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Developmental and cell type-specific regulation of core promoter transcription factors in germ cells of frogs and mice

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Abstract

This article reports on the comparative cell type-specific expression profiles of selected core promoter-associated transcription factors during gametogenesis and embryogenesis in frogs and mice. In frogs we tested TBP, TRF2/TLF, TRF3, TFIIAαβ, and ALF, as well as variant forms of TAFs 4, 5, and 6. Four of these factors, TRF3, TAF4L, TAF5L, and the previously-characterized ALF gene, are preferentially expressed in testis and ovary. In mice we tested TBP, TRF2/TLF, TRF3, TFIIAαβ, and ALF. The results showed that while ALF was present in testis and ovary, as expected, TRF3 could only be detected in the ovary. RT-PCR experiments using RNAs from microdissected ovary tissue, together with in situ hybridization analysis, showed that TRF3 and ALF genes are specifically expressed in oocytes in both adult and prepubertal animals, whereas, their somatic counterparts, TBP and TFIIAαβ, are present in oocytes and in surrounding somatic cells of the follicle. Furthermore, both mice and frogs displayed a reduction in TRF3 and ALF transcript levels around the time of fertilization. In mice, transcripts from these genes could again be detected at low levels in embryonic reproductive tissues, but only reached maximal levels in adult animals. Finally, the results of protein-DNA interaction assays show that all combinations of core promoter complexes can be formed in vitro using recombinant TBP, TRF3, TFIIA, and ALF, including a TRF3-ALF complex. Overall, the diverse gene regulatory patterns observed here and in earlier reports indicate precise control over which transcription factor complexes can be formed in vivo during gametogenesis and early embryogenesis.

Keywords: Germ cell; Testis; Ovary; Gene regulation; Gene expression; Gametogenesis; Embryogenesis; Fertilization; General transcription factor; Core promoter

1. Results and discussion

The interaction between core promoter recognition factors and core promoter DNA is responsible for the accurate initiation of gene expression (Roeder, 1996). In somatic cells, this interaction involves a TFIID complex composed of TBP and TBP-associated factors (TAFs) together with a loosely associated factor TFIIA. In germ cells, regulation of gene expression involves additional core promoter recognition factors, including the TBP variant TRF2/TLF, a TFIIAαβ variant ALF (TFIIAτ), and several TAF variants (Aoyagi and Wassarman, 2000; Veenstra and Wolffe, 2001; Hochheimer and Tjian, 2003). While the details of how these new factors interact and function have yet to be established, they probably assemble into male- and/or female-specific TFIID-like complexes that regulate gene expression during gametogenesis and early embryogenesis. Thus, their existence may explain some of the unusual patterns of gene expression that occur in germ cells (Eddy and O'Brien, 1998; Kleene, 2001).

The regulation of genes that encode variant core promoter factors is surprisingly complex. These regulatory patterns are in some cases organism-specific and may involve preferential expression in male reproductive tissues, female reproductive tissues, somatic cells, and combinations thereof. For example, TAF4b is expressed in granulosa cells of the mouse ovary and in spermatocytes and plays an important functional role in both gametogenic systems (Freiman et al., 2001; Falender et al., 2005). On the other hand, a set of TAF variants identified in *Drosophila* are selectively expressed only in male germ cells and are only important for spermatogenesis (Hiller et al., 2004). In the case of TFIIAαβ and its variant ALF (TFIIAτ)

Abbreviations: BSA, bovine serum albumin; DIG, digoxigenin; EST, expressed sequence tag; GV, germinal vesicle; hCG, human chorionic gonadotrophin; MSCI, meiotic sex chromosome inactivation; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PMSG, pregnant mare serum gonadotropin; RT-PCR, reverse transcriptase-PCR; TAF, TBP associated factor; TBP, TATA binding protein.

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(Upadhyaya et al., 1999; Ozer et al., 2000), it is observed that while both factors are expressed in immature *Xenopus* oocytes, the TFIIAαβ transcript is maternally stored and only the ALF transcript contributes to the production of an active TFIIA-like factor (Han et al., 2003).

Genes of the TBP family are also regulated in complex patterns. For instance, the mouse TBP gene is expressed in all somatic tissues, but is transcribed to higher levels in spermatocytes (Schmidt and Schibler, 1995). Studies in *Xenopus* oocytes show that TBP transcripts are maternally stored, raising the possibility that there is a TBP replacement (Veenstra et al., 1999; Bell and Scheer, 1999; Jallow et al., 2004). One candidate, TRF2/TLF/TRP/TLP (Rabenstein et al., 1999; Maldonado, 1999; Teichmann et al., 1999; Moore et al., 1999; Ohbayashi et al., 1999), is transcribed to high levels in germ cells of mice but has been shown to only play a role during spermatogenesis (Martianov et al., 2001; Zhang et al., 2001). More recent studies have described another TBP-related factor, TRF3 (TBP2) (Jallow et al., 2004; Persengiev et al., 2003; Bartfai et al., 2004). TRF3 has been reported to be widely expressed in rodent and human tissues (Persengiev et al., 2003). However, other studies have suggested that TRF3 has a restricted role in oocytes and early embryos (Jallow et al., 2004; Bartfai et al., 2004), and its exact tissue-specificity is still uncertain. This report is aimed at characterizing and organizing the cell type-specific expression patterns of selected somatic and germ cell-specific core promoter transcription factor genes during gametogenesis and embryogenesis.

1.1. Expression of core promoter factors in Xenopus

Full-length transcripts for *Xenopus* TBP, TRF2, TRF3, TFIIAαβ, ALF, and actin were identified either by homology searches of *Xenopus* EST databases or from their GenBank entries. PCR primers designed against unique regions of each sequence were used to evaluate expression in heart, liver, lung, kidney, spleen, ovary, and testis (Fig. 1A). Results with TBP and TRF2 showed these genes were expressed ubiquitously but were present at higher levels in ovary and testis (lanes 6 and 7). In contrast, expression of the TBP-related factor TRF3 could only be detected in testis and ovary (lanes 6 and 7). Data on other factors provide a basis for comparison.

We also evaluated whether members of the TBP family were differentially expressed in developing oocytes spanning stages I–VI. The results showed transcripts from the TBP, TRF2, and TRF3 genes to be present at similar levels at each stage (Fig. 1B, lanes 1–5). However, when oocytes were matured in vitro by the addition of progesterone, we observed a drop in TRF3 levels (lane 6). To test whether TRF3 levels continued to decline during early development, we examined expression in eggs and in embryos at stages 6.5, 12, and 35. The results show that TRF3 transcripts drop to nearly undetectable levels sometime between embryonic stages 6.5 and 12 (Fig. 1C). Thus, the TRF3 gene appears to be germ cell-specific in *Xenopus* and is precisely coexpressed in a manner similar to that seen with the ALF gene (Han et al., 2003, 2004).

1.2. Expression of alternate Xenopus TAF subunits

TBP associates with somatic TAF subunits to form a large TFIID complex that has roles in promoter recognition and coactivation. Studies in humans, mice, and *Drosophila* have all resulted in the identification of germ cell-specific TAF variants (Freiman et al., 2001; Hiller et al., 2001, 2004; Wang and Page, 2002; Pointud et al., 2003). For this reason, we used database searches to identify possible TAF variants in *Xenopus laevis*.

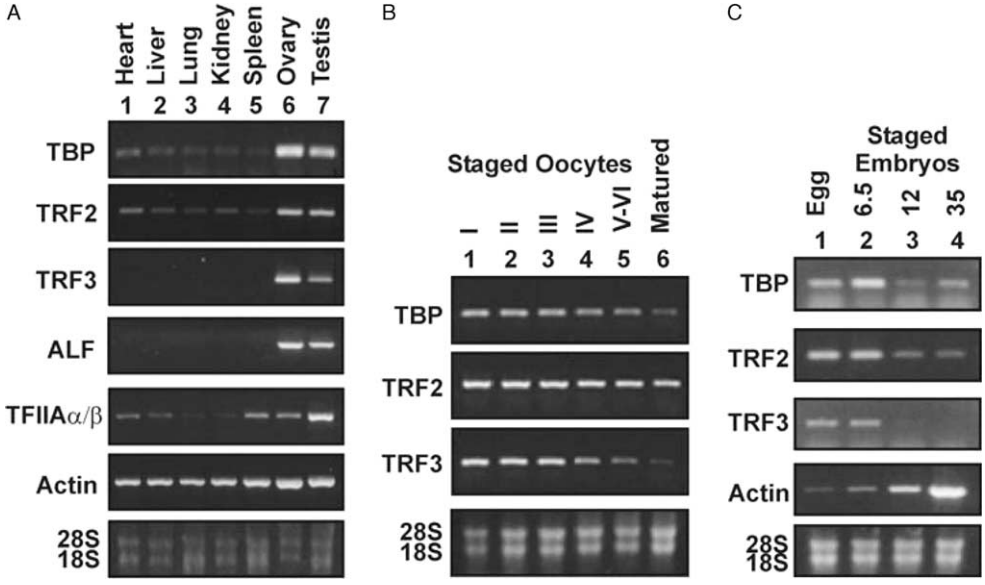

Fig. 1. Expression of somatic and germ cell-specific core promoter recognition factors in *Xenopus*. (A) RT-PCR analysis of TBP, TRF2, TRF3, ALF, TFIIAαβ and actin in heart (lane 1), liver (lane 2), lung (lane 3), kidney (lane 4), spleen (lane 5), ovary (lane 6), and testis (lane 7). (B) Expression of TBP family members in developing oocytes; stage I (lane 1), stage II (lane 2), stage III (lane 3), stage IV (lane 4), and stage V/VI (lane 5). Transcript levels in progesterone-treated (mature) oocytes are shown in lane 6. (C) Expression of TBP family members in eggs (lane 1) and embryos of stage 6.5 (lane 2), 12 (lane 3) and 35 (lane 4). Actin and 28/18S RNA levels are used as controls for normalization of RNA.