INDUCTION OF HYDROXAMATE-SENSITIVE RESPIRATION IN *NEUROSPORA* MITOCHONDRIA

Transcription of nuclear DNA is required

David L. EDWARDS and Barbara W. UNGER
Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, CA 92037, USA

Received 14 October 1977

1. Introduction

A topic of current interest in studies of mitochondrial biogenesis is the regulatory interactions that occur between the mitochondrial and nuclear genomes during this process. Both genomes have been shown to contribute products to the assembly of the mitochondrial inner membrane [1]. Previous studies have shown that inhibition of mitochondrial transcription with ethidium bromide or translation with chloramphenicol leads to an increase in the specific activities of numerous enzymes that function in the mitochondrion but are coded for in the nucleus [2—5]. These enzymes include: DNA-dependent RNA polymerase, elongation factors, methionyl-tRNA transformylase, ribosomal proteins and mitochondrial leucyl- and phenylalanyl-tRNA synthetases. These studies have led to the postulation that the mitochondrial genome acts to regulate the nuclear genome through a repressor protein that affects transcription of the nuclear genes coding for mitochondrial components [1,2].

We have been studying the hydroxamate-sensitive respiratory pathway in mitochondria from *Neurospora crassa*. This pathway has been shown to be present in respiratory-deficient mutants that have defective mitochondrial electron transport [6,7]. This 'cyanide-insensitive respiration' is inhibited by substituted hydroxamic acids [8] and will be referred to here as hydroxamate-sensitive respiration. Mutants with hydroxamate-sensitive respiration have been shown to have a branched mitochondrial electron transport system with one portion being the standard cytochrome chain and the other being the hydroxamate-sensitive pathway of unknown composition [9].

We have previously shown that hydroxamate-sensitive respiration can be induced in wild-type cells by inhibition of mitochondrial transcription or mitochondrial translation [10,11]. Protein synthesis on cycloheximide-sensitive ribosomes is also required for the production of hydroxamate-sensitive respiration in such induction experiments [8]. In this report we present a study with the drug actinomycin-D. This drug is an inhibitor of DNA dependent RNA synthesis in many systems [12] and has been shown to specifically inhibit nuclear transcription in *Neurospora* [13,14]. The data show that transcription of nuclear DNA is required for the production of hydroxamate-sensitive respiration.

2. Materials and methods

Strain *inl*-89601 was obtained from the Fungal Genetics Stock Center, Humboldt State University Foundation, Arcata, CA 95521. Conidia from the cells were inoculated at a concentration of 5×10^5 conidia/ml into Vogels' medium N [7] supplemented with sucrose (2%) and inositol (50 μg/ml) and grown at 30°C in a gyrotory shaker for 13.5 h at 200 rev/min. The mycelium was collected by filtration on a Buchner funnel and resuspended in 20 ml 0.1 M sodium phosphate pH 6.2 containing 0.5% ethylenediaminetetraacetic acid (EDTA). Incubation was

carried out in this solution for 5 min at 30°C with shaking. The permeabilized cells were then collected, washed with distilled water and resuspended in Vogels' medium supplemented as above and also containing chloramphenicol (2 mg/ml) and varying amounts of actinomycin-D. Incubation was carried out at 30°C with shaking. Respiration measurements were made at various times as described [10].

3. Results and discussion

Figure 1 shows the results obtained when EDTA-permeabilized cells of strain *inl*-89601 are treated with chloramphenicol in the presence or absence of actinomycin-D. In the absence of actinomycin-D, hydroxamate-sensitive respiration is induced and can be observed in the culture after approx. 3 h incubation. This lag is considerably longer than that observed in unpermeabilized cells (approx. 30 min. [10]) and is likely due to the drastic nature of the EDTA treatment. Once hydroxamate-sensitive respiration begins to appear in the culture, however, the activity increases in a linear manner for the next several hours. The data in fig.1 show that when the permeabilized cells are treated with chloramphenicol and increasing amounts of actinomycin-D, there is marked inhibition of hydroxamate-sensitive respiration. The activity is inhibited by 90% by 2.5 μg actinomycin-D/ml culture medium and completely by 5 μg/ml.

This result indicates that transcription of nuclear DNA is required for the production of hydroxamate-sensitive respiration in *Neurospora*. In additional experiments, we have attempted to demonstrate the accumulation of the messenger RNA for the hydroxamate-sensitive pathway by sequential incubation of the cells in cycloheximide and then actinomycin-D. These experiments have not been successful and resulted in the death of the cells. The procedure involved permeabilization and blockade with chloramphenicol and cycloheximide followed by a second permeabilization and blockade with chloramphenicol and actinomycin-D.

The nuclear nature of the structural genes for the hydroxamate-sensitive pathway has been inferred from experiments with chloramphenicol and ethidium bromide which inhibit mitochondrial translation and transcription and result in the production of the

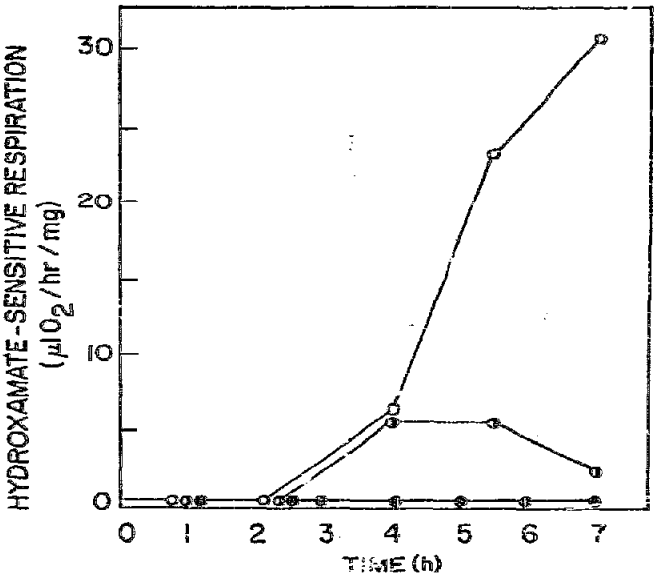

Fig.1. Induction of hydroxamate-sensitive respiration in *Neurospora*. Cells were grown in Vogels' medium N and permeabilized with EDTA as described in Materials and methods. The permeabilized cells were the resuspended in fresh medium containing chloramphenicol (2 mg/ml) and varying amounts of actinomycin-D. Time 0 on the figure is the time when the permeabilized cells were resuspended in fresh medium. Open circles: chloramphenicol only no actinomycin-D. Half-filled circles: chloramphenicol plus 2.5 μg/ml actinomycin-D. Filled circles: chloramphenicol plus 5.0 μg/ml actinomycin-D.

pathway [10,11]. It has also been shown directly by the isolation of mutants which cannot produce the pathway even when challenged with chloramphenicol [15]. These mutations segregate in a Mendelian manner in crosses. Preliminary experiments have assorted them into two complementation groups [15].

The experiments with drugs described [10,11] have shown that a mitochondrial gene product regulates the production of the hydroxamate-sensitive pathway. This product acts in a negative manner to regulate the nuclear genes involved in the production of the pathway [11]. We have also recently described an extrachromosomal mutant, *cni*-3, that has the hydroxamate-sensitive pathway present at all times during the growth cycle although it is not utilized in vivo [9,16]. This mutation appears to be located in the segment of mitochondrial DNA that codes for the regulatory element of the pathway.

The data presented here strongly suggest that the regulation in this system is effected by controlling the transcription of the nuclear genes which code for components of the pathway. Our results lend more experimental support to the reports [1–5,10,11] concerning such a regulatory mechanism. Although we think it unlikely, we cannot, however, rule out the possibility that the mitochondrial product regulates the production of proteins (proteases, nucleases) that modify some pre-existing components which are then utilized to produce hydroxamate-sensitive respiration.

The regulation of gene activity by negative control has been well documented in bacterial systems [17]. In all systems that have been well studied to date, the regulation has been carried out by a protein. Previous studies from our laboratory have indicated that the mitochondrial regulatory molecule for the hydroxamate-sensitive system acts outside of the mitochondrion [11]. The present study indicates that the regulation is at the level of nuclear transcription. We think it most likely that the regulatory element is a protein of mitochondrial origin that acts by inhibiting the transcription of nuclear DNA.

Acknowledgements

This work was supported by research grant GM-24991 from the National Institute of General Medical Sciences to D.L.E. D. L. E. is also the recipient of a Research Career Development Award from the same institute.

References

[1] Schatz, G. and Mason, T. (1974) Ann. Rev. Biochem. 43, 51–87.
[2] Barath, Z. and Kuntzel, H. (1972) Nature New Biol. 240, 195–197.
[3] Barath, Z. and Kuntzel, H. (1972) Proc. Natl. Acad. Sci. USA 69, 1371–1374.
[4] Beauchamp, P. and Gross, S. (1976) Nature 261, 338–340.
[5] Beauchamp, P., Horn, E. and Gross, S. (1977) Proc. Natl. Acad. Sci. USA 74, 1172–1176.
[6] Lambowitz, A. and Slayman, C. W. (1971) J. Bacteriol. 108, 1087–1096.
[7] Edwards, D. L., Kwiecinski, F. and Horstmann, J. (1973) J. Bacteriol. 114, 164–168.
[8] Schonbaum, G. R., Bonner, W. D., Jr, Storey, B. T. and Bahr, J. T. (1971) Plant Physiol. 47, 124–128.
[9] Lambowitz, A., Slayman, C. W., Slayman, C. L. and Bonner, W. D., jr (1972) J. Biol. Chem. 247, 1536–1545.
[10] Edwards, D. L., Rosenberg, E. and Maroney, P. (1974) J. Biol. Chem. 249, 3551–3556.
[11] Edwards, D. L. and Rosenberg, E. (1976) Eur. J. Biochem. 62, 217–221.
[12] Reich, E. and Goldberg, I. H. (1964) in: Progress in Nucleic Acid Research and Molecular Biology (Davidson, J. N. and Cohn, W. E. eds) Vol. 3, pp. 183–234, Academic Press, New York.
[13] Turner, J. R., Terry, K. and Matchett, W. H. (1970) J. Bacteriol. 103, 370–374.
[14] Subramanian, K. N. and Sorger, G. J. (1972) J. Bacteriol. 110, 547–553.
[15] Edwards, D. L., Guzik, H. and Warden, J. T (1976) in: Genetics and Biogenesis of Chloroplasts and Mitochondria (Bucher, Th., Neupert, W., Sebald, W. and Werner, S. eds) pp. 865–872, Elsevier/North-Holland, Amsterdam.
[16] Edwards, D. L. (1977) in: Function of Alternative Oxidases (Degn, H. and Lloyd, D. eds) Pergamon Press, London in press.
[17] Gots, J. and Benson, C. (1974) Ann. Rev. Genet. 8, 79–101.