

Molecular Cloning and Genomic Organization of Mouse Homologue of *Drosophila* *germ cell-less* and Its Expression in Germ Lineage Cells¹

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Primordial germ cells (PGCs) are founder cells of all gametes. A number of genes which control PGCs development have been identified in invertebrates, whereas such genes are by and large unelucidated in mammals. Here we describe cloning, genomic structure and expression of mouse homologue of *germ cell-less* (*gcl*) gene which is required for PGCs formation in *Drosophila*. The mouse *gcl* shows 34% identity compared with *Drosophila* *gcl* protein and contains BTB/POZ domain. The *gcl* gene consists of 14 exons and spans more than 50 kb. The CpG islands are found around exon 1 of the gene. Putative promoter region contains potential binding sites for various transcription factors. Northern blot analysis showed that its mRNA is highly expressed in adult testis with lower expression in ovary, ES (embryonic stem) cells, and various other organs. *In situ* hybridization analysis revealed strong expression of the *gcl* gene in the pachytene stage spermatocytes. The expression was also observed in post-migratory PGCs, but was not apparent in migratory and pre-migratory PGCs. Further studies including gene disruption analysis would provide an important insight into mammalian germ lineage development. © 1999 Academic Press

Key Words: germ lineage; development; primordial germ cell; spermatogenesis.

All gametes are known to arise from primordial germ cells (PGCs) (1, 2). In mouse, PGCs are set aside from other cell lineages during gastrulation and appear as a small population of cells near the base of allantois at

embryonic day 7.5 (7.5E). They become incorporated into the hindgut endoderm and migrate to the genital ridges through dorsal mesentery by 10.5E. PGCs interact with each other and somatic cells in genital ridges to form sex cords which are the pre-structures of seminiferous tubules and ovarian follicles in the male and in the female, respectively. By 12.5E, sex differences become apparent: PGCs are aggregated in a striped pattern along the sex cords in the male gonads whereas they are in a dotted pattern in the female gonads (3). The genes involved in these processes are poorly elucidated.

In contrast, a large number of genes have been identified as regulators of PGCs development in *Drosophila melanogaster* and *Caenorhabditis elegans* (1). In *Drosophila*, PGCs, also called as pole cells, appear at the posterior pole of blastula. During cellularization, nuclei arriving at the posterior pole are directed to enter the germ line by the molecules stored in the posterior egg cytoplasm (the pole plasm). A large number of maternal effect genes are required for the pole cell formation. *germ cell-less* (*gcl*) is one of such genes and has several characteristics of germ cell determinants (4, 5). At first, *gcl* is specifically incorporated into pole cells. Second, its posterior localization requires the function of all the genes necessary for pole cell formation. Most importantly, the reduced *gcl* expression results in failure of pole cell formation and the overexpression of *gcl* results in transient increase of pole cells. Thus, *gcl* is believed to be one of the essential components during germ cell specification pathway.

In this study, we have cloned mouse homologue of *gcl* and determined its gene structure in order to gain more insights into the molecular basis of PGCs development in mammals. The mouse *gcl* was highly expressed in pachytene stage spermatocytes and PGCs in genital ridges of the male and the female, which im-

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A

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10      20      30      40      50      60      70      80      90      100     110     120
GCGAAGCCGGTCCGCTGAGGCGCTACGGGCGGGGCTGAGGATGGAGGTGACTGCGTTCGCGGCAGCGGTGGGGCTGCGCGCAGGCGGCAGCGGAGTAGGCGGTGGAGATACGGGCATG

130     140     150     160     170     180     190     200     210     220     230     240
GCGGGGCGGCCCTCGGTGCTCCCTAGGCGCGGAGCATGGGCGCTCTCAGCAGCGGGTGTCTGCGGCGGCAGGGCGCACAGAGCAGCCGAAACCCACGCCGGGGCTGGGGGCGCGG
      M G A L S S R V L R P A G R T E Q P E P T P G A G G A A

250     260     270     280     290     300     310     320     330     340     350     360
CCCCGAGTTCGGACGCCGCGAAGATGCGGGCCACAGCTTCTGTACTGTCCGGCGGCGCAAGCGCAGCAGCGGCACATTCCTGTACTGTACCCCGACTCCGAGACAGACG
R R S D A G E D A G H S F C Y C P G G R K R K R S S G T F C Y C H P D S E T D D

370     380     390     400     410     420     430     440     450     460     470     480
ACGACGAGGACGAGGCGGACGAGCAGAGGCTGCTGAACACGCCGCGCAGGAAAAATTAAGAGCAGCATCAAAATACATCTACCAACGCTGTTTTGAATGGTGAAGAACAGTGACA
D E D E G D E Q Q R L L N T P R R K K L K S T S K Y I Y Q T L F L N G E N S D I

490     500     510     520     530     540     550     560     570     580     590     600
TTAAGATCTGTGCTCTAGGTGAAGAGTGGAGCTTACACAAATCTACTTATGTCAATCTGGCTACTTTTCTAGTAGTTTCAGTGGTTCTTGGAAAGAAATCCAGCATGAATATTATTGAAC
K I C A L G E E W S L H K I Y L C Q S G Y F S S M F S G S W K E S S M N I I E L

610     620     630     640     650     660     670     680     690     700     710     720
TGGAGATTCTTGACCAAGAACATTGATATAGAAGCACTGCAGGTGCGATTGGATCACTGTATCGAGATGACGCTTAAATAAGCCAGGAGGTGCTTGCCTATTTTGGCAGCAGCTTGCA
E I P D Q N I D I E A L Q V A F G S L Y R D D V L I K P S R V V A I L A A A C M

730     740     750     760     770     780     790     800     810     820     830     840
TGCTGCAATTGATGGTTTGTATACAGCAGTGCCTGAGACAATGAAGGAGACCATCTCTGTGAGAACTGTGTGTGGCTATTACACATCGGCAGGACCTATGGACTAGACTCTGTAAAGA
L Q L D G L I Q Q C G E T M K E T I S V R T V C G Y Y T S A G T Y G L D S V K K

850     860     870     880     890     900     910     920     930     940     950     960
AAAGTGCTCGAGTGGCTGCTGAACAACCTCATGACTCACCAGAGTGTGGAGCTTTTCAAAGAACTCAGTATAAAGCGTCATGAACAGCTCATTGGTTTCGTCTAACTTATTGTGATGC
K C L E W L L N N L M T H Q S V E L F K E L S I N V M K Q L I G S S N L F V M Q

970     980     990     1000    1010    1020    1030    1040    1050    1060    1070    1080
AAGTGGAGATGGATGTATACAGCTCTTAAAAAGTGGATGTTCCTTCAGCTGGTGCCTTCCTGGAATGGGCTTTAAAGCAGCTTTTGACAGAAACAGATGTCTGGTTTCAAAGTGGGA
V E M D V Y T A L K K W M F L Q L V P S W N G S L K Q L L T E T D V W F S K W K

1090    1100    1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AAAAAGACTTTGAAGGACGACTTTCCTTGAAGTGAAGTGAAGGAAACCATTTGCGCCGTGTTTCAGACATTTAAGGCTACAGTACATTCAGTGTGGCTTCTGCAAGGATCATTTG
K D F E G T T F L E T E Q G K P F A P V F R H L R L Q Y I I S D L A S A R I I E

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300    1310    1320
AGCAGGATCTCTGTGCTTACAGTATGGCTGGCGGCGAGTGTATAACAGCAGTGGCTGGCTATGTACGGGCTGAACAGACAGTGAAGTGGGCGCTCAAGAAATCAATAAAGAAAGAAC
Q D S L V P S E W L A A V Y K Q Q W L A M L R A E Q D S E V G P Q E I N K E E L

1330    1340    1350    1360    1370    1380    1390    1400    1410    1420    1430    1440
TTGAGGAAACAGCATGAGGTGTGGTCGAAAGCTTGCCAAAGATGGTGAATGCTGCTGCGCTGGACAGGCTTCAATTTTCGGCTTTGACCTCTTGTGACTTACACCAATCGATACATCA
E G N S M R C G R K L A K D G E Y C W R W T G F N F G F D L L V T Y T N R Y I I

1450    1460    1470    1480    1490    1500    1510    1520    1530    1540    1550    1560
TTTTCAAGCAATACGCTGAACAGCCATGTAGTGGATCTGTGACGCTTACAGCTTCGAAGGAGCATAGCATTTAGATTCGCGCTTGGCTTCTTTTGACAGTAGTGGGAACTCATATGCA
F K R N T L N Q P C S G S V S L Q P R R S I A F R L R L A S F D S S G K L I C S

1570    1580    1590    1600    1610    1620    1630    1640    1650    1660    1670    1680
GTAGAGCAACTGGCTACCAAACTACGACGCTTGAAGAAAGACCAAGAGCAAGTGGTGATGAAGTGGACAGCAGACTTCTGATCTTCCCTCTGTACATCTGCTTAACTTCTTGTATATAT
R A T G Y Q I L T L E K D Q E Q V V M N L D S R L L I F P L Y I C C N F L Y I S

1690    1700    1710    1720    1730    1740    1750    1760    1770    1780    1790    1800
CACCAGAAAAAGAACTGAGAGTAATCGTCACCCAGAAAAACCCAGGACTGAGGCACTCATCAGTGGCCAGTTTAACTTAATGACCTACTGCGTTACAGTCCAAGGTGACTAACAGTG
P E K R T E S N R H P E N P G H *

1810    1820    1830    1840    1850    1860    1870    1880    1890    1900    1910    1920
ACCGGCTTATGAATGTGGGACCTGGAGATGTCTCACCCTCATTACATTTCTATGCACATATGAAAAAGTTTAAAACTGAGAAAGCATCTGTCAAACCATGTTAAAGGATATC

1930    1940    1950    1960    1970    1980    1990    2000    2010    2020    2030    2040
AACTCTTCTTAAATTTAGTAGCAGTAAAAATGCTGTAGGTAATTTCTCATTTCTTTGCAACAAGATATAGATTAAATTTTGGCTTGAATTTGACCTTATCTTAATGTTAGTGAGTT

2050    2060    2070    2080    2090    2100    2110    2120    2130    2140
TACTCATCTGTAATGTGTCTCTGTTTGTAAAGAGAAATGCTAAGGACGGAGTTTAAAGTGGCAATCATAAATGCTCTTTCAATTGGTGCCTTTAAAAAA

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FIG. 1. (A) Nucleotide and amino acid sequences of mouse *gcl*. Sequences in EST clone span nucleotide number 305 to 1029. BTB/POZ domain is underlined. Exon/intron boundaries are shown by vertical lines. GenBank Accession No. of mouse *gcl* is AF163665. (B) Sequence alignment of mouse, *D. melanogaster*, and partial *C. elegans* *gcl*. BTB/POZ domain is underlined. GenBank Accession No. of *D. melanogaster* *gcl* is M97933. *C. elegans* *gcl* is found in the Cosmid clone (clone No. Y62E10, GenBank Accession No. AL031580).

plies its possible function in mammalian germ lineage development.

MATERIALS AND METHODS

Isolation of cDNA and gene for mouse *gcl*. The mouse EST (expressed sequence tags) clone (dbEST Id 641461) was purchased from Genome Systems Inc. (St. Louis, MO). The mouse adult testis cDNA

library derived from B6 mice (6) was screened with the EST clone. The genomic fragments were obtained from two λ phage libraries containing 129J/Sv mice genome [(7), Stratagene, La Jolla, CA]. Sequences were determined using ABI PRIME 310 and 377 automated sequencers. The exons and introns were mapped by standard procedures.

Northern blot analysis. Total cellular RNA was isolated from 8 weeks old C57BL/6J mice using RNeasy Mini Kit (Qiagen, Valencia,

B

C. elegans	1	-----	1
D. melanogaster	1	MCQIVGSMHMNVAEVFS-----NR-----RKRKRS-----	25
M. musculus	1	MGALSSRVLRPAGRTEQPEPTPGAGGAARRSDAGEDAGHSFCYCPGGRKRKRSSGTFYCYC	60
C. elegans	1	-----OLKEIWRFSOISLFFOGNSDVLACCGREWRVF	34
D. melanogaster	26	-TDSSLGKD-----DPAQLDITQEKKKLLTTTCYIYKALPKEEKNSDVAVMALDKVWHLE	80
M. musculus	61	HFDSETDDDEDEGEQRLNLNTERKKLKSISKYIYQTLFLNGNSDITKICALGPEWSEH	120
C. elegans	35	KLYLKQTKRFESMFDCLNTESSNGRVOMETTFENIADGINSVLCSLYHNETETDLDKIE	94
D. melanogaster	81	KVYLSOSPYYHIMFNGTWBAAQNFICITITLDDRTVVASLDAVFGSMMSDETEIESADVI	140
M. musculus	121	KTYLCOSGYBSSMFSCKKSSMTHIELETPCNHLEALQVAFGSLYRCDVLKPSRVV	180
C. elegans	95	GTVAASYSIVLDSVTRCSEMMIEALSTKNVRFYDVSTRYGLKVRKSMFVLLIHOFWK	154
D. melanogaster	141	SVLAIAITLFLHDCIITDKCAVMVDNISPETAIQYEEAACQYGVVGKSTFCQFQINLS	200
M. musculus	181	ATLAAACMLQDLGLHQCCCTMKETLSVRVCCGYTSAGTYGLDSVKKKCLEWLLNNMT	240
C. elegans	155	IMTD-REKINEVDROIEVTTLNSPNLLIEGEFDLYKVVKSMIYMRVDPDCKD-----	207
D. melanogaster	201	IYSKQPNLRRHISTELMSALTASEDLYVMOTEBSLYTILLRTWMFLRLHFDYDPEDPVQRA	260
M. musculus	241	HQS--VEIFKELSLNVMKQIGSSNLFVMOVEMDVYALAKKWMFQLVFSWNG-----	291
C. elegans	208	---DSPETETQN-----ASKRFRESK-----ISMFIKYADIFASLRIEOFL	245
D. melanogaster	261	EALKTQELLVNAGVETHAPSGDVOWTYETTSREERSFLATPEQCPYVKVFQKLRTOYLT	320
M. musculus	292	---SLKQLLETET-----DWWFSKWKKDFEGTIFLETIOCKEFAFVERHLRLQYII	338
C. elegans	246	TCSETIKTVKSDALIFLSIVDEMSTALSSLLLENBESPK--TLEMDDE-FEKRCILRLGR	302
D. melanogaster	321	NHYMDLKIITYNDNLIEKEWYRHHNHMDALLRIDHGOEDCSPOOLDPEQFENCRCGR	380
M. musculus	339	SDLASARILEQSLVSESWLAAYVQKQQLAMLRABQDSE-VGPGCEINKEELEGNSSMRCCR	397
C. elegans	303	SLDS-FPKCWRRLGFGNFGVDLLIHVNDYSVCLKRNCLNCKAPYGVNLSKHVHLHYR----	357
D. melanogaster	381	MULEPGYQKWRWTGFGNFGMDILIMDSRLNLRHHRHEHER-VLSLQTKRKFVMTTIVT	439
M. musculus	398	KLAKDGEYCWRWTGFGNFGFDLLVTYTNVYIIFKRNLLNCPSCGVSLSPPRSIAFLRLA	457
C. elegans	357	-----	357
D. melanogaster	440	SINAQROAVFTQTSEICSLSLEKNEVPLMVLLP-KLVHPLLLISINMVMVFNQSFKEI	498
M. musculus	458	SPDSSGKLICSRATGYQILILEKDDQGVVNLDSRLIFPLYICCNFIYISE-----	509
C. elegans	357	-----	357
D. melanogaster	499	VPLSEEAITSLSIPISEIGANSDRLLSESSADDSAVFIGDSEPSTPSSPAPRPRIAWSAS	558
M. musculus	510	-----EKRIE-----S--NR-----HEENEGH-----	524
C. elegans	357	-----	357
D. melanogaster	559	ETGAICGQLAC	569
M. musculus	524	-----	524

FIG. 1—Continued

CA). Ten micrograms of total RNA were subjected to Northern blot analysis. The 1.7 kb *Sma*I-*Eco*RV fragment was radiolabeled by BcaBest Labeling Kit (Takara, Japan) and used for the probe. Northern blot analysis was performed as described (8).

In situ hybridization analysis. The EST clone (dbEST Id 641461) was used to prepare the sense and antisense cRNA probes. Digoxigenin-11-UTP-labeled cRNA probes were prepared using DIG RNA Labeling Kit (Boeringer Mannheim, Mannheim, Germany). *In situ hybridization* was performed as described (9).

RESULTS AND DISCUSSION

Isolation and sequence of the mouse gcl cDNA. To clone mouse *gcl*, we compared *D. melanogaster gcl* amino acid sequence to conceptual translation of ESTs in the GenBank by use of the National Center for Biotechnology Information's BLAST search program (10, 11), and found highly homologous sequences in mouse, rat and human ESTs. Using one of mouse ESTs (dbEST Id 641461), adult testis cDNA library was screened to isolate the full-length cDNA clone. Deduced amino acid sequences from the cDNA indicate

that it contains open reading frame which encodes a protein of 524 amino acids (Fig. 1A). Thirty-four percent of amino acids are identical to *D. melanogaster gcl*, but N- and C-terminal ends are relatively diverged (Fig. 1B). Database search using full-length mouse *gcl* revealed that it contains BTB/POZ domain. BTB/POZ domains are utilized as protein-protein interaction interfaces and found in transcriptional repressors and actin-binding proteins (12–14). Since *D. melanogaster gcl* is localized in nucleus (5), it might be involved in transcriptional repression in germ line specification pathway. From this point of view, it is noteworthy that *PIE-1*, essential gene for germ line specification in *C. elegans*, acts as a repressor for genes expressing in somatic cell lineage (15, 16). Searching of *C. elegans* database showed that *C. elegans* genome also contain *gcl* sequences (Fig. 1B). Identity of partial *C. elegans gcl* to mouse and *D. melanogaster gcl* are 34 and 30%, respectively. Examination of phylogenetic distances by both UPGMA method and NJ method predicted that

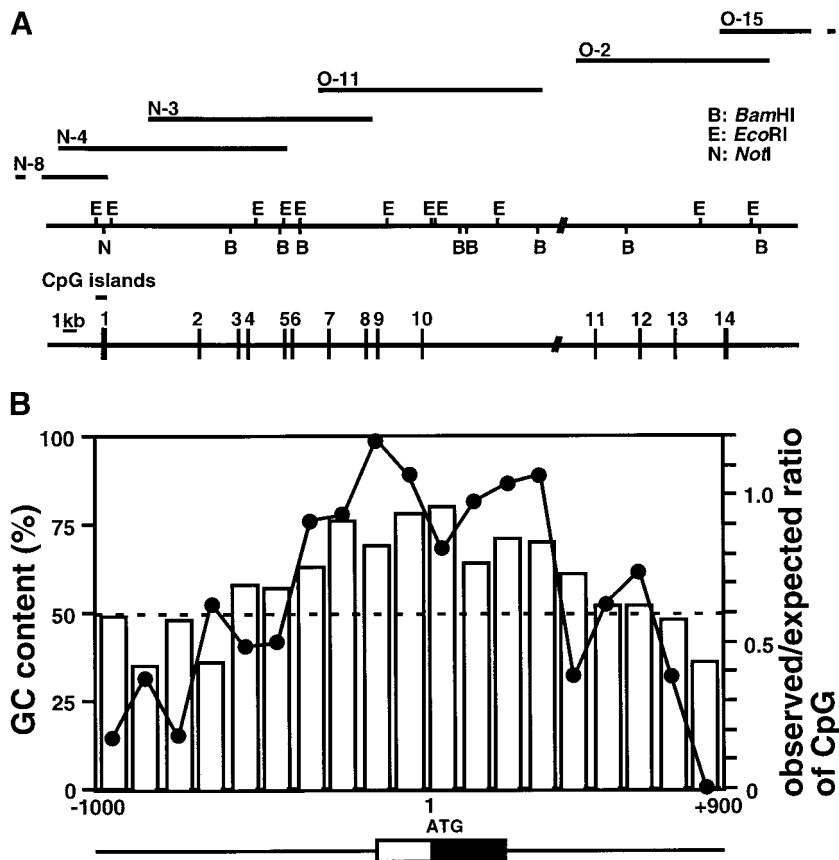


FIG. 2. (A) Genomic organization of mouse *gcl* gene. Position of exons (black boxes) and partial restriction enzyme map are shown. The top six lines indicate the λ phage clones (N-3, N-4, N-8, O-2, O-11 and O-15). (B) The CpG islands. GC contents (open column) and observed/expected ratio of CpG (closed circles) were calculated as described with a window of 100 nucleotides (17). Nucleotide number 1 corresponds to A of initiation codon. The CpG islands have been defined by the nucleotide sequences which contain more than 50% GC contents and more than 0.6 of observed/expected ratio of CpG, thus showing the border by dotted line. Schematic genomic structure is shown below the graph (thin line, 5'-upstream region; open bar, 5'-untranslated region; closed bar, open reading frame). (C) Sequences of putative promoter region and exon 1. CpG is shown in outlined letters. The potential binding sites for transcription factor and its orientation are shown by the arrow below the sequences. Gray arrowhead points to 5' end of cDNA clone which contains the most 5' portion. GenBank/EMBL Accession No. of this sequence is AF163666.

mouse *gcl* is closer to *C. elegans gcl* than to *D. melanogaster gcl*. Homologous sequences were not found in *S. cerevisiae* genome. These results indicate that *gcl* is evolutionarily conserved gene among various multicellular animals.

Genomic organization of the mouse *gcl* gene. We determined genomic structure of *gcl* gene. The mouse *gcl* gene comprises 14 exons, spanning more than 50 kb (Fig. 2A). The sequences around exon/intron boundaries were matched to consensus sequences of splicing donors and acceptors (Table 1). The CpG islands were identified around exon 1 including putative promoter region; GC contents were more than 50% and observed/expected ratio of CpG was above 0.6, which satisfies the criteria for CpG islands (Fig. 2B (17)). TFSEARCH program predicted that putative promoter region contains potential binding sites for various transcription

factors such as Sp1, Sry, CREB (cAMP responsive element binding proteins), Octamer binding proteins and STAT (signal transducers and activators of transcription) [Fig. 2C (18, 19)]. Since some of them are known to play important roles in germ cell development (20–22), further promoter analysis would be required to examine the importance of these elements for *gcl* expression.

Expression of *gcl* mRNA in adult mice and embryonic gonad. Expression of *gcl* mRNA in various adult organs was examined by Northern blot analysis. As shown in Fig. 3A, expression was most prominent in testis where two transcripts, approximately 3 kb and 4 kb, were equally expressed. In contrast, in other organs including ovary, *gcl* expression level was much lower and the size of major transcripts was 4 kb. Low level expression was also observed in D3 ES (embryonic

C

10 20 30 40 50 60
 GAATTCACAGCCAGTAGTAGGTCCACAGGTACTTGTATTGCTCTGTTGAAACATTCTAT
 ← Sry
 70 80 90 100 110 120
 TATAAACATTTTACTTTTGAAGGTATAAAAGGCAGGTATGGTAGCTCAGTGCCTCAACTC
 130 140 150 160 170 180
 CTAGCACTTAGAGGCTGAGATAGGCGGACTTCTGAGAGTTCCAGGGAAGCCTTGGGCTTC
 → GATA
 190 200 210 220 230 240
 AGAGTGACACTTTTCTCTGAAGAAAATTAAAAAAAAAAAAAAAAAAAAATCGAGGCCAG
 → STAT
 250 260 270 280 290 300
 GGATGTAGCTTAGTTGGTAGAATGTTTGCTTAGCATTCAAGAAGCCCTGCATCTGTACT
 → GATA
 310 320 330 340 350 360
 CAGCATTTCAGGTCCAAGAGGTGATGTGACACATACATGGCTGTAATCCCAGCTTTCAG
 → GATA
 370 380 390 400 410 420
 GGGGTAGAAACAGGAGATCAAGAGTTCAAGAGCATCCTATACAGTGAGTCAAGTATGTA
 430 440 450 460 470 480
 GTAAGAGACTGACACAATAAATAAATAAATCAAAACAAGGCAGCAGAAGGAAGC
 → AP-1 → Oct → Sry → Sry
 490 500 510 520 530 540
 CAAGACCTGGAGGCTAGCTGAGGCTCCAGACTGAACCCTCAACACAGCTGGGTGAAGC
 → CREB
 550 560 570 580 590 600
 TCATCTATCTAACCGGCCAGTGGTCCCTAGAGCCCTGGAAGCAAAGGCCATGTTGGCA
 → Sry → NFkB → NFkB
 610 620 630 640 650 660
 AGTCCCTCTCTCTATTAACCGGTACAGGGCTGGATGAGACCCGGAATCGAGGAAGCTT
 → Oct
 670 680 690 700 710 720
 AGCGGAAGAACCAGCCACAGAGATCCCGAGATCTGTTAGGCATAAGCACACAGTGCCCA
 → STAT → Sp1
 730 740 750 760 770 780
 CCCCCCTCCTTGAAGCCGACACCGGCAAGTCAGACACACACCGGCTGAGCCCGCAG
 790 800 810 820 830 840
 GGAAGGCCAGCAGCCTGCGCATGCCAACCTGCAGCCAGGCTCCCTCCTCCCGCCCC
 850 860 870 880 890 900
 CCTTTTCGAGGCTCCCTTGCCCTGCGCCCTGCGCGCGCGCTTTGTTGGGAGAAGG
 910 920 930 940 950 960
 AGGGGGCAGGCTCTAGCGAAGCCCGTCCCTGAGGCGCTAGCGGCGGGCTGAGGATGGA
 → Sp1 → Sp1
 970 980 990 1000 1010 1020
 GGTGACTCGCGTTCCCGCGCAAGGTTGGGGCTGCGCGCGAGGCGCAGCGCAGTAGGCGGT
 1030 1040 1050 1060 1070 1080
 GGAGATACCGGCATGGCGCGCGCCCCCTCGGTGCTCCCTAGGCGCGCAGCCATGGGCG
 M G A
 1090 1100 1110 1120 1130 1140
 CTCTCAGCAGCCCGGTGCTGCGCCCGCAGGGCGCACAGAGCAGCCCGAACCCACCGCCCG
 L S S R V L R P A G R T E Q P E P T P G
 1150 1160 1170 1180 1190 1200
 GGCGTGGGGGCGCGCCCGCAGGTCCGACGCGCGCGAAGATGCGGCCACAGCTTCTGTT
 A G G A A R R S D A G E D A G H S F C Y
 1210 1220 1230 1240 1250 1260
 ACTGTCGCGCGCGCAGAGCGCAAGCGCAGCAGCGGCACATTCTGCTACTGTACCCCG
 C P G G R K R K R S S G T F C Y C H P D
 1270 1280 1290 1300 1310 1320
 ACTCCAGACAGACCGACACAGGAAGGCGCGCAGCAGCAGAGGCTGCTGAACAACCG
 S E T D D D E D E G D E Q Q R L L N T P
 1330
 CCGCCAG
 R

FIG. 2—Continued

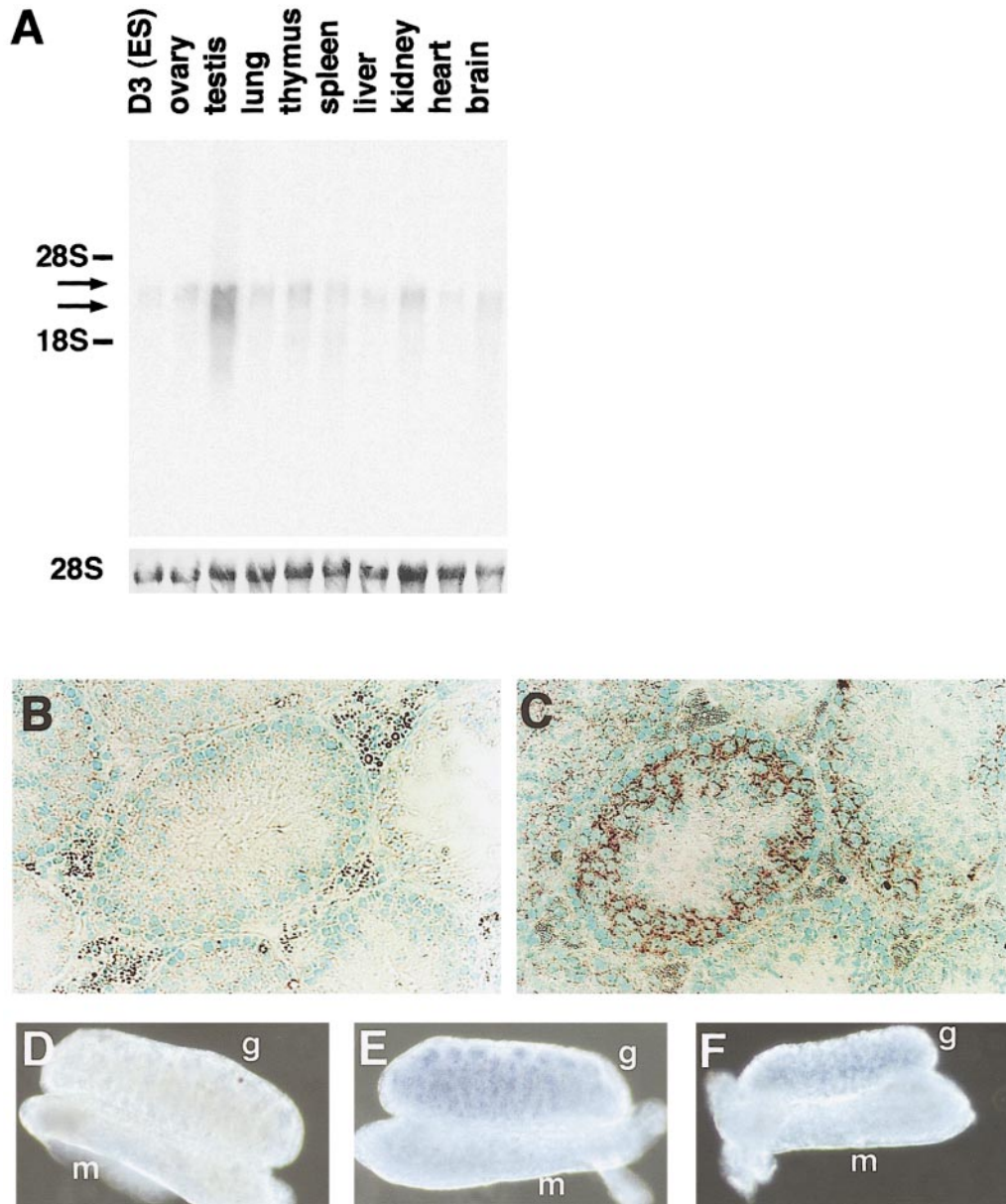


FIG. 3. Expression of mouse *gcl* mRNA. (A) Northern blot analysis of adult mice organs and ES cells. Ten micrograms of total RNA were subjected to Northern blot analysis. Two mRNA species are indicated by arrows. To show the quantity and integrity of RNA, 28S rRNA was visualized by staining the same filter with methylene blue. (B, C) *In situ* hybridization of sections of adult mice testis. Signals were identified in pachytene stage spermatocytes when using antisense probe (C) but not sense probe (B). Nuclei were counterstained with methylgreen. (D to F) Whole mount *in situ* hybridization of 13.5E male (D, E) and female (F) gonads using sense (D) and antisense (E, F) probe. Striped signals and dotted signals were observed in the male (E) and female (F) gonads, respectively, which corresponds to the localization of PGCs in gonads. g, gonad; m, mesonephros.

stem) cells (Fig. 3A). *In situ* hybridization of sections of testis revealed that *gcl* is highly expressed in primary spermatocytes, especially in pachytene stage spermatocytes, but not in spermatogonia and spermatids (Fig. 3C). We also examined expression in PGCs by whole mount *in situ* hybridization. PGCs-specific signal was not observed during pre-migratory and migratory stages (data not shown). However, in the post-

migratory stages, strong signals were visible in PGCs in the gonads. Characteristic striped signals and dotted signals were observed in the male and female gonads, respectively (Figs. 3E, 3F), which corresponds to the localization of PGCs in gonads.

Early stages of germ line specification processes would be rather different between invertebrates and mammals. For instance, germ cell determinants such

TABLE 1
Exon/Intron Boundaries of Mouse *gcl* Gene

(exon 1)	412 GCCGCGCAGgtacggaacg.....ttcttaacagGAAAAAATT	413 536 537	(exon 2)
(exon 2)	TTATGTCAAgtagtatattt.....ttttttccagTCTGGCTAC	633 634	(exon 3)
(exon 3)	ATATAGAAGgtatcacctc.....tgctttcagCACTGCAGG	731 732	(exon 4)
(exon 4)	CTGCAATTGgtaagtagtg.....tagtttacagGATGGTTTG	844 845	(exon 5)
(exon 5)	AAAGAAAAAgtaggccacc.....tggacccagGTGCCTCGA	910 911	(exon 6)
(exon 6)	AGAACTCAGgtatgtgaca.....ctttttacagTATAAACGT	995 996	(exon 7)
(exon 7)	CTTAAAAAGgtattgatgt.....ttctaactagTGGATGTTT	1086 1087	(exon 8)
(exon 8)	GGAAAAAAGgtacattgaa.....tcttaaatagACTTTGAAG	1224 1225	(exon 9)
(exon 9)	TACCTTCAGgtaagaaaac.....tctgttttagAATGGCTGG	1294 1295	(exon 10)
(exon 10)	TGAAGTGGGgtgagtagtgc.....atcatttttagGCCTCAAGA	1370 1371	(exon 11)
(exon 11)	GATGGTGAGgtaggtcttg.....tctctcgtagTACTGCTGG	1516 1517	(exon 12)
(exon 12)	AGCGTTTAGgttaggatggc.....ttaattctagATTGCGCTT	1604 1605	(exon 13)
(exon 13)	AAAGACCAGgtacgtgtgc.....tgtctttagGAGCAAGTG		(exon 14)

Note. Capital letters and lower cases indicate exon and intron sequences, respectively. A at the nucleotide number 1511 is changed to G in 129J/Sv genome, but amino acid is not changed by this polymorphism.

as pole plasm are not stored in mammalian egg cytoplasm (1, 2). Instead of germ cell determinants, signals from extraembryonic ectoderm are considered to direct a small population of cells in extraembryonic mesoderm to enter the germ line (1, 23). It has been postulated that mouse homologues of germ cell determinants in *Drosophila* might play a role in later processes of germ cell specification. Consistent with this hypothesis, mouse *gcl* is expressed in post-migratory stage but not in pre-migratory and migratory stages. Similar expression pattern has been reported in mouse homologue of *vasa* gene which is also required for pole cell formation in *Drosophila* (24). The mouse *gcl* is also highly expressed in pachytene stage spermatocytes, again like mouse *vasa* homologue, suggesting the implication in spermatogenesis.

Recently, other group independently cloned mouse *gcl* as a binding protein of the DP-3 component of E2F transcription factors which regulate G1/S cell cycle transition (25). It is possible that *gcl* might have some roles other than germ cell formation. Here we describe the *gcl* expression in mouse testis and embryonic gonads. At present, it is open question whether its potential role in cell cycle regulation could be involved in germ cell development. Gene disruption analysis is now underway to evaluate the role of mouse *gcl* in the mammalian germ lineage development.

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