

Simultaneous display of different peptides on the surface of filamentous bacteriophage

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ABSTRACT

We have developed a new system for producing hybrid virions of filamentous bacteriophage fd simultaneously displaying two different peptides by infecting cells harbouring a plasmid containing a modified gene VIII with an engineered bacteriophage carrying a second and different copy of a modified gene VIII. The simultaneous display of different peptides has many potential applications in exploring the immune response and studying protein–protein interaction.

Displaying peptides on the surface of filamentous bacteriophage virions (1) has become an important means of generating peptide libraries (2) and of raising anti-peptide antibodies (3). The high copy number (2700) per virion (Fig. 1A), and the ability of displayed epitopes to recruit T-cell help and elicit specific B-cell antibodies without the need of adjuvants, make display on the major coat protein (pVIII) of particular value in exploring the immune response (3,4). Recombinant virions, carrying the same insert on all copies of pVIII, are generated by cloning a DNA fragment encoding the peptide at the appropriate place in gene VIII (Fig. 1B). However, there are limitations on the length and sequence of peptides displayed on recombinant virions (3,5,6). To display larger peptides, hybrid virions with interspersed modified and wild-type pVIII proteins (Fig. 1C) can be generated by simultaneously expressing genes encoding the wild-type and modified coat proteins in the same *Escherichia coli* cell (1,3). We describe here a novel system for creating hybrid virions in which the capsid consists of a mixture of the wild-type and two modified coat proteins carrying different peptide inserts (Fig. 1D).

Bacteriophage fdAMPLAY88 carries two copies of gene VIII: the natural gene VIII, and the modifiable gene, placed under the control of the *tac* promoter, in the intergenic region (Fig. 2A). A β -lactamase encoding gene facilitates selection by providing ampicillin resistance to infected *E.coli* cells. This obviates the need for the plasmid/bacteriophage or phagemid/helper-bacteriophage systems in creating hybrid virions. Hybrid bacteriophages fdAMP88D and fdAMP88MN were generated by cloning the DNA fragments encoding peptides D, PKLRGDLQVLAQ (6), and MN, IHIGPGRFYTT (3), respectively, into fdAMPLAY88. The transformants could be obtained as plaques on a bacterial lawn and as ampicillin-resistant colonies, and infected cells produced hybrid virions displaying the encoded peptide. The

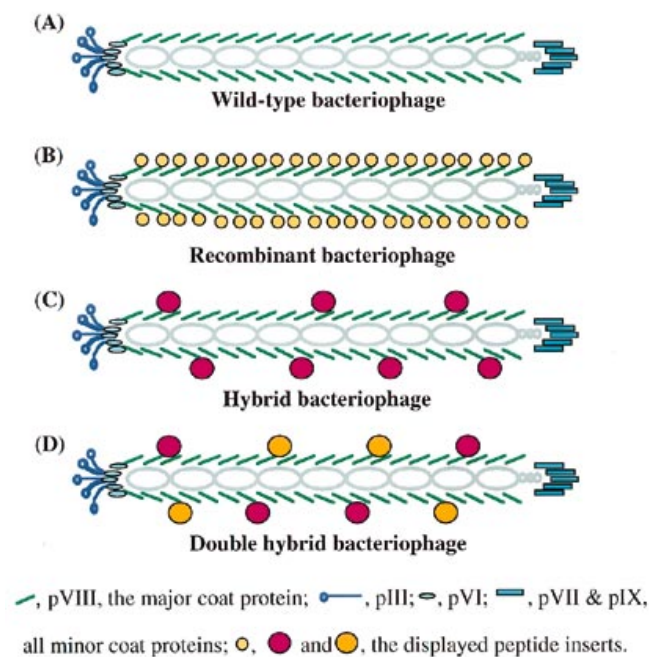


Figure 1. Schematic representation of wild-type bacteriophage fd and different forms of peptide display on pVIII. (A) Wild-type; (B) recombinant virion in which each copy of pVIII has a peptide insert; (C) hybrid virion containing a mixture of wild-type pVIII and pVIII with a peptide insert; and (D) hybrid virion containing a mixture of wild-type pVIII and pVIII proteins displaying different peptide inserts.

simultaneous display of a different peptide was achieved by providing another modified gene VIII under the control of the *tac* promoter in plasmid pTfd8SHU (Fig. 2B), derived from plasmid pfd8SHU (7), which also carries a gene conferring tetracycline resistance. DNA fragments containing the modified gene VIII encoding the peptides T1 (KQIINMWQEVGKAMYA), p-24S (PAGFAILKSNNKTFNY) and p-66 (KDSWTVNDIQKLVGK) were cloned into the vector pTfd8SHU to create plasmids pTfd8p-24S, pTfd8p-66 and pTfd8T1, respectively. Cells transformed with one or other of these plasmids, when infected with the bacteriophage fdAMP88MN or fdAMP88D, produced virions simultaneously displaying two different peptides. This was

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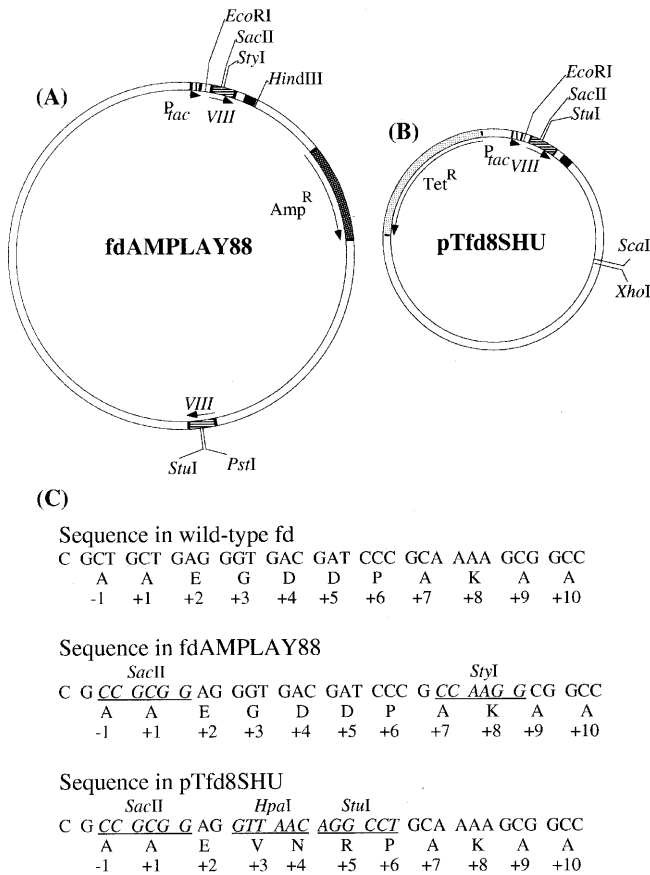


Figure 2. Schematic structures of vectors and the corresponding N-terminal amino acid sequences of pVIII. (A) Bacteriophage fdAMPLAY88. (B) Plasmid pTfd8SHU; *Amp^R*, gene encoding ampicillin-resistance; *Tet^R*, gene encoding tetracycline resistance; *P_{tac}*, *tac* promoter; *VIII*, gene VIII of bacteriophage fd; *III*, gene III of bacteriophage fd. fdAMPLAY88 was constructed by cloning a PCR-amplified fragment, consisting of the *tac* promoter, gene VIII and the β -lactamase-encoding gene from the vector pfd8SY (7), into the intergenic region of a derivative of wild-type fd in which restriction sites *XmaI* and *XbaI* had previously been introduced. pTfd8SHU was constructed by cloning the gene conferring tetracycline resistance into pfd8SHU (7) and deleting a fragment of the β -lactamase gene. (C) The nucleotide and corresponding N-terminal amino acid sequences of the wild-type and modified gene VIII with the cloning sites indicated.

confirmed by SDS-PAGE (Fig. 3) and N-terminal amino acid sequence analysis (6).

The ability to display two different peptides simultaneously offers new possibilities of assembling interacting peptides on the

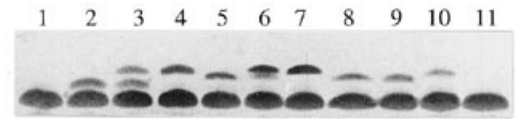


Figure 3. Analysis of hybrid bacteriophage virions. Lanes 1 and 11, wild-type fd; lane 2, fdAMP88D displaying peptide D; lane 3, double hybrid virion simultaneously displaying peptides D and p-66; lane 4, hybrid virion displaying peptide p-66; lane 5, fdAMP88MN displaying peptide MN; lane 6, double hybrid virion simultaneously displaying peptides MN and p-24S; lane 7, hybrid virion displaying peptide p-24S; lane 8, fdAMP88MN displaying peptide MN; lane 9, double hybrid virion simultaneously displaying peptides MN and T1; lane 10, hybrid virion displaying peptide T1. The coat proteins displaying peptides MN and T1 (lane 9) were not resolved; double display was confirmed by N-terminal amino acid sequence analysis. The copy number of the displayed peptide was estimated by N-terminal sequence analysis as ~35% for peptide D in fdAMP88D, 20% for peptide MN in fdAMP88MN, 17% for peptide p-24S and 8% for peptide MN in the p-24S/MN virions and 11% for each peptide in the T1/MN virions; the sequences were too similar to obtain an estimate in the p-66/D virions. The virions were prepared and purified as described elsewhere (6). For double display, *E. coli* cells transformed with the appropriate plasmid were infected with the bacteriophage (fdAMP88D or fdAMP88MN) and grown in medium containing tetracycline and ampicillin.

surface of the virion and has many potential advantages in protein engineering, in the study of protein-protein interaction and in exploring vaccine design and the immune response. Two different epitopes from a pathogen or an effector sequence and a targeting sequence could be displayed on the same capsid; a simple extension of this system is simultaneous display on pIII (1) and pVIII.

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