Potentiation of a Nucleolytic Activity in *Bacillus subtilis*

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In several strains of *Bacillus subtilis* extensive breakdown of chromosomal DNA may be potentiated by osmotic lysis of protoplasts. At its most severe, in strains originating from Farmer & Rothman's thymine auxotroph, the rate of DNA breakdown was greater than 50% per hour at 40 °C. The rate of DNA breakdown in most other strains tested was approximately 5% per hour except for SPβ− strains, in which the rate of DNA breakdown was only 0.3%. DNA degradation was attributed to relaxation of control of a nuclease specified by the prophage of SPβ or a related phage. The most potent nuclease in lysates was an ATP-activated protein of *M*, 280000. Derivatives of Farmer and Rothman's strain containing integrated plasmids had the highest rate of DNA degradation. Although the chromosome was completely destroyed, covalently closed circular plasmids were generated from the integrated sequence. These showed massive deletions of the *B. subtilis* part of the integrated plasmid but the vector sequence remained intact. The nucleolytic activity therefore appears to recognize specific sequences in *B. subtilis* DNA. We suggest that activation of SPβ genes during development of competence may be a cause of deletion of cloned genes in the early stages of establishment of cloned sequences.

**INTRODUCTION**

Although *Bacillus subtilis* and other bacteria contain an abundance of deoxyribonucleases (Kerr *et al.*, 1965; Birnboim, 1966; McCarthy & Nester, 1969; Ohi & Sueoka, 1973; Scher & Dubnau, 1973; Doly & Anagnostopoulos, 1976), their activities are well controlled by protein inhibitors (Strauss & Marone, 1967; McCarthy & Nester, 1969). In non-growing cells and in lysates incubated under physiological conditions, the rate of DNA breakdown is quite low. We have previously found very little DNA degradation in lysates of *B. subtilis* 168 trpC2 prepared from protoplasts by hypotonic shock, incubated at 35 °C in the presence of magnesium ions (Sargent & Bennett, 1982; Sargent *et al.*, 1983). However, in this report we show that the DNA of certain strains may be more unstable than that of the wild-type under the same conditions. This potential instability depends to some extent on the presence of the prophage of the temperate phage SPβ (Warner *et al.*, 1977), and is increased markedly in certain genetic backgrounds. When such strains contain reiterated sequences of an integrative plasmid, the plasmid sequence may be excised from the chromosome by massive deletion or rearrangement directed at *B. subtilis* DNA but not the vector sequences. We believe this activity of the host genome may contribute to the recE-independent instability of autonomously replicating plasmids in *B. subtilis* noted by many authors (Tanaka, 1979; Fugii & Sakaguchi, 1980; Uhlén *et al.*, 1981; Bonamy & Szulmajster, 1982). Strains containing reiterated sequences may provide a sensitive test system for the detection of host activities capable of deleting cloned sequences. Strains containing reiterated sequences can be obtained by transforming *B. subtilis* with non-replicative plasmids containing a chloramphenicol-resistance marker and a piece of *B. subtilis* DNA (Young, 1984; Sargent & Bennett, 1985). These plasmids insert into the chromosome adjacent to the homologous region by a mechanism analogous to Cambell's model for phage λ recombination, and under strong selective conditions become tandemly reiterated.
Fig. 1. Structure and origin of pMS96 and pMS106. Subclones of pMS31 (Sargent & Bennett, 1985) (heavy line) were constructed in pJH101 (Ferrari et al., 1983) as shown. Inner circles show the major Aare II fragments. A, 2.6 kb fragment containing the origin and chloramphenicol marker; B, 2.95 kb fragment of pMS96; C, 1.35 kb fragment of pMS106.

METHODS

Bacteria and plasmids. The two plasmids used for these studies, pMS96 and pMS106, were obtained by constructing deletions of pMS31 (Sargent & Bennett, 1985), as shown in Fig. 1. pMS31 contains a DNA sequence specifying an interaction between the chromosome and the membrane of B. subtilis. It maps close to purA, in the origin region of the chromosome. Plasmids were amplified in Escherichia coli strain DH1 (Hanahan, 1983), and prepared by an alkaline lysis procedure (Ish-Horowicz & Burke, 1981). The plasmids were introduced into B. subtilis by transformation (Wilson & Bott, 1968), selecting for chloramphenicol resistance (5 µg ml⁻¹) on L agar plus glucose. Strains resistant to 100 µg chloramphenicol ml⁻¹ were selected by growing transformants in L broth plus glucose (0.4%, w/v) (Table 1). For studies of DNA degradation, strains (Table 1) were also grown in L broth plus glucose plus growth requirements.

Preparation of lysates and cell breakage. Protoplasts were prepared and lysed using PM and LM respectively as described by Sargent & Bennett (1985). PM contained sucrose (0.5 M), MgCl₂ (0.02 M), Tris/HCl (0.02 M, pH 8.0), sodium azide (1 mM) and phenylmethylsulphonyl fluoride (0.14 mM). LM contained Tris/HCl (0.02 M, pH 7.3), MgCl₂ (0.005 M), NaCl (0.05 M) and phenylmethylsulphonyl fluoride (0.1 mM). Washed cells were resuspended in PM to give 4 mg dry weight ml⁻¹, and lysozyme was added to a final concentration of 500 µg ml⁻¹. Cells were incubated at 40 °C for 10 min, after which protoplast formation was complete, and 3 vols LM were then added. PM and LM also contained thymine (10 µg ml⁻¹) unless stated otherwise.

Radioactive methods. [³H]Thymidine (1 µCi ml⁻¹, 37 kBq ml⁻¹; specific activity 77 Ci mmol⁻¹, 2.85 TBq mmol⁻¹) was added to cultures at OD₅₄₀ = 0.1 and allowed to grow to OD₅₄₀ = 2.0 before harvesting. Bacteria were washed in L broth and resuspended in PM. To follow the time course of DNA breakdown in lysates, 100 µl samples were treated with 300 µl ice-cold TCA (10%, w/v) for 20 min and then centrifuged at 25 000g for 10 min. The supernatant was neutralized with 5 M-NH₄OH and counted in Packard scintillation fluid containing emulsifier.

Assay of DNAase. Phenol-purified chromosomal DNA of B. subtilis 168 trpC2, labelled with [³H]thymidine to give 30,000 c.p.m. µg⁻¹, was dissolved in 0.01 M-Tris/HCl pH 8.0, 0.01 M-NaCl, 0.1 mM-EDTA. Reaction mixtures contained 100 µl sample, 90 µl buffer A (5 mM-MgCl₂, 0.05 M-Tris/HCl pH 8.0, 0.05 M-NaCl), 10 µl 1 mM-ATP and 100 µl substrate. Reactions were incubated at 40 °C for 1 h and were terminated with 100 µl 50% (w/v) TCA. Undigested DNA was sedimented at 25000g for 10 min and the supernatant counted as above.

Fractionation of lysates on Sepharose 4B. Clarified lysates or French pressure extracts were precipitated with ammonium sulphate (0.67 g per ml of sample) and left at 0 °C for 1 h. The precipitate was resuspended in buffer A (4 ml) and loaded onto a Sepharose 4B column equilibrated with buffer A. The column was calibrated with thyroglobulin (Mr, 670000) and bovine γ-globulin (Mr, 165000).
Table 1. Rate of DNA degradation in lysates of *B. subtilis* strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source/construction</th>
<th>Rate of DNA degradation at 40 °C (% per hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td><em>trpC2</em></td>
<td></td>
<td>4-7</td>
</tr>
<tr>
<td>MS247</td>
<td>pMS96 integrated into 168TT</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td>MS254</td>
<td>pMS106 integrated into 168TT</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>MS256</td>
<td>pMS96 integrated into 168</td>
<td></td>
<td>5-5</td>
</tr>
<tr>
<td>MS259</td>
<td>pMS106 integrated into 168</td>
<td></td>
<td>5-0</td>
</tr>
<tr>
<td>MS271</td>
<td><em>ilcA2 thyA5 thyB5 att(SPβ)</em></td>
<td>CU1430 transformed to Trp*</td>
<td>NT</td>
</tr>
<tr>
<td>MS274</td>
<td><em>ilcA2 Thy</em> att(SPβ)</td>
<td>MS247 transduced with MS271</td>
<td>0-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(selection for Trp*; see Results)</td>
<td></td>
</tr>
<tr>
<td>SL989</td>
<td>recE4 metB10 lys-3</td>
<td>Supplied by P. J. Piggot</td>
<td>11</td>
</tr>
<tr>
<td>GSY2251</td>
<td>trpF7 add-5</td>
<td>Doly &amp; Anagnostopoulos (1976)</td>
<td>4</td>
</tr>
<tr>
<td>RB1084</td>
<td>Δ(PBSX phoS proAB metC pyrX)</td>
<td>Buxton (1980)</td>
<td>6</td>
</tr>
<tr>
<td>RB1144</td>
<td>Δ(PBSX phoS proAB metC) <em>ilcA thyA1 thyB1 SPβ</em>-</td>
<td>Buxton (1980)</td>
<td>0-8</td>
</tr>
<tr>
<td>CU1430</td>
<td><em>ilcA2 trpC2 thyA5 thyB5 att(SPβ)</em></td>
<td>Supplied by S. A. Zahler</td>
<td>0-3</td>
</tr>
<tr>
<td>CU1574</td>
<td><em>trpC2 bet SPβc2</em></td>
<td>Supplied by S. A. Zahler</td>
<td>11</td>
</tr>
</tbody>
</table>

NT, not tested.

* Genotype uncertain (*thyA1/C5 thyB1/BS*).

**Agarose gel electrophoresis, restriction enzyme analysis and preparation of DNA.** Standard methods were used and have been described previously (Sargent *et al.*, 1983).

**Genetic methods.** Transducing lysates of phage AR9 (Love *et al.*, 1976) were prepared on strain MS271 (Trp* derivative of CU1430; Table 1), which contains a vacant SPβ attachment site (att) (Zahler *et al.*, 1977). att(SPβ) was transduced into MS247 as described in Results.

**RESULTS**

**Potentiation of nucleolytic activity in MS247**

Strain MS247, which contains pMS96 integrated into the chromosome and reiterated many times, was selected as resistant to 100 μg chloramphenicol ml⁻¹. The copy number of sequences homologous to pMS96 was determined by blot hybridization to be about 20 per chromosome. No difficulty was encountered in preparing DNA which gave good quality restriction patterns, but if lysates were incubated for as little as 20 min in LM at 40 °C, the DNA was sufficiently damaged to totally obscure any restriction patterns. The exact rate of degradation was variable, but in 1–2 h the chromosome became almost completely TCA-soluble.

The trigger that initiated DNA degradation appeared to be the hypotonic shock required to lyse protoplasts (Fig. 2a). Protoplasts of [³H]thymidine-labelled MS247 were prepared in PM and subjected to a hypotonic shock by addition of 3 vols LM, which stimulated the rate of DNA breakdown immediately. If, however, protoplasts were diluted with PM, the rate of DNA breakdown was considerably less. DNA degradation occurred in the presence of sodium azide or rifampicin (data not shown), thus indicating that transcriptional or translational events are not required for DNA degradation.

DNA degradation did not occur at a significant rate in unlysed non-growing cells. Thus, when sodium azide (1 mM), *p*-hydroxyphenylazouracil (50 μg ml⁻¹) (a DNA synthesis inhibitor; Love *et al.*, 1976) or rifampicin (5 μg ml⁻¹) was added to growing cultures of [³H]thymidine-labelled MS247 no significant DNA breakdown occurred over a 2 h period (data not shown).

Once DNA breakdown in MS247 had started, purified DNA that was added to the lysate was broken down at the same rate as endogenous DNA (Fig. 2b).

**Genetic factors affecting the rate of DNA degradation after hypotonic shock**

Although the rate of DNA degradation in lysates of MS247 was considerably higher than in 168 *trpC2* (taken as wild-type with respect to properties studied in this paper), the parental strain...
of MS247 (168TT) also degraded its DNA at a markedly higher rate than 168 trpC2. The rate of degradation in this thymine mutant was also affected by the presence or absence of thymine. Exposure to thymine-less conditions at any time after harvesting of cells doubled the rate of DNA breakdown.

A survey of a number of laboratory strains was therefore undertaken to assess the range of rates of breakdown in lysates (Table 1). Two major sources of DNA-degrading activities could be considered by examining mutant strains. These were the recombination system and the prophages known to be present in B. subtilis.

The two prophages known to be present in B. subtilis 168 are the defective PBSX and the temperate SPP, and strains lacking these are available (Table 1). The strains with deletions of the PBSX region (RB1084, RB1144) showed differences in the rate of DNA degradation. The DNA of RB1084 broke down at the wild-type rate, while in RB1144 the rate of breakdown was less than 1% per hour. As the genetic background of RB1084 appears essentially isogenic with the wild-type (Buxton, 1980), the prophage of PBSX is unlikely to contribute to DNA degradation in lysates. Strain RB1144, on the other hand, lacks the prophages of PBSX and SPβ (Buxton, 1980). This suggests that the prophage of SPβ may determine the nucleolytic activity of wild-type strains (see Discussion for origin of RB1144). An SPβ− PBSX+ strain (CU1430) also showed greatly reduced nucleolytic activity in lysates (Table 1). SPβ therefore appears to encode a gene that causes breakdown of DNA in lysates. Strain CU1574, which contains a temperature-sensitive inducible SPβ prophage (SPβc2), had elevated levels of DNA degradation when incubated at 40 °C (10 °C below the temperature for phage induction).

While these observations suggest that SPβ in some way affects DNA breakdown in lysates of the wild-type, they do not offer a complete explanation of the high rate of DNA breakdown seen in lysates of MS247 or 168TT. This has been studied by transducing att(SPP) into MS247 using the generalized transducing phage AR9. This was done by preparing transducing particles on strain MS271 [ilvA2 thyA5 thyB5 att(SPP)], a trpC+ derivative of CU1430 (Table 1), and using these to transduce MS247 to trpC+. According to Zahler et al. (1977), att(SPP) is 50% transducible with trpC. Furthermore, ilvA2 should be transducible with trpC. Of the Trp+ transductants obtained, 30% were Ilv− and 70% of these were SPβ−. As donor and recipient were both Thy−, so were the transductants. The rates of DNA degradation in lysates of three isolates (MS274; Table 1) were less than 1% per hour as in CU1430. There is therefore a genetic factor located in the trp–SPβ region of MS247 (and presumably 168TT) which gives an elevated rate of DNA degradation in lysates.

The second source of DNA-degrading activities that was considered were those specified by known Rec functions. In a recE4 strain, which is thought to be deficient in a protein comparable
Nuclease potentiation in B. subtilis

Fig. 3. Effects of inhibitors on the degradation of MS247 DNA. Protoplasts of \(^{3}\text{H}\)thymidine-labelled bacteria were prepared and lysed as described in Methods. The time course of release of TCA-soluble radioactivity expressed as a percentage of the initial radioactivity is shown for samples containing no additions (○), EDTA (●), 3 mM-ATP (△), 0·1 mM ATP (▲), 1 mM-S-adenosylmethionine (□), 1 mM-p-chloromercuribenzoate (■) or 1 mM-ADP (◇).

Fig. 4. Fractionation of nucleolytic activity on Sepharose 4B. DNAase activity was measured in the presence (○) and absence (●) of 10 \(\mu\text{M}\) ATP. TG, Thyroglobulin (mol. wt 650000); BGG, bovine γ globulin (mol. wt 165000).

to the \(E.\ coli rec\)A gene product (de Vos et al., 1983), the rate of DNA breakdown was about twice that of the wild-type. In an \(add\)-5 strain, DNA degradation occurs at the same rate as in the wild-type. This strain has a greatly reduced level of the major ATP-activated DNAase.

Properties of the nucleolytic activity

The effect of a variety of inhibitors on the nucleolytic activity in MS247 was examined, the most significant of which are illustrated in Fig. 3. DNA degradation was completely inhibited by EDTA and was 50% inhibited by \(p\)-chloromercuribenzoate. ATP was substantially inhibitory at 3 mM but it slightly stimulated the initial rate of DNA degradation at 0·1 mM. Other compounds that inhibited were S-adenosylmethionine and ADP.

Inhibition and activation by ATP over a small range of concentrations is characteristic of a number of ATP-dependent DNAases (Ohi & Sueoka, 1973; Doly & Anagnostopoulos, 1976; Linn, 1981). The presence of a major ATP-activated DNAase in lysates was demonstrated after fractionating a lysate on Sepharose 4B (Fig. 4). A major peak was observed at a molecular weight of 280000 which was stimulated threefold by 10 \(\mu\text{M}\)-ATP and completely inhibited by 3 mM-ATP. A shoulder to the peak at about 90000 was also observed, but this was not stimulated by ATP.
Table 2. Sizes of major AvaII fragments of plasmids recovered from lysates of MS247 and MS254

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Sizes of fragments (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMS11</td>
<td>MS247</td>
<td>2.6, 1.3</td>
</tr>
<tr>
<td>pMS12</td>
<td>MS247</td>
<td>2.6, 3.15</td>
</tr>
<tr>
<td>pMS13</td>
<td>MS247</td>
<td>2.6, 1.42</td>
</tr>
<tr>
<td>pMS14</td>
<td>MS247</td>
<td>2.6, 2.4</td>
</tr>
<tr>
<td>pMS15</td>
<td>MS247</td>
<td>2.6, 1.1</td>
</tr>
<tr>
<td>pMS96</td>
<td>Parental plasmid in MS247</td>
<td>2.6, 2.95</td>
</tr>
<tr>
<td>pMS117</td>
<td>MS254</td>
<td>2.6, 1.1</td>
</tr>
<tr>
<td>pMS118</td>
<td>MS254</td>
<td>2.6, 1.55</td>
</tr>
<tr>
<td>pMS119</td>
<td>MS254</td>
<td>2.6, 3.15</td>
</tr>
<tr>
<td>pMS106</td>
<td>Parental plasmid in MS254</td>
<td>2.6, 1.35</td>
</tr>
</tbody>
</table>

The DNAase activities observed in lysates of MS247 were only slightly higher than those found in exponential phase cells broken in the French pressure cell (see Methods). Furthermore, they were only slightly greater than those in 168TT and only 50% greater than those in 168 trpC2. Precise quantification for this enzyme probably requires a more elaborate assay system.

Plasmid fraction seen in strains MS247 and MS254

These strains were constructed by the integration of the plasmids pMS96 and pMS106 into the chromosome of 168TT in the purA region (Sargent & Bennett, 1985). Selection for resistance to high concentrations of chloramphenicol caused a tandem reiteration of the inserted sequence. After incubating lysates of MS247 for 1 h there was virtually no DNA of a size greater than 3 kb when examined on agarose gels, except for one or two discrete bands at about 10 kb. The DNA in these bands was probably present as covalently closed circular structures, as it was unaffected by the alkaline denaturation required for preparation of plasmids (Ish-Horowicz & Burke, 1981). Furthermore, chloramphenicol-resistant transformants of E. coli DH1 could be obtained with this material. Restriction patterns of the plasmids found in transformants indicated that the original B. subtilis DNA had been modified by deletion and rearrangement.

A convenient method for analysing the patterns was to digest the plasmids with AvaII. This enzyme gave two major fragments from the parental plasmids, one containing the pJH101 origin and chloramphenicol-resistance marker (2.6 kb) (fragment A; Fig. 1) and the other including the cloned sequence, with AvaII sites 0.1 kb to the left and 0.85 kb (fragment B) or 0.67 kb (fragment C) to the right of the inserted sequence (Fig. 1) (referred to below as the inserted DNA).

Plasmids derived from MS247 or MS254 (Table 2) retained the vector 2.6 kb AvaII fragment intact, whereas the B. subtilis DNA regions were extensively modified. Most plasmids isolated had massive deletions in the inserted DNA, reducing the 2.95 kb fragment to as little as 1-1 kb (pMS115, pMS117), while in others there was an increase in the size of the insertion (pMS112, pMS119). In pMS111 the PstI, HincII, AvaI and EcoRV sites were deleted from the inserted DNA, but the HindIII site was not. Thus the deletion actually extended to the left hand end of the inserted DNA but the right hand end remained intact. In pMS113 the HindIII and PstI sites flanking the inserted DNA were retained, but the other restriction sites that characterize the inserted DNA were deleted (data not shown). Independent experiments (data not shown) indicated that the region between the right hand end of the inserted DNA and the AvaII site (labelled a in Fig. 1) was retained in both plasmids.

The plasmids described in Table 2 were derived from several experiments. Although an exhaustive study of the types of plasmids obtainable has not been made, it seems clear that there are only a limited number of types of deletion possible and consequently that recombination can only take place at a limited number of specific sites. A second point of interest was that the parental plasmid has not been recovered on any occasion from lysates. Plasmids can also be isolated in very low yield from exponential phase cells of MS247, but these are all indistinguishable from pMS96, the parental plasmid. We presume they are generated by homologous recombination between the repeated units.
In our earlier studies of membrane bound DNA, in which lysates of \textit{B. subtilis} were treated with restriction enzymes, the rate of DNA solubilization due to endogenous nucleases had seemed small (Sargent & Bennett, 1982, 1985; Sargent \textit{et al.}, 1983). However, the serendipitous discovery of unusually high rates of DNA degradation in lysates of certain strains of \textit{B. subtilis} had led us to investigate this question further. In a survey of 14 strains, we found that those lacking SP\(\beta\), in both Thy\(^+\) and Thy\(^-\) backgrounds, had the minimum rates of DNA breakdown (i.e. \(< 1\%\) per hour at 40\(^\circ\)C), whereas the SP\(\beta^+\) wild-type degraded its DNA at about five times this rate. Induction of the defective phage PBSX is a well-known cause of DNA degradation in \textit{B. subtilis} (Okamuto \textit{et al.}, 1968; Haas \& Yoshikawa, 1969), but we have found that an almost isogenic strain from which PBSX was deleted (RB1084) had the same rate of DNA breakdown as the wild-type.

In lysates of the Farmer \& Rothman (1965) thy strain, the rate of DNA breakdown was approximately twice that of the wild-type, and was increased further by thymine starvation. These thy mutations were introduced into strain 168 from \textit{B. subtilis} W23 by transformation, perhaps with part of the terminal region of W23 (Callister \& Wake, 1974), thus creating the possibility of substantial non-isogenicity between the two strains.

In other thy auxotrophs (RB1144 and CU1430) the rate of degradation was lower than that of the wild-type, but these strains were also SP\(\beta^-\). Strain RB1144 has apparently lost SP\(\beta\) (Buxton, 1980) in the course of a number of transformation steps (Dedonder \textit{et al.}, 1977). It is not clear whether the non-isogenic region has also been lost during these constructions. Unusually high rates of degradation (40–50\% per hour) were observed in derivatives of the Farmer \& Rothman (1965) strain in which the plasmids pMS96 and pMS106 were present in a tandemly reiterated form. In 168 and other strains (data not shown) integrated plasmids of this type had no effect on the rate of DNA degradation. We believe that the plasmid is unlikely to specify a product that may cause this phenomenon. The entire sequence of pMS106 and most of pMS96 are known, and these contain no possible open reading frames (M. G. Sargent and M. F. Bennett, unpublished observations). A genetic determinant, either in SP\(\beta\) or linked to it, probably determines this instability. Thus SP\(\beta^-\) derivatives of MS247 (i.e. MS274) constructed by transduction showed reduced rates of DNA breakdown. However, if the plasmid does not specify a product, it is extremely difficult to understand why the rate of DNA degradation should be higher in 168TT containing the integrated plasmid. One possibility is that growth in high concentrations of chloramphenicol has an indirect effect on the potential for DNA degradation in the 168TT genetic background. Alternatively, the prophage may be able to respond to the presence of reiterated sequences. Although no satisfactory explanation for this is available, we propose that the prophage encodes a nuclease whose activity is potentiated, without macromolecular synthesis, when protoplasts are osmotically stressed in the presence of magnesium ions, perhaps when an inhibitory factor is inactivated or released from the cell. Although the mechanism is obscure, DNA degradation is clearly initiated only after the osmotic shock, as high molecular weight DNA can be isolated from protoplasts in the presence of EDTA and sodium sarcosylate. Several \textit{B. subtilis} nucleases are controlled \textit{in vivo} by protein inhibitors that can be inactivated by heat treatment (McCarty \& Nester, 1969; Strauss \& Marone, 1967).

Inhibitors of the \textit{E. coli recBC} system are produced by several phages (Sakaki \textit{et al.}, 1973; Van Vliet \textit{et al.}, 1978). We also suggest that genetic factors affecting the state of repression of the prophage genome determine the potential for nucleolytic activity after osmotic shock. Thus temperature sensitive repressor mutations (SP\(\beta\)c2) at 40\(^\circ\)C (10\(^\circ\)C below the temperature required for full induction; Warner \textit{et al.}, 1977) doubled the rate of DNA degradation in lysates.

The genetic factors favouring nucleolytic activity in 168TT can therefore be viewed as either a chromosomal mutation which affects the state of repression of the prophage or a genetic modification of the prophage itself. The observed decrease in nucleolytic activity in SP\(\beta^-\)transductants (MS274) does not distinguish between these possibilities as the amount of DNA transduced is very large (Love \textit{et al.}, 1976). Plaque-forming units of SP\(\beta\) have not been obtained from 168TT, although SP\(\beta\) sequences can be detected by DNA–DNA hybridization (unpublished observations). This suggests that the prophage may be defective and possibly modified with respect to control of the nuclease.
The increased potential for nucleolytic activity in the recE4 strain (believed to be the analogue of recA in *E. coli* (Vos et al., 1983)) is of uncertain significance as it could reflect the 'reckless' phenotype characteristic of recA strains, or may affect SPβ expression. It is clear, however, that the nuclease active in SPβ+ strains cannot be the recE4 protein.

An ATP- and divalent-metal-activated DNAase of *M*, 280000 is responsible for most of the DNA breakdown in lysates of MS247. After partial purification on Sepharose 4B, the enzyme was greatly stimulated at low ATP concentrations and was inhibited at high concentrations. The rate of DNA breakdown in crude lysates was similarly affected. Ohi & Sueoka (1973) and Doly & Anagnostopoulos (1976) have described similar enzymes from the wild-type. However, in lysates of a mutant deficient in this enzyme (Doly & Anagnostopoulos, 1976), the rate of DNA degradation was the same as in the wild-type. The specific activity of the ATP-dependent DNAase in cell-free extracts does not correlate well with the rate of DNA degradation in lysates. Thus the rate of DNA degradation in the wild-type appears substantially less than would be expected from the specific activity of the DNAase. The presence of two enzymes in the extract (one ATP-activated, the other a less active nuclease of lower molecular weight that is unaffected by ATP) further complicates the analysis. The final rate of DNA degradation in MS247 may depend on activities encoded by chromosomal and prophage genes that are regulated by inhibitors. Without further enzymological studies, it is impossible to assess the relationship between SPβ and the two enzyme activities seen in lysates.

After the destruction of most of the DNA in lysates of MS247, one or two discrete ethidium bromide-staining bands, that proved to be plasmids, could be seen on agarose gels. They could be isolated by an alkaline-denaturation procedure and could transform *E. coli* to chloramphenicol-resistance. In these plasmids the vector sequences (pJH101) were intact but there had been massive deletion or re-arrangement of the inserted sequences. We do not believe this occurred after introduction of the plasmids into *E. coli*, because when the *EcoRI* fragment (7.2 kb) containing the reiterated unit of pMS96 was isolated from chromosomal DNA of MS247, circularized and transformed into *E. coli*, it generated plasmids identical to pMS96.

In order to replicate successfully in *E. coli* the sequences containing the pJH101 origin and the chloramphenicol-resistance gene must remain intact. However, from the size of the major *AvaII* and *HaeIII* fragments in the plasmids resulting from this transformation experiment, it was clear that the vector sequences were retained almost to the junction with the insert, while the insert was extensively deleted or rearranged. In addition to this evidence of specificity, the limited number of types of deleted plasmid generated in MS247 seems to indicate that there may be only a small number of recognition sequences for the nuclease in the 2 kb sequence present in pMS96.
The deletion plasmids must have been generated by a specific enzymatic attack on the *B. subtilis* sequences, followed by an illegitimate recombination or ligation step that circularized the released components (Fig. 5). ATP-dependent DNAases such as the *recBC* nuclease of *E. coli* are probably capable of such activities (Linn, 1981; Taylor & Smith, 1980). Furthermore lysogenic phages of *E. coli* (such as the lambdoid phage, *rac*) are known which encode a *recBC* type of activity that is expressed transiently during lytic induction (Kaiser & Murray, 1979).

The ATP-activated DNAase reported here may have been of some importance in the many reports of deletions or rearrangements of cloned sequences in *B. subtilis* (Tanaka, 1979; Fugii & Sakaguchi, 1980; Uhlén et al., 1981; Bonamy & Szulmajster, 1982). Several authors have noted increased activity of prophage genomes during the development of competence in *B. subtilis*. Plaque-forming particles of φ105 may be generated in appropriate lysogens (Yasbin et al., 1975; Yasbin, 1977). PBSX-coded killer protein is expressed during competence without the highly lytic late stages (Ganesan, 1979), and the methyl transferase of SPβ is also expressed (Ganesan, 1979; Trautner et al., 1980). The nuclease could also be expressed under the same conditions and may delete cloned DNA sequences during the early stages of establishment of a plasmid after transformation. Such plasmids would presumably then have a selective advantage. The use of strains containing reiterated sequences such as pMS96 provide a sensitive test system for detecting host activities capable of deleting cloned sequences.

**REFERENCES**


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