EVIDENCE THAT *BACILLUS* PHAGE M2 IS DERIVED FROM Nf BY DELETION IN A PARTICULAR REGION OF THE GENOME

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It has been shown that the restriction maps of *Bacillus* phages M2 and Nf are identical to each other except in the region 32 to 46% from the left ends of the genomes. Furthermore, in this region, genomes of the original stock of phage M2 were heterogeneous in length. We have found that M2 has the same origin as Nf and that the heterogeneity in the region is caused by deletions of various sizes. These results were obtained by electron microscopic observations of heteroduplex molecules between the genomes of Nf and subclones of M2, and by fine physical mapping of the heterologous regions. Finally, it has been shown that these deletions affected phage morphology but not multiplication.

Small *Bacillus* phages M2 and Nf, isolated by IKEDA et al. (1) and SHIMIZU et al. (2), respectively, are closely related to each other in many biological and physicochemical respects such as proteinase-sensitive transfection activity (3, 4), response to phage antiserum (3, 5) and their genome size (6). We have previously reported the physical maps of M2 and Nf with six kinds of restriction endonucleases (6) in which the differences in size of the restriction fragments between M2 and Nf were found only in a region of 32–46% from the left end of the genome (6 and YOSHIKAWA and ITO, personal communication). In the process of constructing the cleavage maps, it was found that the DNAs prepared from the M2 original stock were different in size in the same region (7). This region appeared to be in a *HpaII*-D fragment, since only this fragment showed a difference in size among six subclones of M2, designated H1, H2, H3, H4, H5 and H6 (7). Furthermore, one such subclone, H6, was identical to Nf in the DNA cleavage patterns of the genome with nine kinds of restriction endonucleases. These observations suggest that rearrangements of DNA, such as deletion or insertion, had occurred in a common ancestor of Nf and M2 resulting in the generation of the heterogeneity among the genomes of M2 subclones and Nf.

In this report, we compare five physical maps of these subclones of phages which indicate that the heterogeneous M2 population was a result of deletions of various lengths which occurred in the *HpaII*-D region of the Nf genome. In addition, morphological observations of these phages indicate that such deletions affect the formation of phage head projections.

MATERIALS AND METHODS

Bacterial and phage strains. *Bacillus subtilis* SR22 (*trpC2*, *spo0A12*) (8) was used as the host for growing bacteriophages M2 and Nf. *Bacillus* phage M2 (original stock) was provided by Dr. H. Saito (The Institute of Applied Microbiology, University of Tokyo) and phage Nf by Dr. J. Takagi (Department of Biology, International Christian University). H1, H2, H3, H4, H5 and H6 are subclones of M2 (original stock) isolated in our laboratory (7).

Phage and DNA preparations. The phages and their DNAs were purified by the method of ITO et al. (9). Phage DNA was digested with *HpaII* and the fragments were separated by electrophoresis in 1.0% agarose gel. The *HpaII*-D fragment was eluted from the gel as previously described by YANG et al. (10).

Cleavage maps. Cleavage maps of *HpaII*-D of Nf and three M2 subclones, H3, H5 and H6 were constructed by referring to the *PvuII* and *HindIII* sites in H6 and Nf, whose locations were already known (6, 7). Since the other M2 subclones, H1, H2 and H4, lacked the *PvuII* site in the *HpaII*-D, the resulting cleavage maps of H3, H5 and H6 were referred in constructing those of H1, H2 and H4.

Electron microscopy and gel electrophoresis. Conditions for heteroduplex formation were essentially the same as described by DAVIS et al. (11). Spreading of heteroduplex molecules followed the procedure of KLEINSCHMIDT (12). Purified phages were stuck to carbon grids and negatively stained with 2% uranyl acetate. A Hitachi HS-9 at 75 k volt electron microscope was used. Phage coat proteins were denatured and SDS-polyacrylamide gel electrophoresed according to the methods of CARRASCOSA et al. (13).

RESULTS AND DISCUSSION

It has been shown that the physical maps of M2 subclones and Nf are identical except in the region of *HpaII*-D (6, Yoshikawa personal communication). This evidence was confirmed by DNA-DNA hybridization of the genomes between M2 subclones and Nf. Figure 1 shows an electron micrograph of the heteroduplex molecule between the genomes of H1 and Nf. A single stranded-loop was observed at the position of approximately 40% from one end of the heteroduplex DNA. This in turn, suggested that the genomes of H1 and Nf are identical except in the above region. The same observations were obtained by hybridization experiments using the DNAs of Nf and other M2 subclones (data not shown).

Fig. 1. Electron micrograph of the heteroduplex molecule between genomes of H1 and Nf. Arrow indicates a single stranded-loop.

To determine more precisely the locations of the heterologous region of Nf and six subclones of M2 on their genomes, fine physical maps of the respective *Hpa*II-D fragments were constructed using eight kinds of restriction endonucleases (Fig. 2). By a comparison of these *Hpa*II-D fragments, the heterologous region can be further demarked within a boundary between the *Hha*I site (A_1 in Fig. 2) approximately 290 nucleotides right to the left end of *Hpa*II-D and the *Dde*I site (D_5) approximately 230 nucleotides left to the right end of the fragment. It was shown that there was no difference in Nf and H6 in the location of cleavage sites of all of the restriction enzymes tested, indicating that these two phages are identical. In H3, besides the conserved region as described above, the region from D_5 to D_4 , which exists about 280 nucleotides further to the left of D_5 , was the same as that of Nf. Similarly, the following regions were identical to those of Nf: in H1, the region from A_1 to *Taq*I site (T_2); in H2 and H4, from A_1 to D_2 ; in H5, from A_1 to T_2 and from D_5 to *Pvu*II site (P). These results clearly indicate, first, that M2 is derived from the origin which is identical to Nf by DNA rear-

Fig. 2. Comparison of heterologous region of the M2 subclones and the Nf genomes. a. Physical map of the Nf genome with *Hpa*II. b. Cleavage maps of *Hpa*II-D of the M2 subclones and Nf with restriction endonucleases. A, B, C, D, F, H, P and T represent, respectively, the sites of cleavage for *Hha*I, *Bcl*I, *Hinc*II, *Dde*I, *Hinf*I, *Hind*III, *Pvu*II and *Taq*I. The solid lines indicate the region between the *Hha*I and *Dde*I sites in which the heterogeneity among M2 subclones and Nf occur. Putative endpoints of the deletions are marked with oblique lines.

Fig. 3. The morphological differences among Nf and subclones of M2. Electron micrographs of phage particles of M2 subclones and Nf negatively stained with 2% uranyl acetate. a, Nf; b, H1; c, H2; d, H3; e, H5 and f, H6.

Fig. 4. SDS-polyacrylamide gel electrophoresis of phage coat proteins. The left side slot (lane a) contained ϕ 29 coat proteins as the marker of the molecular weights which are 80k, 71k, 54k, 48k, 40k and 28k corresponding to the proteins of the neck appendage, tail, head major, upper collar, lower collar and head projection, respectively (13). Lane b, Nf; lane c, H3; lane d, H2; lane e, H5 and lane f, H1.

rangement. And second, diverse deletions which have different endpoints and lengths rather than insertions, have occurred on the boundary of the *Hpa*II-D of the origin in the DNA rearrangement.

Since the rearrangement of the genome will cause phenotypical changes, general biological characters, such as multiplication and morphology of phages, were investigated in Nf and the deleted subclones of M2. Although no difference in efficiency of adsorption to the host cells, lytic profile, final yield and burst size of progeny was found among these phages, morphological alteration was observed by electron microscopy (Fig. 3). It appeared that Nf has fiber projections on its head (2), but not the M2 subclones, except H6. This morphological difference between Nf and the deleted M2 subclones was substantially confirmed by electrophoretic analysis of the phage coat proteins. Six bands of polypeptide with molecular weights of 81k, 70k, 53k, 37k, 34k and 24k were revealed by SDS-polyacrylamide gel electrophoresis of heat denatured phage virions of Nf (Fig. 4, lane b). In the deleted M2 subclones, however, only five of the polypeptides were found, or the 24k dalton polypeptide was not detected (Fig. 4, lanes c, d, e and f). A similar electrophoretic pattern was observed in M2Y, the other subclone of the M2 original stock (YOSHIKAWA and ITO, personal communication). These results indicate that the deletion of the nucleotide sequence of the Nf genome is concerned with a morphological alteration of the phage; in other words the protein of the head projection, or alternatively some other polypeptide(s) necessary

for morphogenesis of the head projection, was lacking.

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