STUDIES ON EXTRACELLULAR PROTEASE FORMATION

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1. Extracellular protease synthesis by B. amyloliquefaciens continues for 80 minutes in the presence of rifampicin or actinomycin D concentrations sufficient to prevent mRNA synthesis. Despite this the transcription-independent protease production is inhibited by antibiotics specific for protein synthesis and direct labelling studies have confirmed that de novo synthesis of protease occurs under these conditions.

2. The evidence indicates that there exists in harvested cells, a pool of protease-specific mRNA, capable of supporting protease synthesis, in the absence of RNA synthesis, for up to 80 minutes.

3. The protease mRNA is not intrinsically long-lived and has a half-life of the order of a few minutes.

4. The results imply that the mRNA pool is being constantly turned over by a degradation process unrelated to translation, and therefore the mRNA pool may be a result of a dynamic equilibrium between mRNA synthesis, degradation and translation.

5. The time course of protease production in a medium containing a high level of amino acids is biphasic due to
amino acid repression, while that in the presence of a low level of amino acids is essentially linear. The results presented here are compatible with amino acids acting at the level of transcription of the protease mRNA. The biphasic production of protease in a medium containing a high level of amino acids can be accounted for in the following way. The first phase of synthesis, which is insensitive to rifampicin, is due to amino acid repression of mRNA transcription and the translation to exhaustion of the accumulated pool of mRNA. Subsequent derepression of mRNA synthesis and translation of nascent mRNA accounts for the second phase of synthesis which is therefore sensitive to rifampicin and actinomycin D.

6. The protein synthesis inhibitors, pactamycin and fusidic acid, at certain concentrations, completely inhibit protease production without affecting general intracellular protein synthesis. This is interpreted as supporting the concept that protease synthesis occurs on ribosomes located at the periphery of the cell.

7. Pactamycin, at higher concentrations, inhibits general protein synthesis in B. amyloquefaciens but the cells recover from this inhibition. The recovery is not due to the acquisition of resistance; the results are compatible with the metabolic removal of the antibiotic from the cells.

8. Preliminary attempts to isolate the protease mRNA species have been made. RNA has been recovered apparently
intact from *B. amyloliquefaciens* and from this, fractions containing some mRNA-like RNA species have been isolated. Preliminary attempts to identify the protease mRNA by translating it *in vitro* were unsuccessful.