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NOTES

Crystal Formation by a Ribonucleic Acid Polymerase Mutant of *Bacillus subtilis*

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A crystalline inclusion has been observed in a ribonucleic acid polymerase mutant of *Bacillus subtilis* which is conditionally temperature sensitive only during sporulation. The crystal is formed at the permissive temperature in 1 to 2% of the sporulating (stage III-IV) cells; about 85% of the cells sporulate normally, while the cells with crystals do not sporulate. The wild type does not form crystals at either the permissive (30 C) or the nonpermissive (47 C) temperatures. The crystal may result from altered transcription during sporulation at 30 C.

A conditional temperature-sensitive ribonucleic acid (RNA) polymerase mutant of *Bacillus subtilis* has been isolated recently by Leighton (6). This mutant can grow and sporulate at 30 C, and grow but not sporulate at 47 C. During our ultrastructural analysis of this mutant to determine its sporulation pattern at 30 C and the stage at which sporulation was affected at 47 C (Santo et al., submitted for publication), a crystalline inclusion was observed in a few of these mutant cells sporulating at 30 C. The parasporal crystalline body in *B. thuringiensis* has been thoroughly documented (2, 3, 8) and shown to consist of protein (2), This note reports our preliminary observations on a similar structure present in this *B. subtilis* mutant. We have reported previously on morphologically altered spores produced by RNA polymerase mutants of *B. subtilis* (1, 5).

B. subtilis W168 and W168 *ts*-14 were grown at 30 C in 2 × SG medium as reported (Santo et al., submitted for publication, 1973). Cells were fixed by the Kellenberger technique (4), dehydrated through an ethanol series, and embedded in Epon 812 (7). Ultrathin sections were cut on the Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and Reynolds lead citrate, and examined with an AEI 6B electron microscope.

The inclusion appears as a dark body under the light microscope. It is similar in size to a spore and occurred in approximately 1 to 2% of the sporulating cells at 30 C. No inclusions were observed in cells grown at 47 C; these cells stopped sporulating at stage II and lysed later in the stationary phase.

Figures 2 and 3 are longitudinal sections of the crystal from an 11.5-h culture (Fig. 1, arrow

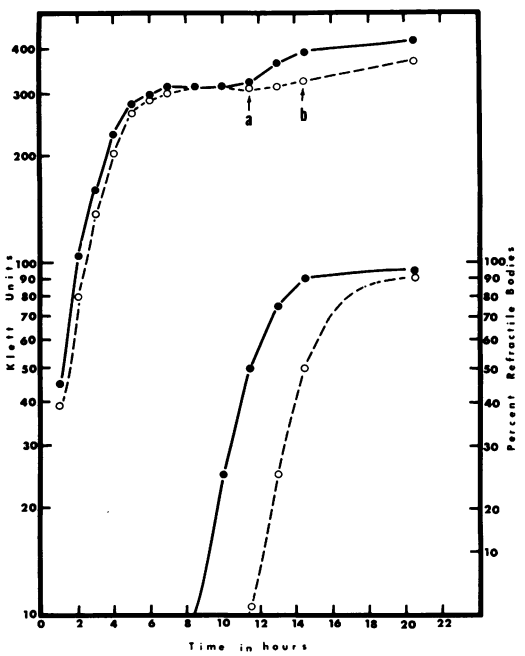


FIG. 1. *Growth and sporulation curve of Bacillus subtilis W168 and W168 ts-14 at 30 C. The closed circles are W168 and open circles are W168 ts-14. The arrows indicate the sample times of the electron micrographs shown in this paper.*

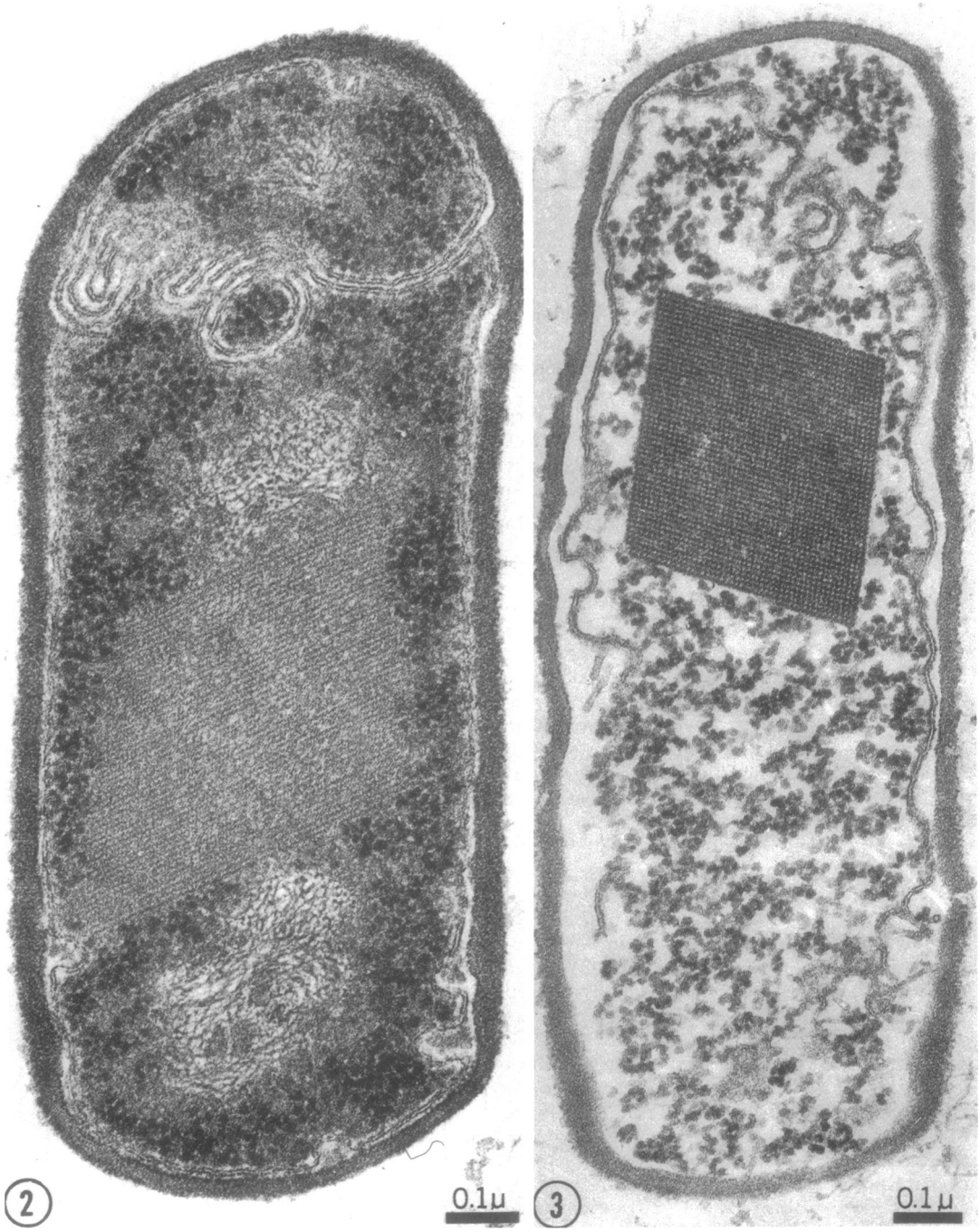
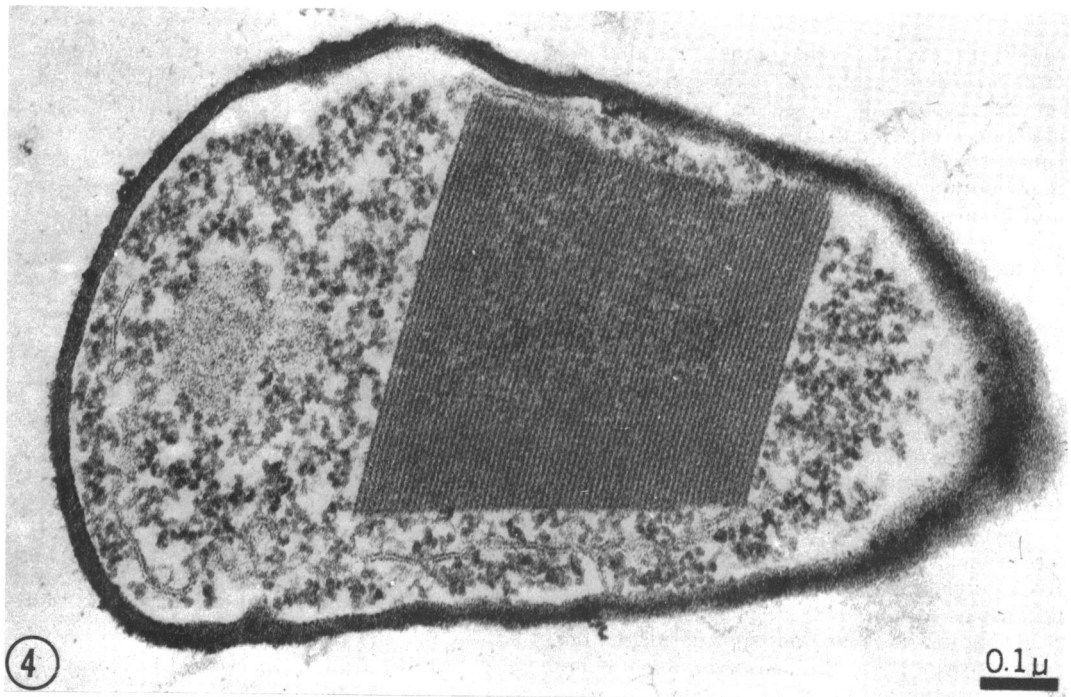


FIG. 2. *Crystalline inclusion in a ts-14 cell (Fig. 1, arrow a) showing the parallel repeating pattern.*

FIG. 3. *A lysing ts-14 cell showing a prominent crystalline inclusion, ribosomes, and membrane remnants. This inclusion is cut in a different plane from Fig. 2 and displays a cross-striation pattern. The inclusion measures approximately 0.41 by 0.37 μm.*



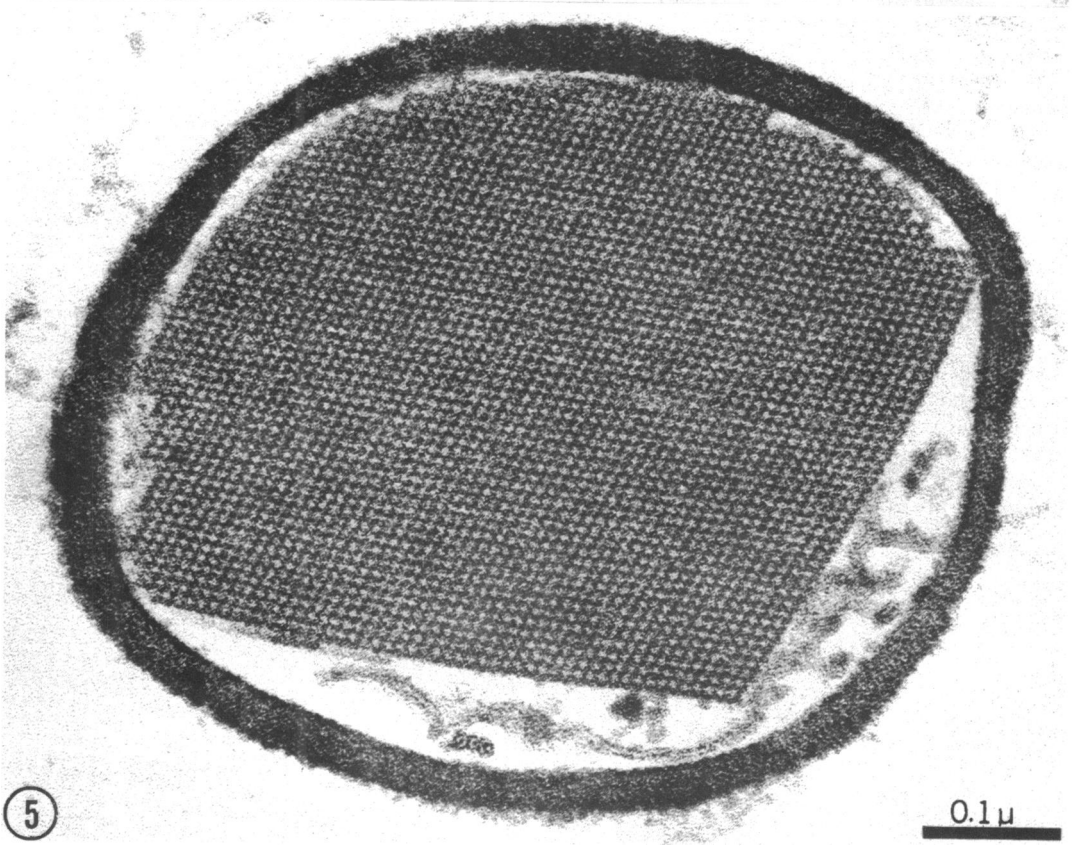


FIG. 4. *Oblique section of a lysing ts-14 cell showing the parallel pattern of the inclusion. The cell is from a 14.5-h culture (Fig. 1, arrow b).*

FIG. 5. *Cross-section of a 14.5-h culture ts-14 cell (Fig. 1, arrow b) where the inclusion extends the width of the cell. Note the cross striation pattern of the inclusion.*

a) cut in two different planes. Parallel and cross-striation patterns were observed depending on the plane of the section. Figures 4 and 5 are from a 14.5 h culture (Fig. 1, arrow *b*). The center-to-center distance of the parallel repeating subunits in the crystalline structure (Fig. 2 and 4) measured approximately 8.4 mm, and the cross-striation units (Fig. 3 and 5) averaged 8.5 by 8.5 nm.

Approximately 1% of the cell profiles in section contained crystalline inclusions at 30 C and less than 1% at 35 C. No inclusions were observed at the nonpermissive temperature in ts-14 cells nor in the wild-type W168 cells at any temperature. The inclusions occurred only in cells with no spores, but we have observed the body in some cells which developed up to stages II to III (forespore stage) in sporulation.

Previous studies (6; Santo et al., submitted for publication, 1973) with this single-point RNA polymerase mutant indicated that sporulation was inhibited at stage II at 47 C. This study shows, however, that sporulation is abortive in about 1% of the cells even at 30 C and that a crystalline inclusion is present in these abortive cells. Since these crystals are only observed in the mutant cells and not in the wild type, it is possible that the mutant RNA polymerase does not function with the usual fidelity even at 30 C, resulting in the over-production of a protein which aggregates into a crystal in the absence of the normal forespore body. Further evidence is required to show a direct relationship between the mutation in the RNA polymerase cistron and crystal formation. Smirnoff (9) also has reported that crystal formation in *B. thuringiensis* occurs at low growth temperatures in the absence of spore formation, suggesting that an alteration of metabolism can also affect normal sporulation. We are currently isolating and characterizing the crystals to determine whether they have any relationship to spore coat proteins since it was proposed previously that the crystal in *B. thuringiensis* may be related to a component of the spore coat (10). If the crystal is related to the spore coat protein, it will indicate that a spore-specific gene(s) can be transcribed and translated in the absence of complete sporulation.

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