

## PRCII, a Representative of a New Class of Avian Sarcoma Viruses

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### A. Abstract

The Poultry Research Center Virus II (PRC II) is a replication-defective avian sarcoma virus with envelope determinants of the A and B subgroups. In nonproducing cells transformed by PRCII the products of the replicative genes *gag*, *pol*, and *env* are not demonstrable, but a single polyprotein of Mr 105,000 (p105) can be detected. P105 contains peptides of the *gag* proteins p19 and p27 plus transformation-specific sequences. It does not contain peptides of gPr95<sup>env</sup> or Pr180<sup>gag-pol</sup> (with the possible exception of one *pol* peptide). The transformation-specific sequences of p105 are distinct from those of p100 of avian carcinoma virus MH2, of p110 coded for by avian myelocytoma virus MC29, and of p75 or p40 of avian erythroblastosis virus AEV. They also show no resemblance to p60<sup>src</sup> of Rous sarcoma virus. P105 is phosphorylated on a tyrosine residue and has an associated phosphokinase activity. P105 appears to be capable of autophosphorylation and of phosphorylating homologous immunoglobulin.

### B. Introduction

Until recently all avian sarcoma viruses were thought to have fundamentally the same genetic structure. The investigated strains, chiefly Rous sarcoma virus (RSV) and its relatives, contain an about 1.8-kilobase (kb) insertion at the juncture of the *env* gene and the C region (Duesberg and Vogt 1970; Wang et al. 1975). This transformation-specific insertion, termed *src*, was found to be of cellular origin and to code for a protein kinase of Mr 60,000 with preference for tyrosine as a phosphoacceptor

(Stehelin et al. 1976; Spector et al. 1978; Brugge and Erikson 1977; Collett and Erikson 1978; Levinson et al. 1978; Hunter and Sefton 1980). In contrast to RSV, avian acute leukemia viruses code for polyproteins that are derived from a gene in which *gag* sequences at the 5' terminal part of the genome are fused to transformation-specific, cell-derived sequences in the middle of the genome. The cell-derived sequences form a substitution for replicative information of the *gag*, *pol*, and *env* genes (Bister et al. 1977; Hu et al. 1979; Lai et al. 1979; Sheiness and Bishop 1979; Roussel et al. 1979; Sheiness et al. 1980). As a consequence of this missing information, the acute avian leukemia viruses are replication defective (Ishizaki and Shimizu 1970; Graf 1975; Bister and Vogt 1978; Hu and Vogt 1979). A genome organization similar to that of avian acute leukemia viruses has also been found in Abelson and radiation leukemia virus of mice and in feline sarcoma virus (Witte et al. 1978; Manteuil-Brutlag et al. 1980; Barbacid et al. 1980; Sherr et al. 1980; Van de Ven et al. 1980).

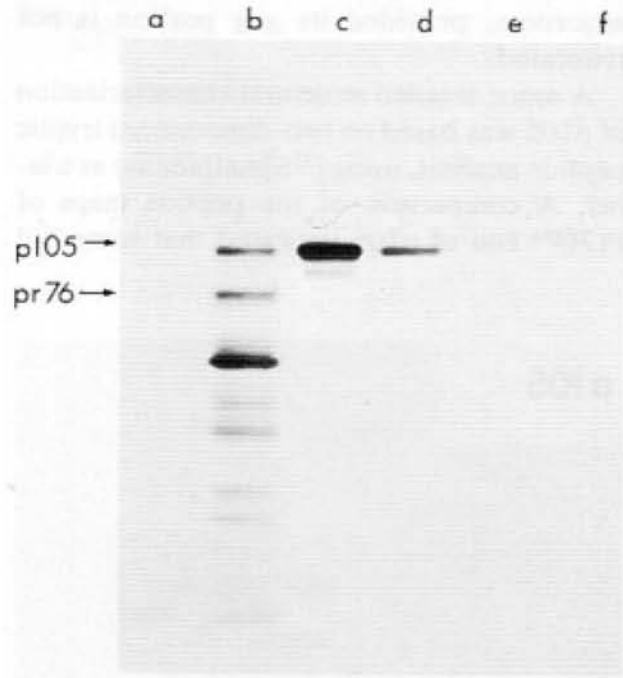
Recently a new class of avian sarcoma viruses has been described which lack *src* and have a genetic structure related to the avian acute leukemia viruses (Breitman et al. 1981; Hanafusa et al. 1980; Kawai et al. 1980; Lee et al. 1980; Neil et al. 1981). The viruses belonging to this group are Fujinami sarcoma virus (FSV), Y73, which is a recent field isolate, and PRCII. Even though these viruses have transformation-specific sequences which are different from the *src* of RSV, their transformation-specific proteins bear some intriguing functional resemblance to p60<sup>src</sup>. This functional similarity suggests possible common elements in the mechanism of trans-

formation by RSV and the new avian sarcoma viruses. The present paper will briefly summarize our studies on PRCII as an example of the new class of avian sarcoma viruses (Breitman et al. 1981; Neil et al. 1981).

### C. Results

PRCII was isolated from a spontaneous mesenteric sarcoma in a chicken and was shown to cause sarcomas which could be distinguished histologically from the growths induced by RSV (Carr and Campbell 1958). The virus belongs to envelope subgroups A and B (Payne and Biggs 1966; Duff and Vogt 1969). PRCII causes only sarcomas in chickens, and compared to RSV, it is a less virulent agent: Of 50 chickens inoculated with about  $10^4$  focus-forming units of virus, only 13 came down with tumors within an observation time of 4 weeks. In chick embryo fibroblast cultures PRCII induced foci of predominantly fusiform transformed cells; but no transformation was seen in cultures of hematopoietic cells derived from chicken bone marrow or chicken embryo yolk sac. Infectious center tests showed that while a focus of PRCII-transformed cells could be induced by a single particle, a focus which also released infectious progeny was the result of double infection with transforming virus and nontransforming helper virus. PRCII-transformed cells which failed to release infectious virus have been isolated. Such nonproducer lines also failed to produce noninfectious virions, an indication that the replication defects in PRCII extend into the *gag* gene (Bister et al. 1977; Bister and Vogt 1978; Hu et al. 1978; Hu and Vogt 1979). Superinfection of these nonproducing cells with avian leukosis helper viruses led to the rescue of infectious PRCII.

In order to search for virus-specific products in PRCII-transformed cells, virus producing and nonproducing transformed fibroblasts were labeled with [ $^{35}$ S]methionine (50 to 20  $\mu$ Ci/ml) and tested in immunoprecipitation using a variety of antisera. The precipitates were resolved by polyacrylamide gel electrophoresis and analyzed by autoradiography (Fig. 1). Rabbit sera prepared against whole virions of the Prague strain of RSV and sera against the structural *gag* protein of avian leukosis viruses precipitated from PRCII-transformed cell lysates a virus-specific protein

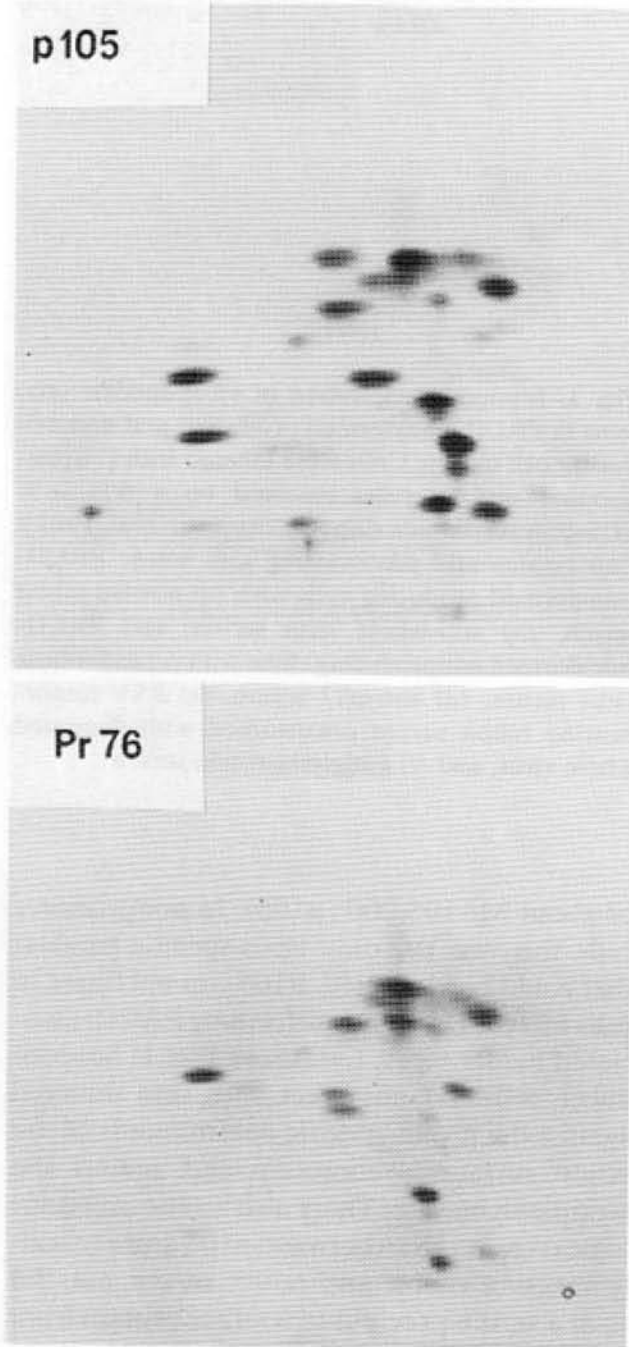


**Fig. 1.** Immunoprecipitation of virus-specific proteins in PRCII-transformed producing and nonproducing cell clones. Cells were labeled with [ $^{35}$ S]methionine and proteins resolved on a 6%–18% SDS-polyacrylamide slab gel (Breitman et al. 1981). Cell lysates and precipitating sera were: PRCII-transformed producing cells with (a) normal rabbit serum, (b) anti-whole virus serum; and PRCII-transformed nonproducing clone with (c) anti-whole virus serum, (d) anti-p27 serum, (e) RSV tumor-bearing rabbit serum preabsorbed with disrupted whole virus, and (f) antiglycoprotein serum

of about Mr 105,000 (p105). In nonproducer cells this was the only virus-specific product that could be identified. P105 was not found in cells infected by nontransforming PRCII-associated helper virus. Lysates of PRCII nonproducer cells did not react with sera directed against the group-specific determinants of the virion surface glycoprotein and against the virion polymerase. There also was no immunologic cross reaction between p105 and a broadly reacting rabbit anti-p60<sup>src</sup> serum nor did such a serum precipitate another protein from PRCII-transformed cell lysates. Therefore, we conclude that PRCII does not code for a functional *env* or *pol* gene product and that although PRCII is a pure sarcoma virus, it does not produce a transformation-specific protein that cross reacts in immunoprecipitation with antisera against p60<sup>src</sup> of RSV. The p105 does, however, contain *gag*-related sequences. But since *gag* codes for a protein of only Mr 76,000, p105 must contain additional non-*gag*

sequences, provided its *gag* portion is not reiterated.

A more detailed structural characterization of p105 was based on two-dimensional tryptic peptide analysis, using [<sup>35</sup>S]methionine as a label. A comparison of the peptide maps of Pr76<sup>gag</sup> and of p105 indicated that some but



**Fig. 2.** Tryptic peptide maps of PRCII p105 and of Pr76 of PRCII-AV. [<sup>35</sup>S]methionine-labeled proteins were separated by PAGE and located by autoradiography. The bands were excised and the proteins eluted and oxidized with performic acid and digested with TPCK-trypsin. The resultant peptides were separated by TLC electrophoresis (pyridine:acetic acid, pH 4.5) at 600 V for 100 min and by chromatography for 4 h in n-butanol:acetic acid:pyridine:water (75:15:60:60)

not all of the *gag* peptides were present in p105 and that p105 contained additional peptides not seen in Pr76<sup>gag</sup> (Fig. 2). Peptide maps of the individual *gag* proteins p19, p27, and p15 were also obtained. The fourth *gag* protein, p12, does not contain useful [<sup>35</sup>S]methionine peptides. These maps of three individual *gag* proteins showed that all of the p19 and p27 but none of the p15 peptides were present in p105. Identification of the common peptides was confirmed by appropriate mixing experiments. These results lead to the conclusion that p105 contains the 5' portion of the *gag* gene encompassing p19 and p27 but lacks the 3' of *gag* with p15. No statement can be made concerning the presence or absence of p12 sequences in p105. However, we found a rabbit antiserum prepared against p27 which did not react with p105, although it precipitated p27. Therefore, not all of the determinants of p27 appear to be present in p105. If the order of the *gag* proteins is N-p19-p27-p12-p15-C (Shealy et al. 1980), then p105 may lack p12 and p15 together with a portion from the carboxyterminus of p27. The *gag*-related part of p105 can then account for roughly half of the p105 molecule. In order to test for the possibility that the remainder of p105 contains partial, immunologically nonreactive sequences derived from the *pol* or *env* genes, tryptic peptide maps of gPr95<sup>env</sup> and of Pr180<sup>gag-pol</sup> of the PRCII helper virus were prepared. There was no overlap between any of the gPr95<sup>env</sup> peptides and those of p105. Of the *pol* peptides in Pr180, one comigrated with a peptide of p105, and this observation was confirmed in a mixing experiment. However, considering the relative complexity of the Pr180 map, this overlap may be fortuitous and requires further examination. We conclude that p105 does not contain significant portions of the helper virus *env* or *pol* genes.

Two-dimensional tryptic maps were also obtained of the transformation-specific proteins of (p110), of carcinoma virus MH2 (p100), of avian erythroblastosis virus AEV strain ES-4 (p75 and p40), and of the p60<sup>src</sup> protein from several genetically distinct lines of the Schmidt-Ruppin strain of RSV as well as of the endogenous p60<sup>src</sup> of chicken cells. The p60<sup>src</sup> proteins, the AEV p40, and the non-*gag* portions of AEV p75, MC29 p110, and MH2 p100 did not contain peptides coinciding with any of p105. We conclude that PRCII contains new transformation-specific sequences not

found in RSV or in avian acute leukemia viruses AEV, MC29, or MH2.

Cells transformed by PRCII were labeled with [<sup>32</sup>P]orthophosphate (1 mCi/ml) and followed by analysis by immunoprecipitation with anti-virion sera. Electrophoresis and autoradiography indicated that p105 was heavily phosphorylated. This degree of phosphorylation was greater than could be accounted for by the presence of the phosphorylated *gag* protein p19 in p105. The most abundant phosphoamino acid in p105 was tyrosine. These observations suggest that p105 is phosphorylated in its non-*gag* portion, probably at one or several tyrosine residues. In order to test for phosphokinase activity associated with p105, immunoprecipitates were incubated with [<sup>32</sup>P]ATP according to published procedures before immunoprecipitation, electrophoresis, and autoradiography. Under these conditions both p105 and the heavy chain of IgG were phosphorylated. We suggest that p105 is a phosphoprotein with associated kinase activity capable of autophosphorylation and of phosphorylating homologous IgG.

#### D. Discussion

PRCII is a representative of a new class of avian sarcoma viruses that lack *src*, have a genetic structure similar to avian acute leukemia viruses, and code for a transformation-specific *gag*-related polyprotein. Other members of this class are FSV and avian sarcoma virus Y73 (Hanafusa et al. 1980; Kawai et al. 1980; Lee et al. 1980). FSV and Y73 have been shown to contain a small (26 S) RNA genome. Our preliminary data suggest that the genome of PRCII has also a size of about 26 S. PRCII and FSV have some common transformation-specific sequences as detected by nucleic acid hybridization (Shibuya et al. 1980), and the proposal has been made to refer to these sequences as *fps* (FSV, PRCII Sarcoma) (J. Coffin et al. to be published). Y73 appears to have no appreciable sequence relationship in its transformation-specific information to either PRCII, FSV, or RSV (Shibuya et al. 1980; M. Yoshida et al. 1980, personal communications). The transformation-specific sequences of FSV have also been found to be related to those of the Snyder-Theilen and Gardner-Arnstein strains of feline sarcoma virus (Shibuya et al. 1980; Barbacid

et al. 1981). Since the latter sequences have been detected in normal feline cells where they code for a cellular protein homologue (Barbacid et al. 1981) one can expect *fps* to be also cell derived. The transformation-specific polyproteins of FSV, Y73, PRCII, and feline sarcoma virus are all phosphorylated and show phosphokinase activity. The predominant phosphoacceptor for these polyproteins is tyrosine (Hanafusa et al. 1980; Kawai et al. 1980; Neil et al. 1981; Barbacid et al. 1980). For PRCII and the Snyder-Theilen strain of feline sarcoma virus it has also been shown that phosphotyrosine levels in transformed cells are substantially increased over normal cells (Barbacid et al. 1980; K. Beemon 1980, personal communication). Phosphorylation and kinase activity are also characteristic of p60<sup>src</sup>, and cells transformed by RSV contain elevated levels of phosphotyrosine (Collett and Erikson 1978; Levinson et al. 1978; Hunter and Sefton 1980). These parallels suggest that even though sarcoma viruses may carry different transforming genes, the mechanisms of viral sarcomagenesis could share common elements in protein function and cellular targets.

#### Acknowledgment

Supported by U.S. Public Health Service Research Grants No. CA 13213 and 19725 awarded by the National Cancer Institute.

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