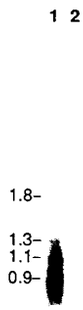

Fig. 3. Southern analysis showing the presence of human repetitive DNA. DNA samples (2.5 μg) extracted from tail tissues were separated by agarose gel electrophoresis, then blotted onto nitrocellulose, and hybridized with nick-translated pBLUR8, a plasmid containing the human interspersed Alu repeats (*15*). Mbo I–digested DNA from E mouse exhibited four distinct bands, thus indicating the retention of some human DNA. Lane 1, E mouse DNA digested with Mbo I; lane 2; undigested E mouse DNA; lane 3, normal mouse DNA digested with Mbo I. Molecular weights indicated are in kilobases.

compared to that found in embryos analyzed at the preimplantation stages. Because the embryo is derived from just a few stem cells (*8*), these results further suggest that cells containing the human centromeric fragment may have been at a selective disadvantage. This might be due to the centromeric origin of the injected fragments. Thus, if the injected fragment retained some centromere activity, mitosis could have been perturbed. Studies in tissue culture cells have shown that mouse chromosomes with multiple centromeres undergo a gradual process of centromere inactivation over many cell divisions (*9*). Perhaps the mosaicism we observed reflects the end point of this inactivation process.

Additional experiments were carried out to examine if noncentromeric fragments might be incorporated into the mouse genome. For these experiments, we dissected and injected random noncentromeric human chromosome fragments into mouse eggs and allowed such eggs to develop to term. Of six eggs injected, three were transferred into surrogate females, from which one mouse was born (referred to as E mouse; Fig. 3). Analysis of DNA from the tail tissue by Southern blot hybridization (Fig. 3) revealed the presence of human Alu repeats (*10*), indicating that at least a portion of the injected chromosome fragment was retained.

Overall, these results suggest that human chromosome fragments introduced into mouse eggs can be propagated through multiple rounds of cell division. It will be necessary to determine whether such fragments can become stably integrated into the germ line so that stable strains of "transomic" animals can be obtained. It may also be feasible to use G-banded metaphase spreads as the source of donor chromosome fragments. With the latter approach, it should be possible to select specific chromosome fragments to be dissected and injected, therefore making it possible to generate animal models for diseases and heritable traits that are cytogenetically localized but have yet to be characterized at the molecular or biochemical level.

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Hyperpolarizing Vasodilators Activate ATP-Sensitive K^+ Channels in Arterial Smooth Muscle

NICHOLAS B. STANDEN, JOHN M. QUAYLE, NOEL W. DAVIES, JOSEPH E. BRAYDEN, YU HUANG, MARK T. NELSON*

Vasodilators are used clinically for the treatment of hypertension and heart failure. The effects of some vasodilators seem to be mediated by membrane hyperpolarization. The molecular basis of this hyperpolarization has been investigated by examining the properties of single K^+ channels in arterial smooth muscle cells. The presence of adenosine triphosphate (ATP)–sensitive K^+ channels in these cells was demonstrated at the single channel level. These channels were opened by the hyperpolarizing vasodilator cromakalim and inhibited by the ATP-sensitive K^+ channel blocker glibenclamide. Furthermore, in arterial rings the vasorelaxing actions of the drugs diazoxide, cromakalim, and pinacidil and the hyperpolarizing actions of vasoactive intestinal polypeptide and acetylcholine were blocked by inhibitors of the ATP-sensitive K^+ channel, suggesting that all these agents may act through a common pathway in smooth muscle by opening ATP-sensitive K^+ channels.

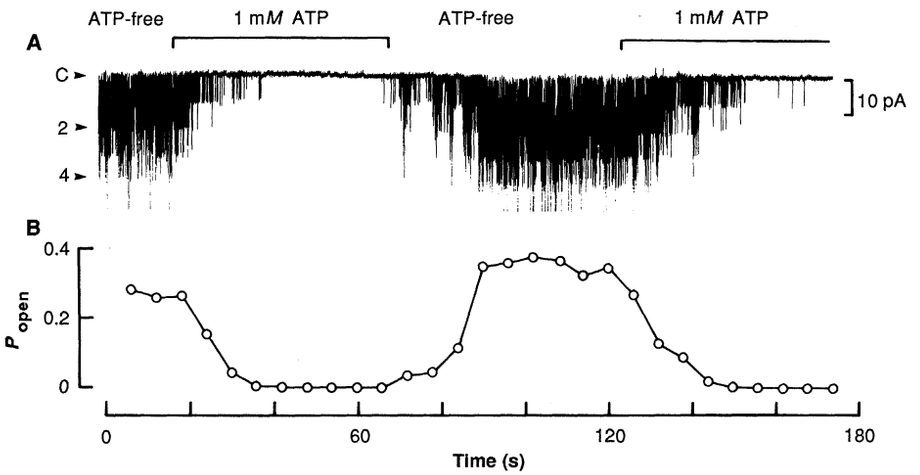
POTASSIUM CHANNELS THAT ARE INhibited by intracellular ATP are present in both vertebrate cardiac and skeletal muscle (*1*), but have not been described in smooth muscle (*2, 3*). However, diazoxide, RP49356 (Rhone-Poulenc), and pinacidil, which activate ATP-sensitive K^+ channels of pancreatic β cells and cardiac muscle (*3, 4*), are vasorelaxing and hypotensive agents, suggesting that such channels may be present in vascular smooth muscle (*3, 5*). Also, the vasorelaxation and increased K^+ conductance produced by RP49356 or by cromakalim (BRL34915, another putative K^+ channel opener) (*6*) are inhibited by the sulfonylurea glibenclamide (*7*), a blocker of ATP-sensitive K^+ channels in pancreatic β cells and heart cells (*8, 9*).

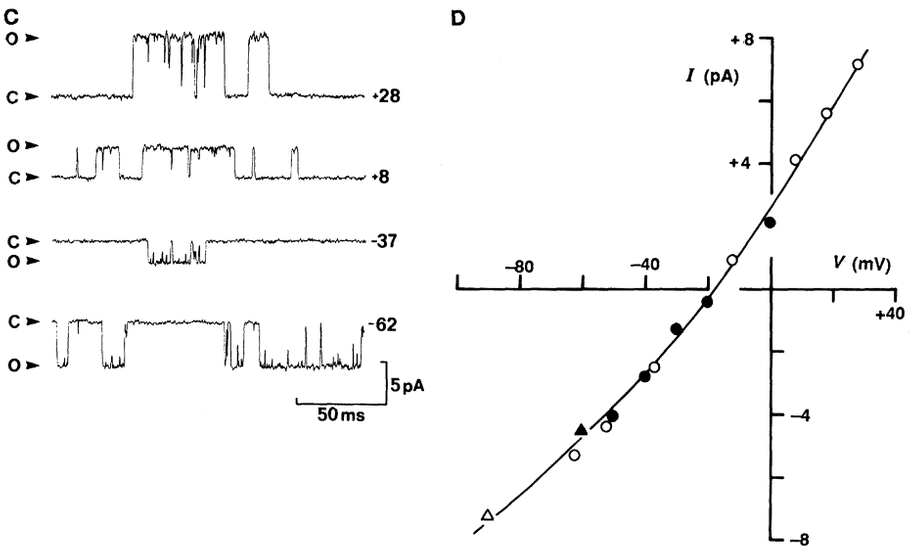
We now describe the recording by patch-clamp methods (*10*) of unitary currents through ATP-sensitive K^+ channels in excised, inside-out membrane patches from smooth muscle cells that had been dissociated enzymatically from rabbit or rat mesenteric arteries (*11*). The external (pipette) solution normally contained 60 m*M* K^+, and the cytoplasmic face of the patch was exposed to a flowing solution with 120 or 6 m*M* K^+, with or without ATP. Activation of Ca^{2+}-activated K^+ channels was minimized by the inclusion of 5 m*M* EGTA in the solution bathing the cytoplasmic face of the patch and by making most recordings at negative membrane potentials. In some experiments charybdotoxin (100 n*M*), a blocker of large-conductance Ca^{2+}-activated K^+ channels (*12*), was included in the pipette solution.

Unitary currents from ATP-sensitive channels were recorded in a patch held at −90 mV, at which channel openings lead to

N. B. Standen and N. W. Davies, Department of Physiology, University of Leicester, P.O. Box 138, Leicester LE1 9HN, United Kingdom.
J. M. Quayle, J. E. Brayden, Y. Huang, M. T. Nelson, Department of Pharmacology, University of Vermont College of Medicine, Burlington, VT 05405.

*To whom correspondence should be addressed.





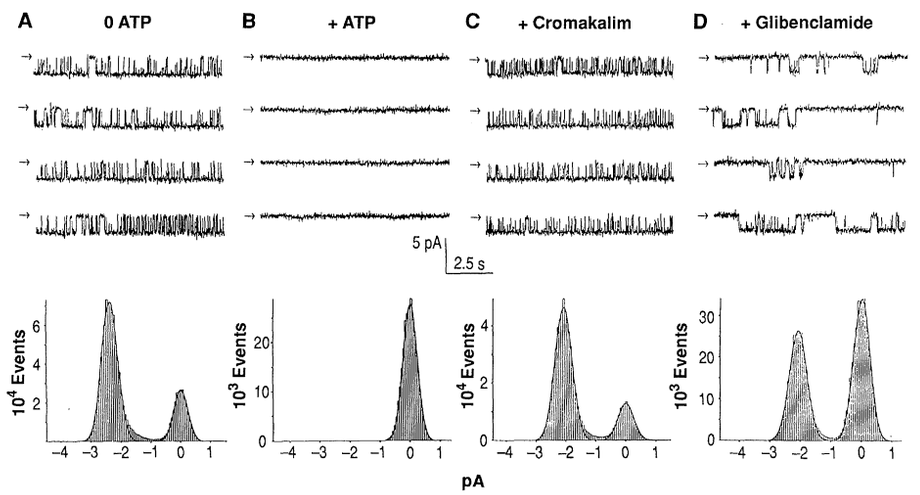


Fig. 2. Effect of cromakalim and glibenclamide on a membrane patch from rabbit mesenteric artery at a holding potential of 0 mV. Single-channel recordings and amplitude histograms are shown: in the absence of ATP in the solution bathing the cytoplasmic face of the patch (**A**); in the presence of ATP (1 m*M*) (**B**); in the presence of ATP (1 m*M*) and cromakalim (1 μ*M*; Beecham Pharmaceuticals) (**C**); and in the presence of ATP (1 m*M*), cromakalim (1 μ*M*), and glibenclamide (20 μ*M*) (**D**). The single-channel recordings are continuous (left to right, top to bottom) and the amplitude histograms were made from longer records; 302.9 s in (A), 77.25 s in (B), 197.7 s in (C), and 196.4 s in (D). The mean unitary currents were −2.36 pA in (A), −2.09 pA in (C), and −2.08 pA in (D) as determined from Gaussian fits to the histograms. The single-channel currents were inward at 0 mV because the bathing solution contained 6 m*M* K^+ instead of 120 m*M* (with Na^+ substituted for K^+). The data were filtered at 1 kHz. The closed level is indicated by arrows.

Fig. 1. Single ATP-sensitive K^+ channels of arterial smooth muscle. (**A**) Continuous record at a holding potential of −90 mV from an inside-out membrane patch excised from a cell that had been isolated from rabbit mesenteric artery. Arrowheads to the left mark the current level where all channels are closed (C) and the levels where two and four channels are open. The pipette solution contained 60 m*M* NaCl, 60 m*M* KCl, 1.8 m*M* $CaCl_2$, and 10 m*M* Hepes-NaOH (*p*H 7.2). The cytoplasmic face of the patch was exposed to a flowing solution containing 104 m*M* KCl, 16 m*M* KOH, 5 m*M* EGTA, 1 m*M* $MgCl_2$, and 10 m*M* Hepes (*p*H 7.2), with or without 1 m*M* ATP (disodium salt). (**B**) P_{open} from the experiment shown in (A) calculated over 6-s intervals as $\Sigma_{j=1}^{N} t_j j/TN$, where the t_j is the time spent (seconds) with j = 1, 2, . . . N channels open; N is the maximum number of channels seen (five in this case); and T is the duration of the measurement. (**C**) Recordings on a faster time scale and at different holding potentials (shown at right in millivolts) of a membrane patch from rat mesenteric artery. The filter frequency was 1 kHz, and C marks the closed and O the open level in each case. Cytoplasmic and pipette solutions were as in (A) (in the absence of ATP). (**D**) *I-V* relation obtained in the solutions given in (A). Symbols show results from one patch from rat (○) and three from rabbit (●, ▲, △) cells.

inward currents (Fig. 1A). After changing the solution bathing the cytoplasmic face of the patch from one that was ATP-free to one containing 1 m*M* ATP, channel activity was inhibited. In this experiment five levels of channel opening were seen in ATP-free solution, whereas in the presence of 1 m*M* ATP channel activity was mostly absent, resulting in a decrease in open-state probability (P_{open}) from 0.268 to 0.002 (Fig. 1B). The P_{open} returned to its previous value after removing ATP from the solution (Fig. 1, A and B). In 12 other patches, ATP (1 m*M*) reduced P_{open} by >95% and in one patch by 79%. These results are consistent with an ATP concentration for half-maximal inhibition of <229 μ*M* for a stoichiometry of 2 and of <53 μ*M* for a stoichiometry of 1, which are similar to values of 10 to 200 μ*M* reported for this channel in other tissues (*2*). In ATP-free solution, P_{open} varied significantly between patches (range of 0.025 to 0.755). In many patches we did not see openings of ATP-sensitive K^+ channels, whereas other patches contained several such channels (Fig. 1A). Maintenance of channel activity in excised patches may require other factors, such as phosphorylation by an appropriate kinase, as has been suggested to explain the variability and loss of channel activity in patches excised from pancreatic β cells (*2*).

Recordings of channel activity on a faster time scale (Fig. 1C) show a similar kinetic pattern to that described for ATP-sensitive channels in cardiac or skeletal muscle (*1*), with openings being interrupted by many short closures and being grouped into

bursts. These recordings and the current-voltage (*I-V*) relation (Fig. 1D) also show that the channel is K^+-selective, with currents reversing close to the calculated K^+ equilibrium potential of −17 mV. The single-channel conductance at 0 mV was 135 pS, a value close to that which we have measured for Ca^{2+}-activated K^+ channels at similar K^+ concentrations. The single-channel conductance that we measured for the ATP-sensitive K^+ channel is greater than that of 40 to 90 pS reported for these channels in pancreatic β cells and cardiac and skeletal muscle (*2*); however, ATP-sensitive K^+ channels of high conductance have also been reported in cortical neurons (*13*). The ATP-sensitive K^+ channel and Ca^{2+}-activated K^+ channel differ in other properties. If we assume that our nominally Ca^{2+}-free cytoplasmic solutions actually contain 10 μ*M* total Ca^{2+}, then the calculated free Ca^{2+} concentration in the presence of 5 m*M* EGTA is 2.87×10^{-10}*M*, and this would be decreased by <2% on the addition of 1 m*M* ATP (*14*); at the negative potentials used, P_{open} for Ca^{2+}-activated K^+ channels would be effectively zero at this free Ca^{2+} concentration (*15*). Ca^{2+}-activated K^+ channels are also strongly activated by depolarization, whereas the ATP-sensitive channels described here showed only slight voltage dependence. Furthermore, when we recorded from Ca^{2+}-activated K^+ channels by increasing the free Ca^{2+} concentration of our cytoplasmic solution (10^{-7} to 10^{-5}*M*), the P_{open} of these channels was not affected by the addition of 1 m*M* ATP, 1 μ*M* glibenclamide, or 10 μ*M* cromakalim.

In the presence of ATP, the activity of ATP-sensitive K^+ channels was increased by cromakalim. In one experiment (Fig. 2A), P_{open} was maintained at a fairly steady high value (0.755) in the absence of ATP; 1 m*M* ATP reduced P_{open} to near zero and addition of 1 μ*M* cromakalim in the continued presence of ATP led to an increase in P_{open} to 0.798. Similar increases in P_{open} with cromakalim (1 μ*M*) have been observed in 11 other patches from smooth muscle. Glibenclamide (20 μ*M*), applied to an excised patch in the presence of cromakalim and ATP, reduced P_{open} from 0.798 to 0.458 (Fig. 2, C and D). In another experiment, glibenclamide (20 μ*M*) in the absence of ATP and cromakalim reduced P_{open} from 0.088 to 0.014, an effect that was reversed on removal of the drug.

The relaxation of arterial smooth muscle by diazoxide, RP 49356, and cromakalim is thus probably mediated by the opening of ATP-sensitive K^+ channels, since these compounds activate this type of channel (*3, 4*) (Fig. 2), and relaxation induced by cromakalim and RP 49356 is blocked by glibenclamide (*7*). To determine whether this might be a more general mechanism for smooth muscle relaxation, we tested the effects of the ATP-sensitive K^+-channel blockers tolbutamide, glibenclamide (*4, 9*), and Ba^{2+} (*16*) on the relaxation induced by a number of vasodilators in rabbit mesenteric arteries that had been constricted with norepinephrine (NE) (*17*). The maintained phase of NE contractions in mesenteric artery depends on Ca^{2+} entry through voltage-dependent Ca^{2+} channels (*18*); therefore membrane hyperpolarization should induce relaxation of these arteries by closing Ca^{2+} channels. Glibenclamide (0.1 to 10 μ*M*), tolbutamide (500 μ*M*), and Ba^{2+} (30 to 100 μ*M*) had no effect on NE-induced contractions in the absence of vasodilators (*19*). However, all these agents reversed the dilator effects of diazoxide, cromakalim, and another hyperpolarizing vasodilator drug, pinacidil (Fig. 3) (*19*). Extracellular Ba^{2+} blocks the ATP-sensitive K^+ channel of skeletal muscle with relatively high affinity [dissociation constant (K_d) = 100 μ*M* at −62 mV) (*16*), as compared to the Ca^{2+}-activated K^+ channel (K_d > 10 m*M*] (*20*). Neither glibenclamide nor tolbutamide have been shown to block any other type of K^+ channel and therefore appear to be specific for ATP-sensitive channels (*2*). The blockers of the Ca^{2+}-activated K^+ channel, charybdotoxin (50 n*M*) and tetraethyl ammonium chloride (TEA) (<1 m*M*) (*21*), did not reverse the effects of vasodilators on muscle tension, indicating that this channel does not regulate membrane potential under these conditions (*19*). Extracellular TEA (<1 m*M*) would not be expected to cause significant block of ATP-sensitive K^+ channels (*22*). These results provide additional support for the role of ATP-sensitive K^+ channels in the vasodilation produced by diazoxide, cromakalim, and pinacidil.

Acetylcholine (ACh) can induce relaxation of blood vessels, probably by stimulat-

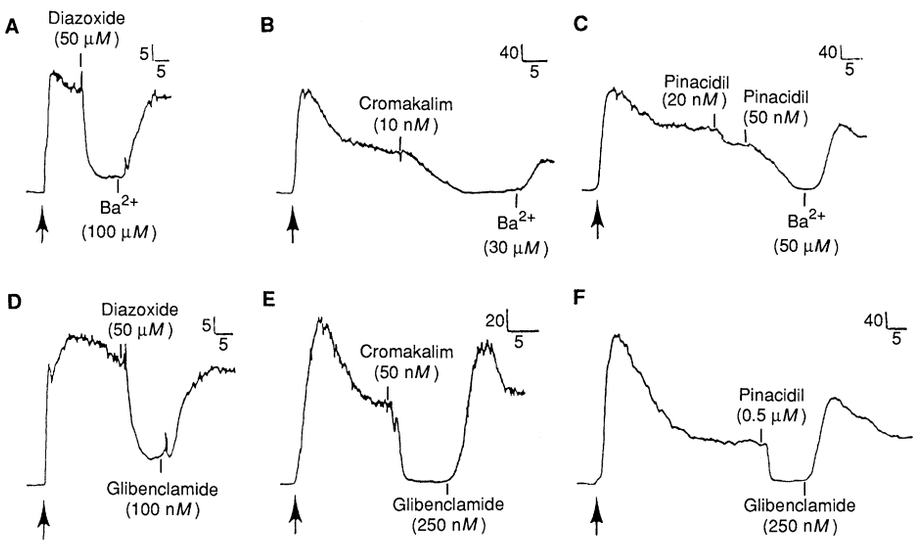


Fig. 3. Reversal of diazoxide (Glaxo)- (**A** and **D**), cromakalim- (**B** and **E**), and pinacidil (Leo Laboratories)-induced (**C** and **F**) relaxations of norepinephrine-contracted rabbit mesenteric artery rings by Ba^{2+} and glibenclamide. Application of norepinephrine (10 μ*M*) is indicated by the arrows. The vertical axis is in milliNewtons and the horizontal axis is in minutes.

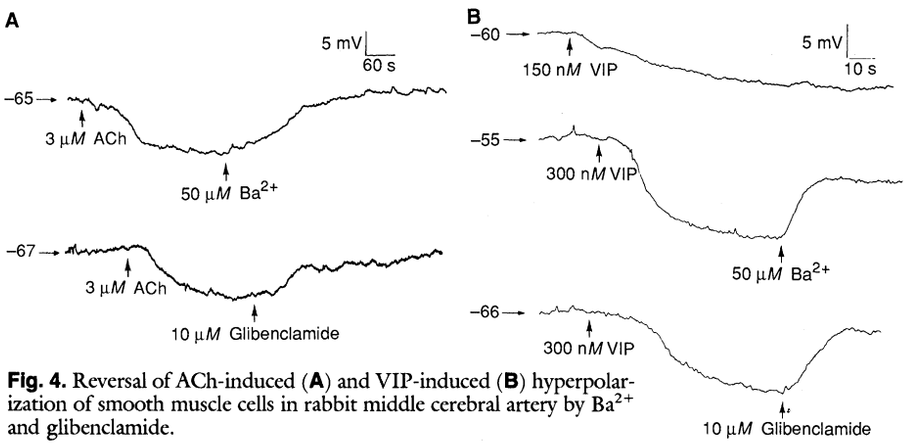


Fig. 4. Reversal of ACh-induced (**A**) and VIP-induced (**B**) hyperpolarization of smooth muscle cells in rabbit middle cerebral artery by Ba^{2+} and glibenclamide.

ing the release of factors from the endothelium [such as endothelium-derived relaxing factor (EDRF) and endothelium-derived hyperpolarizing factor (EDHF)] that cause smooth muscle relaxation. It is thought that EDRF acts by activating guanylate cyclase and EDHF acts by hyperpolarizing the smooth muscle membrane (*23*). The ACh-induced hyperpolarization of the rabbit middle cerebral artery was reversed by glibenclamide (10 μM) (Fig. 4A) or Ba^{2+} (50 μM). Ba^{2+} ($\leq$50 μM) and glibenclamide ($\leq$10 μM) did not affect membrane potential in the absence of ACh. These results suggest that EDHF also opens ATP-sensitive K^+ channels. We also tested whether vasoactive intestinal polypeptide (VIP), an endogenous hyperpolarizing vasodilator that acts directly on smooth muscle (*24*), might also act through the ATP-sensitive K^+ channel. The hyperpolarization of cerebral arterial smooth muscle cells induced by VIP (150 to 300 nM) was reversed by 50 μM Ba^{2+} and 10 μM glibenclamide (Fig. 4B).

Our results provide direct evidence that ATP-sensitive K^+ channels occur in vascular smooth muscle and that these channels are activated by the vasodilator cromakalim. Blockers of ATP-sensitive K^+ channels reverse both the dilation of arteries induced by the drugs diazoxide, cromakalim, RP 49356, and pinacidil and the membrane hyperpolarization induced by the endothelium-dependent vasodilator ACh and the directly acting peptide VIP. Thus, these synthetic and endogenous hyperpolarizing vasodilators seem to act through a common pathway by opening ATP-sensitive K^+ channels in smooth muscle. Cromakalim and pinacidil have been shown to relax airway, intestinal, and uterine smooth muscle (*25*), suggesting that the ATP-sensitive K^+ channel exists in many types of smooth muscle. Since physiological agents such as ACh and VIP appear to open ATP-sensitive K^+ channels, it is possible that these channels regulate membrane potential in vivo. The ATP-sensitive K^+ channel, through its ATP-dependence, may provide a link between metabolism and the regulation of blood flow.

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G_1/S Transition in Normal Human T-Lymphocytes Requires the Nuclear Protein Encoded by c-*myb*

ALAN M. GEWIRTZ, GIOVANNI ANFOSSI, DONATELLA VENTURELLI, SUSANNA VALPREDA, ROBERT SIMS, BRUNO CALABRETTA*

Exposure of peripheral blood mononuclear cells (PBMC) to an 18-base c-*myb* antisense oligomer before mitogen or antigen stimulation resulted in almost complete inhibition of c-*myb* messenger RNA and protein synthesis and blockade of T lymphocyte proliferation. Expression of early and late activation markers, interleukin-2 receptor and transferrin receptor, respectively, by PBMC was unaffected by antisense oligomer exposure as was the expression of c-*myc* messenger RNA. In contrast, histone H3 messenger RNA levels and DNA content were selectively decreased. These results suggest that c-*myb* protein deprivation does not perturb T lymphocyte activation or early molecular events that may prepare the cell for subsequent proliferation. Rather, it appears to specifically block cells in late G_1 or early S phase of the cell cycle.

INDUCTION OF T LYMPHOCYTE PROLIFERATION by mitogen or antigenic stimulation is dependent on a multiplicity of interrelated events such as production of interleukin-2 (IL-2) by T lymphocytes, interaction of IL-2 with its own receptor (IL-2R), and the ensuing activation of numerous genes (*1*), including the transferrin receptor (TFR) and the proto-oncogenes c-

A. M. Gewirtz, Departments of Medicine, Pathology, and Thrombosis Research, Temple University School of Medicine, Philadelphia, PA 19140.
G. Anfossi, D. Venturelli, S. Valpreda, Department of Pathology, Temple University School of Medicine, Philadelphia, PA 19140.
R. Sims, Department of Medicine, Temple University School of Medicine, Philadelphia, PA 19140.
B. Calabretta, Departments of Medicine and Pathology, and Fels Research Institute, Temple University School of Medicine, Philadelphia, PA 19140.

*To whom correspondence should be addressed.