those Ph[1] blast cells containing additional genetic alterations. The mechanism for the suppression of CML-BC colony formation by BCR-ABL antisense oligodeoxynucleotides is not yet known; inhibition of BCR-ABL protein synthesis could remove an essential component in the mitogenic pathway regulated by hematopoietic growth factors as reported (*12*). Less likely, down-regulation of BCR-ABL expression could determine aberrant differentiation of blast cells, resulting in a suppression of colony formation.

We have provided evidence that leukemia growth can be selectively inhibited by synthetic oligomers targeted against a tumor-specific gene involved in the maintenance of the leukemic phenotype. Synthetic oligodeoxynucleotides complementary to BCR-ABL hybrid genes can be synthesized on an individual basis once the specific BCR-ABL junction is identified; this can be done within a few days of diagnosis and offers the prospect, at least in vitro, of a gene-targeted anti-leukemic therapy.

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Differentiation of 3T3-L1 Fibroblasts to Adipocytes Induced by Transfection of *ras* Oncogenes

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Mammalian 3T3-L1 cells differentiate into adipocytes after continuous exposure to pharmacological doses of insulin or physiological doses of insulin-like growth factor I (IGF-1). Expression of transfected *ras* oncogenes led to differentiation of these cells into adipocytes in the absence of externally added insulin or IGF-I. Cells transfected with normal *ras* genes or the tyrosine kinase *trk* oncogene did not differentiate. Transfection with a dominant inhibitory *ras* mutant resulted in inhibition of differentiation. Exposure of untransfected 3T3-L1 cells to insulin stimulated formation of the active Ras·GTP complex. These observations indicate that Ras proteins participate in signal transduction pathways initiated by insulin and IGF-I in these cells.

THE EVOLUTIONARY CONSERVATION of the *ras* gene family suggests that its members have essential cellular functions (*1*). The mammalian Ras proteins are thought to be involved in signal transduction pathways of proliferation or differentiation. However, little is known about the components and mechanisms of the *ras* signaling pathway (*1*). Microinjection of transforming Ras proteins transiently transforms mammalian cells (*2*) and induces meiotic maturation in *Xenopus* oocytes (*3*). Microinjection into *Xenopus* oocytes of the neutralizing Ras antibody Y13-259 or cytosol-localized *ras* oncogene mutants (*4*) inhibits meiotic maturation induced by insulin or IGF-I but not by progesterone. These observations suggest a role of the Ras proteins in the insulin and IGF-I signaling pathways in these amphibian cells.

We examined the function of *ras* in signaling pathways of insulin and IGF in mammalian cells. Differentiation of the murine 3T3-L1 cell line (*5, 6*) into adipocytes shows an absolute requirement for insulin or IGF-I (*5–10*). Exponentially growing and preconfluent 3T3-L1 cells have the morphological and biochemical properties of fibroblasts; however, after reaching confluency, they can be converted to adipocytes (*7*) using protocols that involve prolonged, continuous exposure to pharmacological doses of insulin or physiological doses of IGF-I (*6,8, 9*). Insulin or IGF-I have been shown to be essential regulators of the differentiation process carried out in either serum-supplemented medium (*9*) or serum-free media (*10*). We introduced functionally active *ras* genes into 3T3-L1 cells by means of transfection in order to study their effect on insulin-dependent differentiation into adipocytes (*11*). To assess the specificity of these effects, other oncogenes were also transfected.

The plasmids analyzed in these transfection experiments (Table 1) included pMEXneo H-*ras*^{Gly12} and pMEXneo H-*ras*^{Lys12} (*12*) containing, respectively, the normal and transforming alleles of H-*ras* and pMEXneo H-*ras*^{Lys12,Ser186}, a cytosolic, nontransforming mutant that fails to localize to the plasma membrane because the Ser^{186} mutation precludes posttranslational farnesylation (*13*). Two vectors containing transforming variants of the tyrosine kinase oncogene *trk* were also used. These were pDM16, a pMEX-related plasmid containing the original *trk* oncogene activated through recombination with cellular tropomyosin sequences, and pDM78, a pMEXneo-derived construct containing *trk5*, a different transforming allele activated through a deletion in the extracellular domain of the *trk* proto-oncogene (*14*).

The efficiency of transfection in NIH 3T3 cells, as estimated by the number of neomycin-resistant (neo^r) colonies obtained after selection, ranged from 1 to 5%. As expected, pMEXneo H-*ras*^{Lys12} showed potent trans-

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forming activity that was absent from its normal counterpart pMEXneo H-*ras*^{Gly12} and from the soluble *ras* mutant pMEXneo H-*ras*^{Lys12,Ser186}. The two different variants of the *trk* oncogene showed high transforming activity when transfected into NIH 3T3 cells (*14*) (Table 1). The efficiency of transfection of the same constructs in 3T3-L1 cells was about 100 times lower (Table 1). Transfections with all constructs yielded 1 to 5×10^2 neo^r colonies, with the exception of the pMEXneo H-*ras*^{Lys12} transfections, which showed no surviving colonies after selection in the presence of G418 for 3 weeks (Table 1) (see below). In contrast to NIH 3T3 cells, all 3T3-L1 neo^r clones expressing constitutively the transforming *trk* oncogenes, the nontransforming H-*ras* constructs, or pMEXneo showed the same morphology as untransfected or mock-transfected 3T3-L1 cells. These results indicate that the 3T3-L1 cells are not susceptible to transformation by the same oncogenes as NIH 3T3 cells.

Two criteria were used to estimate adipocyte differentiation in the 3T3-L1 transformants: (i) morphological changes caused by the accumulation of intracellular lipid, apparent under phase contrast microscopy of live cultures or after staining for lipids with oil red O in fixed cultures (*15*) (Fig. 1) and (ii) Northern (RNA) blot analysis of several mRNAs known to be modulated during adipocyte differentiation, including those encoding glycerophosphate dehydrogenase, the fatty acid binding protein aP2, and fatty acid synthase (*16, 17*) (Fig. 2). Hybridization signals were normalized to the amount of β2-microglobulin mRNA rather than β-actin mRNA because the amount of β-actin mRNA changes during differentiation of 3T3-L1 cells (*16–18*). Individual neo^r clones were examined for their ability to undergo differentiation into adipocytes in response to insulin (*6, 8, 9*) (Table 1). The neo^r clones obtained from the transfections with neo alone (pMEXneo), the nontransforming *ras* genes (pMEXneo H-*ras*^{Gly12} and pMEXneo H-*ras*^{Lys12,Ser186}), or the transforming *trk* oncogene forms (pDM16, pDM78) could be induced to differentiate (*9*), indicating that transfection with those constructs did not prohibit differentiation (Table 1).

The same differentiation protocol could not be applied to 3T3-L1 cells expressing H-*ras*^{Lys12} because no clones permanently survived the G418 selection. However, during the initial stages of selection, cells transfected with H-*ras*^{Lys12} showed morphology characteristic of adipocytes (Fig. 1, B and C), including the presence of fat droplets staining with oil red O (Fig. 1, E and F). These neo^r colonies were small in size (less than 100 cells on average) when first identifiable as isolated colonies, about 1.5 weeks after start of selection. The cells within these colonies progressively accumulated fat,

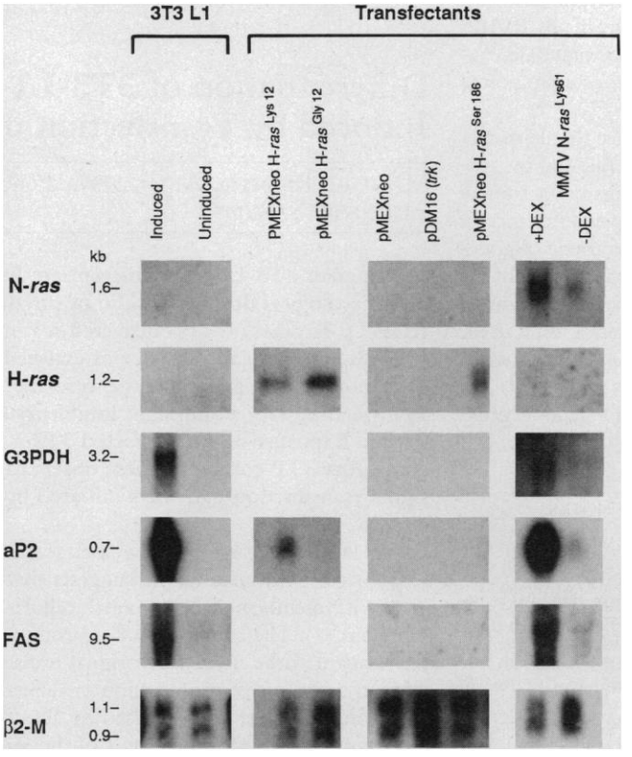

Fig. 2. Expression of *ras* and RNA's characteristic of adipocytes in untransfected and transfected 3T3-L1 cells. Differentiation of untransfected 3T3-L1 cells was induced as described (*9*). Expression of MMTV N-*ras*^{Lys61} was induced by treating cells with Dex (1 μM) for 72 hours. All RNAs were extracted from representative individual clones, with the exception of pMEXneo H-*ras*^{Lys12}, where a pool of colonies surviving after 2 weeks of G418 selection was the source of RNA. For hybridization with the different probes (*17, 18*), Northern blots of total RNA from the indicated cells were stripped and rehybridized repeatedly, or equal portions of the RNA preparations were analyzed in parallel. G3PDH, glycerophosphate dehydrogenase; FAS, fatty acid synthase; β2-M, β2 microglobulin.

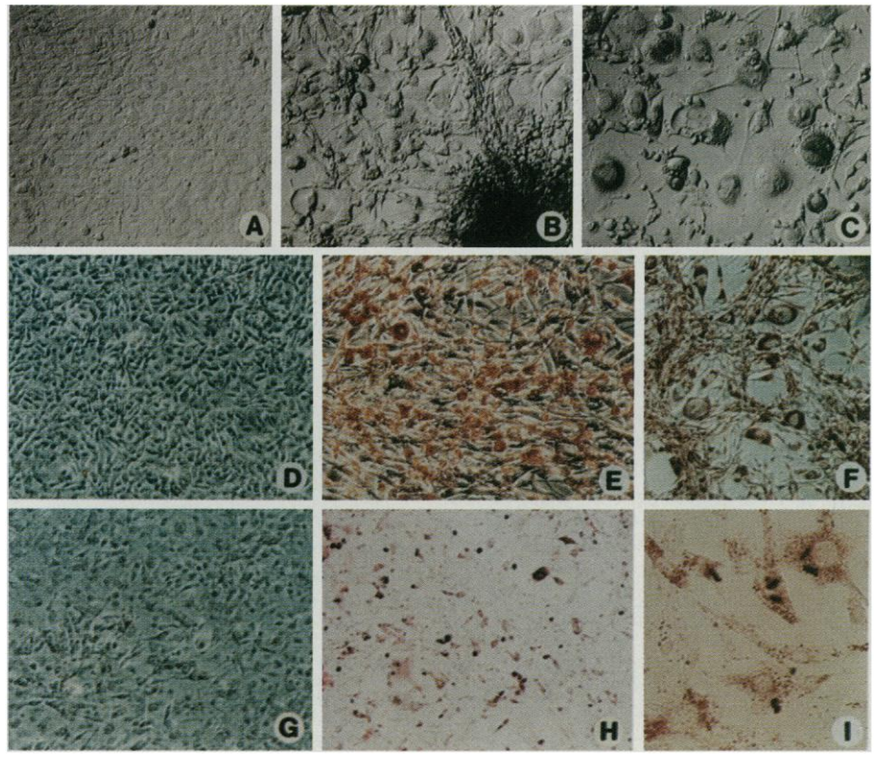

Fig. 1. Differentiation of transfected 3T3-L1 cells. Top row: Nomarsky phase-contrast microscopy of cells transfected with normal H-*ras*^{Gly12} (**A**) or transforming H-*ras*^{Lys12} (**B** and **C**) genes. Middle row: Oil red O staining (*15*) of cells transfected with H-*ras*^{Gly12} (**D**) and H-*ras*^{Lys12} (**E** and **F**). Bottom row: Oil red O staining of MMTV N-*ras*^{Gln61} (**G**) or MMTV N-*ras*^{Lys61} (**H** and **I**) transfectants kept in the presence of Dex for 62 hours.

Table 1. Transfection of 3T3-L1 and NIH 3T3 fibroblasts with constitutively expressed or inducible oncogenes. The constructs (3 μg) were transfected and the transfectants were selected in G-418 (500 μg/ml) by standard procedures (*11*). pMEXneo was cotransfected when the expression plasmids did not carry the neo gene. Results presented are averages of four separate transfections performed in duplicate. Standard deviation for numbers of foci or neo^r colonies was equal or less than 25% of values shown. The clone of 3T3-L1 cells used and the protocol for insulin-induced differentiation have been described (*9*). If viable, at least ten separate neo^r cloned cell lines were kept for analysis from the transfections done with each construct. Differentiation of individual or pooled neo^r clones was evaluated morphologically by phase-contrast microscopy of live cultures and by staining of fixed cells with oil red O (*15*). Expression of MMTV-derived constructs was induced by addition of 1 μM Dex to regular growth medium. Efficiency of differentiation was monitored by counting the number of cells with staining positive for lipid accumulation (−, no staining; ++, less than 50%; +++, ~90%; ++++, >95%). N.A., not applicable.

Transfected DNA	3T3-L1			NIH 3T3 transformation (No. of foci)*	
	Neo^r colonies (No.)*	Differentiation			
		No treatment	Standard treatment		
Mock	0	−	+++	0	
pMexneo	4.5×10^2	−	+++	0	
pMexneo H-*ras*^{Lys12}	0	+++†	NA‡	1.8×10^4	
pMexneo H-*ras*^{Gly12}	5×10^2	−	+++	0	
pMexneo H-*ras*^{Lys12,Ser186}	3.9×10^2	−	++§	0	
pMexneo *trk*5 (*pDM78*)	5.8×10^2	−	+++	2.0×10^4	
pMexneo + pDM16	4.7×10^2	−	+++	1.6×10^4	
		Induction			Induction
		−Dex +Dex			−Dex +Dex
MMTV N-ras^{Lys61} + pMexneo	4×10^2	+ +++	++++	2×10^2	1.5×10^4
MMTV N-ras^{Gln61} + pMexneo	5×10^2	− −	+++	0	0

*Colonies per microgram of neo-containing plasmid surviving after 3 weeks of G418 selection. †As judged by morphology of colonies surviving after 2 weeks of G418 selection. ‡Differentiation protocol could not be applied because no colonies could be rescued after 3 weeks of selection in G418. §A pool of ten separate neo^r clones was examined.

stopped dividing, and eventually detached from the plate. Expression of H-*ras*^{Lys12} correlated with the expression of the specific markers for differentiation (Fig. 2). In contrast, spontaneous adipocytic differentiation was not observed in cells transfected with normal c-H-*ras*^{Gly12} (Fig. 1A, D), the nontransforming H-*ras*^{Lys12,Ser186} mutant, the *trk* oncogenes (pDM16 and pDM78), or pMEXneo alone (Fig. 2).

Because the 3T3-L1 cells carrying H-*ras*^{Lys12} could not be propagated as permanent cell lines, we tried to develop cell lines in which differentiation could be triggered by induced expression of *ras* genes. Clones of normal and transforming N-*ras* placed under the control of the glucocorticoid-sensitive mouse mammary tumor virus (MMTV) promoter (*19*) were cotransfected with pMEXneo, and the neo^r cotransfectants were cultured in the presence or absence of dexamethasone (Dex). In the absence of Dex, MMTV N-*ras*^{Lys61} had weak transforming activity in NIH 3T3 cells (Table 1). After transfection of this vector into 3T3-L1 cells, neo^r clones that expressed low levels of N-*ras* RNA were isolated (Fig. 2). Treatment with Dex increased the expression of N-*ras*^{Lys61} (Fig. 2) and also promoted differentiation into adipocytes as estimated by staining of fat in the cells (Fig. 1, H and I) and by the levels of the specific RNA markers (Fig. 2). As expected, the normal MMTV Nras^{Gln61} construct did not show transforming activity in NIH 3T3 cells, even when the transfected cells were cultured in the presence of Dex, and did not induce adipocytic differentiation in 3T3-L1 cells (Table 1 and Fig. 1G). These results suggest that the induced expression of N-*ras*^{Lys61}, and not any other factor, is responsible for the differentiation observed.

Expression of transforming *ras* genes can apparently substitute for exposure to high concentrations of insulin in the induction of adipocytic differentiation in 3T3-L1 cells. Inasmuch as expression of *trk* oncogenes did not induce adipocytic differentiation, the effect observed seemed to be specific for *ras* oncogenes. These observations are consistent with other studies indicating that *ras* gene products may be mediators in insulin signaling pathways (*4, 20*). Also consistent with this notion is the inhibition of differentiation observed in transfectants expressing the cytosol-localized H-*ras*^{Lys12,Ser186} protein (Table 1). While approximately 90% of untransfected 3T3-L1 cells became adipocytes after undergoing the differentiation protocol (*9*), only about 40% of cells in clones of H-*ras*^{Lys12,Ser186}-transfected cells differentiated. This likely results from the transdominant negative effect of the transfected gene over the endogenous *ras*. Similar inhibition has been reported for dominant inhibitory *ras* mutants in other systems including PC12 cells, NIH 3T3 cells, and *Xenopus* oocytes (*4, 21*). Exposure of untransfected 3T3-L1 cells to insulin also stimulated formation of the biologically active Ras·GTP complex in these cells, strongly suggesting a direct link between endogenous Ras and insulin signaling. The ratio of Ras·GTP to Ras·GDP (*22*) doubled in our 3T3-L1 cells after a 30-min stimulation with insulin. Increases of similar magnitude are produced by platelet-derived growth factor, epidermal growth factor, and oncogenes whose signaling systems involve Ras (*22*). Because the biochemical processes that occur during insulin-induced differentiation of 3T3-L1 cells (*6–10*) are well described, our transfectants may provide a useful system to functionally analyze *ras* gene products at a biochemical level.

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with the calcium phosphate protocol supplied with the Stratagene Mammalian Transfection kit. Transfectants of both cell types were selected in the presence of G-418. Transfection of NIH 3T3 cells allowed assessment of the transforming activity of the constructs and the efficiency of the transfection procedure.
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Visual Motion Commands for Pursuit Eye Movements in the Cerebellum

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Eye movements that follow a target (pursuit eye movements) facilitate high acuity visual perception of moving targets by transforming visual motion inputs into motor commands that match eye motion to target motion. The performance of pursuit eye movements requires the cerebellar flocculus, which processes both visual motion and oculomotor signals. Electrophysiological recordings from floccular Purkinje cells have allowed the identification of their firing patterns during generation of the image velocity and image acceleration signals used for pursuit. Analysis with a method based on a behavioral model converted the time-varying spike trains of floccular Purkinje cells into a description of the firing rate contributed by three visual motion signals and one oculomotor input. The flocculus encodes all the signals needed to guide pursuit.

MANY PRIMATE BEHAVIORS ARE guided by vision. Although much is known about the sensory processing of visual inputs, little is known about the sensory-motor transformations that convert central visual signals into commands for voluntary movements. Pursuit eye movements provide an opportunity to understand this sensory-motor transformation. Pursuit allows primates to use visual inputs related to image motion, defined as target motion with respect to the eye, to drive smooth eye movements that keep the eyes pointed at small moving targets. Previous experiments have led to the delineation of the brain areas that process moving images and the basic anatomical pathways that connect visual motion areas in the extrastriate cortex to the extraocular nuclei (*1*). In addition, our laboratory has developed a quantitative description of how visual motion signals are transformed to generate eye velocity (*2*). The next step is to determine how those signals are represented in the pursuit pathways.

Our experiments focus on the cerebellar flocculus, which is necessary for accurate pursuit (*3*) and is located at the interface between the visual and oculomotor pathways that generate pursuit eye movements. Visual inputs to the flocculus arise from at least the pontine nuclei and the nucleus reticularis tegmenti pontis, whereas inputs related to eye movements arise from the vestibular nuclei and the nucleus prepositus (*4*). Both the visual and oculomotor inputs are reflected in the simple-spike activity of floccular Purkinje cells (P cells), the output neurons of the cerebellum (*5*). In turn, neural activity of floccular P cells causes smooth eye movements within 10 ms by inhibiting the vestibular interneurons in the brainstem that project directly to extraocular motoneurons (*6*).

Our goal was to go beyond the previous demonstration of visual simple-spike responses in floccular P cells and provide a description of how properties of the visual inputs are related to features of pursuit. The design of our experiments was based on behavioral studies in monkeys, which suggested that three different visual signals provide the commands for smooth eye acceleration (*2*, 7). These signals are related to image velocity, the abrupt onset of image motion (image motion transient), and smooth changes in image velocity (image acceleration). Models of pursuit that include a sensitivity to all three visual signals have emergent properties that replicate several distinctive features of pursuit (*8*).

Figure 1 illustrates three target motions that can help dissect the visual inputs for pursuit into its three separate components. When the target is initially stationary and begins to move at a constant speed (Fig. 1A), all three components are available to contribute to pursuit. The image motion transient component, defined as the first derivative of image velocity for abrupt changes in target motion, makes a momentary contribution at the onset of target motion. The image velocity component provides an initial steady input that decreases toward zero as eye velocity increases toward target velocity. The image acceleration component, defined as the first derivative of image velocity for smooth changes in target motion, contributes after image velocity begins to decrease; this component acts in the direction opposite that of image velocity, braking eye velocity as it approaches the target velocity. When the target appears on the screen already moving (Fig. 1B), the image motion transient component does not contribute, and as a result the rising phase of eye velocity is less brisk. This target motion provides image velocity and image acceleration inputs that are qualitatively similar to those in Fig. 1A. When a target starts from rest and accelerates smoothly to a steady target velocity (Fig. 1C), the image velocity and image acceleration pathways contribute synergistically, and the image motion transient is not activated. The accelerating target

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