

β 3 tubulin expression characterizes the differentiating mesodermal germ layer during *Drosophila* embryogenesis

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Summary

During embryogenesis, the β 3 tubulin gene of *Drosophila* is transcribed predominantly in the mesoderm. We have raised antibodies specific to the C-terminal domain of the β 3 tubulin and analysed by immunostaining the distribution of this tubulin isotype during *Drosophila* embryogenesis. The protein is first detectable in the cephalic mesoderm at maximal germband extension. Shortly afterwards, β 3 tubulin is expressed in single cells at identical positions of the thoracic and abdominal segments. We suggest that these cells represent muscle pioneer cells of *Drosophila*. During later embryonic development the somatic muscu-

lature, visceral musculature, dorsal vessel and macrophages contain β 3 tubulin. In dorsalizing mutants dorsal, snail and twist, which do not form a ventral furrow during gastrulation, β 3 expression is greatly reduced but not completely abolished. Our analysis shows that β 3 tubulin immunostaining characterizes the differentiation of mesodermal derivatives during embryogenesis.

Key words: β 3 tubulin, mesoderm, muscle development, *Drosophila*.

Introduction

Tubulins are the main structural components of the meiotic and mitotic spindle, neural processes, cilia and flagella, and form part of the cytoskeleton. In *Drosophila*, as in all higher organisms, α and β tubulins are encoded by gene families (for review see Cleveland & Sullivan, 1985). The β tubulin genes show a clear developmentally regulated mode of expression (Bialojan *et al.* 1984; Natzle & McCarthy, 1984). During early embryogenesis, the β 1 tubulin mRNA is maternal in origin and homogeneously distributed, whereas in later stages it is found mainly in the developing nervous system, presumably due to embryonic expression (Gasch *et al.* 1988). In contrast, *in situ* localization of the β 3 tubulin mRNA revealed restriction to the mesoderm (Gasch *et al.* 1988). Expression of the β 3 tubulin gene in cephalic, somatic and visceral mesoderm, indicates a specificity of this tubulin isotype for mesodermal derivatives such as the musculature. The high tubulin mRNA content in mesodermal derivatives is in agreement with the

cytological observation of numerous microtubules in differentiating muscles during pupal stages of insect development (Crossley, 1978).

In contrast to the comparatively well-characterized processes controlling pattern formation and differentiation of the ectodermal germ layer of *Drosophila*, little is known about the corresponding events leading to the formation of internal structures such as the muscle system (for review see Lawrence, 1985; Martinez-Arias & Lawrence, 1985; Akam, 1987; Technau, 1987). The availability of mutants greatly facilitates studies of differentiation processes. Mutants demonstrating a dorsalizing effect, and thus lacking ventral furrow formation during gastrulation, have been described though it is not clear if all muscle cells are eliminated in these mutants (Anderson & Nüsslein-Volhard, 1986).

We report here the use of an antibody specific for β 3 tubulin to follow the differentiation of mesodermal cells in both wild-type embryos and in dorsalizing mutants. In the wild-type embryo, β 3 tubulin expression is initiated in single cells of the mesoderm in

every segment at maximal germband extension. During later embryogenesis, the $\beta 3$ tubulin is characteristic of mesodermal derivatives such as the somatic and visceral musculature, the dorsal vessel and macrophages. In agreement with the proposed specificity for mesodermal derivatives, $\beta 3$ tubulin expression is greatly reduced in the dorsalizing mutants dorsal, twist and snail.

Materials and methods

Generation and purification of isotype-specific antibodies

The 15 carboxyterminal amino acids were synthesized on an Applied Biosystems peptide synthesizer 430A using the Fmoc-chemistry (Meierhofer *et al.* 1979) and purified by HPLC using gradients of acetonitrile/water containing 0.1% trifluoroacetic acid.

Peptides were covalently linked to Keyhole Limpet Hemocyanin (KLH, Calbiochem.) using 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide as described by Tamura *et al.* (1983), using method 2. Rabbits were injected subcutaneously with these conjugates emulsified in complete Freund's Adjuvant. After two boosts, serum was collected and affinity purified by passing over a peptide-Affigel 15 column as outlined by Carroll & Scott (1985). Collected fractions were tested by ELISA and Western blotting (see below).

Test for specificity of the antibodies

Enzyme-linked-Immunoabsorbent Assay (ELISA) was carried out essentially according to Engvall *et al.* (1971). Preimmunsera were used as negative controls.

For Western blotting, protein extracts prepared from staged *Drosophila* embryos were fractionated on a 11% SDS-polyacrylamide gel and were transferred to nitrocellulose (Towbin *et al.* 1979). Western blots were first incubated with 0.1% Tween 20 in PBS (30 min) and then incubated in the same solution containing $\beta 3$ antibody overnight at 4°C. Blots were washed (10 min room temperature) three times in the Tween-PBS solution and then incubated with phosphatase-conjugated anti-rabbit antibody (DIAVOVA, 1:2000). After repeated washes in PBS-Tween the colour was developed with bromo-chloro-indolyl-phosphate (Boehringer) and nitroblue tetrazolium (Sigma) in substrate buffer (100 mM-NaCl, 10 mM-Tris-HCl, pH 8.8, 5 mM-MgCl₂).

Antibody staining of embryos

Embryos were dechorionated, permeabilized and fixed essentially as described by Freeman *et al.* (1986). After washing and blocking in BBT (0.15% crystalline BSA, 10 mM-Tris-HCl, pH 7.5, 50 mM-NaCl, 40 mM-MgCl₂, 5 mM-CaCl₂, 20 mM-glucose, 50 mM-sucrose, 0.1% Tween 20) they were treated with anti- $\beta 3$ antibody overnight (1:50). The bound antibody was detected with a biotinylated secondary antibody and stained with Vectastain ABC-kit (VectorLabs) using diaminobenzidine following the manufacturers' instructions except that 30 μ l of 1% nickel

sulphate were added per 600 μ l staining mix (essentially according to Lawrence *et al.* 1987).

Individual embryos were mounted on slides in Epon and viewed under a Leitz microscope. All photographs (Kodak, Ektachrome 50) were taken under Nomarski optics.

Results

Generation of specific antibodies against $\beta 3$ tubulin

The amino acid sequences of *Drosophila* β tubulins have been deduced from the DNA sequence (Michiels *et al.* 1987; Rudolph *et al.* 1987) revealing 90–95% homologies between the individual β tubulins. The $\beta 3$ tubulin isotype differs markedly from other *Drosophila* β tubulins by an insertion of six amino acids in the N-terminal region (Rudolph *et al.* 1987; Leiss & Renkawitz-Pohl, unpublished observation). However, the region most generally divergent between the β tubulins is the COOH-terminus (Table 1). Furthermore, it is known from pig β tubulins that the C-terminal domain is accessible to antibodies in the intact microtubules (Breitling & Little, 1986). Therefore, we decided to raise antibodies against synthetic peptides corresponding to the 15 extreme C-terminal amino acids of the $\beta 3$ tubulin isotype (Table 1). The antibody was affinity purified (for details see Materials and methods) and tested for specificity. ELISA (data not shown) as well as Western blot experiments clearly showed specificity of the antibody for the $\beta 3$ tubulin. Total protein extracts of early embryos (Fig. 1A), which contain maternally derived $\beta 1$ tubulin and no $\beta 3$ tubulin mRNA (Gasch *et al.* 1988), do not react with the antibody. In contrast, proteins from 6–10 h old embryos or pupae, which abundantly express $\beta 3$ tubulin (Gasch *et al.* 1988), strongly react with the antibody (Fig. 1B,C). The reacting protein is $55 \times 10^3 M_r$ in size and comigrates with α tubulin (Fig. 1D). These data and the comparison of mRNA and protein *in situ* localization (see below) show that the antibody is specific for the $\beta 3$ tubulin isotype.

At the end of gastrulation, $\beta 3$ tubulin appears in single large mesodermal cells

Our previous *in situ* hybridization studies, the resolution of which was limited by the autoradiographic techniques used, revealed that the expression of the $\beta 3$ tubulin gene during embryogenesis is limited to

Table 1. COOH-terminal β tubulin sequences synthesized *in vitro* to raise isotype specific antibodies

$\beta 1$ tubulin:	D	A	E	F	E	E	E	Q	E	A	E	V	D	E	N
$\beta 2$ tubulin:	E	Q	E	F	D	E	D	E	E	Q	Q	Q	D	E	
$\beta 3$ tubulin:	E	F	D	P	E	V	N	Q	E	E	V	E	G	D	I

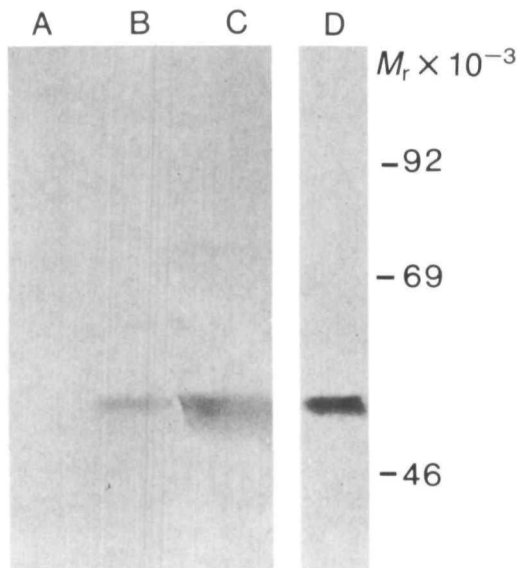


Fig. 1. Anti- $\beta 3$ antibody specifically recognizes the $\beta 3$ tubulin isotype. Western blot of protein extracts from staged embryos reacted with the purified $\beta 3$ -sera (lane A, B and C) and α tubulin antibody as a control (lane D). Lane A: embryos from 0–4 h; lane B: embryos from 6–10 h; lane C: pupae, 2 days old; lane D: pupae, 2 days old.

mesodermal cells (Gasch *et al.* 1988). The comparatively high resolution achieved by whole-mount staining of embryos with the $\beta 3$ specific antibody enabled us to investigate in more detail the precise pattern and progression of $\beta 3$ tubulin expression in early mesodermal cells and their derivatives.

We first detect the $\beta 3$ tubulin at stage 10 in the mesoderm anterior to the cephalic furrow (Fig. 2A) while the other cells of the mesodermal layer are still free of label. Soon after, the antigen is detectable in additional mesodermal cells, being most prominent in the gnathal segments (i.e. mandibular, maxillary and labial bud) as well as in the posterior region of the mesoderm (Fig. 2B). A lateral view of a slightly older embryo clearly shows staining in evenly spaced single cells (Fig. 2C). Although the $\beta 3$ tubulin seems to be initially expressed synchronously in single large cells of every segment, there are subsequent distinct differences. The segments T1 and A8/A9 soon show several stained cells in comparison to the other segments. The $\beta 3$ -positive cells differ from other yet unlabelled mesodermal cells by their large size (Fig. 2C) and have characteristic extended processes, as can be seen by the $\beta 3$ tubulin distribution. The cellular distribution of the $\beta 3$ tubulin reveals a concentration at the cell periphery. We suggest that these cells, which become more abundant later, might represent the *Drosophila* homologue of the muscle pioneer cells as described for the grasshopper (Ho *et al.* 1983; Ball *et al.* 1985).

At stage 12, the splanchnopleura, giving rise to the visceral musculature, has separated from the somatopleura, the progenitor of the somatic musculature and some other mesodermal tissues (Campos-Ortega & Hartenstein, 1985). As is shown in Fig. 2D, this separation process can be followed by the $\beta 3$ tubulin distribution rather well. The outer layer, the somatic mesoderm, is arranged in a segmental order (Fig. 2D). The number of $\beta 3$ stained cells has increased considerably from stage 10, but the cells have not yet elongated. In this embryo, the differentiation state of the splanchnopleura can also be followed by the distribution of $\beta 3$ tubulin, which again concentrates close to the periphery of the cells. In contrast to the somatopleura, the splanchnopleura is visible as a continuous epithelium of palisade-like cells (Fig. 2D).

During proceeding germband shortening (stage 12) further differentiation of myogenic cells occurs in the somatopleura (data not shown, for later stages see below). Three major groups of myoblasts are distinguishable, which will give rise to ventral, pleural and dorsal groups of muscles.

All mesodermal derivatives express $\beta 3$ tubulin

In later embryonic stages, the $\beta 3$ antibody allows one to follow the differentiation of the mesodermal derivatives rather well (Figs 3–5). By stage 14, the midgut is continuous along its entire length and flanked by the visceral mesoderm. In both the visceral and somatic mesoderm, strong expression of the $\beta 3$ tubulin gene is evident by the RNA and the protein distribution (Fig. 4A and B). This comparison of RNA and protein distribution again confirms the specificity of our antibody. The separation between visceral and somatic muscle primordia is clearly visible (Fig. 3A and 4B). Myoblasts of every segment are stained by the antibody. Differentiation of the embryonic muscles by fusion of the myoblasts to syncytial cells starts at stage 13 and continues until stage 15. Differentiation of these muscle fibres gives rise to the final muscle pattern clearly recognizable at stage 16 (Fig. 3B). In a lateral view, the ventral, pleural and dorsal group of muscles are distinguishable as three different groups, the ventral muscles having not yet obtained their final fibrillar organization by this stage (compare Fig. 3C to 3B). The complete somatic musculature of *Drosophila* is characterized by $\beta 3$ tubulin expression (stage 16 and 17, see Fig. 3B and 3C). It is clearly visible that the intersegmental apodemes do not contain detectable levels of $\beta 3$ tubulin. Furthermore the cephalic musculature contains $\beta 3$ tubulin, the parallel arrangement of the pharyngeal muscles being clearly visible (Fig. 3D).

Other cell types also derive from the mesoderm,

including macrophages, which occur predominantly in regions where cell death occurs during embryogenesis (Campos-Ortega & Hartenstein, 1985). In a ventral view of a stage-15 embryo, $\beta 3$ tubulin expression can be seen in distinct large cells, which we suggest to be macrophages (Fig. 5A). Again, the antigen concentrates close to the periphery of the cells and in the fibrillar extensions. For another mesodermal derivative, the fat body, staining could not be clearly visualized due to the strong staining of the muscles. At dorsal closure (stage 14), two rows of cardioblasts migrate to join midsagittally to form the dorsal vessel. These cardioblasts are also of mesodermal origin, and most express $\beta 3$ tubulin as demonstrated in stage 16 when the dorsal vessel is formed (Fig. 5B). The pericardioblasts, which originate from the amnioserosa, are free of label. Four cardioblasts in register with the segments are stained and separated by two cardioblasts free of detectable $\beta 3$ tubulin. In these rather large cells, the $\beta 3$ tubulin is again concentrated at the periphery as observed for the presumptive muscle pioneer cells and the macrophages.

In the head region, we found two additional cell types to be labelled, neither of which are mesodermal in origin. These are a small number of cells of the clypeolabrum, presumably ectodermal in origin, and two prominent axons leading from anterior sense organs to the brain. As described below these structures are much clearer in mutants, lacking the majority of mesodermal cells, than they are in the wild type. The majority of the embryonic nervous system does not contain $\beta 3$ tubulin.

In order to determine if the specificity of $\beta 3$ tubulin for mesodermal derivatives is conserved between different *Drosophila* species, we analysed the $\beta 3$ tubulin distribution in the distantly related species *D. hydei*. As in *D. melanogaster*, the antibody stains the mesodermal derivatives, for example the visceral, somatic and pharyngeal muscles (Fig. 5C). This not only indicates a comparable distribution between the two species, but also demonstrates clearly that the C-terminal domain of the $\beta 3$ tubulin is conserved. Conservation has also been shown for the testis-specific $\beta 2$ tubulins of *D. melanogaster* and *D. hydei*, here the amino acid sequence is completely identical (Michiels *et al.* 1987).

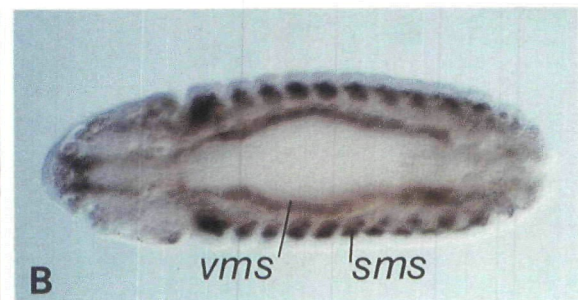
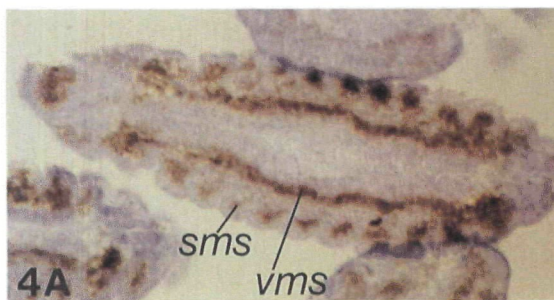
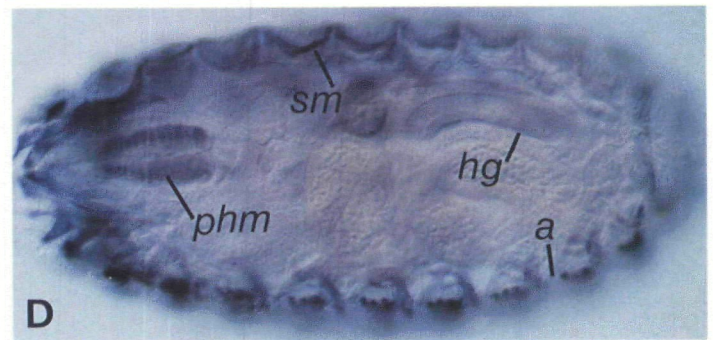
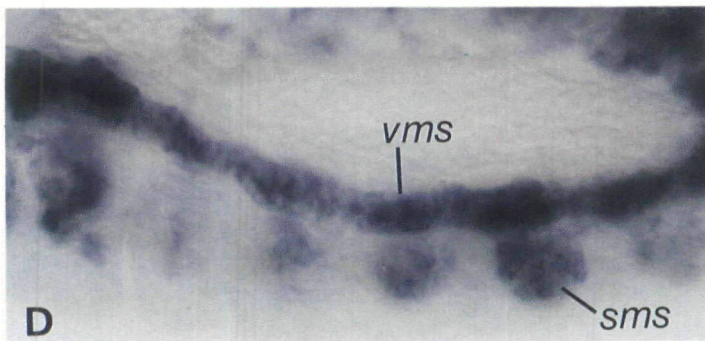
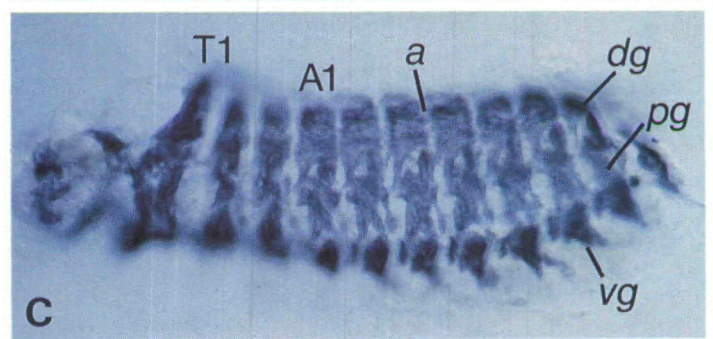
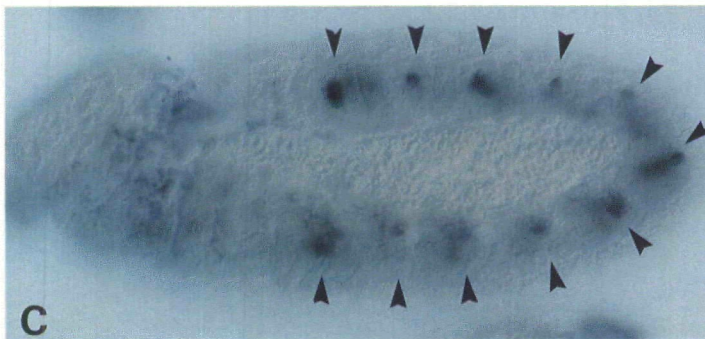
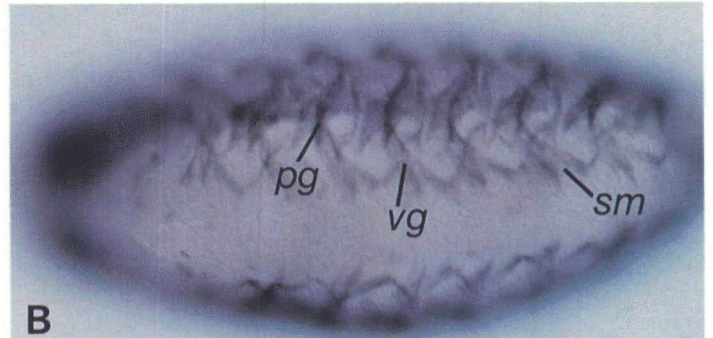
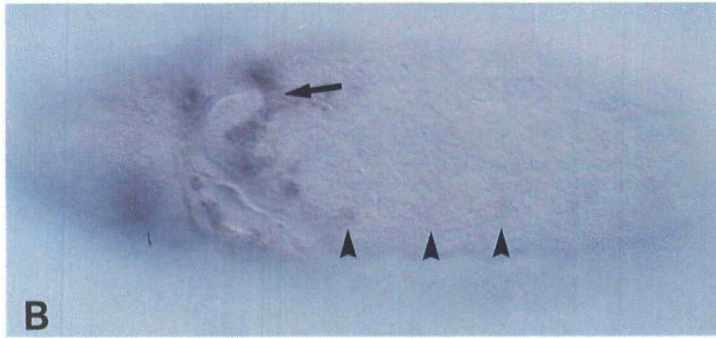
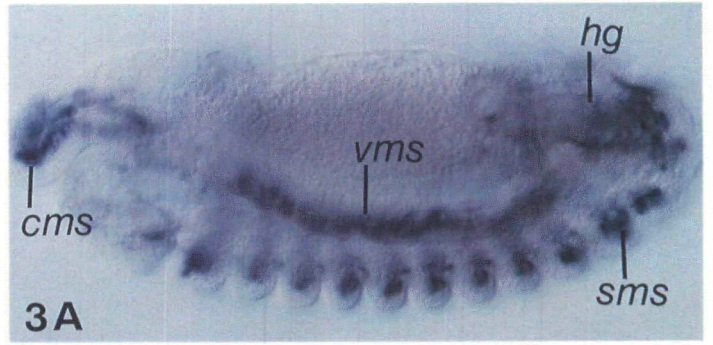
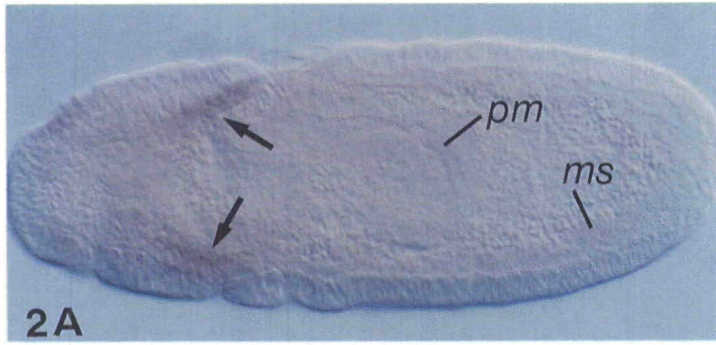
The $\beta 3$ tubulin is present in abundance in mesodermal cells, and is characteristic of the differentiation of mesodermal derivatives during embryogenesis in *D. melanogaster* as well as in *D. hydei*. This may be indicative of a functional role of this tubulin isotype during myoblast migration and fusion.

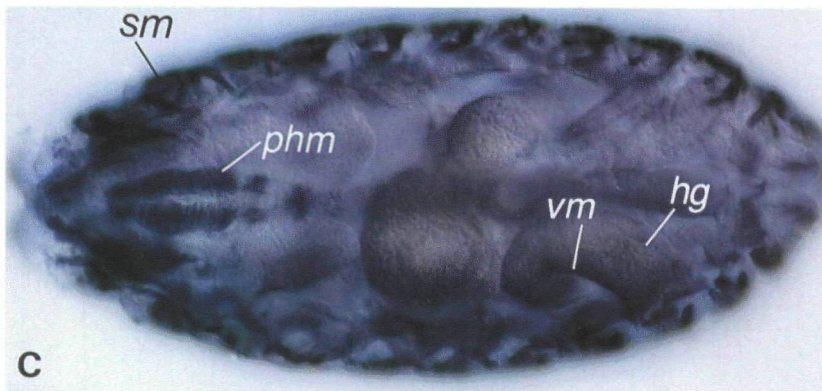
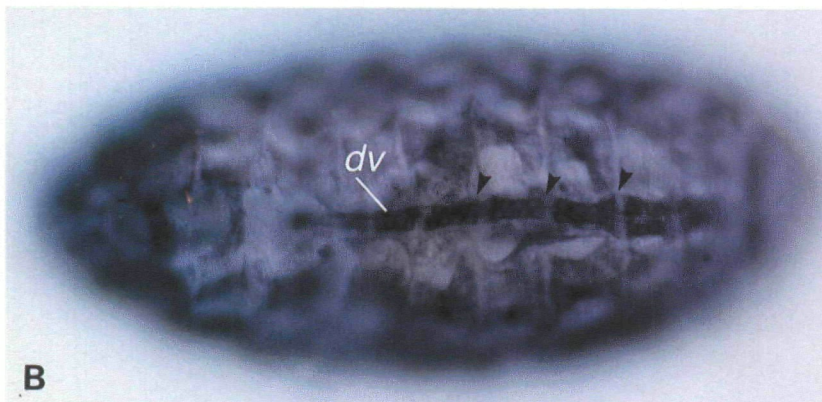
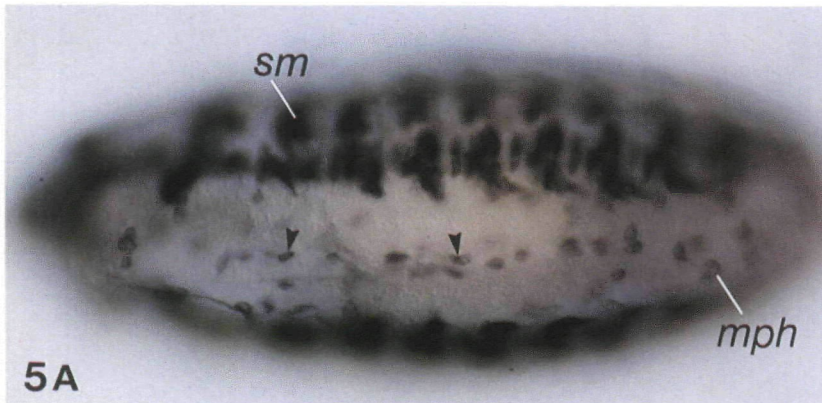
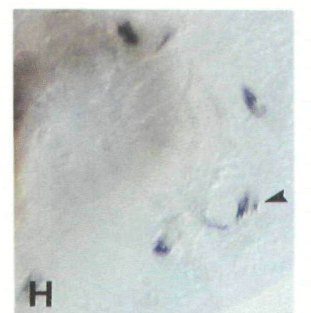
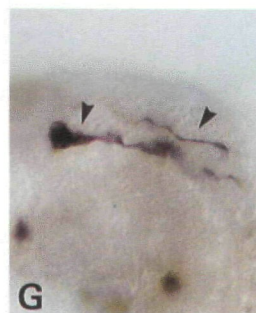
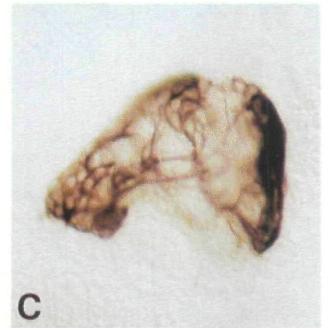
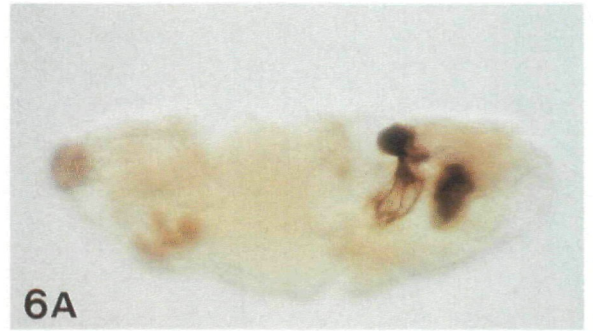
Fig. 2. Localization of $\beta 3$ tubulin in mesodermal cells during stage 10 to 12 of embryogenesis. Whole-mount staining of embryos with anti- $\beta 3$ antibody followed by peroxidase labelling and viewed under Nomarski optics. Orientation of the embryos is anterior to the left. Staging follows Campos-Ortega & Hartenstein (1985).

(A) Horizontal view of an early stage-10 embryo (germband extension) the earliest phase at which $\beta 3$ tubulin is detectable. The antigen is present in the mesoderm anterior to the cephalic furrow (see arrows) but not in the other mesodermal cells (*ms*). (B) Dorsal view of an early stage-11 embryo (appearance of intersegmental furrows in the epidermis, fully extended germ band). Staining is prominent in the mesodermal cells of gnathal segments and in the most posterior part of the mesoderm (arrow). First signs of antigen become apparent in evenly spaced large single cells (small arrows). (C) Midsagittal view of a stage-11 embryo as in B. The presumptive muscle pioneers are clearly visible in every metameric unit (arrows). (D) Higher magnification of a stage-12 embryo. Clearly visible is the separation of the two mesodermal layers visceral- and somatic mesoderm. Focus is on the cells of the visceral mesoderm which attach the midgut as continuous sheet. Abbreviations: *ms*, mesoderm; *pm*, posterior midgut; *sms*, somatic mesoderm; *vms*, visceral mesoderm.

Fig. 3. Localization of $\beta 3$ tubulin in mesodermal derivatives from stage 14 to stage 17 of embryogenesis. (A) Embryo of stage 14, midsagittal plan of focus. The staining pattern in the somatic mesoderm (*sms*) reflects segmental arrangement. Visceral mesoderm (*vms*) and cephalic mesoderm (*cms*) are strongly labelled too. (B) Ventral view of a stage-16 embryo at the point when formation of the musculature is complete. Only the ventral (*vg*) and pleural groups (*pg*) of musculature are visible in this focus. (C) Thoracic and abdominal segment from a late stage 15 embryo. Clearly three groups of muscles are distinguishable: dorsal group (*dg*), pleural group (*pg*), ventral group (*vg*). (D) Horizontal view of a stage-16 embryo. Plane of focus just below the pharyngeal musculature (*phm*) which is strongly stained. Abbreviations: *a*, muscle attachments; *cms*, cephalic mesoderm; *dg*, dorsal muscle group; *hg*, hindgut; *phm*, pharyngeal musculature; *pg*, pleural muscle group; *sm*, somatic musculature; *sms*, somatic mesoderm; *vms*, visceral mesoderm; *vg*, ventral muscle group.

Fig. 4. Comparison of $\beta 3$ tubulin messenger RNA to the isotype distribution at stage 14. (A) Horizontal section through a stage-14 embryo (head involution). $\beta 3$ mRNA is detected by *in situ* hybridization in the visceral (*vms*) and somatic mesoderm (*sms*). *In situ* hybridizations were performed as described previously (Gasch *et al.* 1988). (B) Whole-mount embryo from same stage as in A stained with the $\beta 3$ antibody. The horizontal view shows that the protein and RNA distributions are identical. Abbreviations: *sms*, somatic mesoderm; *vms*, visceral mesoderm.





Distribution of $\beta 3$ tubulin in the dorsalizing mutants, dorsal, snail and twist

The dorsal-ventral pattern of the *Drosophila* embryo is determined by both maternally and zygotically expressed genes (Anderson, 1987; Anderson & Nüsslein-Volhard, 1986). Mutations of any one of these genes results in failure to form a ventral furrow (mesodermal invagination) during gastrulation. All cells continue to develop but the majority obtain epidermal fate (Campos-Ortega, 1983). We addressed the question of whether these mutations completely eliminate cells of the mesodermal pathway or if some mesodermal cells are present despite the inability to form the ventral furrow. Representative of the dorsalizing maternal effect mutants is the mutant dorsal 1 (for review see Anderson & Nüsslein-Volhard, 1986). From molecular studies, it is known that the dorsal (*dl*) gene product is homologous to the *c rel* protein, a vertebrate oncogene known to have a nuclear localization (Steward, 1987). Homozygous *dl*¹/*dl*¹ female flies lay eggs differentiating to an essentially hollow epidermal tube. Besides the posterior midgut and a small group of nerve cells in the head, no organs are built (Campos-Ortega, 1983). In

Fig. 5. $\beta 3$ tubulin in the dorsal vessel and presumptive macrophages during late embryogenesis. (A) Ventral view of a stage-15 embryo when muscle formation proceeds. The somatic musculature (*sm*) is not yet completely differentiated. Macrophages (*mph*) becoming apparent in the paraneural space contain large amounts of $\beta 3$ tubulin. Antigen concentrates to the periphery (arrows). (B) Dorsal view of a stage-16 embryo. Focus is on the dorsal vessel (*dv*). Four large cardioblasts in register with the segments are stained. Notice the absence of $\beta 3$ tubulin in two cardioblasts at the segment boundaries (arrows). (C) Whole-mount staining of a late *Drosophila hydei* embryo with the $\beta 3$ antibody. Comparison with the staining pattern in *D. melanogaster* (compare Fig. 3D) reveals the same specificity of the $\beta 3$ tubulin isotype for mesodermal cells. Abbreviations: *dv*, dorsal vessel; *hg*, hindgut; *mph*, macrophages; *phm*, pharyngeal musculature; *sm*, somatic musculature; *vm*, visceral musculature.

Fig. 6. Localization of $\beta 3$ tubulin in dorsalizing mutants. (A–C) Embryos laid by dorsal (*dl*¹/*dl*¹) females (Nüsslein-Volhard, 1979) (B and C are higher magnifications of the stained structures). Antigen is visible in anterior and posterior regions, but not in a segmental spacing. (D,E) Embryos homozygous for Df(2R)S60, a small deletion encompassing the twist gene (Simpson, 1983; Thisse *et al.* 1987a). $\beta 3$ tubulin is present in the head region and in spindle-shaped cells arranged in several cell clusters (arrow). (F–H) Embryos homozygous for *snail*^{11G}, a strong snail allele (Nüsslein-Volhard *et al.* 1984). The staining pattern is very similar to the twist mutant (D,E), stained cell clusters are visible in a segment-like arrangement. Arrows in G point to the two axons present in wild-type embryos as well (see text).

addition to maternal effect genes, two zygotically expressed genes, twist (*twi*) and snail (*sna*), show a similar dorsalizing effect (Simpson, 1983; Nüsslein-Volhard *et al.* 1984). The ventral cells fail to invaginate in embryos homozygous for the mutations twist and snail (Simpson, 1983; Nüsslein-Volhard *et al.* 1984).

The analysis of the $\beta 3$ tubulin distribution in the mutants dorsal, twist and snail, revealed the common feature of greatly reduced, but surprisingly not completely abolished, $\beta 3$ tubulin expression. As is shown for snail as an example (Fig. 6F and 6G) all three mutants demonstrate two stained axonic cells in the head region, as was observed in the wildtype, and an additional staining of the posterior spiracles. A specific feature of the dorsal 1 mutants are some fibrillar aggregates visible at the anterior and posterior poles (Fig. 6A). The origin of these structures is not clear. Further $\beta 3$ expressing cells are missing, a feature that distinguishes dorsal clearly from the zygotic-effect mutant genes twist and snail. Embryos homozygous for these latter mutations reveal $\beta 3$ tubulin-stained structures organized in a segmental fashion (Fig. 6D and 6F). Higher magnifications show patches of spindle-shaped cells in a parallel arrangement (Fig. 6E for twist, Fig. 6H for snail). The origin of these cells remains to be clarified. From the analysis of the $\beta 3$ tubulin distribution in dorsal, snail and twist mutants, we conclude that, with minor exceptions in neural cells, the $\beta 3$ tubulin expression is specific for the mesodermal germ layer.

Discussion

We have used antibodies against the C-terminal domain of the $\beta 3$ tubulin to follow the distribution of this antigen in the mesoderm and its derivatives.

The main results of our analysis concern the following topics: (1) existence of presumptive muscle pioneer cells in *Drosophila*, (2) the distribution of $\beta 3$ tubulin in differentiating mesodermal tissues.

The $\beta 3$ tubulin expression starts at stage 10 in the cephalic mesoderm, other mesodermal cells being free of staining. This indicates that the process of mesoderm formation during gastrulation is independent of this tubulin species. By the time segmentation is clearly visible, single cells in every metameric unit express $\beta 3$ tubulin. Soon thereafter a group of mesodermal cells become stained. We suggest that these cells are homologous to the muscle pioneer cells described in the grasshopper embryo (Ho *et al.* 1983; Ball *et al.* 1985). In the grasshopper, the muscle pioneer cells erect a scaffold for developing muscles and for motorneurone growth cones. These muscle pioneer cells are distinguishable from their neighbouring mesodermal cells by their large size, a feature

that is shared by the $\beta 3$ -positive cells. So far there is no information about the way in which these $\beta 3$ -positive cells, presumably muscle pioneers, are specified from the other mesodermal cells.

At early stage 12, the splanchnopleura, which give rise to the visceral musculature, is clearly distinguishable from the cells of the somatopleura, which give rise to the somatic musculature. At this stage the cells of the somatic mesoderm start myoblast differentiation, which is characterized by growth, fusion and migration, and which can be followed by $\beta 3$ tubulin staining.

Another antigen has recently been localized in the mesoderm, the invertebrate integrin homologue PS2. PS2 is present in the somatic and visceral mesoderm, and is subsequently found to be concentrated in muscle attachment sites, while the muscles themselves are free of PS2 (Bogaert *et al.* 1987). In contrast, $\beta 3$ tubulin is also expressed in fully differentiated visceral and somatic musculature, as well as cardioblasts and macrophages. The intracellular distribution of $\beta 3$ tubulin at the periphery and in cellular extensions may suggest a functional role during cell migration and cell fusion.

In *Drosophila*, analysis of mutants provides a powerful tool to examine the determination of embryonic cells. Several mutants have been isolated that fail to form a ventral furrow during gastrulation and thus show no invagination of mesodermal cells (for review, see Anderson & Nüsslein-Volhard, 1986; Anderson, 1987). The genes concerned fall in two groups, maternal effect genes (e.g. dorsal) and genes transcribed during embryogenesis (twist and snail).

The snail and twist genes have been cloned, and the molecular analysis suggests a nuclear localization of the gene products in mesodermal cells (Boulay *et al.* 1987; Thisse *et al.* 1987b, 1988). The twist and snail gene products are present earlier than the $\beta 3$ tubulin mRNA. Thus twist and snail are likely to be involved in a regulatory pathway leading to $\beta 3$ tubulin expression, although the residual $\beta 3$ tubulin we found in some nerve cells of these mutants suggests that the $\beta 3$ tubulin gene can be expressed in non-mesodermal derivatives by a pathway independent of twist and snail.

Little is known concerning the differentiation of muscles in *Drosophila*, but few approaches have been taken previously. For example, the homeotic gene *Ultrabithorax* specifies the types of muscles developing in the thoracic segments of the larvae (Hooper, 1986) and for adult muscles it is proposed that there is a dependence on neural cells for the type of muscle developing (Lawrence & Johnston, 1986). Further analysis of $\beta 3$ tubulin expression will enable us to investigate the effect of mutants on the differentiating mesodermal derivatives.

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