

are located on the viral gp120 envelope antigen, and that these epitopes are also found on the recombinant form of the envelope glycoprotein.

These results point to one possible route toward the production of a safe and efficacious vaccine for the prevention of infection by the AIDS retrovirus. While the demonstration here of virus neutralization *in vitro* is encouraging, the use of an animal model, such as chimpanzees, will be a necessary next step (19).

REFERENCES AND NOTES

1. J. Curran *et al.*, *Science* 229, 1352 (1985).
2. F. Barré-Sinoussi *et al.*, *ibid.* 220, 868 (1983).
3. R. Gallo *et al.*, *ibid.* 224, 500 (1984).
4. J. Levy *et al.*, *ibid.* 225, 840 (1984).
5. D. Klatzmann *et al.*, *ibid.*, p. 59.
6. C. Harris *et al.*, *Ann. Intern. Med.* 103, 623 (1985).
7. D. Francis and J. Pettriciani, *N. Engl. J. Med.* 313, 1586 (1985).
8. L. Lasky, D. Dowbenko, C. Simonsen, P. Berman, *BioTechnology* 2, 527 (1984); P. Berman, T. Gregory, D. Crase, L. Lasky, *Science* 227, 1490 (1985).
9. M. Muesing *et al.*, *Nature (London)* 313, 450 (1985).
10. F. Barin *et al.*, *Science* 228, 1094 (1985).
11. G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* 77, 1496 (1980); D. Perlman and H. Halvorson, *J. Mol. Biol.* 167, 394 (1983).
12. R. Eisenberg, D. Long, R. Hogue-Angelleti, G. Cohen, *J. Virol.* 49, 265 (1984).
13. L. Lasky and P. Berman, unpublished results.
14. F. Veronese *et al.*, *Science* 229, 1402 (1985).
15. An immunoaffinity column was produced by incubating the IgG fraction isolated from an AIDS retrovirus-seropositive patient with CNBr-activated Sepharose beads. The serum-free medium conditioned by an envelope-producing cell line was concentrated 100-fold by ultrafiltration using an Amicon ym-10 filter and passed over this column at room temperature at a flow rate of 0.5 ml/min. The column was washed with high and low salt containing buffers and was eluted at pH 3.3 with acetate buffer. The eluted material was immediately neutralized, dialyzed, and then concentrated.
16. M. Robert-Guroff, M. Brown, R. Gallo, *Nature (London)* 316, 72 (1985).
17. P. Clapham and R. Weiss, personal communication.
18. R. Weiss *et al.*, *Nature (London)* 316, 69 (1985).
19. H. Alter *et al.*, *Science* 226, 549 (1984).
20. C. Simonsen and A. Levinson, *Mol. Cell. Biol.* 3, 2250 (1983).
21. M. Lusky and M. Botchan, *Nature (London)* 293, 79 (1981).
22. G. Urlaub and L. Chasin, *Proc. Natl. Acad. Sci. U.S.A.* 77, 4216 (1980).
23. J. Nunberg, R. Kaufman, A. Chang, S. Cohen, R. Schimke, *Cell* 19, 355 (1980); C. Simonsen and A. Levinson, *Proc. Natl. Acad. Sci. U.S.A.* 80, 2495 (1983).
24. We thank D. Gallo of the California State Public Health Department for providing samples of [³⁵S]methionine-labeled AIDS retrovirus. We also thank R. Cazares for expert assistance in the preparation of this manuscript. J.E.G. and P.M.B. were supported in part by grants from the National Heart, Lung and Blood Institute (HL 33774-01) and the New England Deaconess Cancer and Immunodeficiency Research Fund.

1 May 1986; accepted 12 June 1986

The Chronic Myelogenous Leukemia-Specific P210 Protein Is the Product of the *bcr/abl* Hybrid Gene

YINON BEN-NERIAH, GEORGE Q. DALEY,
ANNE-MARIE MES-MASSON, OWEN N. WITTE, DAVID BALTIMORE

Chronic myelogenous leukemia (CML) is a human disease associated with a consistent chromosomal translocation that results in sequences from the *c-abl* locus on chromosome 9 being fused to sequences in a breakpoint cluster region (*bcr*) on chromosome 22. CML cells have two novel products: an 8.5-kilobase RNA transcript containing both *abl* and *bcr* and a 210-kilodalton phosphoprotein (P210) recognized by *v-abl*-specific antisera. To test whether the P210 is the product of the novel 8.5-kilobase *bcr/abl* fusion transcript, antibodies were prepared against *c-abl* and *bcr* determinants. By using these reagents and *v-abl*-specific antisera, it was demonstrated that the P210 in CML cells is indeed the protein product of the 8.5-kilobase transcript. By analogy to the *gag/abl* fusion protein of Abelson murine leukemia virus, the replacement of amino terminal *c-abl* sequences by *bcr* sequences in P210 may create a transforming protein involved in CML. A 190-kilodalton phosphoprotein that is a candidate for the normal *bcr* protein was identified in both HeLa and K562 cells.

CHRONIC MYELOGENOUS LEUKEMIA (CML) cells contain the Philadelphia chromosome, a product of a reciprocal exchange of distal segments of the long arms of chromosomes 9 and 22 (1, 2). The breakpoints of the t(9;22) occur near to but at variable distances from the *c-abl* locus on chromosome 9, and are clustered within a small (6-kb) region of chromosome 22 designated the breakpoint cluster region, or *bcr* (3). The translocation is associated with two novel products: a *bcr/c-abl* transcript of 8.5 kb (4), larger than the normal *c-abl* messenger RNA (mRNA) sizes of 6 and 7 kb seen in most tissues (5); and a phosphoprotein that cross-reacts with *v-abl*-specific antisera and has a molecular mass of 210 kD, larger than the normal *c-abl* protein which has a molecular mass of 145 kD (6). From sequences of complementary DNA (cDNA), the 8.5-kb mRNA represents a

fusion of *bcr* sequences to *c-abl* sequence at just the point where the multiple 5' exons of *c-abl* are fused to the common body of the *c-abl* mRNA. The exon at which the fusion occurs, the most upstream of the common set of *c-abl* exons, has been called the "common exon" (7).

It was suggested that the 210-kD phosphoprotein (P210) found in CML cells is the protein product of the hybrid *bcr/abl* transcript (8). If P210 is the product of the CML-specific 8.5-kb transcript, it should contain, in addition to determinants which cross-react with *v-abl* antisera, *c-abl* common exon determinants as well as *bcr* determinants. We have prepared a set of immunologic reagents specific to either *c-abl* or *bcr* and have used them to test the relationship of P210 to the 8.5-kb *bcr/abl* transcript. The results indicate that P210 represents a fusion of parts of two normal cellular pro-

teins and is the product of the *bcr/abl* hybrid transcript.

In Fig. 1A, the proposed structure of the P210 coding region is shown with the positions of the five peptides used for immunization indicated. The *bcr* antiserum was prepared against a β -galactosidase/*bcr* fusion protein expressed in a pEX vector (9, 10) (Fig. 1B). The antiserum to the *c-abl* common exon was prepared against a synthetic peptide representing the sequence close to the 5' border of the exon (Fig. 1C). The specificity of this serum was demonstrated by radioimmunoassay (11). The *v-abl*-specific antisera pEX2, pEX4, and pEX5 were described previously (12). pEX2 recognizes the protein kinase domain of *v-abl*; pEX4 and pEX5 are directed against carboxyl terminal determinants of *v-abl*.

The specificity of the *c-abl* antiserum was examined by immunoprecipitation of the normal *c-abl* protein from HeLa cells. Extracts immunoprecipitated with *c-abl* antisera were processed for *in vitro* kinase assay with γ -³²P-ATP as described (13) and ³²P-labeled proteins were displayed by electrophoresis. Under these conditions, *v-abl* antisera (pEX4 and pEX5) and the antiserum to the *c-abl* common exon precipitated the same phosphorylated protein of 140 kD previously described as the P145 *c-abl* protein (13) (Fig. 2, lanes 1 and 2). The immunoprecipitation of this 140-kD phosphoprotein with the antiserum to *c-abl* common exon peptide could be completely inhibited by its cognate peptide (Fig. 2, lane

Y. Ben-Neriah, G. Q. Daley, D. Baltimore, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, and Massachusetts Institute of Technology, Department of Biology, Cambridge, MA 02139.

A.-M. Mes-Masson and O. N. Witte, Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA 90024.

3), whereas irrelevant peptides had no effect, implying that the serum specifically recognizes the common exon of *c-abl*. The apparent autophosphorylation of this 140-kD protein reflects the kinase activity of the normal *c-abl* protein (13).

We used these *abl*-specific immunologic reagents to examine the antigenic determinants on the P210 protein from CML cells. Protein extracts from the CML cell line K562 were immunoprecipitated with the different antisera, incubated with γ - 32 P-ATP, and displayed by electrophoresis. As shown previously, the *v-abl*-specific antisera pEX2, pEX4, and pEX5 precipitated a P210 phosphoprotein from K562 cells (Fig. 3, lanes 7 to 9). The pEX2 antiserum inhibits the *in vitro* kinase activity of P210 (6) and therefore results in a reduced signal on autoradiograms (Fig. 3, lane 8). Antiserum to the *c-abl* common exon could efficiently precipitate the 210-kD phosphoprotein from K562 cells (Fig. 3, lane 10). The addition of the cognate common exon peptide to the reaction virtually completely inhibited the immunoprecipitation of P210 (Fig. 3, lane 11), demonstrating the specificity of the immunoprecipitation. In all cases, the 32 P label in the P210 protein was not removed by treating the electrophoretic gels with alkali (Fig. 3B), implying that the protein was phosphorylated on tyrosine residues. Thus, the P210 protein in CML cells shares with the normal *c-abl* P140 the common exon determinant as well as the three *v-abl*-specific determinants, pEX2, pEX4, and pEX5.

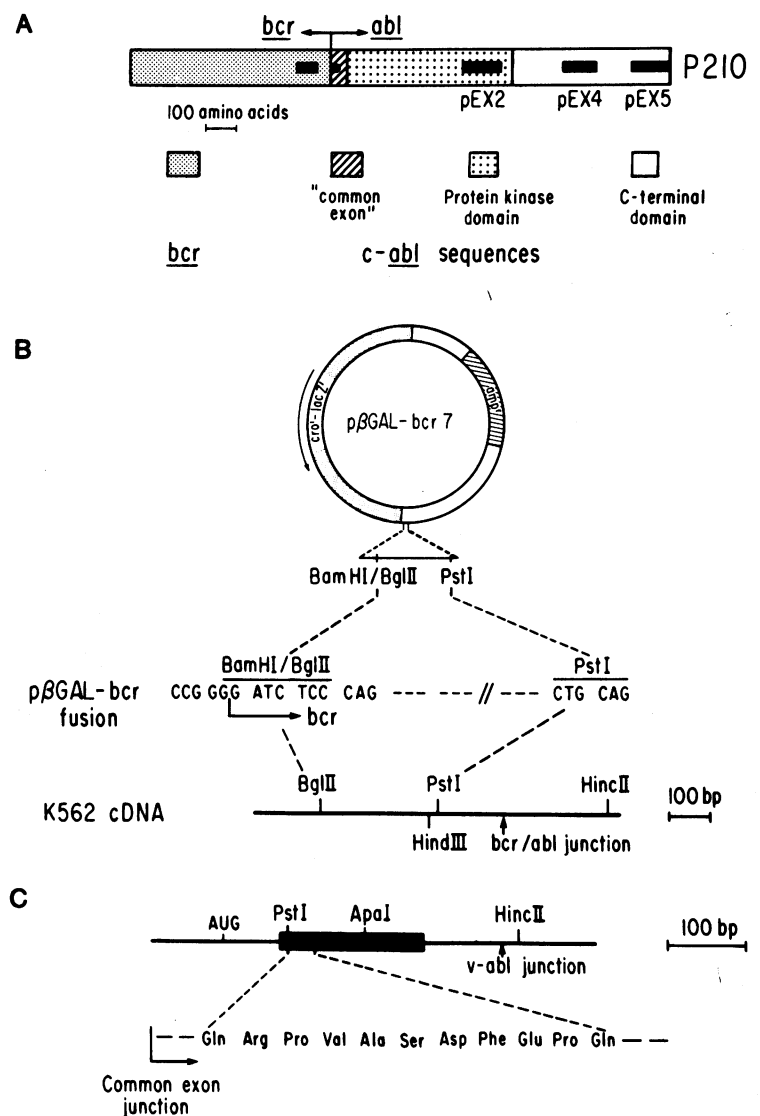
Antisera from two rabbits immunized against the β -galactosidase/*bcr* fusion protein were used to examine whether P210 also contained *bcr* determinants. The preimmune sera precipitated a faintly evident 200-kD background band from both K562 and HeLa cells (Fig. 3A, lanes 1 and 4) which was more evident when immune sera were used (lanes 2, 3, 5, and 6). From K562 cells, the *bcr* antisera precipitated a 210-kD protein (lanes 3 and 6) that was even more clearly evident after alkali treatment of the gel (Fig. 3B). This protein was not immunoprecipitated from HeLa cells (Fig. 3, lanes 2 and 5). Thus, the 210-kD protein precipitated by the *bcr* antiserum had the properties of P210 (6).

To test directly whether the 210-kD protein in K562 cells carried both *bcr* and *abl* determinants, we performed a sequential double immunoprecipitation experiment. K562 cell extracts were first reacted with a mixture of *v-abl* antisera directed against the pEX4 and pEX5 determinants. The precipitated proteins were processed for *in vitro* kinase activity, dissociated from the protein A-Sepharose, and immunoprecipitated a second time with different sera. The *v-abl* antisera (Fig. 3, lane 13), the antiserum to the *c-abl* common exon (lane 14), and the *bcr* antiserum (lane 12) reprecipitated the P210 protein, whereas the preimmune serum did not (lane 15). Again, the precipitated P210 32 P label resisted alkali treatment (Fig. 3B); these results demonstrate that the P210 protein from K562 cells carries *bcr* determinants in addition to *c-abl* determinants.

The *bcr* antiserum also precipitated a 190-kD protein in addition to P210. The protein was evident in HeLa cells (Fig. 3A, lanes 2 and 5) as well as in K562 cells (lanes 3 and 6). The *abl* antisera did not precipitate a band of this molecular weight nor did another antiserum to a β -galactosidase fusion protein unrelated to *bcr* (14). The 32 P label in the P190 band was sensitive to alkali digestion (Fig. 3B) and was shown by phos-

phorylation. The *bcr* antiserum also precipitated a 190-kD protein in addition to P210. The protein was evident in HeLa cells (Fig. 3A, lanes 2 and 5) as well as in K562 cells (lanes 3 and 6). The *abl* antisera did not precipitate a band of this molecular weight nor did another antiserum to a β -galactosidase fusion protein unrelated to *bcr* (14). The 32 P label in the P190 band was sensitive to alkali digestion (Fig. 3B) and was shown by phos-

Fig. 1. Immunogens specific for *c-abl* and *bcr*. (A) Diagrammatic assignment of the different antigenic determinants recognized by the tested antisera to the proposed primary structure of P210. (B) Construction of β -galactosidase/*bcr* expression vector. A 270-bp Bgl II–Pst I fragment from the K562 cDNA clone K28 (4) was cloned into the Bam HI and Pst I sites of the pEX2 vector (9). Sequence analysis around the Bam HI/Bgl II fusion site was performed by the method of Maxam and Gilbert (19) to confirm the proper reading frame. The p β GAL-*bcr*7 plasmid was grown in the bacterial strain POP2136 at 30°C to an OD₆₀₀ of 0.2 and then induced for protein expression at 42°C for 2 hours; under these conditions the fusion protein was expressed at levels >2 μ g per milliliter of culture. The fusion protein was precipitated from lysozyme–Triton X-100 lysates of the bacteria, dissolved in sodium dodecyl sulfate (SDS)– β -mercaptoethanol buffer, and displayed by SDS–polyacrylamide gel electrophoresis. The fusion protein band was cut out of the gel and homogenized in phosphate-buffered saline with a Polytron mixer. Two milliliters of homogenized material containing 20 to 50 μ g of the fusion protein were injected intradermally in New Zealand White rabbits. Blood was withdrawn after the fourth bimonthly immunization. (C) Sequence and position of the *c-abl* common exon peptide. A synthetic peptide corresponding to residues 30 to 41 of *c-abl* type I (7) was purchased from the Peptide Synthesis Unit of Children's Hospital Medical Center (Boston), coupled to KLH (20), and used to raise antiserum (21). The box represents the common exon of *c-abl*. The upstream Gln is the third amino acid of the common exon.



phoamino acid analysis (15) to represent phosphoserine. This protein appears to be the normal product of the *bcr* locus, although further analysis of it is required. It could be a serine kinase that is autophosphorylated or a substrate for a serine kinase that is not itself evident. The individual *bcr* antisera precipitated proteins of lower molecular weight that might be products of the *bcr* locus but, because their occurrence is sporadic, they are likely to be spurious.

The ability of both the *bcr* antisera and the antiserum to the *abl* common exon to immunoprecipitate the P210 protein confirms the previous suggestion (8) that the *bcr/abl* 8.5-kb mRNA found in CML cells encodes the P210 tyrosine protein kinase.

Because the antiserum to the common exon was raised against a 5' terminal peptide (Fig. 1C), the precipitation of P210 is consistent with a fusion point at the 5' end of the common exon. The *bcr* antiserum was raised against a peptide near the 3' end of the *bcr* exon that fuses to *abl* (Fig. 1B), again confirming the proposed structure of P210 shown in Fig. 1A.

The common exon of *abl* is an acceptor for splices from four *c-abl* upstream exons (7) as well as two *bcr* exons (16). The replacement of the normal 5' ends of *abl* with *bcr* sequences is presumed to convert *c-abl* to an oncogene, just as *gag* sequences do for *v-abl* in Abelson murine leukemia virus and the Hardy-Zuckerman-2 feline

sarcoma virus (17). In this regard it is significant that a transforming protein can be created by simply replacing the normal *c-abl* amino terminus with *gag* sequences (18), suggesting that the important differences between *v-abl* and *c-abl* involve the substitution of the amino terminus.

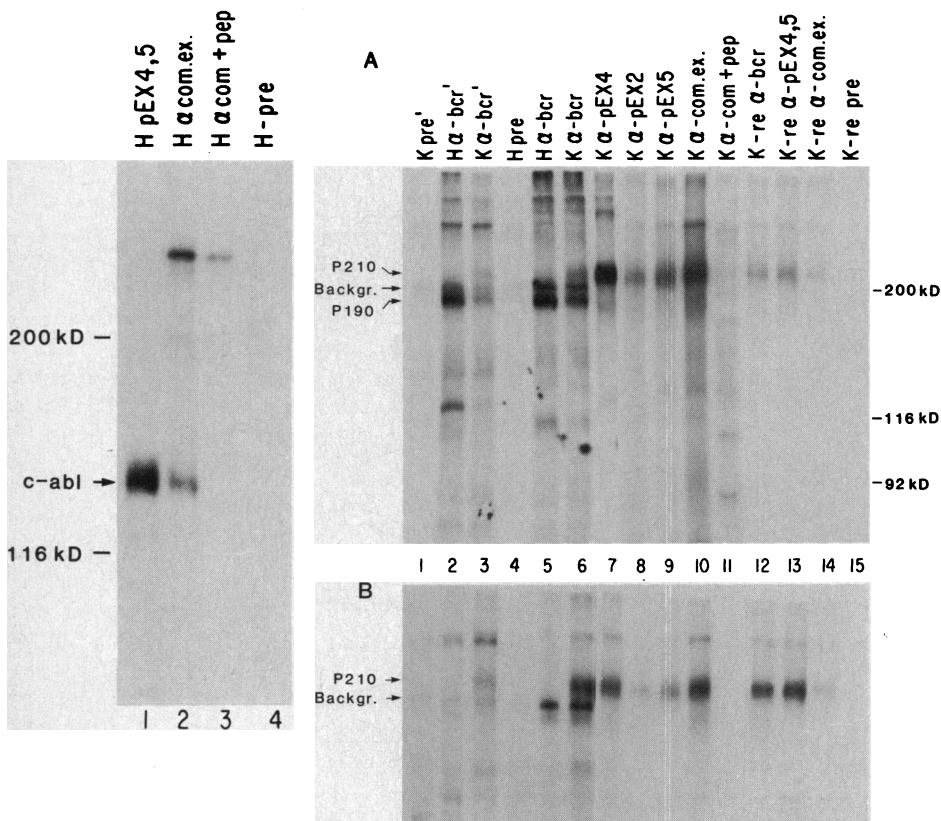


Fig. 2 (left). Immunoprecipitation analysis of the peptide antiserum. Cytosol extracts from HeLa cells (H) were reacted with: Lane 1, a mixture of the *v-abl*-specific antisera pEX4 and pEX5 (2 μ l each) (8); lane 2, antiserum to *c-abl* common exon (10 μ l); lane 3, antiserum to common exon (10 μ l) plus 30 μ g of common exon peptide; lane 4, preimmune serum (10 μ l). Protein A-Sepharose immunoprecipitates were processed for in vitro kinase assay (13), denatured by boiling for 5 minutes in 2% SDS- β -mercaptoethanol sample buffer and subjected to electrophoresis through SDS-polyacrylamide gels (6%). The gel was stained, fixed, dried, and exposed to XAR-5 film (Kodak) for 1 hour at room temperature. Fig. 3 (right). Immunoprecipitation of P210 with *abl*- and *bcr*-specific antisera. Lanes 1 to 11 represent direct immunoprecipitates processed as in Fig. 2. Abbreviations: H, HeLa extract; K, K562 extract; pre and pre', two different preimmune sera; α -com.ex., antiserum to common exon peptide; α -com + pep, antiserum to common exon peptide competed with its cognate peptide (30 μ g); α -pEX4,5, a mixture of the *v-abl* antisera pEX4 and pEX5 (6). (Lanes 12 to 15) K562 cell extracts were precipitated by antisera pEX4 and pEX5 and processed for in vitro kinase assay on the protein A-Sepharose beads. The precipitated proteins were denatured by boiling in 2% SDS with β -mercaptoethanol buffer, diluted 1:20 in buffer containing 1% Triton X-100, 20 mM tris (pH 7.4), 50 mM NaCl, and 1 mg/ml of bovine serum albumin and reprecipitated with different antisera as indicated. (A) Autoradiogram of precipitated products labeled during in vitro incubation, exposed for 15 minutes at room temperature. (B) Autoradiogram of the gel shown in (A) after hot alkali treatment. Treatment was done as described (15) in LM KOH at 55°C for 2 hours.

REFERENCES AND NOTES

1. J. D. Rowley, *Nature (London)* 243, 290 (1973).
2. P. C. Nowell and D. A. Hungerford, *J. Natl. Cancer Inst.* 25, 85 (1960).
3. N. Heisterkamp *et al.*, *Nature (London)* 306, 239 (1983).
4. E. Shrivelman, B. Lifshitz, R. P. Gale, E. Canaani, *ibid.* 315, 550 (1985).
5. R. P. Gale and E. Canaani, *Proc. Natl. Acad. Sci. U.S.A.* 81, 5648 (1984).
6. J. B. Konopka, S. M. Watanabe, O. N. Witte, *Cell* 37, 1035 (1984).
7. Y. Ben-Neriah, A. Bernards, M. Paskind, G. Q. Daley, D. Baltimore, *ibid.* 44, 577 (1986).
8. J. B. Konopka, S. M. Watanabe, J. W. Singer, S. J. Collins, O. N. Witte, *Proc. Natl. Acad. Sci. U.S.A.* 82, 1810 (1985).
9. K. K. Stanley and P. J. Lugio, *EMBO J.* 3, 1429 (1984).
10. The pGAL-*bcr* expression plasmid was constructed by inserting a 270-bp Bgl II-Pst I *bcr* fragment of the K28 cDNA clone (4) into the polylinker region of the pEX2 vector (9) in a manner that maintains the translational reading frame of the normal *bcr* protein. The construction was sequenced to verify the preservation of the proper reading frame (Fig. 1B).
11. Rabbit antisera to residues 30 to 41 of the type I *c-abl* cDNA (7), representing an identical sequence within the human and mouse common exons (4, 7) (Fig. 1C), were raised either by injecting the peptide coupled to keyhole limpet hemocyanin (KLH) alone or in the presence of a peptide representing residues 4 to 16 also coupled to KLH. The peptide alone did not yield useful antibody but (for unknown reasons) the two peptides elicited an antiserum to residues 30 to 41 that has proved very effective. Two rabbits injected with both coupled peptides yielded serum that precipitated the *bcr/c-abl* P210 protein. To determine which population of antibodies was responsible for precipitating P210, the sera were absorbed extensively to the common exon peptide leaving a serum that was only reactive to the peptide residues 4 to 16. This absorption removed all reactivity to P210 showing that only the antibodies to residues 30 to 41 precipitate P210. (The competition experiment in Fig. 3, lane 11, also demonstrates this point.)
12. J. B. Konopka *et al.*, *J. Virol.* 51, 223 (1984).
13. J. B. Konopka and O. N. Witte, *Mol. Cell. Biol.* 5, 3116 (1985).
14. Rabbit antiserum to β -Gal-asialoglycoprotein, a gift of M. Shia.
15. J. A. Cooper, B. M. Sefton, T. Hunter, *Methods Enzymol.* 99, 387 (1983).
16. N. Heisterkamp, K. Stam, J. Groffen, A. de Klein, G. Grosfeld, *Nature (London)* 315, 758 (1985).
17. Y. Ben-Neriah and D. Baltimore, in *Oncogenes and Growth Control*, P. Kahn and T. Graf, Eds. (Springer-Verlag, New York, in press).
18. ———, *Current Topics in Microbiology* (Springer-Verlag, New York, in press).
19. A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* 74, 560 (1977).
20. R. A. Lerner *et al.*, *ibid.* 78, 3403 (1981).
21. Y. Ben-Neriah, P. Lonai, D. Givol, *J. Immunol.* 13, 533 (1980).
22. We thank E. Canaani (Weizmann Institute) for the K562 cDNA plasmid, O. Raibaud (Institut Pasteur) for bacterial strain POP2136, and V. Pierce for typing the manuscript. This work was supported by grants from the National Cancer Institute to O.N.W. and D.B. Y.B.N. was supported by a Fogarty International Postdoctoral Fellowship Award. A.-M.M.-M. is supported by National Cancer Institute of Canada. O.N.W. is a Faculty Scholar of the American Cancer Society.

28 February 1986; accepted 21 April 1986

The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene

Y Ben-Neriah, GQ Daley, AM Mes-Masson, ON Witte and D Baltimore

Science **233** (4760), 212-214.
DOI: 10.1126/science.3460176

ARTICLE TOOLS

<http://science.sciencemag.org/content/233/4760/212>

REFERENCES

This article cites 16 articles, 4 of which you can access for free
<http://science.sciencemag.org/content/233/4760/212#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)