Polarized Injection of the Bacteriophage T5 Chromosome

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Examination of the first-step-transfer DNA of T5ris mutants which carry new EcoRI sites showed that the left end of the chromosome is injected first.

Bacteriophage T5 and its relatives have a unique system for injecting their DNA into their host. Upon adsorption to the surface of the host, T5 injects about 8% of its chromosome. The genes encoded thereon are expressed, and only then is the remaining 92% of the chromosome injected (5, 7). This 8% of the chromosome is called the first-step-transfer DNA, or FST DNA. FST DNA may be prepared by adsorbing T5 to its host under conditions in which phage gene expression is prevented and subjecting the phage-host complexes to mechanical shearing in a blender. This breaks the phage head and the untransferred 92% of the chromosome away from the host and leaves the FST DNA inside the cell. Since the pre-early genes of T5 expressed during the first stage of DNA transfer are duplicated on the two redundant ends of the chromosome, a question arises about the polarity of DNA transfer. Is DNA transfer polarized, and if so, which end of the chromosome enters the cell first? Polarized left-end-first injection of T5 DNA is favored by indirect experimental evidence, but it has never been demonstrated conclusively. The T5 chromosome contains a nick about 8% of its length from the left end (3), and this nick has been suggested as the limit of FST (6). Further studies have shown that there are other nicks closer to the left end (2); therefore, if a nick is the limit of DNA transfer, it is not clear how DNA transfer proceeds past the earlier nicks. Labedan et al. (4) claim, based on studies of sheared DNA from phage-bacteria complexes, that the left end of the T5 chromosome is injected first, but their conclusions have been questioned by McCorquodale (8). The phage tail is attached to the left end of the chromosome (11), and the entire right redundant end has been deleted, although the resulting phage particle is not viable alone (12). Finally, the DNA transfer characteristics of a viable deletion mutant of related phage BP23 which has lost the right 40% of both redundant ends imply polarized DNA injection (13).

The isolation of mutants of T5 with new EcoRI restriction sites within the terminal redundancies provides convenient physical markers that distinguish the left end of the T5 chromosome from the right end. Wild-type T5 DNA has no EcoRI restriction sites within its terminal redundancies, and the phage is insensitive to the presence of EcoRI restriction enzyme in its host. The introduction of EcoRI restriction sites in the FST DNA prevents growth of the mutant phage in hosts carrying the EcoRI restriction system. These mutants are called ris due to their new restriction sites (1, 1a). The new EcoRI sites in T5ris1 and T5ris2 have been mapped both physically and genetically and are shown in Fig. 1. The mutation in T5ris1 is repeated in both redundant ends and causes the release of a 1.7-megadalton (Mdal) fragment and a 4.2-Mdal fragment from the left and right ends, respectively, of the chromosome upon in vitro digestion with EcoRI. Similarly, the mutation in T5ris2 causes the release of a 2.3-Mdal fragment and a 3.6-Mdal fragment from the left and right ends, respectively, of the chromosome. Therefore, the electrophoretic pattern of EcoRI-digested FST DNA from ris mutants prepared by shearing should show a decrease in the size of the internal fragment while the terminal fragment remains unchanged. This would identify the end of the chromosome that enters the host first.

The autoradiogram shown in Fig. 2 shows the EcoRI restriction patterns of whole T5+ DNA, T5+ FST DNA, whole T5ris1 DNA, T5ris1 FST DNA, whole T5ris2 DNA, and T5ris2 FST DNA. The T5+ DNA shows the normal EcoRI pattern (1, 9, 14). The T5+ FST DNA, having no restriction sites, appears as a single fragment of 5.7 Mdal.

The T5ris1 DNA contains two new restriction fragments, 1.7 and 4.2 Mdal, due to the ris1 mutation in the terminal redundancies (1). The T5ris1 FST DNA appears in two fragments, 1.7 and 4.0 Mdal. The 1.7-Mdal fragment corresponds to the left end of the chromosome (Fig. 1) and remains unchanged. The 4.0-Mdal frag-
FIG. 1. Physical maps of the chromosomes of T5+, T5ris1, and T5ris2. Solid horizontal lines represent the DNA duplex. Arrowheads indicate the locations of EcoRI restriction sites, and the numbers above are the sizes in Mdal of the products of EcoRI digestion (3, 14). The solid vertical lines show the limits of the terminal redundancies (tr). The scissors and the broken vertical lines show the possible limits of FST and the points of scission due to mechanical shearing of the phage-bacteria complexes described in the legend to Fig. 2. The numbers with the curved arrows show the locations and sizes in Mdal of DNA fragments resulting from restriction of FST DNA. The mutants ris1 and ris2 have been described previously (1, 1a). All markers are oriented with respect to left and right by the convention used by McCorquodale (8).

FIG. 2. EcoRI restriction patterns of FST DNA. FST DNA was prepared by infecting Escherichia coli W3350 under starvation conditions with 32P-labeled phage, followed by shearing in a blender, sodium dodecyl sulfate-phenol extraction, and dialysis, as described by Shaw and McCorquodale (13). EcoRI digestion, agarose gel electrophoresis, and autoradiography were performed as described by Brunel and Davison (1). The sizes of the restriction fragments are given in Mdal. Unrestricted FST DNA from ris1 and ris2 has the same mobility as T5" FST DNA (data not shown). Samples are as follows: (a) whole T5+ DNA extracted from intact phage; (b) T5" FST DNA; (c) whole T5ris1 DNA; (d) T5ris1 FST DNA; (e) whole T5ris2 DNA; and (f) T5ris2 FST DNA.
sum of sizes of the terminal and internal EcoRI fragments of the FST DNAs of the ris mutants.

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