Distribution of Modules among the Central Regions of the Genomes of Several Actinophages of Faenia and Saccharopolyspora

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The central regions of the genomes of φFR114, φFR113 and Mpl, three temperate phages of the thermophilic actinomycete Faenia, are shown to differ mainly with respect to modules of about 3.5 kb, designated J-module (φFR114) and N-module (φFR113, Mpl). The distribution of J and N was observed amongst 22 phages of Faenia and Saccharopolyspora; J-module homology was found on six phage genomes, whereas homology to the N-module was detected on ten phage genomes.

INTRODUCTION

The evolution of tailed bacteriophages (Reanney & Ackermann, 1982; Ackermann & Dubow, 1987) is thought to be a combination of (1) successive changes of single genes caused by point mutations (Studier, 1979) and (2) the exchange of functional sets of genes (called modules) between not necessarily closely related phages (Botstein, 1980). The latter process facilitates rapid evolution which may enable the phages to adjust to host changes and to overcome host defences. Point mutations may slowly alter the modules (e.g. for host recognition) which, provided they remain functional, can in effect be tested in combination with different existing phage modules (e.g. for tail morphology), perhaps resulting in advantageous new bacteriophage blueprints.

Limited homologies between different phages have often been described and sometimes discussed as possible modules (Brzezinski et al., 1986), but the evolution and distribution of such modules has only been investigated thoroughly with some coliphages (Campbell & Botstein, 1983; Kamp, 1987) or Mu-like phages of Pseudomonas (for a review of the work by Krylov et al. see Dubow, 1987).

φFR114 and φFR113 belong to a group of temperate and lytic phages of several host species within the actinomycete genera Faenia and Saccharopolyspora (Kempf et al., 1987; for host taxonomy see Embley et al., 1988; Greiner-Mai et al., 1988). The two phages were previously described as examples of diverging evolution by point mutations (Schneider et al., 1987). In this paper we demonstrate that they also differ by one module within the central regions; the distribution of the particular modules among both homo- and heteroimmune phages is discussed, including phages of Saccharopolyspora erythraea recently described by others (Brzeziński et al., 1986; Grund & Hutchinson 1987; Smorawińska et al., 1988). Part of this work was presented at the 5th DECHEMA Jahrestagung der Biotechnologen, May 1987, in Frankfurt (Schneider & Kutzner, 1988).

METHODS

Bacteria and phages. For DNA preparation all phages (see Table 1 for origins) were propagated on Faenia sp. TD8, kindly provided by A. Kempf (Darmstadt), whereas for host-range experiments the strains listed in Table 1...

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were used. Phage φSE6 was grown on *S. erythraea* DSM 40517 as it does not infect *Faenia*. Bacteria and phages were grown with GPHF media as described before (Schneider et al., 1987), *Faenia* and *S. hirsuta* at 37 °C or 48 °C and the mesophilic *S. erythraea* at 37 °C. Double-layered plates were inoculated from overnight cultures in GPHF-broth (supplemented with 5% w/v NaCl for *Faenia* and *S. hirsuta* for better formation of submerged spores) rather than with spores.

**DNA manipulations and analysis of phage genomes.** The procedures were as described by Schneider et al. (1987). For relatively accurate mapping of homologies within the central regions of the phage genomes by DNA hybridization, the relevant DNA fragments were cloned in pUC18 (Yanisch-Perron et al., 1985): the recombinant plasmids pJS54 and pJS55 (φFR114) and pJS30 (φFR113) have been described before (Schneider et al., 1987); pJS66 is a subclone of pJS55. pJS82 contains a HindIII–BamHI fragment covering the central region of Mpl.

**DNA–DNA hybridization.** Southern blotting was essentially as described by Wahl et al. (1979) except that biotinylated probes were used (Schneider & Müller, 1988). Filters from the blotting procedure were baked for 60 min at 80 °C and prehybridized at 55 °C in SSCM (6 × SSC with 0.7% dry skimmed milk; 1 × SSC is 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0) and the biotinylated DNA-probe was added to a final concentration of 100–300 ng ml⁻¹. Usually 100 ng biotinylated λ DNA ml⁻¹ was added to visualize λ size standards; this probe did not hybridize with the DNA of the *Faenia* or *Saccharopolyspora* phages. The nick-translation was done as described by Hopwood et al. (1985) using Biotin-11-dUTP (Gibco BRL). Afterwards the hybridization mixture was stored at −20 °C for repeated use. The filter was briefly washed with 2 × SSC + 1% SDS followed by further washes (2 × 30 min) which determined the stringency of the hybridization. The G + C content of the phages was assumed to be 59 mol %, which is the value determined for φFR114 by HPLC (E. Grund & J. Schneider, unpublished data). High stringency was achieved with 0.15 × SSC + 0.1% SDS at 70 °C whereas for medium stringency 0.2 × SSC + 0.1% SDS at 60 °C was used. Finally the filter was blocked with SSCM (20 min, 48 °C) and the biotinylated DNA was detected using the BLUGENE detection system (Gibco BRL).

**RESULTS AND DISCUSSION**

**The J- and N-modules**

φFR114 and φFR113 are closely related temperate phages as shown by similar restriction maps, although the central regions of their linear genomes exhibit considerable differences (Schneider et al., 1987). DNA hybridization revealed that the central region can be further subdivided (Fig. 1).

1) The left part of the central regions differed with respect to restriction pattern but still showed considerable DNA homology under high-stringency conditions, a good example for diverging evolution by point mutations, which may affect some parts of a genome more than others (Campbell et al., 1986). Two independent, non-overlapping deletions within this area of φFR113 are regarded as the origin of φFR371 and φFR755R (Fig. 1; Schneider et al., 1987). Similar deletions, recently described for the *S. erythraea* phage SE-3 and resulting in phage SE-5, were flanked by short repeats (Smorawińska et al., 1988; R. Brzeziński, personal communication); homologous recombination between these repeats could be the cause of the deletions in SE-5.

2) In contrast to the left part, the right half of the central regions showed no homology at all, suggesting that this area corresponded to two distinct modules acquired from different sources. These modules were called J-module (φFR114) and N-module (φFR113) following comparison with virulent φFRv phages (see below). pJS54 (Fig. 4) was used as a probe partially specific for the J-module to screen other phages for its presence. Surprisingly, signals were detected with two of the three virulent φFRv phages, φFRv-J and P113, but not with φFRv-N.

The virulent φFRv phages φFRv-J, φFRv-N and P113 are closely related to each other as shown by restriction fragment pattern (no restriction maps of these phages are available) and DNA hybridization (Fig. 2): under high-stringency conditions φFRv-J and P113 DNA hybridized very well with each other, although a somewhat lower homology was observed within some fragments. φFRv-N, however, shared extensive homology with the genomes of the other two phages, with only a minor part showing no homology. This was not due to a deletion, as φFRv-J and P113 have a region of similar size not hybridizing with φFRv-N. This part of the genome was called the J-module (φFRv-J and P113) and the N-module (φFRv-N).

When total φFR114 DNA was used as a probe, no homology was detected with φFRv-N, and
Fig. 1. The central regions of φFR114, φFR113 and Mpl. The position of the cloned fragments on the respective phage genomes is indicated in Fig. 4. The EcoRV sites marked by an arrow indicate the left border of the central region and are also shown in Fig. 4. The map of pJS54/55 is an improved version of the one recently compared to pJS50 (Schneider et al., 1987). Note that some of the restriction sites on pJS66 had not been recognized before. All three central regions were mapped with the same ten restriction endonucleases: B, BamHI; Bg, BglII; Bs, BstEII; E, EcoRV; H, HindIII; K, KpnI; P, PstI; Pv, PvuII; S, SacI; X, XhoI. There is strong homology between the three central regions except in those areas indicated by labelled lines beneath the respective maps.

Right half: The lines indicate the region with no homology between φFR114 and φFR113 DNA and between φFR114 and Mpl DNA. These areas correspond closely to the homologies found between φFR114 and φFRv-J DNA and between φFR113 or Mpl and φFRv-N DNA and thus were called J- and N-modules.

Left half: The lines indicate the non-homologous region between φFR114 (or φFR113; A-module) and Mpl (M-module) DNA. A371 and A755R mark the fragments of the φFR113 genome which are deleted in φFR371 and φFR755R, respectively (Schneider et al., 1987).

![Diagram of phage genomes and restriction sites](image)

The homology with φFRv-J and P113 was limited to the J-modules of these phages. As expected, labelled φFRv-J or P113 DNA did not hybridize with φFR113 but gave signals with the J-module of φFR114. In spite of their strong homology, the J-modules from φFR114 and the two φFRv phages had no comparable restriction pattern (data not shown). φFR113 DNA as a labelled probe gave no signals with φFRv-J and P113 but hybridized with the N-module of φFRv-N, and labelling of φFRv-N DNA gave signals against φFR113 DNA only in the N-module (data not shown).

Thus it appears that the temperate phage φFR113 and the apparently unrelated phage φFRv-N share a highly homologous region of about 3-5 kb, consequently termed the N-module. Similarly, the temperate phage φFR114 and the virulent phages φFRv-J and P113 (unrelated to φFR114, but closely related to each other and φFRv-N) share a different module, the J-module. The chief differences between φFR114 and φFR113, which are otherwise very closely related, thus seems to be that the former carries the N-module and the latter the J-module.

Distribution of sequences homologous to the J- and N-modules

Whole genomes of φFRv-J and φFRv-N were used as biotinylated probes to screen all available phages known to infect Faenia and Saccharopolyspora for the occurrence of the J- and N-modules. Both phages were used in parallel to ensure that the signals observed were due to one
Fig. 2. Homologies between φFR114, φFRv-J, P113 and φFRv-N. (a) Ethidium-bromide-stained agarose gel of BstEII digests of phage DNAs: (1) φFR114, (2) φFRv-J, (3) φFRv-N, (4) P113. After transfer to nitrocellulose filters the restriction fragments were hybridized with biotinylated probes prepared from phage DNA of (b) φFR114, (c) φFRv-J, (d) φFRv-N, (e) P113. Approximate fragment sizes in kb are shown to the right. The hybridizations were carried out under high-stringency conditions. The fragments which represent the J- and N-modules are indicated on each filter.

of these modules: if the same fragments of a phage were stained with both probes, this was assumed to be caused by genome fragments other than the J- or N-modules. This screening demonstrated a considerable distribution of these modules among the phages tested (Table 1, Fig. 3).

Homology to the J-module was found on the genomes of φFR747 and φFRG9, which are regarded as closely related to φFR114 (Schneider et al., 1987) and on φFRa-C, a temperate phage homimmune with and related to the φFR phages.

Homology to the N-module was found on the genomes of several phages from different phage groups (Table 1), as detailed below.

1. The φFRb phages are temperate Faenia phages which are only distantly related to the φFR phages and unrelated to the φFRv phages (J. Schneider, unpublished data).

2. Phage 121 is an S. erythraea phage which cannot be propagated on host strains with a φFR or φFRb prophage. It was originally isolated from a lysogenic strain of S. erythraea (Retinskaya & Rautenstein, 1960) but later could not be found to lysogenize available hosts (Brzeziński et al., 1986). It might thus be assumed to be a lytic mutant of an unknown temperate phage or to lysogenize only unknown host strains, as was demonstrated for phage Mp1 (see below). Phage 121 is closely related to SE-5, which exhibited no homology to the J- or the N-module. Both
Distribution of modules among Faenia phages

Distribution of the J- and N-module amongst phages infecting Faenia and Saccharopolyspora. (a) Ethidium-bromide-stained agarose gel of BstEII digests of phage DNAs. After transfer to nitrocellulose filters the restriction fragments were hybridized with biotinylated probes prepared from (b) φFRv-J and (c) φFRv-N DNA (performed under medium-stringency conditions). The detections were stopped after 10 min (c) and 20 min (b), which explains the different staining intensities on the two filters. Fragments which were extensively stained by only one of the two probes are assumed to correspond to the J- or N-modules and are indicated by arrows. Lanes: (1) P517, (2) 121, (3) φSE6, (4) φSE60, (5) φFRb-P, (6) φFRb-M, (7) φFRb-D, (8) φFRb-B, (9) φFRA-E, (10) φFRA-C, (11) φFRA-A, (12) φFRG9, (13) φFR747, (14) λ PstI standard.

(3) Mp1 was the first phage described for F. rectiwigula (Prauser & Momirova, 1970) and since then has been a valuable tool in actinomycete taxonomy (e.g. Prauser, 1984). Although it has been considered to be a virulent phage it is not able to lyse strains lysogenic for φFR114 and related phages. The isolation of a new species of Faenia (Faenia sp.; A. Kempf, personal communication) revealed that Mp1 is a temperate phage as well: it easily lysogenized all four strains (e.g. TD8) of Faenia sp. and as a prophage it mediated immunity against φFR114 (Table 1). The restriction map of Mp1 could be aligned with the maps of φFR114 and φFR113 (Fig. 4), although considerably fewer restriction sites were observed with the enzymes used for mapping. DNA hybridization demonstrated a high degree of homology between the three phages with the exception of the central region (Fig. 1): on the right part of the central region the N-module was located, resulting in strong homology with φFR113 DNA and no homology with φFR114 DNA. The left half was different with respect to restriction pattern and revealed an area showing no homology with either φFR114 or φFR113 DNA. These regions were termed A-module (φFR114 and φFR113) and M-module (Mp1).

Conclusion

The idea of modular evolution requires a colinear organization of functional modules within different phages which are able to exchange such modules (Botstein, 1980). This may imply that
Table 1. Characterization of the different Faenia phages

<table>
<thead>
<tr>
<th>Phage</th>
<th>Group*</th>
<th>Homology with φFR114</th>
<th>Life cycle†</th>
<th>CR module‡</th>
<th>Host range§</th>
<th>Origin</th>
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<tr>
<td>φFR114</td>
<td></td>
<td>φFR</td>
<td>t</td>
<td>J</td>
<td>1 2 3 4 5</td>
<td>Kempf et al., (1987)</td>
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<tr>
<td>φFR747</td>
<td></td>
<td>φFR</td>
<td>t</td>
<td>J</td>
<td>1 2 3 4 5</td>
<td>Prauser &amp; Momirova (1970)</td>
</tr>
<tr>
<td>φFRG9</td>
<td></td>
<td>φFR</td>
<td>t</td>
<td>J</td>
<td>1 2 3 4 5</td>
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<tr>
<td>φFR113</td>
<td></td>
<td>Wide at high stringency</td>
<td>t</td>
<td>N</td>
<td>1 2 3 4 5</td>
<td>This paper</td>
</tr>
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<td>φFR755</td>
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<td>φFR</td>
<td>t</td>
<td>N</td>
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</tr>
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<td>φFR371</td>
<td></td>
<td>φFR</td>
<td>t</td>
<td>N</td>
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<tr>
<td>Mpl</td>
<td></td>
<td>φFR</td>
<td>l/t</td>
<td>N</td>
<td>1 2 3 4 5</td>
<td>Kempf et al., (1987)</td>
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<tr>
<td>φFRa-A</td>
<td></td>
<td>φFRa</td>
<td>t</td>
<td>?</td>
<td>1 2 3 4 5</td>
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<td>t</td>
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<td>t</td>
<td>?</td>
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<td>v</td>
<td>J</td>
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<td>Kempf et al., (1987)</td>
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<tr>
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<td>v</td>
<td>N</td>
<td>1 2 3 4 5</td>
<td>Grund &amp; Hutchinsen (1987)</td>
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<td>P113</td>
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<td>One module</td>
<td>v</td>
<td>J</td>
<td>1 2 3 4 5</td>
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<td>Phages isolated for S. erythraeae¶</td>
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<tr>
<td>P517</td>
<td>Partial</td>
<td>φFR</td>
<td>l</td>
<td>?</td>
<td>1 2 3 4 5</td>
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<tr>
<td>121</td>
<td>Partial</td>
<td>φFR</td>
<td>l</td>
<td>N</td>
<td>1 2 3 4 5</td>
<td>Brzeziński et al. (1986)</td>
</tr>
<tr>
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<td>l</td>
<td>pCD</td>
<td>1 2 3 4 5</td>
<td>Smorawinska et al. (1988)</td>
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<td>v</td>
<td>?</td>
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<tr>
<td>SE-60</td>
<td>Partial</td>
<td>φFR</td>
<td>l/t</td>
<td>?</td>
<td>1 2 3 4 5</td>
<td></td>
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</table>

* Phages within one group exhibit a wide overall homology with each other.
† v, virulent; t, temperate; l/t, host-dependent; l, lytic (i.e. no lysogenization observed but phage repressed by other prophages).
‡ ‡ central region (CR) module other than the J-/N-module and no homology with pCD3.
§ 1, F. rectivirgula DSM 43747 or Faenia sp. TD8; 2, F. rectivirgula DSM 43114 or Faenia sp. TD8114 (lysogens of φFR114); 3, Faenia sp. TD8Mp1 (lysogen of Mpl); 4, S. erythraeae DSM 40517; 5, S. hirsuta DSM 43463 (more positives were seen with other strains of S. hirsuta); (+), after overcoming host restriction barriers.
¶ Partial homologies at low stringency.
¶ In contrast to the Faenia phages, the S. erythraeae phages cannot be propagated at temperatures above 43 °C.

DNA homology studies and comparison of restriction maps suggest that at least two more modules analogous to the J- and N-modules, found on corresponding positions of otherwise closely related genomes such as φFR114 and φFR113, or φFRv-J and φFRv-N, represent alternative genetic information responsible for similar functions. Furthermore, the same functions appear to be present in different types of phages such as the temperate φFR and φFRb phages, the lytic phage 121, and the virulent φFRv phages. There is no indication to date of what these functions might be.

In contrast to the Faenia phages, the S. erythraeae phages cannot be propagated at temperatures above 43 °C.
The identification and characterization of modules which are distributed among actino-phages of Faenia and Saccharopolyspora is not only important for the investigation of bacteriophage evolution, but might also be interesting for vector development for this important group of industrial micro-organisms. Transfection experiments by Katz et al. (1988) with Saccharopolyspora erythraea and Streptomyces, and ourselves (Schneider et al., 1988) with F. rectivirgula, Streptomyces and Saccharomonospora, have demonstrated that these phages can be expressed in other actinomycetes and, conversely, that Saccharopolyspora erythraea or F. rectivirgula can express phages from other actinomycete genera, once the phage genome has passed the cell envelope. The combination of, for example, the φC31-derived phage vectors (Chater et al., 1985) with a host recognition module and perhaps an att-module from a Faenia phage could render this versatile set of vectors suitable for a number of species belonging to the Faenia/Saccharopolyspora cluster.

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