

GENETIC CONTROL OF RNA TURNOVER IN SPORULATION MUTANTS  
OF BACILLUS SUBTILIS

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In the preceding paper (Balassa, 1964), it was shown that RNA turnover during sporulation is controlled by the supply of amino acids. It was proposed that this control of RNA turnover is exerted by the formation (or activation) of an intracellular protease during sporulation. A number of asporogenous and oligosporogenous mutants, blocked at various steps of sporulation, have recently been studied in this laboratory (Schaeffer et al., 1963). In the course of studying the behaviour of these mutants with respect to RNA turnover, we have determined the degree of stimulation of uracil uptake by chloramphenicol, added during sporulation, at  $t_2$  and  $t_5$  (2 and 5 hours after growth is stopped). Mutants are considered to have the wild type behaviour ( $TO^+$ ) if they respond similarly to chloramphenicol as to a mixture of chloramphenicol plus the twenty amino acids. Mutants showing no, or only a weak, stimulation by chloramphenicol alone are classed as reduced turnover ( $TO^-$ ) mutants. Control experiments showed that all the  $TO^+$  and  $TO^-$  mutants are stimulated to the same extent of uracil incorporation by the simultaneous addition of both chloramphenicol and the amino acid mixture. The  $TO$  character of the various mutants is determined, in experiments similar to that described in the preceding paper, from the initial rates of  $^{14}C$ -uracil incorporation. In Table I the behaviour of the asporogenous and oligosporogenous mutants used in the study is summarized. It appears that all the mutants

Table I  
Behaviour of sporulation mutants

Mutant (1)	Sp <sup>+</sup>	Sp <sup>-</sup> 1	Sp <sup>-</sup> 3	Sp <sup>-</sup> 5	Osp <sub>α5</sub>	Osp <sub>A</sub>	Osp <sub>N4</sub>	Osp <sub>N1</sub>	Osp <sub>C</sub>	Osp <sub>11</sub>	Sp <sup>-</sup> 12UV	Sp <sup>-</sup> 68	Osp <sub>4UV</sub>	Sp <sup>-</sup> 94	Osp <sub>B</sub>
Morphological block at step (1)	-	0 or I	0	0	0	0	0	0	I	II	II	III	III	III	IV
Antibiotic production (1)	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Turnover behaviour	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+

Sp<sup>+</sup> - wild type ; Sp<sup>-</sup> - asporogenous , Osp - oligosporogenous strains .

(1) See Schaeffer et al. (1963).

(2) The oligosporogenous mutant α5 shows an exceptional behaviour : this mutant, blocked at step 0, seems TO<sup>-</sup> in the beginning of sporulation but changes to TO<sup>+</sup> after t<sub>3</sub>. This behaviour might reflect an abnormal regulatory mechanism in this mutant.

blocked at the late steps (II-V) are  $TO^+$ . Since a mutant, blocked at step II, before  $t_2$ , conserves the  $TO^+$  character up to  $t_5$ , the actual sporulation process is not necessary for stimulation of uracil uptake by chloramphenicol. From the other hand, the fact that the  $TO^-$  character is found only in the early mutants, clearly indicates that the ability of the cells to be stimulated is associated with an early event. It is likely that more than one event is required before the first morphological step of sporulation can be detected. This suggestion is supported by the existence of a series of early mutants, including  $TO^-$ , which are genetically unlinked (Schaeffer et al., 1963). Since it is unlikely that all these genes are involved in the same early event, these genes probably have to do with a variety of functions, all submitted to the general regulatory system of sporulation. Therefore it is possible that all mutants blocked before some early event are  $TO^-$  while all the mutants blocked after this event (late step 0 mutants and step I-V mutants) are  $TO^+$ . The event in question would, according to our hypothesis, be the synthesis (or activation) of an intracellular protease. A similar situation has already been demonstrated for the production of an antibiotic by B. subtilis. The analogy of these two cases can be seen comparing the last two lines of Table I.

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#### References

- Balassa, G., 1964, in press.  
Schaeffer, P., Ionesco, H., Ryter, A. and Balassa, G., 1963, Coll. Intern. sur les Mécanismes de Régulation, Marseille, in press.