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Mutant of *Bacillus subtilis* with a Temperature-Sensitive Lesion in Ribonucleic Acid Synthesis During Germination

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We have isolated a mutant of *Bacillus subtilis* with a temperature-sensitive lesion in the process of spore germination. The temperature-sensitive mutation affects only germination and outgrowth, and the earliest defect observed is an early block of ribonucleic acid synthesis during germination at 46 C. Upon return to 35 C there is a complete repair of the impaired function, even in the absence of protein synthesis. Protein synthesis inhibition during germination of the mutant spores at 46 C has the effect of increasing the amount of ribonucleic acid made. The temperature-sensitive mutation is located near *aroI*.

Bacterial spore germination and outgrowth may represent a simple model system to study the biochemical events that regulate the transition from one cell form to another (10, 12). During the germination process the dormant spore becomes an actively metabolizing cell. The process takes place through a sequence of time-ordered events. First ribonucleic acid (RNA) synthesis starts, followed by protein and, much later, deoxyribonucleic acid (DNA) synthesis (2, 13). RNA synthesis during the first stages of germination occurs in two phases: the first one is mainly due to the synthesis of stable RNA, whereas the second phase corresponds to the synthesis of mRNA (1). Protein synthesis is observed only after the beginning of the second phase. On the other hand, protein synthesis is required to observe the shift in RNA synthesis rate (1). An ordered sequence of protein synthesis during outgrowth as been recognized by Torriani and Levinthal (21) and by Steinberg and Halvorson (19, 20) in *Bacillus cereus* and by Kennet and Sueoka (11) in *Bacillus subtilis*.

The basic nature of the regulation of germination remains to be elucidated and an obvious approach is the study of mutants altered in the germination process. To date few studies have been reported on the genetics of spore germination (6, 8, 9, 23).

In the present paper we describe the isolation of a mutant of *B. subtilis* that is temperature sensitive (ts) in the germination process and shows an alteration in RNA synthesis during spore germination.

MATERIALS AND METHODS

Bacterial strains. The *B. subtilis* strains used in this work are listed in Table 1.

Media. Spizizen (18) minimal medium was used to prepare competent cells. Medium Y (24) was used for phage PBS-1 absorption. Penassay broth (antibiotic medium no. 3, Difco) and Tryptose blood agar base (Difco) were used in transduction experiments. The minimal agar for selection and reisolation of transformants and transductants was that described by Davis and Mingioli (5). PS broth (8) or nutrient agar (Biokar Froissy, Oise), containing 10^{-5} M $MnCl_2$, was used for sporulation. Nutrient broth (Difco) (NB) was used for germination. For RNA synthesis the minimal salts medium M9 (4) was used, after a threefold dilution with distilled water and supplemented with 0.2% Casamino Acids (Difco), 0.5% glucose, and 50 μg of the amino acids/ml required by the strain.

Chemicals. The following chemicals were used: Urografin (Schelabor, Milano); D,L-α,ε,diaminopimelic acid, muramic acid, glucosamine, cyclic 3',5'-adenosine monophosphate (cyclic AMP), cyclic 3',5'-guanosine monophosphate, N^6, O^2, dibutyryl cyclic AMP, egg white lysozyme, and puromycin, all from Sigma Corp. (St. Louis). Chloroamphenicol (CM) and rifampin were a gift of Lancini, Lepetit S.p.A., Milano.

Radioactive chemicals. [^{3}H]uridine with specific activity of 5 Ci/mmol and [U-^{14}C]-labeled protein hydrolysate with specific activity 58 mCi/matom of carbon were from the Radiochemical Centre, Amersham, U.K.

RNA synthesis. Heat-activated spores or vegetative cells were suspended, at an absorbancy at 560 nm (A_{560}) of 0.30, in M9 minimal salts medium containing 1 μCi of [^{3}H]uridine per ml. At intervals, 0.1-ml samples were precipitated with 10% trichloroacetic acid, containing 100 μg of uracil per ml, filtered through glass fiber filters (Whatman, GF/C, 2.5 cm), and washed with cold 5% trichloroacetic acid, followed by cold 0.2 N HCl and cold ethanol.

To measure the rate of RNA synthesis, 2-min pulses of [^{3}H]uridine were performed every 3 min. A sample of 0.2 ml of the culture was incubated with 5

TABLE 1. *Strains Used*

Strain	Genotype[a]	Phenotype[b]	Origin[c]
PB 1424	*hisB2 trpC2 met*		From SB 25 (J. Lederberg)
PB 2421	*hisB2 trpC2 met*	ts-Gsp 0-81; ts-Spo	Derivative of PB 1424
PB 2425	*hisB2 trpC2 met*	ts-Gsp 0-81	Spontaneous from PB 2421
PB 2426	*hisB2 trpC2*	ts-Gsp 0-81	PB 3373 $\xrightarrow{tf}$ PB2425
PB 2427	*hisB2 trpC2*	ts-Gsp 0-81	PB 2426 $\xrightarrow{tf}$ PB 1424
PB 3373	*pyrA26*		BC 34, J. C. Copeland
PB 1711	*aroI906*		WB 906, J. A. Hoch
PB 1714	*sacA321 narB1 mtlB1*		QB 819, J. A. Lepesant

[a] Symbols: *his, trp, met, pyr, cys, thr, aro*, requirement respectively for histidine, tryptophan, methionine, uracil, cysteine, threonine, tryptophan, phenylalanine, and tyrosine. *sac*, Inability to use sucrose as a carbon source; *nar*, no growth with nitrate as sole nitrogen source: *mtl*, inability to use mannitol as a carbon source.

[b] ts-Gsp, ts in spore germination. ts-Spo, ts in sporulation.

[c] An arrow indicates DNA transformation (tf) and points to the recipient strain.

μCi of [^{3}H]uridine per ml. The incorporation was stopped by adding trichloroacetic acid. The samples were then treated as for cumulative RNA synthesis.

Protein synthesis. Heat-activated spores were incubated at an A_{660} of 0.30 in NB, supplemented with 0.5% glucose and ^{14}C-labeled protein hydrolysate (2 μCi/ml). At intervals samples of 0.2 ml were precipitated with 10% trichloroacetic acid, containing 1% Casamino Acids. The samples were then treated as for RNA synthesis. The radioactivity on the filters was measured with a Packard liquid scintillation counter.

Preparation of the spores. Spores were prepared in nutrient agar and purified by centrifugation through a Urografin solution with a density of 1.370 g/cm^3 (17). The pellet was collected and after extensive washing with distilled water was used throughout the work to be described.

Enrichment and isolation of the ts mutant. Enrichment and isolation of thermosensitive mutant, growth curves, temperature-shift experiments, transformation, and transduction were performed as already described (8, 9). The enrichment of ts germination mutants is based on the different densities of the cells at various stages of the process. The density classes are separated by isopycnic centrifugation in Urografin.

Sporulation. The following procedure was used to determine the efficiency of sporulation. Overnight cultures in PS were incubated at 35 C. A sample of 4 ml was centrifuged and the cells were resuspended in 5 volumes of PS broth containing 10^{-5} M $MnCl_2$. The culture was divided into two portions: one incubated at 35 C and the other at 46 C, for 24 h. Samples were heated at 85 C for 15 min to kill vegetative cells and then seeded on nutrient agar plates.

RESULTS

Isolation of a ts germination mutant. A mutant of *B. subtilis* was isolated in which the outgrowth phase of spore germination was ts, after the procedure already described (9). The mutant was designated strain PB 2421. Mutant PB 2421 is also ts for sporulation. The two phenotypes, ts-Gsp and ts-Spo, are due to two different mutations, as shown from the fact that spontaneous ts$^+$-Spo revertants are still ts in the germination process. For this reason we transferred the original mutation giving the ts phenotype during germination of the spores (mutation that will be called O-81), into the parental strain. Spontaneous revertants of strain PB 2421 able to sporulate at 46 C were selected. The strain PB 2425 (*his trp met* ts-Gsp O-81) was then transformed to Met$^+$, using nonsaturating concentrations of DNA from strain PB 3373 (*pyrA*).

The ts-Gsp O-81 marker was then transferred into the parental strain by marker congression, selecting for Met$^+$ transformants.

One of the transformants, strain PB 2427, has been used for all subsequent work. The germination and outgrowth curves at 35 and 46 C in NB containing 0.5% glucose of strain PB 2427 are shown in Fig. 1. At the permissive temperature, initiation, germination, and outgrowth proceed as in the parental strain, albeit more slowly. At 46 C the initial decrease in optical density of the mutant spores is not followed by any further change for at least 300 min. At this temperature the parental strain behaves normally.

Microscopic examination of the mutant spores incubated at 46 C indicate that the initial stages of the outgrowth are not affected, since the spores are stainable and slightly enlarged. According to the suggestion of Young and Wilson (25) the mutant should be classified as ts-Gsp-III.

The mutant phenotype is expressed at 46 and at 44 C, whereas a temperature of 42 C is permissive for germination and outgrowth. When the spores are plated on nutrient agar, the efficiency of plating at 46 C is 50% of the value obtained at 35 C. Thus, in solid media, germination does eventually take place, though

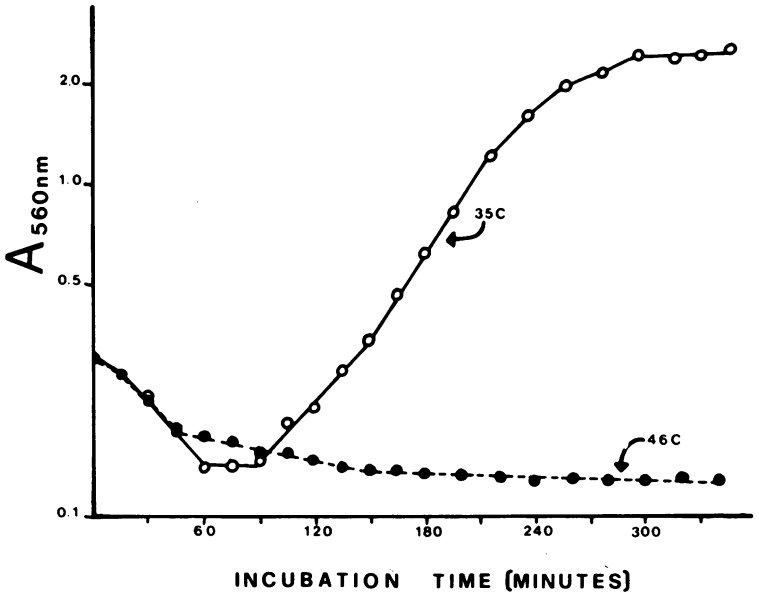

FIG. 1. *Germination and growth in NB of the spores of the mutant strain PB 2427. Spores in distilled water were heat activated at 75 C for 15 min before inoculum. The cultures were incubated at 35 C* (○) *and 46 C* (●).

delayed and only in a fraction of the cells. The colonies formed are smaller than the ones obtained at 35 C. In liquid cultures, no detectable increase in optical density is observed until 5 h of incubation at 46 C; overnight cultures at 46 C show high turbidity. The mutant therefore seems leaky or only quantitatively altered in the germination process.

O-81 mutation affects only germination. Mutant PB 2427 is not grossly affected in the growth of vegetative cells at 46 C, as shown in Fig. 2a. Both at 35 C and 46 C the mutant cells grow more slowly than the parental cells (Fig. 2b). We conclude that the main defect due to the mutation O-81 is a spore outgrowth defect, even though it also affects the vegetative growth. When seeded on agar plates, the vegetative cells give rise to colonies at 46 C with an efficiency of plating of 1. The colonies of the mutant, both at 35 and 46 C, are always of smaller size than the ones of the parental strain. The difference in size is particularly pronounced on minimal agar. To test for the capacity to sporulate at 46 C, vegetative cells were inoculated at A_{560} of about 0.5 in PS medium containing 10^{-5} M $MnCl_2$ and incubated at 35 and 46 C for 24 h. At 46 C the yield of spores was 12% that at 35 C. A similar result was obtained with the parental strain PB 1424.

Temperature-shift experiments. A series of temperature-shift experiments was performed to assess the time at which the ts compound becomes essential for germination. Spores of strain PB 2427 were inoculated in flasks containing 10 ml of NB, plus 0.5% of glucose, and incubation was started at 35 or 46 C. At time intervals, samples were shifted to 46 or 35 C, respectively. The optical density of the cultures was followed for 330 min after inoculating. The shift-down experiments (Fig. 3a) show that the mutant is primarily affected only in the process of spore germination: all the samples shifted from 46 to 35 C show a complete recovery, indicating that the thermolabile component can be reactivated at any time upon incubation at the permissive temperature. In the shift-up experiment (Fig. 3b) the sample shifted to 46 C at 40 min fails to germinate, whereas the samples shifted at 80 min and 150 min show an increase (albeit delayed) in optical density. The increase is delayed with regard to spores germinated at 35 C, possibly owing to the fact that the mutant spores do not show a synchronous germination and outgrowth. To test this possibility a sample was shifted to 46 C much later (265 min) after a 10-fold dilution of the culture. The curve (Fig. 3b) is exactly the same as the one obtained with vegetative cells at 46 C (Fig. 2a). The data indicate that the mutation affects an early stage of the germination process.

Phenotypic repair. As for many other ts mutants (8, 16) the spores of strain PB 2427 incubated in presence of 20% sucrose or 2% sodium chloride recover the ability to grow at

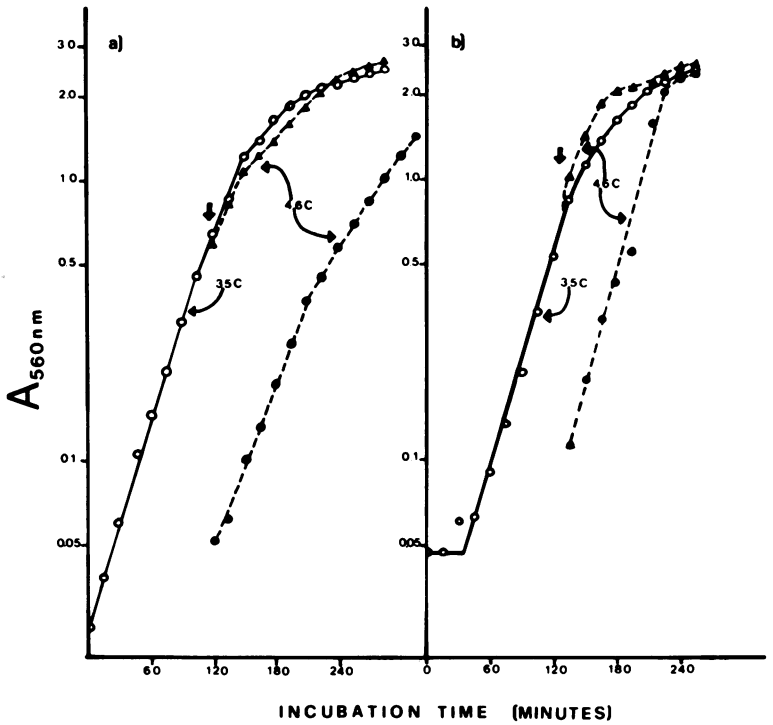

FIG. 2. *Growth curves of vegetative cells of the mutant strain PB 2427 (a) and of the parental strain PB 1424 (b). Cells in logarithmic phase were inoculated into NB. Symbols:* (○) *growth at 35 C,* (▲) *growth after shift to 46 C at the point indicated by the arrow,* (●) *growth at 46 C after 10-fold dilution.*

nonpermissive temperature. Cassier and Ryter (3) have described a mutant of *Clostridium perfringens*, the spores of which require lysozyme to germinate. Our mutant is not helped by the addition of lysozyme at concentrations varying from 0.5 to 10 μg/ml. The parental strain does not leak into the medium any factor that repairs the damage in the mutant; no repair is observed by inoculating the mutant spores into NB filtered after germination of the spores of the parental strain. Conversely, medium in which the mutant spores have been incubated, both at 35 and 46 C, does not inhibit growth of the parental spores. We tested several compounds, i.e., cyclic AMP, butyryl-cyclic AMP, 3',5'-guanosine monophosphate (5×10^{-3} M) and glucosamine, muramic acid, and diaminopimelic acid (10 μg/ml): none had any effect on the germination and outgrowth of the mutant spores at 46 C.

Synthesis of macromolecules. RNA synthesis during germination and growth at 35 and 46 C was measured by incorporation into acid-precipitable material of [^{3}H]uridine. At 35 C (Fig. 4b) the cumulative synthesis follows very closely the incorporation observed with the spores of the parental strain (not shown). At about 35 min a sharp increase in the incorporation can be seen. At 46 C the mutant spores show a very much reduced incorporation of [^{3}H]uridine. The slope of the incorporation curve is similar to the initial slope at 35 C, but it is maintained until 50 min when a plateau is reached. The vegetative cells of the mutant incorporate [^{3}H]uridine to the same level at 35 and 46 C (Fig. 4a), confirming that the defect is only relevant during spore germination and outgrowth.

Protein synthesis was followed during outgrowth, with a mixture of ^{14}C-labeled amino acids. At 35 C (Fig. 5) the incorporation of ^{14}C-labeled amino acids into trichloroacetic acid-precipitable material starts at about 30 min and proceeds exponentially. In agreement with the result on the synthesis of unstable RNA at 46 C (see below), some protein synthesis occurs at the nonpermissive temperature.

RNA synthesis after temperature shift. From the results reported above it seems that the primary defect due to the O-81 mutation is an early block of RNA synthesis during germination. The temperature-shift experiments pre-

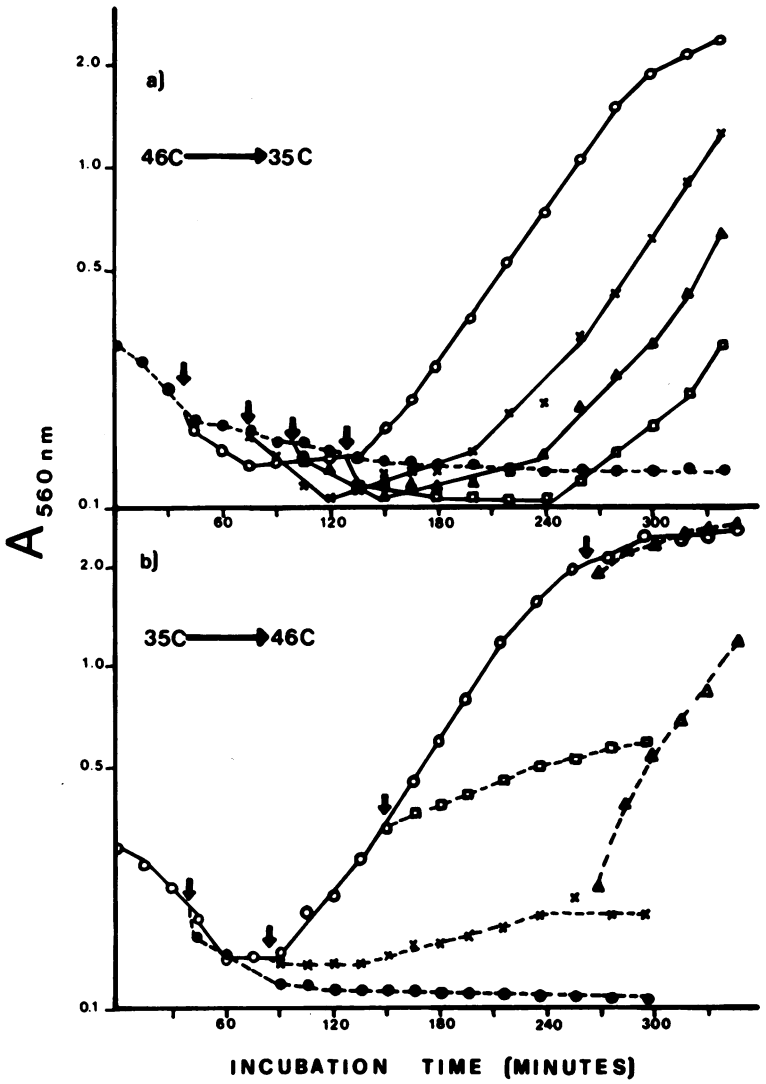

FIG. 3. *Temperature shift of germinating spores of strain PB 2427. (a) Heat-activated spores were inoculated in NB and incubated at 46 C. At time intervals samples were shifted to 35 C. The arrows indicate the time of shifting. Symbols: (●) control, (○) shifted to 35 C at 40 min, (×) shifted at 75 min, (▲) shifted at 100 min, (□) shifted at 130 min. (b) The control culture was incubated at 35 C and samples were shifted to 46 C. Symbols: (○) control, (●) shifted to 46 C at 40 min, (×) shifted at 80 min, (□) shifted at 150 min, (▲) shifted at 265 min, (△) shifted at 265 min after 10-fold dilution.*

viously described indicate an almost complete recovery of the germinating spores, when the culture was shifted from 46 to 35 C. Does the RNA synthesis also recover in these conditions? After 60 min at 46 C the culture was brought to 35 C; as a result of the shift to permissive temperature we observe a pronounced increase in [^{3}H]uridine incorporation (Fig. 6). The initial increase is slow but the rate becomes constant soon and comparable to the one observe in cultures incubated at 35 C from the time of the inoculum. To ascertain if protein synthesis is required for the recovery, a parallel culture was shifted to 35 C and supplemented with 50 μg of CM per ml, a concentration that is completely inhibitory of protein synthesis in the present conditions. The increase in RNA synthesis upon shift to 35 C is not affected by the presence of CM (Fig. 6). Thus, no protein synthesis is required for the recovery of the biosynthetic

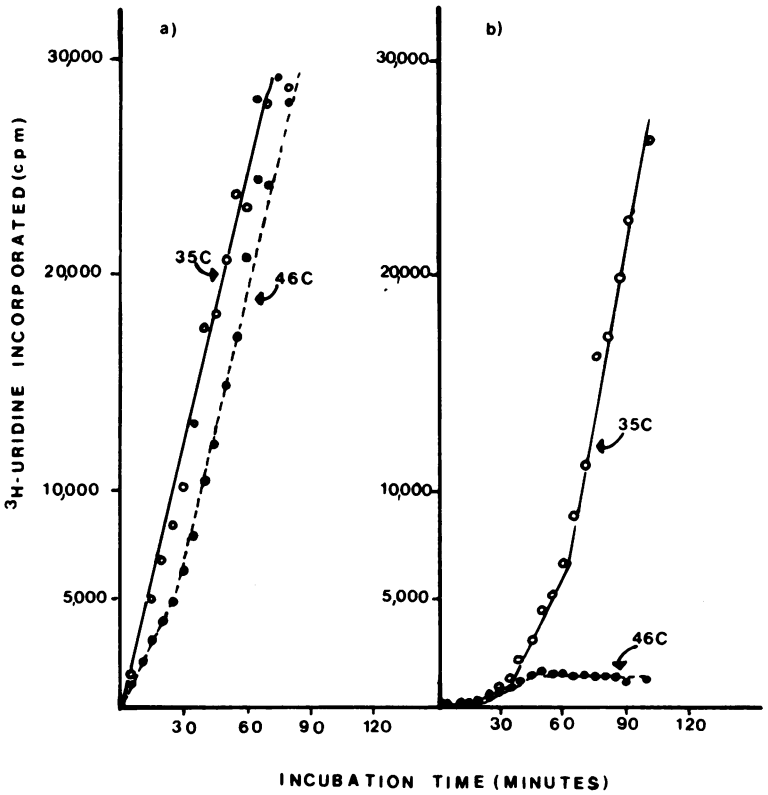

FIG. 4. *RNA synthesis in the mutant strain PB 2427. (a) Vegetative growth, (b) spore germination and outgrowth. Log cells or spores of strain PB 2427 were suspended, at an A_{660} of 0.30, into M9 medium containing 1 μCi of [^{3}H]uridine per ml. At the indicated intervals 0.1-ml samples were precipitated with 10% trichloroacetic acid and processed as described in Materials and Methods. Symbols: (○) culture incubated at 35 C, (●) culture incubated at 46 C.*

abilities of the germinating spores; this confirms that the thermolabile component is reactivated when brought to a permissive temperature.

RNA synthesis stops immediately upon shifting a sample from 35 to 46 C after 50 min of incubation. An increase in incorporation of [^{3}H]uridine is observed only 30 min later. The increase is linear but slower than the one at 35 C, and an extrapolation of the curve to zero incorporation intersects the abscissa at precisely the time of the temperature shift. This increase is probably due to some cells that, owing to the asynchronous germination, at the time of the shift were in a more advanced stage, having thus overcome the ts period. In the sample shifted to 46 C at 70 min, RNA synthesis proceeds as in the control, thus confirming that the mutant is blocked in RNA synthesis only during an early stage of the germination process.

Effect of protein synthesis inhibition on RNA synthesis. Armstrong and Sueoka (1) have shown that the addition of CM to germinating spores has the effect of abolishing the transition from the first phase of RNA synthesis to the second phase, so that RNA is synthesized at a lower level for about 40 min after addition of the drug. With the mutant strain PB 2427, we obtain the same kind of response to CM when the spores are incubated at 35 C (Fig. 7). At a nonpermissive temperature, a completely different picture is found (Fig. 8). In the presence of the drug there is a threefold increase in the amount of uridine incorporated. In both control and CM-containing cultures, incorporation into RNA ceases at around 60 min. The effect is not specific for CM since the same result has been obtained with 100 μg of puromycin/ml and with 200 μg of streptomycin/ml. Thus, the inhibition of protein synthesis during mutant spore development allows more RNA to be synthesized

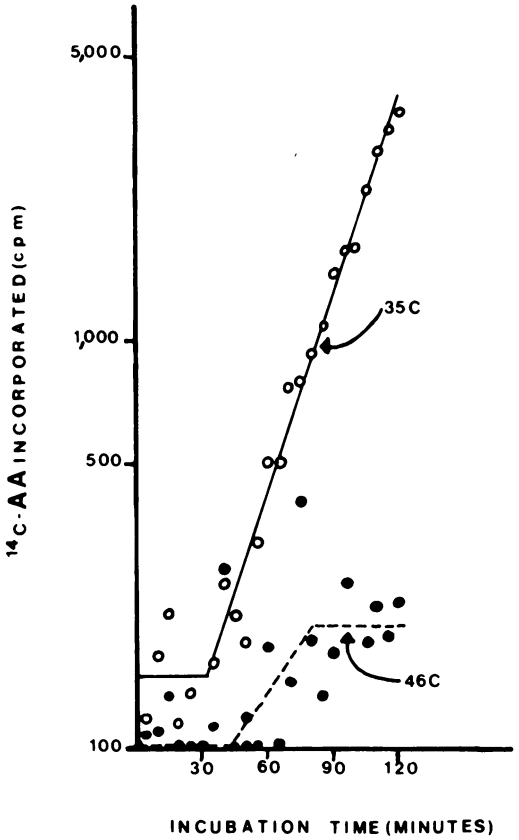

FIG. 5. *Protein synthesis during outgrowth of mutant strain PB 2427. Spores of the mutant were inoculated at an initial A_{660} of 0.30 into NB plus 0.5% glucose. The medium contained 2 μCi of ^{14}C-labeled protein hydrolysate per ml. At intervals 0.2-ml samples were precipitated with 10% trichloroacetic acid and processed as described in Materials and Methods. (O) Culture incubated at 35 C, (●) culture incubated at 46 C.*

than in the presence of protein synthesis. A fraction of the RNA made is unstable, as indicated by the effect of adding 10 μg of rifampin per ml, after further incorporation into RNA has ceased (Fig. 8). The same enhancement in RNA synthesis is obtained when the CM is added at 20 min. Upon adding the drug at later times (35 and 50 min), the stimulation of uridine incorporation becomes less and less pronounced and is almost absent in samples exposed to CM at 60 min. An interpretation of these results is that the protein(s) that blocks RNA synthesis at 46 C is probably synthesized between 20 and 60 min from the beginning of germination. The effect observed upon adding the CM at 60 min could represent the turnover of the protein(s). This is in agreement with the data of the temperature shift from 35 to 46 C, which indicate that the ts period lies between 0 and 80 min.

Rate of RNA synthesis. The rate of RNA synthesis in the mutant strain PB 2427 during germination at 35 and 46 C was measured by 2-min pulses of [^{3}H]uridine. At 35 C (Fig. 9a) the rate of RNA synthesis increases exponentially from 0 to 12 min, it remains constant from 12 to 32 min, and then increases again exponentially but with a rate lower than the initial one. In the presence of CM the rate of synthesis is the same as the one of the control for 24 min when an exponential decrease is observed. The rate does not reach zero, but it levels at a value approximately one-third of the previous one. Thus, protein synthesis is required to obtain the second rate of synthesis of RNA. At 46 C RNA synthesis starts with the same rate as the one at permissive temperature. Then it decreases exponentially to zero value. In the presence of CM at 46 C the constant rate is maintained up to 50 min, then it decreases like at 35 C with CM. The data on the rate of RNA synthesis fit very well with the above reported results on the cumulative synthesis. They also show that the initial incorporation of [^{3}H]uridine is the same at 35 and 46 C.

Map location of the O-81 mutation. The O-81 mutation has been mapped on the chromosome of *B. subtilis* by phage PBS-1-mediated transduction.

Recipient strains were treated with a lysate from the mutant PB 2427 and selection was made to prototrophy for several nutritional markers. The co-transduction of the O-81 mutation was scored by replica plating after the procedure described (9).

Since the strains bearing the O-81 mutation grow slowly, the plates selective for auxotrophic transductants were incubated at 35 C for 3 days to avoid selection against clones with the O-81 mutation.

The O-81 mutation is linked, by PBS-1 transduction, to *aroI* and *narB* (Table 2). No linkage has been found with the markers *cysA14* and *purB6*. The relevant region of the genetic map of *B. subtilis* is reported in Fig. 10.

DISCUSSION

We have described the isolation and the properties of a ts mutant of *B. subtilis* whose spores are unable to germinate and outgrow in rich media at 46 C. The earliest recognizable alteration at nonpermissive temperature is a

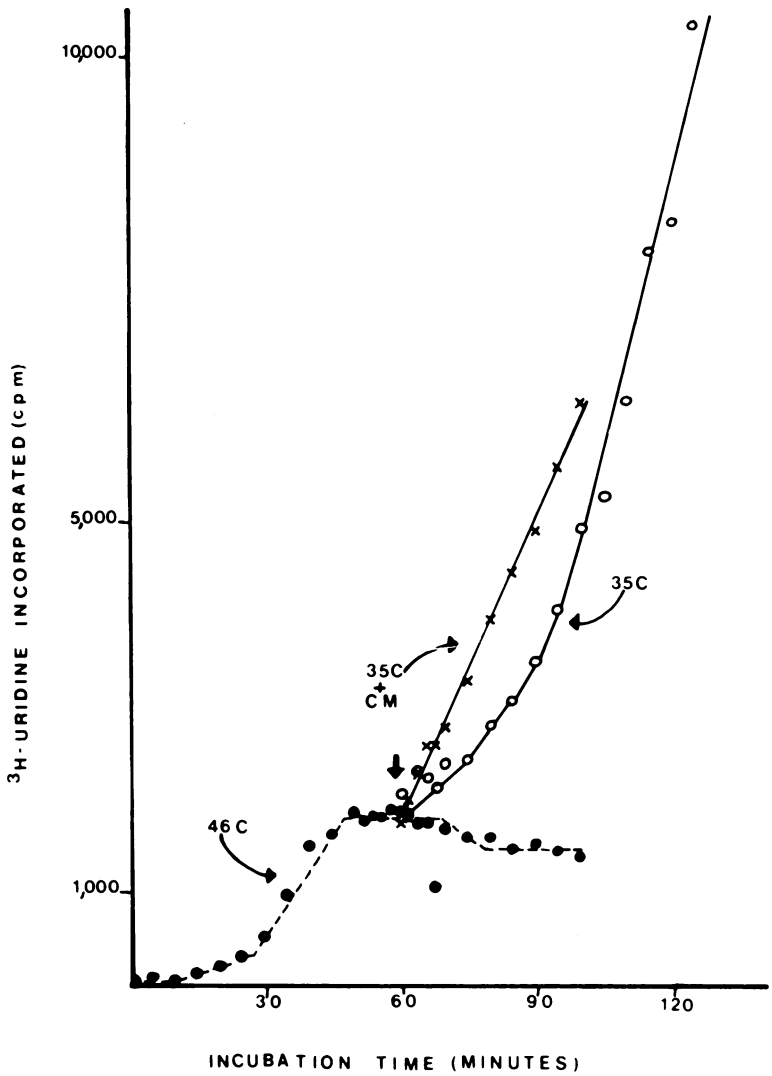

FIG. 6. *RNA synthesis after shifting the germinating spores of strain PB 2427 from 46 to 35 C. A control culture (●) was inoculated with spores of PB 2427, incubated at 46 C and RNA synthesis followed. At 59 min a sample was withdrawn and was divided into two aliquots. One (○) was shifted to 35 C; to the other (×) 50 μg of CM per ml were added and then shifted to 35 C. Other details as in the legend of Fig. 4.*

TABLE 2. *Two- and three-factor transductions involving the ts O-81 marker*[a]

Cross	Donor	Recipient	Classes (phenotypes)	No.	Map distances (min)
1	ts-Gsp 0-81 (PB 2427)	*aroI906* (PB 1711)	Aro$^+$ ts-Gsp	37	
			Aro$^+$ ts$^+$-Gsp	95	*aroI* – O-81 = 38
2	ts-Gsp 0-81 (PB 2427)	*sacA321 narB1 mtlB1* (PB 1714)	Nar$^+$ ts-Gsp Mtl$^+$	10	
			Nar$^+$ ts-Gsp Mtl$^-$	35	
			Nar$^+$ ts$^+$-Gsp Mtl$^+$	37	*narB1* – *mtlB1* = 61
			Nar$^+$ ts$^+$-Gsp Mtl$^-$	38	*narB1* – O-81 = 63

[a] In both crosses the donor was PB 2427, ts for germination (ts-Gsp). In cross 1 selection was for Aro$^+$. Individual colonies were isolated and scored for ts-Gsp. In cross 2 selection was for Nar$^+$. Individual colonies were isolated and scored for *mtl* and ts-Gsp.

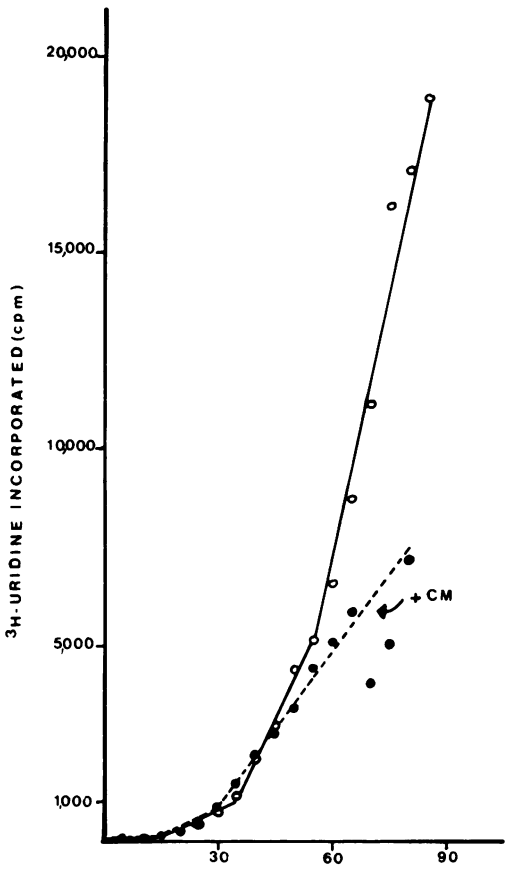

FIG. 7. *Effect of CM on RNA synthesis during germination and outgrowth at 35 C of the mutant strain PB 2427. Spores of the mutant were inoculated into M9 medium containing 1 μCi of [^{3}H]uridine per ml. Symbols: (○) control culture, (●) culture containing 50 μg of CM per ml from zero time. Other details as in the legend of Fig. 4.*

block in RNA synthesis. The mutant is altered only in the germination process: vegetative cells do grow and divide normally at 35 and 46 C although with a longer generation time, at both temperatures, compared to the parental strain.

The thermolabile component present in the mutant can be reactivated after return of the culture to a permissive temperature. Consequently there is a recovery of RNA synthesis at 35 C, even under conditions of inhibition of protein synthesis.

Inhibition of protein synthesis at early stages of the germination has the effect of increasing the amount of transcription observed at 46 C.

This increase cannot be due to a stringency response since streptomycin has the same effect as CM and puromycin (14). Furthermore, in the stringent response the increase in RNA synthesis is observed by also adding the drug after the plateau has been reached. In our case such a late addition has no stimulatory effect.

We suggest two alternative hypotheses to explain our results. The first is based on negative control of protein synthesis during spore germination. The thermolabile component, synthesized between 20 and 60 min, is a repressor-like protein, specific for the RNA made during spore outgrowth. Under permissive conditions

FIG. 8. *Effect of CM on RNA synthesis during germination and outgrowth at 46 C of the mutant strain PB 2427. Spores of the mutant were inoculated into M9 medium containing 1 μCi of [^{3}H]uridine per ml. Symbols: (●) control culture, (○) culture containing 50 μg of CM per ml from zero time. At 70 min for the control (▲) and at 100 min for the CM-treated culture (×) an aliquot was taken and further incubated in the presence of 10 μg of rifampicin/ml. Other details as in the legend of Fig. 4.*

FIG. 9. *Rate of RNA synthesis by 2-min pulses of [^{3}H]uridine in the mutant strain PB 2427. (a) 35 C, (b) 46 C. Spores of strain PB 2427 were inoculated at an A_{660} of 0.40 into M9 medium. At 3-min intervals, 0.2-ml samples were pulsed with 5 μCi of [^{3}H]uridine per ml. After 2 min of incubation the pulses were stopped by precipitation with 10% trichloroacetic acid and the samples were processed as described in Materials and Methods. Symbols: (○) control culture, (●) culture containing 50 μg of CM per ml.*

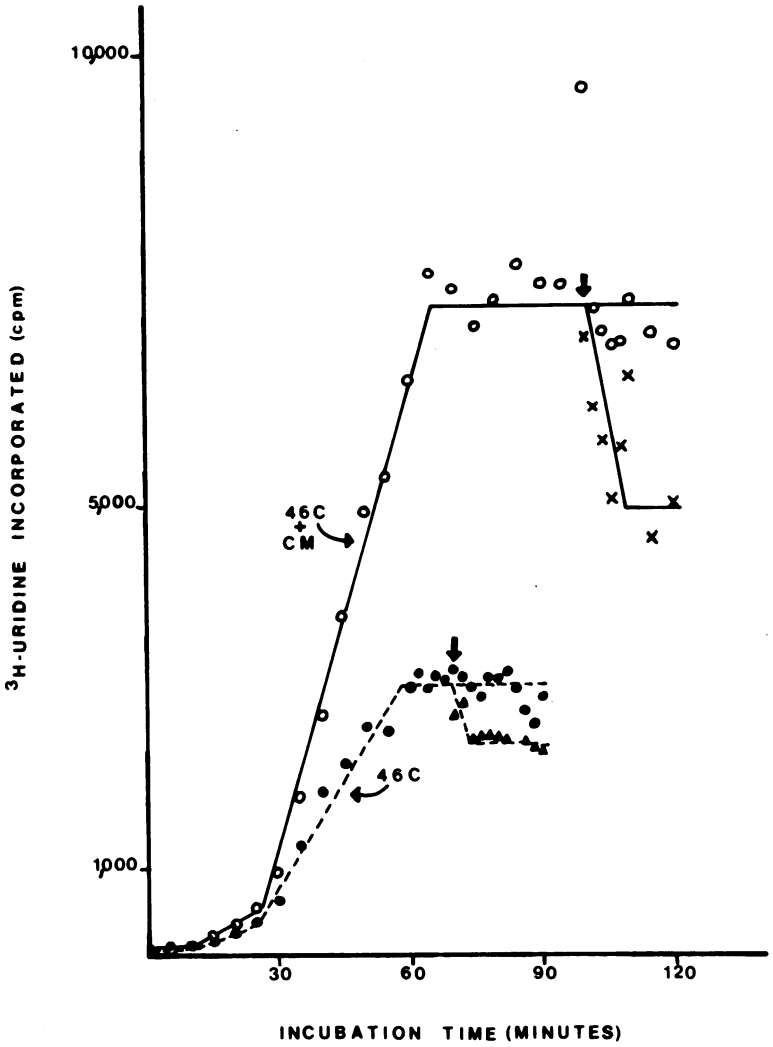
10,000
5,000
1,000
^{3}H-URIDINE INCORPORATED (cpm)
46C
+
CM
46C
30
60
90
120
INCUBATION TIME (MINUTES)

FIG. 8.

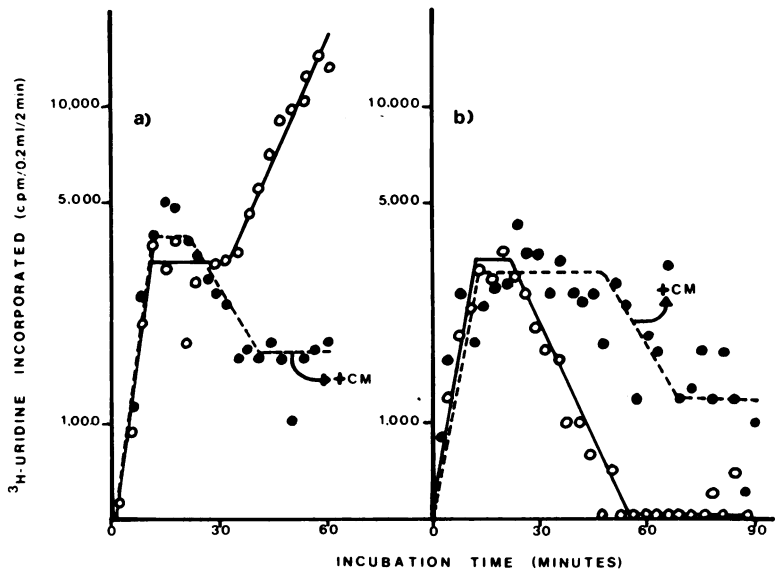
^{3}H-URIDINE INCORPORATED (cpm/0.2ml/2 min)
10,000
5,000
1,000
a)
+CM
10,000
5,000
1,000
b)
+CM
0
30
60
0
30
60
90
INCUBATION TIME (MINUTES)

FIG. 9.

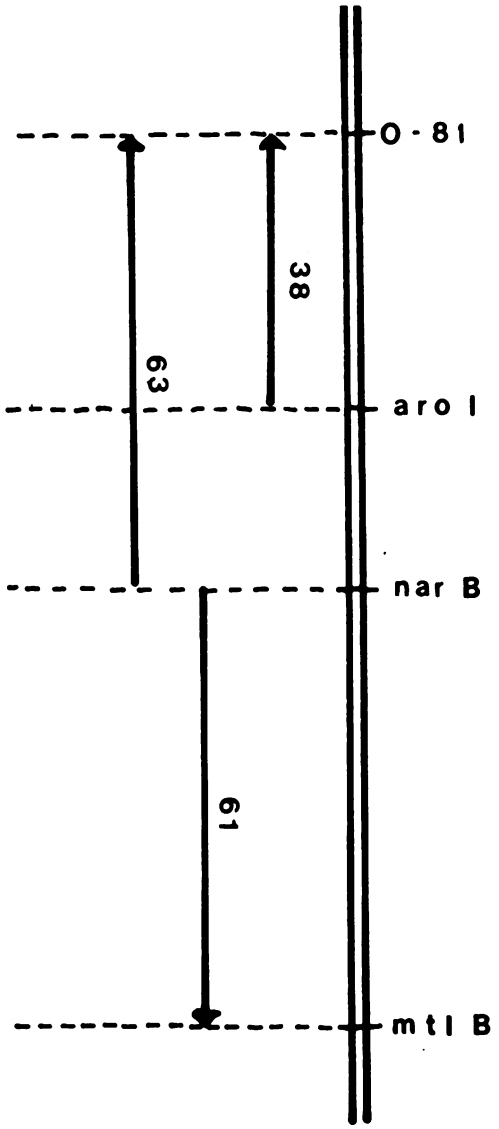

FIG. 10. *PBS-1 transduction map of the mutation O-81. The arrows point to the unselected marker. Distances are expressed as 100 – co-transduction frequency.*

the repressor binds reversibly to one or more operators. At 46 C the repressor is still capable of binding to the operator(s), but it cannot be detached. Inhibition of the repressor synthesis has the effect of increasing the amount of transcription. The second hypothesis takes into consideration a positive type of regulation mediated by the RNA polymerase (22). With this model the thermolabile component is not necessarily synthesized during the ts period. We assume that one of the RNA polymerase subunits is altered in the mutant. Owing to the map location of the mutation, the altered RNA polymerase subunit cannot be β, since the *rif* gene maps near *cysA*, and probably not β', if the location of β and β' genes in *B. subtilis* is similar to that observed in *Escherichia coli* (7, 15).

The altered enzyme can perform all vegetative and sporulation functions. During germination the enzyme present in the spores recognizes a set of promoters. The RNA made is mostly stable but a fraction is messenger RNA. One of the polypeptides made is a factor that must interact with the RNA polymerase so that the latter can recognize new promoters. The presumptive factor is made in the mutant at 35 and at 46 C and interacts with the RNA polymerase. At 46 C the altered enzyme forms with this factor a complex that is unable to transcribe new regions of DNA. RNA synthesis is not completely abolished, although no further increase in radioactivity of the RNA is observed during this period; in fact a plateau is reached in cumulative incorporation experiments. Although no net synthesis is measured, unstable RNA is still made. Stable RNA, however, is no longer made. If the synthesis of the factor is inhibited by CM, the RNA polymerase continues to transcribe the regions of DNA with early promoters that do not require the presence of the factor. Both models can explain our data.

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