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Short Contribution

The meiotic specific synaptonemal complex protein SCP3 is expressed by female and male primordial germ cells of the mouse embryo

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ABSTRACT The synaptonemal complex proteins SCP3 and SCP1 are components of the synaptonemal complex, a meiosis-specific protein structure essential for synapsis of homologous chromosomes. Using polyclonal antibodies raised against SCPs of rat testis, we have studied the expression of these proteins in embryonic germ cells of the mouse embryo using immunohistochemistry and immunoblotting. This investigation provided the first description of the sequential appearance of SCP3 and SCP1 during the different stages of the female meiosis in the mouse. Most importantly, we found that also male primordial germ cells express SCP3 for a short time before undergoing G1 arrest. This strongly supports the hypothesis that primordial germ cells are programmed to enter meiosis unrespective of the sex and that foetal testis produces a factor that inhibits such programme.

KEY WORDS: *synaptonemal protein, meiosis, primordial germ cells, SCP3*

Synaptonemal complexes (SCs) are structures that are formed between homologous chromosomes during the meiotic prophase. They are probably involved in chromosome pairing and recombination. SCs consist of two proteinaceous axes, one along each homologue, referred to as lateral elements (LEs) connected by transverse filaments (TFs). On the TFs there is a third longitudinal element, the central element (CE) (for a review see Heyting, 1996). In rodents, two components of the LEs have been identified, SCP (synaptonemal complex protein) 2 and SCP3 (Dobson *et al.*, 1994; Lammers *et al.*, 1994). Two components of the CE, namely SCP1 (Meuwissen *et al.*, 1992; Dobson *et al.*, 1994) and an unnamed 48 kDa protein (Smith and Benavente, 1992), have also been identified. Moreover, a protein called Xmr seems to be required for the SC assembly of the XY chromosomes, although its localisation in the SC remains to be established (Calenda *et al.*, 1994). The assembly and disassembly of SCs correlate with the successive rearrangements of chromatin. Early in the meiotic prophase (leptotene), LEs are formed along the chromosomes, presumably between the sister chromatids; during zygotene, the transverse filaments connect them, and the CE is formed between them. In pachytene, the homologous chromosomes are connected (synapsed) by the tripartite structure of SCs along their entire length. Subsequently, the SCs are disassembled (diplotene) and chiasmata show up as physical connections between homologues. All identified components of SCs occur specifically in nuclei of meiotic prophase cells, i.e. rat, mouse and human spermatocytes (Heyting *et al.*, 1988; Offenberg *et al.*, 1991; Smith and Benavente, 1992; Liu *et al.*, 1996; Pousette *et al.*, 1997) and rat oocytes (Dietrich *et al.*, 1992).

This work was undertaken to study the expression of SCP1, SCP3 and Xmr in the germ cells of the mouse embryos with the aim to correlate the time at which these proteins are expressed with sex differentiation of gonads and with the stages of meiotic prophase in the mouse oocytes. For this purpose, we used polyclonal antibodies that recognise components of SCs of mouse spermatocytes (Liu *et al.*, 1996). In the mouse embryo, sex differentiation of germ cells in oocytes and prospermatogonia occurs around 13.5 *days post coitum* (dpc) in parallel with the morphological differentiation of gonads in testis and ovary. Until this time, the appearance and behaviour of primordial germ cells (PGCs), the precursors of oocytes and prospermatogonia, seem identical whether they are in female or in male embryos. In female

Abbreviations used in this paper: SCP, synaptonemal complex protein; LE, lateral element; TF, transverse element; CE, central element; dpc, days post coitum; PGCs, primordial germ cells.

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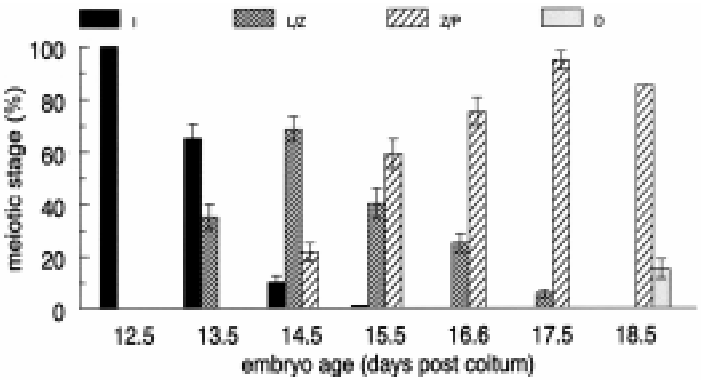

Fig. 1. Meiotic stage composition of the fetal mouse ovary. *Columns represent the mean± standard error of three determinations performed on at least 200 nuclei in ovaries of each age. I, interphase; L, leptotene; Z, zygotene; P, pachytene; D, diplotene.*

embryos, around 13.5 dpc PGCs entering the prophase of meiosis are called primary oocytes: they pass through leptotene, pachytene and zygotene and around birth become arrested at the diplotene stage. At the same time that germ cells in the ovary are entering meiosis, PGCs in the testis of male embryo undergo mitotic arrest: they stop dividing and remain as prospermatogonia in the G1 stage of the cell cycle until birth. Germ cells in the testis do not normally enter meiosis until at least a week after birth. Several evidences indicate that a short-range diffusible factor produced in the male gonadal ridges acts upon germ cells to arrest them in G1 and prevent them entering meiosis (Byskov, 1974; Dolci and De Felici, 1990; McLaren and Southee, 1997). Whether a meiosis-inducing substance (Byskov and Saxen, 1976; Byskov *et al.*, 1995) is required to permit entry of an embryonic germ cell into meiosis or if this is a germ-cell autonomous event (Zamboni and Upadhyay, 1983; McLaren and Southee, 1997), is still controversial.

Figures 1 and 2 show the timing of appearance and disappearance of successive stages during meiotic prophase in the ovary. The results are in agreement with those of Borum (1961) and Bakken and McClanahan (1978). Figure 3 shows the results of the immunofluorescence staining of frozen sections of the gonads of various ages. The antigens recognised by the anti-SCP3 antibody appear around 13.5 dpc as fluorescent dots both in male and female germ cells (Fig. 3A and B). In these latter, a higher number of dots were visible. Similar aggregates of SCP3 immunopositive material have been described in rat leptotene oocytes (Dietrich *et al.*, 1992) and mouse and human spermatocytes (Scherthan *et al.*, 1996). On day 14.5 dpc, SCP3 positive staining was barely visible in prospermatogonia and no staining was present at later stages (not shown). In oocytes at the leptotene/zygotene stage, some fluorescent dots remained and a diffuse immunofluorescence staining of chromosomes could be seen (Fig. 3C and D). Chromosomes resulted more clearly stained with the anti-SCP3 antibody in pachytene oocytes (17-18 dpc) (Fig. 3D and E). As the oocytes reached the diplotene stage chromosome staining became again diffuse and some fluorescent dots reappeared (Fig. 3E and F). Immunoblotting experiments confirmed the expression pattern of SCP3. One specific band (30,000 KDa) and two specific bands (30,000 and 33,000 KDa) were present in extracts from 13.5 dpc ovaries and testes, respectively (Fig. 5A). In 14.5 and 16.5 dpc testes the signals disappear while in the ovaries an intense 30,000 band and a faint 33,000 band were present. The presence of two closely immunologically related SCP3 proteins, probably encoded by the same gene and post translationally modified, has been described in the rat spermatocytes (Lammers *et al.*, 1994, 1995). These proteins are components of the LEs of the synaptonemal complexes that are expressed in the early stages of the meiotic prophase. The finding that SCP3 proteins are expressed both in female and male germ cells indicates that at the end of the proliferation period primordial germ cells of both sexes are preparing to enter into meiosis. In some species, including mouse, in some germ cells a preleptotene condensation stage of chromatin has been described both in female and male embryonic germ cells (Byskov and Høyer, 1994). Hilscher *et al.* (1974) interpreted this stage as atretic mitosis but according to our results it seems more likely that this stage represents an early stage of premeiotic chromosome condensation. In any case, while in the ovary oocytes complete DNA synthesis and enter in the leptotene stage, in the testis prospermatogonia undergo G1 phase arrest and chromatin decondenses.

The antigens recognised by anti-SCP1 antibody appear around 14.5 dpc in zygotene oocytes (Fig. 4A). A clear positive staining appears on chromosomes up to the stage of late pachytene (Fig. 4B). Thereafter, staining becomes diffused and disappears (not shown). No staining was visible in male germ cells. Immunoblotting

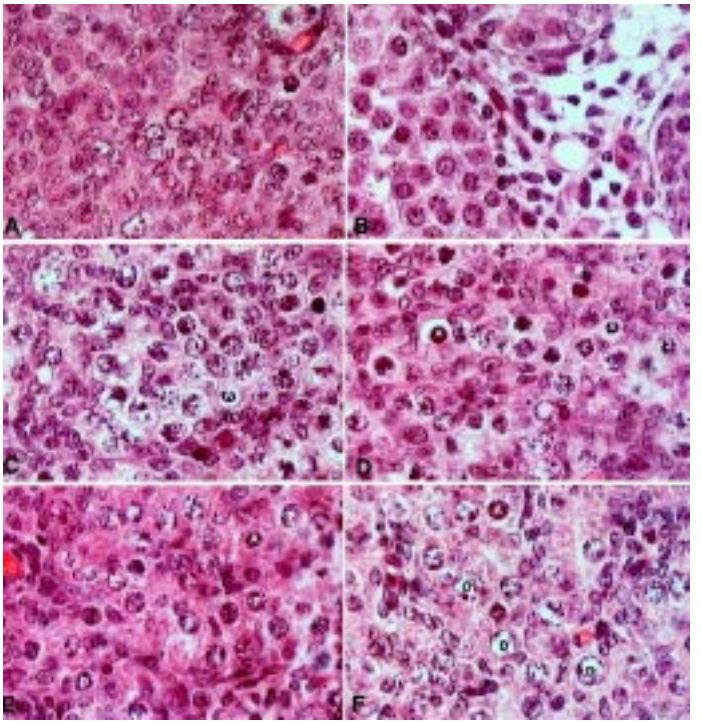

Fig. 2. Paraffin sections of fetal gonads at different developmental stages. *The sequence of sections and the nuclear morphologies parallel those showed in Figure 3.* **(A** *and* **B)** *13.5 dpc ovary and testis, respectively. No meiotic nuclei are clearly distinguishable.* **(C** *and* **D)** *14.5 and 15.5 dpc ovary; most of the nuclei are in the leptotene (L) or zygotene (Z) stage.* **(E** *and* **F)** *17.5 and 18.5 dpc ovary; most of the nuclei are in the pachytene (P) or diplotene (D) stage. A, oocytes in apoptosis. Magnification approximately 300X.*

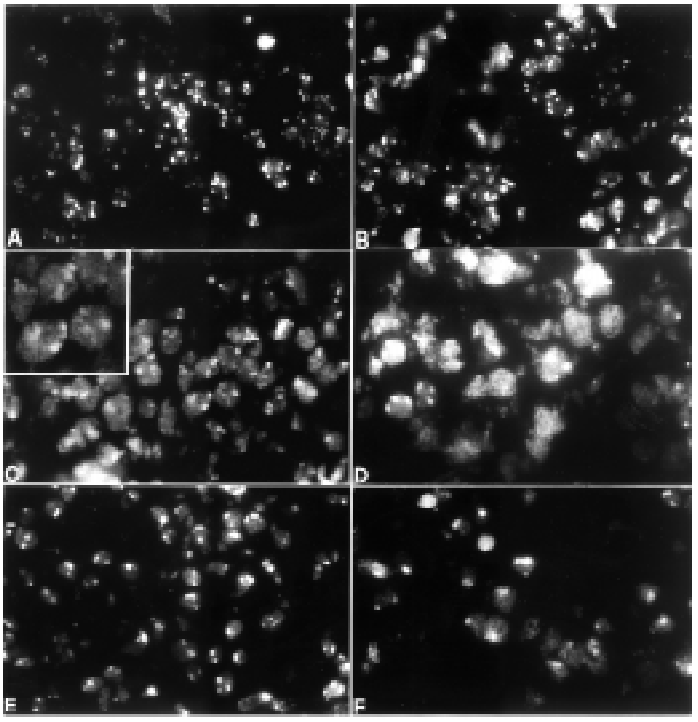

Fig. 3. Details of frozen sections of fetal gonads stained with anti-SCP3 antibody. (A *and* **B)** *13.5 dpc ovary and testis, respectively. Note the presence of fluorescent dots both in female and male germ cells. No meiotic nuclei are clearly distinguishable. Magnification approximately 300X.* **(C** *and* **D)** *14.5 and 15.5 dpc ovary. Most of the nuclei are in the leptotene/zygotene or zygotene/pachytene stage. Magnification approximately 500X. Inset, 750X.* **(E** *and* **F)** *17.5 and 18.5 dpc ovary. Most of the nuclei are in the pachytene or diplotene stage. Magnification approximately 300X.*

confirmed the SCP1 expression pattern found with immunohistochemistry (Fig. 5B).

The anti-Xmr antibody was used for histochemistry only and no specific staining was seen.

In conclusion, our results show that the sequential appearance of components of the synaptonemal complex during meiosis of the mouse oocyte is quite similar to that described in rat oocytes and mouse, rat or human meiotic male germ cells. In addition, the expression of SCP3 reported in this study both in female and male PGCs favours the hypothesis that entering into meiosis is a germ cell autonomous event. Most importantly, our results represent the first evidence that primordial germ cells are programmed to enter meiosis unrespective of the sex of the embryo.

Experimental Procedures

Antibodies

The antibodies used in this paper were generously provided by Dr. Chister Höög (Karolinska Institute, Stockholm). Polyclonal antibodies were risen against recombinant proteins from rat testis. The production and specificity of the antibodies are described in Liu *et al.* (1996).

Gonad histology and immunohistochemistry

Gonadal ridges (11.5-12.5 dpc), ovaries and testes (13.5-18.5 dpc) were dissected from CD-1 mice embryos. One of each pair of gonadal primordia was fixed in Bouin's fluid and the other embedded in Tissue-tek OCT compound and frozen in liquid nitrogen. Gonads fixed in Bouin were subsequently processed following standard paraffin embedding and stained with hematoxilin and eosin to determine the meiotic stages. For immunohistochemistry, sections (8 μm) of OCT embedded samples were cut in a cryostat, air dried onto poly-L-lysine-coated slides and incubated in 1:20 dilution of anti-SCP1, anti-SCP3 or anti-Xmr antibodies (2 h at room temperature) (Liu *et al.*, 1996). After primary antibody incubation, sections were rinsed with PBS containing 20 mg/ml BSA (2 h, 3 changes) and incubated with 1:50 secondary TRITC-anti-rabbit IgG antibodies from sheep (Sigma) (30 min at room temperature). For each age at least 10 gonads were studied. Control experiments were performed using rabbit serum as primary antibodies or the secondary antibody only.

Immunoblotting

Samples of 20-40 gonads were solubilised in Laemmli sample buffer (10% glycerol, 2% SDS, 0.06 M TRIS-HCL-pH 6.8, 5% β-mercaptoethanol, 0.02% bromophenol blue) on ice, sonicated and boiled before submitted (about 50 μg protein/lane) to 12% SDS-PAGE. Proteins were transferred to nitrocellulose membranes, vacant sites blocked with 10% non-fat milk in PBS and 0.01% Tween-20 and the washed membranes incubated for 1-2 h at room temperature with the anti SCP3 or anti-SCP1 antibodies (1:200 dilution). Secondary antibodies and detection reagents were used according to the instructions of the enhanced chemioluminescent (ECL) kit (Amersham). Experiments were repeated at least three times for each embryonic age examined.

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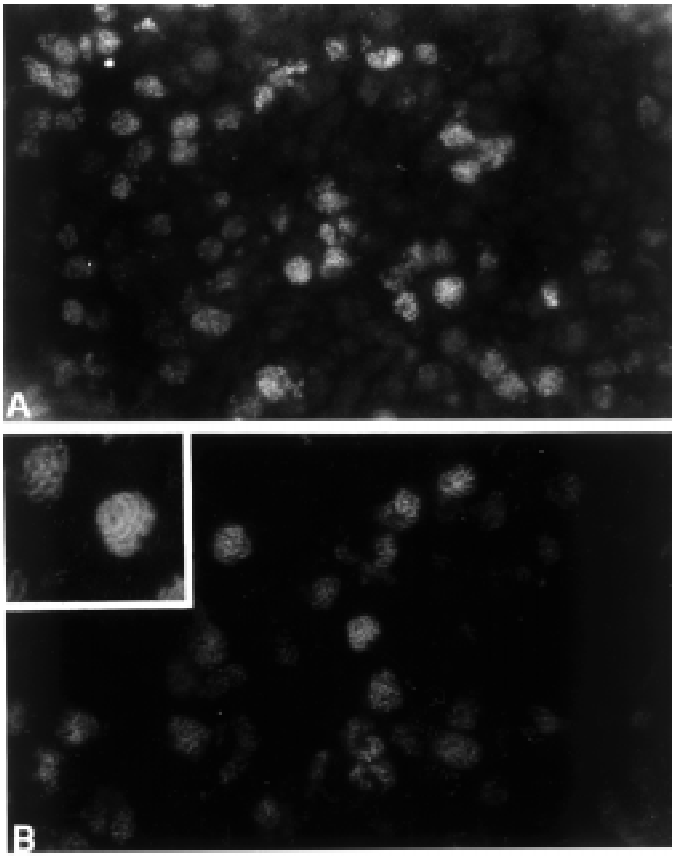

Fig. 4. Details of frozen sections of fetal gonads stained with anti-SCP1 antibody. (A) *14.5 dpc ovary. Most of the nuclei are in the leptotene/zygotene stage.* **(B)** *16.5 dpc ovary. Most of the nuclei are in the pachytene stage. Magnification approximately 300X. Inset, 600X.*

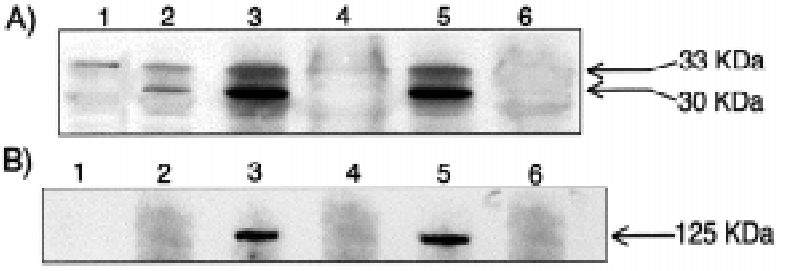

Fig. 5. Immunoblotting of extracts of fetal ovaries and testes of various ages for SCP3 (A) and SCP1 (B) proteins. **(A)** *Lane 1, 13.5 dpc ovaries; lane 2, 13.5 dpc testes; lane 3, 14.5 dpc ovaries; lane 4, 14.5 dpc testes; lane 5, 16.5 dpc ovaries; lane 6, 16.5 dpc testes. Note the presence of one (35 KDa) or two (35 KDa and 30 KDa) specific bands in 13.5 dpc ovaries and testes, respectively. At later stages both specific bands were detected in the ovaries but not in the testes.* **(B)** *Lane 1, 13.5 dpc ovaries; lane 2, 13.5 dpc testes; lane 3, 14.5 dpc ovaries; lane 4, 14.5 dpc testes; lane 5, 16.5 dpc ovaries; lane 6, 16.5 dpc testes. Note the presence of a specific band (125 KDa) in the ovary from 14.5 dpc onward but not in the testes.*

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