# Molecular Mechanisms Controlling the Differentiation of Germ Cells in mammals: Divergent expression patterns of SLBP in male and female meiotic cells and their developmental consequences.

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**Abstract**

The stem-loop binding protein (SLBP) binds to replication-dependent histone mRNA and participates in its processing, stabilization and translation. It has previously been shown that SLBP expression in somatic cells is regulated by the cell cycle. Our work demonstrates that male and female germ line cell expression patterns differ dramatically both from the somatic cell-cycle regulated pattern, and from each other. Using immunofluorescence on a post-natal series of male and female gonads, SLBP was shown to translocate from the cytoplasm of non-growing oocytes to the nucleus upon initiation of growth, followed by re-entry into the cytoplasm upon entry into metaphase II. In contrast, developing male germ cells initially express SLBP at high levels both in the nucleus and the cytoplasm, followed by cytoplasmic segregation and finally complete absence of expression by maturation. We believe that these divergent expression patterns in male and female germ cells reflect the different requirements for SLBP in cell differentiation and early embryogenesis, respectively.

**Introduction**

Understanding how a complex organism results from a single cell is the focus of research in developmental biology, and despite the identification of numerous pathways and processes involved, it remains one of the most persistent and intriguing questions in current biology. A few hundred years ago the prevailing theory for development, known as preformation, was that miniaturized copies of adults existed ready-made inside the egg. In modern times, we have come to view development as a process of epigenesis: the progressive addition and differentiation of characters beginning from an undifferentiated state – ultimately, the fertilized egg. Molecular, genetic, and embryological approaches have all been used to gain a better appreciation of this process, but there remains an overwhelming amount of work to be done, even with model systems such as the mouse.

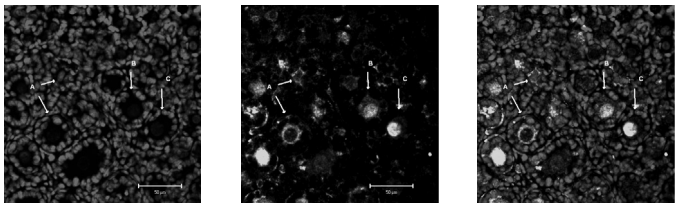
Without the precisely orchestrated process of germ cell specification and maturation, embryogenesis would not occur normally. It therefore stands to reason that in order to fully comprehend animal development, both the haploid (germ cell) and diploid (embryonic; adult) stages of the life cycle must be considered. The molecular mechanisms controlling germ cell development, and their relationship to embryonic development, are active areas of research.

Eukaryotic DNA is packaged as chromatin, a structure consisting of DNA bound to proteins known as histones. In order for cell division to take place, not only must DNA be duplicated, but a sufficient supply of new proteins for DNA packaging must also be available. The stem-loop binding protein (SLBP) is a 31kDa RNA-binding protein that binds to a conserved stem-loop sequence in the 3'-untranslated region of histone mRNA and plays a key role in cellular proliferation by promoting the expression of histones. In somatic cells, most histones are synthesized during S-phase, the stage of the cell cycle where DNA is replicated. SLBP accumulates just prior to the onset of S-phase, and following S-phase, it is rapidly degraded [Whitfield et al. 2000; Marzluff and Duronio 2002].

## Results

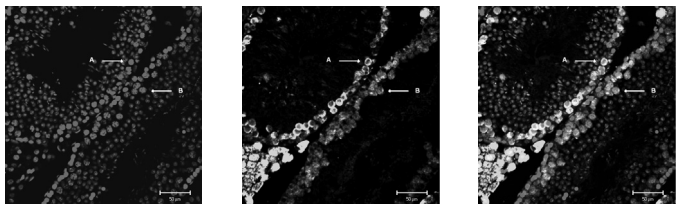
In order to define the role of SLBP in meiotic cells, we used immunofluorescence on sectioned ovarian tissue to detect its expression in the developing oocyte. We found that SLBP is present only the cytoplasm of non-growing oocytes and that upon initiation of oocyte growth, SLBP accumulates to very high levels in the nucleus (figure 1). It remains sequestered in the nucleus during the stage when it would normally be degraded in somatic cells. Only upon progression to metaphase II does SLBP relocate to the cytoplasm where it participates in the translation of histone messages [Allard et al. 2002; our data].

To assess whether SLBP had a conserved role in meiotic cells, we examined the expression of SLBP in developing spermatozoa using immunofluorescence on sectioned testes. Strikingly, the expression pattern was nearly the converse of that seen in oocytes, and markedly different from somatic cells. Early in male meiotic cell development, spermatogonia and spermatocytes expressed SLBP at high levels in both the nucleus and the cytoplasm. Later in development, SLBP becomes sequestered to the cytoplasm in a subset of the spermatocyte population, before its expression is lost completely by the spermatid and spermatozoa (mature sperm cell) stage (figure 2). These results were verified by immunoblotting protein extracts from isolated male germ cell populations, which showed a progressive loss of SLBP as cell populations matured (data not shown).



(1) DNA stain (PI) (II) SLBP (488-tyramide) (III) DNA/SLBP overlay

**Figure 1. Three-day old ovarian section** Cytoplasmic SLBP (A) present in the oocytes in primordial non-growing and early growing follicles. A rapid transition from the cytoplasm to the nucleus appears to occur as SLBP begins to accumulate in the nucleus (B) while some remains cytoplasmic, and finally becomes nearly entirely nuclear (C).



(1) DNA stain (PI) (II) SLBP (488-tyramide) (III) DNA/SLBP overlay

**Figure 2. Seven-week old testis section** SLBP is present exclusively in a subset of the developing male germ line population. SLBP is localized either exclusively in the cytoplasm (A) or is present both in the cytoplasm and the nucleus (B) according to the stage of development of the germ cells. As the male germ cell develops SLBP is lost.

## Discussion

Unlike a somatic cell, the mammalian oocyte must accumulate massive amounts of protein and mRNA necessary for not only one, but several cell divisions in the early stages of embryonic development [Song and Wessel 2005]. We believe that the high level of SLBP in the cytoplasm, followed by its translocation and concentration in the nucleus is necessary in order to safe-guard the histone messages until they are needed, which is upon fertilization. In support of this interpretation, mutant mice expressing RNAi targeted against SLBP, specifically in the growing oocyte, yield embryos whose development arrests at the 2-cell stage [H. Clarke, unpubl. data]. This presumably owes to a deficit of histones, which impedes the ability of the blastomeres to divide beyond the 2-cell stage. Prior to fertilization, the oocyte does not replicate its DNA, and high histone and SLBP levels do not seem to be necessary for oocyte survival, as embryos are able to reach the 2-cell stage. It therefore seems that the reason for the high level of SLBP expression in the oocyte is to facilitate development of the embryo.

The developing male germ cell does not accumulate histones to constitute early embryonic chromatin. In fact, during the process of maturation, the spermatozoa even ultimately replace their own histones with protamines, proteins more efficient at condensing the DNA into a tiny streamlined capsule for efficient transport to the egg [Govin et al. 2004]. Early in this process of chromatin remodeling, however, several histone variants are first synthesized and loaded onto the chromatin. We suggest that the high levels of SLBP expression seen in both the nucleus and cytoplasm during the early stages of spermatogenesis, before the bulk remodeling takes place, is due to the stockpiling of histone variants for this process. Later in development, the attenuation of SLBP may be necessary in order to deplete histone expression and facilitate replacement by the more effective DNA condensing proteins. Experiments are currently being planned to test this hypothesis. Transgenic mice over-expressing SLBP in maturing spermatozoa will be assayed for functional defects in the remodeling process. Because this remodeling process is undone by the oocyte upon fertilization using the stockpile of histones, and no function for highly condensed male chromatin has yet been found for embryonic development, the best way to account for SLBP expression pattern in the male germ line is that it is required for the differentiation of sperm cells. In other words, germ line developmental demands

govern SLBP expression patterns in spermatozoa, as opposed to embryonic development, for which SLBP is required in the oocyte.

**Methods**

**Histology**

Ovaries and testes were dissected from CD-1 mice and fixed overnight in a 10% formalin solution at 4°C with agitation. Tissue was dehydrated through a graded ethanol/xylene series, embedded in paraffin, and stored at -20°C until needed. 5_m sections were cut on a microtome and mounted on slides, and stored at 4°C for up to 24 hrs. Rehydration was followed by antigen recovery in 0.1% sodium citrate at 80-90°C for 15min.

**Immunofluorescence**

Slides were blocked in 0.1% tween-20, 5% BSA, 5% goat serum for 30 min, and then incubated in purified anti-SLBP 1:200 in block overnight at 4°C. Slides were washed in PBS, and then incubated with HRP conjugated secondary antibody diluted 1:200 in block for 1hr at room temperature. Slides were then incubated with Alexa Fluor 488-labeled tyramide for 10 min. Slides were washed, and propidium iodide diluted to 0.1ng/_l was added for 5 min. Slides were mounted in mowiol and stored dark at 4°C. Images were captured using a confocal microscope.

## References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Photograph by Claudio Callagaris