above the rupture temperature of 87°C, and corresponds to a pressure of about −80 MPa.

Comparison of the parallel-polarized Raman spectra of the Sample 2 inclusion in the fundamental -O-H stretching region at 92°C both before and after rupture (Fig. 4) reveals that, despite the ~5% difference in density between the cavitated and stretched samples, their Raman spectra are nearly indistinguishable. This result was not unexpected because both Raman spectra and infrared spectra are known to respond only weakly to positive pressure changes (*22*). Some minor differences in the Raman spectra of the cavitated and uncavitated liquids at 92°C are discernible. For the sample under tension, there is a small loss in intensity of the lower frequency shoulder and a small positive shift of the main band peak relative to the cavitated sample. These differences indicate that, in the temperature range of our study, isothermal stretching has qualitatively the same effect on structure as isobaric thermal expansion (*23*). Computer simulation studies of the stretched state of water (*9*) suggest that this may not be the case at lower temperatures. It should be possible to study this interesting aspect on samples prepared to maximize the tension without provoking cavitation. The relations in Fig. 1 suggest that one should be able to pass solutions through the temperature of maximum density (the temperature of maximum tension in a constant volume experiment) which should be reached at about −150 MPa.

Our observations show that physicochemical studies of liquids are possible in a pressure-temperature regime in which the physical properties are dominated by the attractive component of the intermolecular potential. The low-frequency vibrational modes, the compressibility, and the structural relaxation times of water should be much more sensitive to tension than are the high-frequency vibrational spectra, especially as the spinodal limit is approached. The challenge will now be to exploit microscopic Brillouin scattering techniques developed for ultrahigh-pressure studies in diamond cells (*24*) to explore more fully the properties of water and other liquids in this new and interesting regime.

REFERENCES AND NOTES

1. R. J. Speedy, *J. Phys. Chem.* **86**, 982 (1982).
2. ———, C. A. Angell, *J. Chem. Phys.* **65**, 851 (1976).
3. C. A. Angell, *Annu. Rev. Phys. Chem.* **79**, 3921 (1983).
4. L. Landau and I. M. Lifschitz, *Statistical Physics* (Addison-Wesley, London, 1958), p. 264.
5. L. Haar, J. Gallagher, G. S. Kell, National Bureau of Standards–National Research Council Steam Tables (McGraw-Hill, New York, 1985).
6. C. A. Angell, W. J. Sichina, M. Oguni, *J. Phys. Chem.* **86**, 998 (1982).
7. D. H. Trevena, *Contemp. Phys.* **17**, 109 (1976); *Am. J. Phys.* **47**, 341 (1979).
8. R. J. Henderson and R. J. Speedy, *J. Phys. E* **13** 778 (1980).
9. J. L. Green, A. R. Lacey, M. G. Sceats, S. J. Henderson, R. J. Speedy, *J. Phys. Chem.* **91**, 1684 (1987).
10. S. J. Henderson and R. J. Speedy, *ibid.*, p. 3062.
11. A. Geiger, *Proceedings of the NATO Advanced Study Institute on Hydrogen Bonded Liquids*, J. C. Dore and J. Teixeira, Eds. (Plenum, New York, in press).
12. E. Roedder, *Science* **155**, 1413 (1967); *Fluid Inclusions*, P. H. Ribbe, Ed. (*Rev. Mineral.*, vol. 12, Mineralogical Society of America, Washington, DC, 1984).
13. C. A. Angell and Q. Zheng, *Phys. Rev. Rap. Commun.* **39**, 8784 (1989).
14. G. H. Wolf *et al.*, *J. Chem. Phys.*, in press.
15. S. M. Sternar and R. J. Bodnar, *Geochim. Cosmochim. Acta* **48**, 2659 (1984).
16. M. Berthelot, *Ann. Chem. Ser. 3* **30**, 232 (1850).
17. M. R. Rovetta, J. D. Blacic, R. L. Hervig, J. R. Holloway, *J. Geophys. Res.* **94**, 5840 (1989); M. R. Rovetta, personal communication.
18. T. P. Mernagh and A. R. Wilde, *Geochim. Cosmochim. Acta* **53**, 765 (1989).
19. J. H. Shufle and M. Venogoplan, *J. Geophys. Res.* **72**, 3271 (1967); A. N. Savitskii, *Russ. Colloid. J.* **30**, 199 (1968).
20. G. C. Kennedy, *Econ. Geol.* **45**, 629 (1950); ———, G. J. Wasserburg, H. C. Heard, R. C. Newton, *Am. J. Sci.* **260**, 501 (1962); J. L. Haas, Jr., *U.S. Geol. Surv. Bull. 1421* (1976), data in part C by R. W. Potter II and D. L. Brown.
21. On the basis of compressibility and thermal expansivity data for quartz, we estimate that the ratio of the volume at T_d to the volume at the cavitation temperature, T_h, of the uncavitated fluid will be 1.011 and 1.005 for Samples 1 and 2, respectively. This effect results in approximate reductions of 3% and 10% of the tensions of cavitation indicated on Fig. 3 for Samples 1 and 2, respectively.
22. G. E. Walrafen, *J. Solution Chem.* **2**, 159 (1973); V. Rodgers, thesis, Purdue University, West Lafayette, IN (1981).
23. J. R. Scherer, M. K. Go, S. Kint, *J. Phys. Chem.* **78**, 1304 (1974).
24. A. Polian and M. Grimsditch, *Phys. Rev. B* **27**, 6409 (1983); L. Oliver, C. Herbst, G. H. Wolf, in preparation.

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Direct Cloning of Human Transcripts with HnRNA from Hybrid Cell Lines

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A library of human-derived complementary DNA from a human-hamster hybrid cell line containing the Xq24–qter region has been constructed. Complementary DNA synthesis was primed from heterogeneous nuclear (hn) RNA by oligonucleotides derived from conserved regions of human *Alu* repeats. At least 80% of these cloned sequences were of human origin, providing an enrichment of at least two orders of magnitude. Two clones, one containing a fragment of the primary transcript of the human hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene at Xq26 and another recognizing a family of human genes mapping to two regions of Xq24–qter, were charaterized. Additional hncDNA clones mapped to a variety of sites in the Xq24–qter region, demonstrating the isolation of many transcriptionally active loci. These clones provide probes for identification of genetic loci on the terminal region of the X chromosome long arm, which is the location of a number of inherited disorders.

THE ABILITY TO PURIFY HUMAN transcripts from specific regions of the genome would greatly facilitate its characterization (*1*). The Xq24–qter region of the human X chromosome encodes a minimum of 25 genetic disorders whose genes are unknown (*2*). This region is therefore particularly attractive for the investigation of its gene content. We have isolated transcribed sequences from the human Xq24–qter region using a hybrid cell line, X3000-11.1 (*3*), which retains this human chromosomal region in a hamster background. This approach takes advantage of hybrid cell lines to reduce the complexity of the genomic region under study and exploits the presence of human *Alu* repetitive elements in introns and 3′-untranslated regions of human transcripts.

HnRNA, extracted from purified nuclei of X3000-11.1 and RJK88 (hamster parent of X3000-11.1), was used as template to synthesize first-strand hncDNA. Two oligonucleotides, TC-65 and 517, from the same *Alu* region but in opposite orientations, have been shown to bind specifically to human *Alu* sequences in a hamster or mouse background (*4*). They were used separately to prime the synthesis of human hncDNA

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(Fig. 1). No hncDNA synthesis was detected when hnRNA was treated with deoxyribonuclease (DNase)-free ribonuclease (RNase) before cDNA synthesis, which demonstrates that the cDNA was produced from hnRNA templates and not from contaminating DNA (*5*). The second strand was synthesized according to standard methods (*6*). The double-stranded hncDNA was digested with Hind III or partially digested with Sau 3A and ligated into the plasmid pTZ18. More than 100 independent clones were obtained from 20 μg of hnRNA. The size of hncDNA inserts, analyzed by polymerase chain reaction (PCR) (*7*) with vector primers (see legend to Fig. 2A), ranged from 200 to 1500 bp. These clones constitute a library of transcripts from the human fraction of the hybrid cell genome. To confirm the human Xq24–qter origin of these clones, we radiolabeled the PCR-amplified hncDNA inserts with ^{32}P and hybridized each to a mapping panel consisting of X3000-11.1, RJK88, and human Eco RI–digested DNAs. Twenty (80%) of 25 clones analyzed were Xq24–qter–specific. Of the five remaining clones, two (8%) were clearly of hamster origin, while the other three (12%) hybridized to repetitive elements in both genomes. The relative enrichment in human transcripts from the starting hybrid DNA (0.5% human) is greater than two orders of magnitude. In order to map these clones to subregions of Xq24–qter, they were hybridized to a panel of somatic cell hybrids containing X chromosomes with various breakpoints in Xq24–qter.

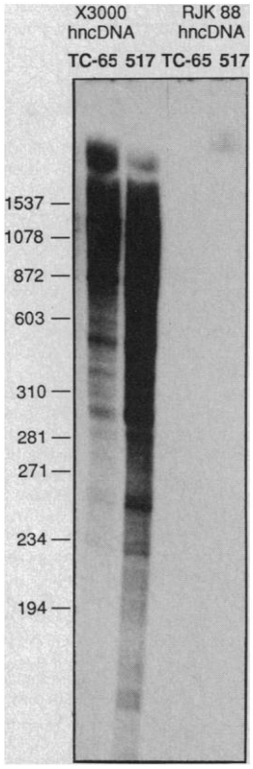

Fig. 1. Gel electrophoresis of single-strand hncDNA synthesized from X3000-11.1 and RJK88 hnRNAs with the use of TC-65 and 517 *Alu*-derived primers (*17*). Aliquots of ^{32}P-labeled first-strand hncDNAs were separated by electrophoresis on a denaturing 8% polyacrylamide–urea gel and visualized by autoradiography. Size markers (in base pairs) are ΦX174 digested with Hae III. We calculated the cloning efficiency on the basis of the amount of ^{32}P-labeled first-strand hncDNA synthesized. About 20 to 40 pg of single-strand hncDNA was obtained from 20 to 40 μg of X3000-11.1 hnRNA.

An example of the localization of one clone to Xq24–q25 is shown in Fig. 2A. A summary of the mapping analysis of the 20 human-derived clones is shown in Fig. 2B. Four subregions distinguishable by the panel are represented by at least one hncDNA clone, indicating the isolation of independent transcripts and the nontrivial complexity of the hncDNA library. In addition, three clones also recognize rodent sequences (*5*). Such cross-hybridization is expected for coding sequences, which are more conserved across species than noncoding sequences.

One such clone, A11, hybridized to three human Eco RI fragments and to one fragment in hamster and mouse DNAs (Fig. 3A). The largest human band (~12 kb) maps to Xq26; the smallest bands (4 to 5 kb) map to Xq24–q25, have variable hybridization signal ratios between different lanes, and demonstrate polymorphic variation between X chromosomes (Fig. 3A). A Northern blot hybridization analysis of RNA prepared from cell lines X3000-11.1 and RJK-88, human liver, and human placenta was carried out with the A11 insert as probe (Fig. 3B). We also probed RNAs extracted from various mouse tissues and from pig and baboon livers. The putative A11 genes appear to be expressed in many of the tissues we examined, with the highest level in liver, brain, and heart (*5*). The expression pattern of A11 genes in human, with two major transcripts (Fig. 3B, lanes 3 and 4), differs from that in rodent with one transcript similar in size to one of the two human products. This additional band is also present in baboon liver (*5*) but is not detectable in X3000-11.1 RNA (Fig. 3B, lane 2); thus the transcript cloned in A11 may correspond to the human RNA comigrating with the hamster product. Together with the Southern (DNA) blot data, these findings demonstrate that clone A11 originates from a member of a human X-linked gene family. Partial DNA sequence data indicated that this transcript is not one of the human genes present in GenBank and revealed a 30-bp region with similarity to the 3′-untranslated region of the human pre-proenkephalin B mRNA (*5*).

To demonstrate the presence of unspliced human transcripts in the library, we searched for an hncDNA clone representing the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene, which maps at Xq26. This was done for several reasons: (i) RJK88, the hamster parent of the hybrid X3000-11.1, has a total deletion of the HPRT gene, and its hybrid derivative was originally isolated by selection for HPRT activity (*3*), therefore the sole HPRT gene of the hybrid cell line is both expressed and of human origin; (ii) λ clones covering the entire gene are available (*8*); (iii) 60 kb surrounding this human gene have been sequenced (*9*); and (iv) the HPRT gene has a high *Alu* content (over 40 copies). To identify the clone or clones carrying transcripts from the HPRT gene, we hybridized

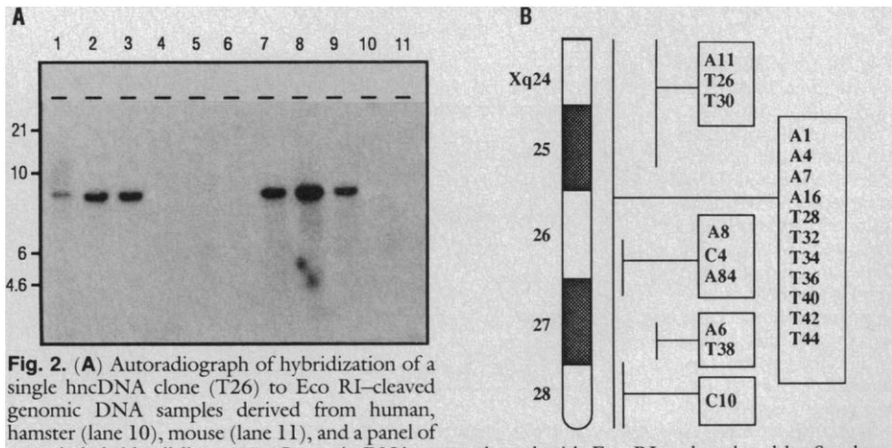

Fig. 2. (**A**) Autoradiograph of hybridization of a single hncDNA clone (T26) to Eco RI–cleaved genomic DNA samples derived from human, hamster (lane 10), mouse (lane 11), and a panel of somatic hybrid cell lines (*18*). Genomic DNAs were cleaved with Eco RI and analyzed by Southern hybridization (*19*). The amount of DNA present in each lane is approximately the same, except the human male line where the amount is half of that present in others. The order of hybrid cell lines is from left to right: 4.12, X3000-11.1, Lowe3, RJK734, 94.3, 8121 Aza 1, 2384 Aza 2, and Micro 28g. Size markers (in kilobases) are Adeno II DNA digested with Bam HI–Eco RI [International Biotechnologies, Inc. (IBI)]. (**B**) Subregional localization of human hncDNA clones.

the hncDNA library to five λ genomic clones covering the entire gene (*10*). This screening identified a single clone whose 1500-bp insert was confirmed by DNA sequencing to derive from the human HPRT gene (Fig. 4). Based on the localization of this clone and the HPRT gene sequence, we can identify four *Alu* repeats with less than two mismatches with primers TC-65 or 517 as potential oligonucleotide priming sites (Fig. 4). The distance from these priming sites to the cloned fragment is quite large; we cannot exclude the priming from *Alu* sequences with more than two mismatches or the removal of an intron or introns from the primary transcript by splicing before hncDNA synthesis.

In summary, we have developed a strategy to clone human transcribed sequences that map to a specific genome region. Evidence that the library contains transcribed sequences includes (i) its origin from hnRNA, which contains gene transcripts at various stages of maturation; (ii) isolation of an hncDNA clone recognizing an X-linked gene family; and (iii) isolation of a clone derived from the human HPRT gene, which is known to be expressed in the hybrid cell line.

Investigation of the expression of further clones by Northern and RNase protection analyses with mRNAs derived from a variety of human tissues is under way. It is likely that many clones derive from intron sequences, as with the HPRT clone, since coding exons are free of *Alu* repeats and constitute only a small portion of primary transcripts. Thus, it will be necessary to isolate genomic sequences related to these clones and search for nearby exons.

This approach has general applicability for cloning transcribed sequences from any region of the human genome for which an appropriate human-rodent cell hybrid is available. It differs from a similar approach described by Liu *et al.* (*11*) by allowing construction of small libraries that are highly enriched for the presence of human clones. Since *Alu* repeats are distributed at an average distance of ~4 kb in the human genome and are present in 23 of the 29 largest human gene sequences of GenBank (*12*), it is likely that many transcribed regions will be amenable to isolation by this method. In addition, recent evidence (*13*) suggests that even tissue-specific genes are transcribed at a basal level in many cell types, which may include hybrid cell lines. Thus, this approach may not be restricted to genes expressed ubiquitously, but may also apply to isolation of tissue-specific genes. It has a substantial potential for the isolation of human-transcribed sequences in rodent cell lines transfected or microinjected (stably or transiently) with large fragments of human DNA (*14*), yeast artificial chromosome clones (*15*), or whole chromosomes (*16*). Application to the study of heritable disease genes with known map position, such as X-linked disorders, is particularly promising.

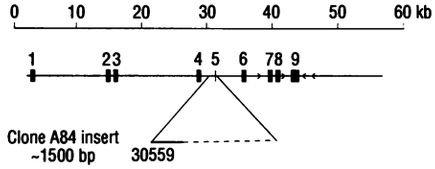

Fig. 4. Localization of hncDNA clone A84 in the human HPRT gene (*10*). Scale map of the HPRT locus showing the positions of the nine exons (boxes), and the positions and orientations of four *Alu* sequences containing less than two mismatches with hncDNA priming oligonucleotides (arrowheads). Since oligonucleotides TC-65 and 517 are complementary, cDNA synthesis may have begun at *Alu* repeats in either orientation. The 400-bp sequenced fragment (bold line) is identical to the corresponding region of HPRT gene. The 5′ end of A84 clone starts at position 30559 of the complete gene sequence (*9*).

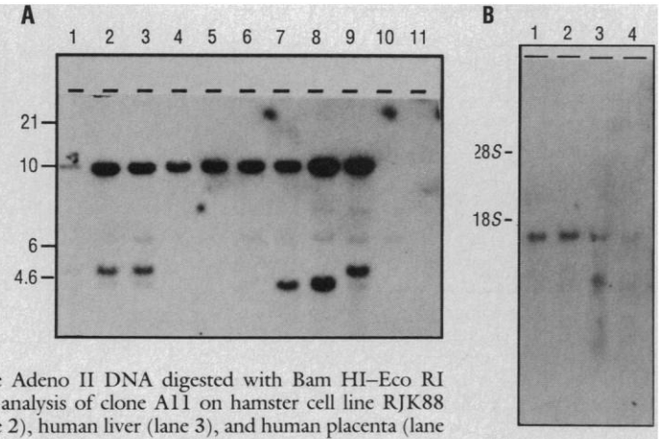

Fig. 3. Characterization of the hncDNA clone A11. (**A**) Autoradiograph of hybridization of clone A11 to Eco RI–cleaved genomic DNA samples derived from the panel of hybrid cell lines described in Fig. 2A. The amount of DNA present in each lane is approximately the same, except for the human male line where the amount is half of that present in others. Size markers (in kilobases) are Adeno II DNA digested with Bam HI–Eco RI (IBI). (**B**) Northern blot analysis of clone A11 on hamster cell line RJK88 (lane 1), X3000-11.1 (lane 2), human liver (lane 3), and human placenta (lane 4) RNAs. Total RNA (20 μg) from cells and from each tissue was prepared by the guanidine isothiocyanate procedure (*20*), separated on formaldehyde gels, and transferred onto nylon membranes, which were hybridized with A11 insert labeled as described (*21*). The positions of the 28*S* and the 18*S* ribosomal RNA bands are indicated.

REFERENCES AND NOTES

1. V. A. McKusick, *N. Engl. J. Med.* **320**, 910 (1989).
2. ———, *Mendelian Inheritance in Man* (Johns Hopkins Univ. Press, Baltimore, MD, ed. 7, 1986).
3. R. L. Nussbaum, S. D. Airhart, D. H. Ledbetter, *Am. J. Med. Genet.* **23**, 457 (1986).
4. D. L. Nelson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6686 (1989); L. Corbo, D. L. Nelson, M. F. Victoria, C. T. Caskey, *UCLA Symp. Mol. Cell. Biol. New Ser.* **126**, 187 (1990).
5. L. Corbo *et al.*, unpublished observations.
6. U. Gubler, *Methods Enzymol.* **152**, 325 (1987).
7. R. K. Saiki *et al.*, *Science* **239**, 487 (1988).
8. P. I. Patel, P. E. Framson, C. T. Caskey, A. C. Chinault, *Mol. Cell. Biol.* **6**, 393 (1986).
9. A. Edwards *et al.*, *Genomics* **6**, 593 (1990).
10. One hundred hncDNA clones were inoculated into wells of microtiter plates containing 200 μl of L broth and ampicillin (at 100 μg/ml) and incubated at 37°C for 16 hours. One hundred microliters of each bacterial culture were mixed in order to prepare ten pools of ten cultures each from which plasmid DNAs were prepared. The hncDNA inserts from each pool were prepared by PCR amplification as described (*21*). Ten microliters of PCR-amplified inserts were labeled by random oligodeoxy-nucleotide priming (*21*) and used to hybridize five λ HPRT clones, bound to nitrocellulose filters. One of the ten groups gave a positive result, while others were clearly negative. We then prepared plasmid DNAs separately from single clones of the positive pool and assayed them for the presence of HPRT gene fragment as above.
11. P. Liu, R. Legerski, M. J. Siciliano, *Science* **246**, 813 (1989).
12. R. K. Moyzis *et al.*, *Genomics* **4**, 273 (1989).
13. G. Sarkar and S. S. Sommer, *Science* **244**, 331 (1989); J. Chelly, J. P. Concordet, J. C. Kaplan, A. Kahan, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2617 (1989).
14. A. Pellicer, in *Gene Transfer*, R. Kucherlapati, Ed. (Plenum, New York, 1986), p. 263.
15. D. T. Burke, G. F. Carle, M. V. Olson, *Science* **236**, 806 (1987).
16. D. E. Housman and D. L. Nelson, in *Gene Transfer*, R. Kucherlapati, Ed. (Plenum, New York, 1986), pp. 95–115.
17. HnRNA was prepared as described [J. R. Nevins, *Methods Enzymol.* **152**, 234 (1987)]. Briefly, semi-confluent cells were harvested in phosphate-buffered saline (PBS), collected by centrifugation at 2000*g* for 5 min, and lysed by mild vortexing in lysis buffer (10 mM tris-HCl (pH 7.5); 4 mM $MgCl_2$; 100 mM KCl; and 0.05% Triton X-100). The nuclei were purified by overlay onto lysis buffer containing 0.3 M sucrose followed by centrifugation at 10,000*g* for 20 min. HnRNA was isolated from the nuclear pellet preparation by the guanidine isothiocyanate procedure (*20*). The hnRNA (20 to 40 μg) and the primer (1 μM TC-65 or 517 separately) were heated at 68°C for 5 min and then added to the reaction mixture containing, in a total volume of 100 μl, 10 mM tris-HCl (pH 8.3); 10 mM $MgCl_2$; 50 mM KCl; 1 mM dithiothreitol; 1 mM EDTA; deoxynucleotide triphosphate (dNTP) (2 mM each); 10 μCi [α-^{32}P]deoxycytidine triphosphate (dCTP); 4 mM sodium pyrophosphate; and 100 units of avian myeloblastosis virus (AMV) reverse transcriptase. Synthesis was performed for 60 min at 42°C. Oligonucleotide sequences are TC-65, AAGTCGCGGCCGCTTGCAGTGAGCCGAGAT; 517, CGACCTCGAGATCT($^{C}_{T}$) ($^{G}_{A}$)GCTC ACTG-CAA; non *Alu*–related tails are underlined.
18. The human-hamster hybrid cell line 4.12 contains the human X chromosome as its only detectable human material [R. L. Nussbaum, S. D. Airhart, D. H. Ledbetter, *Hum. Genet.* **64**, 148 (1983)]. X3000-11.1 (*3*), 94.3, 8121 Aza 1, 2384 Aza 2 [M. Patterson *et al.*, *Genomics* **1**, 279 (1987)], and Micro 28g [S. T. Warren, F. Zhang, G. R. Licameli, J. F. Peters, *Science* **237**, 420 (1987)] are human-hamster

hybrids retaining: human Xq24–qter, Xq26–qter, Xpter–q26.3, Xpter–q27.1, and Xpter–q27, respectively; RJK734 [A. F. Scott, J. A. Phillips, B. R. Migeon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4563 (1979)] and Lowe3 [D. S. Reilly, R. A. Lewis, D. H. Ledbetter, R. L. Nussbaum, *Am. J. Hum. Genet.* **42**, 748 (1988)] are human-mouse hybrid cell lines containing human Xq26–qter and Xq25–qter, respectively.
19. E. M. Southern, *J. Mol. Biol.* **89**, 503 (1975).
20. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
21. All labeling was carried out with [α-^{32}P]dCTP by random oligodeoxynucleotide priming [A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983)]. DNA probes containing repetitive sequences were preannealed with total human DNA before hybridization (0.5 mg/ml, 65°C, and 30 min). Hybridization was carried out in 1% SDS, 1 M NaCl, 10% dextran sulfate, at 65°C overnight, with 0.5 mg of herring sperm or human placental carrier DNA per milliliter. After hybridization, filters were washed at room temperature in 2× saline sodium citrate (SSC) and 0.1% SDS, and at 65°C in 2× SSC and 0.1% SDS or in 0.1× SSC and 0.1% SDS. cDNA insert preparation: Plasmid minipreparation DNA (1 μl, that is, ~100 ng) was used as template in a PCR amplification with the use of M13 universal primer and T7 promoter-derived primer. PCR was performed as described (7). Templates were mixed with 100 pmol of each primer in a total volume of 100 μl containing 50 mM KCl; 10 mM tris-HCl (pH 8.3); 1.5 mM $MgCl_2$; 0.01% (w/v) gelatin; 200 μM each dNTP; and 2.5 units of *Taq* polymerase (Perkin-Elmer Cetus). Amplification consisted of 35 cycles of denaturation (94°C; 1 min); DNA polymerization (68°C; 2 min); and annealing (60°C; 1 min) in an automated thermal cycler (Perkin-Elmer Cetus) and included an initial denaturation of 7 min at 94°C and a final polymerization at 68°C for 7 min.
22. We thank A. Edwards for the unpublished HPRT sequence; C. Schwartz, K. Davies, and S. Warren for unpublished cell lines; D. Ledbetter for use of somatic cell mapping panel; M. F. Victoria for technical assistance; D. Muzny for DNA sequencing; A. Ballabio for encouragement; and P. Patel for critical reading of the manuscript. Supported by the Howard Hughes Medical Institute and grant #DE-FG05-88ER60692 from the U.S. Department of Energy. J. A. Maley was supported by an appointment to the U.S. Department of Energy Alexander Hollaender Distinguished Fellowship program administered by Oak Ridge Associated Universities.

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Two G Protein Oncogenes in Human Endocrine Tumors

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Somatic mutations in a subset of growth hormone (GH)–secreting pituitary tumors convert the gene for the α polypeptide chain (α_s) of G_s into a putative oncogene, termed *gsp*. These mutations, which activate α_s by inhibiting its guanosine triphosphatase (GTPase) activity, are found in codons for either of two amino acids, each of which is completely conserved in all known G protein α chains. The likelihood that similar mutations would activate other G proteins prompted a survey of human tumors for mutations that replace either of these two amino acids in other G protein α chain genes. The first gene so far tested, which encodes the α chain of G_{i2}, showed mutations that replaced arginine-179 with either cysteine or histidine in 3 of 11 tumors of the adrenal cortex and 3 of 10 endocrine tumors of the ovary. The mutant α_{i2} gene is a putative oncogene, referred to as *gip*2. In addition, *gsp* mutations were found in 18 of 42 GH-secreting pituitary tumors and in an autonomously functioning thyroid adenoma. These findings suggest that human tumors may harbor oncogenic mutations in various G protein α chain genes.

MANY PROTO-ONCOGENES encode proteins that transmit signals that regulate normal cell growth. Specific mutations convert these genes into oncogenes, whose mutant protein products are responsible for the abnormal growth of malignant cells. In many human tumors, for example, point mutations convert *ras* genes into oncogenes (*1*). These mutations render $p21^{ras}$ oncogenic by inhibiting its ability to hydrolyze bound guanosine triphosphate (GTP), thus trapping the protein in its active, GTP-bound state (*1*). We reported (*2*) functionally similar GTPase inhibiting mutations that activate the α chain (α_s) of a heterotrimeric G protein, G_s, in human growth hormone (GH)–secreting pituitary tumors; the mutations convert the α_s gene into a putative oncogene, termed *gsp* (*2*).

These findings, combined with conserved stretches of amino acid sequence in a large number of G protein α chains, suggested a novel approach for finding oncogenes. Here we report success of this approach in its first application, to the gene encoding the α chain (α_{i2}) of G_{i2}.

This approach is based on two inferences regarding structure and function of G proteins. First, we infer that the two conserved amino acids whose mutational replacements inhibit GTPase of α_s have similar functional roles in α chains of other G proteins. This inference in turn implies that cognate mutations in other α chains will activate the corresponding G proteins. One set of *gsp* mutations (*2*) substitutes arginine for Gln^{227} of α_s, which is equivalent to Gln^{61} of $p21^{ras}$, a frequent site of GTPase-inhibiting mutations in the *ras* proteins. Other *gsp* mutations (*2*) replace Arg^{201} of α_s with cysteine or histidine. This arginine is the target for cholera toxin-catalyzed adenosine diphosphate (ADP)–ribosylation of α_s and the α chain of retinal transducin, a covalent modification that inhibits GTPase activity of both proteins (*3*). The amino acid sequence in regions surrounding both these codons (Table 1) is conserved from unicellular eukaryotes to mammals, further supporting the inference that the two amino acids play conserved GTPase-catalyzing roles in all G proteins.

The second inference is that α chains of other G proteins, like α_s, are protooncogene proteins—that is, that they mediate signaling pathways coupling external stimuli to stimulation of proliferation. This inference is based on evidence that certain hormones and neurotransmitters promote proliferation via receptors that interact with G proteins and thereby trigger signaling pathways that promote proliferation, including activation of phospholipases C and A_2 (*4*).

This second inference dictates a broad-based search for α chain mutations, because current information does not point to specific cells or tissues in which a specific G protein (other than G_s) is known to mediate a proliferative stimulus. This contrasts with the initial search for *gsp* mutations, which was prompted by abnormally elevated adenylyl cyclase activity and GH secretion in a subset of GH-secreting pituitary tumors (*5*). In normal pituitary somatotrophs, G_s cou-

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