FREQUENT DELETION OF *BACILLUS SUBTILIS* CHROMOSOMAL FRAGMENT IN ARTIFICIALLY CONSTRUCTED PHAGE ρ11p*hisA*$^+$

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1. Introduction

A novel gene-cloning method named 'prophage transformation' has been developed for constructing specialized transducing phages, ρ11 [1] and φ105 [2]. Using this method, several genes of *Bacillus subtilis*, such as *hisA*, *lys* [1], *amyE*, *aroI* [3], *spo0B* [4,5] and *spo0F* [6,7] have been successfully cloned. We have studied the unusual instability of histidine-transducing phage, ρ11p*hisA*$^+$, with regard to His$^+$-transducing ability and found that ~5% of the total phages obtained by induction of either Rec$^+$ or *recE*4 lysogen carrying ρ11p*hisA*$^+$ did not have His$^+$-transducing ability [8]. The loss of His$^+$-transducing ability was not ascribed to the recombination between the prophage and the host chromosomal *hisA*1 gene, since His$^+$-transduction was not restored when a His$^+$ Rec$^+$ lysogen carrying the non-transducer phage was constructed and treated with inducer. These results strongly suggest that ρ11p*hisA*$^+$ prophages, upon induction, lost the *hisA*$^+$ moiety by deletion. The instability of gene-cloning vectors has been a serious problem in DNA recombination, but the mechanism has remained unclear. Here we show that the loss of the His$^+$-transducing ability of ρ11p*hisA*$^+$ is caused by deletion of the nucleotide sequence for the *hisA*$^+$ gene on the ρ11p*hisA*$^+$ genome.

2. Materials and methods

2.1. *Bacterial and phage strains*

Bacillus subtilis BD224 (*trpC*2 *thr*5 *recE*4) and BD366 (*trpC*2 *thr*5 pUB110) were obtained from Dr D. Dubnau. Phages ρ11wt, ρ11p*hisA*$^+$ and ρ11p*hisA*$^-$ were as in [8–10].

2.2. *DNA preparations*

Plasmid DNA was isolated using low melting-point agarose in vertical gels. Phages were induced by treatment with mitomycin C, purified, and their DNA isolated as in [11].

2.3. *DNA–DNA hybridization*

*Eco*RI digests of phage DNA were fractionated on an 0.8% agarose gel and transferred to nitrocellulose filters as in [12]. Nick translation and DNA hybridization were done as in [14].

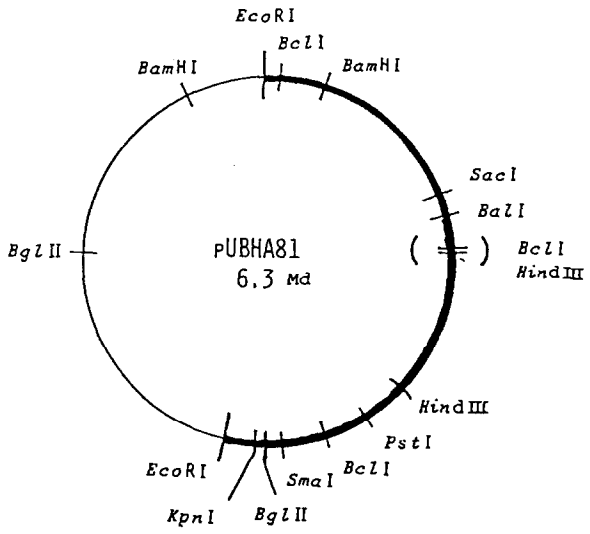

Fig.1. Endonuclease restriction map of plasmid pUBHA81 DNA. The 3.3 × 10^6 M_r *Eco*RI fragment carrying the *hisA*$^+$ gene of *B. subtilis*, which had been cloned in temperate phage ρ11, was inserted into plasmid vector pUB110 [13]. Sites of cleavage for *Eco*RI, *Bam*HI, *Bgl*II, *Sac*I, *Bal*I, *Hind*III, *Sma*I, *Kpn*I, *Pst*I and *Bcl*I are shown: (——) 3.3 × 10^6 M_r *Eco*RI fragment; (——) pUB110 DNA.

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3. Results and discussion

A restriction site map of a 3.3 × 10^6 M_r *Eco*RI fragment bearing the *hisA*$^+$ gene cloned in pUB110 is shown in fig.1. Since a single *Bam*HI site existed on the 3.3 × 10^6 M_r *Eco*RI fragment (fig.1,2), we first analyzed by 0.8% agarose gel electrophoresis the *Bam*HI digests of DNA prepared from ρ11p*hisA*$^+$ and three ρ11p*hisA*$^-$ isolated independently as His$^+$ non-transducer. It is evident from fig.2 that *Bam*HI–A1 and –A2 fragments in the *Bam*HI digest of ρ11p*hisA*$^+$ DNA were not observed in those of all ρ11p*hisA*$^-$ DNA, indicating that at least the nucleotide recognition sequence for *Bam*HI in the 3.3 × 10^6 M_r *Eco*RI fragment carrying the *hisA*$^+$ gene was either deleted or changed to an inert sequence.

As a direct demonstration of deletion of the *hisA*$^+$ gene nucleotide sequence, we analyzed DNA of ρ11p*hisA*$^+$ and DNA of those independent ρ11p*hisA*$^-$ clones with restriction and hybridization techniques. The *Eco*RI fragments of the phage DNA were separated electrophoretically and transferred to nitrocellulose as in [12]. To visualize the 3.3 × 10^6 M_r *Eco*RI fragment carrying *hisA*$^+$ gene, we exposed the nitrocellulose strip to the radioactively labeled plasmid pUBHA81. For ρ11p*hisA*$^+$ DNA, as expected from transformation assay [8], the probe reacted with only an *Eco*RI fragment of 3.3 × 10^6 M_r (fig.3, lanes 2,7). However, radioactive pUBHA81 DNA failed to anneal measurably with any *Eco*RI fragments of three ρ11p*hisA*$^-$ DNAs. Most parts of the 3.3 × 10^6 M_r *Eco*RI fragment containing the *hisA*$^+$ gene were deleted in all three ρ11p*hisA*$^-$ genomes tested.

As a control, *Eco*RI fragments of *B. subtilis* chromosome were reacted with radioactively labeled pUBHA81 DNA (fig.3, lanes 1,6). Unexpectedly, the

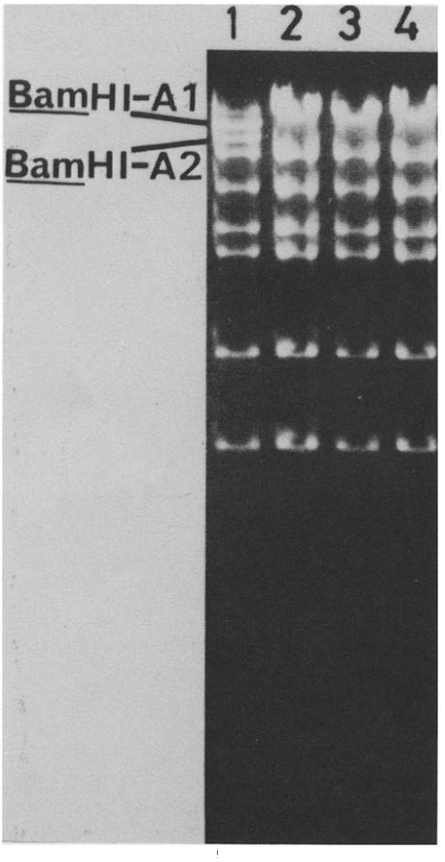

Fig.2. Hydrolysis of ρ11p*hisA*$^+$ and ρ11p*hisA*$^-$ DNA with endonuclease *Bam*HI: (1) ρ11p*hisA*$^+$ DNA; (2–4) ρ11p*hisA*$^-$ DNA. Samples were electrophoresed in 0.8% agarose at 80 mA for 3 h.

Fig.3. Southern hybridization of ^{32}P-labeled pUBHA81 DNA to ρ11p*hisA*$^+$ DNA, ρ11p*hisA*$^-$ DNA and *B. subtilis* chromosomal DNA. Each DNA was digested with *Eco*RI, electrophoresed in 0.8% agarose and blotted onto a nitrocellulose filter as in [12]. (1–5) are UV photographs of ethidium bromide-stained gel, while (6–10) are autoradiographs: (1,6) *B. subtilis* chromosomal DNA; (2,7) ρ11p*hisA*$^+$ DNA; (3–5,8–10) ρ11p*hisA*$^-$ DNA.

probe hybridized with a 4.9×10^6 M_r *Eco*RI fragment, which is 1.6×10^6 M_r larger than the *Eco*RI fragment cloned in the ρ11p*hisA*$^+$ genome. We consider that the deletion took place during either 'prophage transformation' or isolation of ρ11p*hisA*$^+$ phage after 'prophage transformation'.

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References

[1] Kawamura, F., Saito, H. and Ikeda, Y. (1979) Gene 5, 87–91.
[2] Iijima, T., Kawamura, F., Saito, H. and Ikeda, Y. (1980) Gene 9, 115–126.
[3] Nomura, S., Yamane, K., Masuda, T., Kawamura, F., Mizukami, T., Saito, H., Takatsuki, M., Tamura, G. and Maruo, B. (1979) Agric. Biol. Chem. 43, 2637–2638.
[4] Hirochika, H., Kobayashi, Y., Kawamura, F. and Saito, H. (1981) J. Bacteriol. 146, 494–505.
[5] Kobayashi, Y., Hirochika, H., Kawamura, F. and Saito, H. (1981) in: Sporulation and Germination (Levinson, H. S. et al. eds) pp. 114–118, Am. Soc. Microbiol., Washington DC.
[6] Kawamura, F., Saito, H., Hirochika, H. and Kobayashi, Y. (1980) J. Gen. Appl. Microbiol. 26, 345–355.
[7] Kawamura, F., Shimotsu, H., Hirochika, H. and Kobayashi, Y. (1981) in: Sporulation and Germination (Levinson, H. S. et al. eds) pp. 109–113, Am. Soc. Microbiol., Washington DC.
[8] Mizukami, T., Kawamura, F. and Saito, H. (1980) J. Gen. Appl. Microbiol. 26, 307–310.
[9] Mizukami, T., Kawamura, F., Takahashi, H. and Saito, H. (1980) Gene 11, 157–162.
[10] Dean, D. H., Orrego, J. C., Huchison, K. W. and Halvorson, H. O. (1976) J. Virol. 20, 509–519.
[11] Kawamura, F., Saito, H., Ikeda, Y. and Ito, J. (1979) J. Gen. Appl. Microbiol. 25, 223–236.
[12] Southern, E. M. (1975) J. Mol. Biol. 98, 503–517.
[13] Gryczan, T. J. and Dubnau, D. (1978) Proc. Natl. Acad. Sci. USA 75, 1428–1432.
[14] Walz, A., Ratzkin, B. and Carbon, J. (1978) Proc. Natl. Acad. Sci. USA 75, 6172–6176.