Direct evidence that the c-*myb* gene product is required for the formation of normal human hematopoietic colonies of the myeloid, erythroid, and megakaryocytic lineages in vitro is provided by these studies. They suggest an important in vivo role for this nuclear protein as well. The function served by the c-*myb* proto-oncogene remains unclear. Based on the model that hematopoiesis is a developmental continuum (*18*), we hypothesize that the c-*myb* gene product is involved in regulating the very early stages of hematopoiesis, and is probably most relevant to initial progenitor cell proliferation events. We cannot exclude, however, more subtle effects on cells at either earlier (pluripotent hematopoietic stem cell) or later (precursor cell) developmental stages. These studies also imply that it is now possible to employ a powerful yet technically simple in vitro model for studying the effect of deleting any protein encoded by a proto-oncogene (or other specific gene) on the developmental biology of human hematopoietic cells. Finally, since blocking expression of relatively late differentiation markers has no discernible effect on either colony formation or the remainder of the cell's differentiation program, it is also now possible to develop in vitro models for the study of specific enzyme or protein deficiency disorders of hematopoietic cells.

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Transient Expression Shows Ligand Gating and Allosteric Potentiation of $GABA_A$ Receptor Subunits

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Human γ-aminobutyric acid A ($GABA_A$) receptor subunits were expressed transiently in cultured mammalian cells. This expression system allows the simultaneous characterization of ligand-gated ion channels by electrophysiology and by pharmacology. Thus, coexpression of the α and β subunits of the $GABA_A$ receptor generated GABA-gated chloride channels and binding sites for $GABA_A$ receptor ligands. Channels consisting of only α or β subunits could also be detected. These homomeric channels formed with reduced efficiencies compared to the heteromeric receptors. Both of these homomeric GABA-responsive channels were potentiated by barbiturate, indicating that sites for both ligand-gating and allosteric potentiation are present on receptors assembled from either subunit.

THE $GABA_A$ RECEPTOR CONTAINS an intrinsic chloride ion channel that is opened (gated) by GABA, the major inhibitory neurotransmitter in mammalian brain. $GABA_A$ receptor activation stabilizes the neuron's resting potential (*1, 2*). Channel activity can be allosterically modulated by therapeutically useful drugs, for example, barbiturates and benzodiazepines (*1–3*). The affinity-purified receptor contains an α subunit of 53 kD, on which the benzodiazepine binding site is thought to be located, and a β subunit of 57 kD, which can be photoaffinity labeled by GABA agonists (*4–6*).

Human α_1 and β_1 subunit–encoding cDNAs were isolated from a fetal brain library (*7*). The DNA and predicted polypeptide sequences of these cDNAs reveal a high degree of conservation with the corresponding bovine sequences (*1*). The cloned α_1 and β_1 subunit cDNAs were inserted, singly or together (in tandem array), into a eukaryotic expression vector, pCIS2, that contained the human cytomegalovirus promotor-enhancer (*8*). Human embryonic kidney cells (*8*) were transfected with these constructs at high efficiencies, by using a modified $CaPO_4$ precipitation technique (*9, 10*). Cells were harvested 48 hours after transfection for RNA isolation, for pharmacological studies, or were used for electrophysiology. Analysis of total RNA from transfected cells showed high levels of $GABA_A$ receptor subunit mRNA but no such mRNA was seen in untransfected or mock-transfected cells (Fig. 1).

To characterize the transiently expressed heteromeric $\alpha_1 + \beta_1$ receptors, membranes

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prepared from transfected cells (*11*) were analyzed by binding of [^{3}H]muscimol, a GABA analog, and [^{35}S]*t*-butyl-bicyclophosphorothionate (TBPS), a $GABA_A$ receptor channel–specific cage convulsant (*12*). Muscimol bound with a dissociation constant (K_d) of 13 ± 3 n*M* (n = 3) (Fig. 2), demonstrating the presence of a high-affinity binding site for the neurotransmitter on the recombinant $\alpha_1 + \beta_1$ receptors. This value is close to that determined for purified bovine $GABA_A$ receptor (K_d = 12 ± 3 n*M*) (*4*). Muscimol had an average maximum binding (B_{max}) of 23 ± 3 fmol per 10^6 cells (n = 3), corresponding to approximately 6000 receptors per expressing cell. Similarly, TBPS displayed specific binding with B_{max} values of 18 ± 3 fmol per 10^6 cells, based on a K_d of 25 n*M* (*12*). Less than 2 fmol per 10^6 cells of ligand ([^{3}H]muscimol or [^{35}S]TBPS) bound to cells transfected with single subunit cDNAs. These values are not significantly different from those obtained for nonspecific binding (Fig. 2). This may result from reduced numbers of assembled receptors or from the presence of a site of lower affinity than in $\alpha_1 + \beta_1$–transfected cells. No benzodiazepine binding ([^{3}H]flunitrazepam or ^{3}H-labeled Ro15-1788) was observed. This correlates with a lack of potentiation of GABA-induced $\alpha_1 + \beta_1$ channel activity by benzodiazepines (*13*).

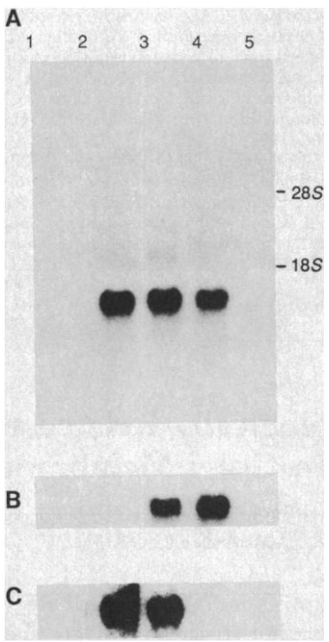

Fig. 1. Blot analysis of RNA from transfected and untransfected cells. Total RNA and polyadenylated [poly(A)$^+$] RNA were prepared from 293 cells transfected with pCIS2 plasmid DNA containing $\alpha_1 + \beta_1$, α_1, or β_1 cDNAs, respectively. No contaminating β_1 or α_1 cDNA sequences (<0.01%) were found in the respective α_1 or β_1 vector preparations. RNA was analyzed by electrophoresis in a 1% agarose/7% formaldehyde denaturing gel and transferred to nitrocellulose. The blot was probed (**A**) with both α_1 and β_1, (**B**) with β_1, and (**C**) with α_1 subunit–specific oligonucleotides (*7*). Total RNA (5 μg) was loaded in lanes 1 to 4 and 2 μg of poly(A)$^+$ RNA was loaded in lane 5 to further demonstrate the absence of endogenous $GABA_A$ receptor subunits in the recipient cell line. Lanes 1 and 5, untransfected cells; lane 2, α_1-transfected; lane 3, $\alpha_1 + \beta_1$–transfected; and lane 4, β_1-transfected.

The GABA agonist muscimol ($10^{-4}M$) (n = 7) mimicked the effect of GABA by inducing an inward current at the resting potential of −60 mV. Picrotoxin ($10^{-4}M$) (n = 7), a specific channel blocker, and bicuculline, a competitive GABA antagonist ($10^{-4}M$) (n = 12), blocked the GABA-induced inward current (Fig. 3A). The barbiturate pentobarbital (50 μ*M*) increased the GABA-induced currents (Fig. 3A). The effect of the barbiturate was most prominent at low (<$10^{-6}M$) GABA concentrations. Pentobarbital alone did not trigger channel activity, as described in cultured spinal neurons (*16*). GABA-induced inward currents were observed at concentrations as low as $10^{-7}M$ GABA in the presence of pentobarbital, whereas GABA alone was in most cases not effective at such low concentrations. These results, in conjunction with those obtained from ligand-binding studies, demonstrate that transient mammalian cellular expression allows for the pharmacological and electrophysiological analysis of ligand-gated ion channel receptors.

GABA-induced current flow was also observed in cells expressing α_1 or β_1 subunits alone (Fig. 3, B and C). Such GABA-responsive channels were not seen on transfection with vector alone, but were specifically generated by the expression of either of the single subunit cDNAs (*17*). The whole-cell currents of the homomeric channels were approximately 10% of those seen with heteromeric receptors; the average saturation response was 40 pA and the maximal response was 100 pA at $10^{-4}M$ GABA (Fig. 3, B and C). Currents were detected with GABA doses as low as $10^{-5}M$, and the reversal potentials for the homomeric channels indicate Cl^- ion flow. The low whole-cell current obtained by expression of homomeric channels may reflect reduced efficiencies of receptor assembly, because cells transfected with cDNAs encoding a single subunit synthesize the respective mRNA in amounts as high as do doubly transfected cells (Fig. 1, B and C). A change in single-channel characteristics, shorter lifetimes for example, may also contribute to the observed reduction in whole-cell current. Homomeric channel formation has not been previously detected in voltage-clamped oocytes (*1, 13*).

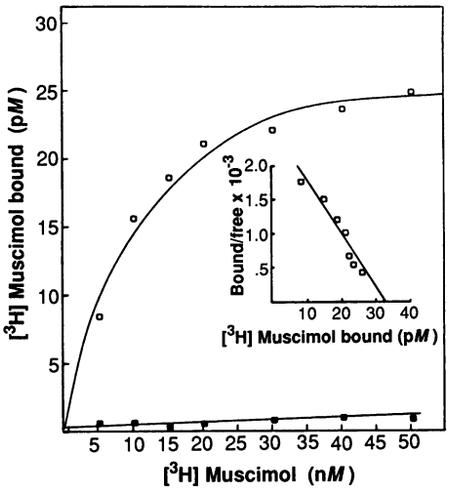

Fig. 2. Binding isotherm and Scatchard plot of [^{3}H]muscimol binding to $\alpha_1 + \beta_1$–transfected cell membranes. Specific binding (□) (*11*) was determined by subtracting the binding in the presence of 1 μ*M* GABA. Untransfected cells showed no specific binding (■). The results shown (K_d = 13 n*M*, B_{max} = 25 n*M*) were from a single transfection experiment and were determined by least-squares analysis of the data. As observed from three different transfections, K_d values were 13 ± 3 n*M* and B_{max} values were 23 ± 3 fmol.

Our finding that $GABA_A$ receptors, assembled from either of the known subunits, can form ligand-gated Cl^- channels suggests that homomeric assembly into functional channels may be a general property of many of the subunits of ligand-gated ion channels (*17*) since it is also true for one subunit of the neuronal nicotinic acetylcholine receptor (*18*). Our results indicate that a site conserved in both the α and β subunits, possibly of lower apparent affinity than the unique photoaffinity-labeled agonist binding site on the β subunit (*6*), may be responsible for agonist gating of the channel. We propose that this site is located extracellulary in a region encompassing the 85–amino acid residues from the invariant disulfide-bonded β-structural loop to the first transmembrane segment (*1*). In addition, the parallel pharmacology of the α_1 and β_1 subunit homomeric channels and the heteromeric $GABA_A$

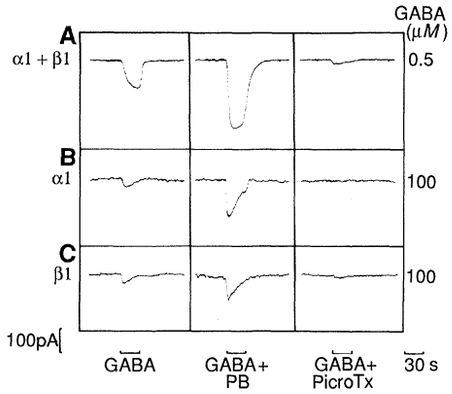

Fig. 3. Pharmacology of the GABA-induced current in cells transfected with either $\alpha_1 + \beta_1$ subunits or the α_1 subunit or the β_1 subunit alone. (**A**) Application of GABA ($5 \times 10^{-7} M$) to a cell transfected with the $\alpha_1 + \beta_1$ subunits and voltage-clamped at −60 mV gave rise to an inward current (left). This current was amplified and increased in duration in the presence of pentobarbital (PB) (50 μM) (middle). After a 5-min washout the current returned to the control level. Picrotoxin (PicroTx) at a concentration of $5 \times 10^{-4} M$ (right) almost completely blocked the GABA-induced current. The same experimental protocol was applied to a cell transfected with the α_1 subunit alone (**B**) or with the β_1 subunit alone (**C**). Note that the concentration used to elicit a detectable inward current in the cells expressing only α_1 or β_1 subunits was $10^{-4} M$ GABA (left traces). Pentobarbital (50 μM) amplified (center traces) and picrotoxin ($5 \times 10^{-4} M$) blocked the inward current (right traces) in cells expressing either the α_1 or the β_1 subunit.

receptor indicates that allosteric sites for barbiturate potentiation are also present on receptors formed from either subunit.

Our results demonstrate the usefulness of expressing neurotransmitter-gated ion channels transiently in mammalian cells. Transient expression, rather than stably expressing cell lines (*19*), should provide for the rapid and simultaneous electrophysiological and pharmacological characterization of many ligand- and voltage-gated ion channels.

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11. Forty-eight hours after transfection, cells from ten plates (10 cm) were washed twice with phosphate-buffered saline (PBS) and scraped into PBS (10 ml). After low-speed centrifugation (500*g*) the cell pellet was homogenized in a Polytron tissue homogenizer (Brinkmann) in 10 ml of 50 m*M* potassium phosphate, *p*H 7.4, and centrifuged at 50,000*g* for 20 min. Membrane pellets were frozen at −20°C overnight and the wash procedure was repeated three times. The final pellet was resuspended in potassium phosphate buffer, *p*H 7.4, containing 100 m*M* KCl. Homogenate equivalent to 10^6 cells (100 μg of protein) was incubated in a 1-ml reaction volume with [^{3}H]muscimol (Du Pont, 23 Ci/mmol) for 60 min at 4°C or [^{35}S]TBPS (Du Pont, 70 Ci/mmol) for 90 min at 27°C. Samples were filtered on GF/B filters with a Bio-Rad vacuum filter apparatus and washed twice with 5 ml of potassium phosphate–KCl buffer. After drying, filter-retained radioactivity was determined by liquid scintillation.
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15. Electrophysiological responses showed a wide variation around the mean. The 293 cells grow in groups and, by Lucifer yellow injection, are electrically coupled. Thus, it is likely that GABA-induced membrane currents generated in transfected cells can also be recorded from an electrically coupled untransfected cell. This would increase the number of apparently transfected cells and may, in part, explain the wide variation of response amplitudes.
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17. These channels were also seen in HeLa cells expressing single $GABA_A$ receptor subunits, suggesting that no cellular component generates these channels or leads to the formation of heteromeric $GABA_A$ receptors. Similar homomeric channels have recently been observed in oocytes with the use of high-sensitivity electrophysiological techniques [L. A. C. Blair, E. S. Levitan, J. Marshall, V. E. Dionne, E. A. Barnard, *Science* **242**, 577 (1988)]. In addition, glycine-gated, GABA-insensitive homomeric receptor channels were formed when either cells (D. B. Pritchett *et al.*, in preparation) or oocytes (H. Betz, personal communication) expressing the 48-kD rat glycine-receptor subunit were analyzed.
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A 115-kD Polypeptide Immunologically Related to Erythrocyte Band 3 Is Present in Golgi Membranes

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Band 3 multigene family consists of several distinct but structurally related polypeptides which are probably involved in the transport of anions across the plasma membrane of both erythrocytes and nonerythroid cells. A novel member of this family of polypeptides that resides in the Golgi complex was identified with antibodies to Band 3. The Golgi antigen had a larger molecular size and was antigenically distinct from Band 3 in the amino-terminal domain. It was expressed most prominently in cells that secrete large amounts of sulfated proteins and proteoglycans. This polypeptide may participate in sulfate transport across Golgi membranes.

ANION TRANSPORT IS A PROPERTY of many cell types and membranes. In red blood cells, the anion exchanger (Band 3) primarily increases the capacity of the blood to transport CO_2 from the respiring tissues to the lungs by exchanging bicarbonate for chloride (*1*). In epithelial cells such as the kidney tubule intercalated

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