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 ϕ FR114, ϕ FR113 and Mp1, 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 (ϕ FR114) and N-module (ϕ FR113, Mp1). 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).

ϕ FR114 and ϕ FR113 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 ϕ SE6 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-layer 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 (ϕ FR114) and pJS50 (ϕ FR113) have been described before (Schneider *et al.*, 1987); pJS66 is a subclone of pJS55. pJS82 contains a *Hind*III–*Bam*HI fragment covering the central region of Mp1.

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 $\times$ SSC with 0.7% dry skimmed milk; 1 $\times$ 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 $\times$ SSC + 1% SDS followed by further washes (2 $\times$ 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 ϕ FR114 by HPLC (E. Grund & J. Schneider, unpublished data). High stringency was achieved with 0.15 $\times$ SSC + 0.1% SDS at 70 °C whereas for medium stringency 0.2 $\times$ 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

ϕ FR114 and ϕ FR113 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 ϕ FR113 are regarded as the origin of ϕ FR371 and ϕ FR755R (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 (ϕ FR114) and N-module (ϕ FR113) following comparison with virulent ϕ FRv 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 ϕ FRv phages, ϕ FRv-J and P113, but not with ϕ FRv-N.

The virulent ϕ FRv phages ϕ FRv-J, ϕ FRv-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 ϕ FRv-J and P113 DNA hybridized very well with each other, although a somewhat lower homology was observed within some fragments. ϕ FRv-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 ϕ FRv-J and P113 have a region of similar size not hybridizing with ϕ FRv-N. This part of the genome was called the J-module (ϕ FRv-J and P113) and the N-module (ϕ FRv-N).

When total ϕ FR114 DNA was used as a probe, no homology was detected with ϕ FRv-N, and

Fig. 1. The central regions of ϕ FR114, ϕ FR113 and Mphi. 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, *Bam*HI; Bg, *Bgl*II; Bs, *Bst*EII; E, *EcoRV*; H, *Hind*III; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*II; X, *Xho*I. 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 ϕ FR114 and ϕ FR113 DNA and between ϕ FR114 and Mphi DNA. These areas correspond closely to the homologies found between ϕ FR114 and ϕ FRv-J DNA and between ϕ FR113 or Mphi and ϕ FRv-N DNA and thus were called J- and N-modules.

Left half. The lines indicate the non-homologous region between ϕ FR114 (or ϕ FR113; A-module) and Mphi (M-module) DNA. $\Delta 371$ and $\Delta 755R$ mark the fragments of the ϕ FR113 genome which are deleted in ϕ FR371 and ϕ FR755R, respectively (Schneider *et al.*, 1987).

the homology with ϕ FRv-J and P113 was limited to the J-modules of these phages. As expected, labelled ϕ FRv-J or P113 DNA did not hybridize with ϕ FR113 but gave signals with the J-module of ϕ FR114. In spite of their strong homology, the J-modules from ϕ FR114 and the two ϕ FRv phages had no comparable restriction pattern (data not shown). ϕ FR113 DNA as a labelled probe gave no signals with ϕ FRv-J and P113 but hybridized with the N-module of ϕ FRv-N, and labelling of ϕ FRv-N DNA gave signals against ϕ FR113 DNA only in the N-module (data not shown).

Thus it appears that the temperate phage ϕ FR113 and the apparently unrelated phage ϕ FRv-N share a highly homologous region of about 3.5 kb, consequently termed the N-module. Similarly, the temperate phage ϕ FR114 and the virulent phages ϕ FRv-J and P113 (unrelated to ϕ FR114, but closely related to each other and ϕ FRv-N) share a different module, the J-module. The chief differences between ϕ FR114 and ϕ FR113, 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 ϕ FRv-J and ϕ FRv-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 ϕ FR114, ϕ FRv-J, P113 and ϕ FRv-N. (a) Ethidium-bromide-stained agarose gel of *Bst*EII digests of phage DNAs: (1) ϕ FR114, (2) ϕ FRv-J, (3) ϕ FRv-N, (4) P113. After transfer to nitrocellulose filters the restriction fragments were hybridized with biotinylated probes prepared from phage DNA of (b) ϕ FR114, (c) ϕ FRv-J, (d) ϕ FRv-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 ϕ FR747 and ϕ FRG9, which are regarded as closely related to ϕ FR114 (Schneider *et al.*, 1987) and on ϕ FRA-C, a temperate phage homoimmune with and related to the ϕ FR 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 ϕ FRb phages are temperate *Faenia* phages which are only distantly related to the ϕ FR phages and unrelated to the ϕ FRv phages (J. Schneider, unpublished data).

(2) Phage 121 is an *S. erythraea* phage which cannot be propagated on host strains with a ϕ FR or ϕ FRb 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

Fig. 3. Distribution of the J- and N-module amongst phages infecting *Faenia* and *Saccharopolyspora*. (a) Ethidium-bromide-stained agarose gel of *Bst*EII digests of phage DNAs. After transfer to nitrocellulose filters the restriction fragments were hybridized with biotinylated probes prepared from (b) ϕ FRv-J and (c) ϕ FRv-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) ϕ SE6, (4) ϕ SE60, (5) ϕ FRb-P, (6) ϕ FRb-M, (7) ϕ FRb-D, (8) ϕ FRb-B, (9) ϕ FRa-E, (10) ϕ FRa-C, (11) ϕ FRa-A, (12) ϕ FRG9, (13) ϕ FR747, (14) λ *Pst*I standard.

phages are distantly related to the ϕ FR phages but not to the ϕ FRv phages (J. Schneider, unpublished data).

(3) Mp1 was the first phage described for *F. rectivirgula* (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 ϕ FR114 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 ϕ FR114 (Table 1). The restriction map of Mp1 could be aligned with the maps of ϕ FR114 and ϕ FR113 (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 ϕ FR113 DNA and no homology with ϕ FR114 DNA. The left half was different with respect to restriction pattern and revealed an area showing no homology with either ϕ FR114 or ϕ FR113 DNA. These regions were termed A-module (ϕ FR114 and ϕ FR113) 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*

Phage	Group*	Homology with ϕ FR114	Life cycle†	CR module‡	Host range§					Origin
					1	2	3	4	5	
ϕ FR114	ϕ FR	Wide at high stringency	t	J	+	-	-	-	-	Kempf <i>et al.</i> , (1987)
ϕ FR747			t	J	+	-	-	-	-	
ϕ FRG9			t	J	+	-	-	-	-	
ϕ FR113			t	N	+	-	-	-	-	
ϕ FR755			t	N	+	-	-	-	-	
ϕ FR371			t	N	+	-	-	-	-	
Mp1			l/t	N	+	-	-	-	-	Prauser & Momirova (1970)
ϕ FRa-A	ϕ FRa	Wide at medium stringency	t	?	+	-	-	+	-	This paper
ϕ FRa-C			t	J	+	-	-	+	(+)	
ϕ FRa-E			t	?	+	-	-	+	(+)	
ϕ FRb-B	ϕ FRb	Partial	t	N	+	+	-	-	-	This paper
ϕ FRb-D			t	N	+	+	-	-	-	
ϕ FRb-M			t	N	+	+	-	-	-	
ϕ FRb-P			t	N	+	+	-	+	(+)	
ϕ FRv-J	ϕ FRv	One module	v	J	+	+	+	-	-	This paper
ϕ FRv-N		None	v	N	+	+	+	-	-	
P113			One module	v	J	+	+	+	-	-
Phages isolated for <i>S. erythraea</i> ¶										
P517	Partial		l	?	+	-	-	+	-	Kempf <i>et al.</i> (1987)
121	Partial		l	N	+	-	-	+	-	Brzeziński <i>et al.</i> (1986)
SE-3	Partial		l	pCD	+	-	-	+	-	Smorawińska <i>et al.</i> (1988)
SE-6	None		v	?	-	-	-	+	+	Grund & Hutchinson (1987)
SE-60	Partial		l/t	?	+	+	+	+	-	

* 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 ϕ FR114); 3, *Faenia* sp. TD8^{Mp1} (lysogen of Mp1); 4, *S. erythraea* 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. erythraea* phages cannot be propagated at temperatures above 43 °C.

the J- and N-modules, found on corresponding positions of otherwise closely related genomes such as ϕ FR114 and ϕ FR113, or ϕ FRv-J and ϕ FRv-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 ϕ FR and ϕ FRb phages, the lytic phage 121, and the virulent ϕ FRv phages. There is no indication to date of what these functions might be.

DNA homology studies and comparison of restriction maps suggest that at least two more modules analogous to the J- and N-module may exist. (1) Several phage DNAs show extensive (e.g. ϕ FRa-E) or partial (e.g. P517) homologies with ϕ FR114 DNA but do not contain the J- or N-module. Homology studies suggest similar genome organization for these phages and at least one more J/N type module (data not shown). The lack of restriction maps and the complex homology patterns do not allow a more precise description. (2) pCD3 (kindly provided by R. Brzeziński, unpublished data) is a probe specific for the central region of SE-5 (Brzeziński *et al.*, 1986). Preliminary data (not shown) indicate that the area from which pCD3 originated corresponds to the N-module of phage 121. pCD3 DNA failed to hybridize with DNA from any of the phages listed in Table 1. The accumulation of sites for particular restriction enzymes which otherwise are not found on SE-5 or 121 may suggest that this module originated from a different evolutionary line of actinophages (R. Brzeziński, personal communication).

Fig. 4. The restriction map of Mpl compared with the maps of ϕ FR114 and ϕ FR113. (a) Restriction map of Mpl (for abbreviation of restriction sites see Fig. 1). (b) Comparison of the restriction maps of Mpl, ϕ FR114 and ϕ FR113 with seven enzymes. Sites which are common to all three phages are named on the map of Mpl and are indicated by long bars on the other two maps. Medium-length bars represent sites which are common to only two phages, and the restriction sites which are specific for each genome are shown by the short bars. The cloned fragments discussed in Fig. 1 are marked by the numbers of the recombinant plasmids. The arrows indicate the left borders of the central regions and are also shown in Fig. 1. For better alignment, the restriction map of ϕ FR114 was drawn as if ϕ FR114 (42 kb) had the same genome length as ϕ FR113 (43 kb); the three small deletions which are responsible for the actual differences (Schneider *et al.*, 1987) were thus ignored. (c) Comparison of HindIII restriction maps of ϕ FR114 and ϕ FR113 which had not been mapped previously. The diagram is to the same scale as the restriction maps recently shown (Schneider *et al.*, 1987).

The identification and characterization of modules which are distributed among actinophages 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 ϕ C31-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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