occurred between the red and green phototopic genes in the lineage leading to humans and at least twice between color phototopic alleles in NWMs. These repeated divergences and the required parallel amino acid substitutions would indicate adaptive evolution. In addition, the antiquity of the alleles implies that they have been maintained by balancing selection; otherwise, one or two of the alleles would have been lost through random drift (20). On the other hand, according to the single origin scenario, the triallelic system should be more ancient than the divergence of the two NWM species, which has been estimated at 16.4 to 19.0 Ma (13). Figure 1B suggests that at least seven gaps have been transferred and homogenized among the three alleles in each of the two species. This implies that the critical amino acids that define these alleles have been maintained in the presence of frequent homogenization events, which again suggests balancing selection. The type of selection is probably not minority advantage because the alleles in each species, with the possible exception of the marmoset P55, are maintained at high frequencies (4, 5). One simple explanation is overdominant selection; trichromacy, which occurs in (female) heterozygotes, is thought to facilitate the detection of colored fruits against dappled foliage (21). The advantage of having three instead of two polymorphic alleles is an increase in the frequency of heterozygotes and thus the chance of overdominant selection. Another possible advantage of polymorphism is that monkeys with different spectral sensitivities may explore visually different environments (3). An additional advantage of the polymorphic system is that dichromats (males and homozygous females) detect color-camouflaged objects better than do trichromats (22). Thus, NWMs, which search for fruits cooperatively in groups, enjoy the advantages of both trichromacy and dichromacy (6).

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8. Introns 4 of each allele in each of the two species were amplified by the polymerase chain reaction (PCR); the primers and the experimental conditions used are available on request. Sequencing reactions were accomplished by dideoxynucleotide chain termination methods on double-stranded plasmid templates with T7 DNA polymerase (Sequenase kits, U.S. Biochemical). All sequences were determined in both directions by a combination of (i) direct sequencing with synthetic oligonucleotide primers and (ii) sequencing of exonuclease III-generated smaller subclones, which contain successively larger unidirectional deletions, with the Erase-a-Base system (Promega). At least three independent PCR clones were sequenced to avoid PCR errors. The GenBank accession numbers are X18888 through X18893.
10. M. Neitz, J. Neitz, and G. H. Jacobs (Science 252, 971 (1991)) found that the three alleles in squirrel monkeys differ at two amino acid residues in exon 5 that are involved in spectral tuning; the amino acids for P534, P550, and P561 are, respectively, Phe, Phe, and Phe at position 277 and Asp, Thr, and Thr at position 285. We had two male squirrel monkeys of Bolivian origin. One of them was determined by electrophototography (ERG) to have a spectral peak at ~560 nm (that is, the P561 allele) and a preliminary ERG study suggested that the other had a spectral peak near 550 nm (that is, the P550 allele), PCR amplification of exon 5 of both alleles and subsequent nucleotide sequencing showed that the exon 5 sequences of the two alleles were identical to Neitz et al.'s sequences of P561 and P550, respectively. To identify P554, we obtained blood samples from 10 male squirrel monkeys of Bolivian origin and from C. R. Abbe of the University of South Alabama. After screening five DNA samples by PCR amplification and sequencing of exon 5 of the X-linked pigment gene, we found an individual with a sequence identical to Neitz et al.'s sequence of P554.
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Functional Characterization and Developmental Regulation of Mouse Telomerase RNA

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Telomerase synthesizes telomeric DNA repeats onto chromosome ends de novo. The mouse telomerase RNA component was cloned and contained only 65 percent sequence identity with the human telomerase RNA. Alteration of the template region in vivo generated altered telomerase products. The shorter template regions of the mouse and other rodent telomerase RNAs could account for the shorter distribution products (processivity) generated by the mouse enzyme relative to the human telomerase. Amounts of telomerase RNA increased in immortal cells derived from primary mouse fibroblasts. RNA was detected in all newborn mouse tissues tested but was decreased during postnatal development.

Telomerases are ribonucleoprotein DNA polymerases that maintain telomere length by adding telomeric sequences onto chromosome ends (1). Human and mouse telomerases differ in both their functional properties and their regulation. Partially purified mouse telomerase adds predominantly only one repeat onto a telomeric primer in vitro, whereas the human enzyme adds hundreds of repeats under identical conditions and mixing extracts does not alter the processivity of either enzyme (2). In contrast to most normal human tissues, some normal mouse somatic tissues have detectable telomerase activity (3). Mouse cells can spontaneously immortalize in culture, whereas human cells rarely, if ever, spontaneously immortalize (4). The differential regulation of mouse and human telomerase may affect the ease of immortalization of mouse cells in culture (3).

To investigate the difference between the human and mouse enzymes, we cloned the mouse telomerase RNA component. A mouse genomic clone was identified (5) by hybridization to a 450-nucleotide (nt) probe from the transcribed region of the human telomerase RNA gene. The transcribed region of the mouse gene was 65% identical to the human telomerase RNA gene (6), which indicates that this clone might be the mouse telomerase RNA gene (Fig. 1) (7). The sequence identity in the transcribed region of the human and putative mouse RNA genes is significantly less than that
found between other small RNA genes in humans and mice (8).

The potential template regions of the human and mouse RNAs were not completely conserved. Eleven nucleotides can serve as potential templates in the human RNA, whereas only nine nucleotides are possible templates in mice, of which only eight probably serve as templates. Using PCR (polymerase chain reaction) and sequencing, we determined that the rat and Chinese hamster telomerase RNAs contain only eight potential template residues (9). The shorter template region in the rodent RNAs may decrease in vitro processivity of the mouse and rat telomerases relative to the human enzyme (2). The probability of dissociation of a growing primer from telomerase may be greater for the rodent than for the human telomerase.

Caliche telomerase is inhibited by, or can be used as primers, antisense oligonucleotides that hybridize at or just 3' of the RNA template (10–12). We tested the effects of antisense oligonucleotides on mouse telomerase activity (Fig. 2A) (13). For the inhibition assays, each oligonucleotide was pre-incubated with mouse telomerase before the primers d(TTAGGG) were added (Fig. 2B). The oligonucleotide, MI-2, that covers the template region efficiently inhibited telomerase. To determine the specificity of MI-2 inhibition, we changed the sequence of this oligonucleotide independently at either the 5' end (generating MI-3) or at the 3' end (generating MI-5), so that complementarity to the RNA was abolished. These oligonucleotides no longer inhibited telomerase, although MI-3 was itself a substrate for elongation, probably because it ends in a telomeric repeat (Fig. 2C).

Incubation with MP-1 or two other oligonucleotides, MP-2 and MP-3 (13), that hybridized to a region 3' to the template did not inhibit elongation (Fig. 2B). However, MP-1, whose 5' end hybridizes just adjacent to the RNA template, was elongated by eight residues (Fig. 2C). When the sequence of MP-1 was lengthened to extend across the template (oligonucleotide MI-4), d(TTAGGG), elongation was inhibited (Fig. 2B) and MI-4 did not serve as a substrate for elongation (Fig. 2C). The inhibition and elongation properties of the antisense oligonucleotides, together with other criteria (14), are consistent with the cloned RNA being a functional component of mouse telomerase.

To determine which potential template residues are copied, we carried out telomerase reactions with MP-1, substituting deoxythymidine triphosphate (dTTP) for deoxythymidine triphosphate (dTTP) (Fig. 2D). The 8-nt labeled product seen with dTTP was reduced to 5 nt with dTTP, which is consistent with the addition of AGGGTTAG. If the entire potential template region of the mouse RNA, 5'-CCUACCCUGCCU-3', were copied, 9 nt should have been added to the MP-1 primer. Thus, the 5'-most C in the mouse RNA, which is not conserved in other rodent RNAs, does not serve as a template residue.

Mutations of the telomerase RNA template in Tetrahymena, yeast, and humans generate telomerase with altered specificity (6, 15, 16). We carried out similar template mutagenesis with the mouse RNA gene (17). The template region of the genomic clone was changed to specify (TTGGGG), repeats instead of (TTAGGG), repeats (18), and after transfection into NIH 3T3 cells, stable clones were selected. If the mutant RNA altered the specificity of telomerase, cell extracts should contain both a wild-type telomerase that will be inhibited by deoxyadenosine triphosphate (dTTP) and a mutant telomerase that should be insensitive to dTTP (6). Telomerase activity was assayed (2) with control and transfected cell extracts with the use of dTTP and deoxyguanosine triphosphate (dTTP) in all reactions and deoxyadenosine triphosphate (dTTP) or dTTP in selected reactions (19). The products from the telomerase reactions were then amplified by PCR (20) in reactions containing all four deoxynucleo-

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**Fig. 1.** Sequence of the genomic clone encoding the mouse telomerase RNA (mTR) gene. The template region is shown with a box. The estimated positions of the 5' and 3' ends of the RNA are designated (7). The primers used to map the 3' end by RT-PCR are shown as arrows above the sequence that they hybridize to. The 3'-most primer that gave a product is shown with a solid line, and two primers that did not give products are shown with dashed lines.

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**Fig. 2.** Inhibition and elongation of oligonucleotides directed against the mouse telomerase RNA. (A) Diagram of the oligonucleotides used; the straight lines indicate that the oligonucleotide sequence is complementary to the RNA. The wavy line indicates an arbitrary sequence that is not complementary to the RNA. (B) Inhibition of mouse telomerase activity. Antisense oligonucleotides (concentrations at top) were first incubated with active telomerase reactions, and telomerase assays were done after the addition of telomeric oligonucleotide d(TTAGGG), (1 μg). Telomerase elongates this telomeric oligonucleotide by addition of 4 nt (2). (C) Elongation of oligonucleotides for 30 min on ice with a fraction (20 μl) purified on a DEAE-airagose column containing telomerase activity that had previously been treated (+) or not (−) with RNase. After the preincubation, the reaction mixture was added and telomerase reactions were carried out as described (2). The numbers on the sides indicate the size in nucleotides of the elongated products. (D) To define the sequence added to the MP-1 oligonucleotide, telomerase assays were carried out with 1 μg of either a telomeric oligonucleotide d(TTAGGG), or MP-1 oligonucleotide as primers. Telomerase assays were done as described above except that dTTP (dT) was substituted with ddTTP (dT) in lanes marked with a ‘+’. Some lanes contain fractions that were first treated with RNase A (+). The number of nucleotides added to each primer is indicated on the sides; arrow on left, 37P [T]Ag3; arrow on right, 34P [T]Ag4.
Fig. 3. Synthesis of TTGGGG repeats by mutant telomerase. Extracts from cells containing the wild-type or mutant telomerase clone were assayed with the conventional telomerase assay and the TS primer in the presence or absence of dATP (dA) or ddATP (ddA). Products were amplified by PCR with all four dNTPs present with the use of the TS primer and either a \( A_8C_{14} \) primer (A) or a \( A_7C_{15} \) primer (B). Each reaction was done with (+) or without (−) a pretreatment with RNase to determine if products were generated by telomerase. The PCR conditions used are described (19). In the presence of ddATP in the telomerase reaction, products were not generated by the wild-type enzyme (A, lanes 5 and 6); however, products were generated by the mutant telomerase (B, lanes 10 to 13). For the wild-type enzyme, addition of both dATP and ddATP did not result in ddATP termination (A, lanes 3 and 4), and simple omission of ddATP did not completely inhibit product synthesis, which suggests that a small amount of ddATP may be present in the extracts used.

side triphosphates (dNTPs) and a return primer specific for either wild-type repeats \( [A_7C_{15}]_n \); Fig. 3A) or for mutant repeats \( [A_7C_{14}]_n \); Fig. 3B). As expected, the wild-type primer amplified telomerase products in both control and transfected cells (Fig. 3A, lanes 1 and 8), and products were not seen in reactions in which ddATP was substituted by ddATP (Fig. 3A, lanes 5 and 12). In contrast, when similar reactions were performed with the mutant PCR primer, only the cells containing the mutant RNA had activity in the presence of ddATP (Fig. 3B, lane 12). Thus, a mutation in the cloned RNA template region generated the expected mutant telomerase products, providing strong evidence that this RNA is the mouse telomerase RNA component (mTR).

To study the regulation of the mouse telomerase, we followed mTR expression in tissues and cell lines. Northern (RNA) blots of total RNA from various tissues in both newborn and adult mice were probed with mTR and with a 55 RNA gene to allow normalization to total RNA (Fig. 4A). In newborn mice, mTR was detectable in intestine, brain, kidney, lung, and liver. However, in adult mice, mTR was detected only in testis, intestine, liver, and spleen. Unlike many human somatic tissues that do not express detectable telomerase activity (20), many adult mouse tissues including testis, liver, spleen, and kidney contain telomerase activity (3, 21). The detection of mTR in some adult tissues correlates with the presence of telomerase activity (22).

To identify when during postnatal development amounts of mTR changed, we prepared RNA from three tissues at different postnatal developmental stages up to 16 days after birth (Fig. 4B). The mTR amounts decreased in the brain (68%), kidney (87%), and liver (77%) (Fig. 4C). However, the mTR amounts in the liver were initially higher than those found in the other tissues, and after day 16 the amount of mTR in the liver was equivalent to that in the newborn brain (Fig. 4C). This larger amount of mTR in adult liver is consistent with the presence of telomerase activity in adult mouse liver.

Primary Mus spretus fibroblasts lack detectable telomerase activity and show telomere shortening. After immortalization, telomerase activity is detected and telomere length is maintained (3, 23). We used these Mus spretus cell lines to follow mTR amounts before and after immortalization. Northern blot analysis showed that the immortalized fibroblasts expressed at least 18 times more mTR than the parental fibroblasts, which indicates that the increase in the amount of mTR correlated with the increase in telomerase activity after immortalization (Fig. 4D). The identification of the human and mouse telomerase RNAs will allow a detailed investigation of the mechanism and regulation of mammalian telomeres.

REFERENCES AND NOTES

An Essential Role for Rho, Rac, and Cdc42
GTPases in Cell Cycle Progression Through G1

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Members of the Rho family of small guanosine triphosphatases (GTPases) regulate the organization of the actin cytoskeleton; Rho controls the assembly of actin stress fibers and focal adhesion complexes, Rac regulates actin filament accumulation at the plasma membrane to produce lamellipodia and membrane ruffles, and Cdc42 stimulates the formation of filopodia. When microinjected into quiescent fibroblasts, Rho, Rac, and Cdc42 stimulated cell cycle progression through G1, and subsequent DNA synthesis. Furthermore, microinjection of dominant negative forms of Rac and Cdc42 or of the Rho inhibitor C3 transferase blocked serum-induced DNA synthesis. Unlike Ras, none of the Rho GTPases activated the mitogen-activated protein kinase (MAPK) cascade that contains the protein kinases c-Raf1, MEK (MAPK or ERK kinase), and ERK (extracellular signal-regulated kinase). Instead, Rac and Cdc42, but not Rho, stimulated a distinct MAP kinase, the c-Jun kinase JNK/SAPK (Jun NH2-terminal kinase or stress-activated protein kinase). Rho, Rac, and Cdc42 control signal transduction pathways that are essential for cell growth.

Constitutively active, V12Cdc42 (Cdc42 with valine substituted for glutamate at position 12), V12Rho, V12Rac, and V12Ras recombinant proteins were each microinjected with rat immunoglobulin G (IgG) into the cytoplasm of quiescent Swiss 3T3 fibroblasts, and the incorporation of bromodeoxyuridine (BrdU) into nascent DNA was measured after 40 to 48 hours (1). Microinjection of rat IgG alone (Fig. 1, A and B) had no effect on DNA synthesis. Of the three microinjected BrdU incorporation (Fig. 1C). Microinjection of V12Cdc42 efficiently stimulated DNA synthesis (Fig. 1, A and B); ~90% of injected cells were positive for BrdU incorporation (Fig. 1C). V12Ras, V14Rho, and V12Rac also stimulated BrdU incorporation in the majority of the injected cells (Fig. 1C).