declined between 2 and 8 h, but during the first hour after addition of α -amanitin the rate of RNA synthesis was consistently found to increase by up to 20% above control.

In a further series of experiments chick-embryo fibroblast cells were treated with various doses of α -amanitin up to 30 μ g/ml, and after 1 h the cells were harvested and the nuclei were purified and assayed for RNA polymerase form I and form II activities (Novello & Stirpe, 1970). After doses of 5 μ g or more/ml there was an 80% inhibition of RNA polymerase form II activity of isolated nuclei, but RNA polymerase I activity was stimulated by 50%. The latter finding may account for the observed stimulation in [3 H]uridine uptake in the pulse-labelling experiments after treatment with α -amanitin (20 μ g/ml) for 1 h.

It is clearly not possible to determine the effects of α -amanitin on cellular RNA synthesis simply by measuring the net incorporation of [3 H]uridine into acid-insoluble material; therefore we have measured the effects of the drug on the synthesis of various types of cellular RNA, and the results of these experiments will be described.

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Polarity in the *gua* Operon of *Escherichia coli* K12

By P. R. LAMBDEN and W. T. DRABBLE (*Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 5NH, U.K.*)

In *Escherichia coli* the purine nucleotide IMP is synthesized in ten steps from 5'-phosphoribosyl 1'-pyrophosphate and is at the branch point for the biosynthesis of AMP and GMP. The biosynthesis of GMP from IMP is catalysed by two enzymes, IMP dehydrogenase and XMP aminase. IMP dehydrogenase is subject to feedback inhibition by GMP (Mager & Magasanik, 1960), and both enzymes are repressed by high intracellular concentrations of GMP (Nijkamp & de Haan, 1967). The structural genes for the two enzymes are linked and are coordinately regulated (Nijkamp & de Haan, 1967).

The present communication gives further information on the regulation of these two enzymes.

A number of mutants blocked in GMP formation were isolated from wild-type cells of *E. coli* K12 and are designated *guaA* (XMP-aminase-less) or *guaB* (IMP-dehydrogenase-less). The mutants, which require guanine for growth, were cultured under conditions where the guanine supply was insufficient to support maximal growth of the cells. Under these conditions enzyme synthesis was no longer repressed.

All *guaA* mutants had de-repressed levels (40–45-fold) of IMP dehydrogenase. By contrast, two classes of *guaB* mutant were obtained. The first class had de-repressed levels (15–25-fold) of XMP aminase whereas the second class had low levels of this enzyme. The latter type of *guaB* mutation showed a polarity effect leading to a low-level expression of the adjacent *guaA* gene. These results are contrary to the findings of Nijkamp & Oskamp (1968), who found that in *E. coli* K12 some *guaA* mutations were polar but that none of the *guaB* mutations showed polarity effects.

Each *guaB* mutant was tested for its ability to produce cross-reacting material. The reaction between anti-(IMP dehydrogenase) serum and cross-reacting material was detected by immunodiffusion in gels on microscope slides. Non-polar *guaB* mutants were either positive or negative with respect to cross-reacting material, whereas all polar *guaB* mutants were negative, as expected if they arose by 'nonsense' mutations.

To establish that the polar mutations lie within the *guaB* gene, a genetic map of the IMP dehydrogenase structural gene was constructed by three-factor reciprocal crosses. The polar mutants were found to lie within the *guaB* gene.

These findings indicate that transcription of the two genes of the *gua* operon proceeds from *guaB* to *guaA*.

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