MOLECULAR AND CELLULAR BIOLOGY, Sept. 1990, p. 5021–5025
0270-7306/90/095021-05$02.00/0

Vol. 10, No. 9

The Testis-Specific Transcript (*ferT*) of the Tyrosine Kinase *FER* Is Expressed during Spermatogenesis in a Stage-Specific Manner

ELI KESHET,[1]* AHUVA ITIN,[1] KINERET FISCHMAN,[2] AND URI NIR[2]

Department of Virology, The Hebrew University-Hadassah Medical School, Jerusalem 91010,[1] and Department of Life Sciences, Bar-Ilan University, Ramat-Gan 52100,[2] Israel

Received 28 March 1990/Accepted 18 June 1990

***ferT* is a testis-specific transcript of *FER* encoding a truncated version of the potential tyrosine kinase. Using in situ hybridization analysis, we found that *ferT* was transiently expressed during spermatogenesis and that expression was restricted to spermatocytes at the pachytene stage of meiotic prophase. This pattern of expression is unprecedented by other tyrosine kinases and suggests a role for *ferT* in a particular stage of spermatogenesis.**

A new member of the tyrosine kinase gene family, termed *FER*, has been recently cloned and characterized (7, 10). *FER* is expressed in a variety of tissues but includes a particular mRNA species detectable only in testes (4, 10). The testis-specific transcript of *FER*, termed *ferT*, has been recently cloned from a mouse testis cDNA library (4). Structural analysis of *ferT* cDNA has indicated that it is generated by an alternative splicing mode and likely also through utilization of a different promoter (4). The encoded 453-amino-acid protein ($p51^{ferT}$) is a truncated version of the *FER*-encoded protein $p94^{fer}$. $p51^{ferT}$ contains a unique N-terminal domain (the first 44 amino acids) that is absent from $p94^{fer}$ (4, 7). A previous study has shown that *ferT* mRNA is undetectable in RNA preparations extracted from testes of newborn mice at times preceding week 3 of postnatal development (4). Since the first wave of differentiating spermatogonia mature into resting spermatocytes at 12 days after birth and primary spermatocytes reach the early pachytene stage of meiotic prophase at about 14 days of age (12), the onset of *ferT* expression in mouse testes at days 18 to 21 after birth (4) was coincidental with the progression of germ cells to mid- and late pachytene stages (duration of the pachytene stage in mice is about 7 days). These findings suggested that expression of *ferT* within the testis is restricted to certain stages of germ cell development. To confirm that expression of *ferT* is transcriptionally activated during spermatogenesis and to more precisely define the particular stage(s) in spermatogenesis at which *ferT* mRNA accumulates, we analyzed *ferT* mRNA in sections of adult testes by in situ hybridization. In situ hybridization of testicular sections has the advantage that hybridization signals can be localized to cells at successive stages of spermatogenesis that are morphologically distinguishable. Moreover, the ability to identify the expressing cells in their authentic cellular contexts aids in their staging along the spermatogenic pathway. As germ cells differentiate, they progressively move from the periphery of the seminiferous tubule toward its central lumen while new spermatogenic cycles are initiated at the periphery. This leads to a cross-sectional arrangement of series of concentric rings of germ cells, each containing cells at the same stage of development. Superimposed on the radially distributed spermatogenic cycle is a longitudinally distributed spermatogenic wave. The spermatogenic wave results from the fact that entry of germ cells into the spermatogenic cycle does not occur simultaneously along the entire length of the tubule but is initiated locally and proceeds as a longitudinally propagated wave. Hence, the wave of the seminiferous epithelium leads to a succession of cell associations along the length of the tubule that follow each other in a sequential order. Each cell association is composed of an ensemble of germ cells in correspondingly different states of differentiation (see reference 14 for a review on the regulation of the seminiferous epithelium).

The *ferT*-specific probe used in this study was a 199-base-pair-long fragment corresponding to the beginning of the 5′ untranslated domain of the *ferT* cDNA clone. The 5′ segment of *ferT* cDNA is composed of a unique sequence not shared by other transcripts of the *FER* gene or by other tyrosine kinase-encoding mRNAs. This ensured that the probe used detected exclusively *ferT* mRNA. An *Eco*RI-*Hin*dIII fragment (the *Eco*RI site is an artificial site marking the 5′ terminus of the cDNA clone that was introduced for cloning purposes, and the *Hin*dIII site is at position 199 of *ferT* cDNA [4]) was subcloned in the pBS vector (Stratagene). The resultant plasmid was linearized with either *Eco*RI or *Hin*dIII to allow synthesis of a ^{35}S-labeled riboprobe in either the antisense or the sense orientation (using T3 or T7 polymerase, respectively). Testes derived from sexually mature BALB/c mice (12 weeks of age) were fixed in 4% paraformaldehyde, embedded, frozen, and sectioned. Frozen sections (10 μm thick) were processed for in situ hybridization and hybridized to *ferT* antisense RNA under stringent conditions as previously described (8).

A cross section through the testis that was hybridized with *ferT* antisense RNA is shown in Fig. 1. *ferT* mRNA was confined to rings of cells residing within the seminiferous tubules and was undetectable in the interstitial stromal cells (Fig. 1A and B). This observation confirmed the germ cell origin of *ferT*-expressing cells. Strikingly, *ferT* mRNA was not detectable in all cross sections, and often positive and negative hybridization signals were detected in adjacent cross sections through the tubule. The most likely interpretation of this pattern is that because of the spermatogenic wave, in combination with the coiling of the seminiferous tubule, different tubule zones are juxtaposed in the level of sectioning. Consequently, adjacent cell associations may be composed of germ cell ensembles from which certain stages of the spermatogenic cycle are excluded. The failure to detect *ferT* mRNA in a considerable fraction of cell associations thus indicates that expression of *ferT* is restricted to particular stages along the spermatogenic cycle.

* Corresponding author.

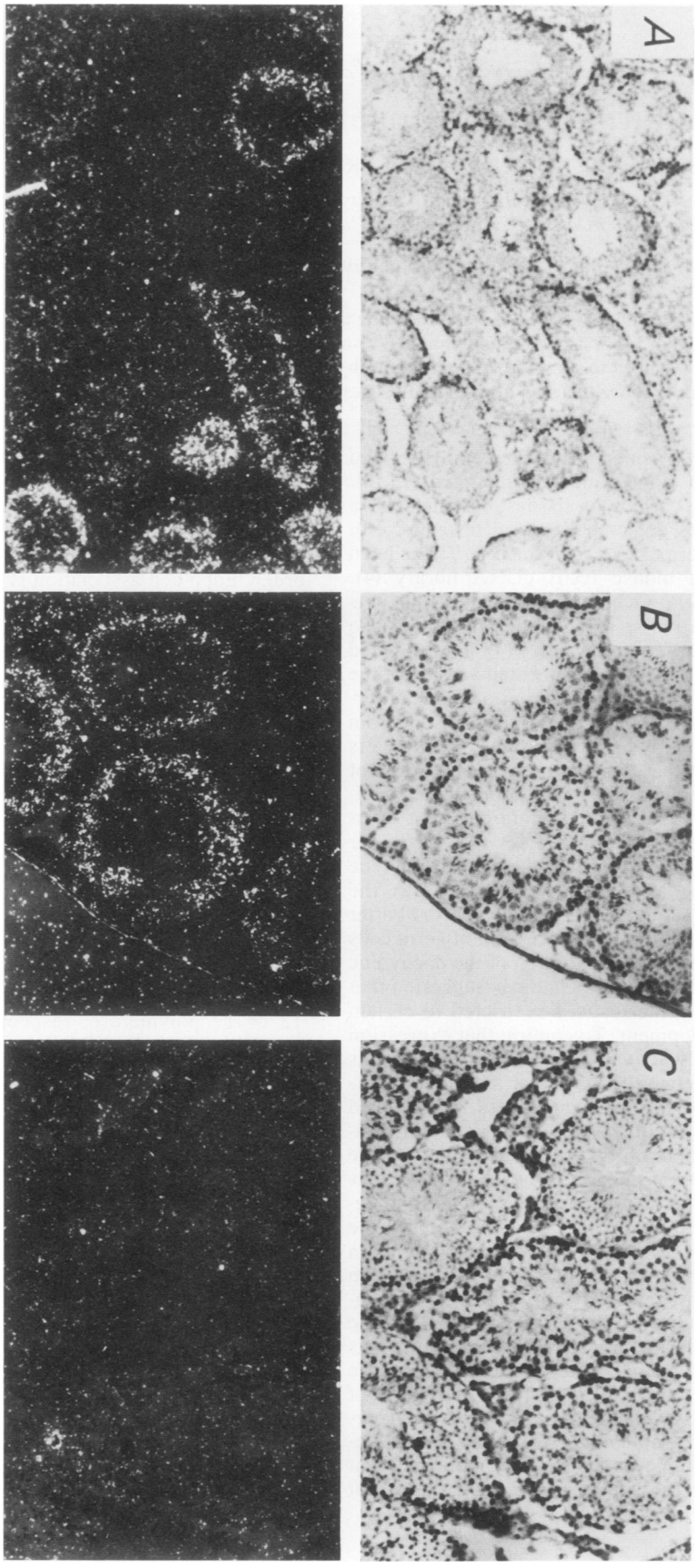

FIG. 1. Localization of *ferT* transcripts in the adult testis by in situ hybridization with the antisense *ferT* probe (A and B) and with the sense *ferT* probe (C). Top and bottom figures show the same cross sections photographed under bright- and dark-field illumination, respectively. Magnifications, ×86 (A) and ×171 (B and C).

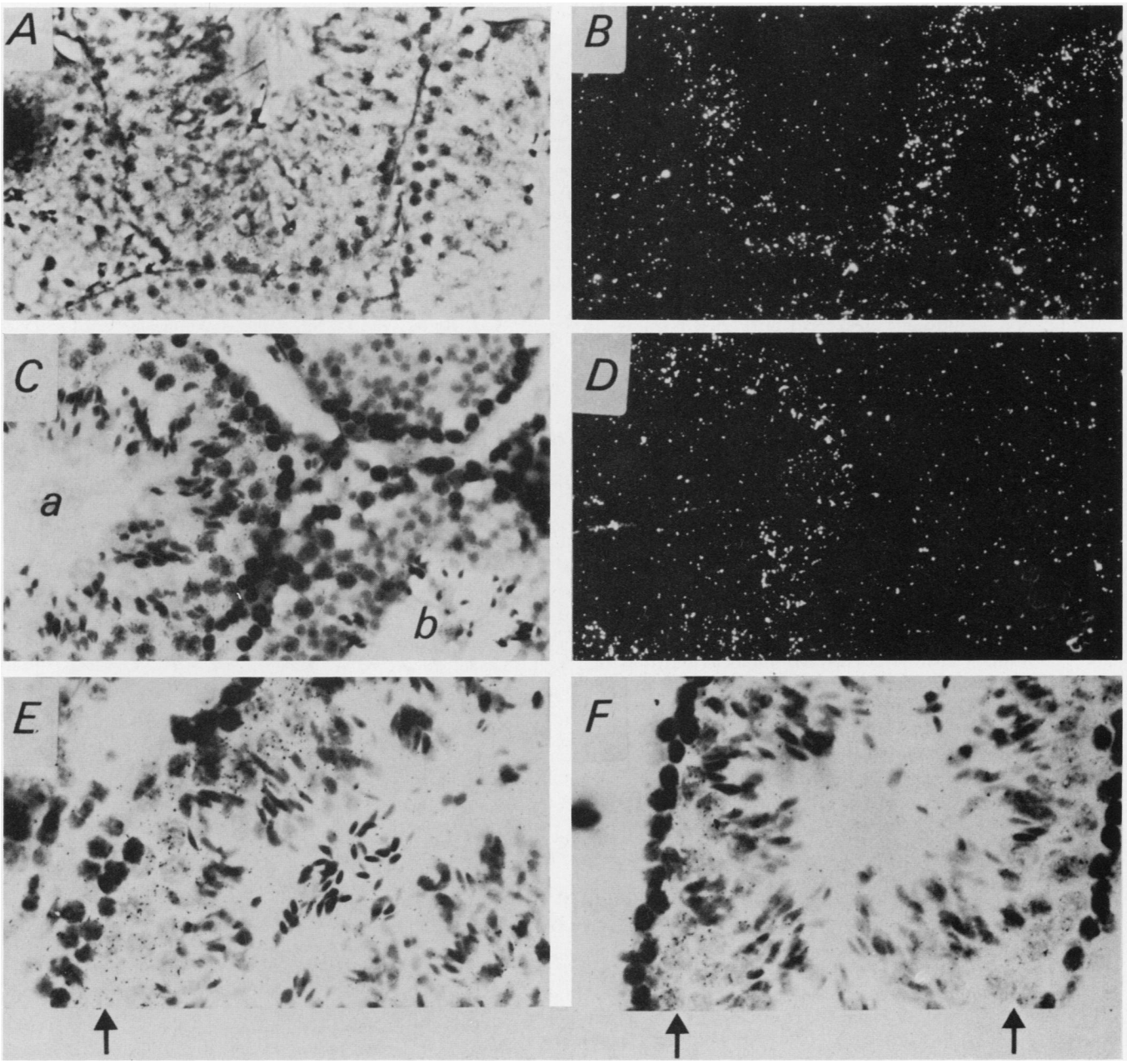

FIG. 2. Identification of cells expressing *ferT* and their cellular associations. High-magnification views of cross sections through the testis that have been hybridized with the antisense *ferT* probe are shown. Panels B and D are the dark-field images of panels A and C, respectively. Two particular cell associations in panel C are marked a and b (see text). Arrows in panels E and F point to the layers of primary spermatocytes expressing *ferT* mRNA. Magnifications, ×288 (A to D) and ×480 (E and F).

A closer view of particular cell associations expressing *ferT* mRNA is provided in Fig. 2. Within each positive cell association, expression of *ferT* was confined to a single or a double ringlike layer of cells adjacent to the spermatogonial layer at the tubule circumference (Fig. 2). Both their morphology as the largest round cells and their position relative to the spermatogonial layers unambiguously identified the *ferT*-expressing cells as primary spermatocytes (this is best seen in the highest magnifications provided in Fig. 2E and F). Clearly, spermatogonia and spermatids at all stages of spermiogenesis showed only background levels of *ferT* mRNA. In the examples shown in Fig. 2, hybridization signals were detectable only over pachytene spermatocytes in cell associations that contained, in addition, peripheral spermatogonia cells and more central elongating spermatids, mostly spermatids at stages 9 and 10 of spermatid development (13). Thus, the cell associations shown in Fig. 2C (association a), E, and F likely represent stage 9 and stage 10 tubules (13). In contrast, background levels of *ferT* mRNA were detected in a cell association that contained, in addition to spermatogonia and primary spermatocytes, round spermatids and spermatids at maturation phase 15 or 16 (association b in Fig. 2C), that is, in a stage 6 or a stage 7 tubule. Since pachytene spermatocytes are present in all cell asso-

ciations excluding tubules at stage 11 (13), accumulation of *ferT* mRNA is likely restricted to a fraction of the pachytene stage. The nature of the positive cell associations suggests that *ferT* accumulation is mostly evident in mid- and late pachytene stages. This notion is also consistent with the failure to detect appreciable levels of *ferT* mRNA by RNA blot analysis before and at day 18 after birth (4). It should be pointed out, however, that the possibility that the methodologies used failed to detect very low levels of *ferT* mRNA present in germ cells at additional developmental stages cannot be excluded. Reasoning that *ferT* might also be expressed during germ cell development in the female gonad, we searched for *ferT* mRNA in ovarian sections, using conditions otherwise identical to those used for in situ hybridization (conditions also previously used by us to detect c-*mos* mRNA in oocytes [9]). *ferT* mRNA could not be detected in oocytes residing in follicles at all stages examined, starting from primordial oocytes and proceeding to oocytes in preovulatory follicles (data not shown). It should be pointed out, however, that by 5 days after birth, all oocytes are in the diplotene stage of the prophase of the first meiotic division. Thus, oocytes have passed the equivalent stage in the developing male germ cell at which *ferT* is expressed. It should also be mentioned that we failed to detect *ferT* mRNA in serial sections obtained from late embryos (>17.5 days postcoitum) (data not shown), thus leaving the adult testis as the only tissue in which *ferT* mRNA has so far been detected.

ferT is the first testis-specific mRNA with a potential tyrosine kinase activity shown to be expressed predominantly in primary spermatocytes. Our analysis showing that *ferT* mRNA accumulates transiently during mid- and late pachytene stages of meiotic prophase, that is, in proximity to the reductive division, suggests a role for this putative tyrosine kinase in a distinct process that precedes or operates in the course of this event. However, until the precise timing for the presence of a biologically active $p51^{ferT}$ protein is established and until its biochemical properties are determined (e.g., its substrates), any suggested role remains speculative. In general, protein kinases have been shown to be involved in regulating the cell division cycle (17, 18, 20), in mediating and transmitting extracellular signals (3, 22), and in regulating differentiative processes in eucaryotic cells (1, 6). Circumstantial evidence suggests that the differentiation of germ cells requires the appropriate cell contacts and is likely dependent on positional information from adjacent cells. Protein(s) with tyrosine kinase activity may play a regulatory role in this process in concert with other regulatory genes. Several genes have been shown to be expressed in germ cells during spermatogenesis (reviewed in references 16 and 23). Among the genes whose participation in control of spermatogenesis has been suggested, one with the most similar pattern of stage-specific expression restricted to pachytene spermatocytes is the gene encoding the recently described zinc finger protein Zfp-35 (2). Certain genes expressed in pachytene spermatocytes display a less-restricted pattern of expression and are also expressed in subsequent spermatid stages (11, 24). Other genes, including *int-1*, c-*mos*, and the testis-specific species of c-*abl*, appear at later stages, where *ferT* mRNA is already undetectable (5, 15, 19, 21). The *ferT*-encoded protein is thus a good candidate for a tyrosine kinase that operates within a network of other regulatory proteins in the coordination of germ cell development.

Work in our laboratories is supported by grants from the Israel Council for Research and Development (to E.K.), the Israel-USA Binational Science Foundation, the Israel Academy of Sciences, and the Israel Cancer Research Fund (to U.N.).

LITERATURE CITED

1. **Bosler, K., and E. Hafen.** 1988. Control of photoreceptor cell fate by the sevenless protein requires a functional tyrosine kinase domain. Cell **54**:299–311.
2. **Cunliffe, V., P. Koopman, A. McLaren, and J. Trowsdale.** 1990. A mouse zinc finger gene which is transiently expressed during spermatogenesis. EMBO J. **9**:197–205.
3. **Ellis, L., E. Clauser, D. O. Morgan, M. Edery, R. A. Roth, and W. J. Rutter.** 1986. Replacement of insulin receptor tyrosine kinase residue 1162 and 1163 compromises insulin-stimulated kinase activity and uptake of 2-deoxyglucose. Cell **45**:721–732.
4. **Fishman, K., J. C. Edman, G. M. Shackleford, J. A. Turner, W. J. Rutter, and U. Nir.** 1990. A murine *fer* testis-specific transcript (*ferT*) encodes a truncated Fer protein. Mol. Cell. Biol. **10**:146–153.
5. **Goldman, D. S., A. A. Kiessling, C. F. Millette, and G. M. Cooper.** 1987. Expression of c-*mos* RNA in germ cells of male and female mice. Proc. Natl. Acad. Sci. USA **84**:4509–4513.
6. **Hafen, E., K. Bosler, J. E. Edstorm, and G. M. Rubin.** 1987. *Sevenless*, a cell specific homeotic gene of Drosophila, encodes a putative transmembrane receptor with a tyrosine kinase domain. Science **236**:55–63.
7. **Hao, Q. L., N. Heisterkamp, and J. Groffen.** 1989. Isolation and sequence analysis of a novel human tyrosine kinase gene. Mol. Cell. Biol. **9**:1587–1593.
8. **Keshet, E., D. Polakiewicz, A. Itin, A. Ornoy, and H. Rosen.** 1989. Proenkephalin A is expressed in mesodermal lineages during organogenesis. EMBO J. **8**:2917–2923.
9. **Keshet, E., M. P. Rosenberg, J. A. Mercer, F. Propst, G. F. Vande Woude, N. A. Jenkins, and N. C. Copeland.** 1988. Developmental regulation of ovarian-specific *Mos* expression. Oncogene **2**:235–240.
10. **Letwin, K., S. P. Yee, and T. Pawson.** 1988. Novel tyrosine kinase cDNAs related to *fps/fes* and *eph*, cloned using anti-phosphotyrosine antibody. Oncogene **3**:621–627.
11. **Nagamine, C. M., K. Chan, L. E. Hake, and Y.-F. C. Lau.** 1990. The two candidate testis-determining Y genes (*Zfy-1* and *Zfy-2*) are differentially expressed in fetal and adult mouse tissues. Genes Dev. **4**:63–74.
12. **Nebel, B. R., A. P. Amarose, and E. M. Hackett.** 1961. Calendar of gametogenic development in the prepubertal male mouse. Science **134**:832–833.
13. **Oakberg, E. F.** 1956. A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous epithelium and germ cell renewal. Am. J. Anat. **99**:391–413.
14. **Parvinen, M.** 1982. Regulation of the seminiferous epithelium. Endocrine Rev. **3**:404–417.
15. **Propst, F., M. P. Rosenberg, A. Ilyer, K. Kaul, and G. F. Vande Woude.** 1987. c-*mos* proto-oncogene RNA transcripts in mouse tissues: structural features, developmental regulation, and localization in specific cell types. Mol. Cell. Biol. **7**:1629–1637.
16. **Propst, F., M. P. Rosenberg, and G. F. Vande Woude.** 1988. Proto-oncogene expression in germ cell development. Trends Genet. **4**:183–187.
17. **Russel, P., and P. Nurse.** 1987. Negative regulation of mitosis by $Weel^+$, a gene encoding a protein kinase homolog. Cell **49**:559–567.
18. **Russel, P., and P. Nurse.** 1987. The mitotic inducer $Nim1^+$ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. Cell **49**:569–576.
19. **Shackleford, G. M., and H. E. Varmus.** 1987. Expression of the proto-oncogene int-1 is restricted to postmeiotic male germ cells and the neural tube of mid-gestational mouse embryos. Cell **50**:89–95.
20. **Simanis, V., and P. Nurse.** 1986. The cell cycle control gene $cdc2^+$ of fission yeast encodes a protein kinase potentially regulated by phosphorylation. Cell **45**:261–268.
21. **Sorentino, V., M. D. McKinney, M. Giorgi, R. Germia, and E. Fleissner.** 1988. Expression of cellular proto-oncogenes in the

mouse male germ line: a distinctive 2.4-kilobase pim-1 transcript is expressed in haploid postmeiotic cells. Proc. Natl. Acad. Sci. USA **85**:2191–2195.

22. **Ulrich, A., L. Coussens, J. S. Hayflick, T. J. Dull, A. Gray, A. W. Tam, J. Lee, Y. Yarden, T. A. Liberman, J. Schlessinger, J. Downward, E. L. V. Mages, N. Whittle, and P. H. Seeburg.** 1984. Human epidermal growth factor receptor cDNA sequences and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature (London) **309**:418–425.
23. **Willison, K., and A. Ashworth.** 1987. Mammalian spermatogenic gene expression. Trends Genet. **3**:351–355.
24. **Wolgemuth, D. J., C. M. Viviano, E. Gizang-Ginsberg, M. A. Frohman, A. L. Joyner, and G. R. Martin.** 1987. Differential expression of the mouse homeobox-containing gene *Hox-1.4* during male germ cell differentiation and embryonic development. Proc. Natl. Acad. Sci. USA **84**:5813–5817.