Expression of β1 tubulin (βTub56D) in *Drosophila* testis stem cells is regulated by a short upstream sequence while intron elements guide expression in somatic cells

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**Abstract.** Stem cell differentiation to mature spermatozoa is a morphogenetic process that is highly dependent on microtubular arrays. In the early, mitotically active stages of spermatogenesis, only the β1 tubulin isotype is expressed. Analysis of transgenic flies containing β1-lacZ gene fusions revealed that this expression is regulated by sequences located between positions −45 and −191 upstream of the transcription initiation site. Furthermore, β1 tubulin is a major component of cyst cells. Expression in these cells is driven by enhancer elements located in the β1 tubulin gene intron. These enhancer elements also guide expression in combination with the hsp70 basal promoter. In addition, redundant enhancer elements in the β1 tubulin gene intron also guide expression in combination with the hsp70 basal promoter. In addition, redundant enhancer elements in the β1 tubulin gene intron also guide expression in combination with the hsp70 basal promoter.

**Key words:** β1 tubulin – Spermatogenesis – Gene regulation – Mitosis

**Introduction**

During spermatogenesis, germ cells develop from undifferentiated stem cells into mature spermatozoa. Germ cells are arranged in the testis according to developmental stage allowing distinction of stem cells at the very tip, followed by spermatogonia which then enter meiotic prophase. Meiosis starts approximately midway along the testis tube, and thereafter sperm morphogenesis occurs. From the spermatogonial stage to the individualization stage, the synchronously developing germ cells are surrounded by two cyst cells. In this morphogenetic process, a number of microtubular structures are involved (for review of *Drosophila* spermatogenesis, see Lindsley and Tokuyasu 1980; Hackstein 1987; Henning 1988). Microtubules, composed of heterodimers of α and β tubulins, and the α and β tubulin gene families that encode them have been intensively studied in *Drosophila*, (Bialojan et al. 1984; Natzle and McCarthy 1984; Theurkauf et al. 1987).

In the testis, β tubulins are well characterized at the genetic, histochemical and molecular levels (Raff 1984; Rudolph et al. 1987; Michiels et al. 1987; Kaltschmidt et al. 1991). A major component of the sperm cells of *Drosophila melanogaster* is the β2 tubulin isotype (βTub85D; for review see Raff 1984), which is the dominant isotype in all microtubular arrays, starting with the mid- spermatocyte stage. Besides the β2 isotype, the β1 (βTub56D) and β3 (βTub60C) tubulins are expressed in the testis (Kimble et al. 1989; Kaltschmidt et al. 1991). The β1 isotype is characteristic for stem cells and spermatogonia as well as for somatic cells like the cyst cells, the testis wall and the paragonia (accessory glands, imaginal disc derivatives). The β3 tubulin is restricted to interstitial and cyst cells (Kaltschmidt et al. 1991), which are of mesodermal origin (Campos-Ortega and Hartenstein 1985). This cell-type-specific expression in the testis is also observed for β1–β3 in the distantly related species *D. hydei* (Glätzer et al. 1992). In addition, the β1 tubulin gene is expressed during oogenesis and supplies the embryo with the β1 tubulin used to form the mitotic spindle apparatus during the synchronous divisions in the pre-blastoderm stage (Buttgereit et al. 1991).

We have investigated how this cell-type-specific expression of individual β tubulin isotypes in the testis is regulated. In the β2 tubulin gene we previously identified a 14 bp promoter element sufficient for cell-type-specific expression in the testis (Michiels et al. 1989, 1991). Here we report that the regulatory regions responsible for expression of the β1 tubulin gene in somatic cells of the testis are located in the single large intron positioned between the codons of amino acids 19 and 20 (Michiels
et al. 1987). In contrast, transcription of the β1 tubulin gene in germ cells is dependent on upstream sequences. By deletion analysis we determined that a short promoter-proximal fragment is responsible for expression in mitotically active male germ cells.

Materials and methods

Construction of deletion mutants. Intron deletions fused to the β1 promoter. The lacZ reporter gene was fused to the β1 tubulin gene at the Sau3A site in the codon for amino acid (aa) 23, according to the strategy applied previously for the β3 tubulin gene (Gasch et al. 1989). Genomic sequences from the intron were used, resulting in the construct Wβ1L (Buttgereit et al. 1991). For Wβ1K, 2082 bp were deleted from the central part of the intron by use of internal restriction sites from bases -2378 to +2856 comprising the first exon, the complete intron and the second exon up to amino acid 23 were used, resulting in the construct Wβ1L (Buttgereit et al. 1991). For Wβ1K, 2082 bp were deleted from the central part of the intron by use of internal restriction sites (AvaII+443 and PstI+2525). Wβ1C was constructed using a β1 cDNA clone (Gasch et al. 1988).

Intron sequences fused to the hsp70 promoter. We used the hsp70 basal promoter as a heterologous promoter in the vector pWHL, which has previously been used successfully (Michiels et al. 1991; Hinz et al. 1992). The constructs Wβ1HI and Wβ1HHI were made by linearizing pWHL with StuI and inserting the intron digested with Asp700 at position +122 (codon for amino acid 4) and Baxl at +2687 (157 bp upstream of the second exon) in both orientations upstream of the truncated hsp70 promoter.

Upstream deletion mutants. Upstream deletions including the first β1 exon and the intron as in Wβ1K were produced by exonuclease III/nuclease S1 digestions. The constructs were analysed by sequencing and subsequently cloned into pW8 (Klemenz et al. 1987).

Intron deletion mutants. The clones containing AS1, AS2, and AS3 were made by successively cleaving the β1 tubulin gene intron with Asp700 (+122) and with PvuII (AS1 +122 to +1433; AS2, +1434 to +2068; AS3, +2069 to +2918; numbers are given relative to the transcription start site). Fragments were isolated and ligated into pWHL that had been linearized with StuI.

P-element-mediated transformations and analysis of transformants. P element transformations and histological staining of isolated testes were performed as published previously (Michiels et al. 1989; Buttgereit et al. 1991).

Results

The male gonad consists of the germ cells and somatic elements such as accessory glands (paragonia), cyst cells, seminal vesicles, sperm pump and testis wall. We previously studied the β1 tubulin distribution with the aid of isotype-specific antibodies and showed that the β1 tubulin isotype is expressed in the somatic components of the testis as well as in the germ line (Kaltschmidt et al. 1991). During the spermatocyte growth phase, the β2 tubulin isotype is synthesized and used as germ line-specific isotype during meiosis and sperm morphogenesis. Within the male germ line, β1 tubulin is characteristic for the cytoskeleton and the mitotic spindle apparatus of stem cells, spermatagonia and early spermatocytes. In this paper we have investigated how the distinct cell-type-specific expression of the β1 tubulin gene is regulated in the male reproductive system of Drosophila.