EVIDENCE FOR THE SYNTHESIS OF STABLE INFORMATIONAL RNA REQUIRED FOR BACTERIAL SPORE FORMATION

Myrtha Rosas del Valle* and Arthur I. Aronson

Department of Biological Sciences

Purdue University

Lafayette, Indiana

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The process of spore formation in bacterial cells provides a useful experimental system for examining the changes in protein and nucleic acid synthesis required for the formation of a unique biological structure. A uniform population in terms of morphological alterations can be obtained by starting with a washed spore inoculum of Bacillus cereus T (about 10^7/ml). Growth is exponential for about 4 hours after an initial 5 hour lag (see Figure 1). The first morphological changes leading to sporulation can be detected at about 12 hours in virtually all of the cells although more subtle changes have been noted at earlier times (Young and Fitz-James, 1959). The first spore structures per se cannot be found until about 19 hours and free spores are formed from 90-95% of the cells at 24 hours.

The important biochemical events must thus take place some time before 12 hours. Since these changes precede any readily detectable morphological alterations, base analogues and other inhibitors were employed to determine the critical period when the synthesis of the informational RNA required for spore protein formation takes place. As seen in Table 1, the addition of 8-azaguanine or 5-fluorouracil before 8 hours completely inhibited spore formation. The analogues could be acting indirectly at these early times

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since there were marked alterations in the growth of the cultures. Between 8 and 9 hours, when growth has slowed down, a more direct effect on a specific RNA fraction probably accounts for the inhibitory action. When added at 12 hours, however, there was no inhibition. If we assume that the capacity of these analogues to be incorporated into RNA is the same at all times, then these results imply that the critical period for RNA synthesis is between 6 and 9 hours and that any synthesis after 9 hours is not relevant to the formation of spore proteins. While the incorporation of 8-azaguanine has been shown to occur as late as 10 hours (Young and Fitz-James, 1959) possible quantitative alterations as well as differences in the position of the analogues within RNA molecules could explain the lack of inhibition. These possibilities must be seriously considered since after 9 hours, when growth stops, there is extensive RNA turnover and even a net loss at later periods (see Figure 1).

TABLE 1

EFFECT OF RNA ANALOGUES AND INHIBITORS ON SPORE FORMATION

Compound	Conc. (μg/ml)	Results as % sporulation* Time of addition+ 6	8	8½	9	12
8-azaguanine	100	0	13	38	49	94
5-fluorouracil	50	0	10	33	33	90
Actinomycin D	10	0	5	10	47	93
Chloramphenicol	50	0	-	-	0	10

+ Time in hours after initial inoculation with spores of Bacillus cereus T.

* Determined by direct microscopic counts and by plating after heating at 24 or 30 hours (Gollakota and Halvorsan, 1960). Control values 90-95% free spores at 24 hours.

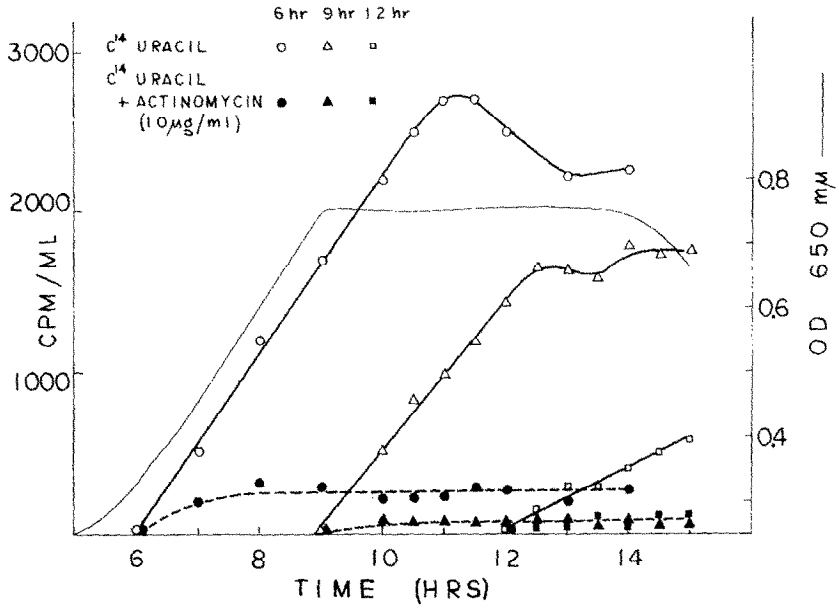

Figure 1 - Incorporation of 2-C^{14} uracil into TCA insoluble fraction in the presence (10 μg/ml) and absence of actinomycin D. One ml samples pipetted into cold 5% TCA and then filtered and washed on membrane filters.

The analogue results were confirmed, however, by employing actinomycin D (gift of Dr. Karl Pfister, Merck, Sharp and Dohme) a potent inhibitor of RNA synthesis (Kirk, 1960, Hurwitz, et al., 1962). As shown in Table 1, this antibiotic also inhibited spore formation at early times but had no effect when added at 12 hours. When added at 9 hours, there was usually a reduction in the number of sporulating cells to 50-60% and these cells did not produce free spores until 30 hours. If the antibiotic is added shortly after 9 hours (Table 2) there is no inhibition of spore formation. The data in Table 2 also show that there is a relatively abrupt change in the sensitivity of the cells to actinomycin D. There thus appears to be a very short but critical period of RNA metabolism at about 9 hours.

As summarized in Figure 1, actinomycin D effectively inhibits C^{14} uracil incorporation when added at any time period. The only detectable incorporation appears to be a small initial burst which is probably due

to residual RNA synthesis before the antibiotic becomes effective. These results thus confirm the observations with analogues.

TABLE 2

THE EFFECT OF THE TIME OF ADDITION OF ACTINOMYCIN D ON SPORE FORMATION

Time of addition[+] (10 μg/ml)	% sporulation[*]
8 3/4	17
9	47
9 1/4	60
9 3/4	89
10 1/4	91
10 1/2	93

[+] See footnote to Table 1.

[*] See footnote to Table 1. The spores were very small, but were refractile and heat resistant.

Also included in Table 1 are some results with chloramphenicol. This antibiotic, an effective inhibitor of protein synthesis, prevents spore formation when added at any time. At later periods, there is a small percentage of sporulating cells probably due to some asynchrony and to synthesis of spore protein prior to the addition of chloramphenicol. The synthesis of protein required for spore formation thus takes place continuously, probably starting at about 9 hours.

These results suggest that the informational RNA required for spore protein synthesis is formed for a short time at about 9 hours when active growth has stopped. This RNA, presumably associated with or as an integral part of ribosomes, can apparently function over an extended period in the absence of any further RNA metabolism. The possibility that the synthesis

of stable informational RNA is a general phenomenon rather than being confined to cells in a terminal pattern of development (as those committed to sporulation) is under investigation.

References

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