resulting in end-to-end association of somatic chromosomes mainly involves homologous chromosomes (figs 1–3). The intact end-to-end attachments during cell division may result in reduction in chromosome number and change of chromosome morphology, forming dicentric chromosomes. Similarly, the anaphase movement of somatic chromosomes without chromatid separation (fig. 3) may lead to somatic reduction.

The mechanism of whole chromosome unions, however, remains to be defined. One possibility may be the stickiness of telomeres of homologous chromosomes, resulting in dicentric chromosomes. In prophase cells, the end-to-end attachments of somatic chromosomes are frequently observed leading to chain-like interphase/prophase association[7], which helps to maintain the spatial relationship of mitotic chromosomes throughout the division cycle[8]. It is quite likely that the end-to-end terminal fusion of chromosomes giving rise to dicentric chromosomes possibly represents a relic of interphase associations which are more tightly held in this particular variety, either due to specific telomeric gene combinations in these chromosomes or representing a genetically-controlled translocation. The genetically-controlled disturbed polarity and spindle operation may cause the somatic reduction.

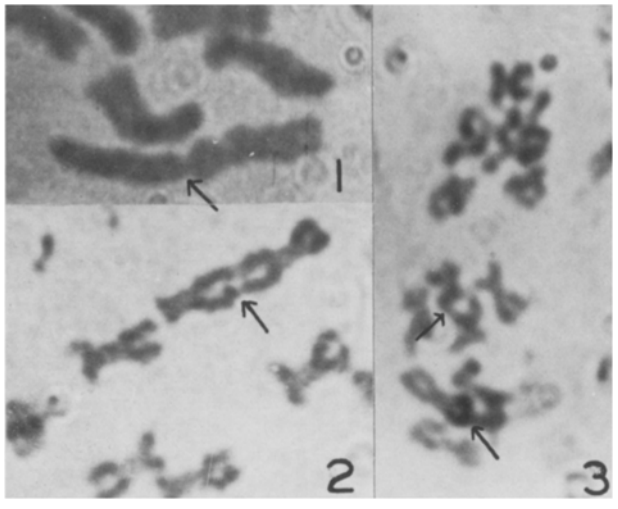

Figure 1 and 2. Chromosome spreads showing end-to-end attachment of somatic chromosomes.

Figure 3. Mitotic anaphase without chromatid separation. Arrows indicate the end-to-end attachment of chromosomes.

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Regulation of ppApp synthesis during sporulation of a conditionally asporogenous rifampin mutant of *Bacillus subtilis*[1]

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Summary. The intracellular synthesis of ppApp in a conditionally asporogenous rifampin-resistant mutant of *Bacillus subtilis* stimulated to sporulate in the Sterlini-Mandelstam (SM) medium supplemented with ppApp is negligible and comparable to that of the non-sporulating culture. The formic acid extract from the sporulating culture stimulates sporulation of the mutant in SM medium.

Bacterial sporulation is a simple model system for differentiation. A rifampin-resistant, conditionally asporogenous mutant of *B. subtilis* (Spo^c Rif^r) which sporulates poorly in Sterlini-Mandelstam[4] (SM) medium but sporulates normally in modified Difco medium[5] was found to regain its ability to sporulate in SM medium supplemented with arginine, methionine, valine and isoleucine[6]. Both the Spo^c and the Rif^r traits were mapped at the same gene. The Rif^r lesion has been shown to be located in the gene encoding RNA polymerase[6]. Several highly phosphorylated nucleotides (HPN) were found in sporulating cells of *B. subtilis*. Only ribosomes from sporulating but not vegetative cells are able to synthesize HPN[7,8]. Asporogenous mutants defective in the synthesis of HPN have been isolated[9]. The stimulation of sporulation by ppApp in the Spo^c Rif^r mutant[10] and the dependence of sporulation on the prior synthesis of pppAppp[11] seem to indicate that these HPN are involved in the initiation of sporulation in *B. subtilis*. This paper reports further evidence that the intracellular synthesis of ppApp is regulated by its exogenous supply during sporulation.

Experimental part. Bacterial strains used, conditions for sporulation, method of determining sporulation frequency have been previously reported[6]. The extraction and quantitation of HPN were done as previously reported[10]. Identification of HPN I was performed by scraping off the radioactive spots from the PEI-cellulose chromatogram. The radioactivities were eluted by 2 M triethylammonium bicarbonate prepared by bubbling CO_2 through a 2 M solution of triethylamine in ice until the pH was 7.5. The eluate was air dried and rechromatographed on PEI-cellulose TLC in 1.5 M KH_2PO_4 at pH of 3.4 for the 1st dimension and in 3.3 M ammonium formate, 4.2% boric acid at pH 7.0. ppApp (adenosine 3′-5′-bis-diphosphate, ICN Pharmaceuticals, Cleveland, Ohio, USA) was also chromatographed in exactly the same fashion on a separate TLC sheet. For ^{3}H-ATP incorporation studies, mutant culture resuspended in SM medium supplemented with amino acids were incubated with $H_3{}^{32}PO_4$ (carrier free, ICN Radiochemicals, Irvine, Cal., USA) or ^{3}H-ATP (adenosine 5′-triphosphate, tetrasodium salt [2,8-^{3}H], ICN Radiochemicals, Irvine, Cal., USA) with cells at final concentrations of 25 µCi/ml and 40 µCi/ml respectively at the time of resuspension. 40 µg/ml of unlabelled ATP was added to the

culture to serve as carriers. Aliquots of the culture were removed at intervals and their formic acid extracts were chromatographed and quantitated as previously described[10]. For large scale extraction of HPN I from mutant culture sporulating for 18 h in SM medium supplemented with amino acids 9 h after resuspension, the procedure of Rhaese et al.[12] was used.

Results and discussion. As shown in figure 1, the level of intracellular HPN I which comigrated with ppApp on PEI-cellulose TLC (spot a as indicated previously[10]) in the ppApp supplemented culture was comparable to that of the unsupplemented culture and were much lower than the amino acid supplemented culture. Both the ppApp supplemented and amino acid supplemented cultures sporulate normally. In order to further identify HPN I, radioactive spots were scraped off from the chromatogram and eluted by triethylammonium bicarbonate and air-dried. The dried extract were rechromatographed on PEI-cellulose sheet developed in a 2 dimensional TLC with ppApp as a marker. There was almost exact superimposition of the radioactivities on the autoradiogram with ppApp. The tentative identification of HPN I as ppApp was also strengthened by the selective uptake of ^{3}H-ATP into the position occupied by HPN I on the PEI cellulose TLC developed in phosphate buffer, as shown in figure 2. The juxtaposition of the peaks of ^{3}H-ATP and the ^{32}P uptake with the position of ppApp on the same chromatogram was highly suggestive that they are probably the same entity. Preliminary alkaline hydrolysis experiment also suggested that HPN I has the same hydrolytic product as that of ppApp. To further test whether ppApp is synthesized during sporulation, 10 l of NS22 cells sporulating in SM medium supplemented with amino acids 9 h after resuspension was extracted with formic acid and DEAE-sephadex ion-exchange chromatography according to Rhaese et al.[12]. Since the heavy deposit of salt during the elution and concentration process of the fractions obscured the UV-light detection of the nucleotides on the TLC, the UV-spectra of various fractions were traced in comparison with known markers. The fraction eluted from the ion-exchanger at 0.45 M phosphate buffer seemed to have similar absorption maxima as ppApp. Fractions eluted at 0.8 M phosphate were pooled and used to supplement the mutant culture resuspended in SM medium. The 0.8 M phosphate pooled fractions were found to stimulate sporulation of the mutant to a level significantly higher than the unsupplemented culture. Their stimulatory effect was comparable to that of the amino acids and was confirmed by both the refractile spore count as well as the heat resistance spore count.

In conclusion, we have presented evidence to implicate that the intracellular synthesis of ppApp is inhibited by its exogenous supply in a sporulating culture. In light of the findings that prior synthesis of pppAppp is needed for sporulation and that the pppAppp synthesizing system is also capable of synthesizing ppApp[11], the possible role of ppApp in the initiation of sporulation can be studied by cloning the Spo^c Rif^r gene from our mutant into the SpoOF mutant which is defective in the pppAppp synthesizing system. The interaction of the gene products of the Spo^c Rif^r and the SpoOF loci will shed light on the regulatory mechanism of gene expression in sporulation. Works in pursuit of this goal are currently underway.

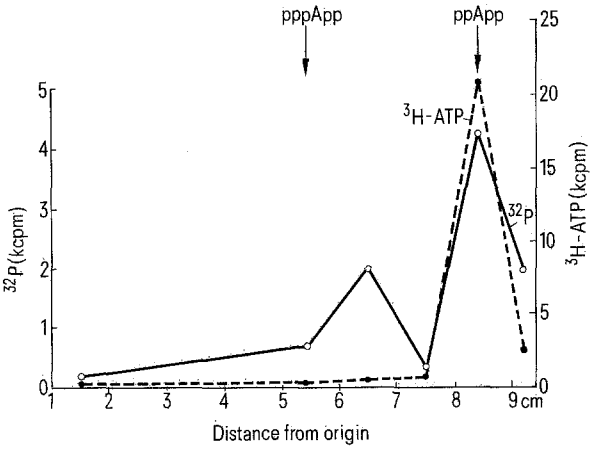

Figure 1. Profile of HPN synthesis and sporulation in strain NS22 resuspended in supplemented and unsupplemented Sterlini-Mandelstam (SM) medium. An exponential culture of strain NS22 was washed and resuspended into SM medium to which amino acid or ppApp were added at the time of resuspension at a final concentration of 50 µg/ml. A culture with no supplement was also included as a control. HPN was extracted and quantitated as described by Rhaese et al.[7]. The ^{32}P cpm was corrected for the background radioactivity of the TLC and normalized against the volume of the cell extract as well as the turbidity of the culture (in Klett units.) □, Supplemented with amino acids (AA); ○, supplemented with ppApp; ●, no addition.

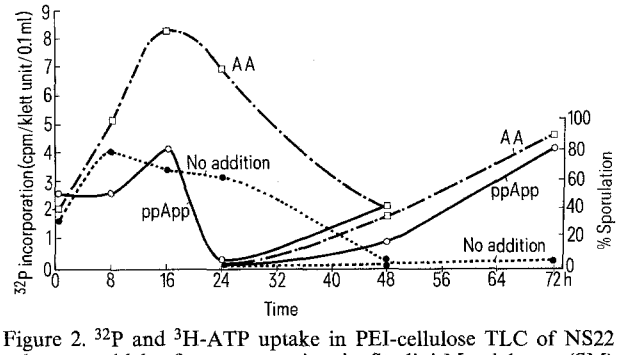

Figure 2. ^{32}P and ^{3}H-ATP uptake in PEI-cellulose TLC of NS22 cultures at 16 h after resuspension in Sterlini-Mandelstam (SM) medium supplemented with amino acids. NS22 cultures resuspended in SM medium supplemented with amino acids were incubated with $H_3{}^{32}PO_4$ or ^{3}H-ATP at the time of resuspension. Aliquots of cultures were removed at intervals and their formic acid extracts were chromatographed on PEI-cellulose TLC developed in phosphate buffer. Spots with radioactivities as indicated from the autoradiograms were cut out from the TLC and quantitated. pppApp and ppApp were also run in the same chromatograms as markers. ○, ^{32}P uptake; ●, ^{3}H-ATP uptake. pppApp (adenosine 3′(2′)-diphosphate-5′-triphosphate) and ppApp (adenosine 3′-5′-bisdiphosphate) were obtained from ICN Pharmaceuticals, Cleveland, Ohio, USA.

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