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NOTES

bcr/abl and *src* but Not *myc* and *ras* Replace v-*abl* in Lymphoid Transformation

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Lymphoid cells transformed by temperature-sensitive Abelson virus die at the nonpermissive temperature. This property was exploited to show that *bcr/abl* and v-*src* but not *myc* and *ras* can replace the transforming signal of v-*abl*, a result suggesting that the former but not the latter oncogenes transform lymphoid cells via a similar pathway.

Expression of many protein tyrosine kinase (PTK) oncogenes, including v-*abl*, *bcr/abl*, v-*src*, v-*fes*, v-*fms*, and v-*erbB*, induces lymphoid cell transformation (2, 16, 20–22, 24). The cooperative action of some oncogenes unrelated to the PTK family, including v-*ras* and v-*myc* or v-*raf* and v-*myc*, can also transform these cells (1, 4, 26). The efficiency of the process varies, but in all cases, both expression of the oncogenes and secondary genetic or epigenetic events are necessary for full malignant growth (6, 10, 11, 15, 18, 28). The mechanism of transformation is not understood in any of these systems, but it is possible that all the PTK family members affect the same pathway and that the others act via a distinct pathway. However, because *ras*, *raf*, and *myc* sometimes appear to act downstream of PTK oncogenes (12, 17, 27) and mutations in *ras* and *myc* may be involved in complete transformation by PTK oncogenes (6, 13, 18), these genes could act at a different point in the pathway affected by PTK oncogenes. In either case, oncogenes using a similar pathway might be complemented by the same types of secondary changes, while those using other pathways might require different secondary events. To begin to dissect these pathways, we examined the ability of oncogenes that transform lymphoid cells to replace v-*abl* in pre-B cells transformed by temperature-sensitive (*ts*) Abelson murine leukemia virus (Ab-MLV) (8; A. Engelman and N. Rosenberg, submitted for publication) (Fig. 1).

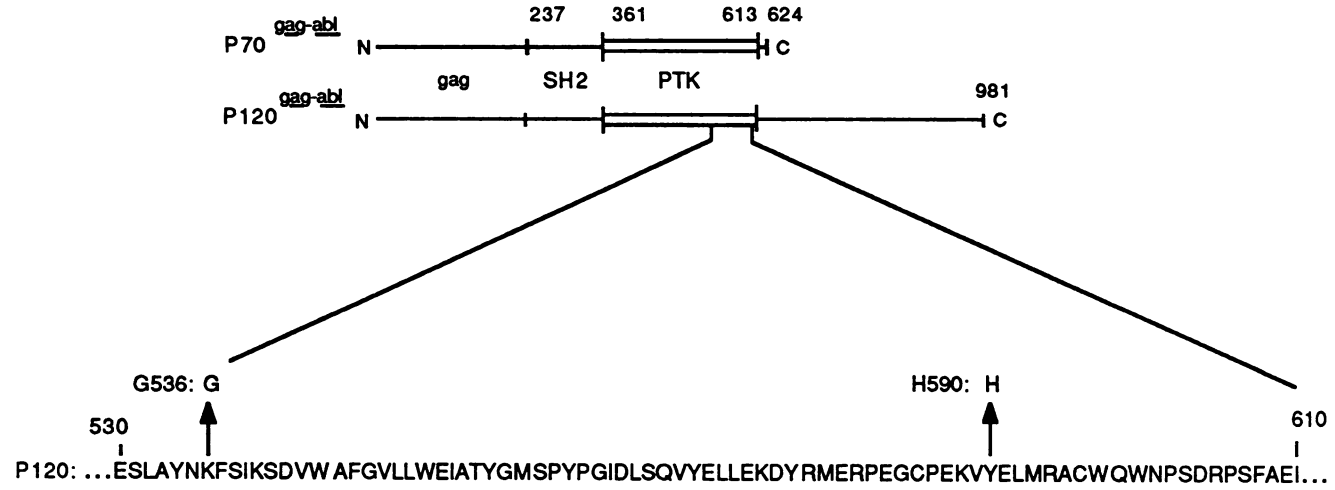

FIG. 1. Structure of Abelson proteins encoded by *ts* Ab-MLV strains. The upper portion of the figure depicts the Abelson proteins encoded by the P70 and P120 strains. Landmarks include the Moloney virus-derived *gag* sequences and the *src* homologous 2 (SH2) and catalytic domains (PTK, open box) encoded by v-*abl*. The lower portion of the figure shows the sequence of the Abelson protein between amino acids 530 and 610. The positions of the two substitutions present in the Abelson proteins encoded by the *ts* strains are noted by the arrows. Each of the strains is named for the size of the Abelson protein and, if present, the amino acid substitution. The P120/G+H strain encodes the P120 Abelson protein containing both the His-590 and the Gly-536 substitutions.

Ab-MLV is required to maintain lymphoid transformation.

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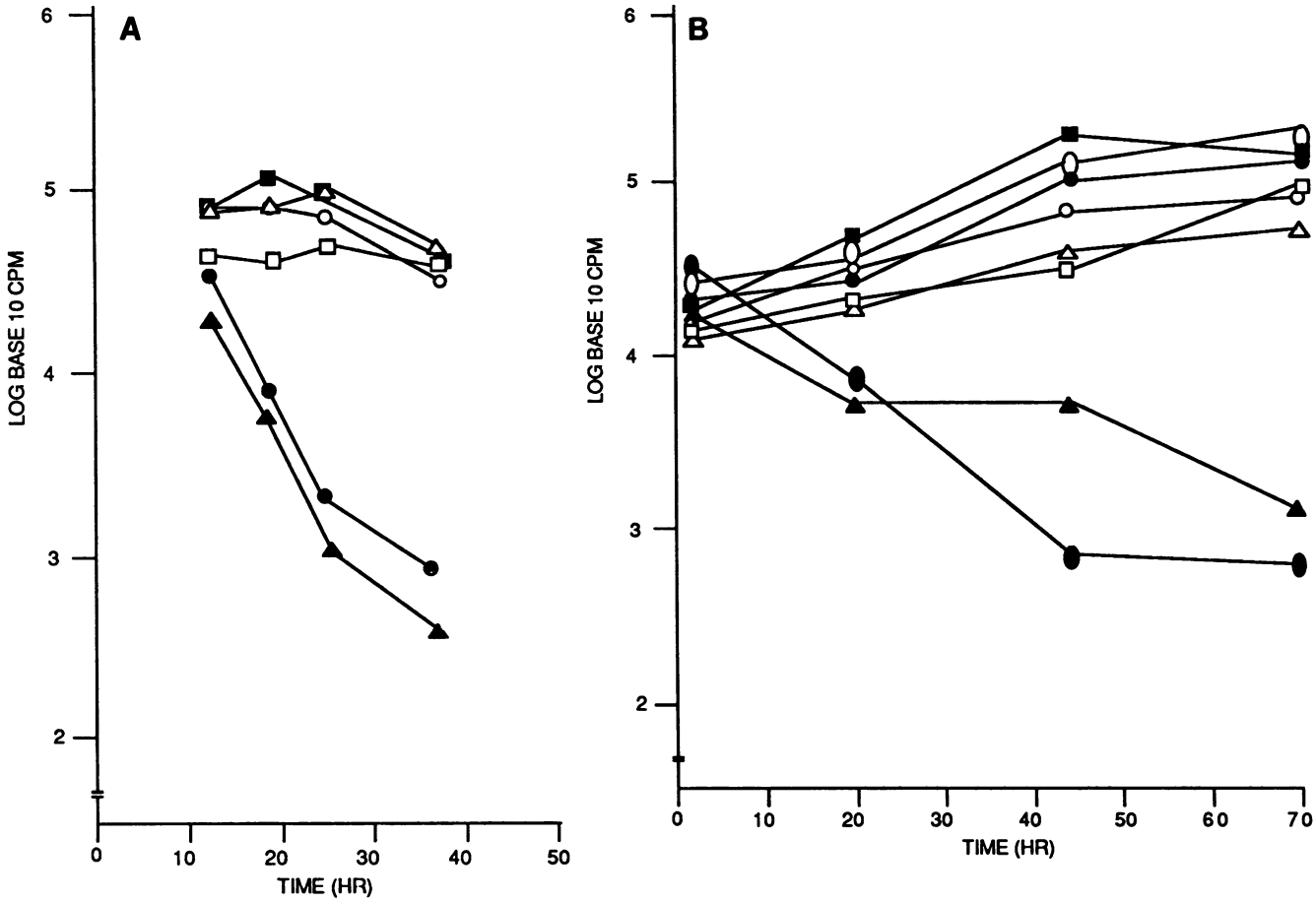


FIG. 2. Growth of lymphoid cells transformed by wild-type and *ts* Ab-MLVs. Lymphoid cell clones transformed by a panel of *ts* Ab-MLV strains were isolated as previously described (23). For the experiment shown in panel A, clones transformed by either the wild-type Ab-MLV P70 strain (□, ■) or the *ts* strains P70/H590 (○, ●) and P70/G536 (△, ▲) were plated at a density of 5×10^5 cells in microtiter wells and grown at 34.5°C (open symbols) or 39.5°C (filled symbols). For each time point, three wells were labeled with [^{3}H]thymidine (10 μCi/ml) for 2 h. The cells were harvested, and DNA synthesis was monitored by scintillation spectroscopy. Each time point represents the average of three wells. (B) Cells transformed by the wild-type strains Ab-MLV P120 (□, ■) and P120/H590 (○, ●) or the *ts* derivatives P120/G536 (△, ▲) and P120/G+H (◊, ⧫) were treated as described for panel A. For both panels, the values shown were less than 1 standard deviation from the mean in repeated experiments. Background values ranged between 2×10^2 and 5×10^2 cpm.

To determine the effect of the *ts* Ab-MLV strains on maintenance of lymphoid transformation, we plated duplicate cultures at 34.5 and 39.5°C. Samples were pulsed with [^{3}H]thymidine at regular time intervals, and incorporation of label into cellular DNA was evaluated. While cells transformed by wild-type Ab-MLV grew well at both temperatures (Fig. 2 and data not shown), those transformed by the *ts* strains P70/H590, P70/G536, P120/G536, and P120/G+H (Engelman and Rosenberg, submitted) grew well only at the low temperature. Analysis of bulk cultures of 50 independently derived lymphoid clones transformed with *ts* viruses and 21 derived with wild-type strains confirmed these findings (data not shown). Thus, a functional Abelson protein is required for maintenance of lymphoid cell transformation.

Expression of wild-type v-*abl*, v-*src*, and *bcr/abl* but not v-*ras* and c-*myc* rescues lymphoid cells transformed with *ts* Ab-MLV. An assay of the ability of other genes to replace v-*abl* was developed with the m5 and 7C411 lymphoid cell clones transformed by Ab-MLV P70/H590. These clones were chosen based on their high susceptibility to ecotropic virus infection (data not shown). Cells were infected with serial dilutions of virus and plated at 39.5°C in microtiter plates under conditions in which all wells contained viable cells at 34.5°C. Plating unmanipulated cells or cells infected with Moloney MLV revealed that 1 in 1×10^6 7C411 cells and 1 in 3×10^6 m5 cells grew at 39.5°C. Cultures infected with either the wild-type Ab-MLV P120 or P160 strain plated with a high efficiency at 39.5°C (Fig. 3). The frequency of positive wells was similar to that expected if every cell infected with a wild-type Ab-MLV survived. Similar results were obtained with MRSV (21), a murine retrovirus expressing the Schmidt-Ruppin A v-*src* gene, and JW-RX (16) (gift of J. McLaughlin, University of California, Los Angeles), a virus expressing P210$^{bcr/abl}$ cDNA (Fig. 3). The RIM virus (5) (gift of J. Devilliers) expressing both *myc* and *ras* and viruses expressing *mos* or *ras* and *myc* individually failed to rescue the cells. Both the m5 and 7C411 cell lines behaved in a similar fashion, suggesting that the response to the different oncogenes was a general property of Ab-MLV-transformed lymphoid cells.

To confirm that survival at 39.5°C correlated with expression of the rescuing virus, [^{35}S]methionine-labeled cellular extracts were immunoprecipitated and analyzed (29). Eighteen cell lines rescued with wild-type Ab-MLV, eight cell

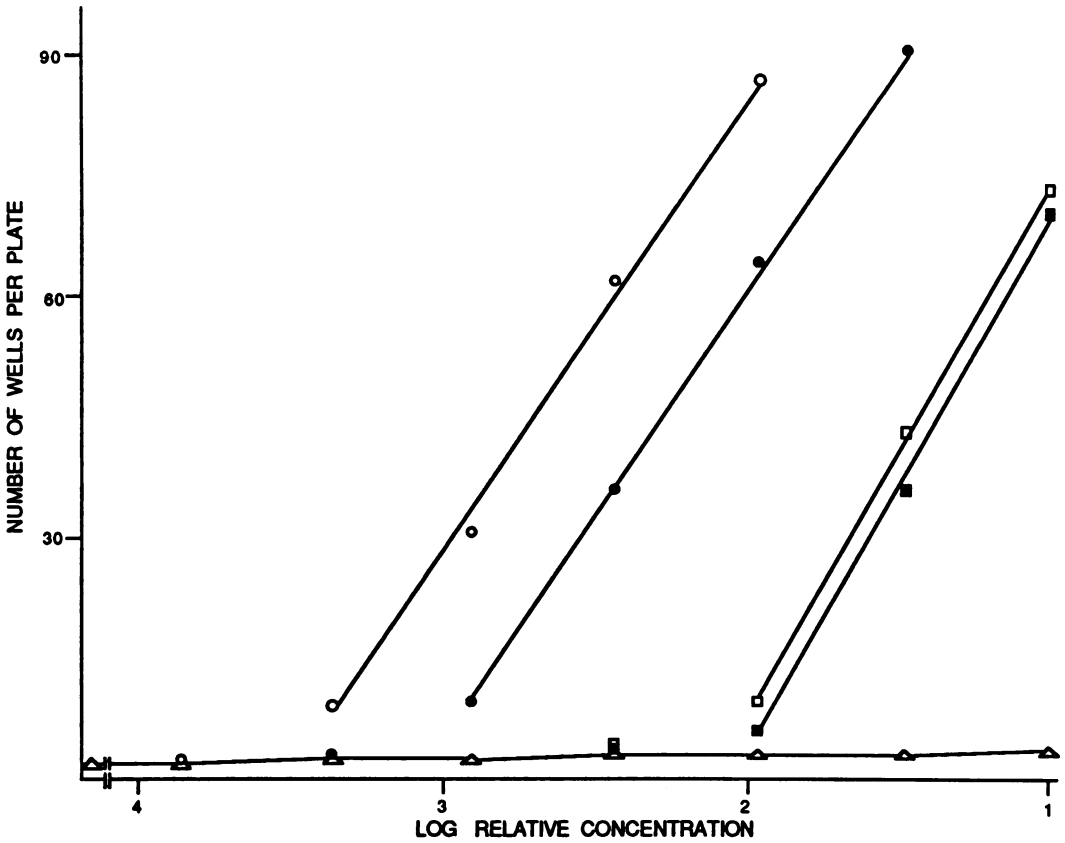


FIG. 3. Rescue of m5 cells by viruses expressing wild-type *abl*, *bcr/abl*, and *src*. To monitor the ability of the various viruses to replace v-*abl* at the nonpermissive temperature, we infected 5×10^6 m5 cells with serial dilutions of Ab-MLV P160 (24) (○), Ab-MLV P120 (24) (●), JW-RX (16) (□), MRSV (21) (■), or RIM (5) or Moloney MLV (both represented by △). After a 2-h adsorption period at 34.5°C, the cells were plated in microdilution plates and incubated at 39.5°C. The wells were fed weekly, and the number of wells containing viable cells was scored 1 month postinfection. Infection with viruses expressing *ras* or *myc* alone or *mos* or mock infection resulted in curves indistinguishable from those shown for RIM and Moloney MLV. The values shown represent the averages obtained in two independent experiments in which the frequency of positive wells varied by less than 10%. The titer of transforming virus in each stock was determined with NIH 3T3 cells. The titers of JW-RX and the *myc* virus were estimated based on formation of G418-resistant colonies of NIH 3T3 cells. All the stocks had titers of approximately 1×10^5 infectious units per ml.

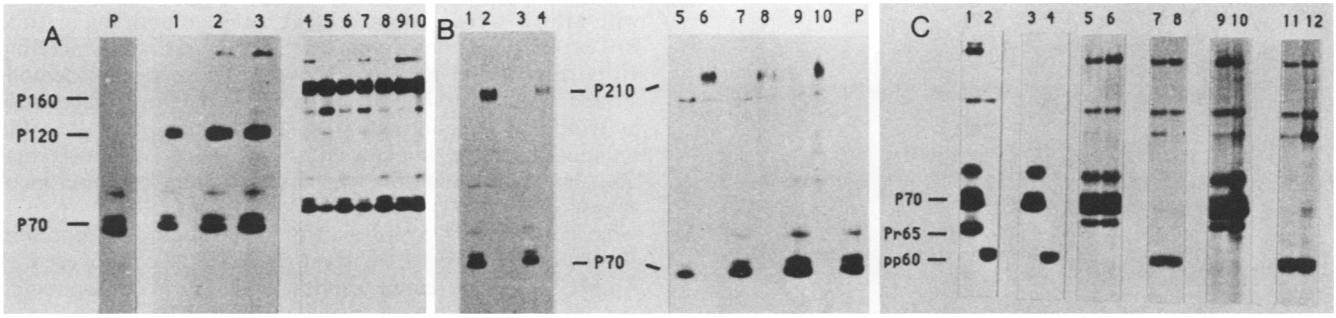


FIG. 4. 7C411 cells rescued at 39.5°C express the protein encoded by the rescuing virus. Clones of 7C411 cells infected with Ab-MLV P120 (A, lanes 1 to 3), Ab-MLV P160 (A, lanes 4 to 10), JW-RX (B), or MRSV (C) were isolated at 39.5°C. The cells were labeled with 100 μCi of [^{35}S]methionine per ml and extracted as described previously (29). Abelson protein was recovered by immunoprecipitation with the H548 monoclonal antibody that reacts with Abelson protein via the p12^{gag} determinants (3) (A and B, odd-numbered lanes; C, lanes 1, 3, 5, 6, 9, and 10); P210$^{bcr/abl}$ was recovered by using the 24-21 anti-*abl* monoclonal antibody (25) (B, even-numbered lanes); pp60$^{v\text{-}src}$ was recovered by using the anti-*src* monoclonal antibody EC10 (19) (C, lanes 2, 4, 7, 8, 11, and 12). The samples were analyzed by electrophoresis through a sodium dodecyl sulfate-10% polyacrylamide gel. The dried gels were treated with sodium salicylate and exposed to Kodak X-AR film at −70°C. Lanes P contain extracts prepared from the 7C411 parent cell line precipitated with H548. The pr65^{gag} gene precursor expressed by Moloney virus is evident in some samples precipitated with H548.

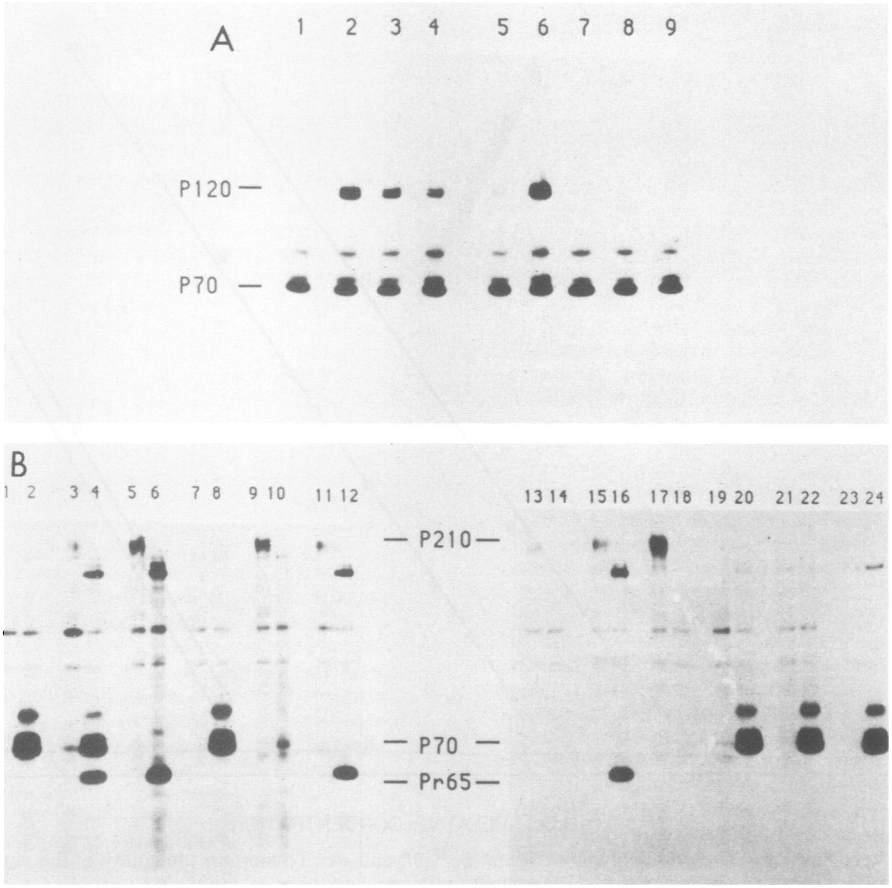
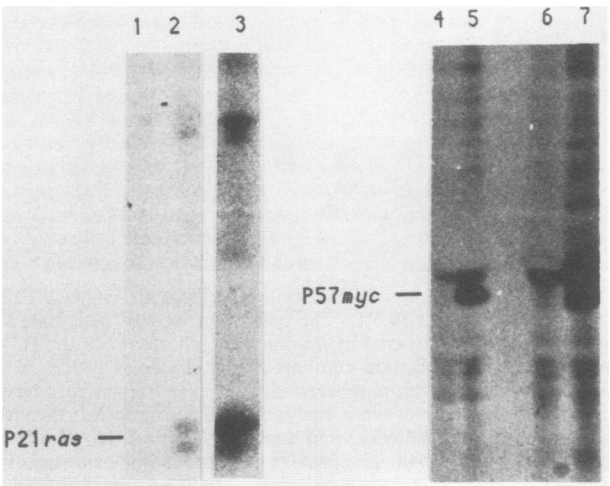


FIG. 5. Expression of *abl* proteins in cells infected with Ab-MLV P120 and JW-RX at 34.5°C. 7C411 cells were infected with either JW-RX or Ab-MLV P120-NEO (a generous gift of M. A. Kelliher, Tufts University), both of which carry a neomycin resistance gene and were selected for their ability to grow in the presence of 2 mg of G418 per ml at 34.5°C. Clones were isolated and categorized based on their ability to survive at 39.5°C. Cell lines from both groups were labeled and processed as described in the legend to Fig. 4. (A) Analysis of representative cell lines isolated after infection with Ab-MLV P120-NEO. The extracts in lanes 2 to 6 are from cells that survived incubation at 39.5°C, while those in lanes 7 to 9 are from cells that did not. The 7C411 parent cell line is shown in lane 1. (B) Analysis of representative cell lines isolated after infection with JW-RX. Abelson protein was analyzed in the even-numbered lanes, and *bcr/abl* protein was analyzed in the odd-numbered lanes. Lanes 1 and 2 contain extracts from the parent 7C411 cell line. The extracts shown in lanes 3 through 6 and 9 through 18 are from cells that survived incubation at 39.5°C, while those represented in lanes 7 and 8 and 19 through 24 are from cells that did not. The $pr65^{gag}$ precursor encoded by Moloney virus is evident in some of the samples.



lines rescued with the *bcr/abl* virus, and six cell lines rescued with MRSV were examined. All expressed both the original P70 protein and the protein encoded by the superinfecting virus (Fig. 4 and data not shown). These results demonstrated that expression of *bcr/abl* and v-*src* can replace v-*abl* in lymphoid transformation systems, suggesting that the secondary changes in place after v-*abl*-mediated transformation also complement the transforming signal of these oncogenes.

To confirm that expression of the rescuing oncoprotein always correlates with survival at 39.5°C, we recovered 21 Ab-MLV P120-infected clones and 26 JW-RX-infected

FIG. 6. Expression of *ras* and *myc* in 7C411 cells. 7C411 infected with RIM and selected for G418 resistance at 34.5°C (lanes 3, 6, and 7) and NIH 3T3 cells transformed by RIM (lanes 1, 2, 4, and 5) were labeled and analyzed as described in the legend to Fig. 4. $P21^{ras}$ was detected with the monoclonal antibody Y13-259 (9) (lanes 2 and 3); $P57^{myc}$ was detected with an anti-*myc* monoclonal antibody (lanes 5 and 7) obtained from B. Bockus, Tufts University. Precipitation of extracts with matched control antibodies is shown in lanes 1, 2, and 6.

clones at 34.5°C using G418 selection. In all cases, clones that survived at the high temperature expressed the protein encoded by the rescuing virus (Fig. 5 and data not shown). Six Ab-MLV P120-infected clones and 16 clones infected with JW-RX failed to express the rescuing protein and did not survive at 39.5°C. This feature probably reflects the presence of deletions in the rescuing viruses present in those cell lines (data not shown). When the JW-RX virus was used, only 3 of the 10 lines that survived at the high temperature retained expression of the original P70 protein (Fig. 5B). All the clones retained a grossly intact P70 provirus, but those that lacked detectable P70 protein expressed greatly reduced levels of P70-specific RNA (data not shown). This phenomenon may reflect the toxic effect of P210 that has been documented in fibroblasts (7, 14). Such an effect would have been difficult to document in other lymphoid transformation systems (16) and would not have been observed in the high-temperature rescue experiments in which the P70 protein is functionally silent.

The neomycin resistance gene in the *myc*- and *ras*-containing RIM virus was used to select infected cells at 34.5°C. Consistent with our earlier results, these cells died at 39.5°C. Because these cells expressed both *myc* and *ras* proteins (Fig. 6), these oncogenes are not sufficient to replace v-*abl*. The absence of a selectable marker gene in the viruses expressing *mos* and *ras* individually prevented confirmation of expression of these genes. However, because *myc* and *ras* were expressed in the RIM-infected cells and both *ras* and *mos* are expressed from ecotropic long terminal repeats, it is likely that these genes do not replace v-*abl* and transform cells via a different pathway.

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