Short Communication

CLOSE RELATIONSHIP BETWEEN *BACILLUS* PHAGES M2 AND Nf: PHYSICAL MAPPING OF THE DNA BY SIX RESTRICTION ENDONUCLEASES¹

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Bacteriophage M2 (1) and Nf (2), both isolated independently in Japan, contain DNA of approximately equivalent size, which has proteinase-sensitive transfection activity (3, 4). The two phages are related serologically, though a slight difference in *K* values of anti-M2 serum (3, 5) and a difference in the response to 6-(*p*-hydroxyphenylazo)uracil on phage growth have been observed (5). A close genetic relationship between the two phages has also been suggested by efficient recombination in a transfection system (5). Restriction endonuclease *Eco*RI cleavage patterns of M2-DNA (4) and Nf-DNA are similar (3). They have recently been revealed to have almost complete homology in DNA-DNA hybridization studies (Mizusawa *et al.*, manuscript in preparation). Recent availability of many different specificities of restriction endonucleases has made easy investigation into the identity of genetic entities possible. This communication describes the physical similarity of M2- and Nf-DNAs *via* a comparison of cleavage patterns and physical maps by using six restriction endonucleases.

Bacteriophage M2 and Nf were provided by Saito (The Institute of Applied Microbiology, University of Tokyo) and Takagi (Department of Biology, International Christian University), respectively. Single plaque isolate (M2H3) of M2 was used for the cleavage mapping experiment, since the original stock of M2 consisted of heterogeneous population (to be published elsewhere). Phages were grown on *B. subtilis* SR22 (4) by the method described previously (6).

In order to clarify the similarity between M2- and Nf-DNAs in physical mapping, both DNAs were digested by the following six restriction endonucleases and their cleavage patterns were compared (Fig. 1a and 1b). In the case of Nf-DNA, only the *Eco*RI-A, *Xba*I-A, *Pvu*II-C, *Bsu*1247I-A and *Ava*I-B fragments

¹ Abbreviation used in this text: Mdal, megadalton.

Fig. 1. Agarose gel electrophoretic patterns of M2H3- and Nf-DNA digested with restriction endonucleases.

a, The restriction endonuclease digests of M2H3-DNA (0.5 μ g) and Nf-DNA (0.8 μ g) were subjected to agarose gel electrophoresis through 0.7% agarose gel (Seakem) in TAE buffer (40 mM Tris-acetate, 5 mM sodium acetate, and 1 mM EDTA, pH 7.8). Methods for preparation of DNA, enzyme digestion, and gel electrophoresis are essentially the same as described in a separate paper (Matsumoto and Hirokawa, submitted to *Mol. Gen. Genet.*).

b, *Hind*III digest, *Hind*III-*Ava*I double digest, and *Hind*III-*Pvu*II double digest of M2H3-DNA (3.0 μ g) and Nf-DNA (2.0 μ g) were subjected to 1.5% agarose gel electrophoresis as described above.

were apparently larger than those of M2H3-DNA. The physical map of M2H3-DNA constructed by these restriction endonucleases is presented in Fig. 2. The molecular weights of the fragments of M2H3-DNA produced by the restriction endonucleases are also presented (Table 1). Since overall cleavage patterns of the two phage DNAs have been shown to be similar, it can be assumed that the physical map of Nf-DNA is quite similar to that of M2H3-DNA.

*Hind*III digestion of Nf-DNA (Fig. 1b) produced two new fragments of *Hind*III-B1 (1.58 Mdal) and *Hind*III-B2 (0.66 Mdal) with concomitant disappear-

Fig. 2. Physical maps of M2H3-DNA and the portion around the additional region of Nf-DNA.

EcoRI map has already been described (4). *EcoRI*, *XbaI*, and *HindIII* maps have been described (Matsumoto, and Hirokawa, submitted to *Mol. Gen. Genet.*). *Bsu*-1247I (a *PstI* isoschizomer) site was determined by double digestion with *EcoRI*. *PvuII* sites were determined by double digestion with *EcoRI* or *XbaI*. *AvaI* site was determined by double digestion with *HindIII*. *XbaI*, *Bsu*1247I, *PvuII*, and *AvaI* maps were consistent with those constructed by Yoshikawa, Elder, and Ito (personal communication). The portion around the additional region of Nf-DNA which corresponds to *HindIII*-B region of M2-DNA is depicted at the bottom. The *HindIII*-site between *HindIII*-B1 and *HindIII*-B2 was determined by double digestion experiments with *AvaI* or *PvuII* (see text and Fig. 1b).

ance of *HindIII*-B (1.85 Mdal) of M2H3-DNA. The sum of the molecular weights of *HindIII*-B1 and -B2 is larger than *HindIII*-B of M2H3-DNA by 0.39 Mdal. The additional region of Nf-DNA was mapped in the corresponding physical map of M2H3-DNA by double digestion (Fig. 1b). The orientation of *HindIII*-B1 and -B2 was determined by double digestion with *AvaI*. The result of *PvuII* and *HindIII* double digestion indicates that the additional *HindIII* site in Nf-DNA is located in *PvuII*-C (0.17 Mdal from the *PvuII* site). The physical mapping study indicates that Nf-DNA is larger than M2H3-DNA by 0.39 Mdal (3.3% of the whole genome) at a particular region (just left of the *PvuII* site including the additional *HindIII* site).

The genetic similarity between the two phages was tested in a recombination experiment using marker rescue of suppressor-sensitive mutant of M2 phage. Phage of M2 *susK436* (Matsumoto and Hirokawa, submitted to *Mol. Gen. Genet.*) was rescued by wild type Nf-DNA with essentially identical efficiency (e.g. 91%) to rescue by M2H3-DNA, in which the mutant was rescued by ϕ 29-DNA with approximately 0.8% of the homologous combination.

These results indicate the very close relationship between the two phages and

Table 1. Molecular weight of restriction endonuclease digested fragments of M2H3-DNA.

Fragment ^a	Enzyme					
	<i>EcoRI</i>	<i>XbaI</i>	<i>HindIII</i>	<i>PvuII</i>	<i>Bsu1247I</i>	<i>AvaI</i>
A	5.1	6.0	3.0	6.0	10.4	6.6
B	2.5	4.4	1.85	2.6	1.4	5.3
C	2.2	1.14	1.47	1.75		
D	1.02	0.17	1.41	1.32		
E	0.98		1.35			
F			0.98			
G			0.80			
H			0.35			
I			0.31			
J			0.31			
Sum	11.80	11.71	11.83	11.67	11.8	11.9

^a Molecular weight (Mdal) was determined by the method described by HELLING *et al.* (7) using SV40 DNA-*EcoRI*, -*Bsu1247I*, -*PvuII*, -*HindIII* fragments (8) and ColEI DNA-*EcoRI* (9) as reference markers. The molecular weights of the larger fragments were calculated from the data of double digestion experiments.

also suggest that one of the two may be the descendant of the other in their evolution.

Recently, YOSHIKAWA and Ito (10) reported that *EcoRI* and *PstI* cleavage maps exhibited similarity between the two phages and that DNA terminal proteins were identical from a peptide mapping study.

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