MOLECULAR BASIS OF NITRATE REDUCTASE INDUCTION IN *CANDIDA UTILIS*

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SUMMARY

Temporal separation of transcription and translation during nitrate reductase induction in *Candida utilis* was achieved by the use of actinomycin D and cycloheximide. The yeast failed to synthesize nitrate reductase when nitrate was not provided during transcription. Nitrate thus appeared to induce during transcription the capacity to synthesize nitrate reductase. Presence of nitrate, on the other hand, was not obligatory during translation except for its essential role in maintaining the stability of nitrate reductase after its formation as well as its mRNA.

The regulation of assimilatory nitrate reductase [NAD(P)H: nitrate oxidoreductase EC 1.6.6.2] has been the subject of intensive investigation in various organisms (2,4-9,11). In most of the systems investigated hitherto, nitrate reductase synthesis has been shown to require nitrate as inducer (9,2). Using different combinations of cycloheximide and actinomycin D, Sorger and Davies (9) demonstrated that NO_3^- ions are essential for the successful translation of NADPH-nitrate reductase mRNA species but not for the transcription of the nitrate reductase gene(s) in *Neurospora*.

We report in this communication the induced transcription-dependent synthesis of nitrate reductase in the yeast *Candida utilis*, and discuss the findings on this important variation in comparison with the other relevant literature reports.

MATERIALS AND METHODS

Chemicals: Actinomycin D and cycloheximide were purchased from Sigma Chemical Co., St. Louis, U.S.A. Rest of the chemicals,

apart from those described earlier (2), were all of analytical grade and obtained from either BDH (INDIA) Ltd., or from Sarabhai M. Chemicals, Baroda, India.

Organism and maintenance: C. utilis CBS 4511, obtained from the Centraalbureau voor Schimmelcultures, Delft, The Netherlands, was used in all the experiments. The media, and procedures for maintenance and growth of the yeast and for induction of nitrate reductase were as described earlier (3).

Permeation of cells and assay of nitrate reductase: The procedures followed to permeate induced cells of C. utilis to assay nitrate reductase activity in situ and for analytical assays, and the definitions of enzyme units and specific activity were as described previously (2,3).

RESULTS

Effect of actinomycin D and cycloheximide: In inducible enzyme systems in bacteria, the inducer is known to enhance the enzyme levels by boosting the synthesis of the specific mRNA. An inducible enzyme can be synthesized from the preformed mRNA even if simultaneous transcription is blocked, and also in the absence of the inducer, provided the mRNA is stable enough. Basing on this fact, Turner et al. (12) devised a simple technique to achieve temporal separation of transcription and translation during induction of kynureninase in Neurospora and then continuing the incubation in inducer-free minimal medium without the drug.

Adopting a similar procedure to separate transcription and translation during nitrate reductase induction in C. utilis, an attempt was made to identify the specific step of the enzyme synthesis during which nitrate, as inducer, exerts its influence on the overall regulation of nitrate reductase induction.

Table 1. Effect of actinomycin D and cycloheximide on nitrate reductase induction in C. utilis

Antibiotic included in minimal medium	Final concentration (mg/ml)	Preincubation with inhibitor (min)	Addition of inducer[a]	Continued incubation period (min)	Nitrate reductase specific activity
A. Nil (Control)		30	+	120	19.0
B. Actinomycin D[b]	0.05	30	+	120	*
C. Cycloheximide	1.00	30	+	120	*
	5.00	30	+	120	*
	10.00	30	+	120	*
D. A portion of cells from system 'C', washed free from drug and inducer, and on continued incubation			-	120	6.3

Ammonia-grown cells were exposed to the drug in minimal medium for 30 min at 30°C. Then 1% (w/v) KNO_3 was added and shaking was continued at 30°C. After 2 h, an aliquot of each culture was drawn, washed and examined for nitrate reductase activity *in situ*. Cells from an aliquot of culture 'C' (exposed to cycloheximide, 1 mg/ml) were washed and transferred to fresh minimal medium without the drug as well as inducer, and the shaking at 30°C was continued for additional 2 h, at the end of which they were examined for nitrate reductase activity.

[a] KNO_3, 50 mM(N).

[b] The uptake of actinomycin D by the cells was facilitated by inclusion of 0.1% (v/v) toluene in the medium.

+ present; - absent; * not detectable.

Actinomycin D, known to prevent DNA-dependent RNA synthesis in eukaryotes as well as in prokaryotes, at 50 μg/ml concentration, completely inhibited induction of nitrate reductase in C. utilis. A relatively high concentration of actinomycin D was used in view of the earlier reports on fungi (6,10). Similarly, cycloheximide, a potent inhibitor of translation, at all concentrations ranging from 1 to 10 mg/ml, effectively blocked nitrate reductase induction (Table 1).

However, cells exposed to nitrate in presence of cycloheximide, after removal of the drug and inducer followed by incubation for 2 h at 30°C in fresh minimal medium, showed considerable levels of nitrate reductase activity (Table 1). On the contrary, the cells exposed to actinomycin D, after similar procedure, failed to show any nitrate reductase activity.

DISCUSSION

The total failure of nitrate in the presence of actinomycin D to induce nitrate reductase in C. utilis indicates that nitrate reductase is synthesized de novo and that the induced synthesis of the enzyme is transcription-dependent. The cells exposed to nitrate in presence of cycloheximide had apparently accumulated the capacity to synthesize nitrate reductase which was translated in minimal medium once the drug was removed. These results suggest that the presence of nitrate is absolutely necessary only for formation of the specific mRNA but not necessary during translation of the mRNA. However, the fairly low level of nitrate reductase activity of these cells compared to those of the control ones indicates the high lability of nitrate-reductase-mRNA species and possibly of the enzyme also, as in the case of Neurospora (10,9); we have already reported that

nitrate reductase in C. utilis is rapidly inactivated in vivo in the absence of nitrate (2).

Thus, these results clearly establish that nitrate ions are required during transcription for accumulation of the capacity to synthesize nitrate reductase in C. utilis, contrary to the situation in Neurospora, where the presence of nitrate was reported to be essential for successful translation of nitrate reductase-mRNA type(s) but not for the transcription of nitrate reductase gene(s) (9). However, the important role of nitrate in maintaining the stability of nitrate reductase, after its formation, in C. utilis is analogous to the regulation of the enzyme in Neurospora (1).

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