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The BCR-ABL Oncogene Transforms Rat-1 Cells and Cooperates with v-*myc*

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The tyrosine kinase P210 is the gene product of the rearranged BCR-ABL locus on the Philadelphia chromosome (Ph[1]), which is found in leukemic cells of patients with chronic myelogenous leukemia. It has a weakly oncogenic effect in immature murine hematopoietic cells and does not transform NIH 3T3 cells. We have found that P210 has a strikingly different effect in Rat-1 cells, another line of established rodent fibroblasts. Stable expression of P210 in Rat-1 cells caused a distinct morphological change and conferred both tumorigenicity and capacity for anchorage-independent growth. The introduction of v-*myc* into Rat-1 cells expressing P210 led to complete morphological transformation and enhanced tumorigenicity. No such interaction took place in NIH 3T3 cells. Thus, Rat-1 cells can be used to detect cooperation between BCR-ABL and other oncogenes and may prove useful for the identification of secondary oncogenic events in chronic myelogenous leukemia.

The Philadelphia chromosome (Ph[1]) is a diagnostic karyotypic abnormality found in almost all cases of chronic myelogenous leukemia (CML) (25, 31). A reciprocal translocation between chromosomes 9 and 22 fuses exons from *bcr*, a gene of unknown function, with exons of the c-*abl* proto-oncogene into a hybrid transcription unit that directs the production of a novel 210-kilodalton phosphoprotein tyrosine kinase, P210 BCR-ABL (3, 11, 17). CML typically begins with a chronic phase characterized by the clonal expansion of a multipotent hematopoietic stem cell in the bone marrow which retains the capacity to terminally differentiate (for a review, see reference 4). This chronic phase may continue for months or years before progressing to the more acute blast crisis phase. Ph[1] and BCR-ABL expression appear early in the chronic phase with striking reproducibility. During the acute phase, Ph[1] persists and additional karyotypic abnormalities, including duplication of Ph[1], often occur, suggesting that secondary genetic events are involved in the progression to full malignancy.

The P210 protein resembles the product of v-*abl*, the transforming gene of Abelson murine leukemia virus (Ab-MuLV) (1, 2). Ab-MuLV is an acutely oncogenic retrovirus that causes the development of a pre-B-cell leukemia within 2 to 3 weeks after injection into susceptible mice (30). Both P210 and the v-*abl* gene product are chimeric proteins in which the NH_2-terminal sequences of the c-*abl* protein have been replaced by sequences from the *BCR* gene and the viral *gag* gene, respectively (3, 16, 29, 38). Both proteins have tyrosine kinase activity derived from the *abl* gene segment. These structural and biochemical similarities between BCR-ABL and a viral oncogene product are a second line of evidence implicating BCR-ABL in the pathogenesis of CML.

A complete cDNA copy of the message that encodes P210 has been introduced into a murine retroviral vector, pJW-RX, to study the effect of P210 expression in several types of cultured cells (7, 23, 24). The infection of fresh bone marrow with pJW-RX led to the outgrowth of clonal cell lines resembling pre-B lymphoid cells. These cultures were characterized by an increase in average cell size and saturation density; some of them exhibited enhanced cloning efficiencies in soft agar and were tumorigenic. The introduction of pJW-RX into NIH 3T3 cells resulted in high levels of P210 expression but not morphological transformation. Rare transformants were found, but in these cells the P210 protein was altered. It was found to contain sequences derived from the viral *gag* gene at its N terminus, presumably as a result of recombination between the pJW-RX vector and the Moloney murine leukemia virus (Mo-MuLV) helper. Only this trimeric *gag*-BCR-ABL protein had transforming activity in NIH 3T3 cells. The limited oncogenic effect of BCR-ABL in these culture systems contrasts with that of v-*abl*, which is capable of acutely transforming established rodent fibroblasts, including NIH 3T3 cells, and causes a rapid shift to a high saturation density in cultured hematopoietic cells (for a review, see reference 41).

The observation that recombination of BCR-ABL with Mo-MuLV *gag* sequences enhanced its transforming activity in NIH 3T3 cells suggested that it might be possible to identify factors in addition to P210 expression that are required for transformation in fibroblasts. To investigate this possibility, we constructed clonal cell lines of NIH 3T3 and Rat-1 fibroblasts that each contain a single integrated copy of pJW-RX and express high levels of P210. We found that these two cell lines differed dramatically in their response to BCR-ABL. The Rat-1 cells underwent a subtle but distinct morphological change and became partially transformed according to several criteria. The NIH 3T3 cells were not transformed, conforming to previous observations (7). The subsequent introduction of v-*myc* into the P210-expressing Rat-1 cells converted them to a fully malignant phenotype, capable of producing invasive tumors in animals. This interaction between BCR-ABL and v-*myc* did not occur in NIH 3T3 cells. The response of Rat-1 cells to BCR-ABL alone and in combination with v-*myc* reproduces certain aspects of tumor progression in vitro and provides a model for the interaction of oncogenes of the tyrosine kinase class with nuclear oncogenes such as *myc*.

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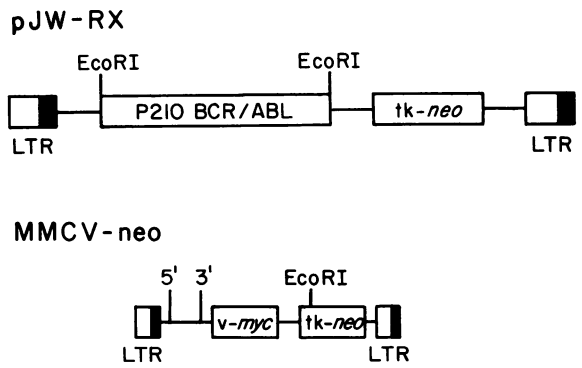


FIG. 1. Structure of the retroviruses described in references 7 (top) and 40 (bottom). LTR, Long terminal repeat.

MATERIALS AND METHODS

Viruses and viral infections. The virus pJW-RX (7) (Fig. 1) was constructed by inserting the complete cDNA encoding BCR-ABL (24) into the unique *Eco*RI site of the Mo-MuLV-based vector pMV6(TKneo) (21) (provided by B. Weinstein, Columbia University). The BCR-ABL transcript is expressed from the viral 5′ long terminal repeat and includes approximately 150 base pairs of 5′-untranslated sequence. pJW-RX also contains the dominant selectable Tn*5* *neo* gene, which confers G418 resistance in mammalian cells, expressed from an internal herpes simplex virus thymidine kinase (TK) gene promoter. A stock of pJW-RX free of helper virus was prepared by transfection into Ψ-2 packaging cells (22).

MMCV-neo virus (40) (Fig. 1) contains the 2.5-kilobase (kb) *Bam*HI-*Eco*RI *myc* fragment of OK10 virus as well as the TK-*neo* gene. This virus expresses *myc* as a subgenomic mRNA from the 5′ long terminal repeat. MMCV-neo viral stocks containing replication-competent Mo-MuLV helper were rescued by cotransfection with Mo-MuLV DNA into NIH 3T3 cells.

Viral infections were performed by exposing cells to virus stock plus 8 μg of Polybrene per ml for 3 h at 37°C. Selective medium containing the antibiotic G418 at a final concentration of 250 μg of active drug per ml was applied 48 h later.

Nucleic acid hybridization analyses. High-molecular-weight DNA was prepared from cells lysed in the presence of 0.5% sodium dodecyl sulfate. After overnight digestion at 37°C with 0.2 mg of proteinase K per ml, the DNA was extracted sequentially with phenol and chloroform-isoamyl alcohol (24:1) and was isolated by precipitation with ethanol in the presence of 2.5 M ammonium acetate. Cytoplasmic RNA was prepared by the sodium dodecyl sulfate-urea lysis procedure of Schwartz et al. (35). Southern and Northern (RNA) blot transfers were performed as described previously (36).

Hybridization probes were prepared by random-primed DNA synthesis (9) in the presence of [α-^{32}P]dATP. The *abl* probe consisted of a 2.3-kb *Hin*c-cII DNA restriction fragment spanning the BCR-ABL junction. The *neo* probe was the 2.0-kb *Cla*I TK-*neo* insert from pJW-RX. The v-*myc* probe was the plasmid p*myc*, containing a 1.5-kb *Pst*I fragment corresponding to the v-*myc* sequences of MC29 virus (39). The probe pRGAPDH, encoding a rat cDNA for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (27), was used on Northern blots to control for variations in the amounts of RNA loaded.

Protein kinase assays. Assays for P210 autophosphorylation were performed as described elsewhere (17). Clarified lysates of cells from one 10-cm plate of fibroblasts or 5×10^6 K562 control cells were incubated overnight at 4°C with 6 to 8 μl of antiserum per ml of lysate. The immune precipitates were collected on protein A-Sepharose beads and were reacted with γ-^{32}P-labeled ATP for 30 min at 30°C. The reaction products were denatured and separated on 7% polyacrylamide gels containing sodium dodecyl sulfate. Site-directed antisera are described in the legend to Fig. 4.

Assay for growth in soft agar. Trypsinized cells were suspended in Iscove medium containing 0.3% noble agar and 20 or 5% fetal calf serum. A layer of this suspension was plated between layers of medium containing 0.6% agar and 12 or 6% serum. Cells were plated at a density of 10^3 or 10^4 cells per 6-cm dish. The plates were refed after 7 days and were scored for colonies at 14 days.

Tumor challenges. Athymic BALB/c (*nu/nu*) mice, 4 to 7 weeks old, were injected subcutaneously with 1.5×10^6 cells that were suspended in 0.2 ml of culture medium plus 5% serum. The animals were observed for the appearance of tumors for 12 weeks following injection. Animals that developed tumors were killed and autopsied when they became moribund; invasiveness of tumors was assessed by gross dissection.

RESULTS

Construction of clonal fibroblast cell lines that stably express BCR-ABL protein. In fibroblasts, the BCR-ABL gene product has a chronic deleterious effect similar to that produced by v-*abl* in certain lines of 3T3 cells (7, 43). Clones of such cells which are morphologically transformed immediately following v-*abl* infection frequently revert to a flat, nontransformed shape after 2 or 3 weeks in culture. Cell death and loss of v-*abl* expression both play a part in this reversion. This toxicity poses several technical problems, including reduced recovery of transformants following transfection with BCR-ABL or v-*abl*, a tendency for transformed lines to grow poorly, and the frequent appearance of outgrowths with deletions of the *abl* sequence.

We therefore chose to use pJW-RX (Fig. 1), a defective murine retrovirus, as an extremely efficient means of introducing BCR-ABL into fibroblast populations from which we could isolate clones that stably expressed P210. pJW-RX expresses a full-length P210 message derived from a cDNA library of the human CML cell line K562. It also expresses the bacterial neomycin resistance gene (*neo*), driven from an internal herpes simplex virus thymidine kinase gene promoter. To avoid the problem of helper virus block to subsequent superinfection, we used a helper-free stock of pJW-RX prepared in Ψ-2 packaging cells.

Monolayer cultures of Rat-1 and NIH 3T3 cells were infected with pJW-RX, and infected clones were selected for *neo* expression in the presence of the antibiotic G418. Individual G418-resistant colonies were screened for tyrosine kinase activity precipitable by antiserum directed against *abl*. One of ten Rat-1 clones and one of twelve NIH 3T3 clones expressed a protein, with autophosphorylation activity, that was similar in size and immunoreactivity to the P210 protein in K562 cells (see below). These two clones, designated Rat-1/P210 and NIH/P210, have stably maintained expression of this protein through months of continuous culture. pJW-RX virus that was rescued from both of these clones by superinfection with Mo-MuLV helper remained toxic to newly infected fibroblast populations (re-

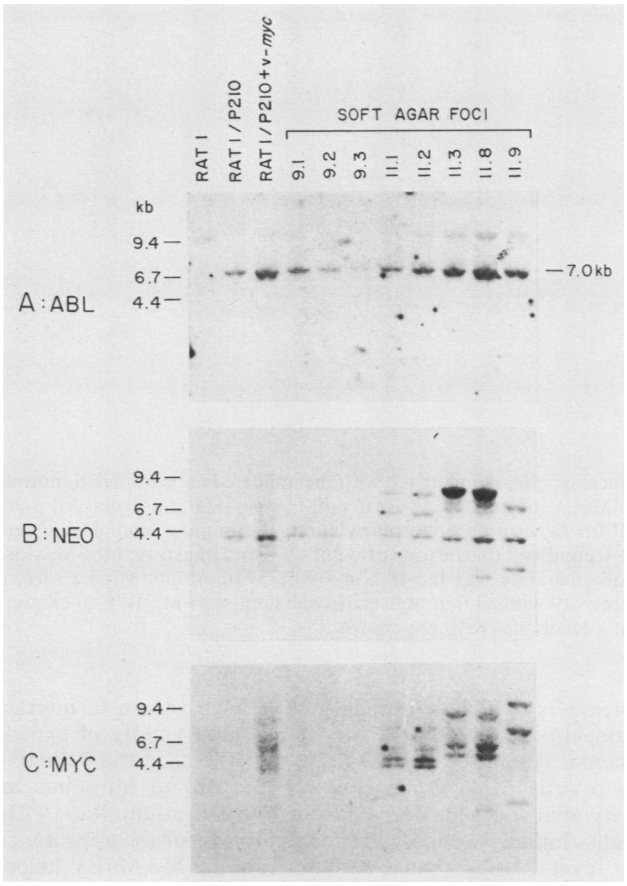


FIG. 2. Proviral integration. High-molecular-weight DNA samples from cells of the indicated cultures were digested with *Eco*RI, electrophoresed through 0.5% agarose (10 μg per lane), and transferred to a Biodyne membrane. Fragments of a *Hin*dIII digest of bacteriophage lambda served as size markers. Digests were hybridized with an *abl* probe (A), a *neo* probe (B), and a *myc* probe (C). The position of the BCR-ABL insert from pJW-RX is marked at 7.0 kb (A). The presence of DNA in lane RAT 1 (Rat-1 cells) was confirmed by ethidium bromide staining.

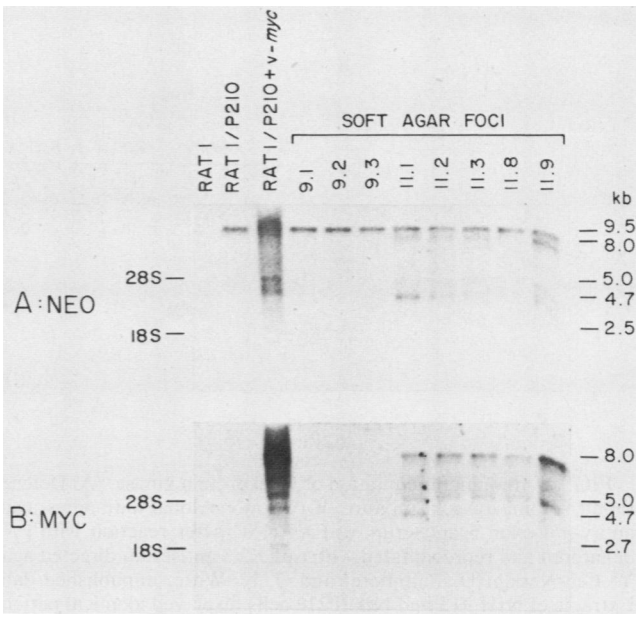


FIG. 3. Transcription of BCR-ABL and v-*myc*. Denatured cytoplasmic RNA (20 μg) from the indicated cell lines was electrophoresed in a 1.0% agarose gel containing 2.2 M formaldehyde and was transferred to nitrocellulose. The positions of ethidium bromide-stained 18S and 28S rRNAs are marked. (A) RNAs hybridized with a *neo* probe. The RNA species with the position marked at 9.5 kb is genomic-length pJW-RX mRNA. The RNAs at 8.0, 5.0, and 4.7 kb are derived from MMCV-neo. Both viruses encode a 2.5-kb *neo* message. (B) The same RNAs hybridized with a *myc* probe. Only genomic (8.0 kb) and subgenomic (5.0 and 4.7 kb) MMCV-neo mRNAs hybridize with this probe (40). The more intense hybridization signal in lanes RAT 1/P210 + v-*myc* (A and B) was caused by overloading with RNA, as confirmed by hybridization to a pRGAPDH (housekeeping gene) probe.

sults not shown). The kinase-negative clones presumably sustained deletions or other mutations in the ABL portion of pJW-RX.

Analysis of pJW-RX proviral integration, transcription, and protein expression showed that the BCR-ABL loci in Rat-1/P210 and NIH/P210 were intact. Southern hybridization of *Eco*RI-digested DNA from both lines with an *abl* probe revealed a single restriction fragment of 7.0 kb, the size of the original BCR-ABL insert cloned into the *Eco*RI site of pJW-RX (Fig. 2). The blots were rehybridized with a *neo* probe, which detected in each DNA a single restriction fragment of unique size, extending from the *Eco*RI site at the 3′ end of the BCR-ABL insert through the *neo* gene into flanking cellular DNA. The presence of unique fragments indicated that both cell lines were clonal, each with a single integrated copy of pJW-RX.

Northern hybridization analysis indicated that the expected messenger RNAs were being transcribed from pJW-RX (Fig. 3). Hybridization of RNA from Rat-1/P210 with a *neo* probe detected the 9.5-kb genomic mRNA and the 2.5-kb TK-*neo* mRNA. A third minor species was seen at 4.9 kb; the presence of this RNA is unexplained at present, but it may arise from cryptic splice sites within the vector.

The protein kinase in Rat-1/P210 and NIH/P210 was tested with a panel of site-specific antisera. An autophosphorylated protein could be immunoprecipitated from extracts of both cell lines by antisera directed against both BCR and ABL determinants (Fig. 4). The labeled protein comigrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis with authentic P210 expressed from Ph^1 in K562 cells. The level of autophosphorylation activity exhibited by P210 in both cell lines was comparable to that in K562 and was much greater than that of the endogenous c-*abl* protein. A mixture of two monoclonal antibodies directed against Mo-MuLV *gag* proteins did not react with P210 in either cell line, although it very effectively precipitated P160, the *gag-abl* protein expressed by an Ab-MuLV-infected cell line (results not shown).

Rat-1 cells, but not NIH 3T3 cells, are partially transformed by BCR-ABL. Both NIH 3T3 cells and Rat-1 cells are established lines of rodent fibroblasts that have been selected for a flat shape and contact inhibition of growth. Malignant transformation of either cell line by a variety of agents, including v-*abl*, leads to loss of contact inhibition and characteristic changes in cellular morphology (28, 33). Expression of BCR-ABL in NIH/P210 cells had none of these effects. The cells were morphologically indistinguishable from uninfected NIH 3T3 cells and remained contact inhibited (results not shown). The only evident effect of P210 expression in these cells was an increase of 10 to 20% in doubling time, probably a manifestation of the toxic effect.

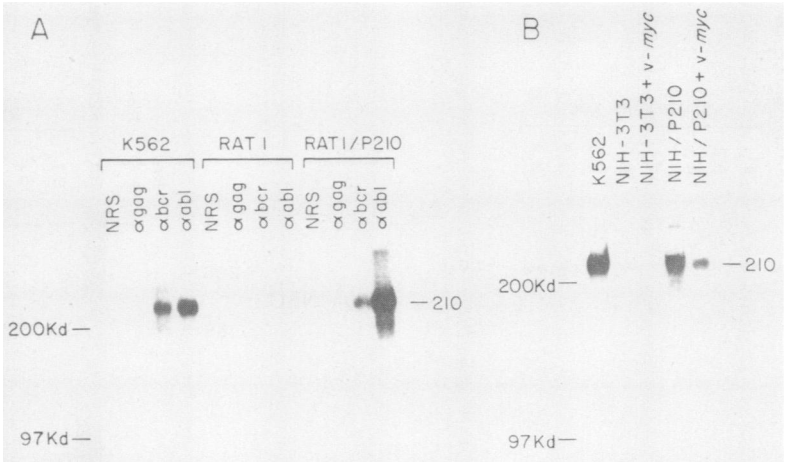


FIG. 4. Immunoprecipitation of P210 protein kinase. (A) Detergent extracts of cytosol incubated with the indicated antisera: NRS, normal rabbit serum; αgag, a mixture of two monoclonal antibodies against Mo-MuLV p12 and p15; αbcr, anti-*bcr* peptide antiserum (5); αabl, anti-v-*abl* exon 5 antiserum αpEX5 (15). After reaction with [γ-^{32}P]ATP for in vitro autophosphorylation, the immune complexes were denatured and reprecipitated with αpEX2/3 antiserum directed against the second and third exons of v-*abl* (A. Pendergast, A. Mes-Masson, Y. Ben-Neriah, D. Baltimore, and O. N. Witte, unpublished data). Autoradiography was for 48 h at −70°C with an intensifying screen. Extracts of NIH 3T3 and NIH/P210 cells displayed identical patterns of reactivity with all four antisera (results not shown). (B) Cell extracts reacted with αpEX5. Exposure time was 1 h. Molecular mass markers (in kilodaltons [Kd]) are shown.

This result was consistent with previous observations that BCR-ABL does not transform NIH 3T3 cells (7).

The Rat-1/P210 cells, however, underwent a subtle morphological change. They became more refractile and slightly less adherent than uninfected Rat-1 cells (Fig. 5) and occasionally formed patches of local dense growth resembling transformed foci. These cells also suffered a decrease in growth rate similar to that of the NIH/P210 cells.

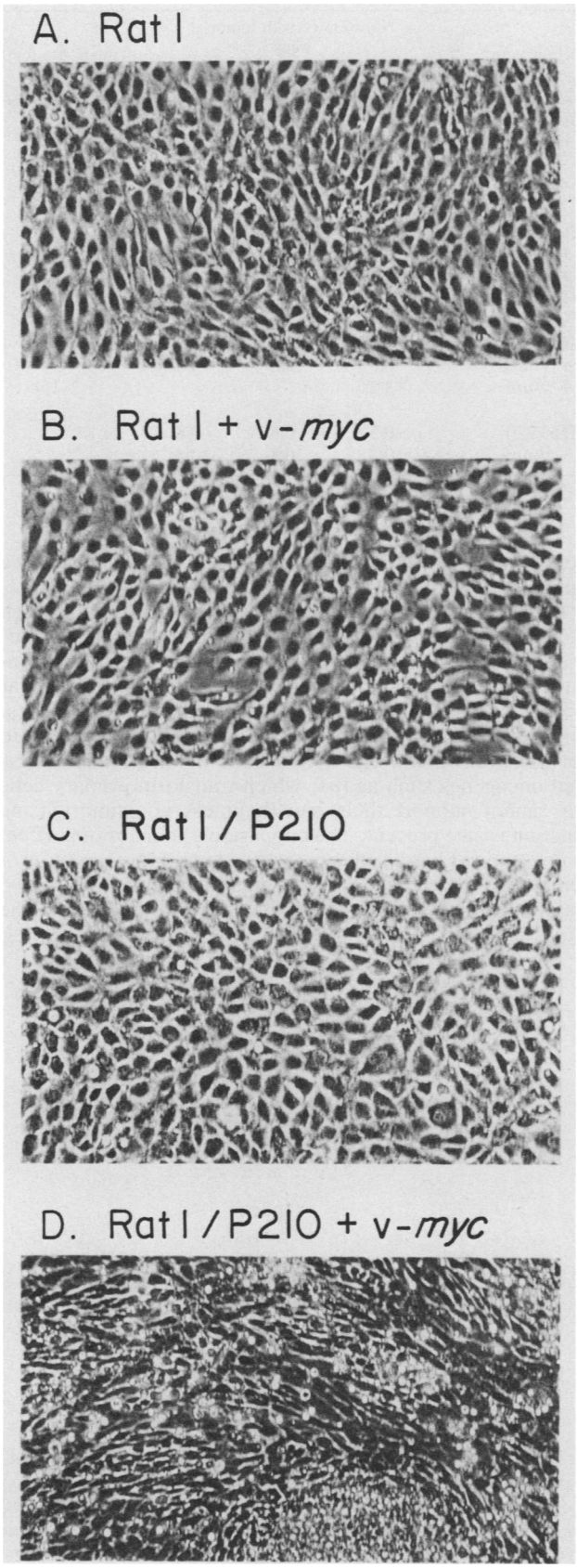
The Rat-1/P210 cells had acquired a significant degree of anchorage independence (Fig. 6). Their plating efficiency in soft agar was comparable to that of Rat-1 cells acutely transformed by Ab-MuLV, although the colonies were not as large. NIH/P210 cells, on the other hand, exhibited a measurable but very slight increase in plating efficiency in soft agar and produced tiny, barely macroscopic colonies. Three colonies formed in soft agar by Rat-1/P210 cells were picked, established in culture, and examined to determine if they differed significantly from the parental cells. These three cultures, 9.1, 9.2, and 9.3, had morphologies similar to that of Rat-1/P210 cells, and analysis of their DNA and RNA did not reveal alterations in either proviral structure or transcription (Fig. 2 and 3), nor was there any change in P210 protein expression (data not shown).

The tumorigenicity of Rat-1/P210 and NIH/P210 was assessed by injecting the cells subcutaneously into nude mice. Rat-1/P210 cells formed tumors at the site of injection with a latent period of 2 to 3 weeks (Table 1). Some of these tumors grew very large (>2 cm) but remained encapsulated and did not invade the surrounding tissue. Uninfected Rat-1 cells produced slow-growing, noninvasive tumors with a latent period of 6 to 7 weeks. Mice injected with NIH/P210 cells or with NIH 3T3 cells remained free of tumors within the 12-week observation period, with the exception of one mouse injected with NIH 3T3 cells that developed a tumor at 6 weeks.

BCR-ABL interacts synergistically with v-*myc* in Rat-1 cells. The range of partially transformed characteristics exhibited by Rat-1/P210 cells suggested that a second oncogene might be required to produce complete malignant transformation in these cells. The *myc* oncogenes were candidates, because members of this gene family have been shown to interact cooperatively with other oncogenes in a variety of experimental systems (18, 19, 34, 36, 42). We used the defective retrovirus MMCV-neo (Fig. 1) (36, 40) to introduce an activated *myc* gene derived from OK10 virus into Rat-1/P210 cells. Initially we monitored the cultures for the appearance of reverse transcriptase activity from the Mo-MuLV helper as an indication that infection with MMCV-neo was proceeding successfully. Later, Northern analysis revealed the appearance of three new messenger RNAs reactive with *neo* and v-*myc* probes: a genomic RNA of 8.0 kb and two subgenomic species of 5.0 and 4.7 kb (Fig. 3 and 7). Hybridization of *Eco*RI-digested DNA from the superinfected cells with the v-*myc* probe resulted in a diffuse pattern, indicating that there were numerous different MMCV-neo integration sites in the population (Fig. 2).

The introduction of v-*myc* into Rat-1/P210 cells caused dramatic, overt transformation. The cell morphology was grossly altered: the cells became rounded, piled on top of each other, and were very easily detached (Fig. 5D). The sizes of colonies formed in soft agar were significantly increased (Fig. 6), and the concentration of serum required to support growth in soft agar was decreased (Table 2). In nude mice, the cells formed aggressive tumors which became visible in 8 to 10 days, grew rapidly, and in several instances invaded the body wall, musculature, and retroperitoneal space around the kidneys. Control experiments that included Rat-1/P210 cells superinfected with Mo-MuLV alone indicated that these changes could not be ascribed to the presence of the helper virus. Infection of Rat-1 cells with MMCV-neo did not change their flat shape, nor did it confer anchorage independence. The behavior of MMCV-neo-infected Rat-1 cells in nude mice was very similar to that of uninfected cells.

Five soft-agar colonies of v-*myc*-infected Rat-1/P210 were established in culture as cell lines 11.1, 11.2, 11.3, 11.8, and 11.9. DNA analysis showed that each of these lines retained an intact pJW-RX provirus and had become pauci-clonal with respect to MMCV-neo integration. An apparent amplification of *neo* but not of *myc* sequences had occurred in



cultures 11.8 and 11.9, which shared an identical set of restriction fragments. Analysis of RNA and protein expression did not reveal any changes in the P210 mRNA or protein molecules, and levels of expression remained similar to those of the parental cells. There was no indication that the highly transformed properties of these cells were due to recombination of BCR-ABL with *gag* sequences. The P210 protein in these cell lines did not react with a mixture of αgag monoclonal antibodies that effectively precipitated the trimeric *gag*-BCR-ABL proteins previously found in transformed NIH 3T3 cells (7).

We carried out an experiment to determine whether this interaction between BCR-ABL and v-*myc* was a general phenomenon rather than a specific property of the particular clone of Rat-1/P210 cells that we had selected for study. We established a population of Rat-1 cells infected with a helper-free stock of MMCV-neo virus and then measured the appearance of fully transformed cells (able to form colonies in soft agar in reduced serum) after acute superinfection with a high-titer stock of pJW-RX. Although the frequency of P210-infected transformants recovered was small, as expected due to the toxicity of BCR-ABL, their plating efficiency in reduced serum was equal to that in the normal concentration of serum (Table 2). Thus, virtually every cell that was successfully infected by pJW-RX underwent complete transformation due to the concomitant expression of v-*myc*.

In striking contrast to the results obtained with Rat-1/P210 cells, the introduction of v-*myc* into NIH/P210 cells caused no obvious effect on cellular morphology or tumorigenicity. RNA expression of v-*myc* in these cells was comparable to that in Rat-1 cells, and the culture supernatant had a high level of reverse transcriptase activity, indicating successful virus spread. The cells continued to produce P210 kinase. The only evidence of any interaction between BCR-ABL and v-*myc* in NIH/P210 cells was a slight enhancement of the plating efficiency of the cells in soft agar, from 0.03 to 0.4%; the colonies remained tiny.

DISCUSSION

We have found that Rat-1 cells and NIH 3T3 cells differ markedly in their response to the transforming effect of BCR-ABL and to the combined action of BCR-ABL and v-*myc*. The differential response of these two cell lines may be due to species-specific or other differences in the biochemical expression of BCR-ABL or v-*myc*. For example, it has been shown that the human c-*fps* gene is nontransforming in rat cells because of a host cell restriction on its tyrosine kinase activity (10). Another possibility is that the intracellular compartmentalization of BCR-ABL differs between Rat-1 cells and NIH 3T3 cells. The *gag*-BCR-ABL fusion protein that is capable of transforming NIH 3T3 cells contains a putative myristylation site derived from *gag*; it has been proposed that N-terminal myristylation of this protein might elicit transforming activity by redirecting its localization within the cell (7). Finally, it is possible that NIH 3T3 cells fail to express some factor that is required to interact with BCR-ABL. We are transfecting genomic DNA

FIG. 5. Phase-contrast micrographs of Rat-1 and NIH 3T3 cell lines expressing BCR-ABL and v-*myc*. Magnification, ×100. (A) Uninfected Rat-1 cells. (B) Rat-1 cells infected with v-*myc* virus, MMCV-neo. (C) Rat-1/P210 cells. (D) Rat-1/P210 cells superinfected with v-*myc*, showing grossly altered morphology.

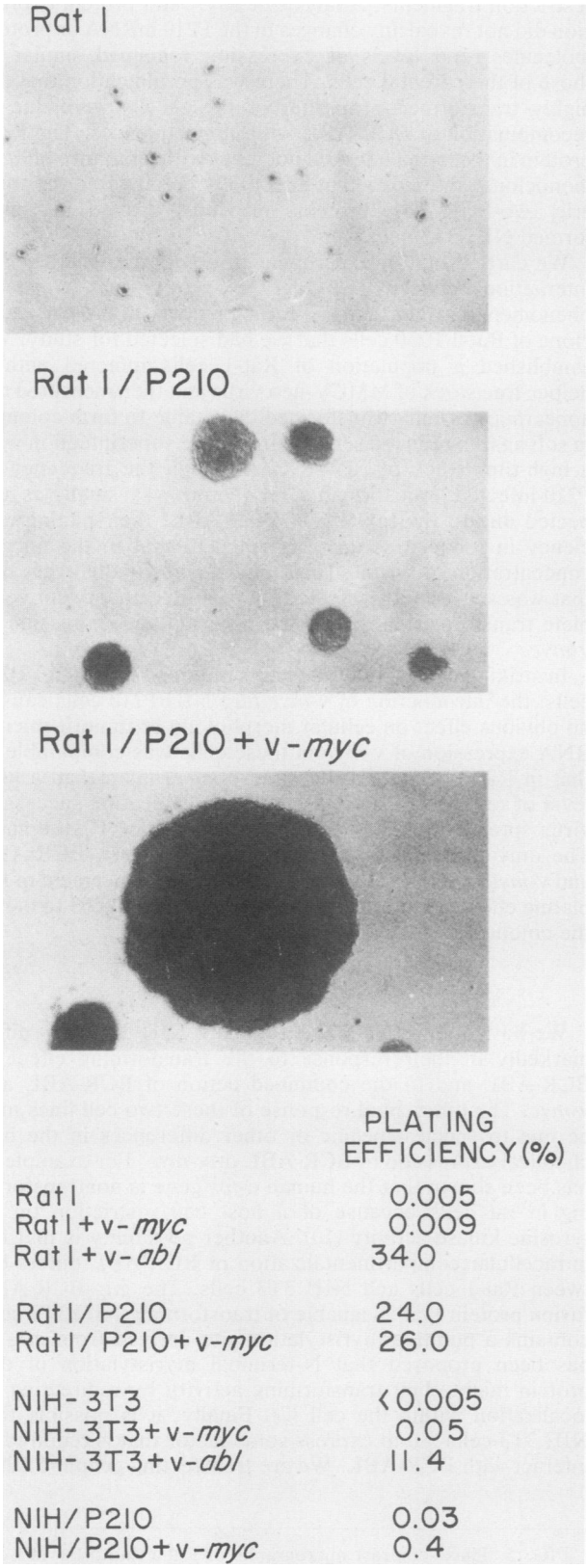


FIG. 6. Colony size and plating efficiency in soft agar. Photomicrograph magnification, ×40.

TABLE 1. Tumor challenges in nude mice

Inoculum[a]	No. of mice with tumor at week			Tumor size (cm)
	2	4	6	
Rat-1	0/8	0/8	0/7	<0.5
+ Mo-MuLV	0/3	0/3	0/2	
+ v-*myc*	0/8	0/8	2/8	0.6–1.7
+ v-*abl*	3/3			1.2–1.5
Rat-1/P210	9/11	11/11		0.4–2.5
+ Mo-MuLV	1/3	3/3		0.5–0.9
+ v-*myc*	11/11			1.0–3.0
+ v-*abl*	3/3			0.6–1.0
NIH 3T3	0/8	0/7	1/7	0.5
+ v-*myc*	0/8	0/8	0/8	
+ v-*abl*	3/3			1.2–1.4
NIH/P210	0/8	0/8	0/8	
+ v-*myc*	0/8	0/8	0/8	

[a] Inoculum = 1.5×10^6 cells injected subcutaneously.

from uninfected Rat-1 cells into NIH/P210 cells to see if we can identify such an intracellular substrate.

The biological significance of the interaction between BCR-ABL and v-*myc* requires further investigation. Experiments with primary rodent fibroblasts (18, 32) have delineated two classes of oncogenes with different subcellular locations and complementary functions: nuclear oncogenes, exemplified by *myc*, which immortalize primary cells but do not transform them, and cytoplasmic or membrane-associated oncogenes such as *ras*, which transform primary cells but cannot support their growth unless an immortalizing function is also present. The cooperative transforming effect of *myc* and *ras* has also been observed in primary cultures of hematopoietic cells (36). The relationship of these two classes to the tyrosine kinase oncogenes is not clear. The

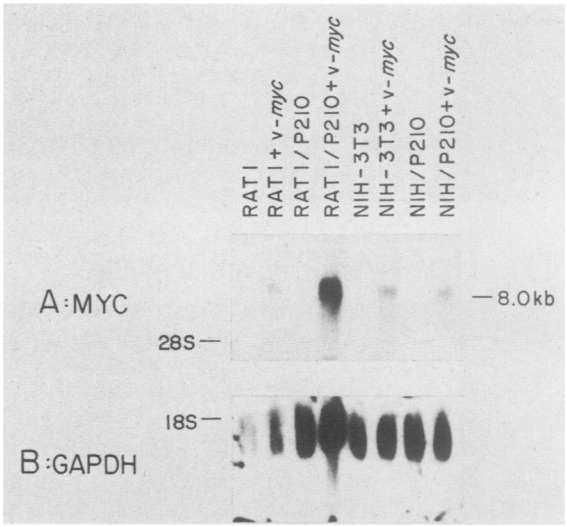


FIG. 7. Expression of v-*myc* by MMCV-neo virus. Cytoplasmic RNAs were processed for Northern analysis as described in the legend to Fig. 3 and were hybridized with probes for *myc* (A) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (B) to control for variations in loading. The position of genomic MMCV-neo mRNA is marked at 8.0 kb.

TABLE 2. Plating efficiency of Rat-1 transformants in soft agar in reduced serum

Transformant	No. of colonies/10^4 cells plated in FCS[a]	
	20%	5%
Rat-1/P210[b]	2,000	<10
Rat-1/P210 + v-*myc*	1,450	950
Rat-1 (v-*myc*)[c]	2	<1
Rat-1 (v-*myc*) + P210	30	29

[a] FCS, Fetal calf serum.
[b] Clonal line.
[c] Mass population.

prototypical tyrosine kinase oncogene, v-*src*, is capable of both immortalizing and transforming primary rat embryo fibroblasts in the absence of any additional oncogenes (13). On the other hand, the addition of v-*myc* or c-*myc* enhances the focus-forming activity of c-*src*, which by itself will not completely transform cells even when overexpressed (37).

We are exploring the interaction of other nuclear-type oncogenes, particularly activated forms of c-*myc*, with BCR-ABL in cultures of fibroblasts and hematopoietic cells. Alterations in c-*myc* expression have been observed in many natural and experimental hematopoietic tumors (for a review, see reference 6), and there is evidence that activation of c-*myc* may play a role in Ab-MuLV-induced malignancy. Plasmacytomas can be induced rapidly in susceptible mice by intraperitoneal injection of pristane and Ab-MuLV. The resulting tumors consistently have chromosome rearrangements involving translocation of the c-*myc* locus (26). We have preliminary evidence that activated forms of both murine and human c-*myc* genes can cooperate with BCR-ABL in Rat-1 cells in a manner similar to that of v-*myc*.

The response of Rat-1 cells to BCR-ABL alone and in combination with a second oncogene constitutes a model for some aspects of malignant progression which we are developing into an assay for the identification of secondary malignant changes in CML. DNAs from clinical isolates of CML and from CML cell lines have been tested for the presence of active oncogenes by the standard NIH 3T3 transfection assay (8, 12, 14, 20). Activated *ras* genes have been detected in a small minority of CML samples in this way, but the results have generally been negative. Our results indicate that the NIH 3T3 cell line is not the most sensitive indicator. We anticipate that the use of Rat-1/P210 cells as transfection recipients will enable us to detect transforming activity in CML cells that may become manifest only when the BCR-ABL function is already present.

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