JOURNAL OF VIROLOGY, Aug. 1988, p. 2696–2700
0022-538X/88/082696-05$02.00/0

Vol. 62, No. 8

Activity of Avian Retroviral Protease Expressed in *Escherichia coli*

MOSHE KOTLER,† RICHARD A. KATZ, AND ANNA MARIE SKALKA*

Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111

Received 5 January 1988/Accepted 22 April 1988

The 3′ end of the avian sarcoma leukosis virus (ASLV) *gag* gene encodes a 124-amino-acid protease (PR) responsible for processing the *gag* and *pol* polyprotein precursors into the mature virion structural proteins and the reverse transcriptase. Here we report the synthesis of the mature ASLV PR and a nucleocapsid (NC)-PR *gag* precursor fragment in *Escherichia coli*. *E. coli* extracts containing mature PR correctly cleaved a synthetic decapeptide homologous to a known ASLV cleavage site. Also, the NC-PR precursor fragment appeared to be correctly processed to produce NC and PR in the bacterial cells. This cleavage was blocked by a mutation in the putative active site of PR. These results strongly support the hypothesis that PR is involved in cleaving itself from the *gag* precursor.

The retroviral *gag*, *pol*, and *env* genes encode precursor polypeptides, which are cleaved to produce the mature forms of the proteins found in virions (6). The processing of *gag* and *pol* precursors is directed by a virus-encoded protease (PR) (7, 8, 18, 26–28) that belongs to the family of aspartic proteases (20, 22, 23). (We have used the recently proposed nomenclature for retroviral proteins [14]: PR [avian sarcoma leukosis virus {ASLV} p15] and nucleocapsid protein [NC] [ASLV pp12].) The PR-directed processing presumably occurs just before or after budding (6). PR is essential for infectivity, although viruses that contain mutations in PR are able to assemble into morphologically immature particles (4, 8, 9, 16, 29, 30).

Unlike the mammalian retroviruses in which PR is encoded in the *pol* gene and thus contained in the *gag-pol* precursor (4, 13, 15), the PR of ASLV is encoded in *gag*. Therefore, the PR domain is contained in both the C terminus of the $Pr76^{gag}$ precursor and the $Pr180^{gag\text{-}pol}$ precursor (6). For both avian and mammalian retroviruses, mature PR is released from precursor proteins by proteolysis during the morphogenesis of viral particles. Since 20 to 50 times more of the *gag* than the *gag-pol* precursor is produced, the bulk of mature ASLV PR is formed as a consequence of cleavage between PR and the adjacent NC (pp12), located upstream in the *gag* precursor (21). In contrast, release of PR in mammalian viruses, such as Moloney murine leukemia virus and human immunodeficiency virus, requires cleavage at both N and C termini of the PR in the *gag-pol* precursor (13, 15).

It is not known exactly how PR is released from these precursors. At least two mechanisms are possible. (i) Cellular proteases may be responsible for the initiation of the process, resulting in the release of a few mature PR molecules. The released PR might then complete the processing, producing more PR and the other mature viral proteins as well. (ii) PR might cleave itself from the precursor via either an inter- or intramolecular mechanism. Similar mechanisms of activation are known for a number of aspartic proteases, in which an inactive zymogen is converted into an active enzyme by autocatalysis. One example is the autocleavage of pepsinogen to pepsin (22).

In this report, we describe the expression in *Escherichia coli* of the predicted ASLV PR-coding region. A protein structurally and immunologically indistinguishable from PR was produced, and a proteolytic activity analogous to the viral PR was detected in *E. coli* extracts. These results confirm the location of the ASLV PR-coding region. In addition, we show that an NC-PR *gag* fragment undergoes processing in *E. coli*, resulting in the formation of a mature PR that appears to be identical to virion PR. Mutation of a highly conserved aspartic acid residue that is thought to be part of the PR active site eliminates proteolytic activity. This demonstrates that the ASLV PR is responsible for releasing itself from the NC-PR *gag* precursor fragment in *E. coli*.

MATERIALS AND METHODS

Bacterial cells. *E. coli* MC1061 (3) was used as a host for all expression vectors. The cells contained the plasmid pRK248cIts, which directs expression of a temperature-sensitive bacteriophage lambda repressor protein (5).

Construction of the bacterial expression clones. The bacterial expression clones were constructed by using the pEV-vfr expression vectors which supply a lambda phage promoter-operator region (P_LO_L), a ribosome-binding site, and a translational initiation codon (5). The pΔNC-PR clone was constructed by replacement of a pEV-vrf2 *Bam*HI-*Pvu*II fragment with a viral 817-base-pair *Bam*HI-*Hpa*I fragment (Fig. 1) derived from an infectious clone of the avian sarcoma virus PrC strain, pATV-8 (11). The viral DNA fragment contains the C-terminal two-thirds of the NC-coding region, all of the PR-coding region including the translational termination codon for $Pr76^{gag}$, and the 5′ end of the *pol* gene. The construction of pNC (formerly called pRKP12) was described previously (10). The pNC-PR plasmid was constructed by substitution of a ca. 1-kilobase-pair pNC-derived *Pst*I-*Bam*HI fragment into pΔNC-PR (Fig. 1). This fragment contained the P_LO_L region and the ribosome-binding site ATG region properly aligned for expression of the native N terminus of NC (10). The pPR expression clone was constructed from pΔNC-PR by using oligonucleotide-directed, site-specific mutagenesis (10, 19) to delete the NC region and to precisely align the ATG codon in the vector with the first codon of the mature PR, a strategy used previously to construct pNC (10). The oligonucleotide used for deletion mutagenesis was 5′-AGGAGGAATTAAT ATGTTAGCGATGACAATGG-3′. A mutation was introduced into the putative active site region of PR (20, 23) by using site-directed oligonucleotide mutagenesis (19). The construct coding for the mutant PR [pNC-PR(S-I)] was

* Corresponding author.

† Permanent address: Department of Molecular Genetics, the Hebrew University, Hadassah Medical School, Jerusalem, Israel.

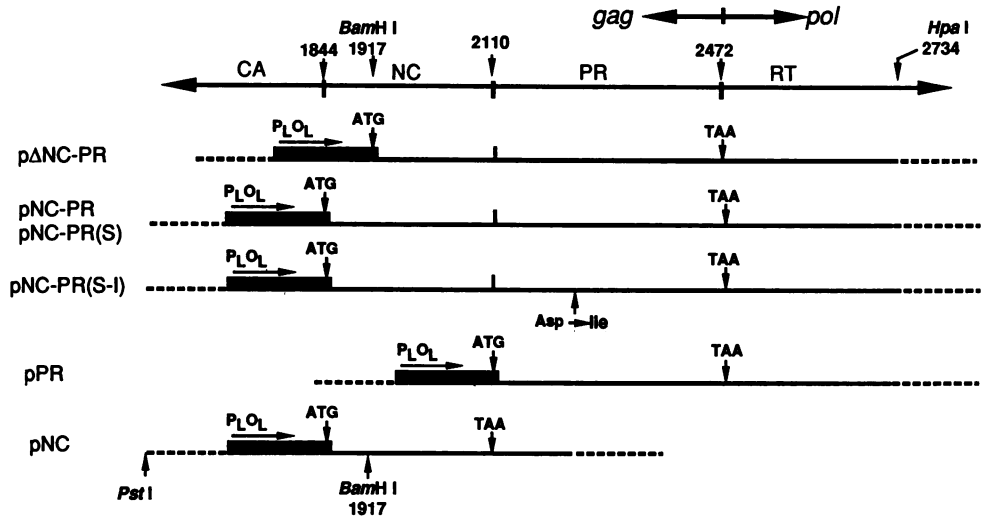

FIG. 1. Maps of PR, NC-PR, and NC *E. coli* plasmid expression clones. All clones are derivatives of pEV-vrf vectors (5). A partial map of the viral DNA clone pATV-8 (11), from which PR and NC sequences were derived is shown at the top; the flanking capsid (CA) and reverse transcriptase (RT) regions are indicated. Nucleotide positions indicated correspond to the avian sarcoma virus sequence described by Schwartz et al. (21). Symbols: – – –, pBR322-derived vector sequences; ■, bacteriophage lambda promoter-operator region (P_LO_L) (not drawn to scale). The initiation codon provided by the expression vector is indicated (ATG). The position of the termination codon for the PR-coding region is indicated (TAA). The site of the Asp→Ile mutation introduced into the putative active site of PR is shown. Numbers indicate nucleotide positions.

provided by V. M. Vogt. It was derived by site-directed mutagenesis at the GAC codon that encodes aspartic acid 37, which converted this codon to an ATA (isoleucine). In this construct, a portion of NC-PR sequences are derived from the Schmidt-Ruppin strain of avian sarcoma virus. A wild-type version of this clone was also constructed [pNC-PR(S)].

Analysis of NC and PR expressed in *E. coli*. The ASLV NC and PR expressed in *E. coli* were detected by immunoblotting with rabbit sera directed against NC (10) and PR. Anti-PR antibody was prepared by immunization of rabbits with purified viral PR provided by J. Leis. Expression in cells containing the various vectors was induced by shifting cultures from 30 to 42°C for 2 to 4 h, and virus-related proteins were detected by immunoblotting as described elsewhere (1, 10, 24).

Preparation of bacterial extracts. Induced and uninduced *E. coli* cultures (1.75 ml) were pelleted and suspended in 400 μl of 1% toluene–10 mM Tris hydrochloride (pH 8.0)–5 mM mercaptoethanol–0.1 mM EDTA–0.1% Triton X-100. Bacterial cell suspensions were vigorously agitated by vortexing (3 min) and were incubated for 20 min at 37°C. Unbroken cells and cell debris were pelleted by centrifugation. Supernatants were freeze-dried and dissolved in 50 μl of water, from which 2 μl was assayed.

Synthesis and cleavage of decapeptide substrates. The synthesis and purification of the decapeptides Thr-Phe-Gln-Ala-Tyr-Pro-Leu-Arg-Glu-Ala (wild type) and Thr-Phe-Gln-Ala-Ala-Pro-Leu-Arg-Glu-Ala (variant) are described elsewhere (M. Kotler, R. A. Katz, W. Danho, J. Leis, and A. M. Skalka, Proc. Natl. Acad. Sci. USA, in press). ASLV PR cleaves the wild-type peptide between Tyr and Pro (Kotler et al., in press). Proteolytic reactions were carried out in 10 μl of 0.1 M sodium citrate buffer (pH 5.5) containing 1.1 mM of decapeptide substrate and 2 μl of bacterial extract. The reactions were incubated at 37°C for 10 h. The cleavage products were analyzed by a thin-layer electrophoresis assay (Kotler et al., in press). Samples of 4 μl were spotted on cellulose plates (Art 5577, 20 by 20 cm; E. M. Reagents, Federal Republic of Germany). Plates were subjected to 45 mA for 1 h in pyridine-acetic acid-acetone-distilled water (volume ratio, 1:2:8:40). They were then air dried and sprayed first with 1% triethylamine (Pierce Chemical Co., Rockford, Ill.) in acetone and then with fluorescamine (Hoffmann-La Roche Inc., Nutley, N.J.) at 0.1 mg of acetone per ml. Photographs were taken under UV light.

RESULTS

Expression of PR in bacteria. ASLV PR is an abundant virion protein formed by proteolytic processing; it is derived from the C terminus of the $Pr76^{gag}$ precursor. For expression of the mature PR in *E. coli*, a translational initiation codon provided by the expression vector was introduced immediately preceding the PR reading frame (Fig. 1, PR). No alteration was necessary at the 3′ end, since the termination codon of PR can also function in bacteria (Fig. 1). The same vector was also used for expression of a viral NC-PR *gag* precursor fragment derived from SR-A avian sarcoma virus [pNC-PR(S)] or PR-C avian sarcoma virus (pNC-PR) (Fig. 1). A truncated version of the pNC-PR clone which is missing the N-terminal one-third of NC was also constructed (pΔNC-PR).

E. coli transformed with pPR was grown to mid-log phase at 30°C. Expression from the P_L promoter was then induced by shifting the cultures to 42°C. Cells were harvested 3 to 4 h postinduction, and proteins were analyzed by polyacrylamide gel electrophoresis and immunoblotting, with antisera directed against the purified ASLV PR and NC. A protein that comigrated with viral PR was produced in the induced but not in the uninduced cells (Fig. 2A, lanes 1 and 2). Neither the viral PR nor the bacterially produced PR reacted with anti-NC serum (Fig. 2B, lanes 1 and 2), as expected. From Coomassie blue staining, it can be estimated that PR represents 5 to 10% of the total protein in induced bacteria (data not shown).

Synthesis and cleavage of NC-PR in bacterial cells. Immunoblot analysis of the proteins produced after induction of

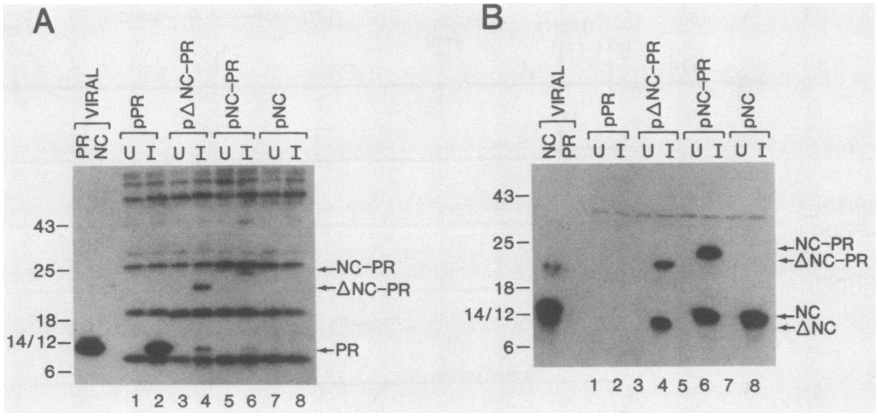

FIG. 2. Immunoblot analysis of PR and NC products expressed in *E. coli*. *E. coli* lysates were fractionated on a 15% polyacrylamide-sodium dodecyl sulfate gel, and virus-related proteins were detected by using rabbit antiserum directed against ASLV PR (A) or NC (B) and ^{125}I-labeled protein G as described elsewhere (1). Lysates were prepared from *E. coli* containing pPR (lanes 1 and 2), pΔNC-PR (lanes 3 and 4), pNC-PR (lanes 5 and 6), and pNC (lanes 7 and 8). Lanes 1, 3, 5, and 7, lysates from uninduced (U) cultures; lanes 2, 4, 6, 8, lysates from cultures induced (I) for 3 h at 42°C. Molecular size markers are indicated in kilodaltons. Viral NC and PR markers are shown. The anti-PR serum (A) cross-reacts with a number of *E. coli* proteins, as seen in lysates from uninduced cultures (lanes 1, 3, 5, and 7). The anti-NC serum (B) cross-reacts with an *E. coli* protein of ca. 35 kilodaltons.

the pNC-PR vector identified a protein of the mass predicted for the NC-PR precursor fragment (ca. 23 kilodaltons); it reacted with both anti-PR and anti-NC (Fig. 2A and B, lanes 5 and 6). Two smaller proteins which had migration coefficients corresponding to those of proteins produced by pNC and pPR and those of the analogous purified viral proteins were also identified. One of these two proteins reacted with anti-NC, while the other reacted with anti-PR, indicating that the NC-PR precursor underwent cleavage at (or near) the site recognized during virus maturation (Fig. 2A and B, lanes 5 and 6). Analysis of proteins produced after induction of pΔNC-PR gave similar results (Fig. 2A and B, lanes 3 and 4). As expected, both the precursor fragment and the NC-related product were shorter than those produced by pNC-PR, while the PR product was unaffected. This result confirmed the identities of the small anti-NC and anti-PR reactive bands.

One explanation for the production of small, discrete-sized products is that the NC-PR and ΔNC-PR precursors were cleaved specifically by PR. To test this hypothesis, we used a mutated clone in which the codon for the highly conserved Asp residue (amino acid 37 in PR) located in the putative active site of PR (20) was changed to an Ile codon [pNC-PR(S-I), Fig. 1]. Expression of proteins from this plasmid was assayed by immunoblotting with the anti-NC serum. The results showed that the NC cleavage product was produced from the wild-type construct, pNC-PR(S), but not from the mutant, pNC-PR(S-I) (Fig. 3, compare lanes 6 and 8). Thus, we conclude that bacterially produced PR activity is responsible for specific cleavage of the NC-PR *gag* precursor fragment. The protein which contained the Asp to Ile mutation migrates more rapidly than the wild-type NC-PR protein (Fig. 3, lane 6; see arrows), indicating that this change may have a significant effect on the protein conformation.

Detection of PR activity in cell extracts. To confirm that an active PR was produced in *E. coli*, proteolytic activities encoded by all six plasmids described in Fig. 1 were tested in cell extracts by using a synthetic peptide cleavage assay (Kotler et al., in press). We have previously shown that decapeptides which are composed of a known ASLV proteolytic processing site in *pol* (between the α and pp32^{pol} domains) are precisely cleaved by ASLV PR in vitro. A change in one of the amino acids which flanks the cleavage site (e.g., Tyr to Ala) abolishes this cleavage (Kotler et al., in press).

Extracts prepared from induced cells which express processed PR [from plasmids pPR, pNC-PR, pΔNC-PR, and pNC-PR(S)] contained activities which cleaved the susceptible peptide. Extracts from cells which express pNC or pNC-PR(S-I), which do not produce processed PR, showed

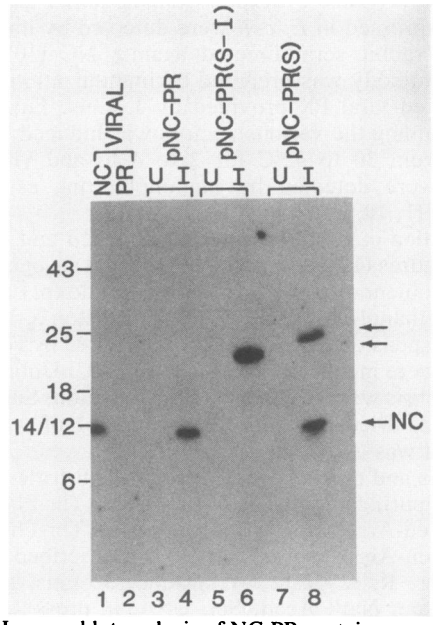

FIG. 3. Immunoblot analysis of NC-PR proteins expressed in *E. coli*. Analysis was carried out as described in the legend to Fig. 2, except that cultures were induced for 4 h. NC-related proteins were detected by incubation with rabbit anti-NC, followed by ^{125}I-labeled protein G. Lysates were prepared from cells containing pNC-PR (lanes 3 and 4), pNC-PR(S-I) (lanes 5 and 6), and pNC-PR(S) (lanes 7 and 8). Lanes 4, 6, and 8 contain lysates from cells that were induced for expression (I); lanes 3, 5 and 7 contain lysates from uninduced (U) cells. Lanes 1 and 2 contain authentic viral proteins.

no cleavage (Fig. 4A). As a control, a peptide containing a single amino acid change at the cleavage site (Tyr to Ala) was incubated with the same *E. coli* extracts. No cleavage was seen even after a 10-h incubation. By using this sensitive peptide assay, low levels of PR activity could be detected in some extracts prepared from uninduced cells which contained plasmids with the wild-type PR sequence, although no PR-related proteins could be detected by immunoblotting (Fig. 2 and 3). We presume that this is due to low expression (leakiness) of the respective plasmids under uninduced conditions. Since the peptides are quite stable in crude extracts, the reaction mixtures can be incubated for very long periods of time, thus further increasing the sensitivity of the assay.

DISCUSSION

In this report, we describe expression of a retroviral protease in bacteria. This enzyme is active in bacterial cells as well as in cell extracts. A mutation in the putative active site of PR (Asp to Ile) in the pNC-PR construct resulted in a failure to release NC and PR. This result suggests that PR is responsible for releasing itself from the *gag* precursor. However, the experiments with the mutated PR cannot exclude limited participation of cellular proteases. For example, it might be imagined that limited proteolysis by a cellular protease could release some mature PR molecules, and these might then be responsible for additional processing

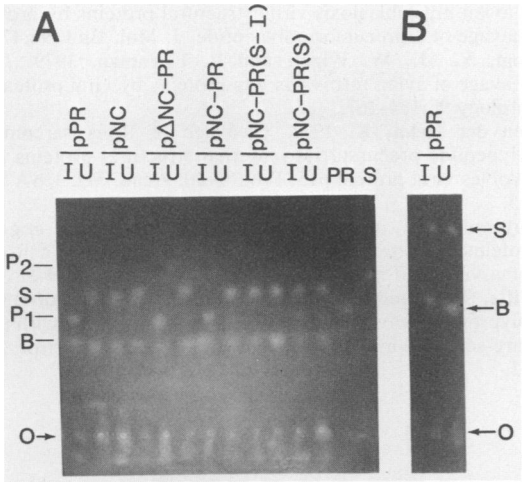

FIG. 4. Detection of PR-specific activity in bacterial extracts by using a thin-layer electrophoresis assay. Synthetic decapeptides containing Tyr-Pro (wild type) (A) or Ala-Pro (mutant) (B) cleavage sites were incubated with bacterial extracts prepared from induced (lanes I) and uninduced (lanes U) cultures. As a control, the Tyr-Pro peptide was subjected to purified viral PR (lane PR) or was incubated with no PR (lane S). The extracts were prepared from cultures containing the indicated clones. After incubation of peptides with extracts, the mixtures were fractionated by thin-layer electrophoresis (see Materials and Methods). The mobilities of peptide substrates (S), products (P1 and P2), and an unidentified spot associated with the bacterial extract (B) are indicated. The origin is indicated (O). P1 is a pentapeptide cleavage product corresponding to the N terminus of the decapeptide substrate. P2 (dark spot) corresponds to a pentapeptide derived from the C terminus. Products were located by staining with fluorescamine, followed by visualization with UV light (see Materials and Methods). The P2 spot appears dark because of the N-terminal Pro which produces chromophores that absorb UV light. Details of the methodology and characterization of the products identified by the thin-layer electrophoresis assay are provided elsewhere (Kotler et al., in press).

which would release more active PR molecules. If the processing is due entirely to PR activity, it would be of interest to determine whether the first mature molecules are released as a consequence of inter- or intramolecular interactions. Introduction of mutations into the NC-PR cleavage site may provide some insight into this question.

Our finding that the PR-NC *gag* precursor fragment is cleaved intracellularly is consistent with earlier reports of proteolytic processing of the ASLV *gag* precursor in *E. coli* (17) and human immunodeficiency virus *gag-pol* precursors in yeast cells (13). In contrast, the ASLV $Pr76^{gag}$ precursor synthesized in vitro by using a rabbit reticulocyte system does not undergo processing unless PR is added (28). Also, $Pr76^{gag}$ is not processed in mammalian cells infected by avian sarcoma virus (25), and these cells are unable to support assembly of infectious particles (2, 12). There are several possible explanations for these differences. (i) Avian, bacterial, and yeast cells, but not mammalian cells, might contain a protease which is able to activate PR by releasing it. The active PR molecules might then complete the processing as mentioned above. (ii) Mammalian cells may contain an inhibitor of ASLV PR which prevents autocleavage or subsequent enzymatic activity. (iii) The release of PR may require dimerization of the precursor (20) or an intermolecular reaction which occurs only when a high concentration of precursor is present, as in bacteria, yeast cells, and the permissive avian cells. Threshold concentrations may not be achieved in vitro or in mammalian cells. (iv) The $Pr76^{gag}$ may be folded in a specific structure which prevents autocleavage until the time of virion budding. Subsequent activation of the protease might occur in virions produced in avian cells but not in mammalian cells. Precursor proteins produced in bacteria might not fold in the manner required to prevent cleavage. Some of these models may be testable by using methods described in this paper.

The bacterial expression systems described here will allow us to produce large amounts of normal and altered ASLV PR proteins. These should be useful for studying the biochemical properties of PR and for providing material for biophysical analyses. The *gag* precursor fragment (NC-PR) will be a useful substrate to test the effect of various cleavage site mutations. Since cleavage occurs in bacteria, it should be possible to study the effect of such mutations without purification of the PR or substrates.

ACKNOWLEDGMENTS

We wish to thank W. Danho for the synthesis of peptides, J. Leis for supplying purified viral PR, F. Erlitz for the synthesis of the oligonucleotides, R. Crowl for pEV-vrf DNA, and D. Markham for advice on extract preparation. We gratefully acknowledge V. Vogt, K. Jones, J. Kulkosky, and P. Tsichlis for critical reviews of the manuscript. We also thank V. Vogt and G. Schatz for providing mutant constructs and for helpful and stimulating discussions.

This work was supported by Public Health Service grants CA-06927 and RR-05539 from the National Institutes of Health, a grant from the Pew Charitable Trust, and an appropriation from the Commonwealth of Pennsylvania. Partial support for R.A.K. was provided by the W. W. Smith Charitable Trust.

LITERATURE CITED

1. **Alexander, F., J. Leis, D. A. Soltis, R. M. Crowl, W. Danho, M. S. Poonian, Y.-C. E. Pan, and A. M. Skalka.** 1987. Proteolytic processing of avian sarcoma and leukosis viruses *pol-endo* recombinant proteins reveals another *pol* gene domain. J. Virol. **61**:534–542.
2. **Altaner, C., and H. M. Temin.** 1970. Carcinogenesis by RNA sarcoma viruses. XII. A quantitative study of infection of rat

cells *in vitro* by avian sarcoma viruses. Virology **40**:118–134.
3. **Casadaban, M. J., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *E. coli*. J. Mol. Biol. **138**:179–207.
4. **Crawford, S., and S. P. Goff.** 1985. A deletion mutation in the 5′ part of the *pol* gene of Moloney murine leukemia virus blocks proteolytic processing of the *gag* and *pol* polyproteins. J. Virol. **53**:899–907.
5. **Crowl, R., C. Seamans, P. Lomedico, and S. McAndrew.** 1985. Versatile expression vector for high-level synthesis of cloned gene products in *Escherichia coli*. Gene **38**:31–38.
6. **Dickson, C., R. Eisenman, H. Fan, E. Hunter, and N. Teich.** 1984. Protein biosynthesis and assembly, vol. 1, p. 513–648. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Dittmar, K. J., and K. Moelling.** 1978. Biochemical properties of p15-associated protease in an avian RNA tumor virus. J. Virol. **28**:106–118.
8. **Eisenman, R. N., W. S. Mason, and M. Linial.** 1980. Synthesis and processing of polymerase proteins of wild-type and mutant avian retroviruses. J. Virol. **36**:62–78.
9. **Katoh, I., Y. Yoshinaka, A. Rein, M. Skibuga, T. Odaka, and S. Oroszlan.** 1985. Murine leukemia virus maturation: protease region required for conversion from "immature" to "mature" core form and for virus infectivity. Virology **145**:280–292.
10. **Katz, R. A., X.-D. Fu, A. M. Skalka, and J. Leis.** 1986. Avian retrovirus nucleocapsid protein, pp12 produced in Escherichia coli has biochemical properties identical to unphosphorylated viral protein. Gene **50**:361–369.
11. **Katz, R. A., C. A. Omer, J. H. Weiss, S. A. Mitsialis, A. J. Faras, and R. Guntaka.** 1982. Restriction endonuclease and nucleotide sequence analyses of molecularly cloned unintegrated avian tumor virus DNA: structure of large terminal repeats in circle junctions. J. Virol. **42**:346–351.
12. **Kotler, M.** 1971. Interaction of avian sarcoma virus with rat embryo cells in culture. J. Gen. Virol. **12**:199–206.
13. **Kramer, R. A., M. D. Schaber, A. M. Skalka, K. Ganguly, F. Wong-Staal, and E. P. Reddy.** 1986. HTLV-III *gag* protein is processed in yeast cells by the virus *pol*-protease. Science **231**: 1580–1584.
14. **Leis, J., D. Baltimore, J. M. Bishop, J. Coffin, E. Fleissner, P. Goff, S. Oroszlan, H. Robinson, A. M. Skalka, H. M. Temin, and V. Vogt.** 1988. A standardized and simplified nomenclature for proteins common to all retroviruses. J. Virol. **62**:1808–1809.
15. **Levin, J. G., S. C. Hu, A. Rein, L. I. Messer, and B. I. Gerwin.** 1984. Murine leukemia virus mutant with a frameshift in the reverse transcriptase coding region: implication for *pol* gene structure. J. Virol. **51**:470–478.
16. **Lu, A. H., M. M. Soong, and P. K. Y. Wong.** 1979. Maturation of Moloney murine leukemia virus. Virology **93**:269–274.
17. **Mermer, B., M. Malamy, and J. M. Coffin.** Rous sarcoma virus contains sequences which permit expression of the *gag* gene in *Escherichia coli*. Mol. Cell. Biol. **3**:1746–1758.
18. **Moelling, K., A. Scott, K. E. J. Dittmar, and M. Owada.** 1980. Effect of p15-associated protease from an avian RNA tumor virus on avian virus-specific polyprotein precursors. J. Virol. **33**:680–688.
19. **Morinaga, Y., T. Franceschini, S., Inouye, and M. Inouye.** 1984. Improvement of oligonucleotide-directed site specific mutagenesis using double-stranded plasmid DNA. Bio/Technology **2**: 636–639.
20. **Pearl, L. H., and W. R. Taylor.** 1987. A structural model for the retroviral protease. Nature (London) **329**:351–354.
21. **Schwartz, D. E., R. Tizard, and W. Gilbert.** 1983. Nucleotide sequence of Rous sarcoma virus. Cell **32**:853–869.
22. **Tang, J., and R. N. S. Wong.** 1987. Evolution in the structure and function of aspartic proteases. J. Cell. Biochem. **33**:53–63.
23. **Toh, H., M. Ono, K. Saigo, and T. Miyata.** 1985. Retroviral protease-like sequence in the yeast transposon Ty-1. Nature (London) **315**:691.
24. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
25. **Vogt, V. M., D. A. Bruckenstein, and A. P. Bell.** 1982. Avian sarcoma virus *gag* precursor polypeptide is not processed in mammalian cells. J. Virol. **44**:725–730.
26. **Vogt, V. M., R. Eisenman, and H. Diggelmann.** 1975. Generation of avian myeloblastosis virus structural proteins by proteolytic cleavage of a precursor polypeptide. J. Mol. Biol. **96**:471–493.
27. **Vogt, V. M., W. Wight, and R. Eisenman.** 1979. *In vitro* cleavage of avian retrovirus *gag* proteins by viral protease p15. Virology **98**:154–167.
28. **Von der Helm, K.** 1977. Cleavage of Rous sarcoma viral polypeptide precursor into internal structural proteins *in vitro* involves viral protein p15. Proc. Natl. Acad. Sci. USA **74**:911–915.
29. **Voynow, S. L., and J. M. Coffin.** 1985. Truncated *gag*-related proteins are produced by large deletion mutants of Rous sarcoma virus and form virus particles. J. Virol. **55**:79–85.
30. **Witte, O. N., and D. Baltimore.** 1978. Relationship of retrovirus polyprotein cleavages to virion maturation studied with temperature-sensitive murine leukemia virus mutants. J. Virol. **26**:750–761.