Cloning and characterization of genes expressed during gametogenesis of female and male mice

D. M. López-Alan and J. del Mazo*

Centro de Investigaciones Biolgicas, Velzquez 144, 28006-Madrid, Spain

Isolation and initial characterization of clones from a mouse fetal ovary cDNA library are reported. In mammals, the development of female gametogenesis, including the prophase of the first meiotic division, begins in the fetus. A parallel conserved process occurs in males, from puberty onwards. We cloned cDNAs preferentially expressed in both sexes during gametogenesis considering both the meiotic programme and the non-germ cells participating in the developmental regulation of gametogenesis. From 183 clones that showed positive hybridization with adult testis and negligible hybridization with somatic probes, 32 clones were selected. After partial sequencing, 16 were found to correspond to known genes that are expressed during other programmes of differentiation and development. Sixteen clones represent novel sequences. The accumulation of corresponding transcripts was analysed by developmental northern blots during spermatogenesis, and genes expressed during gametogenesis were characterized together with others showing pleiotropic expression in different tissues. Additional specific, gametic transcripts were also detected.

Introduction

Differential screening of cDNA libraries has been carried out to isolate genes expressed in a defined developmental process and at a specific stage (Hg, 1991; Nedivi *et al.*, 1993). In this context, and consistent with the goals of the Human Genome Programme (Adams *et al.*, 1991, 1993), different tissues and systems are being assessed to isolate specific genes involved in the gene regulation of the various developmental stages of tissues and organisms. This approach contributes potential new tags for genome mapping and the characterization of unknown proteins and associated functions.

Gametogenesis is a developmental process that involves cascades of regulatory gene expression. In mammals, as a result of terminal differentiation, the gamete is the product of differentiation events in germ cells, including meiosis, and regulatory interaction with somatic components of the gonads, coordinated by endocrine signals. The origin of gametocytes is common to both sexes: they are derived from primordial germ cells after they reach and colonize the genital ridges, in mice, 11 days after coitus (Mintz and Russell, 1957).

In mammalian fetuses, the programme of gonad sex-specific differentiation is followed by a defined pathway of entry into meiosis to generate oocytes in females and prospermatogonia in males. The timing of meiosis and the development of gametogenesis is different between the sexes. In females, germ cell development is characterized by early onset of the meiotic prophase, which starts in the mouse fetus 13 days after coitus, and progresses under partial synchrony until the dictyotene stage at birth. In contrast, spermatogenesis is a continuous

process, in which the onset of the first meiosis occurs at puberty and continues throughout adult life. In addition, the development of gametogenesis is controlled by complex mechanisms of endocrine, paracrine and autocrine regulation (Pescovitz *et al.*, 1994), which also includes active cell to cell signalling, which can modulate the process both in oogenesis and spermatogenesis. Sertoli cells in the testes (Djakiew and Dym, 1988) and granulosa cells in the ovaries (Eppig, 1991) could play a similar role in such regulation. Thus, the same or related genes could be expressed during the corresponding stages of development in both sexes. In addition to isolating genes involved in the regulation of oocyte progression in the mammalian fetus, interest in characterizing genes expressed preferentially, both in fetal ovaries and adult testes, is based on (i) genes expressed in the same process, e.g. meiosis; (ii) genes involved in regulation by interaction between somatic and germ cells; and (iii) genes involved in mechanisms of terminal differentiation.

In female mouse fetuses, about 75% of the oocytes are at the pachytene stage 16 days after coitus (Speed, 1982). Transcripts corresponding to products involved in the same functions and expressed at this stage, in which a high rate of transcription occurs (Kierszenbaum and Tres, 1974; Hecht, 1986), should be present in adult testes, in which about 30% of the seminiferous tubule cells are also at the pachytene stage (Bellv *et al.*, 1977). Thus, to identify clones corresponding to transcripts present in the development of male and female gametogenesis, including those controlling meiosis, a cDNA library has been generated from fetal ovaries at 16 days after coitus. A two-step differential screening method has been developed, using as probes single strand cDNAs reverse transcribed from somatic tissue mRNAs versus testis mRNAs. After this double differential screening, 32 clones were isolated, 50% of which represented

*Correspondence.

Received 19 October 1994.

genes not previously described. Developmental expression analysis during gametogenesis of novel genes shows possible roles for these genes expressed during gametogenesis.

Materials and Methods

Construction of a fetal ovary cDNA library

Female embryos were selected from Swiss mice at 16 days of pregnancy and, after dissection, 300 ovaries from those embryos were collected. Total RNA was purified from the ovaries as described by Chomczynski and Sacchi (1987). Poly(A) + RNA was selected using biotinylated oligo(dT) as primer, and captured with streptavidin coupled to paramagnetic particles (López-Fernández and del Mazo, 1993) in a magnetic separation stand (Promega, Madison). The cDNA was synthesized from 5 µg of ovary poly(A) + RNA, using an oligo (dT)₁₂₋₁₆ as primer having a XhoI site at the 3' end. Fractioning of double-stranded, [³²P] cDNA, on 3.5% acrylamide gels, indicated that cDNA ranged from 900 to 4500 base pairs and most were about 1300 base pairs. Directional cDNA inserts were generated into λ uni-Zap XR vector (Stratagene, La Jolla), after ligation of EcoRI linkers and digestion with EcoRI and XhoI. Ligated DNA was packaged *in vitro* using a commercial extract from Stratagene.

Selection of cDNA clones by differential screening

After infection of SURE cells (Stratagene) by phages, approximately 10 000 plaques were screened at a density of 1000 pfu per nitrocellulose filter (Schleicher and Schuell, Dassel). The probes of single-stranded cDNA used in the differential screening were obtained from 5 µg of an equivalent mixture of poly(A) + RNAs prepared from kidney, muscle, intestine and liver from embryos at day 16 after coitus, or alternatively from 5 µg of poly(A) + RNAs prepared from adult testes. The single-stranded cDNAs were synthesized by starting the reaction with an oligo(dT)₁₂₋₁₆ primer by adding AMV reverse transcriptase (Promega, Madison). The single stranded cDNAs were labelled by incorporation of [α -³²P]dCTP (3000 Ci mmol⁻¹) in the reverse transcription reaction. The differential screening was performed by hybridization at 42°C for 72 h on duplicate filters with somatic versus testis probes in: 50% (w/v) formamide, 0.25% (w/v) SDS, 5 × SSC (1 × SSC is 150 mmol l⁻¹ NaCl/15 mmol sodium citrate l⁻¹, pH 7.0), 5 × Denhardt's solution (1 × Denhardt's solution is 0.02% (w/v) bovine serum albumin/0.02% (v/v) Ficoll/0.02% (w/v) polyvinylpyrrolidone) and 100 µg of denatured salmon spermatozoa DNA ml⁻¹, using 10⁶ c.p.m. probe ml⁻¹. The filters were washed at 65°C for 30 min in 0.1 × SSC, 0.1% (w/v) SDS (Sambrook *et al.*, 1989). The autoradiographic film was developed after exposure for 3 days. The pfus that exhibited positive hybridization with testis probes and null or negligible hybridization with somatic probe single stranded cDNAs were selected and suspended in 1 ml SM buffer (0.1 mol NaCl l⁻¹, 10 mmol MgSO₄ l⁻¹, 50 mmol Tris-HCl l⁻¹ pH 7.5, gelatin 0.01% w/v) with 20 µl chloroform (Sambrook *et al.*, 1989).

Fig. 1. Example of the second differential screening using PCR amplified cDNA inserts. (a) Ethidium bromide staining of PCR products after agarose gel electrophoresis. (b) Southern blot of PCR products from gel (a), after hybridization using single strand DNAs from adult testis RNA as probe. (c) Blot from a duplicate gel hybridized with single-stranded cDNAs from somatic RNA. A differential signal is observed in the clone GEG-108.

Second screening of clones

The second screening was performed by Southern blot analysis (Sambrook *et al.*, 1989) of PCR-amplified insert DNAs from initially selected clones. For this, 20-base primers corresponding to the opposite side of the cDNA flanking the cloning site in the vector were synthesized: primer (5'-TGAAAACGACGGCCAGTGA-3') and reverse primer (5'-TGACCATGATTACGCCAAGC-3'). For the polymerase chain reaction (PCR) 25 pmol of each primer and reverse primer

Table 1. Similarities of the clones of mouse gametogenesis expressed genes with sequences in the EMBL database

GEG clone no.	Putative identification (species)	Per cent similarity 5' end (base pairs)	Per cent similarity 3' end (base pairs)	EMBL accession no.
GEG-98	surf-3 (M)	92% (172)	86% (148)	M20741
GEG-25	Collagen (VI), $\alpha 2$ (M)	98% (149)	98% (205)	X62332
GEG-61	Pyruvate kinase-M1, M2 (R)	87% (132)	$\leq 40\%$	M24359
GEG-139	Insulin-like growth factor II (M)	95% (160)	81% (118)	M36332
GEG-72	Laminin B2 (M)	96% (97)	98% (193)	J03484
GEG-164	Na, K-ATPase, $\alpha 1$ (H)	$\leq 40\%$	85% (148)	X04297
GEG-178	c-jun (M)	92% (148)	90% (164)	J04115
GEG-89	Histone H3.3, pseudogen (M)	93% (114)	85% (145)	M18678
GEG-92	Histone H3.3, pseudogen (M)	78% (75)	91% (180)	M18678
GEG-17	Mitochondrial genome (M)	93% (127)	94% (121)	V00711
GEG-102	Mitochondrial genome (M)	97% (111)	90% (122)	V00711
GEG-70	Globin, α (M)	85% (130)	89% (209)	V00714
GEG-90	Globin, α (M)	98% (88)	85% (80)	V00714
GEG-104	Globin, α (M)	$\leq 40\%$	95% (173)	V00714
GEG-146	Ubiquitin (M)	98% (144)	98% (180)	X51703
GEG-159	Ubiquitin (M)	85% (76)	90% (171)	X51703

GEG: gametogenesis expressed gene; M: mouse; R: rat; H: human.

(Saiki *et al.*, 1985) were combined with the following reagents in a final volume of 100 μ l: 10 μ l of the phage suspension, 0.2 mmol l^{-1} of each deoxynucleotide, PCR buffer (50 mmol KCl l^{-1} , 10 mmol Tris-HCl l^{-1} , pH 8.8 at 25°C, 1.5 mmol $MgCl_2$ l^{-1} and 1% (w/v) Triton X-100) and 2.5 U of TaqDNA polymerase (Promega). After 35 cycles of PCR, the lengths of the cDNAs amplified were analysed by electrophoresis in a 1% (w/v) agarose gel in duplicate. The gels were blotted to nylon filters and the blots hybridized with the same first single stranded cDNA probes from the somatic tissues and adult testis, and under the same conditions as in the first screening. The cDNA clones that showed strong hybridization with adult testis cDNA and null or negligible hybridization with somatic cDNA probes were finally selected (Fig. 1).

In vivo excision of pBluescript containing the cDNA inserts

The cloned inserts contained within the λ uni-ZAP XR vector were excised *in vivo* following the commercial protocol (Stratagene). The rescued phagemid was plated on Luria Bertani (LB)/Ampicillin plates and incubated overnight at 37°C. The colonies were isolated, incubated in LB-Ampicillin media and stored at $-80^\circ C$ with 50% (v/v) glycerol.

DNA sequencing

Dideoxy-terminator sequencing reactions were performed with [α - ^{35}S]dATP and using both primers (to sequence the 3' end) and reverse primers (to sequence the 5' end) synthesized for the DNA amplification of the inserts, following the instructions of the T7-Sequencing Kit purchased from Pharmacia Biotech (Uppsala).

Northern blot analysis

Total RNA was isolated from 100 mg of tissues using the acid phenol-guanidinium method (Chomczynski and Sacchi, 1987). Human testis RNA was obtained from orchidectomy biopsies from patients with normal spermatogenesis development. The informed consent of the patients and approval of the local ethical committee were obtained. The RNA was separated by electrophoresis in 1% (w/v) agarose with 17% formaldehyde (v/v) under denaturing conditions and transferred onto nylon membranes by a vacuum blotter. The gametogenesis expressed genes and β -actin-containing plasmids were labelled with [α - ^{32}P]dCTP by random hexanucleotide priming and hybridized to the membranes at 42°C overnight. Blots were washed twice at room temperature in $2 \times SSC/0.1\%$ (w/v) SDS and twice at 55°C in $0.1 \times SSC/0.1\%$ (w/v) SDS. Autoradiographs were obtained after exposure of blots to Kodak-XAR film with intensifying screens.

Results

Analysis and isolation of cDNA clones

The cDNA library contained 5×10^5 primary plaques and was amplified to a titre of 3×10^9 pfu ml^{-1} . The library was evaluated by screening lifts from 20 000 plated clones and scoring positive clones on films after hybridization with a β -actin probe. The frequency of actin cDNA clones was 0.37%. This result is similar to that obtained in a similar fetal ovary cDNA library from day 17 after coitus (0.25%), but different from an adult testis cDNA library (0.07%; unpublished data). Consequently, the results are internally coherent and, as they are in the range of actin-hybridizing clones found in cDNA

Table 2. Developmental gene expression for novel gametogenesis expressed genes by northern analysis

GEG clone number	Transcript size detected in adult testis (kb)	Level of expression during testis development from day 6 to adult	Transcript size detected in somatic tissues (kb)
GEG-60	1.0	+	None detected
	1.9	+	1.9
GEG-65	2.4	=	2.4
	6.0	=	6.0
GEG-68	1.6	+	1.6
	1.9	+	1.9
	3.0	+	None detected
	4.2	=	4.2
GEG-91	5.0	=	5.0
GEG-93	2.0	=	2.0
	2.4	=	2.4
	5.2	+	None detected
GEG-97	1.9	=	1.9 (none detected in brain)
	5.3	—	5.3 (high level in brain and none detected in kidney)
GEG-108	7.0	=	None detected
GEG-121	2.4	=	2.4
	4.8	—	4.8
GEG-123	1.2	+	None detected
	1.9	=	1.9 (very low expression)
	5.0	—	5.0
GEG-136	1.8	=	1.8
	3.8	+	None detected
	4.7	—	4.7
GEG-152	2.4	=	2.4 (very low expression)
	6.0	+	6.0
GEG-154	3.0 (only detected in day 22 and adult)	+	None detected
	4.2	+	None detected
GEG-168	2.0	+ (maximum at day 14)	None detected
	5.4	=	5.4 (very low expression)

The level of transcript accumulation during testis development is represented as (—): decreasing; (=): constant; or (+): increasing. GEG: gametogenesis expressed gene.

libraries from different mammalian tissues (Ausubel *et al.*, 1990), could be considered in the evaluation of similar libraries. The proportion of recombinants was 70%; both after *in vivo* excision of a sample of the cDNA library and scoring the percentage of white/blue colonies plated on X-gal/IPTG medium.

Approximately 10 000 clones were distributed onto 10 plates. The abundantly expressed cDNA clones (Sambrook *et al.*, 1989) were marked by hybridizing duplicate filters with a mixture of single-stranded cDNA prepared from a mix of fetal kidney, muscle liver and intestine RNAs or with single stranded cDNA prepared from adult testes. From this screening, 183 clones that were positive with testis and negative with somatic probes were selected.

These cDNA clones were rescreened after PCR amplification, blotted on nylon filters and screened by somatic versus testis probes. The size of the 183 cDNA cloned inserts ranged from 300 to 3000 base pairs, with an average of 1600 base pairs. After this second screening, 32 clones showed strong

hybridization with testis cDNA and negligible hybridization with somatic cDNA probes (Fig. 1).

Characterization of GEG clones

At least 150–200 base pairs of high quality sequence data from both 3' and 5' ends, corresponding to the partial sequencing of the 32 gametogenesis expressed gene cDNAs were compared to the GenBank and EMBL databanks using the FASTA program (Pearson, 1990). Fifty per cent of the gametogenesis expressed gene clones revealed no significant similarities (less than 85%, sequencing more than 300 base pairs for each clone) to any sequence included in both databases. Therefore, these clones could be novel mouse cDNA sequences. Sixteen of these mouse cDNA clones shared similarities with genes previously characterized in mice, humans or rats (Table 1). Redundancy appeared in some gametogenesis expressed gene clones including histone H3.3 (GEG-89, 92),

Fig. 2. Northern blot of total RNA from muscle, adult testes and fetal ovary after hybridization, with GEG-108 clone as probe. The ethidium bromide staining of the gel shows equivalent loading of the samples. A low level of transcript accumulation is observed in the fetal ovary sample compared with the adult testes.

ubiquitin (GEG-146, 159), α -globin (GEG-70, 90, 104) and mitochondrial genome (GEG-17, 102). However, while in all redundant clones the 5' ends were different, internal redundancy, as a consequence of library amplification, cannot be considered. Consequently, the multiple presence of these clones represents the relative abundance of the corresponding transcripts.

Developmental gene expression was investigated on northern blots for novel gametogenesis expressed genes. Initially, the analysis was performed on samples of testes, considering both the cumulative process of cell differentiation and the relative accessibility of testis RNA samples compared with those of fetal ovaries. In this analysis, samples of adult brain, kidney or muscle RNAs were included, together with RNA from human testes (Table 2).

For expression during testis development, different patterns of expression were observed: (i) clones, such as GEG-108, showed high expression during testicular development (Fig. 2); (ii) clones, such as GEG-123, displayed changes in the pattern of transcripts during gametogenesis, in which the main transcript accumulated during prepubertal stages, but decreased in adults, when a new transcript corresponding to 1.2 Kb appeared (Fig. 3); and (iii) in clones such as GEG-154, profiles of the development expression of transcripts occurring first in prepubertal testes coincidently with the onset of meiosis accumulated in later stages of development, in some cases concomitantly with the appearance of new transcripts (Fig. 4).

The specificity of expression of these fetal ovary clones in testis varied for the different novel genes. In general, co-expression was frequent in adult brain and kidney. However, in some clones, no signal was detected in somatic tissues or was barely detected in some specific somatic RNA analysed, as in

Fig. 3. Pattern of gene expression within developing mouse seminiferous epithelium, fetal somatic tissues and human testis. Northern hybridization uses a cDNA probe of clone GEG-123. Total RNA (15 μ g) was isolated from mouse testis for the indicated days (lanes 1–5), human adult testis (lane 6), mouse fetal kidney (lane 7) and fetal brain (lane 8). Estimated size in bases is indicated. The filters were stripped from the probe and rehybridized with a β -actin probe as control.

Fig. 4. Pattern of mRNA accumulation within mouse seminiferous epithelium, and somatic muscle tissue. Northern blot hybridization uses a probe of clone GEG-154 cDNA. Samples of mRNA were isolated from the ages designated in Fig. 3. The β -actin signal was obtained from stripping the filter and rehybridization with the corresponding probe.

the case of GEG-154 (Fig. 4) or GEG-108. In the case of GEG-108, fetal ovary at day 16 after coitus was also analysed, and showed a much lower expression than that detected in adult testis (Fig. 2).

Signals were also generally detected in RNA derived from human adult testis. A recurrent observation in human material was that the corresponding transcripts were slightly bigger.

Discussion

Although genes that are expressed specifically during spermatogenesis have been studied (Wolgemuth and Watrin,

1991), genes expressed during the fetal development of oocytes have not been investigated. The development of female gametogenesis in the mammalian fetus is a singular process of differentiation starting with the fetal development of oocytes but with a programmed arrest at the dictyate stage. The terminal differentiation in adult life continues in a cyclic manner and is associated with ovulation. The analysis of the genes expressed, both in oogenesis and spermatogenesis, can help in understanding the mechanisms that genetically regulate the complex programme of gametogenesis.

In the study reported here, the strategy used to find genes expressed more abundantly during both gametogenesis processes was based on a double differential screening of fetal ovary clones by single-stranded cDNA from adult testis versus single stranded cDNA probes from a pool of somatic tissue RNAs. However, in the analysis of novel gametogenesis expressed gene clones by developmental northern blots of testes and somatic tissue samples, hybridization signals were also found in some specific somatic tissues, mainly the fetal brain. In addition, Starborg *et al.* (1992) reported similar gene expression in the testes and the nervous system of mice, including specific expression in both tissues of genes encoding isoenzymes of glutathione S-transferase (Campbell *et al.*, 1990), neuropeptides (Kilpatrick and Millette, 1986; Gizang-Ginsberg and Wolgemuth, 1987; Kilpatrick *et al.*, 1987) and growth factor (Ayer-LeLievre *et al.*, 1988). Therefore, in this study, brain samples were included in the analysis of expression.

The results of this study indicated qualitative and quantitative differences in some specific transcripts expressed in gametic tissues and somatic tissues. Similar results were reported in studies of gene regulation during spermatogenesis. These include the use of different promoters (Howard *et al.*, 1990), alternative splicing (Haugen *et al.*, 1990; Foulkes *et al.*, 1992, 1993) and alternative polyadenylation (Meijer *et al.*, 1987). The signal observed in the northern analysis could also reflect the possible presence of gonad-specific isoforms, as demonstrated in differentiating spermatocytes (McCarrey and Thomas, 1987; Thomas *et al.*, 1990) with specific gene regulation (Robinson *et al.*, 1989).

A pleiotropic effect could be exhibited by some genes, affecting multiple functions of cell differentiation and development. Some clones corresponding to known genes with a defined participation in differentiation programmes could be included in this category. An abundance of ubiquitin as free protein (Agell and Mezquita, 1988) or ubiquitin-histone conjugates (Agell *et al.*, 1983) was reported during spermatogenesis, and Loir *et al.* (1984) propose a putative role for these. Nevertheless, the abundance of ubiquitin during oogenesis has not been detected before. Since a relatively high proportion of different ubiquitin clones was found in our library, a transcript accumulation during fetal oogenesis similar to that observed on adult testis can be inferred. Likewise, the variant histone H3.3 could have an important function during the gametogenesis differentiation. Two different clones similar to a H3.3A pseudogene were found in the cDNA library described here. The mouse histone variant cDNA for H3.3A has not been cloned and sequenced until now. Sequencing and characterization of these clones is now in progress and a domain of the 5' untranslated regions of this gene has already been found to have a high sequence homology with the 3' region of

a pseudogene of the α -globin gene (D. M. López-Alañón, L. A. Lopez-Fernández, V. Castañeda, D. B. Krimer and J. del Mazo, unpublished). This could explain the presence of clones homologous to α -globin in the library. However, a spermatid-specific histone is described by Moss *et al.* (1994), suggesting the synthesis of other histones at specific stages of gametogenesis.

The molecular approach presented here, based on the comparative analysis and cloning of genes expressed in female and male gametogenesis, can elucidate regulatory mechanisms involved in shared processes of differentiation such as meiosis. These studies also contribute to the analysis of developmental functions and their regulatory effect on cell systems that control the differentiation of germ cells such as Sertoli and granulosa cells. In addition, considering the complex mechanisms of gene expression required to form gamete cells, these studies provide tools of molecular analysis that have possible implications in other processes of terminal differentiation.

The novel sequences reported in this paper have been deposited in the EMBL database accession nos. X71637–X71652, inclusive.

These studies were supported by grants from DGICYT (PB89-0053), (PB 92-0063) and CAM (C077/91).

References

- Adams MD, Kelly JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merrill CR, Wu A, Olde B, Moreno RB, Kerlavage AR, McCombie WR and Venter JC (1991) Complementary DNA sequencing: expressed sequence tags and Human Genome Project *Science* **252** 1651–1656
- Adams MD, Kerlavage AR, Fields C and Venter JV (1993) 3400 new expressed sequences tags identify diversity of transcripts in human brain *Nature Genetics* **4** 256–267
- Agell N and Mezquita C (1988) Cellular content of ubiquitin and formation of ubiquitin conjugates during chicken spermatogenesis *Biochemical Journal* **250** 883–889
- Agell N, Chiva M and Mezquita C (1983) Changes in nuclear content of protein conjugate histone H2A-ubiquitin during rooster spermatogenesis *Letters of the Federation of European Biochemical Societies* **155** 209–212
- Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (1990) *Current Protocols in Molecular Biology*. Greene Publishing Associates and Wiley-Interscience, New York
- Ayer-LeLievre C, Olson L, Ebendal T, Hallbook F and Persson H (1988) Nerve growth factor mRNA and protein in the testis and epididymis of the mouse and rat *Proceedings National Academy of Sciences USA* **85** 2628–2632
- Bellvé AR, Cavicchia JC, Millette CF, O'Brien DA, Bhatnagar YM and Dym M (1977) Spermatogenic cells of the prepubertal mouse: isolation and morphological characterization *The Journal of Cell Biology* **74** 68–85
- Campbell E, Takahashi Y, Abramovitz M, Peretz M and Listowsky I (1990) A distinct human testis and brain μ -class glutathione S-transferase *Journal Biological Chemistry* **265** 9188–9193
- Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction *Analytical Biochemistry* **162** 156–159
- Djakiew D and Dym M (1988) Pachytene spermatocyte proteins influence Sertoli cell function *Biology of Reproduction* **39** 1193–1205
- Eppig JJ (1991) Intercommunication between mammalian oocytes and companion somatic cells *BioEssays* **13** 569–574
- Foulkes NS, Mellstrom B, Benusiglio E and Sassonecorsi P (1992) Developmental switch of CREM – function during spermatogenesis – from antagonist to activator *Nature* **355** 80–84
- Foulkes NS, Schlotter F, Pevet P and Sassone-Corsi P (1993) Pituitary hormone FSH directs the CREM functional switch during spermatogenesis *Nature* **362** 264–267

- Gizang-Ginsberg E and Wolgemuth DJ (1987) Expression of the pro-opiomelanocortin gene is developmentally regulated and affected by germ cells in the male mouse reproductive system *Proceedings National Academy of Sciences USA* **84** 1600–1604
- Haugen T, Paulssen E, Gordeladze J and Hansson V (1990) A unique mRNA species for the α subunit of Gs is present in rat haploid germ cells *Biochemical and Biophysical Research Communications* **168** 91–98
- Hecht NB (1986) Regulation of gene expression during mammalian spermatogenesis. In *Experimental Approaches to Mammalian Embryonic Development* pp 151–193 Eds J Rossant and RA Pedersons. Cambridge University Press, Cambridge
- Höög C (1991) Isolation of a large number of novel mammalian genes by a differential cDNA library screening strategy *Nucleic Acids Research* **19** 6123–6127
- Howard T, Shai S, Langford T, Martin B and Bernstein K (1990) Transcription of testicular angiotensin-converting enzyme (ACE) is initiated within the 12th intron of the somatic ACE gene *Molecular and Cellular Biology* **10** 4294–4302
- Kierszenbaum AL and Tres LL (1974) Nucleolar and perichromosomal RNA synthesis during meiotic prophase in the mouse testis *The Journal of Cell Biology* **60** 39–53
- Kilpatrick DL and Millette CF (1986) Expression of pro-enkephalin messenger RNA by mouse spermatogenic cells *Proceedings National Academy of Sciences USA* **83** 5015–5018
- Kilpatrick DL, Borland K and Jin DF (1987) Differential expression of opioid peptide genes by testicular germ cells and somatic cells *Proceedings National Academy of Sciences USA* **84** 5695–5699
- Loir M, Caraty A, Lanneau M, Menezo Y, Muh JP and Sautiere P (1984) Purification and characterization of ubiquitin from mammalian testis *Letters of the Federation of European Biochemical Societies* **169** 199–204
- López-Fernández LA and del Mazo J (1993) Construction of subtractive cDNA libraries from limited amounts of mRNA and multiple cycles of subtraction *BioTechniques* **15** 654–658
- McCarrey JR and Thomas K (1987) Human testis-specific PGK gene lacks introns and possesses characteristics of a processed gene *Nature* **326** 501–505
- Meijer D, Hermans A, Von Lindern M, Van Agthoven T, De Klein A, Mackenbach P, Grootegoed A, Talarico D, Della Valle G and Grosveld G (1987) Molecular characterization of the testis specific *c-abl* mRNA in mouse *EMBO Journal* **6** 4041–4048
- Mintz B and Russell ES (1957) Gene-induced embryological modifications of primordial germ cells in the mouse *Journal of Experimental Zoology* **134** 207–238
- Moss SB, Ferry AR and Groudine M (1994) An alternative pathway of histone mRNA 3' end formation in mouse round spermatid *Nucleic Acids Research* **22** 3160–3166
- Nedivi E, Hevroni D, Naot D, Israeli D and Citri Y (1993) Numerous candidate plasticity-related genes revealed by differential cDNA cloning *Nature* **363** 718–722
- Pearson WR (1990) Rapid and sensitive sequence comparison with FASTP and FASTA *Methods in Enzymology* **183** 63–98
- Pescovitz OH, Srivastava CH, Breyer PR and Monts BA (1994) Paracrine control of spermatogenesis *Trends in Endocrinology and Metabolism* **5** 126–131
- Robinson MO, McCarrey JR and Simon MI (1989) Transcriptional regulatory regions of testis specific PGK-2 defined in transgenic mice *Proceedings National Academy of Sciences USA* **86** 8437–8441
- Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA and Arnheim N (1985) Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia *Science* **230** 1350–1354
- Sambrook J, Fritsch EF and Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, New York
- Speed RM (1982) Meiosis in the foetal mouse ovary I. An analysis at the light microscope level using surface spreading *Chromosoma* **85** 427–437
- Starborg M, Brundell E and Höög C (1992) Analysis of the expression of a large number of novel genes isolated from mouse prepubertal testis *Molecular Reproduction and Development* **33** 243–251
- Thomas K, del Mazo J, Eversole P, Bellvé A, Hiraoka Y, Li SS-L and Simon M (1990) Developmental regulation of expression of the lactate dehydrogenase (LDH) multigene family during mouse spermatogenesis *Development* **109** 483–493
- Wolgemuth DJ and Watrin F (1991) List of cloned mouse genes with unique expression patterns during spermatogenesis *Mammalian Genome* **1** 283–288