

### Integration of an RP1 derivative into the *Pseudomonas aeruginosa* chromosome results in the formation of stable HFR strains

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We used an RP1 derivative which is temperature-sensitive for replication and has an inactivated resolvase gene in its transposon TnI. When selecting for plasmid markers at nonpermissive temperature in a Rec<sup>-</sup> *P. aeruginosa* strain we obtained plasmid integration due to TnI transposition. The strains thus constructed were stable and showed good Hfr donor properties. The origins for chromosomal transfer lay at different sites on the chromosome, giving a useful system for genetic mapping. We observed rearrangements in the integrated plasmid that seem to be necessary or at least advantageous for RP1 maintenance in the chromosome. In many strains we found an intramolecular transposition of IS21 into the *trfA* region, which is required for autonomous RP1 replication. When such plasmids were excised from the chromosome they could replicate autonomously only if the *trfA*<sup>+</sup> replication function was provided in *trans*.

### IS-like repeated sequences clustered around the NIF region of the *Rhizobium japonicum* genome

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Two different repeated sequences (RS) were discovered in the *Rhizobium japonicum* genome: RSRj $\alpha$  is 1126 bp long, and is reiterated 12-fold; RSRj $\beta$  is approximately 950 bp long, and is repeated at least 6 times. Their arrangement in root nodule bacteroid DNA is the same as in DNA from bacteria grown in culture. Deletion analysis has shown that many copies of  $\alpha$  and  $\beta$  are clustered around the nitrogenase genes *nifDK* and *nifH*, or in general, they are found within a genomic region harboring genes which are nonessential for growth. One copy each of  $\alpha$  and  $\beta$  are located upstream of *nifDK*, and adjacent to each other. Neither of them, however, is involved in the expression of *nifDK*. Nucleotide sequence analysis of three copies of RSR $\alpha$  has revealed many characteristics of prokaryotic IS elements: potential inverted repeats at their ends, potential target site duplication, and large open reading frames. Despite this, their genomic positions appear to be stable, as we could not demonstrate any transposition event. One possible function of RS is in deletion formation, probably via recombination between them rather than induced by an IS-like mechanism.

### The genomes of the morphologically different bacteriophages LP52 and theta are related

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Bacteriophages LP52 and theta replicate in several strains of *Bacillus licheniformis*. In spite of different virion morphology these phages form viable recombinants, and two long regions of homology within the genomic DNA have been determined by restriction fragment analysis and blot hybridization (Forstová et al., Molec. gen. Genet. 187 (1982) 138). The denaturation maps also reflect this DNA relationship. Mapping of sequence homology between LP52 and theta DNA by electron microscopic heteroduplex analysis revealed about 50% homologous segments interspaced by 14 nonhomologous regions. This illustrates the importance of DNA rearrangements in these phages' evolution. Several recombinant phages of LP52 virion architecture were analyzed and shown to contain DNA consisting of a large interior segment of theta DNA flanked on either side by the respective LP52 DNA. All hybrid genomes contained within

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the theta DNA a 2 kb DNA segment which was nonhomologous to either parental DNA at this position, indicating that they resulted from a complex recombination process.

### The spreading of an unknown plasmid in a children's hospital

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While typifying *E. coli* strains isolated in a newborn unit of a children's hospital we observed periodically strains with resistance against all colicines and phages. We therefore searched for a plasmid responsible for these properties. Conjugation experiments between the wild strains and laboratory strains lacking colicine- and phage-resistance proved the existence of the postulated plasmid. The plasmid formed (40 kbp) belongs to the F 4-type. Beside the properties mentioned above it also contains genes encoding (an) enzyme (s) for lactose fermentation and for flagella formation (mobility). At present we do not know the origin of this plasmid. Currently further studies on the epidemiology as well as biochemical and genetic studies are under investigation.

### An epidemiological study of two *S. typhimurium* outbreaks by means of plasmid fingerprints

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An outbreak of *S. typhimurium* infection occurred during November 1984 in an old age home. Three persons became ill, and 6 clinically inapparent carriers were found from 25 fecal samples examined. At the same time 16 of 21 cows, belonging to the old age home, were observed to have enteritis. In the feces from all the sick animals *S. typhimurium* could be isolated. Within 4 weeks a second outbreak occurred with 2 cases of *S. typhimurium* enteritis in the butchery of the same village.

By means of plasmid fingerprinting we could demonstrate that all the *Salmonella* strains isolated carried the identical plasmids. The identity of the strains was further confirmed by phage lysotyping and antibiotic susceptibility testing. These findings suggest that both outbreaks might have a common source.

### Insertions of ampicillin transposons into the plasmid DNA of *N. gonorrhoeae*

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Penicillinase producing *Neisseria gonorrhoeae* (PPNG) strains isolated up to now throughout the world harbor a 7.3 kb resistance plasmid or its 5.1 kb deletion derivative: both genetic elements contain 40% of the transposon TnA.

In order to better understand the emergence of such plasmids and to establish whether these can originate from multiple events of TnA insertions into a specific site of a core plasmid, we constructed *bla*<sup>-</sup> derivatives of the PPNG resistance elements. Genetic experiments using phage  $\lambda$ :TnA showed indeed that these recombinant plasmids have a hot-spot insertion site for the transposon TnA.

Experiments are in progress to ascertain whether, when introduced in *Neisseria gonorrhoeae*, these TnA-inserted plasmids will show a specific deletion process resulting in the stable presence of the 40% moiety of the TnA transposon, similar to that found in the naturally occurring resistance genetic elements isolated from PPNG.