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Characterization and developmental expression of β tubulin genes in *Drosophila melanogaster*

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Genomic clones containing β tubulin sequences were isolated from a λ library of *Drosophila melanogaster*. *In situ* hybridization localized three genes to 56D and 60B on chromosome 2 as well as to 85D on chromosome 3. The latter was known through genetic analysis to be specifically expressed during spermatogenesis. The genomic clone, pTu85, derived from this region contains one complete β tubulin coding region as well as the 3' end of an additional so far unidentified β tubulin gene. Genomic Southern hybridizations reveal a total of five fragments with β tubulin homology. Clone pTu56 codes for an RNA of 1.8 kb which is expressed in all developmental stages. Clone pTu60 codes for a 2.5-kb RNA expressed during embryogenesis and pupation. In testes RNA we detected a 2.2-kb message homologous to pTu85.

Key words: β tubulin genes/developmental expression/ *Drosophila melanogaster*

Introduction

Tubulins represent the major component of microtubules in eucaryotic cells. They are essential for meiotic and mitotic spindles, cilia and flagella, elongated neural processes and cell shape. The general structure of tubulin polymers consists of heterodimers of α and β tubulin. Although tubulin assembly and function have been studied extensively, far less is known concerning the regulation of their expression.

α and β tubulin genes have been characterized from several species (for review, see Cleveland, 1983). In multicellular organisms they are encoded by small multigene families. By cross-hybridization with chicken tubulin cDNA clones, Sanchez *et al.* (1980) showed that there are at least four members of each gene family in *Drosophila melanogaster*. The extensive protein and genetic work with *Drosophila* showed that at least two members of the β tubulin gene family have distinct developmental specificities. Mutation of the β2 locus leads to severe distortion of spermatogenesis (Kemphues *et al.*, 1979, 1980, 1982). Furthermore, Raff *et al.* (1982) describe a β3 tubulin specifically expressed during mid-embryogenesis, and they proposed β1 to be the generally expressed form.

Thus β tubulin synthesis is regulated during development as well as in a tissue-specific manner. As a prerequisite to investigate the regulation of members of the β tubulin gene family we isolated and characterized three individual genes and were able to distinguish between the expression of β1, β2 and β3 tubulin genes.

Results

Identification of a β tubulin gene of D. melanogaster

To characterize the individual genes for β tubulin we enriched poly(A)$^+$ RNA coding for β tubulin by sucrose gradient centrifugation and synthesized complementary DNA (see Materials and methods). With this cDNA we screened a λ library of *D. melanogaster* genomic DNA. The final identification of β tubulin coding clones was done by hybrid selected translation experiments. One clone was found to code for β tubulin. Further analysis of the *Eco*RI fragments from this clone localized the gene region within a 6.2-kb fragment. This fragment was subcloned into pUC8. For hybrid selection, sheared plasmid DNA was coupled to Sephacel beads and hybridized to poly(A)$^+$ RNA from embryos (Bünemann, 1982). The bound RNA was eluted and translated in a rabbit reticulocyte lysate system (Figure 1). *In vitro* translation products were separated on SDS-polyacrylamide gels (Laemmli, 1970) in parallel with native α and β tubulin from pig brain (Figure 1A, lane b). The 6.2-kb *Eco*RI fragment specifically selects the mRNA for a 53 000-dalton protein as expected for a β tubulin clone (Figure 1A, lane a). We named this clone pTu56 according to the *in situ* localization on polytene chromosomes (see below).

To show more clearly that we were indeed dealing with a β tubulin gene, we compared the translation products on two-dimensional gels (Figure 1B) with unlabelled α and β tubulin from pig brain (generous gift of Dr. E.-M., Mandelkow, MPI, Heidelberg). α as well as β tubulins are conserved proteins so that the pIs of vertebrate and *Drosophila* tubulins are very similar (Roberts and Hyames, 1979). The clone pTu56 appears to select mRNA for two different polypeptides which are not separable by size (Figure 1A, lane a) but differ slightly in their pI (~5.3, Figure 1B,d). It is known from protein data that embryos express one form of β tubulin stage-specifically (Raff *et al.*, 1982), together with a generally expressed form. As the RNA used in the hybrid-selected translation experiments was obtained from embryos, one of the two spots in Figure 1B,d probably results from this stage-specific β tubulin RNA present in our preparation, whereas the major spot corresponds to the generally expressed form. Similarly, an α tubulin clone (Mischke and Pardue, 1982) used as a positive control selects the message for the two known isoelectric variants of α tubulin in embryos (Figure 1B,c; Raff *et al.*, 1982). The selection of two messages with pTu56 is probably due to considerable cross-homology between sequences coding for β1 and β3 tubulin. Furthermore, we confirmed the identity of at least part of the β tubulin genes by DNA sequencing showing an extreme amino acid conservation compared with vertebrate tubulin sequences (Falkenburg *et al.*, in preparation).

β tubulin genes are members of a small dispersed gene family

To compare structures and developmental expression of the individual members of the β tubulin gene family, we screened a λ library of *D. melanogaster* with pTu56 and picked several positive clones showing differences in restriction enzyme pattern compared with the original clone. *In situ* hybridization with DNA of these phages to polytene chromosomes of Can-

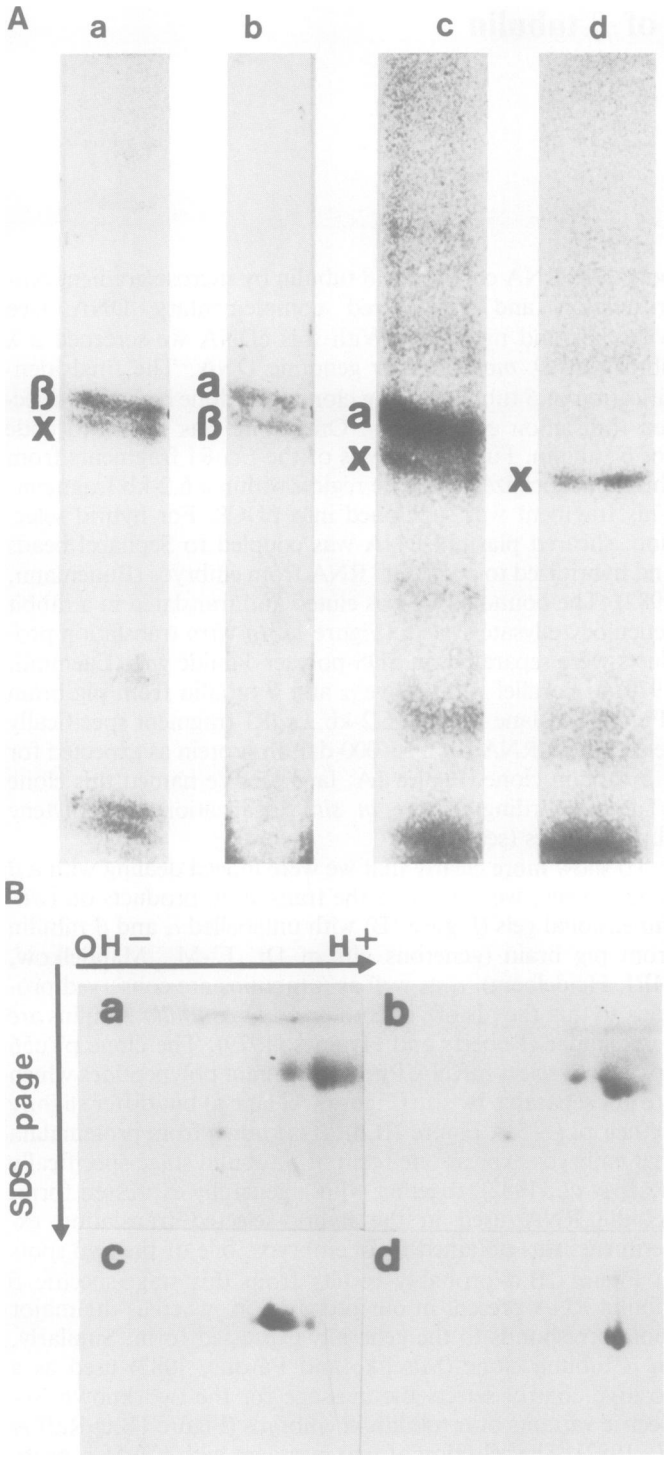

Fig. 1. Hybrid-selected translation experiments identify a β tubulin coding clone. DNA was coupled to Sephacel beads and hybridized to poly(A)$^+$ RNA from embryos (0–10 h). Specifically bound RNA was recovered and translated in a rabbit reticulocyte *in vitro* translation system. **(A)** Translation products were separated on 10% SDS acrylamide gels and exposed to X-ray film after fluorography. **(a)** Translation products of the poly(A)$^+$ RNA selected by pTu56; **(b)** pig tubulin, Coomassie stained; **(c)** translation products of poly(A)$^+$ RNA selected by an α tubulin gene as a positive control (Mischke and Pardue, 1982); **(d)** translation products of internal mRNA (x) of the reticulocyte lysate. **(B)** Translation products were run together with unlabelled tubulin on two-dimensional gels. The 1st dimension was an electrofocussing gel with a pH gradient from 4.0 to 7.0 (O'Farrell, 1975). The 2nd dimension was a 10% SDS acrylamide gel. **(a)** Coomassie stained tubulin run together with α tublin hybrid selection products; **(b)** Coomassie stained tubulin run together with β tubulin (pTu56) translated products; **(c)** fluorography of **a**; **(d)** fluorography of **b**.

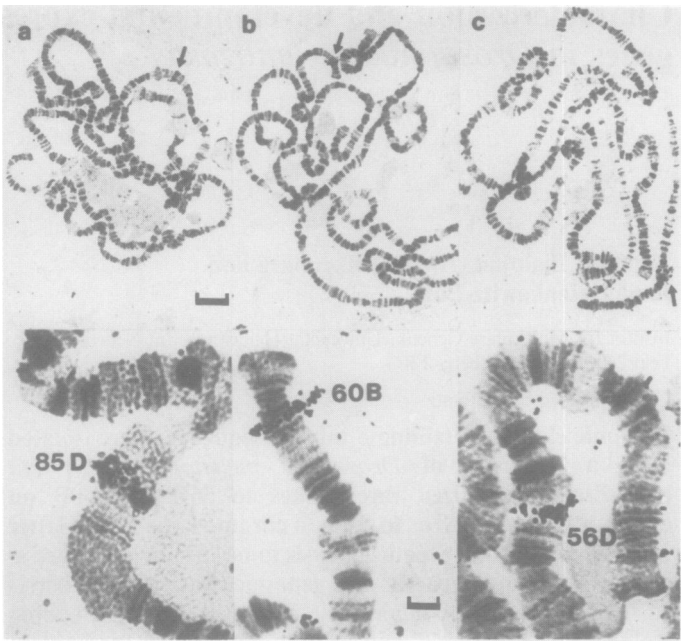

Fig. 2. Detailed *in situ* localization of three β tubulin genes. β tubulin genes were localized in a Canton S strain of *D. melanogaster* by hybridizing nick-translated [^{3}H]DNA to polytene chromosomes. **(a)** Tu85; **(b)** Tu60; **(c)** pTu56. The upper part gives an overview of the complete chromosome set. Arrows indicate regions of hybridization. The lower part enables identification of the hybridizing band at higher magnification. Exposure time was 8–12 days with probes of 2 x 10^7 c.p.m./μg DNA. Bars represent 40 μm (top) and 8 μm (bottom).

ton S larvae revealed two dispersed loci: 85D on chromosome 3 (Figure 2a) and 60B on chromosome 2 (Figure 2b). These are the loci which have been suggested to code for β tubulin (Sanchez *et al.*, 1980), whereas our initial clone pTu56 maps to a so far unknown β tubulin locus at 56D (Figure 2c). We decided to name our clones according to the *in situ* localization. Although there is homology between the different genes (see below), we observed no cross-hybridization at the exposure times used because the sequences surrounding the coding regions are not homologous. In the clones only ~10% of the *Drosophila* inserts represent β tubulin coding regions. We have no indication for repetitive sequences included in the clones, suggesting that only very small repetitive sequences if any are present close to the β tubulin genes investigated here. Southern-type hybridizations of pTu56 to *Eco*RI-restricted DNA of positive phages localized the coding parts to different sized *Eco*RI fragments: 5.3 kb in the case of Tu60 and 3.3 kb in the case of Tu85 (data not shown). For further analysis we cloned the *Eco*RI fragments including the coding regions in to pUC8.

Assignment of β tubulin clones to genomic EcoRI fragments

DNA of nuclei from Canton S and Oregon R flies was restricted with *Eco*RI, separated by gel electrophoresis and transferred to nitrocellulose. After Southern transfer hybridizations with pTu56, pTu60 and pTu85 were performed (Figure 3). All three clones hybridize to a very similar pattern of restriction fragments with one predominant band in each lane, indicating the genomic fragment from which the clone was derived. As the λ library was constructed by partial *Sau*3a digestion, only 6.2 kb of the 7.2-kb genomic *Eco*RI fragment are present in pTu56. pTu60 and pTu85 hybridize to a 5.3-kb and 3.3-kb genomic *Eco*RI fragment. Thus both

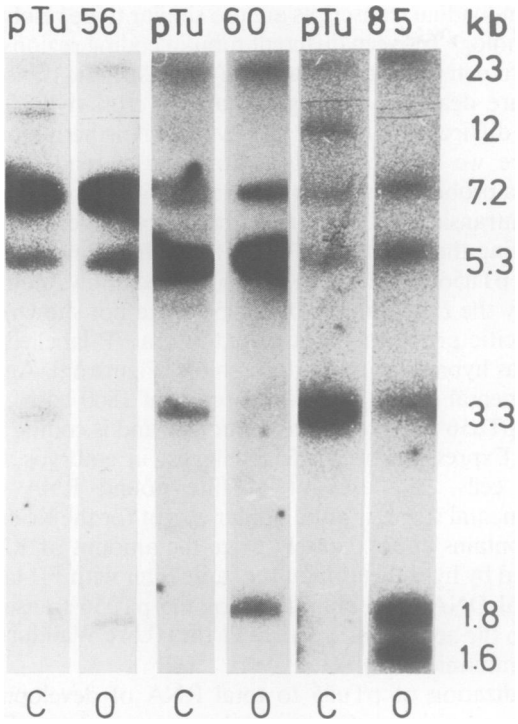

Fig. 3. Assignment of cloned β tubulin genes to genomic *Eco*RI fragments. 2 μg of DNA each of Canton S (C) and Oregon R (O) flies were digested with *Eco*RI, separated on 0.8% agarose gels. After Southern transfer individual strips were hybridized with ^{32}P-labelled pTu56, pTu60 and pTu85 respectively. *Hind*III-digested DNA was the size marker. Exposure 2 days.

genomic fragments are completely included in our clones. Cross-hybridization reveals additional fragments of 1.8, 12 and 23 kb. Tu60 and Tu56 show no strain difference between Oregon R and Canton S flies in their major hybridization components. pTu85 hybridizes mainly to a genomic fragment of 3.3 kb in Canton S flies. In Oregon R flies of our strain this fragment is split into 1.8 and 1.6 kb due to an additional *Eco*RI site. In cross-hybridizations with pTu60 and pTu85 only the 1.8-kb fragment hybridizes with Oregon R DNA, indicating that the bulk of homology to pTu56 and pTu60 is included in this fragment (Figure 3). In addition, a fragment of 3.3 kb as in Canton S flies is retained in Oregon R DNA. This is not due to a contamination with DNA from Canton S flies as the 12-kb *Eco*RI fragment of Canton S DNA is not visible in our Oregon R DNA. Tu85 originates from an Oregon R library and is missing the internal *Eco*RI site. These results argue for a restriction site polymorphism in the Oregon R strain.

In both strains a 23-kb long *Eco*RI fragment possesses sequence homology to β tubulin. We presently do not know which of these additional fragments represent functional genes.

Restriction enzyme analysis and polarity of the coding region in β tubulin genes

We performed restriction enzyme analysis of our clones. To determine the coding regions and to identify the polarity of the genes we selected cDNA clones from an embryonic cDNA library (generously provided by Goldschmidt-Clermont) by hybridization to pTu56. We isolated DNA from several phages giving positive signals and subcloned their complete inserts into pUC8, insert sizes varying between 800 and 1450 bp. One cDNA clone, pc56, contains some untranslated

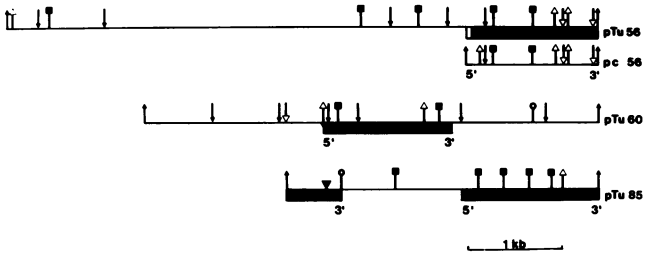

Fig. 4. Restriction maps of the *Drosophila* DNA in β tubulin clones pTu56, pTu60 and pTu85. Restriction endonuclease sites [*Eco*RI (↑), *Pst*I (↓), *Bam*HI (⇕), *Hind*III (▼), *Sac*I (⚲), *Pvu*II (⇑), *Sal*I (⇓)]. ■ Amino acid coding region. The transcribed regions of the three clones were determined by hybridization with the cDNA clone pc56 (see Figure 5a). hybridization with only the 3′ part of pc56 (pc56-3′, see Figure 5b) revealed the polarity of transcription in the clones. This was confirmed by partial sequencing of pc56 and pTu60 (Falkenburg *et al.*, in preparation). pc56 is almost totally co-linear with pTu56. We do not find any introns, although we cannot exclude the existence of very small intervening sequences. The 5′ end of pc56 contains a *Bam*HI site which is not found on the co-linear genomic DNA of pTu56. As a 70-bp subclone of the very 5′ end of pc56 (see below) still hybridizes to the 0.39-kb internal *Pst*I fragment in pTu56 we are obviously dealing with a restriction site polymorphism. Hybridizing pc56 and pc56-3′ to pTu85 revealed two β tubulin-like sequences with only one of them being completely encompassed in the clone pTu85.

regions of the 3′ and 5′ ends as shown by sequence analysis (see below and Falkenburg *et al.*, in preparation) and comparison with human β tubulin amino acid sequence (Cowan and Dudley, 1983). Hybridization of pc56 to Southern blots of *Eco*RI-digested DNA from Canton S flies assigned this cDNA to the 7.2-kb *Eco*RI genomic fragment (Figure 5A).

Hybridization of pc56 to fragments of pTu56, pTu60 and pTu85 allowed us to define the homologous regions in these clones (Figure 4). In addition we partially sequenced pTu56 and pTu60 and compared the amino acid sequences with human β tubulin (Falkenburg *et al.*, in preparation). Furthermore we analysed several cDNA clones of the same type differing in length. The common part is expected to contain the 3′ end of the β tubulin message. This was confirmed by sequence analysis (Figure 5b) which places the rightmost *Sal*I site 12 bases in front of the stop codon. We subcloned the 340-bp *Eco*RI-*Bam*HI fragment containing the 3′ end of the coding region (Figure 5a). Hybridization of genomic Southern blots with this probe (pc56-3′) also labels all four *Eco*RI fragments, the 7.2- and 5.3-kb fragments apparently have the strongest homology at the 3′ ends (Figure 5a). Thus hybridizing pc56-3′ to various restriction fragments of the β tubulin clones allowed us to define the 3′ end and, in combination with the hybridization data from pc56, to determine the polarity of the transcripts in all genes cloned (Figure 4). To our surprise pTu85 includes two 3′ end sequences, suggesting that there may be an additional β tubulin-like sequence. The complete sequence of this putative β tubulin gene is not contained in the original λ clone. Mapping of the original clone placed this additional 3′ end to the right border of the *Drosophila* insert. The identity of this additional β tubulin sequence has to be clarified.

The majority of the cDNA clones correspond to pTu56. The cDNA clones derive from poly(A)$^+$ RNA of 0−5-h old embryos, a time when only β1 tubulin is expressed (Raff *et al.*, 1982). We therefore suggest that pTu56 is the β tubulin gene expressed in all developmental stages. This is confirmed by using gene-specific probes for Northern blot analysis (see below).

Expression of β tubulin genes during development

To visualize the expression of β tubulin genes during development we performed Northern blot analysis with total RNA from different developmental stages and Kc cells (Figure 6). Hybridization with pTu56 revealed a message of 1800 bases in all developmental stages. There are no tubulin messages of other sizes detectable. This may mean either that messenger sizes of individual transcripts are too similar to be resolved, or that homology between different tubulin coding regions is not enough to detect minor amounts of stage-specific transcripts. As we are dealing with a gene family, cross-hybridization makes it difficult to interpret clearly these Northern blot data. Therefore we sequenced pc56 from the 5′ end and constructed a probe containing a 70-bp *Eco*RI-*Sau*3a fragment of the 5′-untranslated region in the transcription vector pSP64. Hybridizing the ^{32}P-labelled DNA from this clone to *Eco*RI-digested pTu56, pTu60 and pTu85 indeed shows homology with only the *Eco*RI insert of pTu56 (data not shown). This gene-specific probe was transcribed into a ^{32}P-labelled RNA which was hybridized to Northern blots (Figure 6a). Again all developmental stages show a transcript of 1800 bases, proving that pTu56 is expressed continuously and is coding for β1 tubulin. Expression of pTu56 is highest in embryos, adults and Kc cells. The amount of filter-bound RNA of all developmental stages is quite similar except for the Kc cell line which contains approximately twice the amount of RNA as controlled by hybridization of the same filter with ^{32}P-labelled ribosomal DNA. The abundance of the pTu56 transcript is similar to the actin message in adult flies (Uwe Walldorf, personal communication).

Hybridization of pTu85 to total RNA of developmental stages gave no signal. Genetic and protein analysis of Kemphues *et al.* (1979, 1982) revealed a β2 tubulin specifically expressed in spermatogenesis; the gene for β2 tubulin was localized to 85D. This corresponds to the *in situ* localization of pTu85. Therefore we isolated RNA from testes of adult wild-type flies. In this RNA we indeed find a 2.2-kb RNA hybridizing with pTu85 (Figure 6b). pTu85 contains one complete β tubulin gene and a 3′ end of another tubulin-like sequence; further experiments are necessary to clarify whether both are functional genes.

We hybridized pTu60 to RNA extracted from the same developmental stages. With this probe a transcript of 2.5 kb was found in the RNA of 0–10 h embryos as well as in early and late pupae. Therefore we suspected that pTu60 represented the β tubulin gene coding for β3 tubulin expressed in mid-embryogenesis (Raff *et al.*, 1982). In a more detailed analysis we examined RNA of different embryonic stages in comparison with larval stages, pupae and adult flies

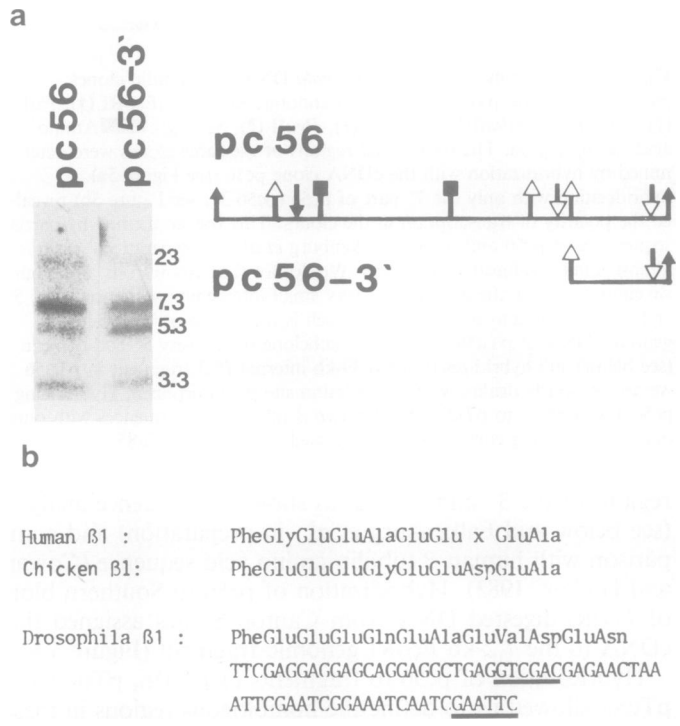

Fig. 5. (**a**) Assignment of cDNA clones to genomic *Eco*RI fragments. 5 μg *Eco*RI-digested Canton S DNA were hybridized after Southern transfer to pc56 and pc56-3′. Exposure 2 days. Restriction endonucleases sites [*Eco*RI (↑), *Pst*I (↓), *Bam*HI (ϕ), *Pvu*II (ψ), *Sal*I (ϕ)]. (**b**) Determination of the 3′ end of pc56. pc56 was sequenced from the rightmost *Eco*RI site using the Maxam and Gilbert technique. Comparison of the amino acid sequence with human and chicken β tubulin (Cowan and Dudley, 1983) revealed strong conservation between vertebrates and insects. A 3′-specific probe pc56-3′ was constructed by subcloning the *Eco*RI-*Bam*HI fragment at the right end of pc56 into pUC8 (see **a**). Underlined sequences encompass the *Sal*I site and the *Eco*RI site respectively.

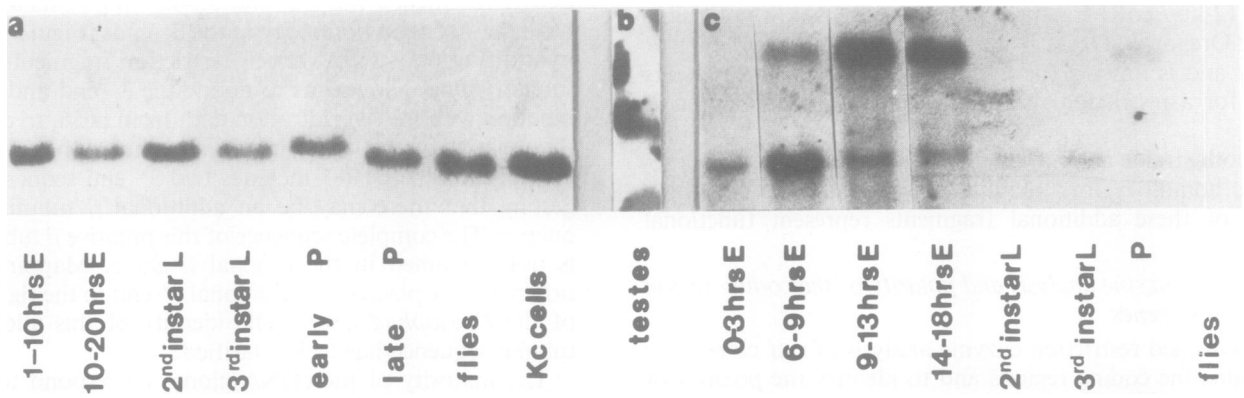

Fig. 6. Developmental expression of β tubulin genes pTu56, pTu85 and pTu60. (**a**) The 70-bp *Eco*RI-*Sau*3a fragment of the cDNA clone pc56 was cloned into the transcription vector pSP 64 (Melton *et al.*, in preparation). The recombinant plasmid was cut with *Pvu*II and transcribed into ^{32}P-labelled RNA which was used as a probe for RNA from different developmental stages and KC cells (10 μg/lane). The transcript has a length of 1.8 kb. (**b**) pTu85 was nick-translated and hybridized to RNA from testes of adult flies (5 μg/lane). Transcript length was 2.2 kb. (**c**) pTu60 was nick-translated and hybridized to RNA from different embryonic stages as well as from larvae, pupae and adult flies (10 μg/lane). The pTu60 transcript is 2.5 kb in length; the 1.8-kb transcript of pTu56 is detected by cross-homology. (E, embryos; L, larvae; P, pupae).

(Figure 6c). By cross-hybridization the continuously expressed β tubulin transcript is also detected. In 0–3 h embryos only the β1 tubulin message is visible, in 6–9 h embryos we recognize the 2.5-kb transcript but with far less abundance compared with the β1 message in the same lane. In 9–13 h as well as in 14–18 h embryos the signal corresponding to the 2.5-kb message is very strong in relation to the β1 transcript. From these data it is not possible to give exact quantitative relations between the two β tubulin messages as the β1 transcript is only detected by cross-hybridization. However, the expression of pTu60 during embryogenesis agrees well with the developmental synthesis of β3 tubulin. In addition pTu60 is expressed in pupae.

Discussion

In *Drosophila*, four genes are coding for α tubulin (Sanchez *et al.*, 1980; Kalfayan and Wensink, 1981). Similarly, β tubulins are encoded by a small gene family. We have analysed three members of this family in detail (pTu56, pTu60 and pTu85). It has to be clarified whether the 12-kb and the 23-kb fragments are additional functional β tubulin genes in *D. melanogaster*. The three genes cloned have distinct specificity: there is a generally expressed gene (Tu56, β1), a spermatogenesis-specific gene (Tu85, β2) and Tu60 (β3), expressed in early development as well as during pupation. Kalfayan and Wensink (1982) and Mischke and Pardue (1982) investigated the developmental specificity of the individual α tubulin genes. The abundance of transcripts of the individual genes varies during development. Different α tubulins show mainly quantitative differences during development, whereas β tubulins exhibit a far more stage-specific expression. β1 is present in all developmental stages with a maximal expression in 6–9 h embryos. This is very similar to the developmental pattern we observed with Tu56. It has long been speculated that multiple genes for α and β tubulin provide distinct functional products for the individual tubulin functions. The unicellular organism yeast has a simple non-motile life cycle. Neff *et al.* (1983) found only one β tubulin gene in yeast, it is likely that this gene relates to meiotic and mitotic cell division. *Chlamydomonas*, a unicellular motile organism, has two α and two β tubulin genes (Silflow and Rosenbaum, 1981; Brunke *et al.*, 1982). All higher organisms investigated so far have at least four α and β tubulin genes (for review, see Cleveland, 1983).

During morphogenesis and cellular differentiation, the expression of distinct tubulin subunits by members of this gene family may well be an important mechanism to control the assembly of functionally specialized microtubules. There are at least four β tubulin genes in *D. melanogaster*. Tu56 is generally expressed and probably is responsible for microtubular structures needed in every cell for cell division and cell shape. Tu60 is expressed for only a few hours during embryogenesis and in pupal stages, when important morphogenetic processes like organogenesis and metamorphosis are taking place. Tu85 most probably is essential for spermatogenesis (Kemphues *et al.*, 1979, 1980, 1982). Now that we have identified the individual β tubulin genes and correlated gene and developmental expression, we wish to study the mode of regulation. The next step is to define the sequences responsible for regulation of developmental timing and tissue specificity of the expression of distinct β tubulin genes. We think that β tubulin genes of *D. melanogaster* provide an excellent model system to investiate regulation of members of multigene families during development.

Materials and methods

Isolation and purification of DNA

Nuclei from frozen Canton S and Oregon R adult *D. melanogaster* were isolated, DNA was extracted and purified by CsCl density gradient centrifugation as described previously (Renkawitz-Pohl *et al.*, 1980).

Enrichment of tubulin sequences

Poly(A)$^+$ RNA specific for tubulins was enriched by sucrose gradient centrifugation, rRNA positions were determined in a parallel gradient. This approach has been successful for the isolation of α tubulin sequences (Kalfayan and Wensink, 1981). RNA of fractions on the light side of the 18S rRNA show an enrichment for tubulin mRNAs as tested by *in vitro* translation experiments. We used these fractions for the synthesis of single-stranded cDNA. With this cDNA we screened a genomic λ library of *D. melanogaster*. We picked only strongly hybridizing clones. The first 19 clones analysed by hybrid-selected translation revealed one β tubulin clone (see below).

Recombinant phages and plasmids

Screening of the EMBL1 and EMBL4 phage library of *D. melanogaster* (kindly provided by V. Pirrotta, EMBL, Heidelberg) and of a cDNA library (generous gift of Goldschmidt-Clermont) was performed according to Benton and Davis (1977).

We subcloned *Eco*RI-fragments of positive clones into the plasmid vector pUC8 treated with alkaline phosphatase (Ullrich *et al.*, 1977). *Escherichia coli* HB101 was transformed, clones were selected by ampicillin resistance and checked for the appropriate insert by restriction enzyme analysis.

Restriction endonuclease digestion, gel electrophoresis of DNA and Southern transfer were performed as described earlier (Renkawitz-Pohl and Bialojan, 1984).

DNA sequencing

Restriction fragments were treated with calf intestinal phosphatase, ^{32}P-end-labelled, separated on agarose gels and electroeluted. The DNA sequences were established using the Maxam and Gilbert method (1980).

Hybrid-selected translation and protein analysis

Cloned DNA was sheared and coupled to Sephacel-S 500 beads (Pharmacia) according to Bünemann (1982) and Bünemann *et al.* (1982). 0.5–1 mg *D. melanogaster* embryonic poly(A)$^+$ RNA was hybridized to the immobilized DNA at 37°C in 55% formamide (deionized by passing through Amberlite), 0.6 M NaCl, 1 mM EDTA, 0.2% SDS, 0.1 M Pipes pH 6.9 (W. Schmidt, personal communication). After washing in 55% formamide, 2 x SSC, 0.2% SDS at 37°C and in 10 mM Tris-HCl pH 8.0, 2 mM EDTA, 0.2% SDS at 60°C specifically bound RNA was eluted with water at 100°C and ethanol precipitated.

Hybrid-selected RNA was translated in a rabbit reticulocyte *in vitro* translation system (Amersham). The translation products were analysed by SDS-PAGE (Laemmli, 1970) as well as two-dimensional gel electrophoresis (O'Farrell, 1975). Purified pig brain α and β tubulin (generous gift of Dr E.-M. Mandelkow) were used as markers.

In situ hybridizations were performed as described previously (Renkawitz-Pohl and Bialojan, 1984).

RNA preparation and RNA blots

RNA was prepared from Kc cells as well as from different developmental stages of *D. melanogaster* according to Le Meur *et al.* (1981). To enrich poly(A)$^+$ RNA, total RNA was passed through a poly(U) Sepharose column (Pharmacia).

[^{32}P]cRNA was synthesized using the transcription vector pSP64 (Riboprobe, Gene Analysis System, Promega Biotec) constructed by Melton *et al.* (in preparation).

RNA blots and DNA hybridizations were performed as described by Goldberg (1980).

cRNA-RNA hybridizations were done in 50% formamide, 50 mM Na-phosphate (pH 6.5), 0.8 M NaCl, 1 mM EDTA, 0.1% SDS, 2.5 x Denhardt's solution, 250 μg/ml denatured salmon sperm DNA and 500 μg/ml yeast RNA at 55°C.

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