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Genetic Economy of Polyoma Virus: Capsid Proteins Are Cleavage Products of Same Viral Gene

(peptide maps/virus maturation)

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ABSTRACT Two-dimensional tryptic peptide maps of the nonhistone proteins of purified polyoma virus show marked similarities. Protein P_1 is a nondisaggregated, possibly covalent, dimer of the major capsid protein P_2, whereas P_3 and P_4 share several new peptides as well as many of the peptides derived from P_2. Extensive use of this kind of processing of viral proteins during the biosynthesis of DNA-containing animal viruses has not been reported previously.

Extensively purified virions of polyoma virus have been found to contain at least seven major protein components (1, 2), three of which are histone-like proteins probably coded for by the host-cell genome (3). The four nonhistone proteins are P_1–P_4, with molecular weights of approximately 86,000, 48,000, 35,000, and 23,000, respectively (1). If each of the four nonhistone virion proteins were unique gene products, then more than the approximately 150,000 daltons of protein that are potentially encoded by the viral genome would be committed for virion structural proteins, leaving no genetic information for other virus functions related to replication and cell transformation (4, 5). Proteolytic cleavage of precursor protein is a potential mechanism for the economic use of minimal viral genetic information, and has been demonstrated during maturation of polio and other picorna viruses, phages, and recently of avian myeloblastosis virus (6–9). Structural studies of polyoma virus now provide evidence for a precursor–product relationship between the nonhistone proteins of polyoma virus, making possible the synthesis of multiple distinct proteins from a single cistron.

MATERIALS AND METHODS

Large-plaque polyoma virus was prepared and purified from baby mouse kidney (BMK) cells by described methods (10). Virus was labeled with [^{14}C]leucine, [^{14}C]lysine, and [^{14}C]-valine, and purified virus was stored at 4°C in 0.05 M Tris·HCl, pH 8.0 (10).

Na Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Analytical and preparative electrophoresis of virus in 12.5% Na dodecyl sulfate–polyacrylamide gels was performed by the method of Neville (11). Radioactive protein bands were made visible by autoradiography using Kodak Royal Blue x-ray film.

Preparation of Purified Viral Proteins. Purified virion proteins were obtained from the dried gel slices used for autoradiography by locating protein bands precisely under the aligned x-ray film and eluting the cut-out gel segments with 0.05 M NH_4HCO_3 in 0.1% Na dodecyl sulfate. Fifty micrograms of bovine-serum albumin (fraction V, Pentex Corp.) were added to each sample, and the purified proteins were dialyzed against 0.05 M NH_4HCO_3. Each sample was precipitated at 0°C with 10% trichloroacetic acid, sedimented in a Sorvall HB-4 swinging bucket rotor at 10,000 rpm for 10 min, washed twice with cold 5% trichloroacetic acid, twice with acetone, and air dried.

Peptide Mapping. Isolated proteins were dissolved with 50 μl of 98% formic acid and then oxidized for 3 hr at 0°C with performic acid (12). After lyophilization, the oxidized proteins were dissolved in 0.5 ml of 0.1% NH_4HCO_3, pH 8.5, and L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone (TPCK) treated trypsin (Worthington Biochemicals) was added to an estimated ratio of 25:1 substrate to trypsin. Incubation was done for 12 hr at 37°C, and the resulting hydrolysate was lyophilized and redissolved in 10 μl of water. Samples of 5–10 μl were spotted onto glass thinlayer plates coated with cellulose (EM Laboratories). Electrophoresis in 8% acetic acid–2% formic acid, pH 1.9, was done at 900 V, 10 mA for 50 min. The dried plates were then rotated 90°, and ascending chromatography was performed in butanol–acetic acid–water pyridine (150:30:120:100) for 5–6 hr (13). Radioactive peptides were located by autoradiography on Kodak Royal Blue x-ray film after exposure for 2–3 months.

RESULTS

Virion proteins resolved by high-resolution SDS–polyacrylamide gel electrophoresis under the conditions described by Neville are similar to those described by Roblin and his colleagues (1), although proteins in the regions of both P_3 and P_4 are resolved into two or more distinct bands (Fig. 1). In most cases, the lower band of P_4 region is the major band in that region, and there are generally one or two major bands in the series of proteins in the P_3 region.

Peptide maps of the isolated, purified proteins designated P_1, P_2, P_3, and P_4 (Fig. 1) are given in Fig. 2. The patterns are reproducible in peptide maps of preparations separately oxidized and trypsinized. The distribution of peptides of protein P_1 is similar, or identical, to that of protein P_2, which is about half the molecular weight of P_1 (1). Protein P_3 contains many or most of the same peptides found in P_2, and about six major new peptides. In addition, several of the peptides seem to be present in reduced amounts compared with P_2. Most or all of the peptides of P_4 correspond to those found in P_3, although

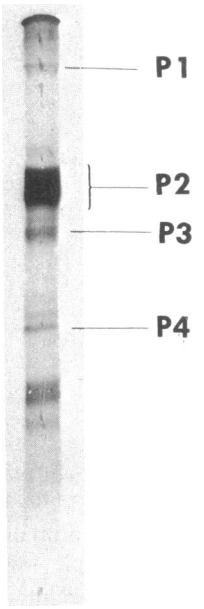

FIG. 1. Polyoma virion proteins after electrophoresis in 12.5% polyacrylamide gel by the method of Neville. Purified polyoma was precipitated with 10% cold trichloroacetic acid in the presence of 50 μg of bovine Fraction V, washed twice with cold 5% trichloroacetic acid, twice with acetone, and air dried. The sample was dissolved in Neville sample buffer containing 1% Na dodecyl sulfate, boiled for 1 min, and applied over 0.25 ml of stacking gels. Electrophoresis was at 2 mA per tube for 4 hr. Longitudinal slices of gels were dried and exposed to Kodak Royal Blue x-ray film.

about 10 to 12 are missing from P_4, accounting for the molecular weight difference of approximately 12,000 (1).

DISCUSSION

The results of these studies do not distinguish between overlapping proteolytic cleavage of a large precursor viral protein, and translation from several classes of related viral messenger RNAs generated by transcriptional or post-transcriptional mechanisms. Analysis of the species of messenger RNAs synthesized during infection of permissive cells with either polyoma or simian virus 40 has failed to demonstrate molecules of messenger RNA that are small enough to code uniquely for proteins of less than about 50,000 daltons (P. Rudland, personal communication), unless only a portion of the large messenger RNA is translated into protein. It is not possible to demonstrate or exclude these possible mechanisms by peptide maps alone. To date, the only known example of the latter mechanism is the report that A_1 protein of Qβ arises from translation of the coat cistron followed by further read-through (14).

Enzymic proteolytic cleavage leading to unique and distinct virion proteins is well known for picorna viruses. In that case, the polypeptides that are generated have unique and distinguishable sequences, and have in common only their origin in the precursor protein. For polio virus, the cleavage of NCVP-1 to produce VP1 at times involves multiple nonunique cleavages, which lead to cleavage products with slightly different molecular weights (15).

Protein P_1 of polyoma virus has a molecular weight approximately twice that of P_2 (1), while their peptide maps are very similar. The persistence of constant amounts of P_1 in all preparations of purified polyoma virions even after the denaturation conditions used for gel electrophoresis suggests that P_1 might be a dimer of P_2 held together through a covalent bond, perhaps a disulfide bond, which resists reduction in conditions used to prepare samples for gel electrophoresis.

The mechanism of the possible proteolytic cleavage that generates product P_4 and P_3 from P_2 or its precursor and the relationship of cleavage to virus maturation are unknown. These peptide maps do not allow the unequivocal identification of the primary gene product, but suggest that it could not be smaller than P_2, since the minimum molecular weight of P_2 from the amino-acid composition (Murakami, personal communication) shows 41 lysine + arginine residues per 40,000 daltons, similar to the number of tryptic peptides found in protein P_2 in this study. Most of the peptides that are present in protein P_2 are also seen in P_3, and there do not seem to be sufficient differences in the patterns to account for the molecular weight differences of approximately 13,000 (1). There are at least six major new peptides in P_3 compared with P_2, and several peptides that are major ones in P_2 seem to be minor spots in P_3. These findings are consistent with the existence of several nonunique cleavages on each end of the precursor protein, leading to a small number of new peptides and a number of normal peptides present in reduced amounts. By this scheme, P_4 is composed of B, P_3 contains families of molecules containing pieces A+B+C and B+C+D, and P_2 contains A+B+C+D (Fig. 3). Alternatively, or, in addition, the new peptides in P_3 could arise by failure of cleavage of the X–A bond and inclusion of piece X in protein P_3 X+A+B (Fig. 3). However, this model is less likely, since many normal P_2 peptides contained in the region C+D would be absent in a peptide map of P_3. Still another explanation for the new peptides of P_3 compared with P_2 could involve the presence in authentic viral P_3 of a contaminating protein. This seems to be unlikely since such a protein having a molecular weight of 34,000 would have an unusually low lysine + arginine content of 5 or 6, as shown by the low number of new peptides. A further problem with this interpretation comes from the necessary presence of the same contaminating protein, in approximately the same amounts, in P_4, since the new P_3 peptides are also present in P_4. The one striking feature of the peptide maps that cannot easily be explained by the overlapping cleavage model is the fact that the new P_3 peptides are present in large amounts, as judged by their relative intensity. However, unequivocal evidence for any of the above models is lacking. The mechanisms for regulating the markedly different numbers of molecules of the individual virion proteins from the equimolar amounts predicated by a proteolytic cleavage model are not clarified by this study. Presumably, physicochemical and thermodynamic constraints involved in self-assembly processes could be sufficient to allow the assembly of particles with nonequimolar ratios of protein components.

The topology and possibly unique structural roles of the various products of the primary gene product, and the limited genetic information of the viral genome suggest that protein components of a small icosohedral virus such as polyoma should retain sufficient sequence identity to permit quasi-equivalent interactions according to the mechanisms elucidated by Casper and Klug (16) while permitting specific and differentiated structural roles in the capsid for the different

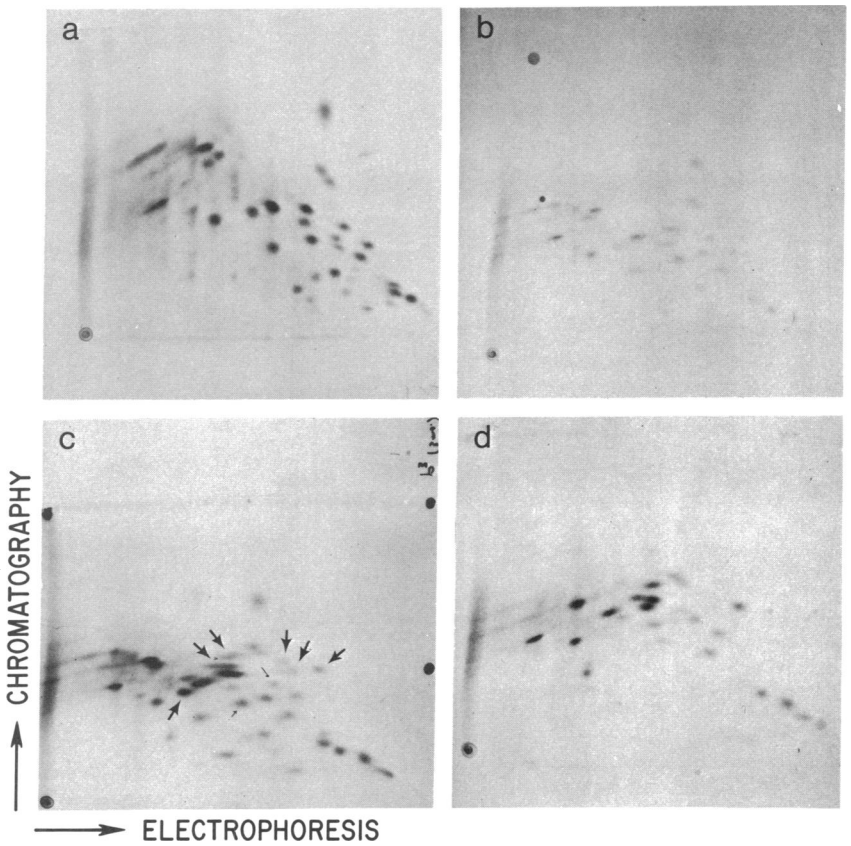

FIG. 2. Peptide maps of isolated purified polyoma proteins. Proteins were purified from dried longitudinal slices, and two-dimensional peptide maps were made (see *Methods*). (*a*) Major capsid protein P_2; (*b*) protein P_1: some of the peptides corresponding to the minor peptides of P_2 are not visible because of the small amount of P_1 available and the prolonged exposure time required; (*c*) protein P_3: *large arrows* indicate major new peptides not found in P_2; *small arrows* indicate normal peptides present in reduced amounts (*d*) protein P_4.

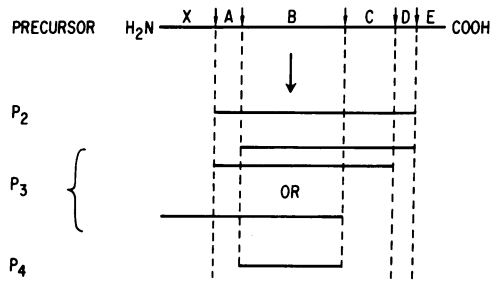

FIG. 3. Possible scheme for the generation of polyoma proteins from a common precursor. *Arrows* indicate theoretical sites of proteolytic cleavage.

cleavage products. It is clear now that polyoma virus makes more frugal use of its limited genetic information than has previously been thought, by the generation of several polypeptides either from several overlapping classes of messenger RNA, or more likely, by proteolytic cleavage of a precursor protein molecule.

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1. Roblin, R., Härle, E. & Dulbecco, R. (1971) *Virology* **45**, 555–566.
2. Friedmann, T. & David, D. (1972) *J. Virol.* **10**, 776–782.
3. Frearson, D. M. & Crawford, L. V. (1972) *J. Gen. Virol.* **14**, 141–155.
4. Sambrook, J. (1972) *Advan. Cancer Res.* **16**, 141–180.
5. Eckhart, W. (1972) *Annu. Rev. Biochem.* **41**, 503–516.
6. Jacobson, M., Asso, J. & Baltimore, D. (1970) *J. Mol. Biol.* **49**, 652–669.
7. Kiehn, E. D. & Holland, J. (1970) *J. Virol.* **5**, 358–367.
8. Laemmli, V. K. (1970) *Nature* **227**, 680–685.
9. Vogt, V. & Eisenman, R. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1734–1738.
10. Friedmann, T. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2574–2578.
11. Neville, D. M. & Glassman, H. (1971) *J. Biol. Chem.* **246**, 6335–6338.
12. Hirs, C. H. W. (1956) *J. Biol. Chem.* **219**, 611–621.
13. Friedmann, T., Taylor, S. & David, D. (1971) *Fed. Proc.* **30**, 1181 Abstr.
14. Moore, C. H., Farron, F., Bohnert, D. & Weissmann, C. (1971) *Nature New Biol.* **234**, 204–206.
15. Cooper, P., Summers, D. & Maizel, J. V. (1970) *Virology* **41**, 408–418.
16. Casper, D. L. D. & Klug, A. (1962) *Cold Spring Harbor Symp. Quant. Biol.* **27**, 1–24.