Control of Nucleic Acid Synthesis in *Aspergillus nidulans*

By H. N. ARST and C. SCAZZOCCHIO (introduced by D. J. COVE) (*Department of Genetics, University of Cambridge, Cambridge CB4 1XH, U.K.*)

A method for measuring nucleic acid synthesis in *Aspergillus nidulans* *in vivo* by using protein synthesis as a measure of growth has been developed. The method involves double-labelling (with ^3H and ^{14}C) with leucine-pyrimidine double auxotrophs. Incorporation of labelled L-leucine and differently labelled D-uridine into trichloroacetic acid-non-extractable material monitors protein and nucleic acid synthesis respectively. Both leucine and uridine were chosen for metabolic inertness in *Asp. nidulans* (i.e. neither is a carbon or a nitrogen source). Label reversal has confirmed the absence of labelling artifacts. The contribution of DNA synthesis during a 2 h incorporation period (roughly equal to a doubling time under these conditions) to total nucleic acid synthesis can be estimated at 13% by comparing parallel experiments with [5- ^3H]uridine and [6- ^3H]uridine, assuming the DNA synthesized to have a 50% G+C content [as compared with published values of 47% (Dutta *et al.*, 1967) and 54% (Storck & Alexopoulos, 1970) for *Asp. nidulans*] and the RNA synthesized to have a 50% pyrimidine content. Except when very short incorporation periods are used, the method should underestimate RNA synthesis owing to degradation of labile RNA.

Starvation experiments with strains requiring leucine, uridine and a third metabolite (arginine, histidine, methionine, proline, adenine, inositol or putrescine) have given the following results. Control of RNA synthesis is stringent in wild-type *Asp. nidulans* during amino acid starvation, and preferential cessation of nucleic acid synthesis relative to growth also results from adenine or inositol starvation. Putrescine starvation or high-concentration (20 mM) supplementation does not affect the proportionality of nucleic acid synthesis and growth. Moreover, putrescine starvation or high-concentration supplementation during amino acid starvation is equivalent to amino acid starvation alone.

A number of possible inhibitors of nucleic acid synthesis have been tested *in vivo*. Actinomycin D, cycloheximide and rifampicin are all inhibitory, whereas α -amanitin is not. Inhibition by rifampicin is additive with that due to amino acid starvation, whereas that by cycloheximide is not. Cycloheximide prevents the inhibition by rifampicin, possibly suggesting that protein synthesis is required. A time-course shows that cycloheximide preferentially affects synthesis of stable nucleic acids.

Selection of relaxed mutants is in progress.

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Regulation of the Pentose Phosphate Pathway in the Fungus *Aspergillus nidulans*

By O. HANKINSON and D. J. COVE (*Department of Genetics, University of Cambridge, Cambridge CB4 1XH, U.K.*)

During the reduction of nitrate to ammonium by *Aspergillus*, NADPH is oxidized to NADP^+ . Action of the pentose phosphate pathway probably affords the major means whereby the organism reduces NADP^+ back to NADPH.

Addition of nitrate to *Aspergillus nidulans* growing with urea as nitrogen source causes a 100-fold induction of nitrate reductase and nitrite reductase (Pateman *et al.*, 1967). This treatment also leads to an approximate twofold increase in the activities of four enzymes of the pentose phosphate pathway and a threefold increase in that of phosphoglucose isomerase, whereas those of three other glycolytic enzymes are unaffected. Osmond & ap Rees (1969) described a similar response to nitrate by *Candida utilis*.

Cove, Pateman and associates have isolated mutants at several loci that are abnormal in the induction of nitrate reductase and nitrite reductase. All these mutations are believed to produce their effects on the induction process by leading either to abnormal activation of an inducer molecule specified by the *nir* gene or to the synthesis of a defective *nir* molecule (Pateman *et al.*, 1967; Pateman & Cove, 1967; Cove & Pateman, 1969; Cove, 1969).

Possession of the higher activities of the four pentose phosphate-pathway enzymes and of phosphoglucose isomerase does not depend on the metabolism of nitrate, but only occurs under genetical and environmental conditions in which an active *nir* molecule can be inferred to exist.

Mutants of the pentose phosphate pathway have been isolated. These map at two loci, and all grow poorly on nitrate and nitrite, but normally on other nitrogen sources. One class has lower activities of the four enzymes of the pentose phosphate pathway and of phosphoglucose isomerase, but retains inducibility by nitrate for these enzymes. Another class lacks transaldolase. These latter mutants accumulate sedoheptulose 7-phosphate when grown with urea to an extent that is increased 20-fold by the addition of nitrate. This increased accumulation is abolished in double mutants that also lack nitrate reductase, but in which the nitrate-mediated increase in the pentose phosphate-pathway enzymes occurs.