The intracellular localisation of TAF7L, a paralogue of transcription factor TFIID subunit TAF7, is developmentally regulated during male germ-cell differentiation

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Summary

Transcription regulation in male germ cells can involve specialised mechanisms and testis-specific paralogues of the general transcription machinery. Here we describe TAF7L, a germ-cell-specific paralogue of the TFIID subunit TAF7. TAF7L is expressed through most of the male germ-cell differentiation programme, but its intracellular localisation is dynamically regulated from cytoplasmic in spermatogonia and early spermatocytes to nuclear in late pachytene spermatocytes and haploid round spermatids. Import of TAF7L into the nucleus coincides with decreased TAF7 expression and a strong increase in nuclear TBP expression, which suggests that TAF7L replaces TAF7 as a TFIID subunit in late pachytene spermatocytes and in haploid cells. In agreement with this, biochemical experiments indicate that a subpopulation of TAF7L is tightly associated with TBP in both pachytene and haploid cells and TAF7L interacts with the TFIID subunit TAF1. We further show that TAF3, TAF4 and TAF10 are all strongly expressed in early spermatocytes, but that in contrast to TBP and TAF7L, they are downregulated in haploid cells. Hence, different subunits of the TFIID complex are regulated in distinct ways during male germ-cell differentiation. These results show for the first time how the composition of a general transcription factor such as TFIID and other TAF-containing complexes are modulated during a differentiation programme highlighting the unique nature of the transcription regulatory machinery in spermatogenesis.

Key words: X chromosome, Spermatogonia, TBP, Spermatogenesis, Haploid cells, Meiosis

Introduction

Accurate transcription initiation at protein coding genes by RNA polymerase II (pol II) requires the assembly of a multiprotein complex around the mRNA start-site (Hampsey, 1998). One of the most critical general factors involved in this process is TFIID, comprising the TATA-binding protein (TBP), responsible for specific binding to the TATA element found in many pol II promoters, and a set of 13-14 TBP-associated factors (TAFs) (Gangloff et al., 2001a; Veenstra and Wolffe, 2001). A subset of TAFs is present not only in TFIID, but also in the SAGA, PCAF, STAGA and TFTC complexes and the *Drosophila* PRC1 complex, which are involved in pol II transcription, but lack TBP (Brand et al., 1999; Grant and Workman, 1998; Martinez et al., 2001; Ogryzko et al., 1998; Saurin et al., 2001).

Genetic and biochemical investigations in several organisms have shown that TAFs play important roles in transcriptional regulation. Mutation of TAFs in yeast and in mammalian cells leads to cell cycle arrest and gene-specific transcriptional effects. TAFs have also been shown to play a role in promoter recognition and selectivity and to interact directly with transcriptional activators (for reviews, see Albright and Tjian, 2000; Green, 2000). These interactions are thought to contribute to recruitment of TFIID to specific promoters.

While the majority of TAFs are 'ubiquitously' expressed in all tissues, albeit at different levels (Perletti et al., 1999), two examples of TAFs with tissue-restricted expression and function have been more recently described. In *Drosophila melanogaster*, Cannonball (can, dTAF5L) is a testis-specific paralogue of the somatic TAF5 protein. Mutation of this gene in *Drosophila* leads to arrest of germ-cell development and male sterility (Hiller et al., 2001). In mouse, the TAF4b protein, a paralogue of somatic TAF4, is expressed in a tissue-restricted fashion with high expression in the granulosa cells of the ovary and the testis (Dikstein et al., 1996; Freiman et al., 2001). Female TAF4b$^{-/-}$ mice show defective oocyte maturation leading to sterility, while males are fertile and show no spermatogenesis abnormalities. These two examples indicate that, in addition to the somatically expressed TAFs, there are tissue-specific paralogues that have much more specific functions.

Spermatogenesis is a cyclic process in which diploid

spermatogonia differentiate into mature haploid spermatozoa. Spermatogonia committed for differentiation give rise to spermatocytes that undergo two meiotic divisions, to generate haploid round spermatids. During the process of spermiogenesis the haploid round spermatids undergo an elongation phase, during which they are sculptured into the shape of mature spermatozoa. This entails a major biochemical and morphological restructuring of the germ cell in which the majority of the somatic histones are replaced by protamines to pack the DNA into the sperm cell nucleus.

Several lines of evidence point to specialised transcriptional regulatory mechanisms in spermatogenesis (for a review, see Sassone-Corsi, 2002). Components of the general transcription machinery such as TBP and TAF_{II}s are strongly upregulated in testis (Perletti et al., 1999; Schmidt and Schibler, 1995). In the case of TBP, upregulation takes place in late pachytene spermatocytes and in haploid round spermatids (Martianov et al., 2002). In addition, male germ cells express paralogues of the general transcription factors such as TFIIAτ/ALF, a paralogue of TFIIA, and TLF/TRF2, a paralogue of TBP (Ozer et al., 2000; Upadhyaya et al., 1999). $TLF^{-/-}$ male mice are sterile as a result of a complete arrest of spermatogenesis resulting from germ-cell apoptosis at the transition from round to elongating spermatids (Martianov et al., 2001; Zhang et al., 2001). Hence, spermatogenesis is characterised by overexpression of somatically expressed transcription factors and in some cases expression of germ-cell-specific paralogues.

To add to this list, murine TAF7L (TAF2Q) is a testis-specific protein with sequence similarity to somatic TAF7 [formerly $TAF_{II}55$ (Lavigne et al., 1996; Tora, 2002)] encoded by the X chromosome (Wang et al., 2001). RT-PCR experiments have indicated that TAF7L expression is restricted to the germ-cell lineage, where its mRNA is strongly expressed in spermatogonia.

While the TAF7L mRNA has been shown to be expressed in spermatogonia, nothing is known about its expression at other stages of spermatogenesis and whether it is associated with TBP as a bona fide TAF. We have investigated expression of the TAF7L protein during spermatogenesis and show that it is expressed in primary spermatocytes and haploid cells, but that its intracellular localisation is regulated during differentiation. A fraction of the TAF7L present in pachytene and haploid cells is tightly associated with TBP and in two hybrid screens TAF7L interacts with the TFIID subunit TAF1. These results are consistent with the idea that TAF7L is a bona fide TFIID subunit that replaces TAF7 as TFIID composition is remodelled during spermatogenesis. Analysis of the expression of other TAFs during spermatogenesis unexpectedly revealed that they are expressed differently both from each other and from TBP. Therefore, while TBP and TAFs are overexpressed in testis, they are overexpressed at distinct stages of the differentiation process indicating a dramatic remodelling of the composition of TFIID and other TAF-containing complexes during male germ-cell differentiation.

Materials and Methods

Cloning of TAF7L

A cDNA encoding full length TAFL7 was amplified from a murine cDNA library using oligonucleotide primers located at the 5′ and 3′ ends as deduced from the reported data base entry and BLAST searches of mouse EST data bases. Primers contained *Bam*HI and *Not*I restriction sites. The 1425 bp fragment was digested by *Bam*HI and *Not*I and cloned in a modified pXJ41 expression vector containing a FLAG epitope linearised with the same enzymes. Transfection of Cos cells and preparation of transfected cells extracts were as previously described (Mengus et al., 1995).

Immunoblots and immunohistochemistry

Mouse seminiferous tubule segments at defined stages were isolated using the transillumination-assisted microdissection method (Parvinen and Hecht, 1981). Immunohistochemistry was performed on fixed sectioned seminiferous tubules or from staged squash preparations of microdissected tubules from wild-type C57 BL/6 mice as previously described (Martianov et al., 2001; Nantel et al., 1996). Antibodies against TBP, TAF3, TAF4, TAF6, TAF7, TAF10, TAF12, and TAF13 are as previously described (Bell et al., 2001; Brou et al., 1993; Gangloff et al., 2001b; Lavigne et al., 1996; Mengus et al., 1997; Mengus et al., 1995; Metzger et al., 1999; Perletti et al., 1999). Monoclonal (46TA) and polyclonal antibodies were generated against the indicated TAF7L peptide coupled to ovalbumin as previously described (Mengus et al., 1997).

Extract preparation and immunoprecipitation

Extracts were prepared from frozen mouse testis ground in a mortar in the presence of liquid nitrogen. Cytoplasmic and nuclear extracts were then prepared essentially as previously described (Mengus et al., 1995). Briefly, the powdered material obtained was resuspended in buffer A (50 mM Tris-HCl, pH 7.9; 20% glycerol; 1 mM dithiothreitol; 0.5 mM EDTA) in the presence of 0.01 M KCl. The resuspended material was dounced and the nuclei collected by low speed centrifugation. The supernatant fraction (cytoplasmic extract) was recovered and the nuclei resuspended in buffer A with 0.5 M NaCl. After douncing and a 30 minute incubation in ice the nuclei were spun out (20 minutes at 30,000 ***g***) and the supernatant fraction (nuclear extract) recovered. The nuclear extract was precipitated by addition of 0.33 g/ml ammonium sulphate and the protein recovered by centrifugation. The protein was resuspended in buffer A with 0.05 M KCl and dialysed overnight against the same buffer. Typically 10 ml of cytoplasmic extract (around 100 mg of protein) and 4 ml (around 6 mg) of nuclear extract were recovered from 20 mouse testes. Purified populations of pachytene spermatocytes and haploid spermatids were prepared by centrifugal elutriation as described (Fimia et al., 1999; Meistrich et al., 1981). Cell extracts were prepared from each population by several cycles of the freeze thaw in buffer A with 0.5 M NaCl and 0.1% NP40 followed by incubation for 30 minutes on ice and microfuge centrifugation for 10 minutes at 4°C. Immunoprecipitation of TBP with monoclonal antibody 2C1 and elution with peptide were as described (Mengus et al., 1995). Immunoblotting and chemiluminescence detection were performed by standard methods.

Yeast two hybrid screen

A yeast two-hybrid screen was performed essentially as described (Fimia et al., 1999). As bait, full length TAF7L was cloned in frame with the DNA-binding domain of the yeast activator GAL4 (GAL4 DBD). This plasmid was transformed in the *Saccharomyces cerevisiae* strain sc1945 with a murine adult testis cDNA library (Clontech). Approximately 2×10^6 transformants were plated on selection medium lacking Leu, Trp and His. Clones expressing HIS3 were isolated, plasmid DNA was recovered and transformed in the *E. coli* HB101 strain to isolate the prey plasmid. The specificity of each clone was tested further by co-transformation of the different clones with the TAF7L bait or the vector encoding only the GAL4 DBD in yeast strain Y190, containing an integrated GAL4 responsive β-galactosidase

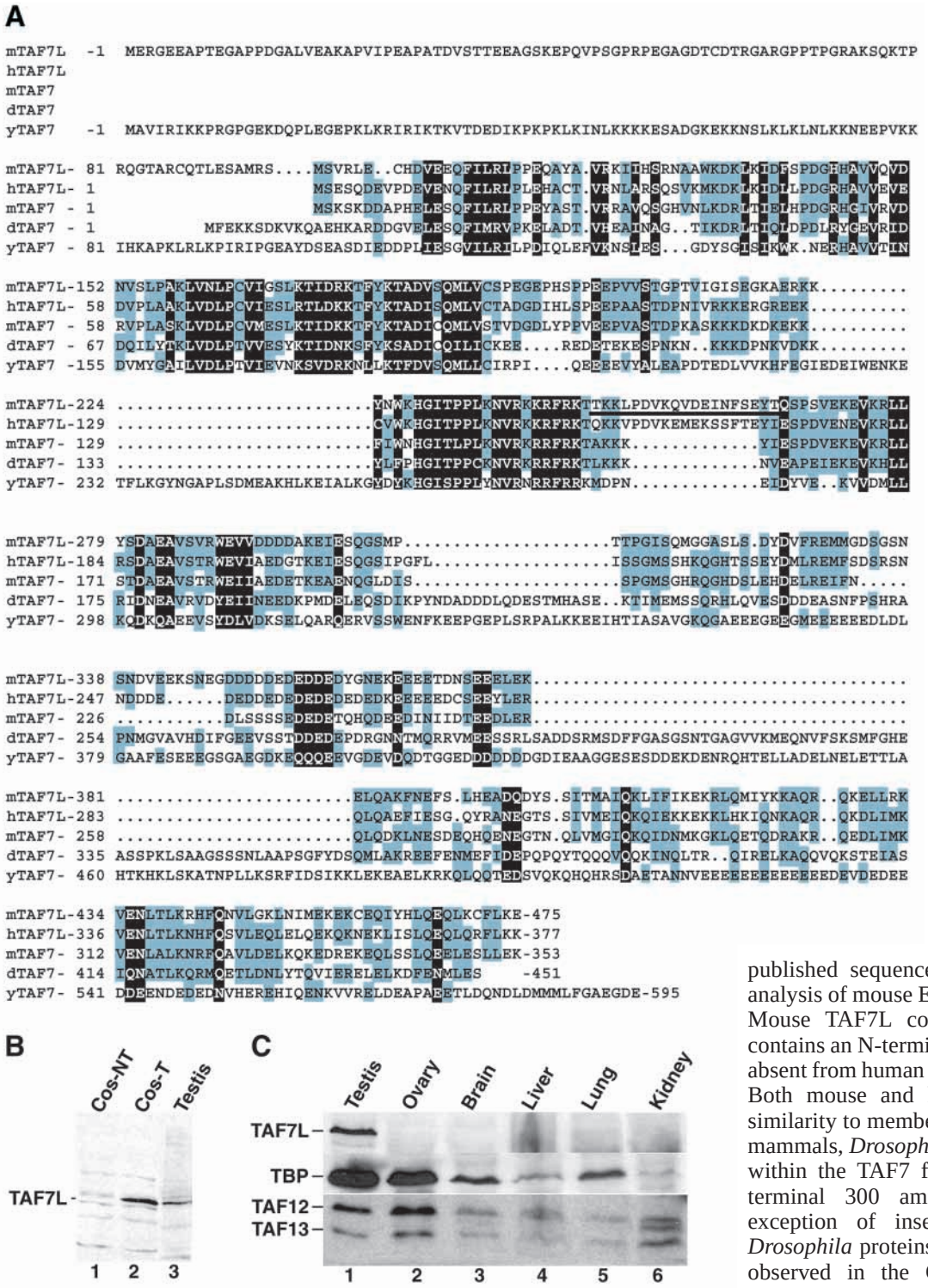

Fig. 1. Cloning of TAF7L and characterisation of anti-TAF7L antibodies. (A) Alignment of the sequences of TAF7L from human and mouse with those of TAF7 from mouse, *Drosophila melanogaster* and yeast (*Saccharomyces cerevisiae*). Highly conserved amino acids are shown in white on a black background. Positions conserved in at least three of the proteins are boxed in grey. Amino acids were classified as follows. Small residues: P, A, G, S, T; hydrophobic: L, I, V, A, F, M, C, Y, W; polar/acidic: D, E, Q, N; basic: R, K, H. The peptide used to generate the antibodies is underlined. (B) Cos cells were transfected with a TAF7L (lane 2) or empty (lane 1) expression vector and the recombinant TAF7L detected with monoclonal antibody 46TA. TAF7L is also visible in the testis nuclear extract (lane 3). (C) TAF7L, TBP, TAF12 and TAF13 were detected in total extracts from the tissues shown above each lane.

gene. Quantitative β-galactosidase assays were performed as described (Fimia et al., 1999).

Results

Cloning of murine TAF7L

A cDNA encoding full length TAF7L was isolated from a mouse testis cDNA library by PCR using primers based on the published sequence (Wang et al., 2001) and analysis of mouse ESTs in the public databases. Mouse TAF7L comprises 475 residues and contains an N-terminal 96 amino acid extension absent from human TAF7L and TAF7 (Fig. 1A). Both mouse and human TAF7L show high similarity to members of the TAF7 family from mammals, *Drosophila* and yeast. The similarity within the TAF7 family is highest in the N-terminal 300 amino acids, but with the exception of insertions in the yeast and *Drosophila* proteins a weaker similarity is also observed in the C-terminal portion of the proteins (Fig. 1A).

To selectively detect TAF7L, monoclonal and polyclonal antibodies were generated against a synthetic peptide (underlined in Fig. 1A). These antibodies recognised a 70 kDa protein in extracts from Cos cells transfected with a TAF7L expression vector and in extracts from mouse testis (Fig. 1B and data not shown). In agreement with the published RT-PCR analysis (Wang et al., 2001), TAF7L was not detected in extracts from several other organs and is therefore testis-specific, whereas as described, TBP and other TAFs are widely

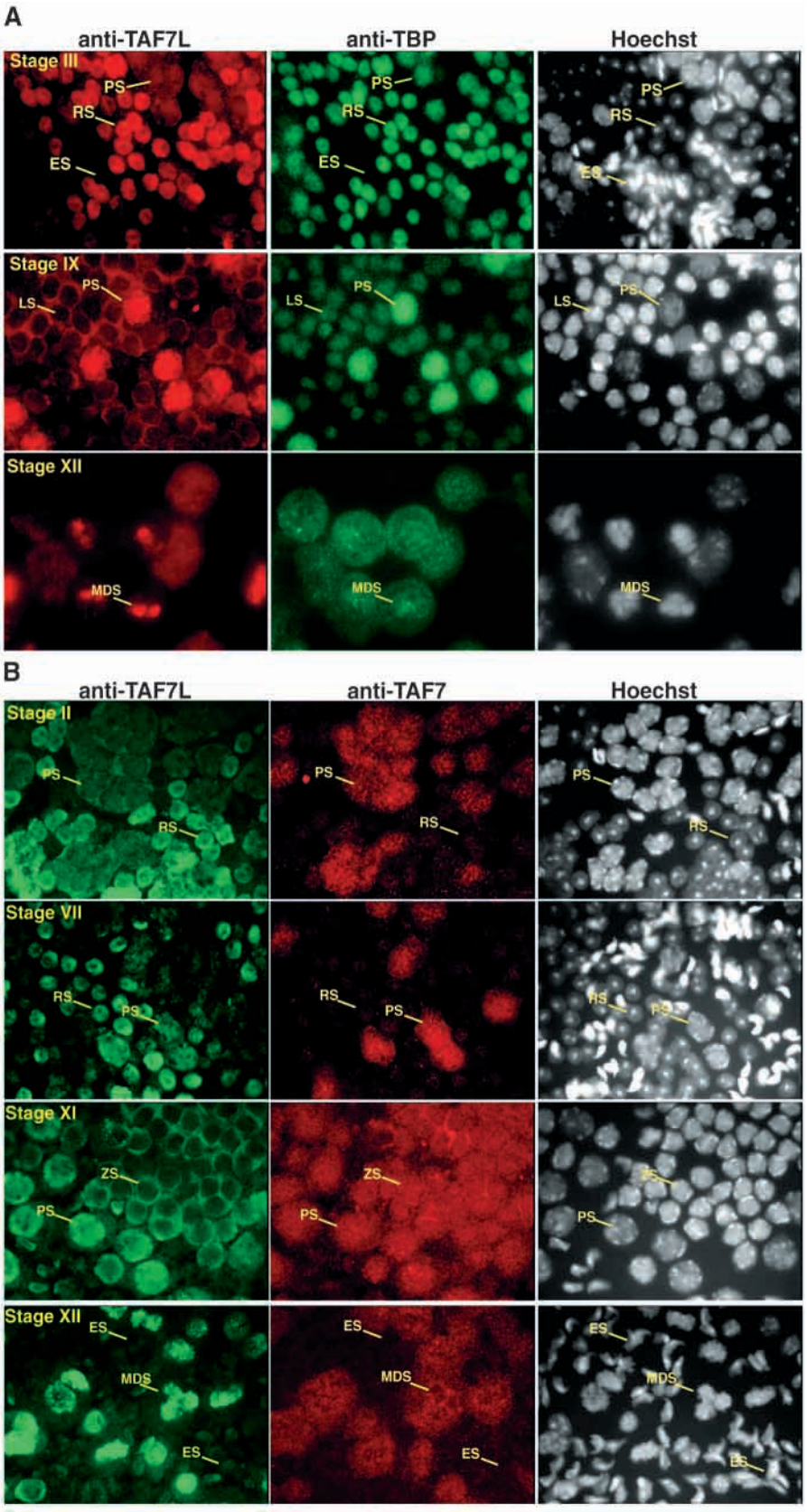

Fig. 2. Developmental expression profiles of TAF7L, TBP and TAF7. (A) Double labelling immunodetection of TAF7L and TBP in developing male germ cells. TAF7L and TBP are detected in microdissected segments of seminiferous tubules from the stages shown in each panel. (B) Double labeling immunodetection of TAF7L and TAF7. Representative examples of cell types are indicated. ES, elongating spermatids; LS, leptotene spermatocytes; MDS, meiotic dividing spermatocytes; PS, pachytene spermatocytes; RS, haploid round spermatids; ZS, zygotene spermatocytes. Magnification, 40×.

expressed although at different levels (Fig. 1C) (Perletti et al., 1999).

The intracellular localisation of TAF7L is regulated during male germ-cell differentiation

The polyclonal antibodies were used to examine the expression of TAF7L during spermatogenesis. Segments of mouse seminiferous tubules corresponding to each developmental stage were isolated by transillumination-assisted microdissection to make squash preparations where the exact stage of the cycle was identified (Parvinen and Hecht, 1981). Double immunostaining was performed with the polyclonal anti-TAF7L antibodies and the 3G3 monoclonal antibody against TBP (Lescure et al., 1994). We have previously used this antibody to establish a precise map of TBP expression in male germ cells (Martianov et al., 2002).

TAF7L was strongly expressed in the nucleus of haploid round spermatids, but was excluded from the heterochromatic chromocenter (Fig. 2A, red image and 2B, green image, see also Fig. 4A). Expression in these cells was seen between stages I and VII and disappeared early in the elongation phase (Fig 2A,B, Fig. 3, and data not shown, summarised in Fig. 5A). Strong nuclear TAF7L expression at these stages coincides with strong TBP expression [Fig. 2A green image (see also Martianov et al., 2002); summarised in Fig. 5B]. TAF7L is also strongly expressed along with TBP in the nuclei of late pachytene spermatocytes from stage VIII onwards (Fig. 2A,B). Strikingly however, at stage XII, all the cellular TAF7L is found associated with the condensed chromatin in meiotically dividing cells, while TBP is excluded (Fig. 2A, Fig. 3). Thus, at this stage TBP and TAF7L do not colocalise and hence cannot be associated. The differential localisation of TAF7L and TBP at this stage can be more clearly seen in confocal sections (Fig. 4B). Close examination of TAF7L localisation indicates that it is excluded from the most dense

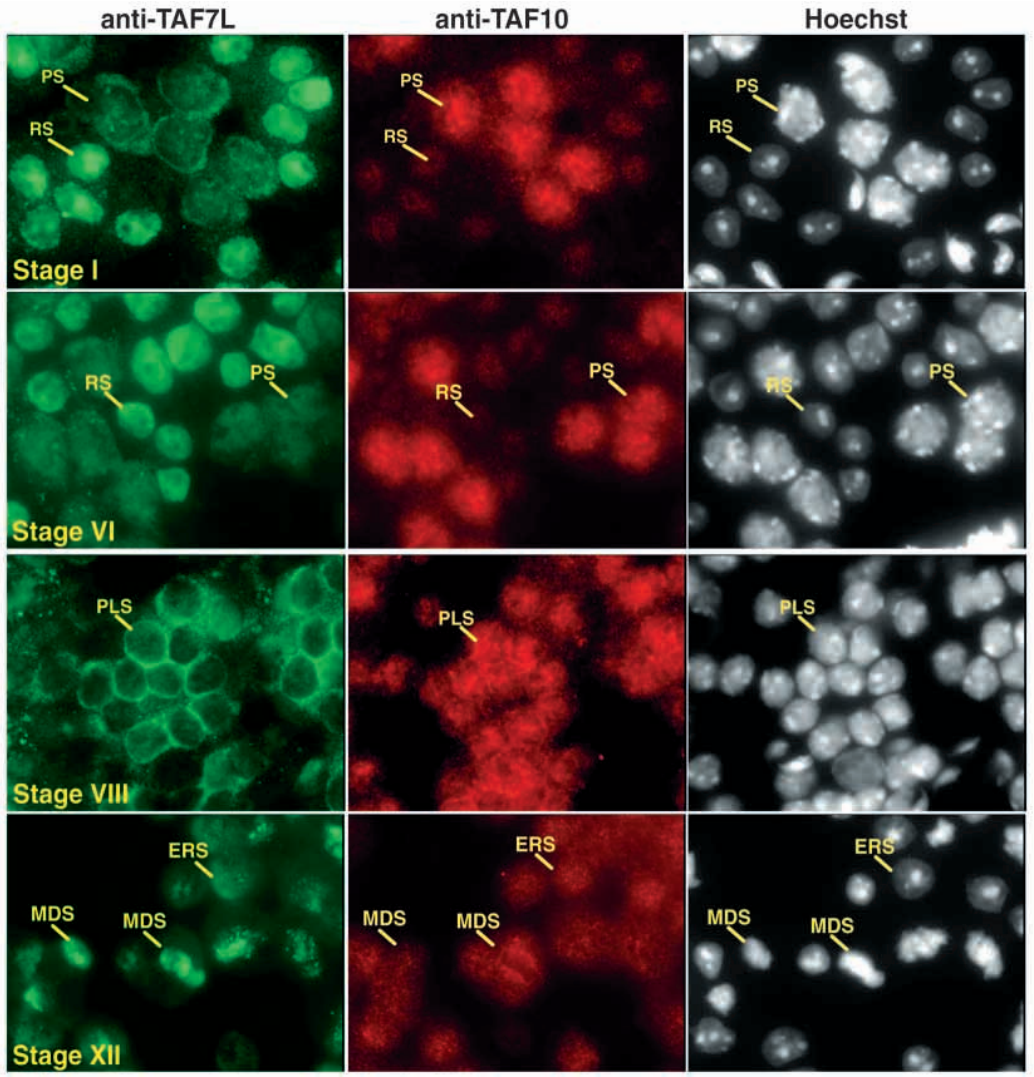

Fig. 3. Developmental expression profiles of TAF7L and TAF10. Double labeling immunodetection of TAF7L and TAF10 in developing male germ cells. Representative examples of cell types are indicated. ERS, early haploid round spermatids; MDS, meiotic dividing spermatocytes; PLS, preleptotene spermatocytes; PS, pachytene spermatocytes; RS, haploid round spermatids. Magnification, 40×.

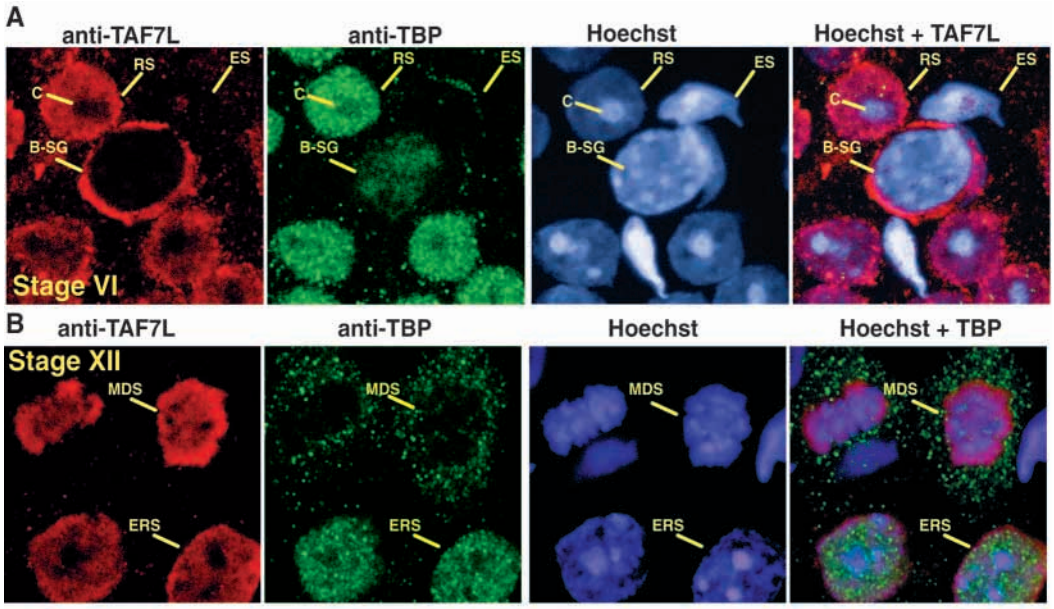

Fig. 4. Changes in intracellular localisation of TAF7L during differentiation. (A) Confocal images of staged squash sections. Double staining with TBP and TAF7L. The right hand panel shows the overlayed image of TAF7L labeling and Hoechst stained DNA. (B) Confocal image of meiotic dividing spermatocytes showing different cellular localisations of TAF7L and TBP. Magnification 100×. Representative examples of cell types are indicated. B-SG, type B spermatogonia; C, chromocenter; ERS, early haploid round spermatids; ES, elongating spermatids; MDS, meiotic dividing spermatocytes; RS, haploid round spermatids. Magnification, 40×.

heterochromatin. In agreement with this TAF7L is obviously excluded from the heterochromatic foci in early round spermatids (Fig. 4B) in which the chromocenter is forming and is excluded from the chromocenter in later stage spermatids (Fig. 4A). Localisation of TAF7L with what appear to be specific chromatin domains can also be seen in the stage XII sections of Fig. 2A,B and Fig. 3.

Contrary to later stage pachytene spermatocytes, where TAF7L is nuclear, in spermatogonia, preleptotene, leptotene and zygotene cells, TAF7L is restricted to the cytoplasm and is excluded from the nucleus (Fig. 2A,B and Fig. 3, and data not shown). This can be clearly seen in confocal sections of stage VI tubules, where TAF7L is present in the cytoplasm of a type B spermatogonia (Fig. 4A), but is present in the nucleus of round spermatids (Fig. 4A). As previously reported (Martianov et al., 2002), perinuclear TBP expression in late elongating spermatids can also be seen in these images.

In mid-stage pachytene spermatocytes, there is a switch in TAF7L localisation as it begins to enter the nucleus and becomes uniquely nuclear only in late stage pachytene cells. This change in TAF7L intracellular localisation shows an interesting correlation with the increase in TBP expression. In early primary spermatocytes, TBP expression is low and nuclear [see double staining in Fig. 2A (see also Martianov et al., 2002)], while TAF7L is cytoplasmic. Soon after TAF7L enters the nucleus in mid-stage pachytene cells, TBP expression strongly increases (compare TBP expression in zygotene and pachytene cells at stage IX in Fig. 2A) such that both proteins are found strongly expressed in the nucleus in later stage pachytene and haploid cells (summarised in Fig. 5A,B).

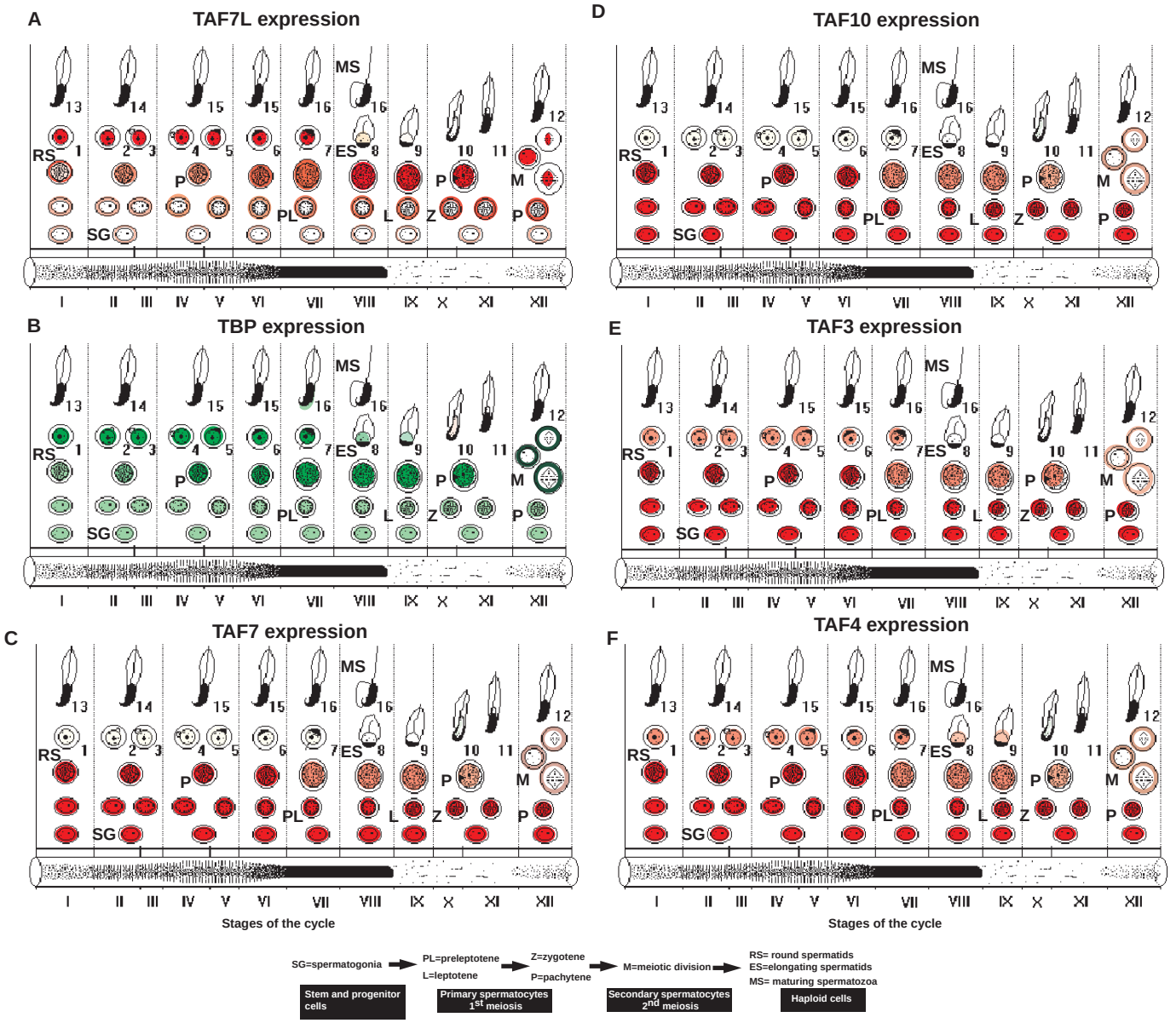

Fig. 5. (A-F) Summary of TBP and TAF expression during spermatogenesis. The spermatogenic differentiation programme is illustrated and the representative cell types are indicated below the bottom panels. Expression of TAFs is indicated in shades of red and that of TBP in panel B in shades of green.

Differential expression of TAF7 and TAF7L during spermatogenesis

We next compared the testis expression of TAF7L with that of its somatic paralogue TAF7. Staged squash preparations were double stained with a polyclonal antibody against TAF7L (green, in Fig. 2B) and the previously characterised anti-TAF7 monoclonal antibody [(Lavigne et al., 1996) red in Fig. 3B].

While TAF7L is uniquely located in the cytoplasm in early primary spermatocytes, TAF7 is strongly expressed in both the cytoplasm and the nucleus at this stage (Fig. 2B, stage XI, see zygotene spermatocytes, summarised in Fig. 5A,C). TAF7 is expressed in both the cytoplasm and the nucleus up until the late pachytene stage, where its expression declines (Fig. 2B, stages II and VII). In marked contrast to TAF7L, which is associated with the condensed chromatin in stage XII dividing spermatocytes, TAF7, like TBP, is excluded from the chromatin (Fig. 2B, stage XII). Furthermore, while TAF7L is strongly expressed in haploid cells, TAF7 expression is virtually undetectable in these cells. Hence, TAF7 and TAF7L exhibit a reciprocal mirror-like expression pattern. At early stages TAF7, but not TAF7L, is present in the nucleus, while at the pachytene stage both proteins are nuclear and in haploid cell nuclei TAF7L is strongly expressed, whereas TAF7 disappears (summarised in Fig. 5A,C).

TBP-associated and TBP-independent forms of TAF7L

To confirm the differential expression of TAF7 and TAF7L and to determine whether TAF7L is associated with TBP, extracts from mouse testis and from centrifugal elutriation purified populations of spermatocytes and haploid cells were prepared. The presence of TBP, TAF7L and TAF7 in cytoplasmic and nuclear extracts from whole mouse testis was verified. TAF7L and TAF7 were present in the nuclear extract and a significant fraction was also present in the cytoplasmic extract (Fig. 6A). While some TBP was also detected in the cytoplasmic extract, the majority was present in the nuclear extract (Fig. 6A). In addition, TBP, TAF7 and TAF7L could all be readily detected in extracts from purified spermatocytes, but, in agreement with the immunofluorescence, significantly lower amounts of TAF7 were seen in haploid cell extracts (Fig. 6D). As a control, the transcriptional coactivator ACT was detected only in the haploid cell extract showing efficient separation of the two cell polulations (Fimia et al., 1999).

To determine whether the cytoplasmic TAF7L is associated with TBP, this fraction was precipitated with the anti-TBP antibody

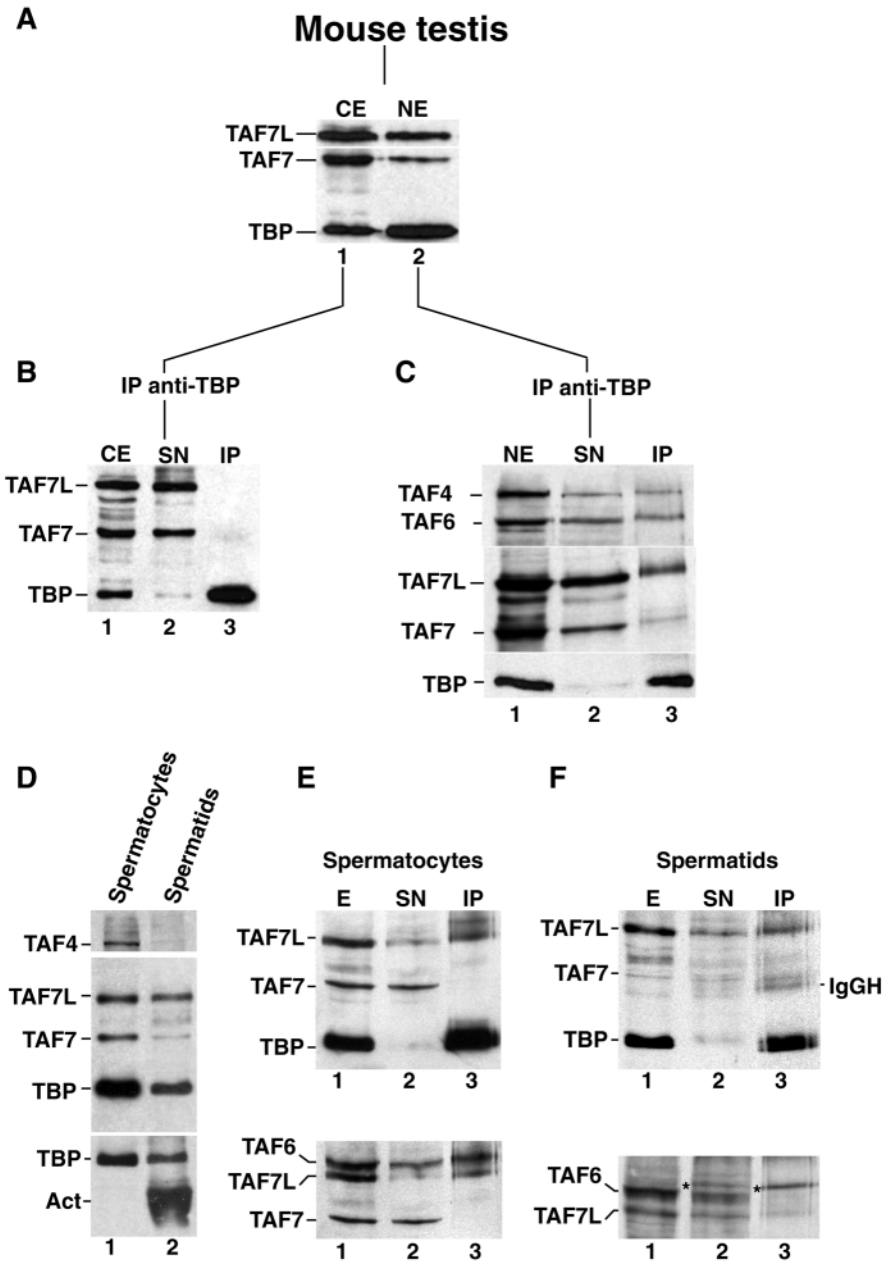

Fig. 6. Co-immunoprecipitation of TBP and TAF7L from testis extracts. (A) Immunoblots detecting TBP, TAF7 and TAF7L in cytoplasmic extract (CE) and nuclear extract (NE). (B) Immunoprecipitation of TBP from cytoplasmic extract. SN is the immunoprecipitate supernatant; IP the immunoprecipitated peptide-eluted fraction. (C) Immunoprecipitation of TBP from nuclear extract. (D) Detection of the presence of TBP and TAFs in total extracts from elutriation purified pachytene spermatocytes and haploid cells. The blot was first probed with antibodies against TBP, TAF7 and TAF7L and then reprobed with antibody against ACT and subsequently with TAF4. (E-F) The spermatocyte and haploid cell extracts (E) were immunoprecipitated with anti-TBP antibody. SN and IP are as described above. A small amount of IgG heavy chain which has leaked from the resin is visible in panel F, lane 3. The immunoblots were first probed with antibody against TBP, TAF7 and TAF7L (upper panel) and then reprobed with anti-TAF6 (lower panel). The presence of non-specific signal in lower panel F is indicated by asterisks (*).

that has been previously used to purify and characterise TFIID in HeLa cell extracts (Brou et al., 1993; Mengus et al., 1995). Immunoprecipitation of the cytoplasmic extract depleted almost all the TBP which was eluted using a peptide corresponding to the antibody epitope (Fig. 6B). Neither the cytoplasmic TAF7L nor TAF7 were coprecipitated with TBP (lane 3), showing that they are not associated with TBP. In contrast, a fraction of both TAF7L and TAF7 was coprecipitated with TBP from the nuclear extract (Fig. 6C, lane 3), indicating that a sub-population of these molecules is tightly associated with TBP. However, a significant amount of these proteins remains in the supernatant showing the existence of a TBP-independent form(s). Similar results were seen with other known TFIID subunits, TAF4 and TAF6, which were also present in both the supernatant and immunoprecipitated fractions from total testis extracts (Fig. 6C).

To determine whether TAF7L is associated with TBP in spermatocytes and/or haploid cells, the corresponding extracts were precipitated with the anti-TBP antibody. In extracts from spermatocytes, TAF7L was coimmunoprecipitated along with TBP, but no TAF7 was precipitated (Fig. 6E, upper panel). Similarly, TAF7L was also coprecipitated with TBP in extracts from haploid cells, however only trace amounts of TAF7 present in this extract were precipitated with TBP (Fig. 6F, upper panel). These results show that TAF7L, but not TAF7, is associated with TBP in spermatocytes, and in haploid cells much more TAF7L than TAF7 is precipitated with TBP. However, in each immunoprecipitation, a significant amount of TAF7L remains in the supernatant fraction indicating the existence of a TBP-independent form of TAF7L in both cell types.

The above immunoblots were reprobed for the presence of TAF6. Unlike TAF7 and TAF4, comparable amounts of TAF6 were present in both the spermatocyte and haploid cell extracts (Fig. 6E,F, lane 1, lower panel). TAF6 was coprecipitated with TBP from the spermatocyte extract, but little or no TAF6 was precipitated with TBP from the haploid cell extract and in both extracts a TBP-independent population of TAF6 exists.

Interaction of TAF7L with TAF1

To identify potential molecular targets of TAF7L a two hybrid screen was performed using a fusion of TAF7L to the DNA-binding domain of the yeast GAL4 and a testis cDNA library fused to the GAL4 activation domain (Fimia et al., 1999). The cDNA library was cotransformed along with the vector expressing the GAL4-TAF7L fusion into a yeast strain harbouring an integrated HIS3 gene under the control of GAL4 DNA-binding sites. Transformants were grown in the absence of histidine to select for clones that had activated the HIS3 gene. From 2×10^6 transformants, more than 180 HIS3-expressing clones were isolated. However, only two of these clones, which also grew most rapidly, displayed a significant expression of an integrated β-galactosidase gene under the control of GAL4 binding sites (Fig. 7A). A strong interaction of TAF7L with the proteins encoded by each of these clones was observed, while no significant β-galactosidase activity was observed in control experiments with the GAL4 activation domain alone, or when each clone was expressed in the presence of only the GAL4 DNA binding domain (Fig. 7A, and

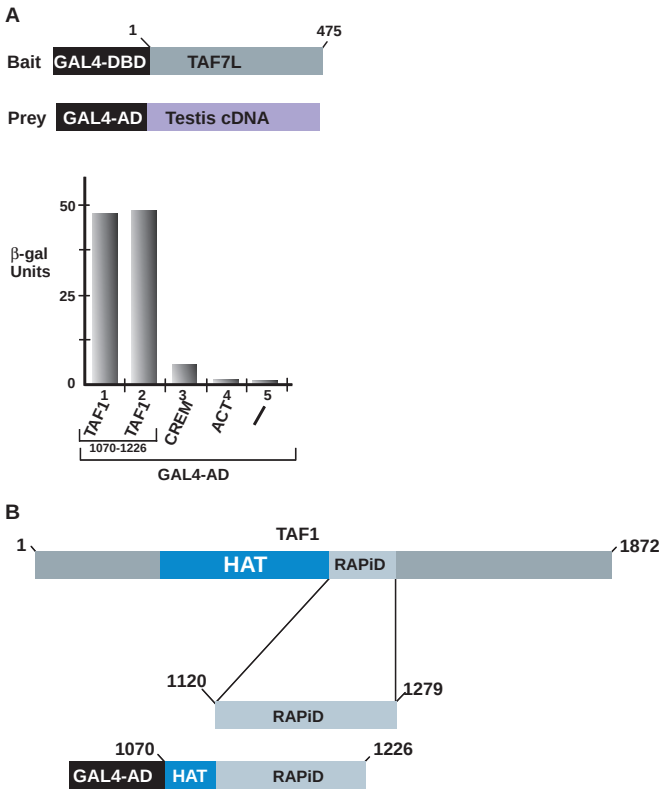

Fig. 7. Two hybrid interactions. (A) The TAF7L bait and the prey cDNA library are shown schematically. AD, activation domain; DBD, DNA binding domain. Graphical representation of quantitative two hybrid β-galactosidase assays. The GAL4-DBD TAF7L chimera shown above the graph was tested with the GAL4-AD chimeras shown below each column. The results obtained with the two independent TAF1(1070-1226) clones are shown. – indicates negative controls with the GAL4-AD alone. The values represent the average of two independent experiments. (B) Schematic representation of TAF1 and the isolated clones. TAF1 is shown along with the histone acetyl-transferase (HAT) and RAPiD domains. A comparison of the TAF1 RAPiD domain and the region found in the two hybrid clones is also depicted. The numbers above represent the TAF1 amino acids at the N and C-termini of the domains.

data not shown). Sequencing of these clones revealed them to be identical, both encoding amino acids 1070-1226 of TAF1, the largest TFIID subunit. This region overlaps with the previously described RAPiD domain (Fig. 7B), which interacts with the RAP74 subunit of transcription factor TFIIF (Ruppert and Tjian, 1995)

We also tested the ability of TAF7L to interact with the testis-specific activator CREM and its coactivator ACT (Fimia et al., 1999; Sassone-Corsi, 1998). However, in two hybrid assays, neither of these proteins showed significant interaction with TAF7L (Fig. 7A).

Differential expression of TAFs and TBP during male germ-cell differentiation

TBP and many TAFs are overexpressed in testis (Perletti et al., 1999). However, as shown above, TAF7 and TBP are strongly expressed at different stages of differentiation. This is contrary

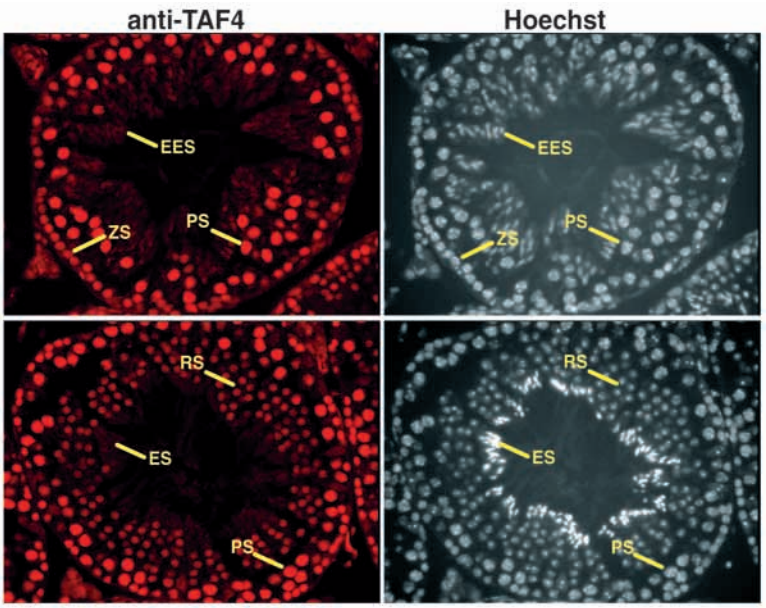

Fig. 8. Immunodetection of TAF4 in developing male germ cells. Immunofluorescence on sectioned mouse seminiferous tubules. TAF4 is shown in red. Representative examples of cell types are indicated. EES, early elongating spermatids; ES, elongating spermatids; PS, pachytene spermatocytes; RS, haploid round spermatids; ZS, zygotene spermatocytes. Magnification, 40×.

to what one may have expected for two subunits of the same complex. We therefore decided to investigate the expression of another TFIID component to evaluate whether its expression was coordinated with that of TBP. TAF10 is a subunit of both TFIID and the TFTC/STAGA complexes (Jacq et al., 1994; Martinez et al., 2001; Wieczorek et al., 1998). A monoclonal antibody against TAF10 (red), was used to determine its expression in staged squash preparations where strong expression is seen in leptotene, zygotene and early-stage pachytene spermatocytes, whereas it is only very weakly expressed in haploid cells (Fig. 3 and data not shown). At stage XII, TAF10 is not associated with the condensed chromatin of meiotically dividing cells and therefore cannot be associated with TAF7L at this time. Hence, TAF10 expression differs significantly from that of both TAF7L and TBP (summarised in Fig. 5D).

Lastly, we also investigated the expression of TAF4, a subunit of TFIID and TFTC (Brand et al., 1999; Gangloff et al., 2001b; Mengus et al., 1997). TAF4 is strongly expressed in the nucleus of early stage spermatocytes, however a weaker expression is visible in haploid round spermatids (Fig. 8, data not shown). The downregulation of TAF4 is also seen by comparing its presence in extracts from purified pachytene spermatocytes and haploid spermatids (Fig. 6D, summarised in Fig. 5E). Similar results were observed for TAF3 (data not shown summarised in Fig. 5F). Therefore, the TAF3 and TAF4 expression profiles are similar to those of TAF7 and TAF10, but are different from that of TBP.

Discussion

TAF7L a novel testis-specific protein with a developmentally regulated intracellular localisation

Here we describe the first characterisation of a male germ-cell-specific TAF paralogue in mouse. We show that TAF7L can associate with TBP, but that this interaction is developmentally regulated through differential intracellular localisation of the two proteins.

We report here the amino acid sequence of mouse and human TAF7L. The sequence of Wang et al. predicted the translation of a mouse TAF7L protein beginning at M94 or M97 that would have a molecular mass similar to that of TAF7 or human TAF7L (Wang et al., 2001). However, our sequence predicts the presence of an upstream in-frame methionine encoding a mouse TAF7L with an additional N-terminal region not found in human TAF7L. The molecular mass of the endogenous mouse testis TAF7L is consistent with the use of this methionine, but not with that of the downstream methionines.

Besides this domain, the amino acid sequences of both human and murine TAF7L show strong similarity to those of other TAF7 family proteins. The apparently minor differences in the sequences of TAF7 and TAF7L do however lead to a differential intracellular localisation of these proteins in primary spermatocytes and in meiotically dividing cells. One of the most striking features is the dynamic control of TAF7L intracellular localisation. In early stage spermatocytes, TAF7L is excluded from the nucleus and begins to enter only at the pachytene stage, whereas TAF7 is always present both in the cytoplasm and nucleus. Entry of TAF7L into the nucleus coincides with increased TBP expression and a wave of transcriptional activity in the pachytene cells. Both TAF7L and TBP are subsequently strongly expressed in the nucleus of haploid round spermatids, where they associate to form a TFIID complex with a unique composition specifically adapted to control the gene expression programme in these cells. Developmental regulation of intracellular localisation is unique to TAF7L as expression of TBP, TAF10, TAF4 and TAF3 are nuclear at all stages.

It is also striking that somatic TAF7 expression is strongly downregulated in haploid cells leading to a developmental switch between the somatic and germ-cell specific paralogues. In terms of association with TBP, this switch takes place in pachytene cells where TAF7L, TAF6 (and TAF11 and 13; J.-C.P., G.M. and I.D., unpublished), but not TAF7, is associated with TBP. This indicates that TFIID composition has been remodelled in these cells to include TAF7L and exclude TAF7. Thus, if TAF7 plays a role in pachytene cells, this function is TBP-independent. The function of TAF7L is likely to be predominant in haploid cells where its expression is significantly higher than that of TAF7, and much more TAF7L than TAF7 is associated with TBP. This suggests that TAF7L has a unique function in these cells that cannot be fulfilled by somatic TAF7. A unique function for TAF7L is also implied by the observation that it remains associated with the condensed chromatin during meiotic division, while TAF7 and TBP do not. TAF7L associates with specific chromatin domains at this time, but not with the dense heterochromatin. While its function at this stage is unknown, it is interesting to

speculate that it may 'bookmark' specific promoters or chromatin domains for activation (or repression) in haploid cells as has been suggested for TBP in somatic cells (Christova and Oelgeschlager, 2002).

TAF7L was first reported to be expressed in spermatogonia (Wang et al., 2001). The detailed study presented here shows that it is present in spermatocytes and in round haploid spermatids. Its expression and potential function at these later stages are interesting as the gene encoding TAF7L is present on the X chromosome (Wang et al., 2001), which is transcriptionally silenced in the XY body, possibly by the *Xist* RNA (Ayoub et al., 1997) in pachytene cells. As a consequence, at later developmental stages the TAF7L must be translated from a pre-existing mRNA store. Moreover, only around 50% of the haploid spermatids carry an X chromosome. This unique regulatory mechanism allows cells to express a potentially critical factor, despite the loss of the corresponding gene.

TAF7L has TAF-like properties

As discussed above, a TBP-TAF7L complex exists in both pachytene and haploid cells. The existence of these complexes is supported by biochemical analyses of pachytene spermatocyte and haploid cell extracts indicating that a sub-population of TAF7L is tightly associated with TBP. As TAF7 does not directly interact with TBP (Lavigne et al., 1996), it is probable that TAF7L interacts indirectly with TBP in the context of a complex with other TAFs. The TBP-TAF7L association may also be dynamic, as these proteins are physically separated during the meiotic divisions. In contrast, however, TAF7 is not associated with TBP in pachytene cell extracts. This shows that a specialised TFIID complex containing TAF7L, but lacking TAF7, exists in these cells. Hence, during spermatogenesis, TFIID composition is modulated by the inclusion of TAF7L and the exclusion of TAF7 and this process is probably regulated through changes in the intracellular localisation of TAF7L.

Further evidence for association of TAF7L with a TBP-TAF complex comes from a two-hybrid screen revealing a TAF7L-TAF1 interaction. TAF1 is the largest TFIID subunit and is TFIID-specific not being present in the TFTC-type complexes (Brand et al., 1999). The TAF7L bait independently isolated twice the same clone encoding the central region of TAF1 containing the RAPiD domain that has been shown to interact with the RAP74 subunit of transcription factor TFIIF (Ruppert and Tjian, 1995). A region of TAF1 overlapping with RAPiD was previously used in a screen of a HeLa cell cDNA library which isolated somatic TAF7 (Gegonne et al., 2001). The study of Gegonne et al. showed that TAF7 interacts with TAF1 amino acids 1120-1279 (Gegonne et al., 2001). The clone isolated here encodes amino acids 1070-1226, thus more precisely mapping the required TAF1 region to amino acids 1120-1226. The ability to interact with TAF1 is therefore common to TAF7 and TAF7L.

Together, the conserved TAF7L-TAF1 interactions and the coprecipitation of TAF7L with TBP indicate that TAF7L is a TFIID subunit in pachytene and haploid cells where the two proteins colocalise. This is the first demonstration that the testis-specific TAF paralogues are in fact bona fide TAFs. It remains to be determined whether the *Drosophila* Can protein (TAF5L), which shares strong sequence similarity with TAF5, is also associated with TBP (Hiller et al., 2001).

Although TAF7L can be coprecipitated with TBP, a significant fraction of TAF7L is not associated with TBP and hence may also function in a TBP-independent manner (we excluded the possibility that TAF7L associates with TLF, as these two proteins do not co-purify with one another; J.-C.P., G.M. and I.D., unpublished). Gel filtration analysis of this TAF7L fraction suggests that it is present in a high molecular mass complex(s) (J.-C.P., G.M. and I.D., unpublished). Purification of this complex(s) will allow the characterisation of its subunit composition and its functional properties.

Somatic TAF7 has been reported to interact with several transcriptional activators in vitro (Chiang and Roeder, 1995), however the functional significance of these interactions has not been demonstrated in vivo. Additionally TAF7 interacts with the nuclear receptors for vitamin D3 and thyroid hormone in mammalian cells (Lavigne et al., 1999). One possibility therefore is that TAF7L exerts its function by interacting specifically with haploid cell-specific transcriptional activators or coactivators such as CREM or ACT (Fimia et al., 1999; Sassone-Corsi, 1998). However, two-hybrid assays with these factors failed to reveal such interactions. Moreover, our two-hybrid screen with TAF7L did not isolate any known transcriptional activators, nor other obvious targets for TAF7L (J.-C.P., G.M. and I.D., unpublished). Although, by analogy with the somatic TAF7, we are presuming that TAF7L action is limited to the nucleus, we cannot exclude that TAF7L also has a function in the cytoplasm of early spermatocytes. Further genetic studies will be required to understand the role of TAF7L during spermatogenesis.

Remodelling of TFIID composition during spermatogenesis

Previous reports have shown that both TBP and TAFs are strongly overexpressed in testis (Perletti et al., 1999; Schmidt and Schibler, 1995). As these proteins are all part of the TFIID complex in somatic cells, it may have been assumed that they were overexpressed at the same developmental stage leading to a net increase in TFIID concentration. Here we show that this is not the case. TAF3, TAF4, TAF7 and TAF10 are strongly expressed in early stage spermatocytes and are downregulated in haploid cells, just the opposite of TBP. While the diminished levels of TAF7 may be compensated by the presence of TAF7L, no testis-specific paralogues of the other TAFs have yet been described in mammals which could substitute for the somatic TAFs in haploid cells. The low TBP/TAF ratio suggests that the above TAFs may in part function independently from TBP in primary spermatocytes. These TAFs are present in the TFTC-type complexes (Brand et al., 1999; Martinez et al., 2001; Ogryzko et al., 1998), suggesting that these complexes play an important role at this stage. However, the existence of novel TAF-containing complexes with specialised functions during germ-cell differentiation cannot be excluded.

An opposing situation is observed in haploid cells where expression of a key TFIID and TFTC component TAF10 is strongly diminished, while TBP is strongly upregulated. These changes in protein abundance suggest that the compositions of TFIID and TFTC in haploid cells may radically change both by the presence of TAF7L and the absence of TAF10. Given the key

role of the histone-fold-containing TAF10 in TFIID organisation (Gangloff et al., 2001a; Gangloff et al., 2001b; Gangloff et al., 2001c; Kirschner et al., 2002; Leurent et al., 2002; Mohan et al., 2003), the loss of this protein as well as the diminished expression of the other TAFs should lead to a disruption of TFIID structure and a consequent reduction in the amount of TFIID with the full complement of TAFs in haploid cells. Direct evidence for this comes from the observation that, while TAF7L can be found associated with TBP in haploid cell extracts, almost no TAF6 coprecipitates with TBP, whereas TAF6 is found associated with TBP in spermatocyte extracts. Hence, in haploid cells, partial TBP-TAF sub-complexes exist and TBP may also act in a TAF-independent manner, either alone or complexed with other factors. This is the first documentation of how the composition of a general transcription factor can change during a developmental process. The changes in TBP and TAF expression and the resulting remodelling of TFIID composition and that of other TAF-containing complexes highlight the unique nature of the general transcriptional machinery in haploid cells.

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