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Infertility in Male Transgenic Mice: Disruption of Sperm Development by HSV-tk Expression in Postmeiotic Germ Cells[1]

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ABSTRACT

Previous experiments revealed that male transgenic mice bearing a cosmid that included the Class II E_α gene, about 35 kb of 5′ flanking DNA, and the cosmid vector sequences were sterile. To ascertain the cause of the sterility, various subfragments of the cosmid were tested in transgenic mice. Only those pieces of DNA that included some of the E_α flanking chromosomal DNA and the herpes simplex virus (HSV)-thymidine kinase (tk) gene that was in the vector resulted in male sterility. Histological analysis revealed abnormalities in nuclear morphology of elongating spermatids and retention of mature spermatids within the seminiferous epithelium. Immunocytochemical studies showed that the HSV-tk gene was expressed at low levels in postmeiotic round spermatids and at higher levels in more mature elongating spermatids. To determine whether expression of HSV-tk in spermatids might be responsible for the sterility, the protamine gene promoter was used to direct the expression of HSV-tk to postmeiotic germ cells. Since the mice so treated were also sterile, the data suggest that expression of this enzyme in spermatids is responsible for the sterility phenotype.

INTRODUCTION

We previously described an unexpected reproductive phenomenon in transgenic male mice harboring a cosmid carrying the major histocompatibility complex (MHC) Class II E_α gene with 35 kb of 5′ flanking sequences [1]. Infertility or lack of transgene transmission to offspring occurred in three different male (C57BL/6 × SJL) F_2 founder transgenic mice. In contrast, a female founder mouse was fertile and transmitted the gene to offspring. Furthermore, whereas all of her female and nontransgenic male offspring were fertile, male transgenic offspring were not. Male transgenic offspring had normal libido and formed vaginal plugs after mating with control females, but microscopic examination showed that normal spermatozoa were absent or greatly reduced in number in both the vaginal plugs and uterine fluid.

Results from parallel studies suggested that the Class II gene itself was not responsible for causing infertility in those transgenic males. When the MHC Class II E_α gene was transferred into the mouse germline with 5 kb of 5′ flanking DNA [2] or with 3.2 kb or less of 5′ flanking DNA [3–5], no effect on male reproductive function or transmission of the transgene was detected.

To understand the factors responsible for the infertility in transgenic males, we analyzed the transgenic males in more detail using functional, morphological, and molecular assays. In addition, using subclones of the original transgene construct, we generated additional transgenic lines in an attempt to identify the sequences responsible for causing sterility in transgenic males. Our results indicate that expression of the herpes simplex virus (HSV)-thymidine kinase (tk) gene present in the cosmid vector was most likely responsible for the infertility.

MATERIALS AND METHODS

Microinjection

The pronuclei of fertilized eggs derived from (C57BL/6 × SJL)F_1 females mated to identical hybrid males were microinjected with about 2 picoliters of DNA solution, as described by Brinster et al. [6]. Eggs that survived microinjection were implanted into the oviducts of pseudopregnant Swiss foster females.

DNA and RNA Analysis

DNA manipulations were performed using standard procedures. DNA fragments for injection into mouse eggs were isolated from Sea Plaque low gelling temperature agarose

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(FMC Corp., NY, NY). RNA used for Northern blot analysis was extracted by homogenization in guanidinium isothiocyanate, followed by precipitation with lithium chloride as described by Cathala et al. [7]. RNA samples were electrophoresed in agarose-formaldehyde gels, transferred to nitrocellulose paper, and hybridized with radiolabeled probes as described by Peschon et al. [8]. A 500 base pair *Bgl*II-*Nco*I and an 800 base pair *Eco*RI-*Hin*dIII restriction fragment specific for protamine-1 (Prm-1) and Hox 1.4, respectively, were labeled by nick translation and used for hybridization.

A small piece of tail from a weaned mouse was analyzed for the presence of the transgene DNA by dot hybridization as previously described by Brinster et al. [6].

Histology

For histology, tissues were fixed in Bouin's or Carnoy's fluid, embedded in paraffin, then sectioned and stained with hematoxylin and eosin or periodic acid-Schiff (PAS). For electron microscopy, fresh tissue was immersion-fixed in 5% cacodylate-buffered glutaraldehyde [9] overnight at 4°C in centrifuge tubes. For the studies on sperm, the tissue was then pelleted with a DYNAC II centrifuge at 2 000 RPM. The pellets were removed, minced to 0.5-mm cubes, and postfixed in 2% osmium tetroxide for 2 h at 4°C. The postfixed cubes were dehydrated through alcohol and embedded in Spurr's low viscosity embedding medium. Embedded blocks were sectioned to 70 nm on a Sorvall MT2-B ultramicrotome. The thin sections were then stained in uranyl acetate and lead citrate and photographed on a Zeiss EM9-S electron microscope.

Staining for HSV-tk was done on paraffin-embedded sections using a rabbit anti-HSV-tk antibody (gift from S. Kit) and a peroxidase-aminoethylcarbazole staining system.

RESULTS

Infertility in E_α Cosmid Transgenic Mice

The structure of the original cosmid that contains the E_α structural gene (designated H-7 in this study) is shown in Figure 1. In addition to the E_α structural gene, the cosmid contains 35 kb of 5′ flanking chromosomal DNA and 8.3 kb of vector sequence. The vector includes the HSV-tk gene, the β-lactamase gene, the pBR322 origin of replication, a bacteriophage λ packaging site (*cos*), and the SV40 origin of replication. Table 1 lists the founder animals established with construct H-7 and transmission of the transgene to their offspring.

Founder males were either sterile or failed to transmit the transgene. The latter phenomenon is most easily explained by the fact that many founder transgenic mice are mosaics, with the transgene in only a fraction of the somatic

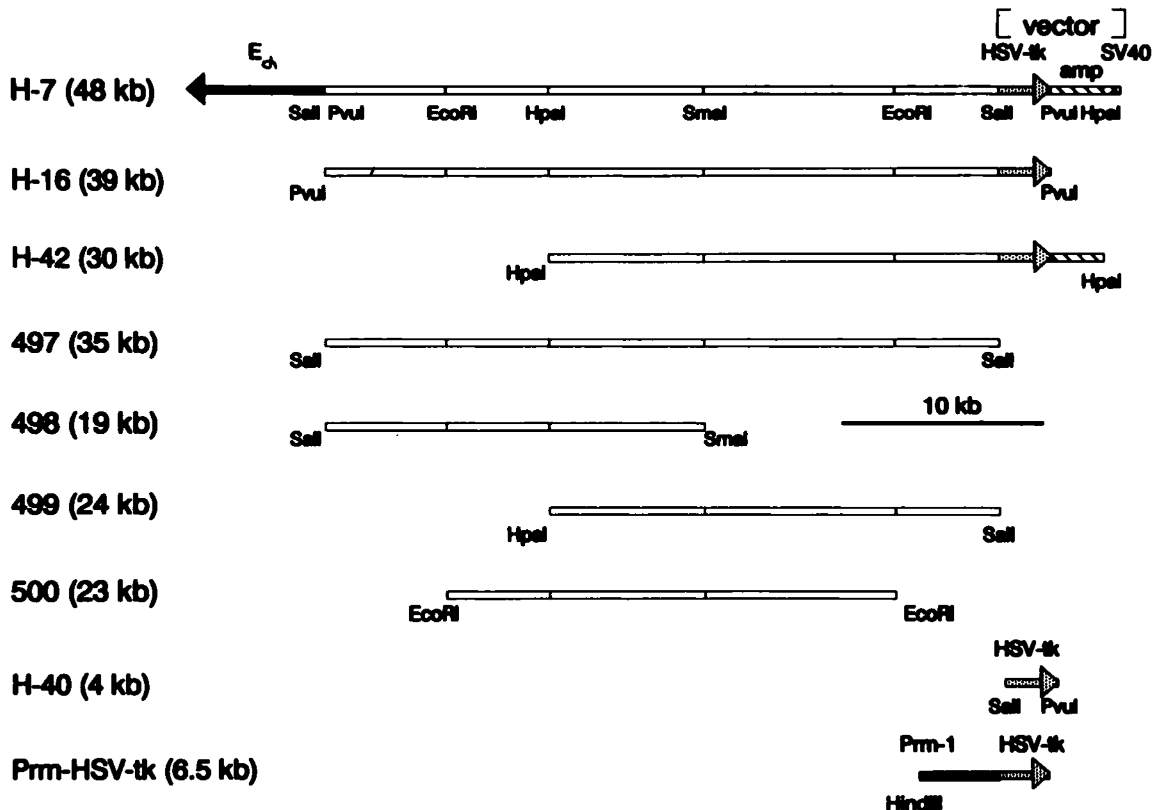

FIG. 1. Transgene constructs used in this study. Important restriction sites are indicated. HSV-tk, herpes simplex virus thymidine kinase; amp, ampicillin resistance gene; SV40, Simian virus 40 origin of replication; for details, see Hyldig-Nielsen et al. [28].

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TABLE 1. H-7 and H-16 founders.

Gene[a]	Founder animal number[b]	Gene copy number[c]	Transmission frequency[d]
H-7	24-3 M	4	0/22
	25-4 M	19	infertile
	27-2 F	2	6/11
	27-3 M	2	0/17
H-16	63-2 M	3	0/23
	63-3 F	10	4/9
	64-6 M	8	infertile
	69-9 F	2	3/4

[a]See *Figure 1* for map of gene.
[b]Founder transgenic mice were generated from (C57BL/6 × SJL)F_2 fertilized eggs microinjected with the respective transgene. The founder animal and subsequent transgenic offspring constitute a line.
[c]Gene copies per cell, as determined by dot hybridization.
[d]Numerator, offspring harboring the transgene; denominator, total number of offspring examined. Infertile: more than three observed plugs failing to result in pregnancy, and failure to produce litters after being kept with two or more females for more than 4 months. At cessaton of breeding, females were killed to confirm absence of pregnancy.

and germ cells. This is due to stable genomic integration of the transgene after one or more rounds of DNA replication [10]. Consequently, the testis could be composed of clones of normal germ cells and affected germ cells. If the deleterious effect of the transgene were germ-cell-autonomous, then the normal germ cells would give rise to wild-type spermatozoa while the transgenic ones would be mutant. All F_1 transgenic males derived from female founder mice would be hemizygous for the transgene and would be expected to be sterile according to this hypothesis.

To confirm that the E_α structural gene was not responsible for the male infertility, transgenic mice were generated using a construct containing only the 35 kb of 5′ flanking DNA with 4 kb of vector sequences. This construct, designated H-16, is shown in Figure 1. Both of the resulting founder transgenic males exhibited a reproductive dysfunction similar to that observed in H-7 males, and transgenic male offspring from one of the founder transgenic females were sterile (Table 1). Offspring from the other founder female were not tested. The results of this experiment raised the possibility that the 35 kb of 5′ flanking DNA may contain a previously unidentified gene that is inappropriately expressed in male germ cells, leading to infertility.

Because the injected DNA was derived from the H-2^d haplotype, it was formally possible that the host (H-2^b × H-2^s)F_2 genome lacked some factor present in the H-2^d haplotype necessary for blocking the deleterious effect of the transgene. However, when transgenic H-7 and H-16 females were bred to B10.D2 (H-2^d) males, nine of ten male offspring carrying the transgene were infertile (data not shown). The tenth male carrying the H-7 construct sired a single nontransgenic pup, but failed to sire any subsequent litters.

The major feature of the sterility phenotype is a marked decrease in the number of spermatozoa. Vaginal plugs produced by transgenic males at the time of mating generally had few if any sperm, and uterine flushings from plugged females yielded only occasional nonmotile sperm. In contrast, plugs from control males contained a high density of nonmotile sperm, and uterine flushings generally yielded hundreds of motile sperm per high-power field versus <1 sperm per high-power field from females plugged by transgenic males.

Eggs from females plugged by transgenic males were not fertilized. Thus, the eggs did not progress to the blastocyst stage either in vivo or in vitro (0/351 eggs from 15 plugged superovulated females). No sperm heads adhering to any eggs could be visualized by staining with Hoechst dye 33258 [11], and none of the eggs contained two pronuclei. Sperm isolated from the epididymis of transgenic males were also unable to fertilize eggs in vitro (data not shown). Eggs from females plugged by control males were generally fertilized (117/142 from 6 females). In this case, sperm heads adhering to the zona pellucida or two pronuclei could be readily visualized using Hoechst dye, and these eggs proceeded to the blastocyst stage both in vitro and in vivo.

Transgenic Testis and Sperm Morphology

Histologic examination of testes from two sterile 36-wk-old H-16 founder males and several 6- and 12-week-old transgenic H-7 and H-16 male progeny revealed mild-to-severe degeneration and dysplasia of the testis seminiferous tubules. Figure 2 shows characteristic sections of testis from control (Fig. 2A) and transgenic (Fig. 2B) males. In the transgenic testis, spermatogonia, spermatocytes, and round spermatids appeared to be normal in morphology and number. In contrast, elongating spermatids appeared to be fewer, and abnormally shaped sperm heads were consistently seen. As expected, in control testis, mature spermatids were not observed in the later stages (stages IX through XII) of the cycle of the seminiferous epithelium [12]. However, in transgenic males, mature spermatids were observed in the later stages of the cycle (Fig. 2B), suggesting that there was a failure of the release of elongated spermatids from the seminiferous epithilium at the completion of spermatogenesis. Some seminiferous tubules from younger transgenic males possessed normal-appearing spermatozoa. The presence of sperm was age-dependent in these two transgenic lines, because seminiferous tubules from older males (>2 mo of age) rarely contained normal-appearing spermatozoa.

Electron microscopic evaluation of testis also revealed abnormal sperm development in three transgenic animals analyzed from three different lines, but not in age-matched control males. As in the light microscope sections, the first step obviously affected was the elongating spermatid (Fig. 2C,D). Persistent acrosomal abnormalities were accompanied by incomplete condensation of nuclear chromatin.

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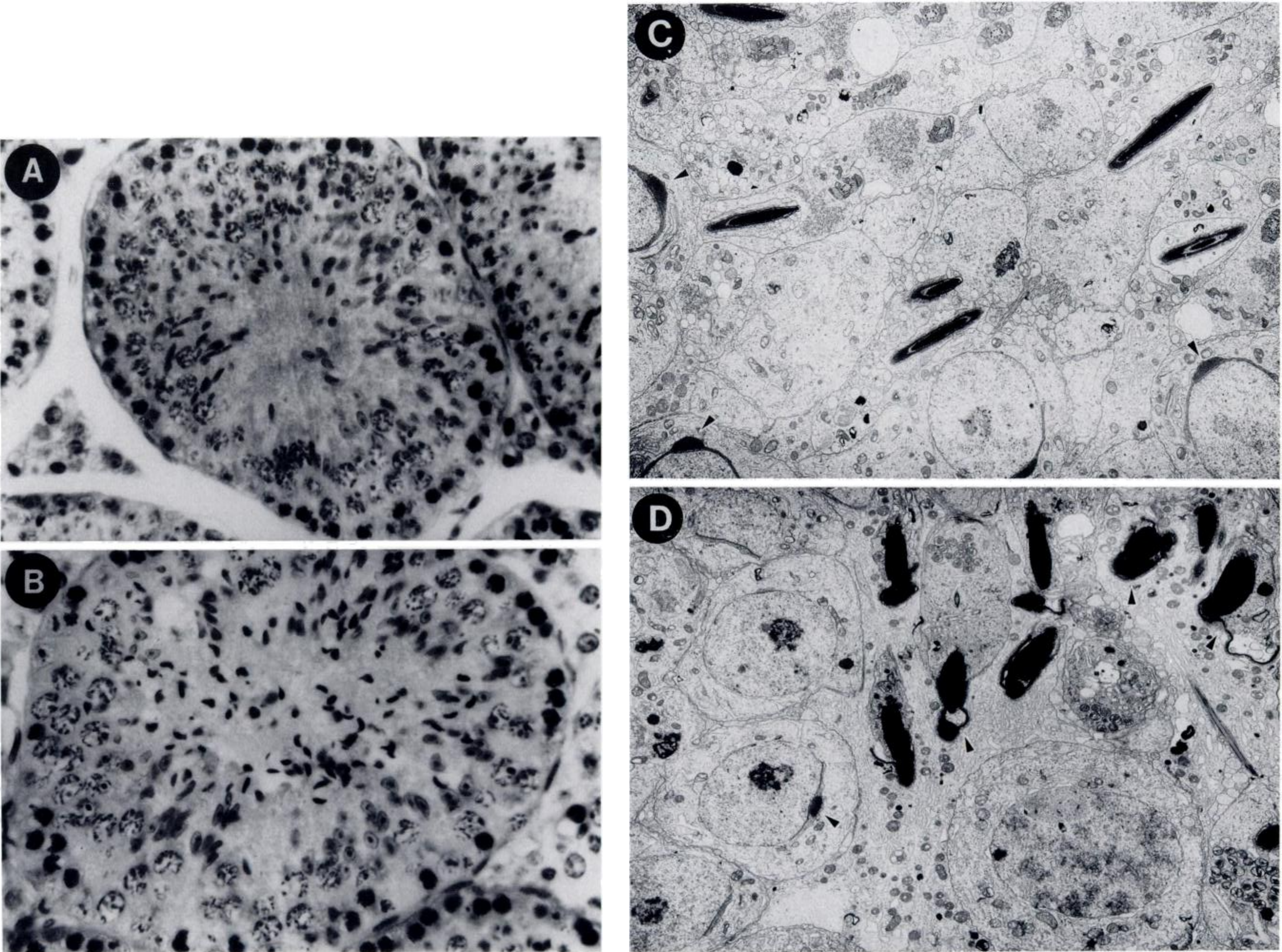

FIG. 2. Histological analysis of testis. (A) Testis for 6-wk-old control stained with PAS. Seminiferous tubule at about stage IX. Tubules at this stage contain a single population of elongating spermatids [12]. ×400. (B) Testis from 6-wk-old transgenic harboring the H-16 construct. Seminiferous tubules also at about stage IX. Note the abnormally shaped sperm heads and the presence of two populations of elongating sperm heads. ×500. (C) Electron micrograph of control testis. Developing acrosomes in round spermatids are marked by arrowheads. ×4 000. (D) Electron micrograph of testis from transgenic harboring the H-16 construct. Note the abnormally shaped elongating sperm heads and the irregular shape of the developing acrosomes (arrowheads). ×3 700.

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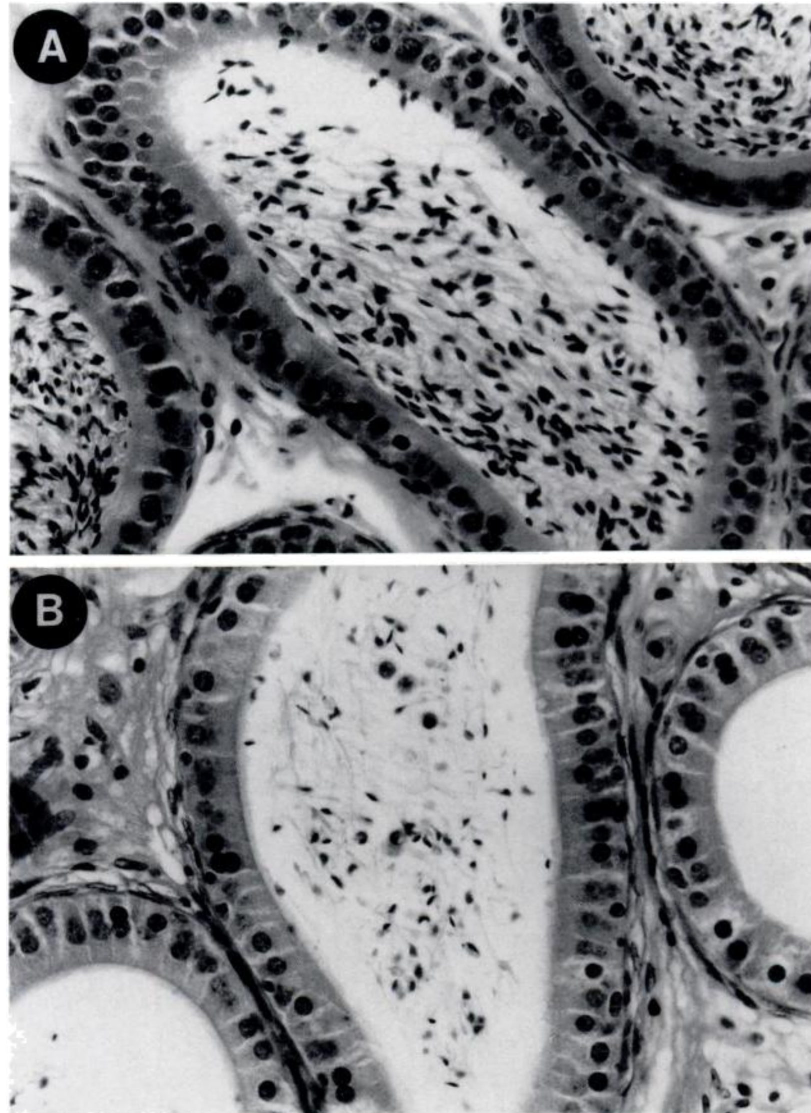

FIG. 3. Histological analysis of epididymis. (A) Control epididymis. ×400. (B) Epididymis from six week old transgenic. Numerous degenerating spermatids and abnormal sperm can be seen. ×400. (C) EM of control sperm. ×7 600. (D,E) EM of sperm from six week old transgenic epididymis (H-16). Note the abnormal condensation of sperm nuclei (N), and abnormal microtubule formation in the tails (arrowhead). D, ×25 200; E, ×6 000. (F) EM of degenerating cells in old transgenic epididymis. ×10 000.

In some round spermatids, acrosomal development also appeared to be disrupted, resulting in irregular membrane figures and incomplete formation of the acrosomal granule.

The cauda epididymis of transgenic males contained a preponderance of empty ducts or ducts with degenerating spermatids and spermatozoa as well as multinucleated giant cells (Fig. 3B). This was especially pronounced in older transgenic males, whose epididymides contained few if any recognizable sperm. For a closer examination of the sperm abnormalities, epididymal contents from transgenic males were studied by use of electron microscopy (Fig. 3D–F). As in the testis, sperm heads were abnormal in shape, chromatin was incompletely condensed, and the acrosomal vesicle was irregular. In addition, many of the sperm tails had abnormal axonemes. In the epididymides of older transgenic males, numerous large degenerating cells were found, with features resembling those of round spermatids (Fig. 3F). Despite the loss of most cell structures, some suggestion of acrosome formation could be seen in many of these cells. In these animals, sperm were rare or entirely absent.

Molecular Analysis of the Infertility Phenotype

Since sperm development appeared to be affected during the haploid phase of spermatogenesis, we sought information on whether gene expression was also affected. It has previously been shown that expression of the mouse Prm-1 gene can be used as a molecular marker for spermatid development [13]. The Prm-1 gene is first transcribed in round spermatids, yet its mRNA is not translated until approximately one week later in elongating spermatids [14].

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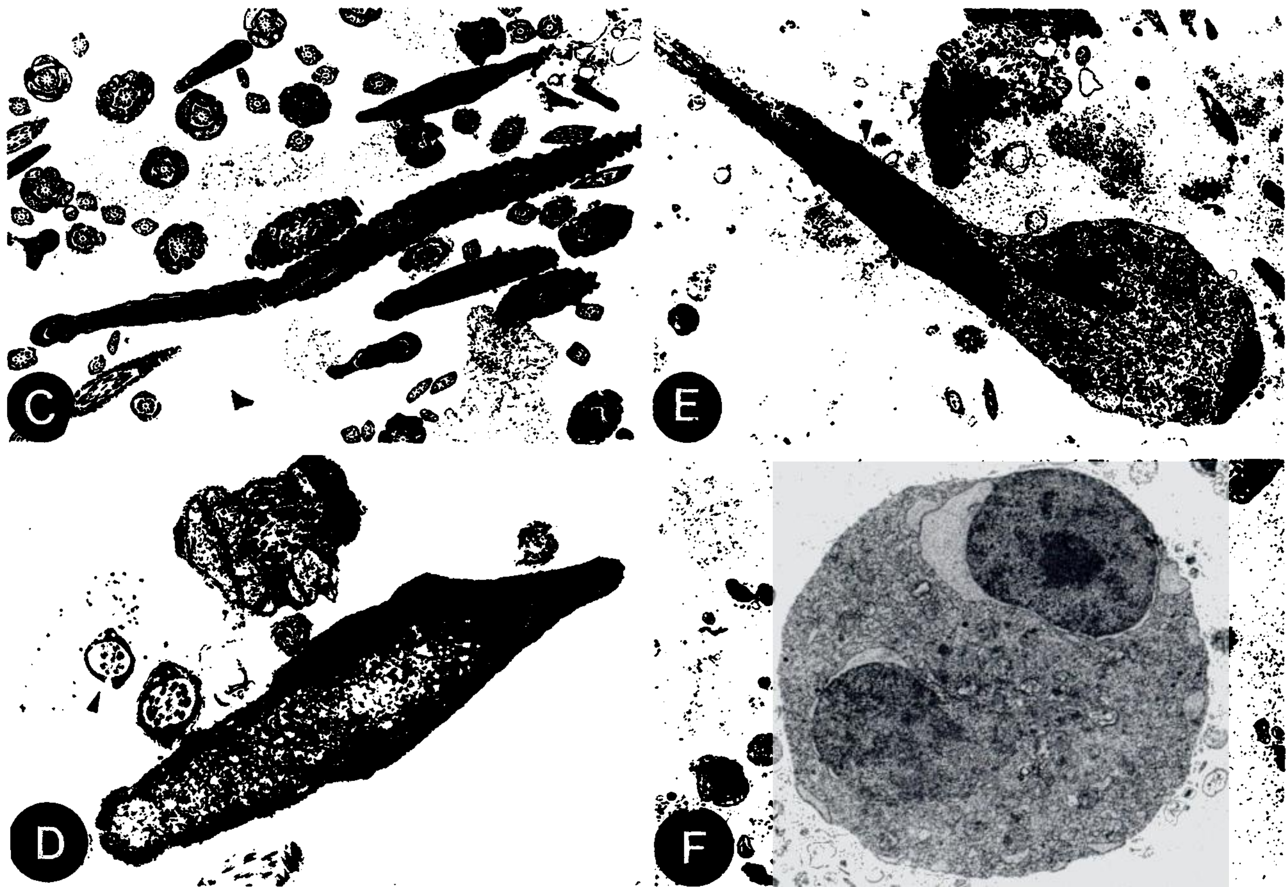

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Translation of protamine mRNA is associated with extensive deadenylation of the transcript. When Northern blots of testis RNA from normal and transgenic mice were examined for Prm-1 transcripts, no differences in abundance or size were seen (Fig. 4). Similarly, expression of the male germ-cell-specific gene Hox 1.4, normally expressed in pachytene spermatocytes [15], appeared to be unaffected. These results indicate that the transgene effects on spermatid differentiation are independent of their effects on Prm-1 gene expression.

Subclones of the E_α Cosmid and Infertility

In an attempt to identify the region of the cosmid responsible for the fertility defect, we generated additional lines of transgenic mice carrying subclones of the cosmid as shown in Figure 1. Construct H-42 combined a shorter piece of E_α 5′ sequence (26 kb *Hpa*I-*Sal*I) with the 4-kb vector fragment (Fig. 1). Line 687-3, generated with this construct, showed the most severe histological effects on spermatid development of all transgenic lines. Testis sections were devoid of any normal-appearing mature sperm even in young transgenic males; epididymal contents consisted entirely of degenerating spermatids and cell debris.

Transgenic lines were generated from four additional subclones, one of which included the entire 35 kb of E_α 5′ flanking sequence without vector (Fig. 1). In all cases, transgenic lines carrying each construct yielded fertile males capable of transmitting the transgene to progeny (Table 2). Because each of these clones lacked the cosmid vector sequence, we examined whether vector sequences might contribute to infertility. We tested the 4 kb of vector DNA (included in the H-16 construct) in transgenic mice (construct H-40). This *Sal*I-*Pvu*I fragment of the vector contains the HSV-tk gene and a portion of the β-lactamase (ampicillin resistance) gene. Three H-40 transgenic lines were tested for fertility in males. All three lines showed both normal fertility and transgene transmission to offspring, suggesting that the vector sequences alone were insufficient to cause male infertility.

Infertility in transgenic mice occurred only in mice carrying both vector sequences and E_α 5′ sequences (transgenes H-7, H-16, and H-42). This combination could cause infertility by either of two mechanisms. First, the E_α 5′ sequences might affect the expression of the HSV-tk gene, which in turn may have detrimental effects on sperm development. Although HSV-tk mRNA was detected in testis from both fertile and infertile transgenic mice carrying an HSV-tk transgene (Fig. 5), it was possible that cell- and stage-specific enhancer activity could determine the effects of HSV-tk on sperm development. An alternative possibility is that

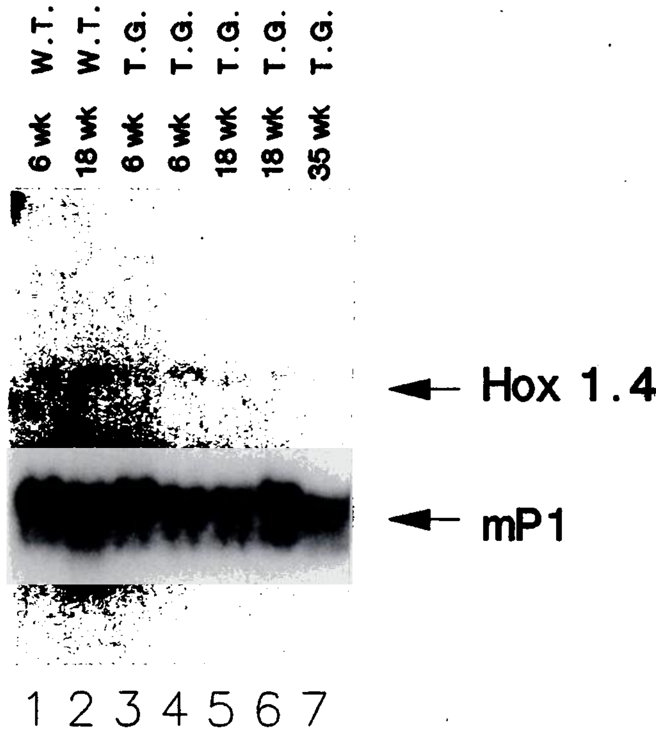

FIG. 4. Northern analysis of testis RNA for protamine 1 (designated mP1 in figure) and Hox 1.4 transcripts. Each lane contained approximately 10 μg total RNA isolated from the testes of 6-, 18-, or 35-wk-old mice; either control (W.T.) or transgenic (T.G.) animals from H-7 and H-16 lines were used. After separation of the RNAs on a 2.0% agarose-formaldehye gel, the filter was hybridized with a 500 base pair *BglII-NcoI* and an 800 base pair *EcoRI-HindIII* restriction fragment specific for Prm-1 and Hox 1.4, respectively.

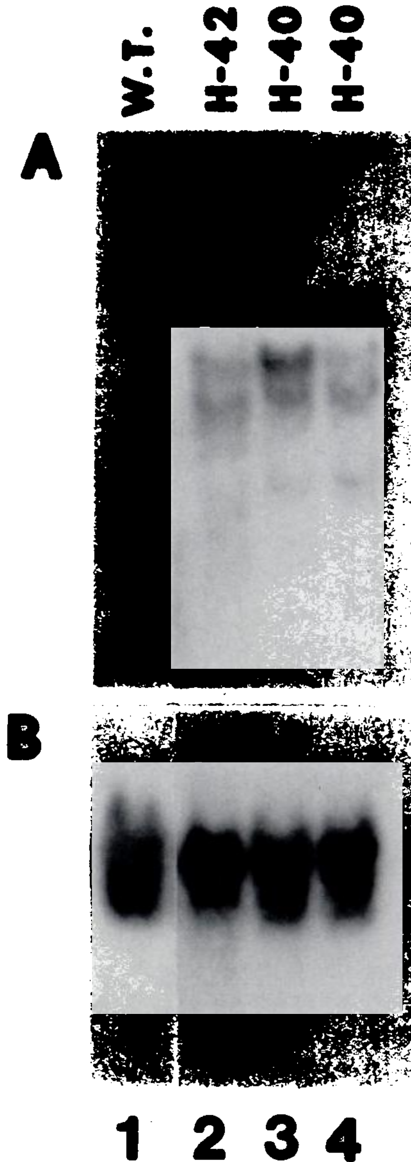

FIG. 5. Northern analysis of transgenic lines for HSV-tk expression. Total RNA (15 μg) extracted from adult testis was separated on a 1.5% agarose-formaldehyde gel, transferred to nitrocellulose paper, and hybridized with a [^{32}P]-labeled (A) 2.3 kb *EcoRI* fragment that contains the HSV-tk gene, or (B) a 175 bp *NcoI* fragment that contains a portion of the Prm-1 gene. Lane 1, control; Lane 2, H-42 (line 687-3); Lane 3, H-40 (line 640-7); Lane 4, H-40 (line 640-8). All samples were hybridized under the same conditions.

TABLE 2. Fertility in males transgenic for subclones of cosmid H-7.

Gene[a]	Transgenic line animal number[b]	Transmission frequency
497	100-5-1	6/10
	238-4-2	11/19
	407-6-4	2/5
498	311-3 founder	4/11
	311-5 founder	11/18
	315-4 founder	4/23
499	294-5 founder	6/11
500	161-1 founder	4/11
	161-9-9-5	fertile[c]

[a]From *Figure 1*.

[b]Founder transgenic mice were generated from (C57BL/6 × SJL)F_2 fertilized eggs microinjected with the respective transgene. As a marker gene, an inactive human growth hormone gene (hGH) was coinjected along with the subclone, and initial screening of transgenic animals used a probe for hGH. Integration of the subcloned E_α 5′ fragment was confirmed by Southern blot. The founder animal and subsequent transgenic offspring constitute a line.

[c]Male gave rise to at least one litter, but transmission of the transgene was not tested.

the vector sequences might affect the expression of some unidentified gene carried in the E_α 5′ sequences. However, various attempts to identify transcripts from this E_α 5′ region have been unsuccessful.

To assess whether HSV-tk expression may be responsible for male infertility, sections of testis from various transgenic lines were stained for the presence of HSV-tk protein (Fig. 6A–C). Thymidine kinase protein could be detected in the germ cells of all transgenic lines carrying the HSV-tk gene, but the strongest staining was seen in the round spermatids and elongating spermatids of testis from the

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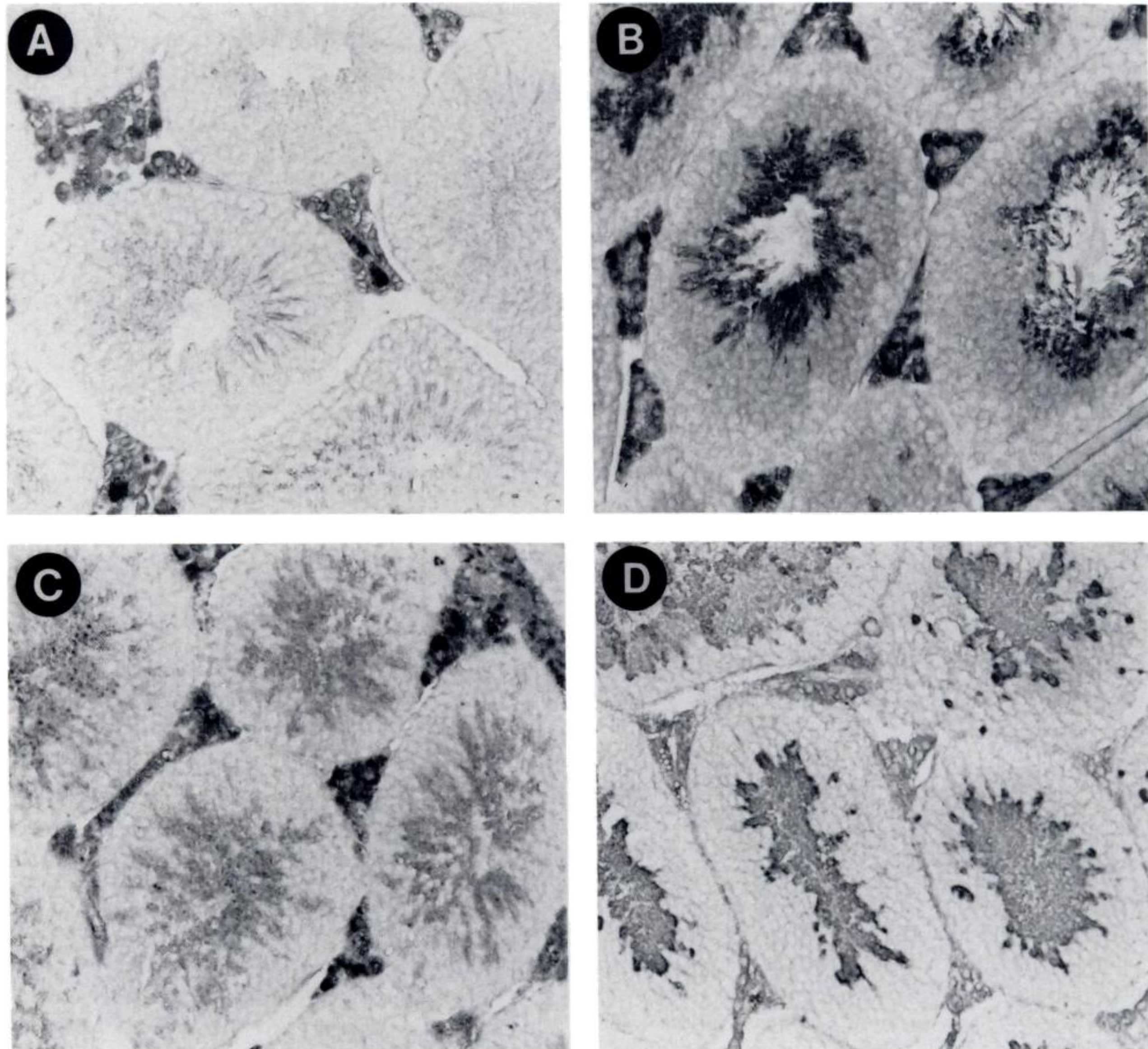

FIG. 6. Immunocytochemical analysis of thymidine kinase expression. Testis from adult animals were fixed, sectioned, and stained as described in *Materials and Methods*. (A) Control testis; (B) H-42 testis (line 687-3); (C) H-40 testis (line 640-7); (D) Prm-HSV-tk testis (line 2120-2). Sections in Panels A–C were stained on the same day for the same duration. Staining of thymidine kinase in H-40 testis was always less intense than in H-42, H-16, and H-7 testes (four experiments). To identify spermatocyte, round spermatid, and elongating spermatid nuclei, adjacent sections were counterstained with hematoxylin (data not shown). ×200.

sterile lines. In contrast, testis from fertile mice bearing only the H-40 HSV-tk construct stained only weakly in elongating spermatids. These data therefore suggest that either low levels of HSV-tk protein in round spermatids and/or high levels of expression in elongating spermatids may adversely affect sperm development.

Infertility in Prm-HSV-tk Transgenic Mice

To test directly whether expression of HSV-tk was by itself sufficient to cause infertility in the absence of E_α 5′ sequences, transgenic mice were generated using a hybrid construct containing the mouse Prm-1 5′ regulatory elements fused to the HSV-tk coding sequences (Fig. 1). The Prm-1 gene is expressed exclusively in postmeiotic spermatids, and hybrid transgene constructs containing the protamine 5′ regulatory sequences retain this tissue-specific expression [8, 16]. In this way HSV-tk expression could be directly targeted to round spermatids.

Of nine male transgenic founder mice generated with the Prm-HSV-tk transgene, eight were fertile, but failed to transmit the transgene (Table 3). The ninth founder male was infertile. Immunohistochemical staining of testis from fertile Prm-HSV-tk founders revealed a patchy distribution of thymidine kinase protein, suggesting that these mice were

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TABLE 3. Fertility in males transgenic for Prm-HSV-tk.

Founder animal number	Transmission frequency
1540-5 M	0/10
1540-9 M	0/17
1542-3 F	no progeny
1543-1 M	0/14
1543-6 M	0/7
2118-1 M	0/9
2118-4 M	0/10
2119-3 M	infertile
2120-2 F	4/6, male offspring infertile
2120-3 F	0/15
2120-7 M	0/11
2122-2 M	0/11

mosaics (data not shown). Thus, fertility was probably obtained through production of normal sperm generated by nontransgenic germ cells. A line of Prm-HSV-tk transgenic mice was established from one of the female founder mice. Transgenic male offspring from this line expressed HSV-tk in round and elongating spermatids (Fig. 6D), and they were all sterile. Thus, by intentionally targeting expression of HSV-tk to postmeiotic male germ cells, sperm development was severely affected.

DISCUSSION

We have explored a defect in male fertility resulting from integration of DNA cloned from the mouse H-2 locus plus vector sequences. Transgenic males appear to be normal except for a defect in sperm production. The range of spermatogenic cells affected in the transgenic males indicates a defect in maturation beginning with the postmeiotic spermatid stages. The major histological defect observed was abnormally shaped sperm heads and retention of elongating spermatids within the seminiferous epithilium. Curiously, the marked histological alterations in spermiogenesis were not accompanied by significant alterations in the accumulation and maturation of the Prm-1 mRNA.

Some of the founder H-7, H-16, and Prm-HSV-tk transgenic males were fertile but did not transmit the gene to offspring. These were probably mosaic animals, having some normal (nontransgenic) tissue as well as transgenic tissue [10]. Germ cells populate the testis stroma in a random manner [17], so some transgenic germ cells should have encountered normal testis stroma. This expected combination did not result in production of functional transgenic sperm, since the transgene was not transmitted by the fertile male founders. Thus, the defect induced by the transgene must be intrinsic to germ cells.

Our results show that E_α 5′ chromosomal DNA is not sufficient to cause the defect in sperm development; only those constructs that included the HSV-tk gene resulted in sterility or lack of transgene transmission. That HSV-tk alone did not affect fertility despite the presence of HSV-tk mRNA and protein in germ cells is probably due to the lower level of expression of this gene by itself compared to the E_α-HSV-tk constructs. The increased HSV-tk expression observed when E_α sequences were present might be due to an enhancer in the E_α region, or it might reflect the greater spacing of the HSV-tk genes that reduces promoter interference. Alternatively, HSV-tk expression may be prone to activation in the testis when integrated as a transgene in tandem with other unrelated DNA combinations [18, 19]. Although there were comparable levels of HSV-tk mRNA in testes of H-40 (fertile) and H-42 (infertile) mice (Fig. 5), there was always more tk protein in round and elongating spermatids of H-42 mice, perhaps because of greater stability of the protein or differences in translational capability of the mRNA in the H-42 mice.

In hemizygous transgenic males, the effects of HSV-tk expression on spermiogenesis were seen in all developing spermatids, even when HSV-tk expression was targeted to postmeiotic germ cells by the Prm-1 promoter. This result suggests that HSV-tk expression exerts its effects on all of the haploid cells even though the transgene would be present in only half of them. Because the male germ cells are connected by intercellular bridges [20], it is likely that the effect in all haploid cells reflects movement of HSV-tk mRNA, protein, or secondary metabolic products of thymidine kinase expression between cells through the intercellular bridges. On the basis of our previous findings, movement of HSV-tk mRNA through syncytial bridges is the most likely possibility [21].

At the outset of these experiments, we failed to appreciate the potential deleterious effects of HSV-tk because a number of transgenic mice bearing HSV-tk alone or driven by the metallothionein promoter (which is expressed in male germ cells) showed normal fertility [18, 22, 23]. The level of expression in testis was not monitored in these animals, and it may have been below the threshold necessary for infertility. However, in the ensuing years, several other examples have accumulated. In one case, the regulatory elements of the mouse major urinary protein (Mup) gene were fused to the HSV-tk structural gene to see if thymidine kinase expression would be directed to the liver [24]. The resulting Mup-tk transgenic male mice were sterile, attributed to ectopic expression of thymidine kinase in the testis. In another case, transgenic male mice bearing an IFN-γ transgene driven by the mouse metallothionein promoter were sterile [25]. Here, too, HSV-tk gene expression (from the plasmid vector) was found in the testis. Finally, reduced male fertility was also reported in transgenic mice bearing HSV-tk driven by an immunoglobulin gene enhancer and promoter [26], although the researchers did not assess the expression of HSV-tk in the testis.

How could HSV-tk inhibit male germ cell development? One possibility is that HSV-tk can metabolize novel substrates into toxic compounds. HSV-tk has a broader substrate specificity than endogenous mammalian thymidine

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kinase, a fact used to advantage for metabolic labeling of HSV-infected cells with iodocytidine, or killing of cells with ganciclovir [26], an analog that is incorporated into DNA to arrest replication. There may be novel substrates in post-meiotic germ cells that are metabolized into nucleotide analogs that might interfere with macromolecular synthesis. Because the major histological defect appears to be abnormal nuclear morphology, these results suggest that nucleotide biosynthesis may be important during nuclear shaping and condensation. Alternatively, considering that spermatids have completed DNA replication, it is possible that enzymes are induced that degrade deoxynucleotide monophosphates. Consequently, the expression of HSV-tk might establish a futile cycle of thymidine phosphorylation and dephosphorylation at the expense of ATP.

We have presented evidence that expression of HSV-tk in male germ cells results in disruption of sperm development, a phenomenon that may be useful in further analysis of male germ cell development and metabolism. In addition, we found that HSV-tk expression in germ cells was most disruptive in the presence of E_α 5′ DNA sequences. Considering that the E_α gene maps within the t-complex, a region of chromosome 17 that contains numerous genes expressed in germ cells [27], these studies may also lead toward identification of new germ-cell-specific genes or regulatory elements.

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