunoblotting analysis of embryonic extracts with affinity-purified anti-*sry* α antibodies specifically revealed a 58-kD protein (Fig. 4, lanes b and c). This band is specific of blastoderm-stage embryos (2–4 hr). When one precisely staged individual embryo of the *Df(3R)X3F* stock is loaded in each slot, this band is detectable in some lanes but not in others, suggesting that this protein is absent in *Df(3R)X3F* homozygous embryos (data not shown). This protein comigrates with the *in vitro* translation product of *sry* α synthetic RNA (Fig. 4, lane a). Its size, 58 kD, as measured by SDS-PAGE, is close to that predicted from the nucleic acid sequence of the gene (59 kD). We conclude that this 58-kD protein is the *sry* α protein. Following fractionation of an embryonic extract by centrifugation, the *sry* α protein is found in both cytosolic and crude membrane fractions (Fig. 4, lanes b and c).

Table 1. Rescue of the cellularization defect associated with the *Df(3R)X3F* deficiency by one copy of the *sry α* gene

P[*Jan/sry, ry⁺*]

P[*sryα, ry⁺*]

A

line	genotype	cellularization defects at cycle 14
<i>Df(3R)X3F</i>	$\frac{+}{+}, \frac{Df(3R)X3F}{TM3}$	14 / 56
S2	$\frac{P[Jan/sry, ry^+]}{P[Jan/sry, ry^+]}, \frac{Df(3R)X3F}{TM3}$	0 / 33
Sα1	$\frac{P[sry\alpha, ry^+]}{P[sry\alpha, ry^+]}, \frac{Df(3R)X3F}{TM3}$	0 / 52

B

(A) Genomic and transcriptional organization of the *Jan/sry* locus regions (Vincent et al. 1985; Yanicostas et al. 1989) included in the P elements *P*[*Jan/sry, ry⁺*] and *P*[*sry α, ry⁺*]. Intergenic regions are represented as a single line, coding sequences are boxed, and open reading frames are blackened. Transcripts are drawn as arrows, indicating the direction of transcription. Bar, 0.5 kb. (B) The genotypes of lines listed in the left column are given in the central column. The *Df(3R)X3F* stock is indicated on the first line as a control experiment. Note that the *Df(3R)X3F* chromosome also carries a *P*[*rp49, ry⁺*] element at 84F on the cytological map (Kongsuwan et al. 1985). Phenotype refers to the hexagonal F-actin distribution around each nucleus in cellular blastoderm embryos (interphase of cycle 14). The number of embryos showing cellularization defects is given with reference to the total number of embryos examined. In the *Df(3R)X3F* stock only, one-quarter of the progeny, corresponding to the *Df(3R)X3F* homozygous embryos, show cellularization defects.

Expression of the *sry α* protein during early embryogenesis

The *sry α* protein was detected by indirect immunostaining on whole-mount embryos. The absence of staining over one-quarter of the progeny of *Df(3R)X3F* heterozygous parents (Fig. 5G,H) confirms the specificity of our sera.

In wild-type embryos no signal is detectable before cycle 12 (Fig. 5A). Very weak staining is observed at nuclear cycle 12 and 13 (Fig. 5B). The signal intensity increases sharply during the interphase of mitotic cycle 14 (Fig. 5C,D). The staining is evenly distributed, along both axes, over the entire surface of the embryos, and is also detectable around yolk nuclei, whereas pole cells are not stained. The signal disappears rapidly as gastrulation proceeds (Fig. 5E,F).

The *sry α* protein is associated with the invaginating membrane

The subcellular localization of the *sry α* protein was examined by indirect immunofluorescence on cryostat sections throughout cellularization (Fig. 6). No immuno-

fluorescence signal was observed in syncytial blastoderm embryos before cycle 13. A faint signal could then be seen in the cap region (Fig. 6A). At the beginning of the interphase of mitotic cycle 14, the *sry α* protein accumulates close to the folds and microprojections of the plasma membrane (Fig. 6B). During both the slow (Fig. 6C) and fast (Fig. 6D) phases of cellularization, the *sry α* protein is associated with the invaginating plasma membrane. Fluorescent dots are detectable at the cellularization front, at the level of the furrow canals (Fig. 6C). The localization of the *sry α* protein, associated with the membrane in the cortex and along the furrow, and differentially accumulated at the cellularization front, is similar to that of F-actin (cf. Figs. 1E and 6C). A weaker signal is also detectable in the cytoplasm during cellularization (Fig. 6C–E). In contrast, the signal becomes essentially cytoplasmic at the end of cellularization (Fig. 6E) and is also detected beneath the cell bases (Fig. 6E); then it fades away during gastrulation.

Discussion

Hypotheses concerning the molecular role of the *sry α* protein stem from an analysis of the early phenotype of *Df(3R)X3F* homozygous embryos, from its rescue by a transposed *sry α* gene, and from the subcellular immunolocalization of the *sry α* protein. The phenotype associated with *Df(3R)X3F* may be considered as a null phenotype, because it corresponds to a complete lack of function. At the blastoderm stage, embryos homozygous for this deficiency display disruptions of the hexagonal

Figure 2. Spectrin as a marker of the F-actin meshwork. F-actin (A and B) and spectrin (C and D) localization are shown in surface views in wild-type (A and C) and *Df(3R)X3F* embryos (B and D). The focal plane is just above the cellularization front. Embryos were maintained at 29°C, with increased temperature resulting in a more severe phenotype; almost no single mononucleate cells are observed under these conditions. Bar, 5 μ.

Figure 3. Initial cellularization defects in an early cycle 14 *Df(3R)X3F* embryo. A double immunofluorescence-labeling experiment reveals the nuclei (B), spectrin (C), and tubulin (D) distribution. The white arrowheads indicate cytoplasmic internuclear spaces where tubulin is detectable (D) and where spectrin is absent (C). The precise developmental stage of the embryo is determined according to nuclei elongation (black arrow) and to membrane invagination (black arrowhead), which is visible in a transverse view of the same embryo, using Nomarski optics (A). Embryos were maintained at 22°C in this experiment. Bar, 15 μ (A); 5 μ (B–D).

F-actin array. This phenotype is fully rescued by a single copy of the *sry* α gene. However, the embryonic lethality associated with the deficiency was not rescued in these P-element rescue experiments, indicating that embryonic lethal genes other than the *sry* and *Jan* genes map within the boundaries of the deficiency breakpoints (99 D1–2; 99 E1). Whether the sole lack of *sry* α activity may lead to the death of the embryo is still an open question in the absence of a *sry* α null mutation. These

P-element rescue experiments also provide a functional definition of the *sry* α gene that is consistent with our previous mapping analysis of the *cis*-acting regulatory elements of the *sry* α gene (Vincent et al. 1986; Schweisguth et al. 1989).

The *sry* α protein is mostly localized along apical and furrow membranes and accumulates at the level of the furrow canal. Although membrane associated, the *sry* α protein is probably not an integral membrane protein. First, the *sry* α protein is also detected in the cytoplasm on cryostat sections. Second, it is present in both the crude membrane and cytosolic fractions, and when the crude membrane fraction is submitted to Triton X-114 phase separation (Bordier 1981), the *sry* α protein is recovered in the aqueous phase (data not shown). Finally, there are no putative membrane-spanning domains nor signal sequence in the *sry* α protein sequence (Vincent et al. 1985). The localization of the *sry* α protein to the inner face of the membrane may be mediated by protein interactions.

The accumulation of the *sry* α protein along the furrow and close to the furrow canal is reminiscent of the localization of the actin filaments (Warn and Magrath 1983), myosin (Warn et al. 1980), and particular actin-binding proteins (Miller et al. 1989). The distribution of the *sry* α protein differs from those of spectrin, which is not detectable at the furrow canal (Pesacre et al. 1989), and tubulin (Warn and Warn 1986).

The colocalization of the *sry* α protein and F-actin raises the hypothesis that *sry* α could be an actin-binding protein required for the initial positioning and/or the stability of the F-actin network. The essential role of the F-actin in the inward extension of the growing plasma membrane and the distribution of the nuclei in an even cortical monolayer was demonstrated by the injection in early cycle 14 embryos of cytochalasin B, an inhibitor of actin polymerization (Foe and Alberts 1983; Edgar et al. 1987). Noticeably, the number and size of multinucleate cells do not increase between mid-cellularization and the end of cellularization in embryos homozygous for *Df(3R)X3F* (data not shown). As disruptions do not progressively arise throughout cellu-

Figure 4. Western blot analysis of the *sry* α protein. In vitro-translation products of the synthetic *sry* α RNA in a rabbit reticulocyte lysate (lane a) and embryonic extracts of blastoderm-stage embryos (lanes b–e) were separated by SDS-PAGE. Ten micrograms of protein was loaded per slot. The 58-kD *sry* α protein is specifically detected by the affinity-purified anti-*sry* α antibodies in the cytosolic (b) and crude membrane (c) fractions. The other visible bands are due to nonspecific binding of the secondary antibody, as revealed by incubating the same extracts with the secondary antibody alone (lanes d and e). Nonspecific binding to the 130-kD doublet and the 80-kD proteins has been reported previously (Driever and Nüsslein-Volhard 1988; Piovan and Lena 1988). Molecular weight markers (in kD) are indicated at left.

Figure 5. Pattern of accumulation of the *sry* α protein in whole-mount embryos. Wild-type embryos at different stages were stained for the *sry* α protein. (A) Pole-cell formation (nuclear cycle 10; stage 3). (B) Syncytial blastoderm (nuclear cycle 12; stage 4). At this stage, the signal is too weak to be visualized with Nomarski optics. (C) Cellular blastoderm (mid-interphase cycle 14; stage 5). Note the absence of staining over pole cells. (D) Early gastrulation (stage 6). (E) Gastrulation (transition from stage 6 to 7). (F) Germ-band extension (late stage 8). Immunostaining specificity is given by comparing, in the same batch, the staining of embryos homozygous for *Df(3R)X3F* (G; stage 4) with heterozygous embryos (H; stage 4). In all cases, anterior is at left, dorsal at top. Staging is according to Campos-Ortega and Hartenstein (1985).

larization, the *sry* α protein is probably not required for the stabilization of the filaments of actin along the furrow during membrane invagination. The initial abnormal positioning of F-actin in embryos homozygous for *Df(3R)X3F* suggests that the *sry* α protein participates in cell demarcation either by properly positioning the F-actin array at the beginning of cycle 14 or by stabilizing the newly formed furrows.

A number of actin-binding proteins have now been sequenced (for review, see Tellam et al. 1989). The absence of sequence similarity between the *sry* α protein and previously identified actin-binding proteins raises the possibility that proteins specifically involved in reorganizing the cytoskeleton at unique developmental stages have yet to be identified. Indeed, ~40 actin-binding proteins have been isolated from the *Drosophila* embryo by

Figure 6. Localization of the sry α protein on cryostat sections of wild-type embryos. Immunofluorescence data are shown below a schematic representation of the cellularization process. (A) The sry α protein is first seen in the cap region as early as nuclear cycle 13. The plasma membrane already forms numerous folds and microprojections over nuclei. (B) At the beginning of the interphase of cycle 14, the sry α protein is detectable close to the plasma membrane microprojections. (C and D) The sry α protein is primarily associated with the invaginating membranes during both the slow and fast phases of cellularization. During the slow phase (C), the plasma membrane invaginates synchronously between adjacent nuclei. Although the egg surface is progressively flattened, microvilli are still abundant. During the fast phase (D), the membrane appears to be pulled down from the remaining microvilli into the invaginating cleavage furrows. The accumulation of the sry α protein at the level of the furrow canal is indicated by arrows in C. A weak signal is also detectable in the cortical cytoplasm but not in the yolk. (E) Cellularization is almost complete prior to gastrulation: Only cytoplasmic bridges remain, connecting the cytoplasm with yolk until mid-gastrulation. The sry α protein is then redistributed in the cytoplasm where it is presumably degraded.

using F-actin affinity chromatography (Miller et al. 1989). However, the *sry* α protein does not correspond to any of these most abundant proteins identified by Miller et al. (1989) in the F-actin column eluates, based on Western blot analysis with anti-*sry* α polyclonal antibodies (C. Field and B. Alberts, pers. comm.).

An abnormal distribution of F-actin has been also described in *nullo-X* embryos (Wieschaus and Sweeton 1988), which is associated with a deficiency uncovering the 6F1–2 region. Local disruptions of the F-actin array are also part of a more complex phenotype observed in embryos homozygous for deficiencies uncovering the 100B4–C2 region (Merrill et al. 1988). This suggests that the *sry* α gene and specific genes of the 6F1–2 and 100B4–C2 regions act in the same cellular process during transition between the syncytial and cellular blastoderm stages. Although phenotypically similar, these genes probably do not encode homologous proteins, because low-stringency Southern blot analysis of *D. melanogaster* genomic DNA failed to reveal sequences homologous to the *sry* α gene (data not shown).

Germinal cells bud off from the posterior pole at cycle 9, whereas somatic nuclei are cellularized at cycle 14. Pole cells and somatic blastoderm cells are formed under separate genetic control (Rice and Garen 1975; Merrill et al. 1988; Wieschaus and Sweeton 1988). Pole cells normally form in homozygous *Df(3R)X3F* embryos, thereby showing that the *sry* α protein is not required for pole-cell budding. This conclusion is supported by the temporal accumulation of the *sry* α protein, which is not detectable until mitotic cycle 12.

To our knowledge, *sry* α is the first gene to be identified that is specifically required for the cellularization of the *Drosophila* embryo. In contrast to ubiquitous cytoskeletal components, the *sry* α protein is specifically accumulated at the blastoderm stage. Immunodetection of the *sry* α protein thus parallels the accumulation of *sry* α transcripts as seen by *in situ* hybridization. *sry* α transcripts are first detectable over nuclei in syncytial blastoderm embryo at nuclear cycle 11, transiently accumulate in all somatic cells at the interphase of cycle 14, and are no longer detectable during germ-band elongation (Schweisguth et al. 1989). The temporal restriction of *sry* α protein accumulation is thus achieved through a strict blastoderm-specific promoter (Schweisguth et al. 1989) and by the high instability of both the *sry* α mRNA and protein. This study provides a remarkable example of developmental control of cell architecture by a short-lived protein, whose transient accumulation is tightly regulated at the transcriptional level.

Material and methods

Drosophila stocks

The Oregon R stock of wild-type flies was used for immunocytochemistry. The *Df(3R)X3F* stock was obtained from Dr. K. Kongsuwan. The *ry⁵⁰⁶* strain used in the transformation experiments was obtained from Dr. W. Bender. Balancers *CyO*⁺ *TM6* *Ubx/ap^{xa}* and *CyO*⁺ *TM3* *Sb Ser ry^k/ap^{xa}* were obtained from

Dr. J. Merriam and Dr. J. Deutsch and were used to construct strains S2 and S α 1.

P-element rescue

The strain S2 was directly established from transformant line CHs829 (Kongsuwan et al. 1985), which carries a *P[*Jan/sry*, *ry*⁺]* element (see Table 1) on the second chromosome. The *P[sry* α , *ry*⁺] element was obtained by subcloning a 3-kb *XhoI*–*DraI* DNA segment, which contains the *sry* α gene under its own control elements (Schweisguth et al. 1989), into the pDM 23 P-element vector (Mismar and Rubin 1987) opened by *XbaI*, filled in by Klenow enzyme, and subsequently digested by *Sall*. Handling of DNA followed standard protocols. P-element-mediated transformation was carried out according to Rubin and Spradling (1982), by use of P π 25.7wc as the helper (Karess and Rubin 1984). Appropriate crosses of transformed flies carrying a *P[sry* α , *ry*⁺] element on the X chromosome with balancer stocks and the *Df(3R)X3F* line gave rise to the strain S α 1. S α 1 is heterozygous for *Df(3R)X3F* and homozygous for *P[sry* α , *ry*⁺]. Strains S2 and S α 1 were shown by genomic Southern blot analysis to contain the reintroduced P-element, and to have retained an embryonic lethal locus on the third chromosome. Cellular blastoderm embryos were analyzed by staining F-actin and nuclei, and their phenotypes were scored under the microscope.

Staining of F-actin and DNA

Staining was performed as described by Wieschaus and Sweeton (1988) except that glutaraldehyde was replaced by 7% formaldehyde by using fluorescently labeled (TRITC or FITC) phalloidin (Molecular Probe), diluted 1 : 150. Nuclei were stained with DAPI (Sigma). The stained embryos were examined and photographed with a Zeiss microscope equipped with epifluorescence at 25 $\times$, 40 $\times$, and 100 $\times$.

Production and purification of anti-*sry* α antibodies

Two *sry* α polypeptides, SX (37 kD) and SB (14 kD), consisting of 36- and 13-kD *sry* α fragments fused to a 10-amino-acid phage peptide, were produced in *E. coli*, by use of the T7 expression system (Rosenberg et al. 1987). Neither the 23-kD amino-terminal fragment nor the full-length protein were accumulated in *E. coli*. The *XmnI*–*XmnI* and *BamHI*–*BamHI* DNA fragments, coding for *sry* α amino acids 229–530 (SX) and 265–370 (SB), respectively, were subcloned in-frame in the T7 expression vectors pET3 (Rosenberg et al. 1987). The resulting plasmids were introduced into the *E. coli* strain K38 pGP 1.2 (Tabor and Richardson 1985).

Bacteria were grown in L-broth supplemented with 50 μ g/ml ampicillin, 70 μ g/ml kanamycin, and 0.5 mM IPTG at 30°C to an OD₆₀₀ of ~0.8. Synthesis of T7 polymerase was induced after bacteria had been washed and resuspended in L-broth prewarmed at 42°C. Rifampicin (150 μ g/ml) was added 20 min later. After a 45-min-long heat shock, bacteria were allowed to grow for 90 min at 30°C. A relatively weak polypeptide synthesis was obtained upon induction. After centrifugation and resuspension in 50 mM Tris (pH 7.5), 150 mM NaCl, and 1 mM PMSF, bacteria from a 10-liter induced culture were sonicated (3 $\times$ 30 sec, with 50% duty cycle, output 6, Vibra cell, Sonics and Materials) at 4°C. The lysate was centrifuged twice (rotor Sorvall HB4, 4°C) at 4000 rpm and 9000 rpm. A large fraction of enriched polypeptides was recovered in the second pellet and was subjected further to preparative SDS-PAGE. The gel slice

containing the SB polypeptide was visualized by incubating the gel in 0.25 M KCl at 4°C, excised, and electroeluted. After extensive dialysis against PBS, the purified SB antigen was mixed with one volume of complete Freund's adjuvant (incomplete for booster injections). Alternatively, both SB and SX polypeptides were electroblotted onto a nitrocellulose filter. After visualization of proteins with Ponceau Red, nitrocellulose strips were excised and sonicated in PBS until a fine powder was recovered (5 × 30 sec, pulsed at 4°C, with 50% duty cycle, output 6, Vibra cell, Sonics and Materials). Four rabbits were injected at multiple subcutaneous sites and boosted every 3 weeks (repeated five times) until a high titer was reached.

A β -galactosidase-sry α fusion protein, consisting of a carboxy-terminal 34-kD fragment (amino acids 247–530) fused to the *E. coli* β -galactosidase, was used for the purification of the antibody. The corresponding *Bgl*II–*Hind*III sry α DNA fragment was inserted in-frame in the pUR 290 expression vector (Rüther and Müller-Hill 1983), opened by *Bam*HI and *Hind*III. Synthesis of the fusion protein under control of the *lac* promoter was induced for 2 hr with 1 mM IPTG at room temperature to minimize aggregation of the induced protein. Bacteria were sonicated and centrifuged as above. The fusion protein was affinity-purified from the supernatant on an APTG agarose column (Sigma) and used to prepare an affinity column (Affi-Gel 10, Bio-Rad), according to the manufacturer's protocol. Specific anti-sry α antibodies from the IgG fraction of the DEAE-purified sera were affinity-purified, as described by Carroll and Scott (1985). Antibodies raised against bacterial antigens were absorbed on an affinity column prepared with *E. coli* extracts. The specificity of the purified anti-sry α antibodies was determined first on immunoblots of bacterial extracts. Each serum raised against one antigen specifically recognized both SX and SB polypeptides. All sera gave qualitatively similar results on embryos. Western blot analysis was carried out with immunopurified antibodies from a serum raised against the electroeluted SB antigen, and immunostaining and immunofluorescence data were obtained by using antibodies purified from a serum raised against the SX protein bound to a nitrocellulose support.

Western blot

Embryonic extracts were prepared according to Bastiani et al. (1987). The proteins present in the postmitochondrial supernatant partition in a 2 : 3 ratio after centrifugation at 100,000g between the pellet (12 mg of total protein) and the supernatant (18 mg of total protein) that constitute the crude membrane and the cytosolic fractions, respectively. In vitro transcription and translation were performed according to the manufacturers' indications (Stratagene and Promega, respectively). Proteins separated on 10% SDS-PAGE were electroblotted onto nitrocellulose filters. Incubation with the first antibody, anti-sry α at a 1 : 500 dilution, was overnight at room temperature. Subsequent steps were carried out as indicated by the manufacturer of the blotting detection kit (Amersham), except the secondary antibody was diluted 1 : 20,000 instead of 1 : 500.

Whole-mount staining

Embryos were incubated overnight at room temperature with anti-sry α antibodies, diluted 1 : 200. Whole-mount preparations of embryos were stained by using the Vectastain ABC-peroxidase "Elite" system (Vector Laboratories), according to Macdonald and Struhl (1986). Stained embryos were mounted in Epon 812. Photographs were taken with a Zeiss microscope equipped with Nomarski optics.

Immunofluorescence

Embryos of the appropriate stage were dechorionated with commercial bleach, fixed at the interphase of heptane and 4% paraformaldehyde in 10 mM KPO₄ (pH 6.8), 45 mM KCl, 15 mM NaCl, and 15 mM MgCl₂, and devitellinized with vigorous shaking in methanol at room temperature. After progressive rehydration in PBS, embryos were embedded in Tissue-Tek OCT (Miles). Frozen sections of ~5 μ m were air-dried for 1 hr, rehydrated for 10 min in PBS, incubated first in PBS (pH 7.5), 1% BSA (grade V), and 0.1% Tween 20 and then overnight with affinity-purified antibodies at a 1 : 100 dilution in the same buffer containing 1% goat serum. The TRITC-labeled goat anti-rabbit second antibody (Cappel) was incubated 2 hr. All steps were carried out at room temperature. Double immunofluorescence labeling was carried out on whole embryos prepared according to Karr and Alberts (1986), and incubated overnight at 4°C using rabbit anti-spectrin polyclonal antibodies (serum 905: Pesacreta et al. 1989) and monoclonal anti- α -tubulin (Amersham), both diluted 1 : 1000. Secondary antibodies, FITC-labeled goat anti-mouse antibodies (KPL) and TRITC-labeled goat anti-rabbit antibodies (Cappel), were applied for 1 hr at room temperature. Sections and whole-mount embryos were mounted in 1 : 1 PBS/glycerol and photographed with a Zeiss photomicroscope equipped with epifluorescence at 40×, 63×, and 100×, using Kodak Tri-pan film (400 ASA).

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F Schweisguth, J A Lepesant and A Vincent

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