References and Notes

1. For visual cortex, see C. D. Gilbert, *Annu. Rev. Neurosci.* **6**, 217 (1983).
2. J. B. Angevine, Jr., and R. L. Sidman, *Nature (London)* **192**, 766 (1961); P. Rakic, *Science* **183**, 425 (1974); M. B. Luskin and C. J. Shatz, *Soc. Neurosci. Abstr.* **8**, 3 (1982).
3. M. Shimada and J. Langman, *J. Comp. Neurol.* **139**, 227 (1970).
4. E. G. Jones, K. L. Valentino, J. W. Fleshman, Jr., *Dev. Brain Res.* **2**, 425 (1982); V. S. Caviness, *ibid.* **4**, 293 (1982); K. F. Jensen and H. P. Killackey, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 964 (1984).
5. R. D. Lund and S. D. Hauschka, *Science* **193**, 582 (1976); C. B. Jaeger and R. D. Lund, *Exp. Brain Res.* **40**, 265 (1980).
6. "Proliferative zone" here denotes both the ventricular and subventricular zones [Boulder Committee, *Anat. Rec.* **166**, 257 (1970)] in which neurons undergo their terminal mitotic division before migration.
7. C. A. Jackson, J. D. Peduzzi, T. L. Hickey, *Soc. Neurosci. Abstr.* **10**, 519 (1984).
8. P. Rakic, *J. Comp. Neurol.* **145**, 61 (1972).
9. Some labeled cells were also found in this region in adult ferrets injected with [^{3}H]thymidine on postnatal days 1 or 4.
10. Labeled cells were counted from a one-in-five series of autoradiographs for each host brain—thus, allowing for the remaining sections and estimating that only cells in the upper third of each 20-μm section were detected autoradiographically, it was calculated that on average 300 labeled neurons (range, 25 to 795 cells) were present in each host. Typically, 2000 to 3000 cells were injected (calculated from the volumes used and density of the suspensions and from counts of cells in host animals killed shortly after transplantation); of these roughly 600 to 900 would be labeled with [^{3}H]thymidine. The estimated survival rates ranged from 6 to 80 percent and represent a minimum survival, since some labeled cells may have reentered the mitotic cycle and diluted out their label after transplantation.
11. D. K. Morest, *Z. Anat. Entwicklungsgesch.* **128**, 271 (1969); *ibid.*, p. 290.
12. I am grateful to S. LeVay and J. Huettner for many helpful discussions; S. LeVay, M. Luskin, and B. Stanfield for comments on the manuscript; K. Allen for histological assistance; and N. Goodknight for preparing the manuscript. This work was supported by PHS grant EY-05551 to S. LeVay.

22 April 1985; accepted 1 July 1985

Differential Control of U1 Small Nuclear RNA Expression During Mouse Development

Abstract. *During normal mouse development the relative amounts of two types of U1 small nuclear RNA's (U1 RNA) change significantly. Fetal tissues have comparable levels of the two major types of mouse U1 RNA's, mU1a and mU1b, whereas most differentiated adult tissues contain only mU1a RNA's. Those adult tissues that also accumulate detectable amounts of embryonic (mU1b) RNA's (for example, testis, spleen, and thymus) contain a significant proportion of stem cells capable of further differentiation. Several strains of mice express minor sequence variants of U1 RNA's that are subject to the same developmental controls as the major types of adult and embryonic U1 RNA. The differential accumulation of embryonic U1 RNA's may influence the pattern of gene expression during early development and differentiation.*

ELSEBET LUND
Department of Physiological Chemistry, University of Wisconsin, Madison 53706
BRENDA KAHAN
Department of Zoology, University of Wisconsin
JAMES E. DAHLBERG
Department of Physiological Chemistry, University of Wisconsin

U1 small nuclear RNA (U1 RNA) (*1*) is essential in the processing of messenger RNA precursors (pre-mRNA's) (*2*). The U1 ribonucleoprotein particle (U1 snRNP), which contains U1 RNA plus a set of seven to nine proteins (*3*), appears to function in the recognition of 5′ splice sites of pre-mRNA (*4*).

Since pre-mRNA splicing occurs in all eukaryotic cells (*5*) it has generally been assumed that the genes for U1 RNA and snRNP-associated proteins are expressed constitutively as "housekeeping" genes (*6*). We demonstrated, however, that a class of the *Xenopus laevis* U1 RNA genes (which we called embryonic U1 RNA genes) was subject to control of expression during development (*7*). On the basis of that observation, we proposed that differential expression of certain genes for U1 RNA's or U1 snRNP proteins might result in changes in pre-mRNA splicing patterns. We now report that the expression of mouse U1 RNA genes is also developmentally controlled and that two types of mouse U1 RNA's exist; one is found in all cells, whereas the other is present primarily in embryonic tissues.

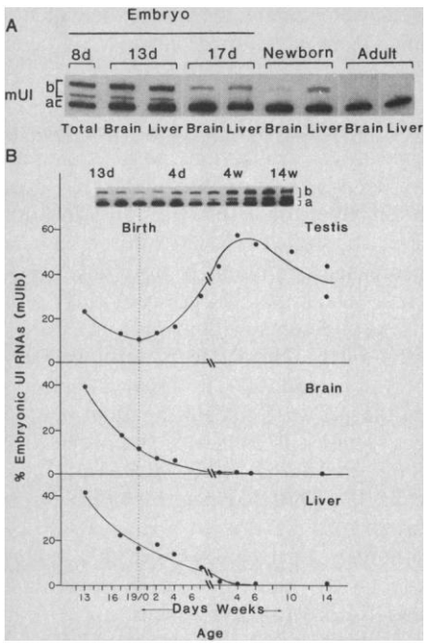
The U1 RNA's in brain, liver, and testis isolated from mouse embryos or adult mice were analyzed by Northern blot hybridization (*8*) (Fig. 1, A and B). At all stages of fetal development, these tissues contained mU1a and mU1b, the two major types of mouse U1 RNA that had previously been identified in mouse tumor cells (*3*) (see also below). In contrast to fetal tissues or the testis, the brain and liver of newborn or adult mice contained primarily mU1a RNA and little or no mU1b RNA. The relative amounts of the U1 RNA's in brain and liver were unaffected by the sex of the mice from which these tissues had been isolated.

The changes in the relative amounts of mU1a and mU1b RNA's, as a function of time after fertilization, were examined by analyses of RNA's isolated from brain, liver, or testis (Fig. 1B). In both liver and brain the level of mU1b RNA's decreased continuously from day 13 after fertilization (day 0 is day of plug) until it was undetectable at about 1 or 2 weeks after birth. The same pattern was observed for kidney. The apparent half-life of the mU1b RNA's was about 4 or 5 days, which is similar to the previously reported half-life of U1 RNA in HeLa cells (*1*). This decrease in mU1b RNA levels may be due to a higher rate of turnover of mU1b RNA's or to a reduction in the number of cells containing mU1b RNA in these tissues.

In testis, a different pattern of differential accumulation was observed (Fig. 1B). Shortly after birth of the mice, the mU1b RNA's began to increase rapidly so that in testes of 4- to 6-week-old mice, mU1b RNA's accounted for about 50 percent of the total U1 RNA. Since the number of germ cells in testes show similar changes during late fetal and postnatal development (*9*), the differential accumulation of embryonic U1 RNA's in the developing testis is consistent with the notion that mU1b RNA's are expressed in the pre-spermatid cells but not in other types of cells in the testis.

We showed that the presence of mU1b RNA in the embryos resulted from the state of differentiation of the tissues rather than from the rapid rate of cell division in embryonic tissues. Three secondary cultures of skin fibroblasts, all growing with a generation time of about 24 hours, were analyzed for their ability to synthesize mU1b RNA. Fibroblasts from day 13 embryos produced both mU1a and mU1b RNA's, whereas fibroblasts from late embryos (days 16 to 18) or neonates synthesized only mU1a RNA.

A minor variant of mU1b RNA (middle band in Fig. 1, A and B) is also evident in fetal tissues from inbred strain ICR/Au mice (*10*). This variant (called mU1b-3 RNA) and additional variants of mU1b and mU1a RNA's found in tissues from other strains of mice (Fig. 2) were tentatively classified as mU1a (adult) or mU1b (embryonic) RNA's according to their patterns of differential accumulation; variants present in both adult and fetal tissues were called mU1a RNA's, whereas those species found only in fetal



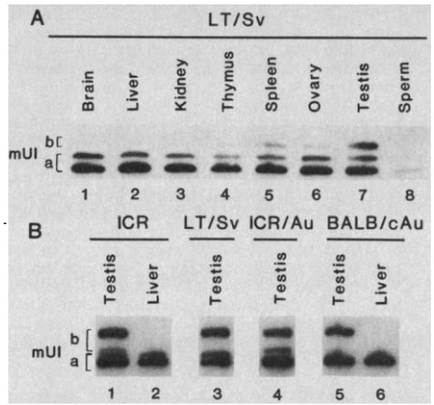


Fig. 1 (left). Differential accumulation of U1 RNA's during normal mouse development. (A) Northern blot analysis (*8*) of accumulated U1 RNA's in total embryos or brain and liver tissues from ICR/Au mice and embryos (*10*). The U1 RNA in the minor band (mU1b-3) was identified by nucleotide sequence analysis of ^{32}P-labeled U1 RNA isolated from tissue culture cells (Fig. 2). (B) Time course of expression of embryonic U1 RNA's during mouse development. Testis, brain, and liver tissues, isolated at the indicated times during fetal and postnatal development of ICR/Au mice, were assayed for the relative levels of mU1a (adult) and mU1b (embryonic) RNA's as above (insert). The percentage of the total U1 RNA (mU1a plus mU1b) corresponding to embryonic (mU1b) RNA's was determined by densitometric scanning of autoradiograms.

Fig. 2 (right). Tissue and strain specificities of the expression of mouse U1 RNA genes. (A) The U1 RNA's of different tissues isolated from one female (lanes 1 through 6) or one male (lanes 7 and 8) LT mouse (*10*) were analyzed as in Fig. 1A. Lanes 1 through 7 contained the U1 RNA's corresponding to about 0.5 to 1.0 × 10^6 cells (*8*), whereas lane 8 contained the RNA's of about 10^6 mature sperm. The different species of mU1a and mU1b RNA's observed in tissues from LT mice were identified by nucleotide sequence analyses of U1 RNA's isolated from LT-C18 cultured cells (Table 1). (B) Liver and testis RNA's, isolated from different strains of mice (*10*), were analyzed for U1 RNA's by Northern blot hybridization, as above.

tissues were termed mU1b RNA's. These assignments were confirmed by nucleotide sequence analyses of uniformly labeled RNA's.

The variant U1 RNA sequences are summarized in Fig. 3. The major mU1a species that was originally characterized, (*3*), is referred to as mU1a-1, and the minor mU1a-like variant is referred to as mU1a-2. Likewise, the major mU1b species (*3*) is called mU1b-1, and the mU1b variants identified by us are called mU1b-3 and mU1b-6 (Fig. 3A). Additional mU1b variants, which were recently identified by Kato and Harada (*11*) in cultured cells from different strains of mice, were called mU1b-2, -4, and -5. The seven nucleotide differences distinguishing all mU1b RNA's from the mU1a RNA's are clustered in the region between positions 60 and 77. As shown in the insert of Fig. 3B, these changes probably allow for the formation of an alternate stem-loop structure. Significantly, digestion with RNase T2 revealed that the ribose-methylation of residue A_{70} in mU1a RNA's was absent from all mU1b RNA's. It is unclear whether this altered methylation pattern results directly from RNA structural changes (for example, the more stable stem or the extra nucleotide in the loop) or indirectly from a change in the way one or more snRNP-associated proteins binds to the RNA. The significance (if any) of the additional scattered nucleotide differences between variants is not clear. Because the minor variants of mU1a and mU1b RNA's are strain-specific (rather than common to all mice), we feel that these heterogeneities of U1 RNA sequences probably do not have a functional role; more likely, they reflect polymorphisms of the U1 RNA genes. As such, the minor variants may be useful for chromosomal mapping of mouse U1 RNA genes or as a measure of the relatedness of strains of mice.

We screened a variety of other adult tissues to determine if any of them accumulated mU1b RNA's (Fig. 2A). No mU1b RNA's were present in adult kidney, but small amounts were consistently found in several other tissues that contain a significant proportion of germline or immature stem cells capable of further differentiation, such as thymus, spleen, and ovary. Adult testes had a

Table 1. Levels of somatic (mU1a) and embryonic (mU1b) U1 RNA's in cultured mouse cells. For the sources of various cell lines, see (*21*). Abbreviations: EC, embryonal carcinoma; non-EC, differentiated cells (including a variety of embryo-derived tissue types); PE, primitive endoderm; VYS, visceral yolk sac; and PYS, parietal yolk sac.

Cell line	Cell type	Mouse strain	mU1a		mU1b	
			Percent	Type*	Percent	Type*
		Teratocarcinoma-derived cells				
LT-Cl8	EC	LT	67	1, 2	33	1, 6
LT0-1	EC	LT	56	1, 2	44	1, 6
LT0-1 DIFF	Non-EC	LT	93	1, 2	7	1†
F9	EC	129	46	1, 2	54	1, 3, 6
F9 + RA	PYS	129	45	1, 2	55	1, 3†
129 DIFF	Non-EC	129	87	1, 2	13	1, 3†
1H5	PE	LT	70	1, 2	30	1†
1H5 cysts	VYS	LT	70	1, 2	30	1†
		Transformed cells				
D7	Fibrosarcoma	C3H	64	1	36	1†
C1300	Neuroblastoma	A	64	1	36	1, 3†
B16	Melanoma	C57BL/6	82	1, 2	18	1†
RAG	Renal adenocarcinoma	BALB/c	88	1	12	1†
1848	Lymphoma	BALB/c	88	1	12	1
E102	Lymphoma	C57BL/6	80	1, 2	20	1
		Untransformed fibroblasts				
NIH 3T3		Swiss	>98	1, 2	<2	
BALB/c 3T3		BALB/c	>98	1	<2	
C3H/10T1/2-Cl8		C3H/He	>95	1	<5	
C127		RIII	>99	1	<1	

*The types of U1 RNA variant (see Fig. 3). †The Northern blot hybridization used for these analyses does not distinguish between mU1b-1 and mU1b-6 RNA's.

high level of mU1b RNA (lane 7 in Fig. 1B); in contrast, mature sperm were devoid of U1 RNAs (lane 8) as well as other snRNA's (data not shown). Testes contain high levels of germ cells (*9*); ovaries contain a lower fraction of germ cells and they have a lower level of mU1b RNA's. Thus, these observations indicate that mU1b RNA's may be expressed in adult tissues only in certain cells of the hematopoietic and germlines.

The generality of the differential expression of mU1b RNA's during development was established by analyses of the U1 RNA's in other strains of mice (Fig. 2B). In all cases, high levels of embryonic (mU1b) RNA's were observed only in fetal tissues and in adult testes; small amounts were found in spleen, thymus, and ovary.

Certain strains of mice, including LT and 129, develop spontaneous teratocarcinomas or teratomas at high frequencies (*12, 13*). Established embryonal carcinoma (EC) cell lines derived from the pluripotent stem cells of such tumors provide a model system for early mouse embryogenesis (*14*). The relative amounts of mU1a and mU1b RNA's in cells derived from these lines are summarized in Table 1. As expected, cultured EC cells express very high levels of embryonic mU1b RNA's (for example, lines LT-Cl8, LTO-1, and F9 in Table 1). In contrast, non-EC cells isolated from a highly differentiated primary LT teratocarcinoma or 129 teratoma tumor contain only small amounts of mU1b RNA's (LTO-1-DIFF and 129-DIFF in Table 1) (*15*).

In vitro differentiation of some EC cells produces a limited number of cell types, which may correspond to only early stages of embryonic development. Treatment of cultured F9 EC cells with retinoic acid plus cyclic adenosine monophosphate (cyclic AMP) (resulting in the formation of parietal yolk sac) (*16*) did not lead to changes in the pattern of U1 RNA accumulation (F9 versus F9+RA in Table 1). In addition, visceral yolk-sac cysts of 1H5 cells that develop in vitro (*17*) accumulated the same high level of mU1b RNA's as the progenitor monolayer of 1H5 primitive endoderm cells (Table 1). Thus it appears that the mU1b RNA genes remain fully expressed during the early stages of embryonic differentiation represented by these teratocarcinoma-derived cells. These results are consistent with our observation that mU1b RNA's are abundant in 7- to 8-day embryos (Fig. 1A), which are still in early stages of development.

All of the established lines of neoplastically transformed cells assayed in this study accumulate varying but significant amounts of mU1b RNA's (Table 1). In contrast, contact-inhibited, untransformed NIH-3T3, BALB/c-3T3, C3H/10T1/2, and C127 fibroblasts express mU1a RNA's almost exclusively. Since C127 mouse fibroblasts transformed by bovine papilloma virus do not accumulate detectable levels of mU1b RNA's (*18*), morphological transformation in itself does not result in the expression of genes for mU1b RNA's.

These data extend our earlier observation (*7*) that expression of some U1 RNA genes is developmentally controlled. The fact that this control occurs in both frogs and mice suggests that it may be functionally significant, perhaps by modifying the pattern of pre-mRNA splicing. Another possibility is that embryonic U1 RNA's represent fetal or developmental markers with no inherent functional difference from the adult U1 RNA's. For this reason, it will be important to determine whether developmentally controlled U1 RNA's participate with comparable efficiency and specificity in mRNA processing reactions.

A common feature of the embryonic U1 RNA's of mice (this study) and frogs (*7*) is the absence of methylation of nucleotide A_{70}. It is possible that in some tissues or in other species, the control of U1 RNA function could be modulated by post-transcriptional nucleotide modification of a single product rather than by transcription of groups of genes. RNA base modification could influence (or be influenced by) the way these U1 RNA's interact with specific proteins to form functional U1 snRNP particles (*3*). In *X. laevis*, the accumulation of a group of Sm-antigenic snRNP-associated proteins is modulated during development (*19*) in a pattern that is coincident with the

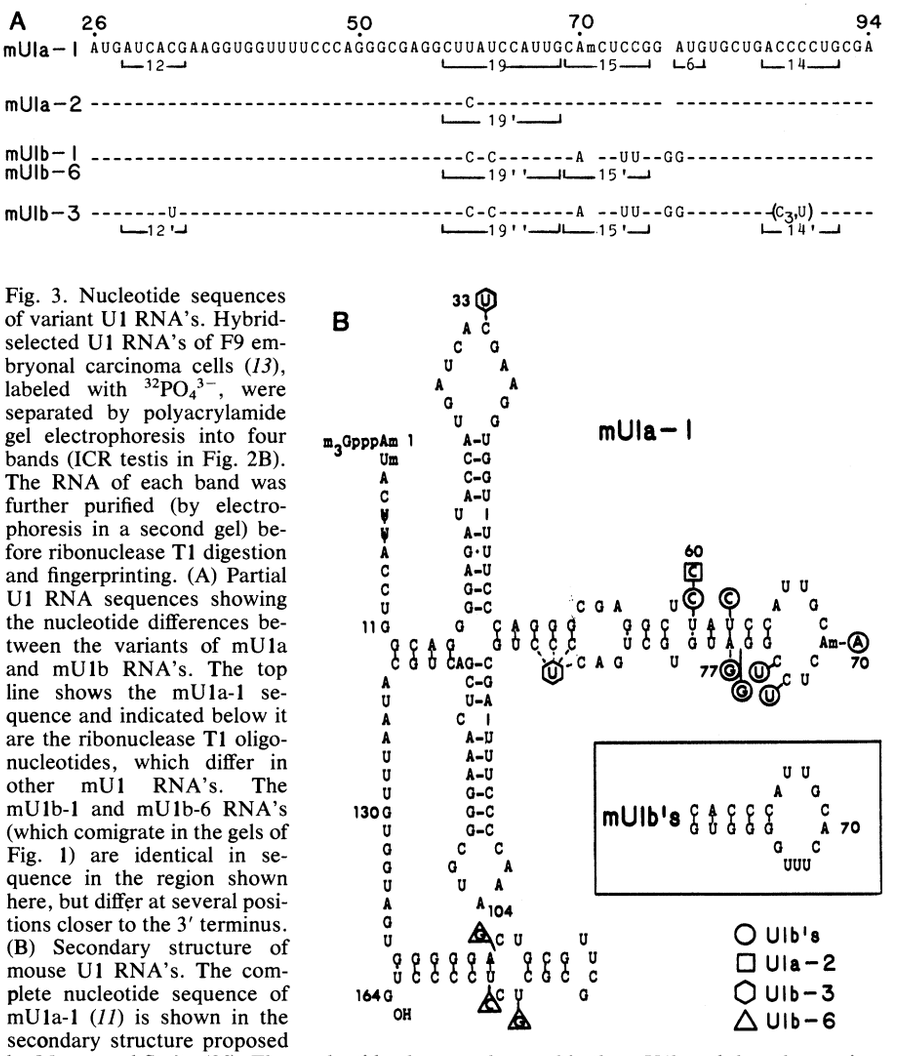


Fig. 3. Nucleotide sequences of variant U1 RNA's. Hybrid-selected U1 RNA's of F9 embryonal carcinoma cells (*13*), labeled with $^{32}PO_4^{3-}$, were separated by polyacrylamide gel electrophoresis into four bands (ICR testis in Fig. 2B). The RNA of each band was further purified (by electrophoresis in a second gel) before ribonuclease T1 digestion and fingerprinting. (A) Partial U1 RNA sequences showing the nucleotide differences between the variants of mU1a and mU1b RNA's. The top line shows the mU1a-1 sequence and indicated below it are the ribonuclease T1 oligonucleotides, which differ in other mU1 RNA's. The mU1b-1 and mU1b-6 RNA's (which comigrate in the gels of Fig. 1) are identical in sequence in the region shown here, but differ at several positions closer to the 3′ terminus. (B) Secondary structure of mouse U1 RNA's. The complete nucleotide sequence of mU1a-1 (*11*) is shown in the secondary structure proposed by Mount and Steitz (*20*). The nucleotide changes observed in the mU1b and the other variant mU1 RNA's are indicated. The insert shows the alternative stem-loop structure of the sequences around residue A_{70} in the mU1b RNA's. The nucleotide sequence of the 3′ terminus of mU1b-6 RNA was determined by Kato and Harada (*11*).

differential synthesis of embryonic U1 RNA's (*7*). It remains to be established how—or whether—these changes in composition of U1 snRNP's might alter the specificity of splice site recognition in different tissues or during development (or both).

References and Notes

1. See review by H. Busch, *Annu. Rev. Biochem.* **51**, 617 (1982).
2. V. W. Yang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1371 (1981); R. A. Padgett *et al.*, *Cell* **35**, 101 (1983); A. Krämer *et al.*, *ibid.* **38**, 299 (1984); C. L. Moore, and P. A. Sharp, *ibid.* **36**, 581 (1984).
3. M. R. Lerner and J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5495 (1979); M. Hinterberger, I. Petterson, J. A. Steitz, *J. Biol. Chem.* **258**, 2604 (1983); C. S. Kinlaw *et al.*, *ibid.*, p. 7181.
4. M. R. Lerner *et al.*, *Nature* (*London*) **283**, 220 (1980); J. Rogers and R. Wall, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1877 (1980); S. M. Mount *et al.*, *Cell* **33**, 509 (1983).
5. See minireviews by P. A. Sharp [*Cell* **23**, 643 (1981)] and W. Keller [*ibid.* **39**, 423 (1984)] and references therein.
6. "Housekeeping" genes encode common functions and are active in all cells in contrast to "tissue-specific" genes that are expressed only in some cells or at certain stages of development [B. Lewin, *Gene Expression*, vol. 2, *Eucaryotic Chromosomes* (Wiley, New York, ed. 2, 1980), p. 259]. In vertebrates the highly conserved U1 RNA genes constitute large multigene families with between 30 and 100 (human and mouse) and 1000 (frog) copies per haploid genome (E. Lund and J. E. Dahlberg, *J. Biol. Chem.* **259**, 2013 (1984); T. Manser and R. F. Gesteland, *Cell* **29**, 257 (1982); W. F. Marzluff *et al.*, *Nucleic Acids Res.* **11**, 6255 (1983); E. Lund, J. E. Dahlberg, D. J. Forbes, *Mol. Cell. Biol.* **4**, 2580 (1984); R. Zeller *et al.*, *EMBO J.* **3**, 1075 (1984). The copy numbers of genes for snRNP proteins are not known.
7. D. J. Forbes *et al.*, *Cell* **38**, 681 (1984); E. Lund and J. E. Dahlberg, in preparation.
8. For Northern blot analyses, total cellular RNA's were prepared from fresh or quick-frozen tissues by homogenization at 0°C in 10 to 20 volumes of urea lysis buffer [7*M* urea, 0.3*M* NaCl, 2 percent sodium dodecyl sulfate (SDS), 2 m*M* EDTA, and 10 m*M* tris-HCl, *p*H 8.0] [J. Ross, *J. Mol. Biol.* **106**, 403 (1976)], followed by one or two extractions with an equal volume of a mixture of phenol, chloroform, and isoamylalcohol (25:24:1). Nucleic acids were precipitated with two volumes of ethanol and redissolved in 20 m*M* tris-HCl, *p*H 7.6, and 1 m*M* EDTA so that 10 μl contained the RNA of 10^5 to 10^6 cells (total RNA from tissue culture cells were used as standards). After fractionation of total RNA's in 12 percent (30:0.8) polyacrylamide gels containing 7*M* urea and 90 m*M* tris-borate, *p*H 8.3, plus 2.3 m*M* EDTA (run at room temperature for 18 to 22 hours at 10 V/cm), the U1 RNA's were transferred electrophoretically to GeneScreen Plus membrane (New England Nuclear). ^{32}P-labeled U1 RNA specific probes were prepared by transcription of pSP64/-U1 (E. T. Schenborn and R. C. Mierendorf, *Nucleic Acids Res.*, in press) with SP6 RNA polymerase [D. A. Melton *et al.*, *ibid.* **12**, 7035 (1984)] by use of the Riboprobe system (Promega Biotec). After hybridization in 0.9M NaCl, 1 percent SDS, 10 mM tris-HCl, *p*H 7.6, and 1 m*M* EDTA for 18 to 24 hours at 68°C, the blots were washed twice for 20 minutes in 3× standard saline citrate (SSC) and 1 percent SDS at 60°C and then rinsed briefly at room temperature in 0.1× SSC. Autoradiograms were obtained by exposure of XR5 Kodak x-ray film (without intensifying screen) for 1 to 10 hours.
9. Mitotic division of germline cells in the developing testis stops around day 12 or 13 of gestation and is not resumed until shortly after birth. During that period the actual number of germ cells in the testis decreases by about 50 percent. Spermatogenesis is initiated a few days after birth coincident with the beginning of mitotic division of germ cells [A. R. Bellvé *et al.*, *J. Cell Biol.* **74**, 69 (1977); H. Peters, *Philos. Trans. R. Soc. London Ser. B*, **259**, 91 (1970)].
10. Inbred ICR/Au mice [R. Auerbach, J. B. Hay, L. Kubai, *J. Physiol.* (*London*) **307**, 465 (1980)] and BALB/c Au mice were obtained from R. Auberbach (Department of Zoology, University of Wisconsin, Madison). LT mice were from a closed breeding colony of LT-Sv mice (*12*) maintained at the same place. Noninbred ICR mice were purchased from Harlan Sprague-Dawley.
11. N. Kato and F. Harada, *J. Biol. Chem.* **260**, 7775 (1985).
12. L. C. Stevens and D. S. Varnum, *Dev. Biol.* **37**, 369 (1974).
13. L. C. Stevens, *Cold Spring Harbor Conf. Cell Proliferation* **10**, 23 (1983).
14. B. Kahan and B. Ephrussi, *J. Natl. Cancer Inst.* **44**, 1015 (1970).
15. Similarly, the level of embryonic mU1b RNA's was very low in highly differentiated tumors obtained by injection of LTO-2 EC cells into syngeneic LT mice (B. Kahan, unpublished). LTO-2 EC cells cultured in vitro contain a high level of mU1b RNA, comparable to that of LTO-1 cells.
16. S. Strickland and V. Mahdavi, *Cell* **15**, 393 (1978). F9 cells were plated at 2×10^5 cells on gelatinized (0.1 percent) 60-mm culture plates in Dulbecco's minimum essential medium (DMEM) containing 15 percent fetal bovine serum (FBS). After 6 hours, the medium was placed with DMEM containing either 2 or 15 percent FBS, $10^{-7}M$ retinoic acid (RA) (all *trans*), $10^{-4}M$ dibutyryl cyclic AMP, and $10^{-4}M$ isobutylmethylxanthine [M. Kurkinen *et al.*, *Cold Spring Harbor Conf. Cell Proliferation* **10**, 389 (1983)]. At 7 to 14 days, cells were analyzed for U1 RNA's by Northern blot analysis (*8*).
17. E. Adamson *et al.*, *Differentiation* **29**, 68 (1985).
18. E. Lund *et al.*, *Mol. Cell. Biol.* **3**, 221 (1983).
19. A. Fritz *et al.*, *J. Mol. Biol.* **178**, 273 (1984).
20. S. M. Mount and J. A. Steitz, *Nucleic Acids Res.* **9**, 6351 (1981).
21. The sources of the cell lines used are as follows: LT EC lines: B. Kahan, *Somat. Cell Genet.* **5**, 763 (1979); 1H5: B. Kahan and E. Adamson, *Cold Spring Harbor Conf. Cell Proliferation* **10**, 131 (1983); F9: E. G. Bernstine *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3899 (1973); D7: B. Kahan and R. DeMars, *ibid.* **72**, 1510 (1975); B16 and C1300 tumors were obtained from the Jackson Laboratory, Bar Harbor, Maine; the RAG cell line was acquired from the American Type Culture Collection, CCL 142; C3H/10T1/2 C18: C. Reznikoff *et al.*, *Cancer Res.* **33**, 3231 (1973) (these cells were obtained from C. Reznikoff, University of Wisconsin in Madison); 1848 and E102 cells (Abelson virus–transformed pre-B cells) and NIH- and BALB/c-3T3 cells were provided by R. Risser, University of Wisconsin, Madison; C127: D. R. Lowy, E. Rands, E. M. Scolnick, *J. Virol.* **26**, 291 (1978).

22. Supported by NIH grant GM 30220 to J.E.D. and E.L. and NIH grant CA 33453 to B.K.. We thank K. Mosher for providing 1H5 monolayers and cysts and J. L. Mitchen for technical assistance with other cultured cells.

5 March 1985; accepted 2 July 1985

Molecular Cloning of Complementary DNA for the Alpha Subunit of the G Protein That Stimulates Adenylate Cyclase

Abstract. *A complementary DNA clone encoding the α subunit of the adenylate cyclase stimulatory G protein (G_s) was isolated and identified. A bovine brain complementary DNA library was screened with an oligonucleotide probe derived from amino acid sequence common to known G proteins. The only clone that was obtained with this probe has a complementary DNA insert of approximately 1670 base pairs. An antibody to a peptide synthesized according to deduced amino acid sequence reacts specifically with the α subunit of G_s. In addition, RNA that hybridizes with probes made from the clone is detected in wild-type S49 cells; however, cyc$^-$ S49 cells, which are deficient in $G_{s\alpha}$ activity, are devoid of this messenger RNA.*

Bruce A. Harris
Janet D. Robishaw
Susanne M. Mumby
Alfred G. Gilman
Department of Pharmacology, Southwestern Graduate School, University of Texas Health Science Center, Dallas 75235

The activity of hormone-sensitive adenylate cyclase is regulated by at least two guanine nucleotide–binding proteins (G proteins) (*1*). The stimulatory G protein, G_s, which has been purified to homogeneity (*2*), is a heterotrimer (*2, 3*). The guanine nucleotide–binding α subunit exists as two predominant species (M_r, 52,000 and 45,000), which are thought to be products of a single gene. The α subunit of G_s is the activator of the catalytic moiety of the adenylate cyclase complex. The molecular sizes of the β and γ subunits are approximately 35,000 and 8,000 daltons, respectively. A homologous oligomer, G_i, mediates hormonal inhibition of adenylate cyclase (*4*); it has a distinct α subunit (41,000 daltons), while its β and γ subunits are functionally indistinguishable from those of G_s. Dissociation of the shared βγ subunit complex of G_i and its subsequent interaction with $G_{s\alpha}$ are thought to be responsible for inhibition of adenylate cyclase (*4, 5*). A third G protein, G_o, is abundant in brain, and its α subunit is 39,000 daltons. The α subunits of G_i and G_o may interact with other specific effectors that are distinct from adenylate cyclase (*1*). G_o may also function as an inhibitor of adenylate cyclase, since it too shares an indistinguishable βγ subunit complex (*6*). The G protein family also includes transducin, which mediates the activation by rhodopsin of a cyclic GMP–specific (guanosine monophosphate) phosphodiesterase in the retinal rod outer segment (*7*). The sequences of complementary DNA's (cDNA's) for the α and γ subunits of transducin have been reported (*8, 9*). Structural and functional relations between G proteins and the products of the *ras* genes in yeast and in higher organisms have also been noted (*10–12*).

Comparison of partial amino acid sequences of polypeptides near the NH_2-termini of the α subunits of bovine transducin and G_o (*12*) reveals a region con-