be freely substituted to produce highly functional mutant proteases with generally more narrowed substrate specificities. However, a broadly specific subtilisin (Gly^{166}) is probably more biologically adaptive because, unlike many other microbes that secrete a myriad of proteases with narrowed and different specificities, *Bacillus* species secrete only two major extracellular proteases, subtilisin and neutral protease.

Engineered proteases tailored to have highly restricted specificities should be very useful in protein chemistry, synthetic chemistry, and industry. In addition, they contribute to the database needed for protein design.

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13. Peptide substrate nomenclature can be diagrammed as

$$NH_2\text{-Pn} \ldots \text{P2-P1-}\underset{\|}{\overset{O}{C}}\text{-}\overset{H}{\underset{|}{N}}\text{-P1'-P2'} \ldots \text{Pn'COOH}$$

where the scissile peptide bond is between P1 and P1′ [I. Schechter and A. Berger, *Biochem. Biophys. Res. Commun.* **27**, 157 (1967)].
14. According to transition state theory [see equation 12.4 in reference (*2*)], the free energy difference between the free enzyme plus substrate (E + S) and the transition state complex ($E \cdot S^{\ddagger}$) can be calculated from Eq. 1,

$$\Delta G_T^{\ddagger} = -RT \ln k_{cat}/K_m + RT \ln kT/h \quad (1)$$

in which k_{cat} is the turnover number, K_m is the Michaelis constant, R is the gas constant, T is the temperature, k is Boltzmann's constant, and h is Planck's constant. Specificity differences are expressed quantitatively as differences between transition state binding energies ($\Delta\Delta G_T^{\ddagger}$) and can be calculated from Eq. 2.

$$\Delta\Delta G_T^{\ddagger} = -RT \ln (k_{cat}/K_m)_A/(k_{cat}/K_m)_B \quad (2)$$

A and B represent either two different substrates assayed against the same enzyme or two mutant enzymes assayed against the same substrate.
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A Mouse Homeo Box Gene Is Expressed in Spermatocytes and Embryos

MICHAEL R. RUBIN, LESLIE E. TOTH, MAYURI D. PATEL, PETER D'EUSTACHIO, M. CHI NGUYEN-HUU

The MH-3 gene, which contains a homeo box that is expressed specifically in the adult testis, was identified and mapped to mouse chromosome 6. By means of in situ hybridization with adult testis sections and Northern blot hybridization with testis RNA from prepuberal mice and from *Sl/Sl*^{d} mutant mice, it was demonstrated that this gene is expressed in male germ cells during late meiosis. In the embryo, MH-3 transcripts were present at day 11.5 post coitum, a stage in mouse development when gonadal differentiation has not yet occurred. The MH-3 gene may have functions in spermatogenesis and embryogenesis.

AMONG THE GENES THAT CONTROL development in the fruit fly, the homeotic and segmentation genes seem to control the identity, polarity, and number of body segments (*1–4*). A characteristic component of such genes in the Antennapedia, Bithorax, and Engrailed complexes is a 180–base pair sequence called the homeo box (*5–9*). The sequence of the carboxyl half of the homeo box shows homology to the helix-turn-helix domain of several DNA binding proteins that control developmental processes in bacteria and yeast, such as the λ *cro* and *c*I proteins and the yeast mating type a1 and α2 proteins (*10–13*). It has been suggested that the homeo box proteins exert their function by regulating in *trans* batteries of genes involved in cell differentiation (*10*, *11*).

Homeo box sequences have also been identified and isolated from the DNA of frogs, mice, and humans (*14–23*). The understanding of the function of mammalian homeo box–containing genes requires a detailed knowledge of where, when, and how these genes are expressed. The differential expression of several homeo box–containing genes in frog embyros, mouse embryos, and mouse adult tissues has been recently reported (*15, 18, 21–23*). Expression of homeo box–containing genes has also been found in frog oocytes (*16*) and during the differentiation of several mouse teratocarcinoma cell lines (*20–23*). We report here the identification and chromosomal assignment of a new mouse homeo box gene, MH-3, and the analysis of its expression in the adult and in the embryo.

A large complementary DNA (cDNA) library from adult mouse testis was constructed in the vector λgt10 (*24*). To ask whether homeo box sequences are expressed in the adult testis, we screened this library with mixtures of the homeo box probes Hu-1, Hu-2 (*17*), *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*) (*5*), *engrailed* (*en*) (*7*), and

M. R. Rubin, L. E. Toth, M. D. Patel, M. C. Nguyen-Huu, Departments of Microbiology and Urology, Columbia University, College of Physicians and Surgeons, New York, NY 10032.
P. D'Eustachio, Department of Biochemistry, New York University Medical School, New York, NY 10016.

MH-1 (*25*) at low stringency conditions. Several clones contained a 0.22-kb Eco RI insert fragment designated MH-3 that hybridized with the *Antp* and *Ubx* probes. This fragment contained 126 nucleotides of homeo box sequences and 99 nucleotides of sequences 3′ to the homeo box (Fig. 1). At the nucleotide level, the MH-3 homeo box is 79% and 66% homologous to the *deformed* (*Dfd*) and *Antp* homeo box, respectively (*5*, *39*). For the predicted amino acid sequence, the homologies were 83% and 75%, respectively (*5, 39*). The sequence of the MH-3 homeo box is homologous to, but clearly distinct from, previously described murine homeo boxes (*18–23*). No termination codon was found in the sequences 3′ to the MH-3 homeo box. Two distinguishing features of the MH-3 gene were observed: (i) a codon has been deleted in the MH-3 homeo box and (ii) the five amino acids immediately 3′ of the MH-3 homeo box are identical to those 3′ of the *Dfd* homeo box.

Under very stringent conditions of hybridization and washing, MH-3 detected a single Eco RI and Hind III restriction fragment in restriction endonuclease-digested mouse genomic DNA (Fig. 2A). To assign the MH-3 gene to a mouse chromosome, genomic DNA's were prepared from a panel of 11 mouse-hamster cell hybrids (*26*). Eco RI–digested DNA's were hybridized to the MH-3 insert probe (Fig. 2B). A 1.0-kb hybridizing band was found with mouse DNA, as expected, and a larger and distinct band was found with hamster DNA. The presence of the 1.0-kb band was concordant with the presence in the cell hybrids of mouse chromosome 6 and discordant with the presence of all other mouse chromosomes. Therefore, the MH-3 gene maps to mouse chromosome 6. Two other homeo box genes, Mo10 (*19*) and MH-1 (*25*), have been mapped to mouse chromosome 6.

To determine the tissue specificity of the MH-3 gene in the adult, we prepared Northern blots containing total or polyadenylated RNA from adult testis and other adult mouse tissues. A 1.5-kb transcript was detected at high levels in the testis but not in the brain, colon, heart, kidney, liver, lung, muscle, pancreas, or ovary (Fig. 3A). Hybridization to probes that contain mostly homeo box sequences (the 0.17-kb Eco RI–Hae III fragment, Fig. 1) demonstrated that these sequences were present within the 1.5-kb transcript (*27*). This was not cross-hybridization with other closely related homeo box sequences as shown by the detection of the 1.5-kb transcript by a probe that does not contain the homeo box (the 0.05-kb Hae III–Eco RI fragment, Fig. 1). Hybridization to single-stranded RNA probes (*28*) demonstrated that the 1.5-kb transcript is derived from the DNA strand that contains the homeo box in the correct orientation (*27*).

The observed expression of the MH-3 gene in the adult testis could result from expression either in germ cells or in somatic cells or in both. To distinguish among these possibilities, we analyzed the expression of the MH-3 gene in testes from *Sl*/*Sl*^{d} mutant mice. Mutations at the *Sl* (*steel*) locus on mouse chromosome 10 cause deficiencies in gonadal germ cells that can be traced back to primordial germ cells (*29, 30*). The testes of *Sl*/*Sl*^{d} compound homozygous males are normal with respect to their somatic cells but severely deficient in germ cells (*30*). Expression of the MH-3 gene was readily detected by Northern blot analysis of wild-type testis but was not detectable in the mutant testis (Fig. 3B). Therefore, the MH-3 gene is not expressed in somatic cells but is expressed in germ cells of the adult testis.

The organization of germ cells within the seminiferous tubules of the testis reflects the different stages in their differentiation: the stem cells are located at the basal compartment of the tubule, spermatogenic cells of progressively more advanced stages are found further inside, and the highly differentiated spermatozoa are deposited in the lumen (*31*). Spermatogenesis may be divided into three phases: spermatogonial proliferation, meiosis, and spermiogenesis. We used the technique of in situ hybridization (*32*) to correlate the expression of the MH-3 gene with the different cells and stages in spermatogenesis. The 0.22-kb MH-3 insert was subcloned into the Eco RI site of the pGEM-1 vector (*33*) in both orientations. We used SP6 RNA polymerase to synthesize both antisense and sense MH-3 probes (*34*) from templates linearized by Pvu II digestion. Frozen sections of testis from 8-week-old mice were prepared and hybridized to the single-stranded ^{35}S-labeled MH-3 probes. The density of grains observed with the sense probe was not above background (Fig. 4, C and D). Therefore, the minus strand of the MH-3 gene is either not

```
A
          1
MH-3      CCT GAG CGC TCT CGA ACC GCC TAT ACC CGG CAG CAA GTC TTG GAA CTG GAG AAG
Dfd       --A A-A --- CAA --C --- --- --C --A --C --T --G A-- C-- --- --- --A ---
Antp      -GC A-A --- GAA A-G CAG A-A --C --- --- T-C --G ACT C-A --G --A --- ---

       55 Eco RI
MH-3    GAA TTC CAC TTT AAC CGC TAC CTG ACC del CGG CGG CGC ATC GAG ATC GCC CAC ACG
Dfd     --G --- --- -AC --- --- --- --- --G CGT --- --- --- --- --- --T --- --T ---
Antp    --G --T --- --C --T --- --- T-- --- CGT --- --A A-G --- --- --- --- --- G-C

      112
MH-3    CTC TGC TTG TCG GAG CGC CAG GTC AAG ATC TGG TTT CAG AAC CGG AGA ATG AAG TGG
Dfd     T-A GTT C-C --- --- --G --- A-- --- --- --- --C --- --- A-- C-C --- --- ---
Antp    --G --- C-C A-- --- --- --- A-A --- --T --- --C --- --T -G- C-C --- --- ---

      169                 183                                                Hae III
MH-3    AAG AAA GAC CAC AAA CTT CCC AAC ACC AAG ATG CGA TCT TCC AAC ACT GCC TCG GCC
Dfd     --- --G --- A-- --G --G --- --- --- --- -AC GTG CGC AAG --G --G
Antp    --- --G --G A-- --G ACG AAG GGG GAG CCG GAT

      226       Hae III                                                        Eco
MH-3    CCT GCC GGC CCG CCT GGG AAA GCA CAA ACT CAC AGC CCA CAC CAC CAT CCC CCG GAA

B         1
MH-3      Pro Glu Arg Ser Arg Thr Ala Tyr Thr Arg Gln His Val Leu Glu Leu Glu Lys
Dfd        - Lys  -  Gln  -   -   -   -   -   -  His Gln Ile  -   -   -   -   -
Antp      Arg Lys  -  Gly  -  Gln Thr  -   -   -  His Gln Thr  -   -   -   -   -

       19
MH-3    Glu Phe His Phe Asn Arg Tyr Leu Thr del Arg Arg Arg Ile Glu Ile Ala His Thr
Dfd      -   -   -  Tyr  -   -   -   -   -  Arg  -   -   -   -   -   -   -   -   -
Antp     -   -   -   -   -   -   -   -   -  Arg  -   -   -   -   -   -   -   -  Ala

       38
MH-3    Leu Cys Leu Ser Glu Arg Gln Val Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp
Dfd      -  Val  -   -   -   -   -  Ile  -   -   -   -   -   -   -   -   -   -   -
Antp     -   -   -  Thr  -   -   -  Ile  -   -   -   -   -   -   -   -   -   -   -

       57                  61
MH-3    Lys Lys Asp His Lys Leu Pro Asn Thr Lys Met Arg Ser Ser Asn Thr Ala Ser Ala
Dfd      -   -   -  Asn  -   -   -   -   -   -  Asn Val Arg Lys Lys
Antp     -   -  Glu Asn  -  Thr Lys Gly Gly Pro Gly Ser Gly Gly Glu

       76
MH-3    Pro Ala Gly Pro Pro Gly Lys Ala Gln Thr His Ser Pro His His His Pro
```

Fig. 1. Sequence of clone MH-3 and comparison to *Dfd* and *Antp* homeo boxes. Homologies are shown by dashes. (A) The sequence of the MH-3 cDNA clone is 225 nucleotides long. It begins at the Eco RI site at position 55 of the homeo box, according to the numbering system previously described (*5*) and terminates with an Eco RI site that may be part of the DNA linker used in cloning. The sequence of the first 55 nucleotides of the MH-3 homeo box was determined from a mouse genomic MH-3 clone isolated from a C57BL/6 DNA library (*27*). The MH-3 homeo box sequence (nucleotides 1 to 183) as well as sequences immediately 3′ are compared to the corresponding regions of the *Dfd* and *Antp* cDNA's (*39*). A codon was found deleted in the MH-3 homeo box (del). The position of the Hae III sites are also shown. (B) Predicted amino acid sequence of the MH-3 homeo box (amino acid 1 to 61) as well as the ten amino acids immediately 3′ as compared to the corresponding regions of the *Dfd* and *Antp* proteins.

transcribed or is transcribed at very low levels.

In contrast, specific patterns of hybridization grains were observed with the antisense probe (Fig. 4, A and B). Identical results were found with an antisense probe homologous only to the non–homeo box portion of clone MH-3 (the 0.05-kb Hae III–Eco RI fragment, Fig. 1). No hybridization was found in interstitial cells outside the tubules. Hybridization grains could be found in situ in regions of the adult testis containing pachytene spermatocytes and round spermatids and could not be found in regions containing spermatogonia. A lower density of grains was found over condensing spermatids and over the lumen of tubules, raising the possibility that some MH-3 transcripts are present during spermiogenesis. Our data indicate that the MH-3 gene is not expressed during spermatogonial renewal but that it is expressed during meiosis. MH-3 transcripts were found in spermatogenic cells at late meiotic prophase and at both meiotic divisions. It is not clear whether transcripts were present in cells at early meiotic prophase because of the difficulty in identifying these cells.

To confirm and extend the findings described above, we analyzed the expression of the MH-3 gene during development of the prepuberal testis. The appearance of the various spermatogenic cells in the prepuberal testis occurs in a defined temporal sequence (*35*). No MH-3 transcripts were detected in the mouse testis at day 10 but high levels were found at days 17, 24, and 30 after birth (Fig. 5A). In comparison, this level was only three- to fourfold lower than that found in the adult testis. Equal amounts of total RNA were present on the blots, as judged by the staining intensity of the 28*S* and 18*S* ribosomal RNA (rRNA). To define the time at which the MH-3 gene is activated, we analyzed RNA of testis from day 8 to 18 postnatal (Fig. 5B). The 1.5-kb MH-3 transcript was first detected in the prepuberal testis at day 14. The amount of transcripts then increased to high levels by day 18. Since Sertoli cells, spermatogonia, and spermatocytes at early meiotic prophase comprise high percentages of total cells in the seminiferous epithelium at days 8 to 13, our data indicate that these cells do not express the MH-3 gene. Thus the activation of the MH-3 gene in male germ cells seems to occur at the pachytene stage of meiotic prophase.

To determine whether the MH-3 gene is expressed at various stages of embryogenesis we analyzed polyadenylated RNA isolated from embryos at different midgestation days. A 1.5-kb MH-3 transcript was readily detected at day 11.5 post coitum (Fig. 5C).

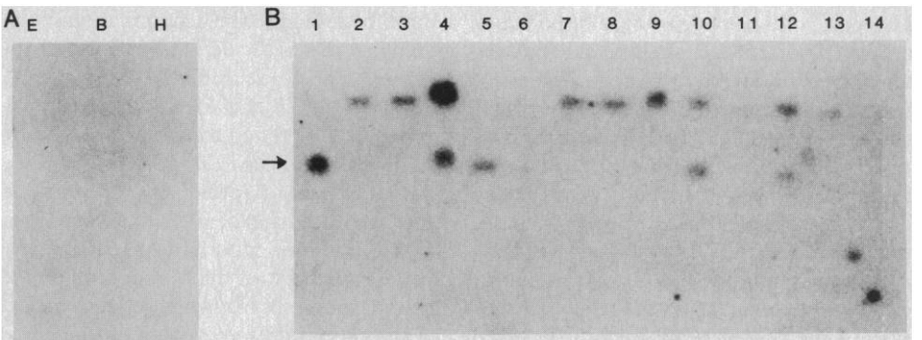

Fig. 2. Chromosomal assignment of the MH-3 gene. (A) Southern blot containing murine cell (F9) DNA cleaved with Bam HI (B), Eco RI (E), or Hind III (H) and hybridized to the 0.22-kb MH-3 cDNA. (B) Southern blot showing hybridization of the MH-3 cDNA probe to Eco RI–digested DNA from C57BL/6J mouse liver (lane 1); the Chinese hamster cell line E36 (lane 2); and the hybrid cell lines ABm11 (lane 3), ABm14 (lane 4), F(11)U (lane 5), no DNA (lane 6), MAE28 (lane 7), MACH4A63 (lane 8), MACH4B31Az3 (lane 9), MACH2A2B1 (lane 10), MACH2A2C2 (lane 11), MACH2A2H3 (lane 12), ECm4e (lane 13), and R44-1 (lane 14). Somatic hybrid cell lines contained various numbers of mouse chromosomes on the background of a Chinese hamster genome, with the exception of lane 5, in which the background was a complete rat genome (*26*). An arrow marks the mouse MH-3 band. For (A) and (B), digested mouse genomic DNA (10 μg) was fractionated by electrophoresis on a 1.0% agarose gel along with ^{32}P-labeled Hind III fragments of λ DNA as size markers. The DNA's were blotted onto nitrocellulose filters (*40*). Filters were hybridized for 18 hours at 68°C to nick-translated MH-3 DNA (10 ng/ml) in 6× standard saline citrate (SSC), 0.1% bovine serum albumin (BSA), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, salmon-sperm DNA (sonicated and denatured; 100 μg/ml), 1 m*M* EDTA, 10 m*M* $NaPO_4$ (*p*H 7.4) and were washed to a final stringency of 0.1× SSC, 0.1% sodium dodecyl sulfate (SDS) at 68°C.

The relative amount of this transcript decreased about fivefold at day 12.5, another fivefold at day 13.5, and remained at this level for the next 3 days. Equal amounts of RNA were present on the filter, as shown by rehybridization with an H-*ras* probe. Our data demonstrate the stage-specific expression of the MH-3 gene during embryogenesis.

The 11.5-day embryo contains primordial germ cells that are completing their migration from the yolk sac into the genital ridges (*36*). At this stage, the gonads begin to form from the genital ridges but differentiation into testis or ovary occurs a few days later. The expression of the MH-3 gene observed at day 11.5 may be due to expression in the primordial germ cells, or to expression in somatic tissues or extra-embryonic tissues. In either case, the expression observed at day

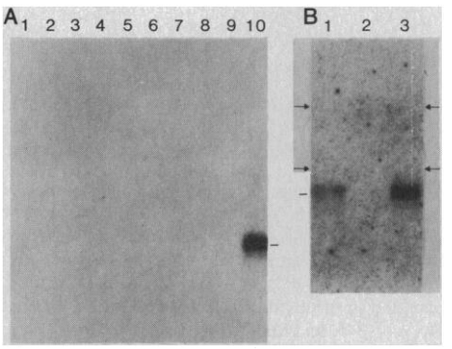

Fig. 3. Expression of the MH-3 gene in male gonads and germ cells. (A) Northern blots containing 20 μg of total RNA isolated from 8-week-old ICR mouse brain (lane 1), colon (lane 2), heart (lane 3), kidney (lane 4), liver (lane 5), lung (lane 6), muscle (lane 7), pancreas (lane 8), ovary (lane 9), and testis (lane 10). (B) Northern blots containing 20 μg of total testis RNA isolated from the following 4-week-old mice: +/+(lane 1), *Sl*/*Sl*^{d} (lane 2), and ICR (lane 3). Blots were hybridized to the MH-3 probe as described below. *Sl*/*Sl*^{d} and +/+ mice were obtained by crossing WC/REJ *Sl*/+ with C57/BLJ *Sl*^{d}/+ (Jackson Laboratory). For (A) and (B) total cellular RNA was extracted from pulverized frozen tissues; lysed in 5*M* guanidinium thiocyanate, 50 m*M* tris-HCl (*p*H 7.5), 10 m*M* EDTA, and 5% 2-mercaptoethanol; homogenized (*41*); and isolated by precipitation in LiCl (*42*). RNA (20 μg) was fractionated by electrophoresis on 1.2% agarose gels containing formaldehyde and blotted onto nitrocellulose filters (*43*). Blots were hybridized to nick-translated MH-3 probes (10 ng/ml) in 5× SSC, 0.1% BSA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 1 m*M* EDTA, 10 m*M* $NaPO_4$ (*p*H 7), plus 0.5% SDS, salmon sperm DNA (sonicated and denatured; 200 μg/ml), 50% deionized formamide, and 10% dextran sulfate at 42°C for 12 to 16 hours. Blots were washed to 0.1× SSC, 0.1% SDS at 68°C. The integrity and amount of RNA on the blot was confirmed by the intensity of the 18*S* and 28*S* rRNA bands (location shown by arrows) after ethidium-bromide staining; horizontal lines indicate a size of 1.5 kb.

11.5 post coitum appears not to be linked to meiosis, since this event is initiated at a later age, namely at day 13 of gestation in female embryos or at day 10 postnatal in males.

The data presented here defined the specific expression in the male germ line of a mouse homeo box–containing gene that we designate MH-3. During spermatogenesis, the MH-3 gene was expressed in spermatocytes undergoing meiosis but not in mitotically proliferating spermatogonia. Expression of the MH-3 gene was also detected during embryogenesis and at developmental stages that precede gonadal and sexual differentiation. Thus, the MH-3 gene may have functions in spermatogenesis as well as embryogenesis.

Why is a homeo box–containing gene expressed during spermatogenesis? The MH-3 gene was not expressed in proliferating spermatogonia and was first activated in pachytene spermatocytes, at late meiotic prophase. At this time, the fate of male germ cells has been fully determined and their entry into meiosis has been initiated. Therefore, the expression of the MH-3 gene seems not to be related to the determination of the germ line nor to the decision to enter meiosis, but may be related to later events in meiosis. Perhaps the MH-3 protein, with some DNA-binding properties mediated by the homeo domain (*10, 11*), may be involved in events specific to late meiotic prophase such as general genetic repression and chromosome condensation. Alternatively, the expression of the MH-3 gene in late meiotic germ cells may be functionally related to postfertilization events.

What are the possible roles of the MH-3 homeo box gene in embryogenesis? Since high levels of MH-3 RNA were found in round spermatids, the possibility exists that MH-3 gene products may be present in mature spermatozoa. If so, paternally derived MH-3 gene products may be found in the zygote and early embryo and may participate in early developmental events. Recent nuclear transplantation experiments have shown that the presence of the male pronucleus is indispensable for normal development of the mouse zygote and have indicated that paternally derived nuclear factors may play a crucial role in early embryogenesis (*37, 38*). The earliest embryonic stage we have studied with respect to MH-3 gene expression is at day 11.5 post coitum. At this stage, the organ rudiments derived from the three embryonic germ layers (ectoderm, mesoderm, and endoderm) are undergoing extensive morphogenesis and the primordial germ cells have almost completed their migration from the yolk sac into the genital ridges (*36*). If the expression of the MH-3 gene observed at this stage is also restricted to the germ line, as in the male adult, then the gene has to be expressed in primordial germ cells. In this case, the MH-3 gene may have a role in the ontogeny of the germ line or n e used to trace the origin of

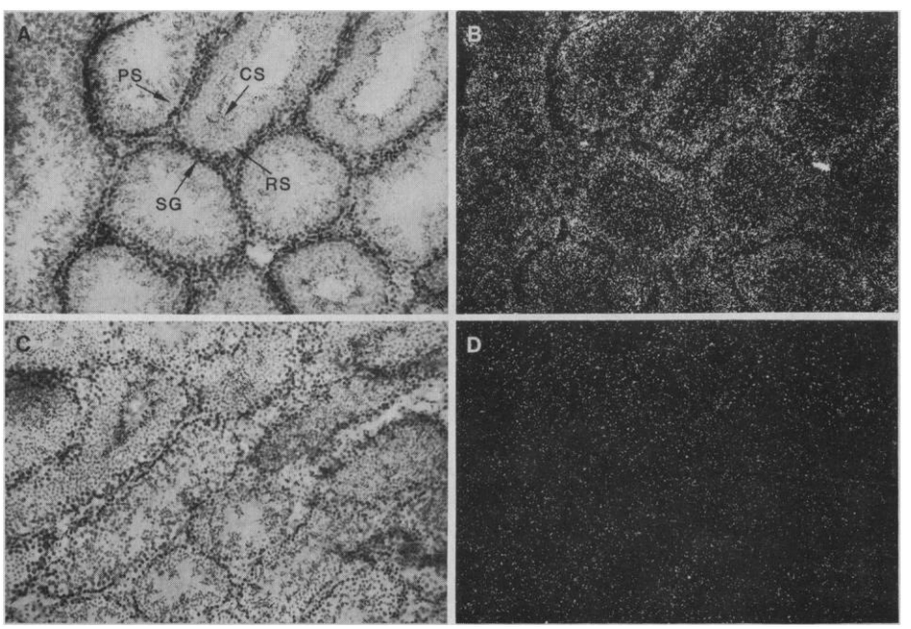

Fig. 4. In situ hybridization to sections of mouse testis. MH-3 RNA probes (^{35}S-labeled) detecting the sense MH-3 RNA (panels A and B) or the antisense MH-3 RNA (panels C and D) were hybridized to 8-μm sections of adult mouse testis. Panels A and C show bright field photomicrographs and panels B and D show dark field photomicrographs. The arrows identify spermatogonia (SG), pachytene spermatocytes (PS), round spermatids (RS), and condensing spermatids (CS). Spermatogenesis may be divided in three phases: In phase 1, stem cells proliferate and self-renew (spermatogonial proliferation). In phase 2, spermatogonia divide to form preleptotene spermatocytes that undergo a final round of DNA replication before entering meiotic prophase. Meiotic prophase proceeds through the leptotene, zygotene, and pachytene stages and terminates in the first meiotic division with the formation of secondary spermatocytes. The latter cells quickly enter the second meiotic division to form round spermatids (meiosis). In phase 3, round spermatids undergo morphological changes to form condensing spermatids and spermatozoa (spermiogenesis). Testes from adult ICR random-bred albino mice were fixed in 4% paraformaldehyde in phosphate-buffered saline (130 m*M* NaCl, 7 m*M* Na_2HPO_4, 3 m*M* $NaH_2PO_4 \cdot H_2O$) for 24 hours at 4°C, incubated in 0.5*M* sucrose in phosphate-buffered saline for 24 hours at 4°C, and mounted in Tissue Tek II O.T.C.; sections (8 μm) were cut and placed on subbed slides. Slides were prehybridized, hybridized, and washed as described (*32*). After a 1- to 2-week exposure to a 1:1 dilution of Kodak NTB-2 emulsion, the slides were developed in Kodak D19 and Rapid Fix, and stained with Giemsa solution.

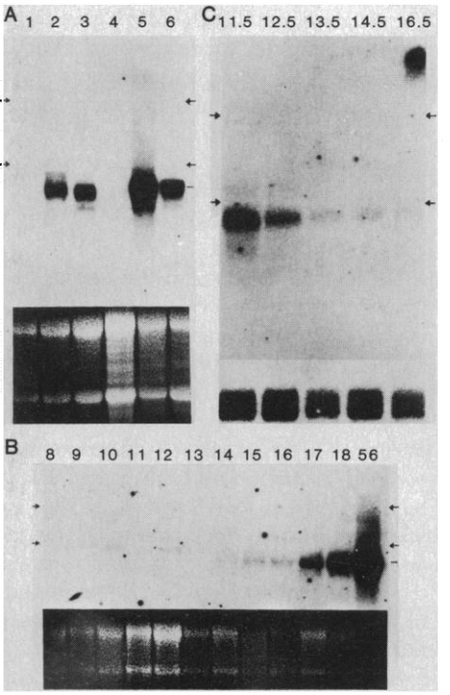

Fig. 5. Expression of the MH-3 gene in (A and B) prepuberal murine testis and (C) mouse embryos. (A) Northern blots containing 20 μg of total RNA from ICR mouse testis at days 10 (lanes 1 and 4), 17 (lane 2), 24 (lane 3), 30 (lane 6), and 56 postnatal (lane 5). (B) Northern blots containing 20 μg of total RNA from ICR mouse testis at days 8 to 18 (lanes 8 to 18) and day 56 (lane 56). Blots were hybridized to the MH-3 cDNA probe as described (Fig. 3). (C) Northern blots containing 5 μg of polyadenylated RNA isolated from ICR mouse embryos at days 11.5, 12.5, 13.5, 14.5, and 16.5 post coitum were hybridized to the MH-3 cDNA probe (top panel). Equal amounts of RNA were present in the lanes as determined by hybridization to an H-*ras* probe (bottom panel). Embryos were obtained by mating ICR mice. Total cellular RNA was extracted from embryos dissected from implantation sites. The day of detection of the vaginal plug was defined as day 0.5 of gestation. Polyadenylated RNA was selected by chromatography onto oligodeoxythymidylate-linked cellulose. Arrows indicate the positions of 18*S* and 28*S* rRNA bands; horizontal lines indicate a size of 1.5 kb.

primordial germ cells. Alternatively, the MH-3 gene may be expressed and play a role in embryonic cells that are not of the germ cell lineage.

In contrast to the defined functions of *Drosophila* homeo box genes, nothing is known about the functions of the 10–20 homeo box genes in mice. Their relationship to the control of development remains to be determined.

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Bright Light Resets the Human Circadian Pacemaker Independent of the Timing of the Sleep-Wake Cycle

CHARLES A. CZEISLER,* JAMES S. ALLAN, STEVEN H. STROGATZ, JOSEPH M. RONDA, RAMIRO SÁNCHEZ, C. DAVID RÍOS, WALTER O. FREITAG, GARY S. RICHARDSON, RICHARD E. KRONAUER

Human circadian rhythms were once thought to be insensitive to light, with synchronization to the 24-hour day accomplished either through social contacts or the sleep-wake schedule. Yet the demonstration of an intensity-dependent neuroendocrine response to bright light has led to renewed consideration of light as a possible synchronizer of the human circadian pacemaker. In a laboratory study, the output of the circadian pacemaker of an elderly woman was monitored before and after exposure to 4 hours of bright light for seven consecutive evenings, and before and after a control study in ordinary room light while her sleep-wake schedule and social contacts remained unchanged. The exposure to bright light in the evening induced a 6-hour delay shift of her circadian pacemaker, as indicated by recordings of body temperature and cortisol secretion. The unexpected magnitude, rapidity, and stability of the shift challenge existing concepts regarding circadian phase-resetting capacity in man and suggest that exposure to bright light can indeed reset the human circadian pacemaker, which controls daily variations in physiologic, behavioral, and cognitive function.

IN THE 25 YEARS SINCE DECOURSEY discovered the phase response curve to light in the flying squirrel (*1*), the resetting of biological clocks by light has been characterized in nearly all species studied except man. Synchronization of the human circadian system, which usually has an intrinsic period greater than 24 hours (*2, 3*), to a 24-hour day implies that our biological clocks are reset daily. Yet, a specific resetting stimulus that shifts the phase of the human circadian pacemaker has not been identified. In a controlled case study, we have demonstrated that critically timed exposure to bright indoor light can rapidly reset the human circadian pacemaker by about 6 hours, even when the timing of the sleep-wake cycle is constant.

Despite documentation of human neuroanatomic structures analogous to those subserving circadian rhythmicity and photic entrainment in other mammals (*4*), attempts to assess the specific role of light in the synchronization of the human circadian system have been methodologically difficult. In contrast to the results of animal studies, the light-dark cycle was reported to be too weak a synchronizing cue to entrain human circadian rhythms (*5*); however, these experiments were confounded by the subjects' access to auxiliary lighting. In 1981, we demonstrated that a true light-dark cycle could entrain human circadian rhythms (*6*). However, studies of light-dark cycle entrainment in humans cannot distinguish whether synchronization occurs (i) directly through an action of light on the endogenous circadian pacemaker or (ii) indirectly by an influence on the behavioral rest-activity cycle (*7*). Because subjects attempt to sleep when it is dark and are awakened by light, the light-dark cycle influences the timing of the subjects' sleep-wake cycle, which itself may be a synchronizing agent (*6, 8*).

Having demonstrated that bright light must exceed a minimum threshold (>2500 lux) to suppress melatonin secretion (*9*), Lewy has suggested that bright light may

C. A. Czeisler, J. S. Allan, J. M. Ronda, R. Sánchez, C. D. Ríos, W. O. Freitag, G. S. Richardson, Neuroendocrinology Laboratory, Division of Endocrinology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 221 Longwood Avenue, Boston, MA 02115.
S. H. Strogatz and R. E. Kronauer, Division of Applied Sciences, 324 Pierce Hall, Harvard University, Cambridge, MA 02138.

*To whom requests for reprints should be addressed.