Bacteriophage P1 pac sites inserted into the chromosome greatly increase packaging and transduction of Escherichia coli genomic DNA

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**Abstract**

The Escherichia coli bacteriophage P1 packages host chromosome separately from phage DNA, and transfers it to recipient cells at low frequency in a process called generalized transduction. Phage genomes are packaged from concatemers beginning at a specific site, pac. To increase transduction rate, we have inserted pac into the chromosome at up to five equally spaced positions; at least this many are fully tolerated in the absence of P1 infection. A single chromosomal pac greatly increases transduction of downstream markers without decreasing phage yields; 3.5 × as much total chromosomal DNA is packaged. Additional insertions decrease phage yield by > 90% and also decrease phage DNA synthesis, although less dramatically. Packaging of chromosomal markers near to and downstream of each inserted pac site is, at the same time, increased by greater than 10 fold. Transduction of markers near an inserted pac site can be increased by over 1000-fold, potentially allowing identification of such transductants by screening.

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**Introduction**

Bacteriophage P1 is a temperate DNA coliphage with a double-stranded, linear, cyclically permuted, terminally redundant chromosome (Lehnerr, 2005, Yarmolinsky and Sternberg, 1988). The full P1 genome sequence has been published (Lobocka et al., 2004; Yarmolinsky, 2004). The phage DNA consists of the 94 kb long full P1 genome sequence has been published (Lobocka et al., 2004; Yarmolinsky, 2004). The phage DNA consists of the 94 kb long genome plus a 10–15 kb variable terminal redundancy, together equivalent to 2.2 *Escherichia coli* map units (minutes). The maximum distance between cotransduced host DNA markers is thus limited to this length.

The phage chromosome is circularized by recombination early after infection allowing the later formation of phage concatemers by rolling-circle replication (Cohen, 1983; Bornhoft and Stodolsky, 1981). Concatemers are cut once by the phage-encoded enzyme, Pacase, at a specific site, pac (packaging), from which the filling of phage heads is initiated (Sternberg, 1990 (review), Skorupski et al., 1994, Skorupski et al., 1992, Sternberg and Coulby, 1987a, 1987b). Sequential encapsidation from this cut yields the set of redundant, permuted molecules found in progeny phages, a process termed "circular permutation". Each repeat contains a GATC Dam methylation site, and methylation appears essential for recognition and cleavage by Pacases (Sternberg and Coulby, 1990). The unpackaged end of the cut pac site is degraded by host nucleases (Sternberg and Coulby, 1987a).

To prevent understuffing of the phage prohead and to ensure circular permutation, the cleavage of phage concatemers at more than one pac site must be prevented. Although hypotheses have been offered (Yarmolinsky and Sternberg, 1988; Skorupski et al., 1994a) the actual mechanism that prevents additional Pacase cleavages within concatemers is unknown.

P1’s widespread familiarity and usefulness results from its ability to mediate generalized transduction (Masters, 1985, 1996, 2000), but the mechanism by which transducing phages arise is not fully understood. Since transducing particles contain only host DNA (Ikeda and Tomizawa, 1965), which lacks pac sites (Hanks, 1988), and packaging of host sequences is mainly proportional to their intracellular concentrations (Masters, 1970, 1977, 1985; Masters et al., 1984), packaging must also be able to begin from free host DNA ends. Such ends are found at replication forks or could result from nuclease action. Sequential packaging from pac-free chromosomes has been reported (Harriman, 1972; Hanks et al., 1988; Sternberg and Coulby, 1987b) and it appears to proceed much farther than the 3–5 headfuls packaged from phage concatemers. When transducing phage are prepared from

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cells in which a single pac site has been inserted in the chromo-
some, processivity from pac can be inferred, confirming that
Pacase does not need to cut at any but the initial pac site.

The number of fully transduced bacterial progeny recovered for
any particular marker is normally between $10^{-5}$ and $10^{-6}$ per
phage even though transducing phages have been reported to
comprise up to 6% of particles in lysates of P1kc (Hanks et al.,
1988). This is thought to be because most transductions are
in abortive transductants is not covalently closed and that circular-
ly inherited stable circle in the recipient cell. Sandri and
incomplete (abortive), terminating with a non-replicating, unili-
1988). This is thought to be because most transductions are
hybridization probes are indicated outside the ring (positions in blue). Genes inside the ring (turquoise) were used as transductional markers.

Strain construction

The argB<sub>+</sub>pac strain, MG(argB<sub>+</sub>pac), was made using the Link
et al., (1997) pKO3 procedure. Crossover PCR was used to construct
a -BamH1-argc-sact-argH-BamH1- fragment that was cloned into
pKO3 to yield pHM2. P1 EcoRI-20 (containing pac) was cloned into
the pHM2 Sacl site to give pHM3, which was then used to replace
argB with pac by transformation followed by temperature and
succrose resistance selection/screen. The four other single pac

Methods and materials

Bacteria, plasmids, bacteriophages and associated techniques

Bacteriophage P1kc, purified from our laboratory stock, was
used throughout this work. All pac-containing strains were
derived from E. coli K12 MG1655 obtained from F. Blattner. Plasmid
pKO3 (Link et al., 1997) was kindly provided by G. Church. Media
and methods for bacterial growth and transductional selections
were as described by Masters (1970). Transductional recipients
were MM303 (Masters et al., 1984), MM38 [MM18 (Jenkins et al.,
1986) amB<sup>+</sup>], AB352 (thr-1 leuB6 lacZ4 purF1 rpsL8 thi-1 supE44);
from CGSC, Yale University, and MG1655Δlac (this lab). Other
plasmids are described in Table 1.
are identiﬁed with reference to the number and identity of pac insertion positions, in bold type, are between the genes in brackets.

A single 2pac strain was made by transducing the KanR (1-pb) replacement to the argB:pac strain. All strains containing more than 2 pac sites are derived from this MG1655(2pac) strain. Strains are named with reference to the number and identity of pac insertions. The number in the brackets is the number of pac sites in the strain. MG (argB:pac), the strain in which argB has been replaced by pac, does not have a numerical designation as it was constructed differently than the others. MGΔargB is a control strain from which argB has been deleted and not replaced by pac. The other four strains with one pac site are identiﬁed by the ﬁrst letters of the genes between which pac has been inserted (see Fig. 1, Table 2). The strains with 3 pac sites are identiﬁed by their single unique site. MG(3-tt) is the parent of MG(4pac) to which the (dy) construct has been added; (5pac) contains (de) in addition.

Table 2
Genes probed and insertion positions of pac sites.

<table>
<thead>
<tr>
<th>Marker/ pac site names</th>
<th>Location on chromosome (min)</th>
<th>Length EcoRV fragment (probe template)</th>
<th>Orientation of pac</th>
</tr>
</thead>
<tbody>
<tr>
<td>nadR</td>
<td>99.7</td>
<td>3.0 k</td>
<td>↓</td>
</tr>
<tr>
<td>hoIC</td>
<td>96.0</td>
<td>0.7 k</td>
<td>↓</td>
</tr>
<tr>
<td>yji</td>
<td>95.0</td>
<td>1.2 k</td>
<td>↓</td>
</tr>
<tr>
<td>psi</td>
<td>91.2</td>
<td>4.6 k</td>
<td>↓</td>
</tr>
<tr>
<td>pac(argB:pac)</td>
<td>89</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>metB</td>
<td>88.9</td>
<td>8.0 k</td>
<td>↓</td>
</tr>
<tr>
<td>squU</td>
<td>87.7</td>
<td>2.4 k</td>
<td>↓</td>
</tr>
<tr>
<td>hemD</td>
<td>85.9</td>
<td>2.0 k</td>
<td>↓</td>
</tr>
<tr>
<td>waaS</td>
<td>82.0</td>
<td>1.4 k</td>
<td>↓</td>
</tr>
<tr>
<td>yhiY</td>
<td>80.0</td>
<td>1.7 k</td>
<td>↓</td>
</tr>
<tr>
<td>zntA</td>
<td>77.7</td>
<td>10.8 k</td>
<td>↓</td>
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<tr>
<td>yhiX</td>
<td>75.6</td>
<td>3.0 k</td>
<td>↓</td>
</tr>
<tr>
<td>rrID</td>
<td>73.8</td>
<td>7.0 k</td>
<td>↓</td>
</tr>
<tr>
<td>pac(dacB-ybbZ)</td>
<td>71.75</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>dacB</td>
<td>71.7</td>
<td>3.8 k</td>
<td>↓</td>
</tr>
<tr>
<td>ssaA</td>
<td>65.0</td>
<td>2.5 k</td>
<td>↓</td>
</tr>
<tr>
<td>hycE</td>
<td>61.6</td>
<td>3.1 k</td>
<td>↓</td>
</tr>
<tr>
<td>hemF</td>
<td>55.9</td>
<td>13.5 k</td>
<td>↓</td>
</tr>
<tr>
<td>pac(dsdA-emrY)</td>
<td>53.4</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>intS</td>
<td>53.2</td>
<td>4.7 k</td>
<td>↓</td>
</tr>
<tr>
<td>rnhA</td>
<td>50.9</td>
<td>1.4 k</td>
<td>↓</td>
</tr>
<tr>
<td>yeeA</td>
<td>44.8</td>
<td>1.8 k</td>
<td>↓</td>
</tr>
<tr>
<td>rspR</td>
<td>35.1</td>
<td>2.2 k</td>
<td>↓</td>
</tr>
<tr>
<td>pac(tyrR-tpX)</td>
<td>29.9</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>trpK(trpC)</td>
<td>28.3</td>
<td>5.9 k</td>
<td>↓</td>
</tr>
<tr>
<td>ptkG</td>
<td>24.9</td>
<td>6.7 k</td>
<td>↓</td>
</tr>
<tr>
<td>hcr</td>
<td>19.6</td>
<td>2.3 k</td>
<td>↓</td>
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<tr>
<td>ubiqu</td>
<td>15.0</td>
<td>4.6 k</td>
<td>↓</td>
</tr>
<tr>
<td>ybvV</td>
<td>10.0</td>
<td>1.8 k</td>
<td>↓</td>
</tr>
<tr>
<td>pac(phoR-brnQ)</td>
<td>9.0</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>phoA</td>
<td>8.6</td>
<td>9.7 k</td>
<td>↓</td>
</tr>
<tr>
<td>metI</td>
<td>4.8</td>
<td>1.2 k</td>
<td>↓</td>
</tr>
</tbody>
</table>

*a Markers in plain type are contained within EcoRV fragments (lengths listed in column 3). Pac insertion positions, in bold type, are between the genes in brackets. Two-letter designations are used in strain names and on ﬁgures.

Results and discussion

Packaging from a single pac site on the chromosome

In order to determine the effect of a chromosomal copy of pac on transduction we inserted the pac-containing P1 EcoRII-20 fragment (Bachi and Arber, 1977) into the chromosome in place of the argB gene (89.5 min). We chose argB for replacement as strains mutated in nearby genes were available, facilitating later experiments.

We ﬁrst conﬁrmed that the pac insert increases the transduction frequency of a downstream chromosomal marker. The metB
gene, at 88.9 min (Fig. 1, Table 2), is about 0.6 min. from argB; trpA at 28.3 min is not expected to be packaged from this pac site. Surprisingly, argB<>pac increased the transduction frequency of metB by as much as 1000 relative to the pac-free strain, whereas the transduction frequencies of trpA from pac+ and pac-free donors were similar (ratio of 0.92). These data are included in Fig. 4B, below.

P1 packaging of MG1655 (argB<>pac) DNA

Increased packaging of chromosomal DNA downstream from pac has previously only been inferred from increases in transduction frequency (Sternberg and Coulby 1987a, Hanks, 1986, PhD Thesis). To demonstrate enhanced packaging directly we analyzed three separately prepared P1 lysates (MG1655(argB<>pac) and MG1655(ΔargB)) using EcoRV-cut, processed and hybridized to probe mixtures. Bands are identified with the names of probed genes followed by their map positions. (B) Data from 5 experiments (each with a different symbol and different probe mixes), are plotted against chromosomal positions. Ordinate values are band densities of P1(argB<>pac) relative to P1(ΔargB), each normalized to trp hybridization.

Fig. 2. Hybridization to transducing DNA from a single pac donor. (A) Chromosomal DNA and DNA extracted from three pairs [(MG1655(argB<>pac) and MG1655(ΔargB)] of separately prepared P1 lysates were EcoRV-cut, processed and hybridized to probe mixtures. Bands are identified with the names of probed genes followed by their map positions. (B) Data from 5 experiments (each with a different symbol and different probe mixes), are plotted against chromosomal positions. Ordinate values are band densities of P1(argB<>pac) relative to P1(ΔargB), each normalized to trp hybridization.
for markers distributed over the chromosome and is plotted against marker position. Clearly, the DNA encoding markers to one side of pac is more highly represented within P1(ΔargB) DNA and packaging is increased by as much as 30-fold for markers closest to the inserted pac. This decreases rapidly over 20 map units, but some increase in packaging is detectable as far as 35 min downstream from the site of packaging initiation. Packaging from the chromosomal pac site is clearly directional, since the packaging of markers to only one side of pac is increased. Packaging of markers to the other side appears markedly reduced, consistent with the finding that the packaged DNA end is protected from cellular nucleases, while the other end is degraded (Sternberg and Coulby, 1987a). The reduction in packaging we see between 91 and 100 min (Fig. 2B) could be accounted for by such degradation. Markers on about half of the chromosome (from 50 min counterclockwise (passing trp, to 91 min) are packaged between 90% and 50% as efficiently as from P1(ΔargB). This may be because the number of phage heads within an infected cell is limiting and packaging from bona-fide pac sites favored over those originating from random cuts.

We noted above that metB transduction can be increased 1000-fold by contiguity to pac; how this occurs despite an only 15-fold increase in actual packaging is unknown but it is also observed for other markers close to a pac site (see below). One possibility is that many likely abortive transductants are now capable of undergoing recombination, perhaps because there is not enough of the putative protein presumed to maintain circular DNA available for the 15-fold increased amount of packaged host DNA (see below). Although quantifying abortive transduction could be informative, we could find no simple and reproducible way to do so.

A single pac at other chromosomeal locations

To introduce pac sites at other locations on the chromosome, four additional single pac-containing strains were made by inserting pac in the same orientation as (argB pac) between genes in four new positions at 20 min intervals (Table 2 and Fig. 1). P1 transduction was again used to test whether transduction frequency from the new strains with single pac sites was stimulated to one side of pac (Fig. 4B, below). A marker within 2 min of pac was tested for three of these, (the nearest marker then available for (1-de) was 8 min from pac). Transduction was again greatly increased [up to 1300-fold for ArgG - from MG1655(1-dy)]. In Fig. 4B (below) transduction frequency is plotted vs. distance from the nearest pac site with the leftmost five Xs representing markers packaged early from the five single-pac strains. We conclude that the effect of pac on packaging of transducing DNA is independent of the location of pac on the chromosome.

Lysate preparation from strains with multiple pac sites

Strains with multiple pac sites were constructed as described above. P1 plate lysates were then prepared on MG1655, the five single-pac containing strains, the 2-pac strain, three strains, each with a different 3rd pac, and the 4-pac and 5-pac strains.

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**Fig. 3.** Effect of multiple donor pac sites on lysis by P1. (A) MG1655 (○), MG1655(argB pac) (●), MG(5 pac) (■), and MG(3-ter) (▲) were grown in LB to an O.D.600 of 0.3 and P1 added at an moi of ~4 in the presence of cyanide. After 10 min, cultures were washed, resuspended in LB and followed until lysis. (B) P1 in the supernatants (closed symbols) and in the CHCl3-lysed pellets (open symbols) were measured by titration in samples taken after resuspension from MG1655(argB pac) (●) and MG(5 pac) (■) cultures. (C) Growth of MG(5 pac) and MG1655 in a similar experiment after P1 adsorption and culture dilution. (D) Samples were taken from (C) during phage development; intact cells were collected by centrifugation and retained for DNA analysis. DNA, purified from cells, was cut with EcoRI and hybridized to labeled probes detecting 16.5 kb and 2.8 kb P1 bands. Results shown are from analysis of the phosphoinomage in (E). (E) Time course of labeling of P1 16.5 kb and 2.8 kb EcoRI fragments from which the time course of P1 DNA synthesis was calculated [plotted in (D)]. The extra band in the 4 μg P1 DNA track results from incomplete hydrolysis. The MB1 ladder shows sizing standards separated by 1 kb.
Surprisingly, although MG1655 and each of the single pac strains yielded plate lysates of P1 with high titers of between $5 \times 10^{10}$ and $10^{11}$ pfu/ml, all multiple pac strain lysates had titers of only $10^3$ to $10^4$ pfu/ml. In liquid broth, before infection, all strains divided with about the same doubling time (Fig. 3A). Thus the multiple pac insertions must interfere with growth after infection or with production and/or release of progeny phage. To investigate further, we followed phage development in single-cycle liquid lysates of MG1655 and its (argB-3pac), (3-tt) and (5pac) derivatives. Growth after infection was at the same rate for all strains (Fig. 3A); however, the pac-free and single-pac strains lysed sooner and more completely than the multi-pac strains, achieving titers greater than $10^9$. The multi-pac strains only reached titers of $< 5 \times 10^8$, fortunately just sufficient for adequate transduction. Fig. 3B shows the accumulation of free phage for the 5pac and pac-free hosts. The 5pac host not only lyses more slowly and releases fewer phage, but it also retains fewer viable phage in the pellet. To see whether DNA is synthesized in the multi-pac strains but not incorporated into phage heads, we measured P1 DNA accumulation in chloroformed lysates of MG1655 and MG1655(5pac) by hybridization. Fig. 3C shows a lysis curve, 3E an autoradiogram and 3D P1 DNA/cell. The 5pac strain lysates later and has less P1 DNA/cell than the pac-free strain (Fig. 3D and E). The difference in P1 DNA content (~1.6-fold) is not so great as is the titer (~7-fold), suggesting that packaging of the DNA rather than its replication is principally responsible for the reduced titer. This may be due either to an insufficiency of heads or a fault in the packaging process. The 1000-fold reduced titers of the plate lysates most probably result from outgrowth of host lawn before sufficient phage are produced to infect all cells, thus drastically reducing the number of cycles of infection and yield of phage.

**Transduction using lysates prepared from strains with multiple pac sites**

In order to determine whether multiple pac sites reduce the amount of packaging initiated at each pac site, single growth cycle transducing lysates were prepared from MG1655 and its 2pac, 3-de and 4pac derivatives. These were used to transduce a set of markers selected so that each is an indicator of packaging initiated from one of the five possible chromosomal pac site locations. Fig. 4A, in which transduction ratios are plotted against chromosomal map position, shows that markers very near to pac sites are transduced with very high frequencies, $10^3$–$10^4$ higher than from MG1655. This frequency drops by at least 90% over 20% of the chromosomal length [see metB to argG for MG1655 (2pac)] and the same again over the next 20%. Subsequent pac sites restore a high level of transduction. If there is a limit to how many pac sites can be inserted before packaging efficiency near pac decreases, it must be greater than four. From Fig. 4B, in which transductant numbers are plotted against distance from the nearest upstream pac site, we can see that transductant numbers decrease exponentially with distance from pac over a greater than $100 \times$ range, irrespective of which pac site is closest.

Note that ilv, marked by * in Fig. 4, is transduced less efficiently than expected. Ilv transduction normally occurs with higher frequency than predicted from the proportion of ilv DNA in a lysate (Hanks et al., 1988), a property shared by nearby markers also able to be cotransduced with oriC. This may be because replication is initiated on abortively transduced fragments containing oriC and the ends thus created stimulate recombination (Masters, 1977). Ilv is 0.6 min from oriC, so that most random 2.2 min P1 “headfuls” of DNA containing one also contain the other. However, in the construct argC+pac, headfuls originating at pac would not include both ilv and oriC, making a pac-free strain an inappropriate control for ilv transduction from an argB+ pac donor.

**Hybridization using DNA prepared from strains with multiple pac sites**

To determine whether competition between multiple pac sites limits chromosomal packaging, DNA prepared from P1 grown on pac containing strains for a single cycle in broth were hybridized to several probes.

Representative Southern blots (Fig. 5A) show relative hybridization of markers from five strains with single, and four strains with multiple pac inserts. In Fig. 5B, band intensities relative to those from a pac-free strain (MG1655) are plotted for six of these (symbols above Fig. 5A). The multi-pac strains hybridize strongly to bands downstream of and close to inserted pac sites, as expected. However, when compared with the hybridization of P1 (argB+pac) to bands originating far from pac, such as trpA (by P1 (3-dy) or P1(3-de) lysates], yhIX, or holC, hybridization of multi-

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**Fig. 4.** Transduction from multi-pac donors. (A) Lysates prepared from MG1655 and 2-pac, 3-pac and 4-pac derivatives were used to transduce six markers selected to be near the origins of packaging from each pac site (positions indicated above figure and by dashed vertical lines). Transductants per phage, relative to those from MG1655 as host, are plotted against chromosomal position in Fig. 5A. (B) The values from Fig. 5A, plus others obtained from 5 single-pac strains and rpaA (Xs), are plotted as a function of distance from the nearest upstream pac site. Note that the X-axis in Fig. 4B is exponential. *indicates ilv+ transductants; see text.
Fig. 5. Transducing DNA packaged from chromosomes with different pac site locations and numbers. (A) DNA from P1 lysates of MG1655 and nine derivatives with combinations of 0–4 pac sites was analyzed. Symbols above the relevant lanes are those used in (B) where the relative hybridization of 6 strains to 12 probes from around the chromosome is shown. Pac sites with positions (peaks) are named above the figure ([argB]/pac−)/argL. (C) Relative hybridization of other lysates to markers between pac−, argB and pac (1-dy) for 5 strains with [pac][argB]: 4pac (–□–, –△–), 3-dy (–○–, –■–), 3-tt (–●–), 2pac (–▲–), pac−/argB (▲–) and 3 without: 1-tt (–○–), 1-pb (■–), 1-dy (●–). Results are corrected for DNA loading. Hybridizations are not shown.

Fig. 6. Total chromosomal DNA packaged from donors with different chromosomal pac site numbers. Different amounts of each DNA confirmed that the signal obtained is proportional to loading. (A) P1 lysates and host DNA were cut with EcoRV and, together with size markers, were separated on gels. (B) DNA was transferred to membranes, hybridized to 32P-labeled random chromosomal probes and radioactivity/lane determined as explained in the text and the legend to Fig. 3. Each lane is labeled with the amount of DNA applied. Labels below indicate relative counts, normalized to 1 μg of P1(1-de).

pac DNAs seems reduced or absent (Fig. 5A). This suggests that processivity of packaging from each pac site is reduced in multipac donors. (Note that the dacB band is reduced in size in strains with the (1-dy) pac insert; this is because the Pac insertion contributes an EcoRV site converting the chromosomal EcoRV fragment into two smaller ones.)

Processivity of packaging might decrease for reasons including chromosomal degradation from the unpackaged end of Pacase-cut DNA or because of insufficient packaging capacity (which would also reduce random initiation of packaging). To learn more DNA extracted from P1 lysates grown on the (argB)/pac−, (1-dy), (1-de), (1-tt), (2pac), 2 (3pac) and the (4pac) strains were hybridized to measure in more detail packaging between adjacent pac insertions at 89.5 and 71.8 min (Fig. 5C, gel not shown). Six of the lysates were from a donor with the argB/pac− insert. These show differences in the rate of packaging decrease amongst them, with packaging from the argB/pac−, 2pac and 3-tt strains, which all have (argB)/pac−, but not pac−/pac−, exhibiting higher transduction of markers closer to the “dy” replacement site, than do 3-dy or 4pac donors, which contain both inserts, consistent with possible degradation of the unprotected DNA end after a cut at pac(1-dy).

Repetition of the experiment with other pairs of pac sites would be desirable to confirm the generality of these hybridization profiles.

Total chromosomal DNA packaged by P1 phage

Although markers to one side of pac are packaged at increased frequency, reduced packaging far from pac could result in total chromosomal DNA packaged remaining the same. To measure the total chromosomal DNA packaged, purified E. coli DNA was used as a template to make random probes covering its entire length. This should generate labeled probes for all chromosome sequences, allowing measurement of the total transducing DNA packaged by P1 despite the presence of excess P1 DNA.

In the gel shown in Fig. 6A, MG 1655 chromosomal fragments vary continuously in size, while in lysates only P1 bands are visible. Fig. 6B shows the hybridization result. All lanes show bands of many sizes but more intense bands appear in each. A band between 2.5 and 3 kb appears to be shared by all lysates and chromosomal DNA, suggesting that it was preferentially amplified during primer synthesis, as template DNAs should be equimolar. The strongly hybridizing bands in the P1 lanes differ from those in the chromosomal lanes, are not coincident with P1 fragments, and are similar in the 3-dy and 5pac lanes, which share three pac locations. The P1(1-de) DNA lacks the most intense of these (ca. 8 kb in size), but displays others shared with 5pac (i.e. 4 kb). These
results are consistent with the hypothesis that the bands represent regions downstream from pac sites. The total amount of hybridizing DNA in each lane in Fig. 6B was determined. All values, expressed relative to that of P1(1-de), are listed below the lanes in Fig. 6B, and, per unit DNA loaded, below this. Using these measurements and those from a second gel (not shown), we can deduce that pac sites on the chromosome increase chromosomal DNA packaging by P1. Inserting a single pac site into the chromosome results in a 3.5-fold increase in the amount of packaged transducing DNA. The total amount of DNA packaged from multi-pac strains increases with the number of pac sites but is not in direct proportion to pac number. Three inserted pac sites doubles rather than triples the amount of chromosomal DNA packaged and five pac sites increases packaging only by a further 15–20%. Since packaging near each pac site, even when there are five, is high, no packaging site appears to be used preferentially. Possible explanations for the lack of direct proportionality between pac site numbers and DNA packaged include: (1) All or many of the pac sites present are used in all cells which package transducing DNA, but there is a reduction in packaging of DNA remote from pac. (2) Only one or two pac sites in an individual cell are used, but the increase in pac site number results in an increase in the percentage of cells that make transducing phage. Distinguishing between these alternatives will require further experimentation.

Conclusions

Physiological effects of chromosomal pac sites

A single chromosomal pac site does not alter the course of phage maturation or lysis. In liquid infections, two or more chromosomal pac sites extend the period before lysis and reduce viable phage yield by about 90%. Phage DNA synthesis is more normal, at 70% of the yield from hosts with pac-free chromosomes, suggesting a reduction primarily of packaging. Growth of all strains is normal in the absence of P1 infection, even with five inserted pac sites.

Packaging

P1 packaging of markers to one side of and near to a chromosomal pac-site is increased more than 10-fold. This effect diminishes with distance but packaging can be detected over 30% of the length of the chromosome. One chromosomal pac site more than triples the amount of chromosomal DNA packaged by P1. Packaging of DNA is high downstream of each pac site of a multi-pac donor, but the total amount of DNA packaged does not increase proportionately with pac site number, perhaps because of increased degradation of donor DNA behind the pac cut.

Competition of transducing and phage DNA for encapsidation

MG1655 (5pac) lysates contain about seven-fold more transducing DNA than those of MG1655, and about equally fewer infectious phage (Figs. 4 and 6). How might this come about? Encapsulation of P1 DNA requires cutting at pac by Pacase, which is synthesized at a low level throughout infection (Skorupski et al., 1992). PacA binds to methylated or hemimethylated sites independently of PacB, but full methylation and PacB is required for cutting (Sternberg, 1990). Since Dam-methylated chromosomal pac sites are present at the time of infection it is possible that Pacase binds to them before fully methylated P1 concatemer is synthesized. This would reduce the amount of Pacase available to initiate phage DNA packaging, and could explain how DNA from chromosomes with multiple pac sites can be packaged at the expense of P1 DNA.

Possible applications and future prospects

Insertion of a pac site near to and in the appropriate orientation could increase transduction sufficiently to allow transfer and identification of an unselectable marker with minimal screening. The intriguing questions of whether and how abortive transductants may be stabilized remain to be addressed.

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