Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 2191–2195, April 1988
Developmental Biology

Expression of cellular protooncogenes in the mouse male germ line: A distinctive 2.4-kilobase *pim-1* transcript is expressed in haploid postmeiotic cells

(meiosis/oncogenes/spermatogenesis)

VINCENZO SORRENTINO*†, MARTIN D. MCKINNEY*, MAURO GIORGI‡, RAFFAELE GEREMIA‡, AND ERWIN FLEISSNER*§

*Molecular Biology Program, Memorial Sloan–Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; and ‡Department of Public Health and Cellular Biology, II University of Rome, Rome, Italy

Communicated by Edward A. Boyse, December 28, 1987

ABSTRACT **We report that a 2.4-kilobase (kb) *pim-1* transcript is expressed in the germ cells of mouse testis. Analysis of purified populations of spermatogenic cell types indicates that the 2.4-kb transcript is selectively expressed in haploid postmeiotic early spermatids. The evidence for a developmentally regulated expression of *pim-1* in haploid spermatids suggests a possible developmental role for this protooncogene product. The 2.4-kb *pim-1* transcript present in postmeiotic cells differs in size from the 2.8-kb transcript usually detected in somatic tissues. Similar testis-specific transcripts have been seen for *mos* and *abl* genes. These data suggest specificity in transcription or processing of certain genes in haploid male germ cells. We have also analyzed other representative protooncogenes, including examples of protein kinases, the *ras* family, and the "nuclear" protooncogenes. The results indicate that additional protooncogenes are preferentially expressed in either meiotic pachytene cells or postmeiotic early spermatids. These findings suggest a differential regulation of gene expression in these two developmental stages of germ cells. In particular, analysis of expression of the three members of the *ras* gene family indicates a distinct temporal differential regulation in the expression of the Harvey, Kirsten, and N-*ras* genes in these germ cells.**

Cellular protooncogenes are the normal alleles of genes found to be involved in cellular transformation via diverse mechanisms of activation (1). Activated oncogenes can cause cells to divide inappropriately, but, other than this *prima facie* involvement in neoplasia, relatively little is known about the mechanisms by which the products of these genes operate. The functions of protooncogenes are generally assumed to be involved in regulation of cellular proliferation. Support for this hypothesis has come from evidence such as the identification of certain oncogenes as genes encoding growth factors or growth factor receptors (2, 3). Genes such as *fos* and *myc* may be involved in transducing growth factor signals that trigger activation of genes needed for cell proliferation (4–8). Some cellular oncogenes are regulated during differentiation (8, 9) and development (10–12). Recent reports have indicated that constitutive expression of some oncogenes can prevent or induce differentiation in at least some *in vitro* systems (13–17). Expression of protooncogenes during development suggests participation of these genes in normal developmental processes. Sequence similarities have recently linked the cellular protooncogenes *int-1* and *rel* with genes known to be involved in *Drosophila* development (18, 19).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The *pim-1* gene has been detected as a preferential site of provirus integration in virus-induced murine T-cell lymphomas (20). The insertion of Moloney murine leukemia virus in the *pim-1* locus enhances *pim-1* transcription, suggesting that the *pim-1* gene can be activated in its transforming potential by a mechanism similar to that reported for other protooncogenes (21). The *pim-1* locus codes for a 2.8-kilobase (kb) transcript in all normal mouse tissue analyzed (refs. 20 and 21; V.S., data not shown).

Male germ-cell differentiation is a complex process that couples mitotic and meiotic cellular divisions with marked structural changes (22). After mitotic spermatogonial proliferation, the last DNA duplication gives rise to tetraploid preleptotene spermatocytes that then enter meiosis. After a prolonged prophase, in which genetic recombination occurs at the pachytene stage, two cellular divisions without chromosome duplication result in haploid early spermatids (postmeiotic cells). Early spermatids undergo complex structural changes (spermatogenesis) to form mature spermatozoa.

Passage of cells from the pachytene to the early spermatid stage is accompanied with selective changes in gene expression. Specific changes in actins and tubulins have been seen (23), and an unusual actin transcript of 1.4 kb has been detected in haploid early spermatids (24). α-Tubulin cDNAs have also been cloned from a mouse testis cDNA library and found to encode polypeptides expressed exclusively in the testis (25). Recently, analysis of gene expression in the testis has revealed that several other genes are also expressed during male germ-cell differentiation (26–31). Some of these genes produce transcripts in germ cells that differ from those seen for the same genes in other tissues.

Analysis of protooncogene expression in mouse tissue by Muller *et al.* (10) indicated that a testis-specific 4-kb c-*abl* transcript was detectable in mouse testis. This transcript has been further characterized as specifically expressed in postmeiotic haploid spermatids (31). More recently c-*mos* and *int-1* have also been found to be actively transcribed in mouse testis (32–36).

We report here that a 2.4-kb *pim-1* transcript is expressed specifically in postmeiotic spermatids of the adult mouse testis. An extension of such analysis to the expression of representative protooncogenes indicates selectivity in the expression of some of these genes in either pachytene spermatocytes or postmeiotic spermatids. The data reported

†To whom reprint requests should be addressed at: European Molecular Biology Laboratory, Postfach 10 2209, 6900 Heidelberg, F.R.G.
§Present address: Office of the Dean, Division of Sciences and Mathematics, Hunter College, 695 Park Avenue, New York, NY 10021.

here suggest a role of these protooncogene products in normal germ-cell development.

MATERIALS AND METHODS

Prepuberal (<10 days old) newborn mice and sexually mature (>2 mo) CD-1 mice were obtained from Charles River Breeding Laboratories. *Sl/Sl^d* mice were provided by R. Stevens (The Jackson Laboratories). *t^{w5}/t^{w12}* mice were provided by K. Artzt and D. Bennett (University of Texas, Austin). Quaking mice were purchased from The Jackson Laboratories.

Purified germ-cell preparations were from mature male Swiss CD-1 mice. Testes were decapsulated, and cell suspensions were obtained by trypsinization of the seminiferous tubules. In each cell preparation (20–25 mice) 10^9 cells were loaded into an elutriation rotor (Beckman model JE-6D with Saunderson elutriation chamber) to yield cells that were sequentially purified by Percoll density gradients according to Meistrich *et al.* (37).

The cells were extensively washed in phosphate-buffered saline and then counted. The homogeneity of each fraction was assessed by both structural and histochemical parameters (37, 38). We routinely obtained a 98% homogeneous pachytene-spermatocyte population and at least 95% purified round-spermatid preparation (Fig. 1). Cell pellets were frozen at −70°C until use for RNA extraction. Frozen pellets were then dissolved in a 4 M guanidinium isothiocyanate solution, and the suspension was homogenized by using a Polytron homogenizer. RNA was isolated according to Chirgwin *et al.* (39). RNA samples were denatured by heating at 65°C in a formamide/formaldehyde solution, and electrophoretic separation was obtained in 1.1% agarose gel. Twenty micrograms of total cellular RNA was loaded in each lane.

RNA was then transferred to a Nytran membrane by overnight blotting as previously described (40). Filters were hybridized overnight with 2–5 × 10^6 cpm of ^{32}P-labeled DNA probe per ml. DNA probes were labeled by random priming to an efficiency of 0.5–1 × 10^9 cpm per μg. Filters were washed at a final stringency of 0.1× SSC (1× SCC is 0.15 M sodium chloride/0.015 M sodium citrate)/0.5% $NaDodSO_4$ at 65°C. Autoradiography was done at −70°C with an intensifying screen. The *pim-1* probe was the *Bam*HI fragment, probe A, described in ref. 20. The *mos* probe was the probe D described in ref. 32. The *abl* probe was the *Sma* I–*Hin*dIII 1.9-kb fragment from pABsub3 (41). The *raf* probe was the *Sac*II–*Xho* I 0.7-kb fragment from p3611 (42). *Ha-ras* probe was the *Bam*HI–*Eco*RI 2.2-kb fragment from pHEE6 (43). Ki-*ras* was an *Eco*RI 1-kb fragment from pHiHi3 (43). N-*ras* probe was a *Bgl* II–*Hin*dIII fragment corresponding to the 3′ untranslated region (A. Pellicer, New York University). The *myc* probe was an *Xho* I fragment of the mouse c-*myc* cDNA described in ref. 6. The *fos* probe was a *Pst* I–*Bgl* II fragment of v-*fos* described in ref. 5.

RESULTS

Expression of Protooncogenes of the Class of Protein Kinases in Germ Cells: A Distinct 2.4-kb *pim-1* Transcript Is Expressed in Postmeiotic Cells. The *pim-1* locus has been found to be activated in murine leukemia virus-induced T-cell lymphomas (20, 21). Sequence analysis indicates that the *pim-1* gene is a member of the protein kinase class of oncogenes (44). RNA blot analysis has indicated that *pim-1* transcripts are detectable mainly in thymus and in spleen

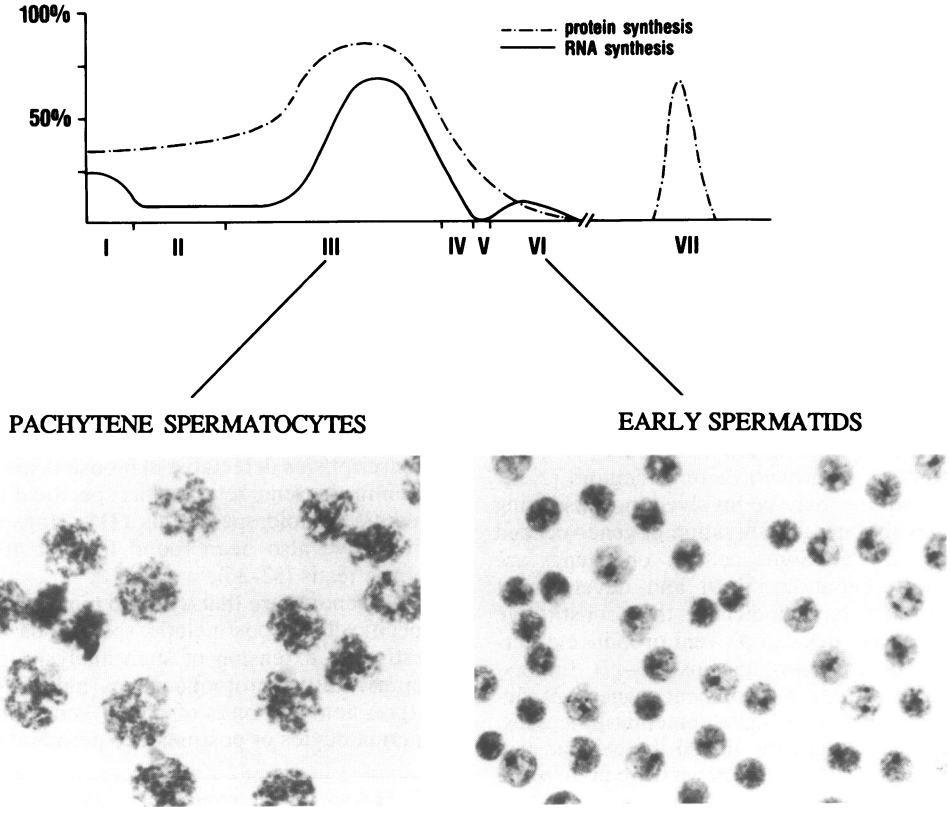

FIG. 1. RNA and nuclear protein synthesis in murine spermatogenesis (modified from ref. 38). Germ cells from adult CD-1 mice were isolated as described. Photographs are ×400 magnifications of cells after fractionation. I, resting stage; II, leptotene and zygotene (3-day) stage; III, pachytene (7-day) stage; IV, diplotene and diakinesis (1-day) stage; V, I and II meiotic division (0.5 day); VI and VII, spermiogenesis (13.5 days)—early spermiogenesis (early spermatids) and late spermiogenesis, respectively.

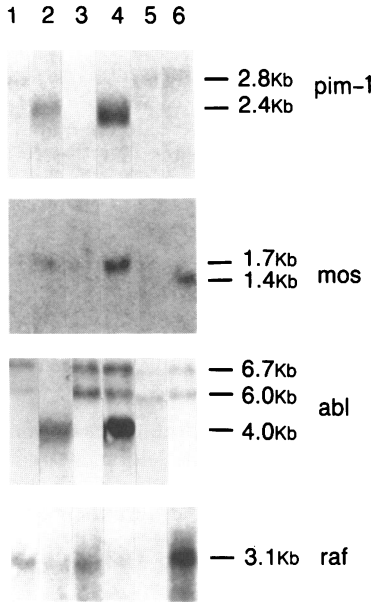

FIG. 2. Expression of *pim-1*, *mos*, *abl*, and *raf* protooncogenes in male germ cells. Total RNA was isolated as described. Lanes: 1, newborn mice testis; 2, adult mice testis; 3, pachytene cells; 4, early round spermatids; 5, adult liver; and 6, adult ovaries.

(20, 21). In these tissues a *pim-1* transcript of 2.8 kb has been described.

Studying the expression of protooncogenes in the male mouse germ line (Fig. 1) we have seen a specific 2.4-kb *pim-1* transcript in testis from adult mice. Fig. 2 shows the results of the analysis of *pim-1* transcripts in newborn and adult mouse testis and in preparations of purified pachytene spermatocytes and postmeiotic early spermatids. The presence of the 2.4-kb transcript appears to correlate with sexual maturation because in testis from newborn mice only a low level of the 2.8-kb *pim-1* transcript is seen (lanes 1, 2). Analysis of total RNA prepared from purified preparations of pachytene spermatocytes and from postmeiotic spermatids indicated that the particular 2.4-kb transcript is expressed only in postmeiotic spermatids (lane 4). In RNA extracted from adult mouse ovaries we saw only the 2.8-kb transcript (lane 6).

Also shown in Fig. 2 are the data on the expression of the *mos* gene in germ cells. The *mos* testis-specific transcript first observed by Propst and Vande Woude (32) is also present only in sexually mature adult animals (lane 2) and is not present in prepuberal mice (lane 1). Analysis of RNA extracted from purified germ cells indicated that the *mos*-related mRNA was specifically detected in postmeiotic cells (lane 4). The observed size of this mRNA species is 1.7 kb, corresponding to the band seen in adult testis. In RNA from cells at the pachytene stage we did not see any *mos* transcript. In ovaries from adult mice a smaller *mos*-specific transcript of 1.4-kb size was seen (lane 6). While this work was in preparation, other groups reported the expression of the *mos* oncogene in purified germ cells (33–35).

For comparison, the specific expression of the *abl* protooncogene is also shown in Fig. 2. We have also analyzed the *raf* protooncogene (42), another member of the protein kinase class, which we found to be transcribed in both newborn and adult testis. However, in contrast with the above-described genes, analysis of purified pachytene cells and early spermatids indicated that the *raf* gene is expressed mainly in meiotic pachytene cells; a significant reduction in the levels of *raf* transcript occurs when the cells complete meiosis and divide to form early spermatids.

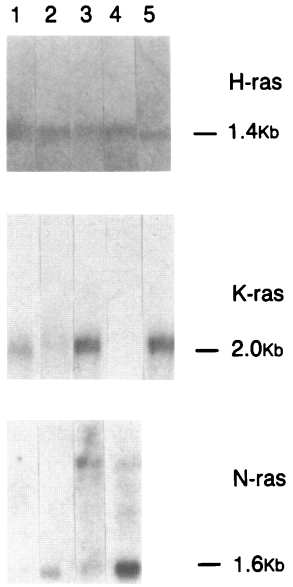

FIG. 3. Differential expression of the members of the *ras* family genes in male germ cells. RNA samples were as follows: newborn testis (lane 1), adult testis (lane 2), pachytene cells (lane 3), early round spermatids (lane 4), and adult ovaries (lane 5). H-ras, Ha-*ras*; K-ras, Ki-*ras*.

Expression of the Three Members of the *ras* Family in Murine Male Germ Cells. Three closely related, evolutionarily conserved *ras* genes are present in the mouse genome (45). Little information is currently available about the physiological roles of these genes. These genes encode highly conserved proteins, differing only at the carboxyl terminus, a region implicated in interaction with the plasma membrane and possibly with other proteins. Using a probe specific for Ha-*ras* we detected low levels of transcript in both meiotic and postmeiotic cells (Fig. 3). As shown in the same figure, Ki-*ras* transcripts were detected only in RNA from cells at the pachytene stage. No hybridization to RNA extracted from spermatids was seen. Analysis of the transcripts specific for N-*ras*, the third member of the *ras* family, was done with a probe specific for the 3′ untranslated region of the mRNA (provided by A. Pellicer, New York University). A high level of N-*ras* transcript is seen in testis from adult mice compared with newborn testis. Consistent with this result, a low level of the N-*ras* gene transcripts is seen in pachytene cells, and a 10- to 20-fold increase in message levels can be seen as the cells differentiate into postmeiotic spermatid cells.

Expression of Protooncogenes Encoding Nuclear Proteins. Another group of protooncogenes that has been defined by

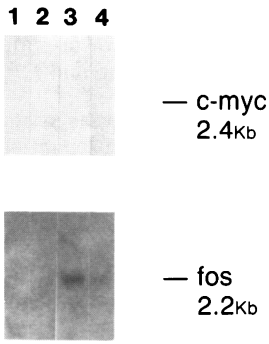

FIG. 4. Expression of protooncogenes encoding nuclear proteins. Total RNA, 20 μg per lane, was from newborn testis (lane 1), adult testis (lane 2), pachytene cells (lane 3), and early spermatids (lane 4).

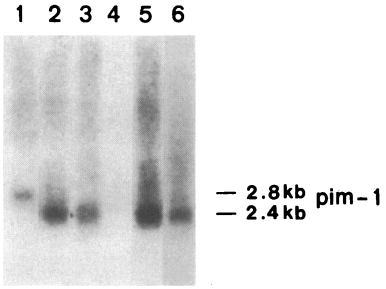

FIG. 5. Detection of *pim-1* transcripts in RNA from testes of mutant mice with defects in germ-cell development. RNA was isolated as described, and 20 μg of total RNA was loaded per lane. Lanes: 1, Sl/Sl^d mice; 2, *qk/qk* mice; 3, t^{w5}/t^{w12} mice; 4–6, pachytene spermatocytes, early spermatids, and residual bodies from CD-1 adult mice, respectively.

their products' functional properties as well as their intracellular location is represented by the "nuclear" protooncogenes. As representatives of this group we have studied the expression of c-*myc* and c-*fos* genes. Analysis of *fos* gene expression indicates that this gene is transcribed in the germ cells at the pachytene stage (Fig. 4). The level of *fos* gene transcripts appears to drop when the cells differentiate to the stage of early spermatids. The level of *fos* transcripts is too low to be detected in newborn and adult testis (lanes 1 and 2). We did not detect c-*myc* transcripts in either newborn and adult testis or in purified pachytene and early spermatid cells, consistent with previous reports (46).

Sterile Mutant Mouse Strains: *pim-1*, *mos*, and *abl* Transcripts as Markers of Germ-Cell Differentiation. Experiments were done to assess whether those genes preferentially expressed in the haploid phase of spermatogenesis may be used as markers of defects in germ-cell differentiation. For this purpose three different strains of sterile mice were used. We first analyzed Sl/Sl^d mice, which completely lack male germ cells (47). The second mutant strain we used was quaking (*qk*) mice, which carry an autosomal recessive mutation that causes defects at the level of myelinization in the central nervous system and almost complete azoospermia in the homozygous male (48). However, in contrast to Sl/Sl^d mice, which completely lack germ cells, *qk/qk* mice possess germ cells that are able to complete the meiotic process and form spermatids but are unable to complete spermiogenesis. The third strain of mutant sterile mice that we examined was t^{w5}/t^{w12} mice (49). These are of particular interest in that they can complete the entire process of spermatogenesis and produce spermatozoa that are infertile. An interesting feature of these latter mice is that they are mutants of the *T* (brachyury) locus, the involvement of which in spermatogenesis has long been studied (49).

Analysis of RNA from these mutants demonstrates that expression of the 2.4-kb *pim-1* transcript correlates with the presence of spermatids in quaking and t^{w5}/t^{w12} mice. Lack of germ cells in Sl/Sl^d mice results in failure to express the 2.4-kb transcript (Fig. 5). Also Fig. 5 shows that the *pim-1* transcript detectable in early spermatids is present in RNA prepared from residual bodies as well (lane 6).

In Table 1 we report a more extensive analysis of genes expressed in mutant mice testes. No testis-specific transcripts for *abl* and *mos* genes could be detected in RNA extracted from the testes of adult Sl/Sl^d mice, confirming that also these genes are specific for germ cells. In RNA preparations from both *qk/qk* and t^{w5}/t^{w12} animals we observed the testis-specific *abl* and *mos* transcripts that correlate with the presence of postmeiotic cells in these animals. The level of expression of the N-*ras* gene was also consistent with the presence or absence of early spermatids in testis of the mutants.

By comparing expression of the genes of *pim-1*, *mos*, and *abl* with stages of germ-cell development reached in each mutant, it is evident that these genes can be used as markers for the presence of germ cells differentiated to the stage of early postmeiotic spermatids. The results also indicate that other unknown defects cause sterility in *qk/qk* and t^{w5}/t^{w12} strains of mice.

DISCUSSION

We report the presence of a 2.4-kb *pim-1* transcript expressed in adult mouse testis, in addition to the known 2.8-kb transcript found expressed in other tissues such as thymus (20, 21, 44). The 2.4-kb *pim-1* transcript is specifically expressed in the germ cells, as indicated by lack of this transcript in testis from adult Sl/Sl^d mice. Analysis of RNA from purified germ cells shows that expression of this transcript is restricted to postmeiotic early spermatids. These data strongly suggest that *pim-1* is implicated in the normal development of male germ cells. An atypically sized testis-specific transcript for this gene also provides a molecular marker for differentiating male gene cells.

We have also studied the expression of a larger group of protooncogenes in murine male germ cells. An interesting example is provided by the expression patterns of the three members of the *ras* gene family. Although low levels of Ha-*ras* transcript are present in both pachytene and postmeiotic cells, a substantial expression of Ki-*ras* can be observed for cells at the pachytene stage but not in early spermatids. In contrast, N-*ras* transcripts, quite low in pachytene cells, are found abundantly expressed in early spermatids.

This is one of the clearest examples of differential expression among members of the *ras* family and is interesting because of recent evidence for *ras* gene involvement in differentiation and development (11, 17). In contrast with genes such as *pim-1*, *abl*, *mos*, and N-*ras*, which are expressed preferentially in postmeiotic haploid cells, we have found that *raf*, *fos*, and Ki-*ras* appear to be expressed preferentially in pachytene cells and that the levels of

Table 1. Expression of protooncogenes in testes from mutant mouse strains with different genetic lesions that affect spermatogenesis

	Mutant mice			CD-1 mice	
	Sl/Sl^d	*qk/qk*	t^{w5}/t^{w12}	Newborn	Adult
Germ-cell developmental stage	Null	Spermatids	Spermatozoa (infertile)	Spermatogonia	Spermatozoa (fertile)
Oncogene					
pim-1	−	+	+	−	+
c-*abl*	−	+	+	−	+
c-*mos*	−	+	+	−	+
N-*ras*	±	+	+	±	+

+, Expression of gene; −, no gene expression; ±, slight gene expression.

transcripts from these genes decline in postmeiotic early spermatids. These results indicate that postmeiotic early spermatids express a pattern of genes different from the one seen in pachytene cells.

Haploid gene expression has been reported for some genes (26, 27, 31, 33–36, 50); some of these genes produce transcripts of unusual size in early spermatids that differ from the transcript expressed in other somatic tissues. Our finding of a specific 2.4-kb *pim-1* transcript in early spermatids, and the similar findings for other genes such as *abl* and *mos* (refs. 31, 33–35; and our results), as well as our data on N-*ras* expression, together suggest that transcripts of these genes result from *de novo* transcription occurring in early spermatids. These data also indicate that in early spermatids some genes undergo a specific initiation or processing of transcripts to produce the observed testis-specific RNA. That different transcripts can be processed from the same gene has been demonstrated; such a process (51) can cause alterations in the coding sequences or affect mRNA functions or stability in another way (52, 53). In postmeiotic cells, the reason for atypically sized transcripts is unknown. Specificity of the cell system suggests that these transcripts may represent more stable messages that can survive for the time needed by early spermatids to mature into fully differentiated spermatozoa (38). In agreement with this hypothesis, we detected the 2.4-kb *pim-1* transcript in the residual bodies (Fig. 5, lane 6) that are released from the differentiated spermatozoa, as has also been observed for other testis-specific transcripts (24, 27, 31, 36).

Detection of a testis-specific 2.4-kb *pim-1* transcript and the demonstration of the regulated expression of other protooncogenes in male germ cells, encoding proteins that are sometimes rarely expressed in somatic tissues, suggests that products of these genes are causally involved in the differentiation of these cells. This developmental system should help illuminate the function of these genes.

We thank Drs. P. Besmer and P. O'Donnell for providing some of the plasmids used and Dr. E. Dmitrovsky for discussing and reading this paper. This work was supported by National Institutes of Health Grant CA-41518 from the National Cancer Institute and Consiglio Nationale delle Ricerce (Italy) Oncologia P.F. 86.00429.44.

1. Varmus, A. (1987) in *The Molecular Basis of Blood Disease*, eds. Stomatoyannopulos, G., Nienhuis, A. W., Leder, P. & Majerus, P. W. (Sanders, Philadelphia), pp. 271–346.
2. Doolittle, R. F., Hunkapillar, M. W., Devare, S. G., Robbins, K., Aaronson, S. & Antoniades, H. (1983) *Science* **221**, 275–277.
3. Sherr, C., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. (1985) *Cell* **41**, 665–676.
4. Greenberg, M. E. & Ziff, E. B. (1984) *Nature (London)* **311**, 433–438.
5. Muller, R., Bravo, R., Burckhardt, J. & Curran, T. (1984) *Nature (London)* **312**, 716–720.
6. Sorrentino, V., Drozdoff, V., McKinney, M. D., Zeitz, L. & Fleissner, E. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8167–8171.
7. Hume, C. R., Nocka, K. H., Sorrentino, V., Lee, J. S. & Fleissner, E. (1988) *Oncogene*, in press.
8. Lachman, H. M., Hatton, K. S., Skoultchi, A. I. & Schildkraut, C. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5323–5327.
9. Muller, R., Curran, T., Muller, D. & Guilbert, L. (1985) *Nature (London)* **314**, 546–548.
10. Muller, R., Slamon, D. J., Tremblay, J. M., Cline, M. J. & Verma, I. M. (1982) *Nature (London)* **299**, 640–644.
11. Leon, J., Guerrero, I. & Pellicer, A. (1987) *Mol. Cell. Biol.* **7**, 1535–1540.
12. Jakobovits, A., Shackleford, G. M., Varmus, H. E. & Martin, G. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7806–7810.
13. Falcone, G., Tato, F. & Alema, S. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 426–430.
14. Coppola, J. A. & Cole, M. D. (1986) *Nature (London)* **320**, 760–763.
15. Dmitrovsky, E., Kuehl, W. M., Hollis, G. F., Kirsch, I. R., Bender, T. & Segal, S. (1986) *Nature (London)* **322**, 748–750.
16. Alema, S., Casalbore, P., Agostini, E. & Tato, F. (1985) *Nature (London)* **316**, 557–559.
17. Bar-Sagi, D. & Feramisco, J. R. (1985) *Cell* **42**, 841–848.
18. Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D. & Nusse, R. (1987) *Cell* **50**, 649–657.
19. Steward, R. (1987) *Science* **238**, 692–694.
20. Cuypers, H. T., Selten, G., Quint, W., Zijlstra, M., Robanus-Maandag, E., Boelens, W. & Berns, A. (1984) *Cell* **37**, 141–150.
21. Selten, G., Cuypers, H. T. & Berns, A. (1985) *EMBO J.* **4**, 1793–1798.
22. Bellvé, A. R. (1979) in *Oxford Reviews of Reproductive Biology*, ed. Finn, C. A. (Clarendon Press, Oxford), pp. 159–261.
23. Hecht, N. B., Kleene, K. C., Distel, R. J. & Silver, L. M. (1984) *Exp. Cell Res.* **153**, 275–279.
24. Waters, S. H., Distel, R. J. & Hecht, N. B. (1985) *Mol. Cell. Biol.* **5**, 1649–1654.
25. Villasante, A., Wang, D., Dobner, P., Dolph, P., Lewis, S. A. & Cowan, N. J. (1986) *Mol. Cell. Biol.* **6**, 2409–2419.
26. Kleene, K. C., Distel, R. J. & Hecht, N. B. (1983) *Dev. Biol.* **98**, 455–464.
27. Zakeri, Z. F. & Wolgemuth, D. J. (1987) *Mol. Cell. Biol.* **7**, 1791–1796.
28. Gizang-Ginsberg, E. & Wolgemuth, D. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1600–1604.
29. Wolgemuth, D. J., Viviano, C. M., Gizang-Ginsberg, E., Frohman, M. A., Joyner, A. L. & Martin, G. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5813–5817.
30. Kilpatrick, D. L. & Millette, C. F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5015–5018.
31. Ponzetto, C. & Wolgemuth, D. J. (1985) *Mol. Cell. Biol.* **5**, 1791–1794.
32. Propst, F. & Vande Woude, G. F. (1985) *Nature (London)* **315**, 516–518.
33. Propst, F., Rosenberg, M. P., Kaul, I. K. & Vande Woude, G. F. (1987) *Mol. Cell. Biol.* **7**, 1629–1637.
34. Mutter, G. L. & Wolgemuth, D. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5301–5305.
35. Goldman, D. S., Kiessling, A. A., Millette, C. F. & Cooper, G. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4509–4513.
36. Shackelford, G. M. & Varmus, H. E. (1987) *Cell* **50**, 89–95.
37. Meistrich, M. L., Longtin, Brock, W. A., Grimes, S. R. & Mace, M. L. (1981) *Biol. Reprod.* **25**, 1065–1077.
38. Monesi, V. (1971) *J. Reprod. Fert. Suppl.* **13**, 1–14.
39. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
40. Sorrentino, V., Drodzoff, V., Zeitz, L. & Fleissner, E. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4131–4134.
41. Goff, S. P., Gilboa, E., Witte, O. N. & Baltimore, D. (1980) *Cell* **22**, 777–785.
42. Rapp, U. R., Goldsborough, M. D., Mark, G. E., Bonner, T. I., Groffen, B. J., Reynolds, F. H., Jr., & Stephenson, J. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4218–4222.
43. Ellis, R. W., De Feo, D., Shih, T., Gonda, M., Young, H. A., Tsuchida, N., Lowy, D. & Scolnick, E. M. (1981) *Nature (London)* **292**, 506–511.
44. Selten, G., Cuypers, H. T., Boelens, W., Robanus-Maandag, E., Verbeek, J., Domen, J., van Beveren, C. & Berns, A. (1986) *Cell* **46**, 603–611.
45. Barbacid, M. (1987) *Annu. Rev. Biochem.* **56**, 779–828.
46. Stewart, T. A., Bellvé, A. R. & Leder, P. (1984) *Science* **226**, 707–710.
47. Green, M. C. (1981) *Genetic Variants and Strains of the Laboratory Mouse* (Fischer, Stuttgart, F.R.G.).
48. Bennett, W. I., Gall, A. M., Southard, J. L. & Sidman, R. L. (1971) *Biol. Reprod.* **5**, 30–58.
49. Bennett, D. (1975) *Cell* **6**, 441–454.
50. Peschon, J. J., Behringer, R. R., Brinster, R. L. & Palmiter, R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5316–5319.
51. Sharp, P. A. (1981) *Cell* **23**, 643–646.
52. Shaw, G. & Kamen, R. (1986) *Cell* **46**, 659–667.
53. Brawerman, G. (1987) *Cell* **48**, 5–6.