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On the Sequential Packaging of Bacteriophage P22 DNA

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Bacteriophage P22 is thought to package daughter chromosomes serially along concatemeric DNA. We present experiments which show that the average DNA packaging series length increases with time after infection, which supports this model. In addition, we have analyzed the effect on average series length of lowering the amount of the various individual proteins involved in DNA packaging. These results support the notion that the protein products of gene 2 and gene 3 are both more stringently required for initiation of sequential DNA packaging series than for their extension, and they are compatible with a model for the control of series length in which that length is determined, at least in part, by a competition between series initiation events and extension events.

Packaging of the phage P22 dsDNA chromosome within the coat protein shell requires only five proteins (1, 4, 13, 22). These are (i) the coat protein, gp5, which forms the protein shell of the virion and of the precursor particle, the prohead, within which the DNA molecule is packaged (2, 7, 13); (ii) the scaffolding protein, gp8, which is present as a core in proheads but exits intact from this structure at or near the time of DNA packaging (4, 11, 13); (iii) gp1, which is present in 8 to 12 molecules per prohead and virion and is required for DNA packaging activity by proheads (13, 17); and (iv and v) gp2 and gp3, which are required for DNA packaging by proheads but are not found in the completed virion (4, 13, 17). Although genetic evidence implicates gp3 in the process of recognition of packagable DNA (9, 18), the detailed roles of gp8, gp1, gp2, and gp3 remain obscure. (The designation gpX refers to the polypeptide gene product of cistron X.)

Phage P22 DNA is thought to be replicated by a rolling circle process, since progeny DNA molecules are present mainly as concatemers (1, 23). During packaging, chromosomes containing 103% of the genome sequence are cut from the concatemers and are placed within the coat protein shell in a coupled process called "headful" packaging (6, 22). This results in chromosomes which are partially circularly permuted and about 3% terminally redundant (19, 20, 26). Tye et al. (26) have presented an elegant model called "sequential packaging," which explains the observed limited circular permutation. They suggest that the phage packaging apparatus recognizes its DNA substrate by the presence of a specific base sequence (now called *pac*) and that packaging initiates and proceeds unidirectionally from that point (or nearby) (8, 27). Two double-strand cuts are made in concert with this process which release the packaged DNA from the concatemer. According to this model, subsequent packaging events begin at the end created by the previous event and proceed in the same direction as the first, with single dsDNA cuts releasing these packaged chromosomes from the concatemer. If these series are limited to about five events before a new sequence recognition and series initiation occurs, the model explains the observed limited extent of the circular permutation (8, 26), the packaging of deletion and insertion chromosomes (8, 25, 26), and certain aspects of the observed low level of packaging of host DNA (5, 14).

Control of the number of series initiation events is not understood (less than one-fourth of the *end* regions are cut [3, 8, 26]). It is known that the DNA cuts made near the *pac* site during the first event in a series can occur at one of several *end* sites which are probably not coincident with the *pac* site (3) but which must be within a few kilobases of the recognition sequence (H. Schmeiger, personal communication). These initial cuts may occur (normally?) before interaction with proheads, and gp2 and gp3 are absolutely required for these cuts to occur (15). In this report, we present evidence that gp2 and gp3 are involved in controlling the ratio of initiation to extension events in DNA packaging series.

If a packaging series proceeds from a *pac/end* region rightward (3; see Fig. 1), the left end of the first molecule packaged, when cut with a restriction enzyme, gives rise to a discrete "restriction" fragment with a packaging cut on its

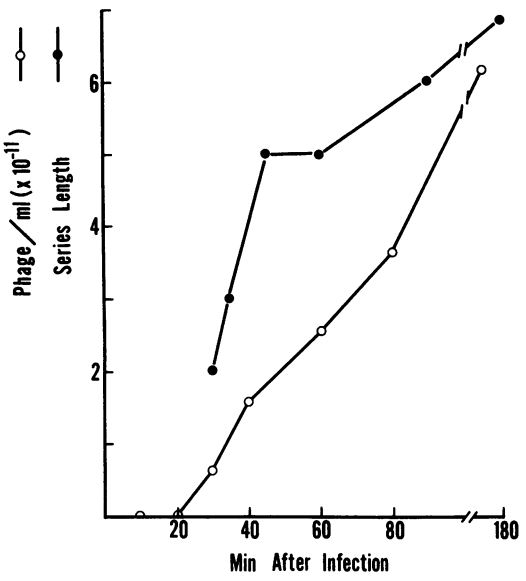

FIG. 1. Increase in sequential packaging series length with time after infection. Exponentially growing *Salmonella* strain DB7000 (sup^0) (2×10^8 cells per ml) was infected with phage P22 *c*1-7 13$^-$ *amH*1301 at a multiplicity of infection of 7 in LB broth at 37°C. At 10 min after infection, the cells were collected by centrifugation for 5 min at 7,000 × *g* and quickly resuspended in fresh, warm LB broth. The phage titer was monitored (○), and at the indicated times, aliquots were removed and immediately lysed with $CHCl_3$. Cell debris was removed by a low-speed centrifugation. Phage particles were pelleted by an overnight 8,000 × *g* centrifugation, suspended in 0.01 M Tris-chloride (pH 7.4)–0.01 M $MgCl_2$, and purified by centrifugation through a CsCl step gradient (11). DNA was purified from phage particles by three phenol extractions followed by several ether extractions and dialysis against 0.01 M Tris-chloride (pH 7.8)–0.001 M EDTA. DNA was cut with *Eco*RI or *Pst*I under the conditions specified by the supplier. The fragments were displayed in 1.0% agarose gels and stained with ethidium bromide. Band intensities were quantitated by scanning photographic negatives of the stained gels with a Joyce-Loebl microdensitometer as previously described (3). The length of an average packaging series (●) was calculated as the molar ratio of *Eco*RI fragments F, G, or H (or their sum) to the *Eco*RI *pac* fragment (8, 10).

left end and a restriction cut on the right end. Apparently headful packaging is imprecise, since the right end of the first chromosome in a series is too variable to give rise to a visible band in an agarose gel (3, 8; our unpublished data). It follows that left and right end fragments from DNA molecules packaged in secondary (noninitial) events are heterogeneous at both ends. Because the sequential packaging process causes all of the molecular ends to fall within the 20% of the genome to the right of the *end* site region (26), the molar ratio of a true restriction fragment from the right 80% of the genome (present intact in all phage chromosomes) to the *pac* fragment (present in only the first DNA molecule packaged in each series) in DNA purified from phage particles is the average length of a packaging series in the infected cell (8). This length has been found to be about three for wild-type P22 infections (8) and up to seven in lysis-defective infections (see below).

There is a monotonic increase in average series length between 30 and 180 min after infection of *Salmonella typhimurim* DB7000 (29) by P22 *c*1-7 13$^-$ *amH*101 (Fig. 1). The *c*1-7 mutation prevents a lysogenic response, and the 13$^-$ *amH*101 mutation blocks cell lysis (23). These results strongly support the sequential packaging model, since it predicts that the initial phage produced in an infected cell should have a *pac* fragment/true restriction fragment ratio of 1 and that series length should increase with time. The fact that the curve does not actually begin at a ratio of one is no doubt due to a combination of the following factors: (i) at early times it is difficult to obtain sufficient pure DNA to perform the analysis accurately using these methods, and (ii) if series initiation is rate limiting in DNA packaging, then it is possible that packaging of different concatemers is very asynchronous in the infected cell.

To begin to determine what controls the rate of series initiation, we have varied the in vivo levels of the proteins required for DNA packaging and analyzed the average series length 100 min after infection. By mixedly infecting cells with a known ratio of phage, one of which has wild-type DNA packaging genes and one of which carries a nonsense mutation in a DNA packaging gene, it is possible to specifically lower the rate of functional expression of that gene in the infected cell (21). This of course assumes that each (wild-type and mutant) cistron is read at a "constitutive" rate, and there are no compensatory regulatory mechanisms which could adjust the rate of expression of that gene relative to the other phage genes. Gene 8 is known to be regulated partially independently from the other morphogenetic genes (12), and so mutants in this gene were not used in this study. We have previously determined that the level of expression of gene 5 is proportional to the fraction of wild-type genes in the infecting phage (12), and there is no evidence for independent mechanisms controlling the expression of gene 1, 2, or 3 (28). Thus, we varied the levels of gp1, gp2, gp3, and coat protein in the infected cell and measured the average sequential packaging series length in each case. The results are shown in Fig. 2. Lowering the level of gp1 or coat

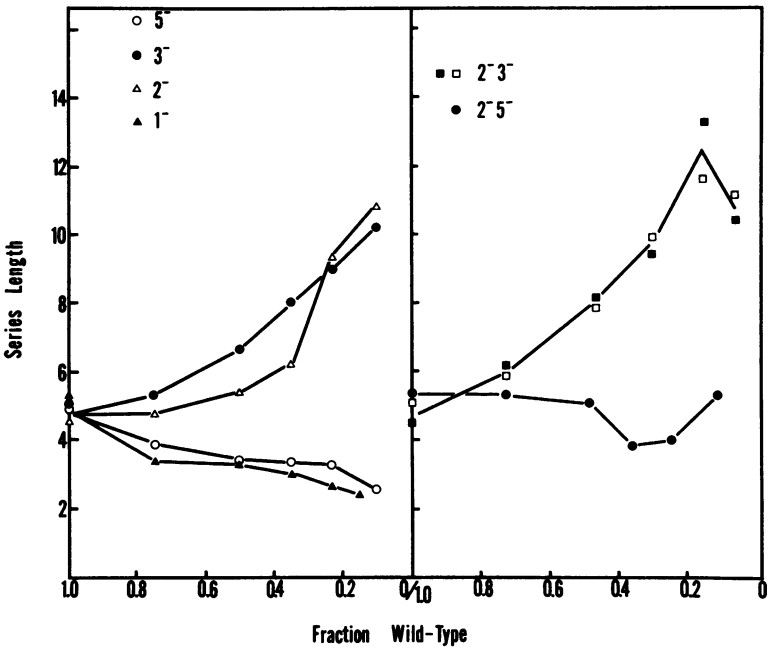

FIG. 2. Length of sequential packaging series as a function of gene dosage of the DNA packaging genes. Aliquots of exponentially growing DB7000 at 2×10^8 cells per ml in L broth at 37°C were infected at a total multiplicity of 20 with various ratios of wild-type and mutant phage as indicated in the figure. All phage carried the *c*1-7 and 13⁻*amH*1301 mutations. The alleles of 1^-, 2^-, 3^-, and 5^- mutations were *amN*10, *amH*200, *amH*317, and *amN*114, respectively. Average series length was determined as described in the legend to Fig. 1, except for the 2^- 3^- curve delineated with open squares, in which the *PstI pac* fragment and fragment E (20) were used for calculations.

protein (gp5) causes a decrease in the length of an average series, whereas lowering the level of gp2 or gp3 or both causes an increase in the series length.

The fact that lowering coat protein or gp1 decreases the series length is easily interpreted in terms of the sequential packaging model and probably reflects the fact that both are structural proteins of the prohead and are not made in great excess. Lowering the number of proheads (total packaging events) in the cell without lowering the number of series initiations would shorten the average series length. The number of phage produced in the 5^- and 1^- mixed infections did not fall until the wild type/total phage ratio decreased to about 0.6 and 0.8, respectively (data not shown). At values below this, there was an approximately linear decrease in progeny phage yield.

On the other hand, lowering the level of gp2 or gp3 or both causes an increase in series length. The data argue that gp2 and gp3 are more stringently required for series initiation than for extension, and they also argue strongly against models for control of series length in which gp2 and gp3 are antagonists, with one protecting *pac* sites and one initiating packaging series (8), since both proteins affect series length in qualitatively the same way. These results are consistent with the facts that (i) gp3 is thought to be involved in the initial recognition event in a packaging series, although this may not be its only function (9, 18, 24); (ii) initiation events, according to current ideas, require two DNA cuts, whereas extension events require only one (6); (iii) gp2 and gp3 are required for making the initial cut in the concatemer (15); and (iv) our own unpublished observations suggest that neither gp2 or gp3 is *cis* acting. These combined results are compatible with a general model in which series length is determined at least in part by competition between series initiation events and extension events. The observation that simultaneously lowering gp2 and gp5 levels causes no substantial change in series length (Fig. 2) is also consistent with this idea.

Although the increases in series length were approximately parallel, the changes in number of progeny phage were not parallel in the 2^- and 3^- mixed infections (Fig. 3). The 2^- mixed infections yielded progeny phage numbers approximately proportional to the ratio of wild-type infecting phage, whereas the 3^- mixed infections did not show a significant drop in

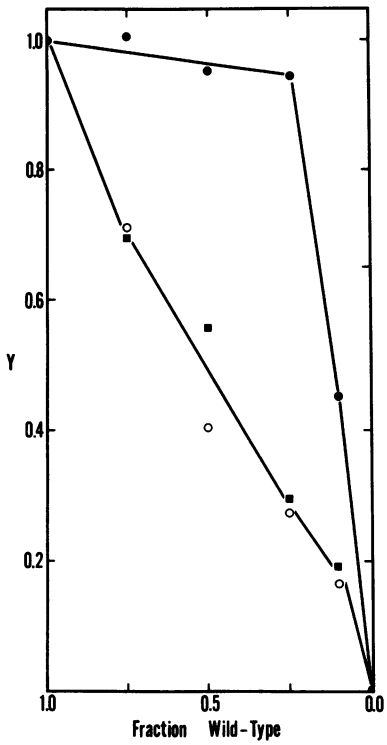

FIG. 3. Relative phage production in mixedly infected cells. Phage were mixedly infected with wild-type and 3^- (●), 2^- (○), or 2^- 3^- (■) phage as described in the legend to Fig. 2, and total phage yield was determined by plaque assay on DB7004 (12) after $CHCl_3$ lysis at 100 min after infection. The results are plotted as fraction of wild-type phage yield (Y) versus fraction of wild-type infecting phage (○).

phage yield until the fraction of wild-type infecting phage decreased to below 0.2. These results are in substantial agreement with previous observations made by J. Edelman and J. King (personal communication). In addition, the 2^- effect on progeny yield is epistatic to the 3^- effect (at least at this time after infection; at much later times, intermediate phage yields were obtained). These observations can be interpreted as follows. At lowered levels of gp3 (down to about 20% of normal), the amount of gp3 is not limiting, suggesting it is made in substantial excess for its essential role in phage production. However, at all lowered levels of gp2, phage production is limited by the amount of gp2 present, even if the level of gp3 is also lowered. These observations are consistent with the discovery by Poteete and Botstein (16) that gp2 and gp3 purify from infected cells as a complex and with our observation that gp3 appears to be made in excess over that required to saturate its gp2 binding sites (H. Brown and S. Casjens, unpublished data). More detailed interpretation of these results is complex, but it does comment on the simple hypothesis that gp2 and gp3 must always perform their functions as the complex described by Poteete and Botstein (16). In particular, this hypothesis predicts that the relative changes in phage yield and series length should be the same whether the level of complex is lowered by lowering gp2 or gp3. However, comparing Fig. 2 and 3 shows that this is qualitatively not the case. Specifically, as gp3 is lowered, the series length is lengthened greatly before changes in phage yield are seen, whereas the reverse is seen as gp2 is lowered. Because the exact relationship between series length and phage yield depends upon the absolute number of series initiation events, we do not yet understand these results in molecular terms, but they do imply that gp3-gp2 function is more complicated than this simplest model.

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