inhibitory effect on the oxidation of the succinyl-phosphatide, and concentrations of malonate that completely inhibit oxidation of succinate only partially inhibited oxidation of equimolar amounts of succinyl-phosphatide (based on phosphorus content).

These results may be accounted for as follows. It is suggested that a first step in the succinate-dehydrogenase reaction is the formation of a succinate ester at the β -position of a phosphoglyceride close to or at the active site for dehydrogenation. The formation of this succinyl-phosphoglyceride intermediate from succinate may be competitively inhibited by fumarate and malonate.

The next step in the reaction is the dehydrogenation of β -succinyl-phosphatide to β -fumaryl-phosphatide, from which fumarate plus phospholipid are released (Scheme 1).

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Biochem. J. (1965) **96**, 12c

Phenotypic Expression of the 'Relaxed' Genotype during Inhibition of *Escherichia coli* by β -2-Thienylalanine

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(Received 21 May 1965)

In bacteria, the synthesis of RNA normally depends on the presence of all the amino acids found in protein. Thus, when the essential amino acid is withdrawn from an amino acid auxotroph, neither protein nor RNA is made. Similarly, bacteria that require an amino acid for growth are unable to synthesize RNA in the presence of low concentrations of chloramphenicol unless trace amounts of the required amino acid are present (Pardee & Prestidge, 1956).

The dependence of RNA synthesis on amino acids is genetically determined, and mutants have been

described in which the normal 'stringent' control of RNA synthesis has become 'relaxed'; the characteristic feature of relaxed mutants that also require an amino acid for growth is that RNA continues to be made when the amino acid is withdrawn (Stent & Brenner, 1961). However, the relaxed genotype is not invariably expressed under conditions in which the rate of protein synthesis is varied. Neidhardt (1963) investigated the behaviour of relaxed strains during balanced growth in different media and found that the ratio of RNA to protein varied in an apparently normal manner with growth rate; similarly, a 'shift-down' transition, i.e. a decrease in growth rate produced by a change in

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carbon and energy or nitrogen source, was accompanied by a cessation of RNA synthesis similar to that observed in stringent strains.

Fig. 1. Synthesis of RNA and protein during inhibition by β -2-thienylalanine of two methionine-requiring mutants of *E. coli* K12: (a) mutant 58-161 ('relaxed'); (b) mutant H239 ('stringent'). The experiments used cultures growing exponentially (mean generation times: 75 min.) at 30° with forced aeration in a mineral salts medium containing 50 mg. of DL-methionine/l. (Dagley *et al.* 1963). Then 15 μ g. of uracil/ml. and 20 μ g. of cytosine/ml. were added to portions (50 ml.) of a culture and aeration was continued. Synthesis of RNA and protein was followed separately by the addition of 20 μ g. of [2- 14 C]uracil/ml. or 30 μ g. of DL-[35 S]methionine/ml. in the presence and absence of 3 μ g. of DL- β -2-thienylalanine/ml. Measurements were made after the addition of the analogue and were of the radioactivity at infinite thinness of cells precipitated with 5% (w/v) trichloroacetic acid at 0°. ■, RNA, and □, protein made by cultures inhibited by thienylalanine. ●, RNA, and ○, protein made by uninhibited controls.

The present communication reports a striking difference between relaxed and stringent strains that is found during the inhibition of growth caused by the analogue of phenylalanine, DL- β -2-thienylalanine. We used exponentially growing cultures of two methionine-requiring mutants of *Escherichia coli* K12; mutant 58-161 continues to synthesize RNA when starved of methionine and is thus 'relaxed'; in the other mutant, H239, RNA synthesis is under normal stringent control by amino acids. Fig. 1 shows the synthesis of RNA and protein by cultures of both mutants after the addition of 3 μ g. of thienylalanine/ml. Protein synthesis was inhibited considerably and to about the same extent in both strains but synthesis of RNA was affected very differently. In the stringent mutant, RNA production was inhibited to a slightly greater extent than the synthesis of protein. Synthesis of RNA by the relaxed mutant was at first stimulated and only after incubation for 2 hr. did the amount of RNA produced in the presence of thienylalanine become less than in the untreated control; similar results were obtained with this mutant in experiments with concentrations of up to 300 μ g. of thienylalanine/ml. This difference in the effect of thienylalanine on RNA synthesis provides a convenient means for identifying organisms that carry the relaxed genotype (R. E. MacDonald & N. Fiil, unpublished work).

Fig. 1 shows that, during incubation for 3 hr. with thienylalanine, the relaxed mutant synthesized about three times as much RNA per unit of newly synthesized protein as did the untreated culture; there was therefore considerable accumulation of RNA during inhibition. This same mutant also accumulates RNA during methionine-starvation; in this case newly made ribosomal RNA is contained in particles whose sedimentation coefficient in crude cell-free extracts is about 15s. These particles are in some respects similar to the ribosome precursors (eosomes) detected in exponentially growing cells (Dagley, Turnock & Wild, 1963; Turnock & Wild, 1965). Extracts were prepared, as described by Dagley *et al.* (1963), from cells of the relaxed mutant after incubation for 90 min. in a medium containing 50 μ g. of thienylalanine/ml. When these extracts were examined in the analytical ultracentrifuge, 15s particles corresponding to those present in extracts of starved cells were not observed. Estimations of RNA and protein by orcinol and biuret methods respectively (Dagley *et al.* 1963) showed that, in a typical experiment, the RNA content of a culture increased by about 100% in 90 min. and protein by about 35%. The protein synthesized during inhibition may be sufficient for the incorporation of newly synthesized ribosomal RNA into ribosomes and their immediate precursors (neosomes); a similar increase in protein content after the addition of methionine to starved cells is

sufficient for the conversion of 15s particles into ribosomes and neosomes to take place (Turnock & Wild, 1965).

Kurland & Maaløe (1962) suggested that transfer RNA is involved in the regulation of RNA synthesis by amino acids. On this view, each species of transfer RNA can inhibit RNA synthesis; the inhibition is released by reaction with the appropriate amino acid to form the corresponding species of aminoacyl-transfer RNA. Thus withdrawal of methionine from mutant H 239 leads to cessation of RNA synthesis because transfer RNA specific for methionine must exist predominantly in the unesterified form during starvation; in a relaxed mutant such as 58-161 this control mechanism is defective. The effects of thienylalanine that we have observed are consistent with this hypothesis. The analogue has been shown to be activated non-additively with phenylalanine in crude cell-free systems from *E. coli* (Wolfe & Hahn, 1960) and to replace phenylalanine in protein synthesized during inhibition (Munier & Cohen, 1959). In experiments with the relaxed mutant similar to that in Fig. 1, the incorporation into 5%-trichloroacetic acid-insoluble material of [^{14}C]thienylalanine ($10\text{ }\mu\text{g./ml.}$ and $2\text{ m}\mu\text{C/ml.}$) was proportional to the amount of protein synthesized, as measured by the incorporation of [^{35}S]methionine. However, the rapid inhibition of growth brought about by thienylalanine is probably due not to the production of

inactive proteins but to inhibition of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase, the enzyme that catalyses the first step in the synthesis of phenylalanine, tyrosine and tryptophan (Ezekiel, 1965). The enzyme present in *E. coli* growing exponentially in minimal medium is strongly inhibited by both phenylalanine and thienylalanine. Incubation with thienylalanine will therefore cause a considerable decrease in the rate of synthesis of both tyrosine and tryptophan; operation of the hypothetical control mechanism outlined above would then lead to inhibition of the synthesis of RNA by the stringent mutant but not in the relaxed strain.

G. T. thanks the Department of Scientific and Industrial Research for a Research Studentship. The skilled technical assistance of Mrs J. M. Midgley is gratefully acknowledged.

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Biochem. J. (1965) **96**, 14c

The Incorporation of [$2\text{-}^{14}\text{C}, 4\text{R-}^3\text{H}_1$]Mevalonate and [$2\text{-}^{14}\text{C}, 4\text{S-}^3\text{H}_1$]Mevalonate into Squalene, Ubiquinone and Ergosterol of *Aspergillus fumigatus* Fresenius

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(Received 21 May 1965)

Intermediate steps in the biosynthesis of polyisoprenoid compounds from mevalonate include the conversion of isopentenyl pyrophosphate into either (a) dimethylallyl pyrophosphate or (b) the isoprenyl derivatives of dimethylallyl pyrophosphate (Scheme 1). Step (a) involves the addition of a proton to what was originally C-2 of mevalonic acid followed by the removal of a proton from what was originally C-4 of mevalonic acid. In step (b) an isoprenyl ion is added instead of a proton.

The work reported by Cornforth, Cornforth,

Donninger & Popják (1965a), Cornforth, Cornforth, Donninger, Popják & Ryback (1965b) and Popják (1965) on the biosynthesis of *trans-trans*-farnesyl pyrophosphate and all-*trans*-squalene shows that the elimination of the proton from what was originally C-4 in mevalonate is stereospecific. When either [$2\text{-}^{14}\text{C}, 4\text{R-}^3\text{H}_1$]- or [$2\text{-}^{14}\text{C}, 4\text{S-}^3\text{H}_1$]-mevalonate was incubated with liver preparations, counting of the farnesol and squalene isolated revealed that the hydrogen eliminated was that which, when replaced by a heavy hydrogen isotope, would confer the 4S