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Letter to the Editor

The checkpoint monitoring chromosomal pairing in male meiotic cells is p53-independent

Dear Editor,

The high incidence of spermatocyte apoptosis described in infertile men is largely attributable to chromosomal asynapsis and underscores the need for better animal models to understand how damaged meiotic germ cells are selectively eliminated.[1] We have previously inactivated the *Scp3* gene (also known as *Cor1*) in mice, which encodes a major structural component of the axial/lateral element of the synaptonemal complex (SC).[2] The spermatocytes in these null animals exhibit extensive chromosomal asynapsis at an early meiotic stage and undergo apoptosis. Several mechanisms may mediate this selective apoptotic response. For example, it is known that p53 is required for checkpoint-induced apoptosis in male germ cells in response to irradiation[1] and in response to loss of the ataxia-telangiectasia protein (ATM).[3,4]

We have now generated *Scp3*$^{-/-}$*Trp53*$^{-/-}$ mice to investigate the involvement of p53 in the apoptotic response seen in SCP3-deficient germ cells (Figure 1A). We find that p53-deficiency fails to rescue the meiotic prophase I defects seen in *Scp3*$^{-/-}$ testes (Figure 1B). While we found a full complement of male germ cells in the testes of wild-type and *Scp3*$^{+/+}$*Trp53*$^{-/-}$ animals in sections stained with hematoxylin and eosin, a drastically different spermatogenic process was observed in *Scp3*$^{-/-}$ and *Scp3*$^{-/-}$*Trp53*$^{-/-}$ mice (Figure 1B). Testes null for both *Scp3* and *Trp53* (the gene encoding p53) are indistinguishable from SCP3-deficient testes, in that both contain spermatocytes which undergo apoptosis coincident

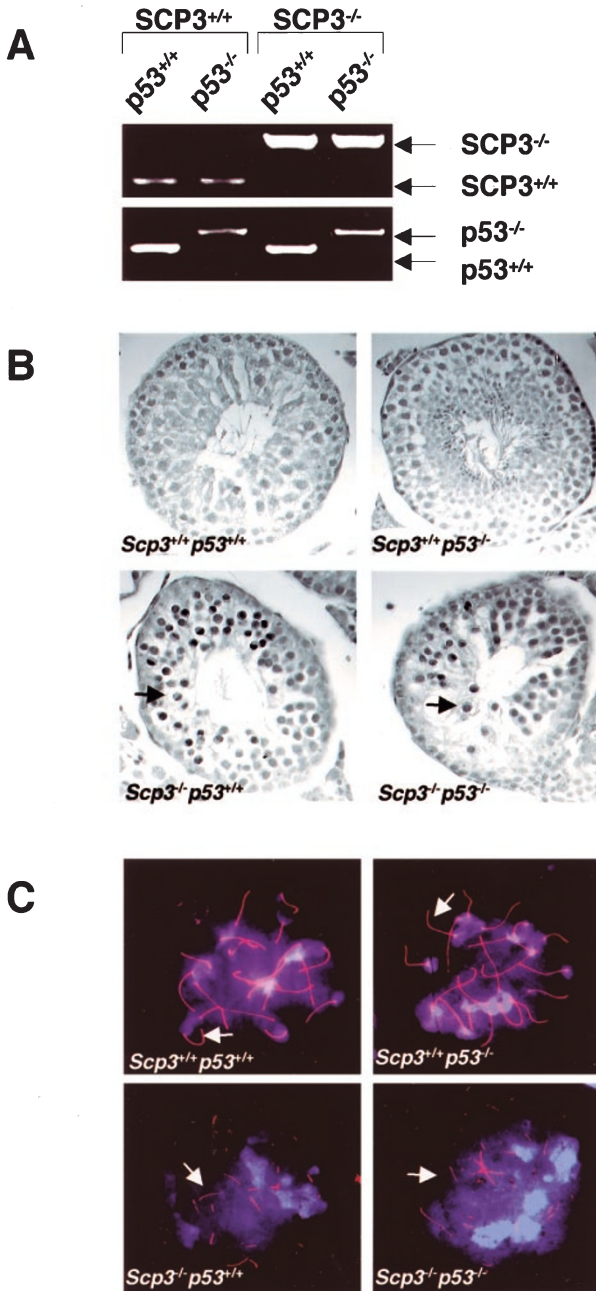

Figure 1 (**A**) Generation of *Scp3*$^{-/-}$*Trp53*$^{-/-}$ mice. *Scp3*$^{+/-}$ and *Trp53*$^{+/-}$ mice were generated as described previously.[2,13] PCR was carried out using tail DNA from representative adult offspring of *Scp3*$^{+/-}$*Trp53*$^{+/-}$ matings. *Scp3* and *Trp53* status were confirmed by PCR using primer sets that distinguished the targeted and wild-type alleles. *Scp3*-specific primers were *ForNeo*, 5′-AAGTGCTGCTTCTCAGGGTAG, *F1Neo1*, 5′-AGAGCAGCC-GATTGTCTGTTG and *SCP3map2*, 5′-ATGTTTGCTCAGCGGCTCCGTGA. Primers *ForNeo* and *F1Neo1* give a 552 bp product, diagnostic of the targeted allele, while primers *ForNeo* and *SCP3map2* yield the normal allele product of 265 bp. Primers specific for *Trp53* were *X6.5*, 5′-ACAGCGTGGTGCTACCTTAT, *Neo18.5*, 5′-TCCTCGTGCTTTACGGTATC and *X7*, 5′-TCCTCGTGCTTTACGGTATC. Primers *X6.5* and *X7* amplify a 470 bp-product specific for the wild-type allele, while primers *Neo18.5* and *X7* generate a 600 bp-product diagnostic of the targeted allele. PCR was performed in a 30 μl reaction containing 100 ng of genomic DNA, and 10 pmol of each primer (20 pmol of primers *ForNeo* or *X7* were added per reaction). Cycling conditions were 94°C for 3 min, 3 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 1 min, 32 cycles of 94°C for 30 s, 56°C for 45 s and 72°C for 1 min. (**B**) Disruption of meiosis in *Scp3*$^{-/-}$*Trp53*$^{-/-}$ male mice. Hematoxylin and eosin stained cross sections of the seminiferous tubules of the testis from adult wild-type and single- or double-mutant animals. Note the vacuolar structures, the multiple layers of spermatocytes and the abnormal germ cells (arrows) in *Scp3*$^{-/-}$*Trp53*$^{+/+}$ and *Scp3*$^{-/-}$*Trp53*$^{-/-}$ tubules. (**C**) Fluorescent immunostaining of spermatocyte spreads from wild-type and single- or double-mutant mice. The cells were fixed and labeled using SCP1 antisera (red fibrillar structures indicated by arrows) and DAPI (blue). The SCP1 fibers are fragmented and much shorter in *Scp3*$^{-/-}$*Trp53*$^{+/+}$ and *Scp3*$^{-/-}$*Trp53*$^{-/-}$ spermatocytes

with entry into pachytene (Figure 1B).[2] Spermatocytes at a more advanced developmental stage have not been observed in *Scp3*$^{-/-}$ and *Scp3*$^{-/-}$*Trp53*$^{-/-}$ mice.

We also monitored meiotic progression in *Scp3*$^{-/-}$ *Trp53*$^{-/-}$ spermatocytes by investigating the expression of the synaptonemal complex protein, SCP1 (a marker for synapsis)[5] and the DNA repair protein, MLH1.[6] The SCP1 protein first appears in zygotene spermatocytes, whereas MLH1 foci become visible at the pachytene stage of prophase I. We have previously shown that the SCP1 antisera used in this experiment labels fiber-like structures in zygotene-pachytene spermatocytes, representing the SC that forms between the meiotic chromosomes as they pair.[2] Indirect immunofluorescent staining of *Scp3*$^{-/-}$ spermatocytes shows a fragmented SCP1-fiber distribution (Figure 1C), suggesting partial synapsis.[2] We find that the distribution of SCP1 in *Scp3*$^{-/-}$*Trp53*$^{-/-}$ spermatocytes is identical to that seen in *Scp3*$^{-/-}$ spermatocytes. This shows that the synaptic process in *Scp3*$^{-/-}$ spermatocytes is not restored by inactivation of p53.

To further confirm this conclusion, we also analyzed the distribution of MLH foci in *Scp3*$^{-/-}$*Trp53*$^{-/-}$ spermatocytes. The number of MLH1 foci on the SCs in pachytene has been reported to correspond to the number of chiasmata.[6] While we observe the expected pattern of MLH1 foci associated with the meiotic chromosomes in pachytene spermatocytes from wild-type and *Trp53*$^{-/-}$ animals, no MLH1 foci are observed in *Scp3*$^{-/-}$ or *Scp3*$^{-/-}$ *Trp53*$^{-/-}$ spermatocytes (data not shown). This confirms that the absence of p53 in SCP3-deficient spermatocytes does not promote further meiotic progression.

In summary, the spermatocytes from *Scp3*$^{-/-}$*Trp53*$^{-/-}$ animals behave in an identical manner, as regards their differentiation capacities, the time-point at which they undergo apoptosis and their abilities to form SCP1 structures and MLH1 foci. Taken together, these results show that the apoptotic mechanism that responds to meiotic disruption and asynapsis in *Scp3*$^{-/-}$ cells is p53-independent.

It has been shown that inactivation of a number of genes encoding DNA recombination/repair proteins results in spermatogenic failure and sterility.[7–10] The spermatocytes in these null animals undergo apoptosis as a result of extensive chromosomal pairing failures in early meiotic prophase I. Similarly, it has been shown that inactivation of the *Atm* gene leads to chromosomal asynapsis at an early meiotic stage, followed by apoptotic germ cell death.[4] In this case, simultaneous inactivation of *Atm* and *Trp53* essentially restores chromosomal pairing, lifting the early meiotic block seen in *Atm*-null spermatocytes and thereby allowing meiosis to proceed to a later developmental stage.[3] This has led to the proposal that ATM takes part in some aspects of chromosomal pairing in meiotic cells, in a process monitored by p53. Our data suggests that deficiency of either ATP or SCP3 affects different molecular mechanisms that regulate chromosomal pairing during meiosis and, implicitly, that the absence of these two proteins could trigger two separate checkpoints.

Odorisio *et al.*[11] have previously observed that a single asynaptic sex chromosome in X*Sxr*^{a}O mice is sufficient to induce p53-independent spermatocyte apoptosis at meiotic metaphase I. Our work extends the findings of Odorisio *et al.*[11] in that more extensive asynapsis, involving a majority of the meiotic chromosomes, induces apoptosis as early as the zygotene stage of meiotic prophase I. This suggests a direct relationship between the degree of chromosomal asynapsis and the meiotic stage at which the aberration is detected. Together, our results support a model that was first proposed by Miklos,[12] in which a quantitative relationship exists between the extent of asynapsis and the temporal loss of spermatocytes during meiosis. The SCP3-deficient mouse model system described here should thus prove invaluable in the elucidation of the mechanisms underlying spermatogenic quality control, and for identification of the proteins that monitor chromosomal pairing during meiosis.

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