QUANTITATIVE REGULATION OF RNA SYNTHESIS DURING SPORULATION OF BACILLUS SUBTILIS

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Received February 13, 1964

Spore formation occurs in the stationary phase of growth, in the absence of any net macromolecular synthesis. A considerable turnover, involving the three principal types of RNA : ribosomal, soluble and messenger, is, however, observed during sporulation (Balassa, 1963). Although such turnover is not restricted to sporulating cells (Mandelstam, 1963), breakdown and resynthesis of RNA is more rapid in sporulating B. subtilis than is some asporogenous mutants. Thus it would appear that a direct correlation exists between sporulation and quantitative control of the rate of RNA turnover. Some evidence on the existence and nature of this correlation will be presented here.

It is well known that bacterial RNA synthesis is dependent upon the presence of amino acids (Gros, 1963). This dependance has been demonstrated in amino acid starvation and in "shift-down" experiments, in which it has been found (Gros and Gros, 1956 ; Neidhardt and Fraenkel, 1961) that the addition of chloramphenicol leads to a stimulation of RNA synthesis, due to an accumulation of amino acids. Since sporulation occurs in exhausted complex media which are unable to support growth, we suspected that the factor which actually limits RNA synthesis under these conditions is the reduction in amino acid supply. In the following experiments, the action of chloramphenicol and of amino acids on RNA synthesis in the sporogenous (Sp^+) Marburg strain was compared with the action of these compounds on the asporogenous mutant

Sp^-_3 (Schaeffer et al., 1963) which is blocked in an early step of sporulation.

^{14}C-uracil, added at t_3 (3 hours after growth has stopped ; at this time Sp^+ bacteria are already actively engaged in the sporulation process, for details see Schaeffer et al. (1963)) is incorporated linearly in both cultures. However, the rate of incorporation is 2-3 times higher in Sp^+ than in Sp^- organisms (figure 1, curve A). Addition of chloramphenicol (curve B), moreover, raises the initial rate of incorporation 8 fold in Sp^+ but results in only weak stimulation of uracil uptake in Sp^-_3. This lack of stimulation in Sp^-_3 suggests that, in this strain, chloramphenicol does not lead to amino acid accumulation. Alternatively, the RNA synthesizing capacity of these cells may be altered by some factor other than the amino acid supply. In order to distinguish between these two possibilities, a mixture of the twenty amino acids were added along with the ^{14}C-uracil and chloramphenicol. As illustrated in curve C, under these conditions the mutant now responds similarly to the wild type strain. Thus it appears that the two strains differ in the amino acid supply during stationary phase.

Since in an exhausted medium, in the absence of net protein synthesis, amino acids are probably furnished by protein degradation, it seems likely that Sp^+ strains degrade proteins more rapidly than the mutant. This degradation presumably results from the formation (or activation) of an intracellular protease, induced by sporulation. Similar observations were made by Aubert and Millet (1963) on degradation of β-galactosidase in B. megaterium Sp^+ and Osp.

In conclusion, the following hypothesis is presented to explain : 1) the more rapid RNA turnover during sporulation in Sp^+ than in Sp^- strains ; 2) the effect of amino acids on this synthesis : 3) the proportionality of RNA and protein synthesis (Spotts and Szulmajster, 1962) ; 4) the coordination of synthesis and degradation of RNA.

As summarized in Figure 2, our hypothesis proposes that : a) RNA synthesis during sporulation is controlled by the supply of amino acids ; b) the

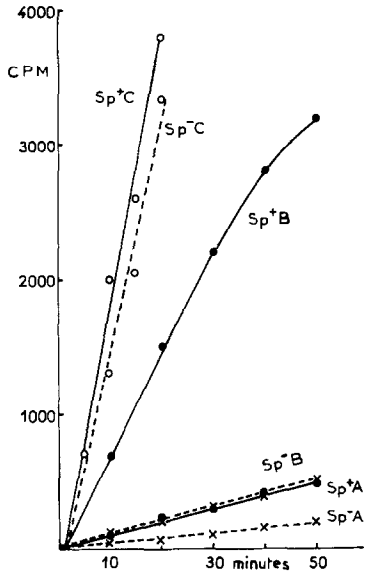

Figure 1

^{14}C-uracil uptake by sporulating cells

^{14}C-uracil (0,2 µC/ml, 4 µC/µM) was added to sporulating cells (Sp^+,—) and to the asporogenous mutant (Sp^-_3,---) at t_3 with : A (x) no supplement, B (o) + chloramphenicol 100 µg/ml, C(o) + chloramphenicol 100 µC/ml + 20 amino acids 8 µg/ml each. Samples of 0,5 ml were pipetted in cold 5 % TCA, filtered on Millipore membranes, washed, and the incorporated radioactivity was counted.

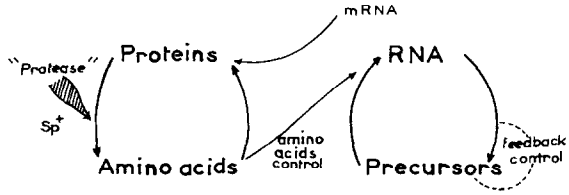

Figure 2

Regulation of RNA and protein turnover

latter are provided by protein degradation, which is itself dependent upon the induction (or activation) of a specific intracellular protease during sporulation ; c) the rate of protein synthesis depends upon the rate of messenger RNA production, which is in turn proportional to the rate of total

RNA synthesis (Balassa, 1963) ; d) finally, the degradation of RNA might be controlled by a feedback-mechanism exercised by the degradation products. This hypothesis provides an explanation of the coordination of the two turnover cycles and further suggests that levels of protease may control the rates of RNA and protein turnover.

Detailed results and discussion will be presented in a later publication

I am grateful to Drs. P. Schaeffer, F. Gros and A. Shedlovsky for discussions and to Miss A. Malhié for valuable technical assistance.

This work has been aided by grants from the "Délégation Générale à la Recherche Scientifique et Technique ", the "Centre National de la Recherche Scientifique", and the "Commissariat à l'Energie Atomique".

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