kov counts of excised zones, averaged 0.5 percent in four experiments, with a range of 0.16 to 1.0 percent.

STobRV RNA does not have detectable messenger activity (*27*). Its nucleotide sequence nevertheless has several functions: it serves as a template for transcription into RNA; it undergoes autolysis; and it is encapsidated. STobRV RNA greatly reduces the yield of TobRV and the severity of symptoms that TobRV alone induces. Because of these multiple functions, probably less than the entire nucleotide sequence of dimeric STobRV RNA is necessary for the autolysis reaction alone.

REFERENCES AND NOTES

1. I. R. Schneider, in *Beltsville Symposia in Agricultural Research*, J. A. Romberger, Ed. (Allenheld, Osmun, Montclair, NJ, 1977), vol. 1, pp. 201–219.
2. A. F. Murant and M. A. Mayo, *Annu. Rev. Phytopathol.* **20**, 49 (1982); J. M. Kaper and M. E. Tousignant, *Endeavor (New Series)* **8**, 194 (1984); R. I. B. Francki, *Annu. Rev. Microbiol.* **39**, 151 (1985).
3. I. R. Schneider, *Science* **166**, 1627 (1969).
4. M. C. Kiefer, S. D. Daubert, I. R. Schneider, G. Bruening, *Virology* **121**, 262 (1982).
5. J. M. Sogo and I. R. Schneider, *ibid.* **117**, 401 (1982); H. J. M. Linthorst and J. M. Kaper, *ibid.* **137**, 206 (1984).
6. P. A. Kiberstis, J. Hazeloff, D. Zimmern, *EMBO J.* **4**, 817 (1985).
7. M. Konarska, W. Filipowicz, H. J. Gross, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1474 (1982).
8. A. D. Branch, H. D. Robertson, E. Dickson, *ibid.* **78**, 6381 (1981); A. D. Branch and H. D. Robertson, *Science* **223**, 450 (1984).
9. R. H. Symons *et al.*, in *Subviral Pathogens of Plants and Animals: Viroids and Prions*, K. Maramorosch and J. J. McKelvey, Jr., Eds. (Academic Press, New York, 1985), pp. 235–263.
10. R. P. Kahn, H. A. Scott, R. L. Monroe, *Phytopathology* **52**, 1211 (1962); I. R. Schneider, R. M. White, G. V. Gooding, *Virology* **50**, 902 (1972).
11. I. R. Schneider, R. Hull, R. Markham, *Virology* **47**, 320 (1972).
12. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 156, 174–177.
13. Zones were located in the electrophoresis gel by autoradiography or by staining with toluidine blue or ethidium bromide. Excised gel was soaked (*14*) overnight in ammonium chloride (0.5*M*), Na_2EDTA (0.5 m*M*), and SDS (0.5 mg/ml). RNA was recovered from the soak solution by precipitation with two volumes of ethanol. Yields were approximately 80 percent for monomeric STobRV RNA and 50 percent for the dimeric form.
14. J. Haseloff and R. H. Symons, *Nucleic Acids Res.* **9**, 2741 (1981).
15. G. K. McMaster and G. G. Carmichael, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4835 (1977).
16. Photochemically activated silver staining detected either RNA or RNA and protein. The procedure was that of C. R. Merril, D. Goldman, S. A. Sedman, and M. H. Ebert [*Science* **211**, 1437 (1981)] except that the rinses that preceded the dichromate oxidation step were either (i) 10 percent methanol for 1 hour or longer, three changes of water over a 15-minute period, 10 percent methanol for 30 and 5 minutes, 40 percent methanol for 20 minutes, and finally water for 10 minutes (for RNA detection) or (ii) 10 percent glutaraldehyde for 30 minutes and three washes with water for 10 minutes each (for detection of both RNA and protein).
17. J. M. Buzayan, W. L. Gerlach, G. Bruening, P. Keese, A. R. Gould, *Virology*, in press.
18. S. D. Daubert and G. Bruening, *Virology* **98**, 246 (1979).
19. S. Nishimura *et al.*, *Biochim. Biophys. Acta* **142**, 133 (1967).
20. H. D. Robertson, D. L. Rosen, A. D. Branch, *Virology* **142**, 441 (1985).
21. C. Guerrier-Takada and S. Altman, *Science* **223**, 285 (1984); M. Baer and S. Altman, *ibid.* **228**, 999 (1985).
22. A. J. Zaug, J. R. Kent, T. R. Cech, *ibid.* **224**, 574 (1984).
23. N. Watson, M. Gurevitz, J. Ford, D. Apirion, *J. Mol. Biol.* **172**, 301 (1984).
24. W. C. Ginoza *et al.*, *Nature (London)* **203**, 606 (1964).
25. K. Haydock and L. C. Allen, *Prog. Clin. Biol. Res.* **172A**, 87 (1985); R. S. Brown *et al.*, *Nature (London)* **303**, 543 (1983).
26. A. D. Branch and H. D. Robertson, personal communication.
27. R. A. Owen and I. R. Schneider, *Virology* **80**, 222 (1977).
28. T. O. Diener, I. R. Schneider, D. R. Smith, *ibid.* **57**, 577 (1974); I. R. Schneider, *ibid.* **45**, 108 (1971).

29. This research was supported in its early phases by a grant from Xenogen, Inc., and throughout by the Competitive Grants Program of the Science and Education Administration of the Department of Agriculture and the Agricultural Experiment Station of the University of California at Davis. All the authors were associated with the Department of Biochemistry and Biophysics of the University of California at Davis during most of the course of the reported research.

30 May 1985; accepted 16 December 1985

HTLV-III *gag* Protein Is Processed in Yeast Cells by the Virus *pol*-Protease

R. A. KRAMER, M. D. SCHABER, A. M. SKALKA, K. GANGULY, F. WONG-STAAL, E. P. REDDY

The *gag-pol* gene of HTLV-III (human T-lymphotropic virus), the virus linked to AIDS (acquired immune deficiency syndrome), was expressed in yeast, and processing of the *gag* precursor into proteins of the same size as those in the virion was observed. Processing of the *gag* gene in yeast cells mimics the process that naturally occurs in mammalian cells during maturation of virions. Therefore it was possible to perform mutational analysis of the virus genome to localize the gene that codes for the protease function to the amino terminal coding region of the *pol* gene. Since this region overlaps the *gag* gene, it is likely that ribosomal frameshifting occurs from *gag* to *pol*. Antibodies in all of the AIDS patients' sera tested recognized the yeast synthesized *gag* proteins, although the sera showed differences in relative reactivity to the individual *gag* proteins and the precursor. This yeast system should be valuable not only for production of viral proteins for diagnostic or vaccine purposes but also for analysis of the genetics and biochemistry of viral gene functions—parameters that are difficult to study otherwise with this virus.

THE RETROVIRUS HTLV-III AND the closely related variants of this virus, LAV and ARV, are the causative agents of the disease acquired immune deficiency syndrome (AIDS) (*1*). Molecular cloning and nucleotide sequence analysis of HTLV-III and its variants have demonstrated that this viral genome exhibits many of the structural features of the avian and mammalian retroviruses. Thus, the viral genome contains the three genes (*gag, pol*, and *env*) characteristic of all retroviruses (*2*). In addition, the HTLV-III genome contains two short open reading frames whose functions are unknown (*2*).

One of the viral genes, *gag*, encodes a precursor which is processed into core proteins during virion maturation. From DNA sequence data and analysis of isolated viral proteins, the HTLV-III *gag* precursor is about 56 kD and is processed into species of approximately 24, 16, and 14 kD (*2*) (Fig. 1A). The protease responsible for this processing is typically encoded by the retroviral genome. It is included in the 3′ end of the *gag* gene in avian retroviruses and in the 5′ end of the *pol* gene in mammalian viruses (*3*). In Moloney murine leukemia virus (MuLV), the protease is a *gag-pol* read through product (*4*) and, for Rous sarcoma virus (RSV), a *gag-pol* fusion protein is produced by a frameshift between overlapping reading frames (*5*). A therapeutic agent that could inhibit this protease might block virus spread. It is, therefore, important to identify the region of the HTLV-III genome that encodes this protease and to develop in vivo and in vitro systems in which the proteolysis of the *gag* gene precursor can be studied. Our results show that the processing reaction is carried out very efficiently in yeast cells and suggest that the yeast system may be used for the development of inhibitors of this process. We have illustrated the utility of the system by map-

R. A. Kramer and M. D. Schaber, Department of Molecular Genetics, Hoffmann–La Roche, Inc., Roche Research Center, Nutley, NJ 07110.
A. M. Skalka, K. Ganguly, E. P. Reddy, Department of Molecular Oncology, Hoffmann–La Roche, Inc., Roche Research Center, Nutley, NJ 07110.
F. Wong-Staal, Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205.

ping the protease region by functional analysis in yeast. In addition, the *gag* proteins made in yeast react with antibodies in sera from a number of AIDS patients and, thus, could also provide useful diagnostic and perhaps vaccine reagents.

To construct plasmids for *gag* gene expression in yeast, a 3.8-kb Cla I–Eco RI fragment of HTLV-III proviral DNA from λ HXB-3 (*6*) was inserted into a yeast expression vector PYE72 (*7*) (Fig. 1). The yeast promoter and translation initiation site of this vector are contained in a 560-bp Bam HI–Aha III restriction fragment derived from the gene for repressible acid phosphatase, *PHO5* (*8, 9*). Ligation of the Aha III end of the *PHO5* fragment to the Cla I end of HTLV-III fragment fused the promoter and first two codons of *PHO5* to the 15th codon of *gag* (Fig. 1). Yeast cells (*10*) transformed with the resulting plasmids, PYE72/*gag*1 were induced by depletion of phosphate in growth medium (*11*) and extracts of the cells were analyzed for the presence of *gag*-specific proteins by immunoblot analysis with rabbit polyclonal antiserum to disrupted virus (Fig. 2). Several reacting species were seen in the extract from induced cells (lane 2), but not uninduced cells (lane 1). As would be expected, the vector with no *gag* gene showed no detectable reaction after induction. The major immunoreactive protein produced by the induced pYE72/*gag*1 seemed to correspond in size to the HTLV-III p24 *gag* protein identified in virions and predicted from the DNA sequence (Fig. 1A). Less reactive species of the sizes expected for the p14 and p16 proteins were also detected as well. A

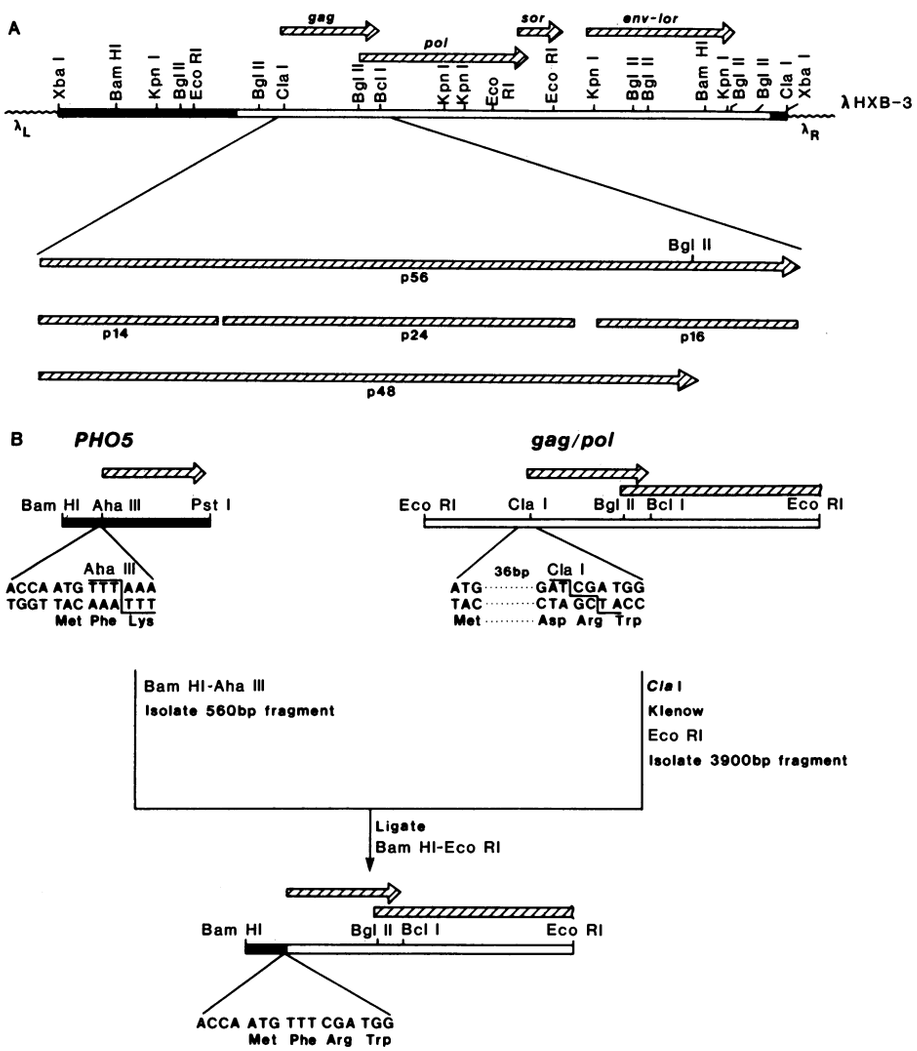

Fig. 1. (A) Map of λHXB-3. The major open reading frames are shown as striped arrows. Bacteriophage λ DNA is indicated by wavy lines, genomic DNA by solid lines, and HTLV-III DNA by an open line. The *gag* region is expanded to show the precursor (p56) and the processed products (p24, p16, and p14). (B) Construction of yeast expression plasmids. The construction of the *gag* expression plasmid pYE72/*gag*1 is described in the text. A deletion that removed the COOH-terminal portion of *gag* and all of *pol* was made by digesting pYE7/*gag*1 with Bam HI and Bgl II and isolating the *PH05-gag* fragment. This was converted to a Bam HI to Eco RI fragment by ligation of the 375-bp Bam HI–Eco RI fragment from pBR322 to the Bgl II site through the identical 5′ overhangs of Bgl II and Bam HI. Insertion of this fragment into pYE7 yielded PYE72/*gag*2. Another mutation was introduced at the Bcl I site in the *pol* gene just downstream of *gag*. Since Bcl I does not cut methylated DNA, pYE72/*gag*1 was introduced into *E. coli* GM119 (*14*), and unmethylated DNA was prepared with *E. coli* GM119 (*14*) as host and digested with Bcl I. The 5′ overhang was filled in with Klenow fragment as above and the plasmid recircularized by blunt-end ligation. The resulting plasmid with a 4-bp insert at the Bcl I site designated pYE72/*gag*3.

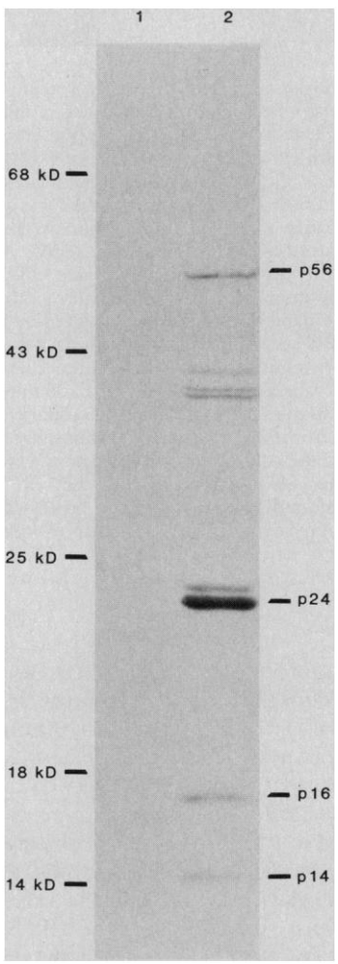

Fig. 2. Immunoblot analysis of yeast lysates. Yeast cells were grown, induced in phosphate-free medium, and lysed as described (*11*). Samples of the lysates were fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to nitrocellulose membranes (*15*). The resulting immunoblots were developed by reacting with rabbit antibodies to total disrupted HTLV-III virus and developed (*16*). Lysates were from (1) cells with pYE72/*gag*1 grown in high-phosphate medium and (2) cells with pYE72/*gag*1 grown in phosphate-free medium. The indicated molecular markers were used to estimate the sizes of the reactive bands. A separate comparison with virus-derived proteins demonstrated that the 24-, 16-, and 14-kD proteins had the same electrophoretic mobilities.

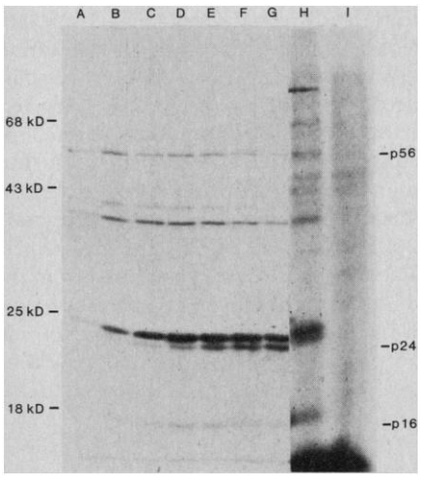

Fig. 3. Processing of the *gag* polyprotein. Yeast cells transformed with pYE72/*gag*1 were induced in phosphate-free medium for 6 hours, then labeled with either [^{35}S]methionine, or [^{32}P]phosphate. For the [^{35}S]methionine pulse-chase experiment, 500 μCi of [^{35}S]methionine was added to the culture medium. After a 2-minute labeling period, unlabeled methionine was added. At intervals, the cells were harvested, lysed, and processed by immunoprecipitation with rabbit antibody (*16*), and the lysates were analyzed by SDS-PAGE and autoradiography. "Chase" times were: lane A, 0 minutes; lane B, 2 minutes; lane C, 5 minutes; lane D, 10 minutes; lane E, 20 minutes; lane F, 30 minutes; and lane G, 45 minutes. For the 3P labeling, 1 mCi of [^{32}P]phosphate was added to the culture medium. Labeling was continued for 30 minutes at 30°C; the cells were harvested and the lysates were processed for immunoprecipitation as described above. Lysates were from: lane H, cells containing pYE72/*gag*1; lane I, cells containing a similar plasmid with no *gag* gene.

larger protein of about 56 kD, the size expected for the entire *gag* precursor, as well as several species of about 40 kD, that might represent processing intermediates, were also seen.

Production of viral-specific proteins was followed in a "pulse-chase" experiment with [^{35}S]methionine. It was not possible to remove all the labeled methionine from the intracellular pool because of the large amount of label used. However, the results (Fig. 3) showed that the larger species (56 kD and 40 kD) acquire radioactivity at the earliest time measured, and the absolute amount of label in this fraction remains constant until the latest time point. With increased time, proportionately more of the radioactivity accumulates in smaller "processed" molecules. An identical processing event appears to take place in HTLV-III infected human cells, since the COOH-terminal sequence of p24 and the NH_2-terminal sequence of p16 determined by direct analysis of viral proteins are separated by about 1 kD in the amino acid sequence of the *gag* precursor predicted from the DNA sequence. A protein of about 25 kD, also seen in the previous experiment, was detected before p24 and thus may be its immediate precursor. A protein migrating as expected of p16 was also detected in yeast. The p14 region of *gag* has no methionine; thus our failure to detect this protein supports its proposed origin.

Phosphorylation of the *gag* proteins made in yeast was examined after growth of cells in low-phosphate medium containing [^{32}P]phosphate. The results of gel analysis of labeled proteins immunoprecipitated with rabbit HTLV-III antibodies are shown in Fig. 3. The 56-kD, the faster migrating of the 40-kD intermediates, the 25-kD, 24-kD, and the 16-kD species all were radioactive. No radioactivity was seen at the position of the 14-kD band when a higher percentage gel was run. Thus, the p24 and p16 *gag* proteins appear to be phosphorylated in yeast.

The presence of a Bgl II site near the COOH-terminal coding region of the *gag* gene provided a way to eliminate about half of the p16 coding region as well as most of the *pol* gene present in the 5.5-kb Eco RI fragment (see Fig. 1A). The COOH-terminal part of the *gag* polyprotein or the NH_2-terminal region of *pol* encodes a processing protease in many retroviruses (*3*). The product formed by the truncated *gag* gene is shown in Fig. 4, A and B (lane 2). The major immunoreactive protein band migrated as approximately 48 kD, the size expected for the unprocessed polyprotein from the Bgl II deletion (Fig. 1A). As expected from the location of the deletion, no p16 was detected. The absence of p24 indicates that processing of the polypeptide is defective. This defect could reflect elimination of the protease or a change in the conformation of the precursor which masks potential cleavage sites. To distinguish between these two possibilities and to examine the role of the *pol* coding region in the processing of the *gag* precursor, we introduced a frameshift mutation in the 5′ end of the *pol* gene in PYE72/*gag*1 at a Bc 1I restriction site located just downstream of the stop codon of the *gag* gene (Fig. 1). Digestion with Bcl I, followed by filling in the overhang and again ligating the plasmid, introduced a 4-bp insert that caused a frameshift in the *pol* reading frame (Fig. 5). The products of this plasmid are shown in Fig. 4, A and B (lane 3). The major immunoreactive protein band was about 56 kD and comigrated with the presumptive precursor protein seen in the cells with induced pYE72/*gag*1. Thus, this mutation also appears to prevent processing of the *gag* precursor. It has recently been noted (*2*) that the amino acid sequence of the *pol* region just upstream of the frameshift

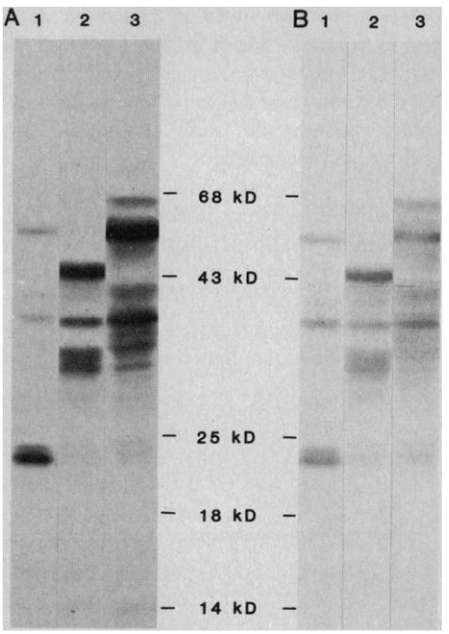

Fig. 4. Immunoblot analysis of *gag* mutations. Samples were prepared as for Fig. 2. The immunoblots were developed with rabbit antibodies to disrupted HTLV-III (A) or with serum from an AIDS patient (B). Lysates were from induced cells with (lane 1) pYE72/*gag*1; (lane 2) pYE72/*gag*2; and (lane 3) pYE72/*gag*3.

mutation (Fig. 5) is homologous with other retroviral *gag* proteases and other pepsin-related proteases (*12*). Thus, it appears likely that the *gag* protease of HTLV-III is encoded in this region. The overlap of the *gag* and *pol* genes in different reading frames suggests that a frameshift gives rise to a *gag-pol* fusion with protease activity. Such a fusion product is expected to be 170 kD in size (*2*). Although a splicing event leading to translation of the *pol* gene cannot be ruled out, this seems likely in light of the failure of most splices from higher eukaryotic messenger RNA's to take place in yeast. A small amount of an approximately 68-kD protein was observed. It seems probable that this represents a frameshift-readthrough into *pol* that is terminated near the filled in Bcl I site. Our failure to detect this precursor previously may have resulted from rapid self-cleavage of the frameshift product when it included an active protease domain. Furthermore, studies with other retroviruses (*5*) have demonstrated that the ratio of *gag-pol* fusion protein to *gag* protein is 1:20 and such a quantity would not be detectable by our assay.

Immunoblots of lysates of yeast cells expressing the *gag* proteins developed with serum of individual AIDS patients are shown in Fig. 6. A sample of normal human serum showed no reactivity. All sera contained some antibodies to *gag* proteins although there were significant qualitative and quantitative variations between pa-

tients. Interestingly, the sera from patients on the East Coast generally reacted more strongly with the mature species, p24, p16, and p14, whereas the sera from patients on the West Coast seemed to recognize the precursor preferentially.

A few sera contained antibodies to several proteins not detected by the rabbit antiserum to HTLV-III, most noticeably at about 60 kD and 35 kD. Since the same proteins were detected in an extract from yeast cells without the *gag* gene, it could be that they are yeast proteins recognized by antibodies in AIDS patient sera. However, most of the bands, including some larger than 56 kD, are only seen in extracts from cells with the *gag* gene. Since this vector also carries part of the *pol* gene, some of these products could be related to *pol* as well as *gag*.

We and others have recently demonstrated that sera from AIDS patients also recognize the *env* gene product made in *Escherichia coli* very efficiently (*13*). It, therefore, appears that a mixture of *gag* and *env* gene derived proteins might constitute appropriate diagnostic reagents for the detection of these antibodies, and that further exploration of the differences in reactivity to various components could provide clues to the epidemiology or progression of the disease.

The rapid synthesis and processing of *gag* products in yeast provides a promising system for the development of therapeutic agents that can block this step in the formation of mature virion particles. Since our studies suggest that an HTLV-III–coded protein made in yeast is the proteolytic enzyme that mediates this process, it should be possible to purify this protein from yeast and study its biochemical properties. Other genetic and biochemical studies on HTLV-III genes should also be possible in yeast. This is of practical importance since the virus is difficult to grow and dangerous to handle. Furthermore, human T cells, the normal host for this virus, are not amenable to easy manipulation as are yeast cells.

```
                    BglII
  1 GAGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAG  69
gag - GluArgGlnAlaAsnPheLeuGlyLysIleTrpProSerTyrLysGlyArgProGlyAsnPheLeuGln
pol -                PhePheArgGluAspLeuAlaPheLeuGlnGlyLysAlaArgGluPheSerSerG

 70 AGCAGACCAGAGCCAACAGCCCCACCATTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAG 138
    SerArgProGluProThrAlaProProPheLeuGlnSerArgProGluProThrAlaProProGluGlu
    luGlnThrArgAlaAsnSerProThrIleSerSerGluGlnThrArgAlaAsnSerProThrArgArgG

139 AGCTTCAGGTCTGGGGTAGAGACAACAACTCCCCCTCAGAAGCAGGAGCCGATAGACAAGGAACTGTAT 207
    SerPheArgSerGlyValGluThrThrThrProProGlnLysGlnGluProIleAspLysGluLeuTyr
    luLeuGlnValTrpGlyArgAspAsnAsnSerProSerGluAlaGlyAlaAspArgGlnGlyThrValS

208 CCTTTAACTTCCCTCAGATCACTCTTTGGCAACGACCCCTCGTCACAATAAAGATAGGGGGGCAACTAA 276
    ProLeuThrSerLeuArgSerLeuPheGlyAsnAspProSerSerGln .
    erPheAsnPheProGlnIleThrLeuTrpGlnArgProLeuValThrIleLysIleGlyGlyGlnLeuL

277 AGGAAGCTCTATTAGATACAGGAGCAGATGATACAGTATTAGAAGAAATGAGTTTGCCAGGAAGATGGA 345
    ysGluAlaLeuLeuAspThrGlyAlaAspAspThrValLeuGluGluMETSerLeuProGlyArgTrpL

                                                    BclI
346 AACCAAAAATGATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAA 414
    ysProLysMETIleGlyGlyIleGlyGlyPheIleLysValArgGlnTyrAspGlnIleLeuIleGluI
                                                   ↓
                                                  ArgSerAspThrHisArgAs

415 TCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAA 477
    leCysGlyHisLysAlaIleGlyThrValLeuValGlyProThrProValAsnIleIleGly
    nLeuTrpThr .
```

Fig. 5. *Gag-pol* overlap region. The carboxyl terminal coding region of *gag* and the amino terminal coding region of *pol* are shown. The region that is homologous to other *gag* proteases (*11*) is underlined. The reading frame to which the *pol* gene is shifted by the Bcl I fill in is shown by the arrow. A indicates a translation termination site.

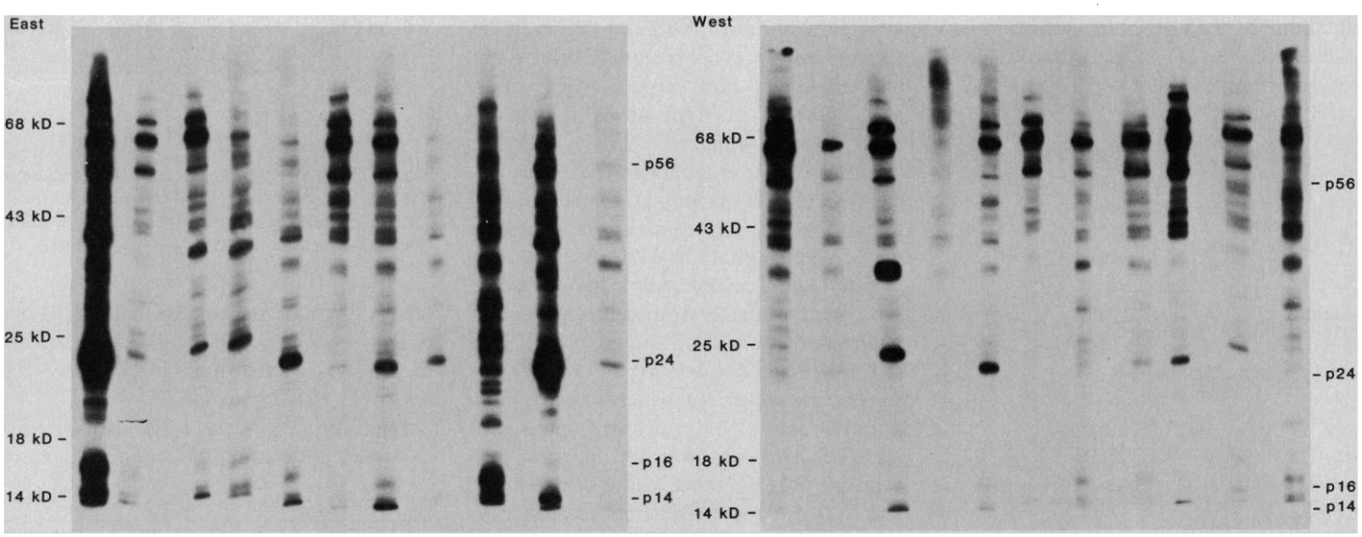

Fig. 6. Detection of *gag* antibodies in sera from AIDS patients. Extracts of induced cells carrying pYE72/*gag*1 were placed across the entire width of an SDS-polyacrylamide gel, fractionated by electrophoresis, and transferred to nitrocellulose. The resulting blot was cut into strips that were reacted with serum from individual patients with AIDS. The strips in the top panel show sera from patients on the East Coast of the United States. The lower panel represents patients from the West Coast. Serum from a normal patient showed no significant reactivity with the yeast lysate. The approximate positions of the *gag*-related proteins made in yeast are indicated. Because the reactions were done on individual strips, bands do not always line up precisely from strip to strip.

REFERENCES AND NOTES

1. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983); J. A. Levy *et al.*, *ibid.* **225**, 840 (1984); M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500; M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *ibid.*, p. 506; J. Schüpbach *et al.* *ibid.*, p. 503.
2. L. Ratner *et al.*, *Nature (London)* **313**, 277 (1985); R. Sanchez-Pescador *et al.*, *Science* **227**, 484 (1985); M. Muesing *et al.*, *Nature (London)* **313**, 450 (1985); S. Wain-Hobson, P. Sonigo, O. Danos, S. Cole, M. Alizon, *Cell* **40**, 9 (1982).
3. C. Dickson, R. Eisenman, H. Fan, E. Hunter, N. Teich, in *Molecular Biology of Tumor Viruses*, R. A. Weiss, N. M. Teich, H. E. Varmus and J. M. Coffin, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), p. 513; B. Mermer, M. Malamy, J. M. Coffin, *Mol. Cell. Biol.* **3**, 1746 (1983).
4. Y. Yoshinaka *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1618 (1985).
5. T. Jacks and H. E. Varmus, *Science* **230**, 1237 (1985).
6. G. M. Shaw, B. H. Hahn, S. K. Arya, J. E. Groopman, R. C. Gallo, F. Wong-Staal, *Science* **226**, 1165 (1984).
7. M. D. Schaber, T. M. DeChiara, R. A. Kramer, *Methods Enzymol.*, in press.
8. G. P. Thill, R. Kramer, K. Turner, K. A. Bostian, *Mol. Cell. Biol.* **3**, 570 (1983).
9. N. Andersen, G. P. Thill, R. A. Kramer, *ibid.*, p. 562.
10. E. Jones, *Genetics* **85**, 23 (1977).
11. R. A. Kramer, T. DeChiara, M. Schaber, S. Hilliker, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 367 (1984).
12. H. Toh, M. Ono, K. Saigo, T. Miyata, *Nature (London)* **315**, 691 (1985).
13. R. Crowl *et al.*, *Cell* **41**, 979 (1985); N. T. Chang *et al.*, *Science* **228**, 93 (1985).
14. J. A. Arraj and M. G. Marinus, *J. Bacteriol.* **153**, 562 (1983).
15. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979); W. N. Burnette, *Anal. Biochem.* **112**, 195 (1981).
16. T. Curran, C. VanBeveren, N. Ling, I. Verma, *Mol. Cell. Biol.* **5**, 167 (1985).
17. We thank Drs. R. C. Gallo and M. G. Sarngadharan for helpful discussions and for providing several of the sera used in these studies.

17 December 1985; accepted 6 February 1986

Human β-Adrenoceptors: Relation of Myocardial and Lymphocyte β-Adrenoceptor Density

OTTO-ERICH BRODDE, ROLAND KRETSCH, KATSUMI IKEZONO, HANS-REINHARD ZERKOWSKI, JÜRGEN CHRISTOPH REIDEMEISTER

In human right atria obtained from 21 patients during open-heart surgery, β-adrenoceptor density [assessed by iodine-125–labeled (−)-cyanopindolol binding] and responsiveness (positive inotropic responses to isoprenaline) were linearly related to the β-adrenoceptor density in the corresponding circulating lymphocytes. This direct relation of human myocardial and lymphocyte β-adrenoceptor alterations, therefore, makes it possible to monitor drug- or disease-induced β-adrenoceptor changes in tissues not easily accessible in humans.

THE DEVELOPMENT OF RADIOLIGAND binding techniques for direct identification of receptors has advanced our knowledge of the molecular pharmacology of β-adrenoceptors (*1, 2*). The number of β-adrenoceptors on cells is dynamically regulated by a variety of drugs, hormones, and physiological and pathological conditions (*3*). Circulating lymphocytes containing a homogeneous population of β_2-adrenoceptors (*4*) excitatorily coupled to adenylate cyclase (*5*) are used to study such alterations of β-adrenoceptor function (*6*) in humans. However, despite similar in vitro properties of β-adrenoceptors (as determined by radioligand binding studies) in blood cells and various tissues, the relation of β-adrenoceptor changes measured in circulating lymphocytes to changes potentially occurring in solid tissues has not been established. We report here that, in human subjects, the density of β-adrenoceptors in circulating lymphocytes is significantly related to the density and responsiveness of β-adrenoceptors in right atrial appendages from the same subjects.

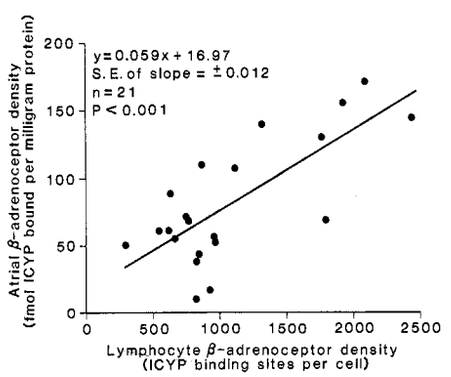

Fig. 1. Relation of β-adrenoceptor density (determined by Scatchard analysis of specific ICYP binding at six to eight concentrations ranging from 10 to 150 p*M*) on membranes from human right atria and on intact cells of the corresponding lymphocytes.

Human right atrial appendages were obtained from 21 patients [18 males and 3 females 54.6 ± 1.3 years old (mean ± standard error; range, 41 to 64 years)] undergoing elective coronary artery bypass grafting. No patient suffered from acute myocardial failure and none had been treated with catecholamines for at least 3 weeks before surgery. However, the patients had received nitrates (n = 19), calcium antagonists (n = 16), and occasionally β-blockers (metoprolol, 50 mg twice daily, two patients and 100 mg twice daily, one patient; and atenolol, 50 mg once daily, one patient and 100 mg once daily, one patient). Preoperative medication consisted of flunitrazepam and atropine; the operation was done under balanced anesthesia with fentanyl, isoflurane, etomidate, and flunitrazepam. In some cases N_2O was added. Pancuronium was used as a muscle relaxant.

In all patients the right atrial appendages were removed under normothermic conditions before cardiopulmonary bypass. Immediately after removal, all specimens were placed in a sealed vial with Krebs-Henseleit solution (119 m*M* NaCl, 2.5 m*M* $CaCl_2$, 4.8 m*M* KCl, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 24.9 m*M* $NaHCO_3$, 10.0 m*M* glucose, and 0.057 m*M* ascorbic acid) aerated by "carbogen" (95 percent O_2 and 5 percent CO_2) at room temperature and transported immediately to the laboratory. The preparation of the tissues was begun within 5 to 15 minutes of surgical removal. The atrial appendages were first divided in two parts: one was used to determine β-adrenoceptor density; the other was dissected to yield trabecular strips (diameter, 1 mm or less) 4 to 5 mm long without endocardial damage for determination of mechanical responses to isoprenaline.

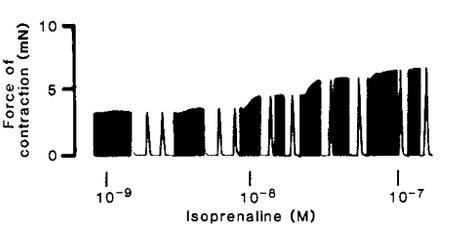

Fig. 2. Positive inotropic effect of isoprenaline on the isolated electrically driven muscle strip of human right atria. The concentration of isoprenaline was increased in steps of 0.5 log units.

The preparations were mounted in a 50-ml organ bath containing Krebs-Henseleit

O.-E. Brodde and R. Kretsch, Biochemical Research Laboratory, Medizinische Klinik und Poliklinik, Division of Renal and Hypertensive Diseases, University of Essen, D-4300 Essen, Federal Republic of Germany. K. Ikezono, H.-R. Zerkowski, J. C. Reidemeister, Division of Thoracic and Cardiovascular Surgery, University of Essen, D-4300 Essen, Federal Republic of Germany.