reaction between bound and exogenous guanine nucleotides (*33*) and blocks GAP interaction with ras (*34*), which may explain why this antibody blocks serum-induced growth in 3T3 cells (*15*). Microinjection of this antibody showed that the transforming signals generated by several growth-factor receptor–like oncogenes (*fms*, *fes*, and *src*) were dependent on the cellular Ras protein. In contrast, the transforming signal generated by cytoplasmic oncogenes (*raf* and *mos*) were not dependent on Ras protein for activity because induction of DNA synthesis by these oncogenes was not blocked by the Y13-259 antibody (*16*). These injection experiments established a hierarchy within signal-transducing biochemical pathways for the oncogenes and showed that a downstream block could stop the transforming signal of an upstream oncogene. The antibody to PIP_2 that inhibits bombesin- and PDGF-induced DNA synthesis (*22*) also blocks mitogenesis when injected into *ras*, *src*, and *erb*B oncogene–transformed NIH 3T3 cells, whereas *myc*-transformed cells are not affected (*30*). In summary, our data indicate that PLC-γ activity is necessary for Ras-mediated induction of DNA synthesis in NIH 3T3 fibroblasts and that Ras may function as a G protein–like molecule in inositol phospholipid signal transduction.

REFERENCES AND NOTES

1. J. L. Bos *et al.*, *Nature* **327**, 293 (1987); L. F. Parada, C. J. Tabin, C. Shih, R. A. Weinberg, *ibid.* **297**, 474 (1982); E. Santos, S. R. Tronick, S. A. Aaronson, S. Pulciani, M. Barbacid, *ibid.* **298**, 343 (1982).
2. M. Barbacid, *Annu. Rev. Biochem.* **56**, 779 (1987).
3. P. H. Seeburg, W. W. Colby, D. J. Capon, D. V. Goeddel, A. D. Levinson, *Nature* **312**, 71 (1984); D. J. Clanton, Y. Lu, D. G. Blair, T. Y. Shih, *Mol. Cell. Biol.* **7**, 3092 (1987).
4. J. P. McGarth, D. J. Capon, D. V. Goeddel, A. D. Levinson, *Nature* **310**, 644 (1984).
5. C. J. Der, T. Finkel, G. M. Cooper, *Cell* **44**, 167 (1986).
6. V. Manne, S. Yamazaki, H.-F. Kung, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 376 (1985).
7. T. Y. Shih, A. G. Papageorge, P. E. Stokes, M. O. Weeks, E. M. Scolnick, *Nature* **287**, 686 (1980).
8. R. W. Sweet *et al.*, *ibid.* **311**, 273 (1984).
9. M. Trahey and F. McCormick, *Science* **238**, 542 (1987).
10. J. B. Hurley, M. I. Simon, D. B. Teplow, J. D. Robishaw, A. G. Gilman, *ibid.* **226**, 860 (1984).
11. E. J. Neer and D. E. Clapham, *Nature* **333**, 129 (1988).
12. D. W. Stacey and H.-F. Kung, *ibid.* **310**, 508 (1984).
13. J. R. Feramisco, M. Gross, T. Kamata, M. Rosenberg, R. W. Sweet, *Cell* **38**, 109 (1984).
14. M. R. Smith, S.-H. Ryu, P.-G. Suh, S.-G. Rhee, H.-f. Kung, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3659 (1989).
15. L. S. Mulcahy, M. R. Smith, D. W. Stacey, *Nature* **313**, 241 (1985).
16. M. R. Smith, S. J. DeGudicibus, D. W. Stacey, *ibid.* **320**, 540 (1986).
17. H.-F. Kung, M. R. Smith, E. Bekesi, V. Manne, D. W. Stacey, *Exp. Cell Res.* **162**, 363 (1986).
18. J. R. Feramisco *et al.*, *Nature* **314**, 639 (1985).
19. M. J. O. Wakelam *et al.*, *ibid.* **323**, 173 (1986).
20. T. Kamata and H.-f. Kung, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5799 (1988).
21. J. Meisenhelder, P.-G. Suh, S. G. Rhee, T. Hunter, *Cell* **57**, 1109 (1989).
22. K. Matuoka, K. Fukami, O. Nakanishi, S. Kawai, T. Takenawa, *Science* **239**, 640 (1988).
23. L. F. Fleischman, S. B. Chahwala, L. Cantley, *ibid.* **231**, 407 (1986).
24. M. J. Berridge and R. F. Irvine, *Nature* **312**, 315 (1984); Y. Nishizuka, *ibid.* **308**, 693 (1984); J. F. Hancock *et al.*, *Oncogene* **3**, 187 (1988).
25. J. C. Lacal, J. Moscat, S. A. Aaronson, *Nature* **330**, 269 (1987); A. Wolfman and I. G. Macara, *ibid.* **325**, 359 (1987).
26. L. Kaczmarek, J. K. Hyland, R. Watt, M. Rosenberg, R. Baserga, *Science* **228**, 1313 (1985).
27. P.-G. Suh, S.-H. Ryu, W. C. Choi, K.-Y. Lee, S. G. Rhee, *J. Biol. Chem.* **263**, 14497 (1988).
28. S. H. Ryu, P.-G. Suh, K. S. Cho, K.-Y. Lee, S. G. Rhee, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6649 (1987).
29. S. G. Rhee, P.-G. Suh, S.-H. Ryu, S. Y. Lee, *Science* **244**, 546 (1989).
30. K. Fukami *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9057 (1988).
31. I. S. Sigal, J. B. Gibbs, J. S. D'Alonzo, E. M. Scolnick, *ibid.* **83**, 4725 (1986).
32. J. C. Lacal and S. A. Aaronson, *Mol. Cell. Biol.* **6**, 1002 (1986).
33. S. Hattori *et al.*, *ibid.* **7**, 1999 (1987).
34. S. K. Srivastava, A. DiDonato, J. C. Lacal, *ibid.* **9**, 1779 (1989).

35. We thank D. Longo, J. Rossio, H. Young, and C. Faltynek for critical review of this manuscript and C. Hemp and T. Hardy for assistance in preparation of this manuscript. Funded in part by the Department of Health and Human Services under contract number N01-CO-74102 with Program Resources, Inc.

20 September 1989; accepted 11 January 1990

Translation Initiation and Ribosomal Biogenesis: Involvement of a Putative rRNA Helicase and RPL46

A. B. SACHS* AND R. W. DAVIS

Cold-sensitive mutations in the *SPB* genes (*spb1–spb7*) of *Saccharomyces cerevisiae* suppress the inhibition of translation initiation resulting from deletion of the poly(A)-binding protein gene (*PAB1*). The SPB4 protein belongs to a family of adenosine triphosphate (ATP)–dependent RNA helicases. The aberrant production of 25*S* ribosomal RNA (rRNA) occurring in *spb4-1* mutants or the deletion of *SPB2* (*RPL46*) permits the deletion of *PAB1*. These data suggest that mutations affecting different steps of 60*S* subunit formation can allow PAB-independent translation, and they indicate that further characterization of the *spb* mutations could lend insight into the biogenesis of the ribosome.

THE FUNCTION OF PROTEINS NECESSARY for the processing and assembly of ribosomal components can be explored through mutational studies. Mutations in these proteins could result in an improperly assembled ribosome capable of bypassing steps normally required for translation to be initiated. The *spb* genes (*spb1* through *spb7*) represent one class of such mutations since they suppress the requirement for the poly(A)-binding protein (PAB) in translation initiation (*1, 2*). This requirement could prevent the efficient translation of partially degraded mRNAs lacking their poly(A) tail and its associated PAB. The 60*S* ribosomal subunit probably mediates the PAB requirement because the *spb* mutations affect the amounts of this particle, and one of them (*SPB2*) encodes ribosomal protein L46 (*2*). The identification of the site of action of these mutations suggested that different alterations in the native form of the 60*S* subunit could lead to a basal level of PAB-independent translation initiation. Here we report that these alterations can result from the aberrant production of 25*S* rRNA or the deletion of *RPL46*.

The gene encoding SPB4 (suppressor of *pab1-F364L*) was identified and isolated from a yeast genomic library (*2, 3*) by its ability to rescue the recessive cold-sensitive phenotype of a *spb4-1* mutant (YAS165) (*2*), and the nucleotide sequence of the 2349-bp fragment containing the complementing activity was determined (Fig. 1). Genetic analysis showed tight linkage between this fragment and *spb4-1*. DNA and RNA blot analysis revealed *SPB4* to be a single-copy gene encoding an mRNA of approximately 2.1 kb (Fig. 2, lanes 1 to 4). A diploid strain heterozygous for a replacement of *SPB4* with *TRP1* and homozygous for *trp1* (YAS325) produced up to 50% viable spores (compared with >95% for controls), and all such spores were tryptophan auxotrophs. Furthermore, a haploid strain containing this genomic replacement could only grow in the presence of *SPB4* on a plasmid (YAS356). These experiments show that *SPB4* is essential for growth, and they imply that at the nonrestrictive temperature *spb4-1* encodes a partially functional protein.

SPB4 is a 69,430-dalton basic protein

Department of Biochemistry, Stanford Medical Center, Stanford, CA 94305.

*To whom correspondence should be addressed. Present address: Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

Fig. 1. SPB4 is homologous to a family of ATP-dependent RNA helicases. A partial nucleotide sequence (numbers 331 to 1430) of *SPB4* and its deduced amino acid sequence are shown (*13*). Positions of amino acids that are similar in all nine of the family members or identical in at least eight of the nine members are boxed (*4*). Amino acids that are absolutely conserved in all but *SPB4* are indicated below the deduced amino acid sequence. The other members are: eIF-4A1, eIF-4A2, and TIF1, which are all translational initiation factors (*14*); PL10, a male germ cell–specific protein (*7*); p68, a human nuclear protein that cross-reactions with SV40 large T-antigen (*15*); vasa, a *Drosophila* protein involved in oogenesis and posterior pole embryonic development (*5, 6*); MSS116, a yeast mitochondrial protein involved in mRNA splicing (*16*); and srmB, an *E. coli* protein that suppresses a temperature-sensitive mutation in *RPL24* when overexpressed (*11*). A minimum subfragment from a 5.3-kb insert in YCP50 that rescued the cold-sensitive growth phenotype conferred by *spb4-1* in strain YAS165 (MAT**a** *spb4-1 his3 leu2 trp1 ura3*) was sequenced by the method of Sanger *et al.* (*17*). Amino acid similarities and identities are based on the previous alignment of Linder *et al.* (*4*).

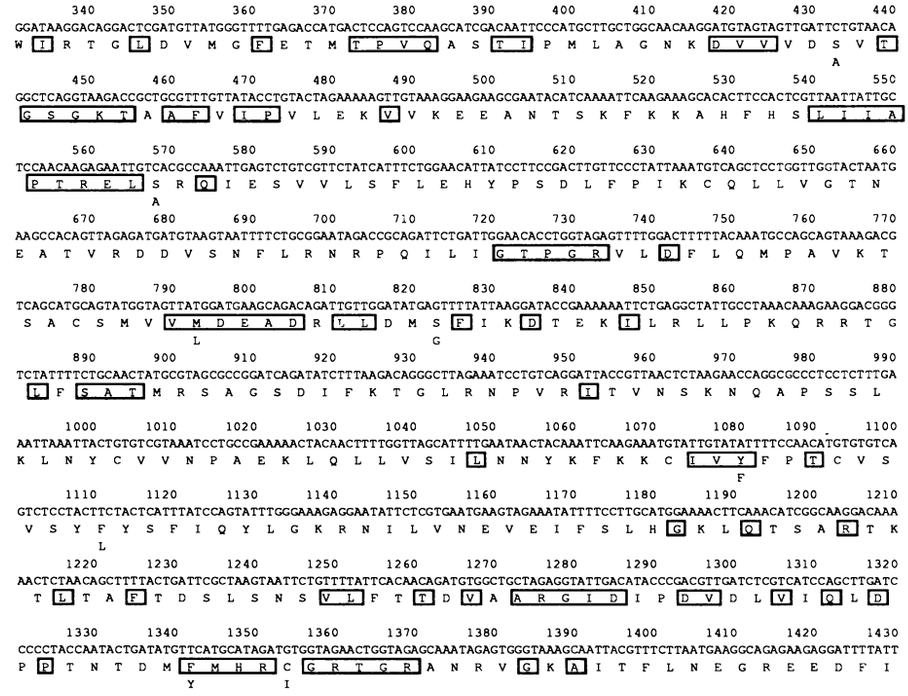

Fig. 2. *SPB4* is essential for growth, whereas a deletion of *RPL46* (*SPB2*) is tolerated and allows a deletion of *PAB1*. DNA blot analysis of *SPB4*: (lane 1) wild-type cells (YAS308, MAT**a**/α *ade2 his3 leu2 trp1 ura3*); (lane 2) diploid cells heterozygous for a genomic replacement of *SPB4* (nucleotides 131 to 1924) with *TRP1* (YAS325, MAT**a**/α *SPB4*::*TRP1*/*SPB4 ade2 his3 leu2 trp1 ura3*); and (lane 3) in haploid cells with this replacement and a centromeric plasmid (YCP50) containing *SPB4* and *URA3* (pAS24) (YAS356, MAT**a** *SPB4*::*TRP1 ade2 his3 leu2 trp1 ura3*). Bands at 5.8, 4.3, and 13 kb represent the wild-type, the deleted, and the plasmid-borne copies of *SPB4*, respectively. The deleted copy of *SPB4* has a weaker hybridization signal because of its limited homology with the radiolabeled probe. RNA blot analysis of *SPB4* (lane 4). RNA size standards in kilobases are shown. DNA blot analysis of *RPL46* in wild-type cells (lane 5) (YAS308) and in haploid cells (lane 6) containing a replacement of *RPL46* (nucleotides 786 to 1358) (*10*) with *LEU2* (YAS282, MAT**a** *RPL46*::*LEU2 his3 leu2 trp1 ura3*). Bands at 5.5 kb and at 4.2 and 3.2 kb represent the wild-type and deleted copies of *RPL46*, respectively. DNA blot analysis of *PAB1* in wild-type cells (lane 7) (YAS308) and in haploid cells (lane 8) in which *RPL46* is replaced with *LEU2* and *PAB1* is replaced with *HIS3* (1, 18) (YAS321, MAT**a** *RPL46*::*LEU2 PAB1*::*HIS3 his3 leu2 trp1 ura3*). The band at 4.3 kb and the 2.0-kb doublet represent the wild-type and deleted copies of *PAB1*, respectively. Yeast genomic DNA was digested with Hind III (*SPB4*), Eco R1 (*RPL46*), or Sal 1 and Pst 1 (*PAB1*), fractionated on a 1% agarose gel in TBE (*19*), transferred to GeneScreen, and probed with radiolabeled *SPB4* (nucleotides 34 to 2348), *RPL46* (a 2-kb fragment containing entire gene) (*10*), or *PAB1* (nucleotides 1 to 3436) (*18*). Hybridization was carried out in 50% formamide, 1*M* NaCl at 42°C, and washes were performed at 65°C in 2× SSC (standard saline citrate) (*19*). For RNA blot analysis, approximately 2 μg of poly(A)$^+$ mRNA was fractionated on a formaldehyde agarose (1.3%) gel (*19*), transferred to Nytran, hybridized to radiolabeled *SPB4* (nucleotides 131 to 1924) under the above conditions, and washed at 60°C in 2× SSC.

(isoelectric point $pI = 9.9$) that is highly homologous to a recently discovered family of ATP-dependent RNA helicases (*4*) (Fig. 1). The homology within this family of eight proteins extends throughout a conserved 370–amino acid domain, and SPB4 shares 38 of their 48 absolute amino acid conservations. None of the characteristic conserved motifs found in this family of proteins is absent in the SPB4 protein, although several of them have amino acid substitutions (Fig. 1) (*4*). Also, the glycine-glycine repeat between two of these motifs (nucleotides 580 to 720) is missing. SPB4 is ~25% identical to each of these proteins, and like two other members of this family (*5–7*), it also contains a nonhomologous basic ($pI = 10.0$) carboxyl terminus. The lack of greater homology to any of these proteins and the presence of a nonhomologous carboxyl terminus suggest that SPB4 has a distinct function within this family.

Mutations in *SPB4* decrease the amount of 60*S* ribosomal subunit (*2*). Because of the homology with proteins involved in RNA metabolism, we investigated whether this effect of the *spb4-1* mutation was due to changes in rRNA processing by performing a pulse-chase analysis (*8, 9*) at 30°C. At this temperature *spb4-1* retains its altered activity, as evidenced by its ability to suppress a null mutation in *PAB1* (*2*). The rate of maturation of 25*S* rRNA relative to 18*S* rRNA was significantly decreased in the *spb4-1* mutant (Fig. 3). Furthermore, mature radiolabeled 25*S* rRNA was approximately 2.5-fold less abundant in the mutant, suggesting that much of the inefficiently processed rRNA was degraded. These data indicate that the *spb4-1* mutation results in alterations in the production of 25*S* rRNA.

The *RPL46* (*SPB2*) gene encodes a 51–amino acid ribosomal protein (*10*), and mutations within it suppress a deletion of *PAB1* and result in cold-sensitive growth (*2*). Replacement of the single *RPL46* gene with *LEU2* in haploid cells is tolerated at 30°C (Fig. 2, lanes 5 and 6), although it prevents cell growth at 18°C in all strains and at 37°C in some strains. All strains containing the *RPL46* deletion are also viable at 30°C when *PAB1* is deleted (Fig. 2, lanes 7 and 8), indicating that removal of RPL46 from the large ribosomal subunit

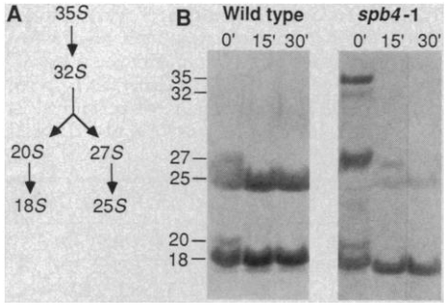

Fig. 3. *SPB4* is involved in the maturation of 25*S* rRNA. (**A**) Schematic representation of the processing of pre-rRNA in *S. cerevisiae*. (**B**) Pulse-chase analysis of pre-rRNA processing in wild-type cells (YAS43, MAT**a** *his3 leu2 trp1 ura3*) and the *spb4-1* mutant (YAS165). The location of the different rRNAs on the gel are shown, and the length of the chase period in minutes is indicated. Approximately 10^9 exponentially growing cells in 45 ml of minimal medium supplemented with the auxotrophic requirements (*20*) were concentrated to 1 ml, and the rRNA was pulse-labeled for 6 min with 50 μCi of [methyl-^{3}H]methionine (Amersham, 75 Ci/mmol) at 30°C (rRNA is selectively labeled in this procedure by methylation through the methyl donor *S*-adenosylmethionine) (*8*). The chase period was initiated by dilution of cells into 45 ml of minimal medium plus 1 mg/ml unlabeled methionine. Samples (10 ml) were withdrawn at the indicated times, and total RNA was prepared as previously described (*18*). RNA quantities yielding equivalent 18*S* rRNA band intensities (~20,000 cpm) were denatured with glyoxal and fractionated on a 1% agarose gel in 10 m*M* sodium phosphate, *p*H 7.0 (*19*), fixed in sodium salicylate (*21*), and visualized by fluorography for 5 days at −70°C. Some mature rRNA appears in the 0′ lanes because of the length of the pulse-labeling. The slightly altered 18*S* rRNA migration seen in the *spb4-1* chase lanes is not reproducible between experiments.

bypasses the cellular requirement for PAB. These results suggest that the *spb2* mutants previously isolated (*2*) are deficient in a functional *RPL46* gene product.

The discovery of a putative RNA helicase that is involved in the maturation of rRNA defines a new substrate for this family of proteins. The availability of the *spb4* conditional mutations, together with the potential of developing an in vitro assay for the protein's putative ATPase and helicase activities, should allow the identification of the essential residues for these functions. The srmB protein of *Escherichia coli* is also a member of this family, and its overexpression allows the assembly of the 50*S* ribosomal subunit in the presence of a temperature-sensitive *RPL24* mutation (*11*). Whether this protein is utilized for unwinding rRNA in a wild-type background, and whether this potential activity is similar to that of SPB4, remains to be determined.

The experiments presented here suggest that the formation of the 60*S* ribosomal subunit involves specific, enzymatic unwinding of the rRNA by SPB4. It is unlikely that SPB4 is part of the mature ribosome since ribosomal proteins of this molecular weight have not been reported (*12*). SPB4 could be part of the preribosomal particle in the nucleus that is involved in ribosomal biogenesis. *Spb4-1* might exert its suppressor effect by changing the regions of rRNA that are unwound during subunit assembly, thereby altering the structure of the subunit and possibly its protein components. For example, RPL46 and other proteins may not associate with this modified subunit, and this could allow a basal level of PAB-independent translation initiation. We suspect that equivalent mutations in helicases involved in the assembly of other ribonucleoproteins, like the splicesome, could have analogous effect on their composition and specificity.

If PAB-independent translation is made possible by altering the native 60*S* subunit structure, then a detailed analysis of the *spb* mutants will yield much information about the multiple gene products that play a role in the assembly of this particle. The ability to positively select for the *spb* mutations (*2*) will greatly simplify these studies. Future work will focus on determining if the 60*S* ribosomal subunits in *spb1–spb7* mutants have distinct compositions of ribosomal proteins, and how the requirements of PAB for initiation is mediated by this subunit.

REFERENCES AND NOTES

1. A. B. Sachs *et al.*, *Mol. Cell. Biol.* **7**, 3268 (1987).
2. A. B. Sachs and R. W. Davis, *Cell* **58**, 857 (1989).
3. M. D. Rose *et al.*, *Gene* **60**, 237 (1987).
4. P. Linder *et al.*, *Nature* **337**, 121 (1989).
5. P. F. Lasko and M. Ashburner, *ibid.* **611**, **335** (1988).
6. B. Hay, L. Y. Jan, Y. N. Jan, *Cell* **55**, 577 (1988).
7. P. Leroy *et al.*, *ibid.* **57**, 549 (1989).
8. D. R. Kief and J. R. Warner, *Mol. Cell. Biol.* **1**, 1007 (1981).
9. D. Finley *et al.*, *Nature* **338**, 394 (1989).
10. J. R. Leer *et al.*, *Nucleic Acids Res.* **13**, 701 (1985).
11. K. Nishi *et al.*, *Nature* **336**, 496 (1988).
12. J. R. Warner, *Micro. Rev.* **53**, 256 (1989).
13. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
14. P. J. Nielsen *et al.*, *Nucleic Acids Res.* **13**, 6867 (1985); P. J. Nielsen and H. Trachsel. *EMBO J.* **7**, 2097 (1988); P. Linder and P. P. Slonimski, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2286 (1989).
15. M. J. Ford *et al.*, *Nature* **332**, 736 (1988).
16. B. Seraphin *et al.*, *ibid.* **337**, 84 (1989).
17. F. Sanger, S. Nicklen, A. R. Coulsen, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
18. A. B. Sachs *et al.*, *Cell* **45**, 827 (1986).
19. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
20. F. Sherman, G. R. Fink, C. Lawrence, *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986).
21. J. P. Chamberlain, *Anal. Biochem.* **98**, 132 (1979).

22. We thank S. Elledge, P. Foreman, S. Klapholz, S. Marqusee, J. McCusker, M. Sachs, and M. Walberg for many helpful discussions. Supported by a Public Health Service grant R37 GM 21891 from NIH to R.W.D. These sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X16147.

3 October 1989; accepted 26 December 1989

Tyrosine Kinase Activity and Transformation Potency of *bcr-abl* Oncogene Products

TRACY G. LUGO, ANN-MARIE PENDERGAST, ALEXANDER J. MULLER, OWEN N. WITTE

Oncogenic activation of the proto-oncogene c-*abl* in human leukemias occurs as a result of the addition of exons from the gene *bcr* and truncation of the first *abl* exon. Analysis of tyrosine kinase activity and quantitative measurement of transformation potency in a single-step assay indicate that variation in *bcr* exon contribution results in a functional difference between p210$^{bcr\text{-}abl}$ and p185$^{bcr\text{-}abl}$ proteins. Thus, foreign upstream sequences are important in the deregulation of the kinase activity of the *abl* product, and the extent of deregulation correlates with the pathological effects of the *bcr-abl* proteins.

AMINO-TERMINAL DELETIONS FREquently activate tyrosine kinase oncogene products [for example, *abl* (*1*), *src* (*2*), and *erb*B (*3*)]. The activation of c-*abl* in human leukemias occurs after a chromosomal translocation that replaces the first exon of c-*abl* with sequences from *bcr*, which results in the generation of a fused *bcr-abl* transcript (*4*). Alternative forms of the *bcr-abl* oncogene product, p210$^{bcr\text{-}abl}$ and p185$^{bcr\text{-}abl}$, are characteristic of chronic myelogenous leukemia (CML) and Philadelphia chromosome–positive acute lymphocytic leukemia (ALL), respectively. In ALL the breakpoint on chromosome 22 occurs within the first intron of *bcr*, whereas in

Department of Microbiology and Molecular Biology Institute and the Howard Hughes Medical Institute, University of California, Los Angeles, CA 90024.