Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Maria Grazia Costantini, Maria Grazia Massari

Shift-up transitions of growth in Neurospora crassa

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **58** (1975), n.3, p. 421–426.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1975_8_58_3_421_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1975.

Biochimica comparata. — *Shift-up transitions of growth in* Neurospora crassa. Nota di MARIA GRAZIA COSTANTINI e MARIA GRAZIA MASSARI, presentata (*) dal Corrisp. E. MARRÈ.

RIASSUNTO. — Sono descritte transizioni nutrizionali per shift-up da acetato a brodo nutriente e da glicerolo a glucosio in culture di *Neurospora crassa.*

In entrambi i casi si osserva un brusco aumento della velocità di accumulo di RNA in seguito all'aggiunta di un nutriente più facilmente utilizzabile. Si suggerisce che tali transizioni possano essere utili per studiare la regolazione della sintesi di RNA in *Neurospora*, già oggetto di studi in questo laboratorio.

INTRODUCTION

The regulation of synthesis of RNA is one of the more important of the metabolic processes which ensure the control of cellular growth. A number of studies on bacterial cells [1–2] have shown that the ribosomal RNA (rRNA) content of exponentially growing cells depend upon the rate of growth of the culture. Recent findings indicate that this is also true for eukaryotic microorganisms [3–4] and, in more general way for cells of higher organisms.

It is clear that in the study of the regulation of the synthesis of RNA and in particular of rRNA, beside the analysis of different conditions of exponential growth, it may be of great interest the use of nutritional shifts which rapidly induce a change in the rate of growth and, with all likehood, of rRNA synthesis. In a study on a shift-down transition of growth of *Neurospora crassa* cells which has been done in this laboratory [5] the exhaustion of the richer carbon source from the culture medium was shown to be accompanied by an abrupt and almost complete inhibition of ribosomal RNA synthesis. Contrary to what has been shown in comparable bacterial systems [6–7], this rapid inhibition of ribosomal RNA synthesis is not accompanied by an increase of guanosine 3′–5′ tetraphosphate [8], and the question is still open on the small molecule which mediates such negative control.

In this paper we present a preliminary report on the study of shift-up transition, which may represent a convenient experimental condition to induce a rapid increase of the rate of synthesis of ribosomal RNA.

MATERIALS AND METHODS

Organisms and growth conditions

The wild type strain 74 A (St. Lawrence) of *Neurospora crassa* has been used for the experiments described in this paper. The growth conditions were indicated in detail in a previous paper [4].

(*) Nella seduta dell'8 marzo 1975.

The growth media were prepared by adding to a Vogel's mineral medium [13] one of the following carbon sources: glycerol (100 μg/ml), sodium acetate (40 mM), glucose 2 % (*w/v*) and in the case of nutrient broth 0.75 % (*w/v*) of nutrient broth (Biolife), 0.75 % (*w/v*) of yeast extract (Biolife) and 2 % (*w/v*) of sucrose.

The shift-up experiment were performed by dilution as indicated in a previous paper [4].

700 ml flasks containing 100 ml of the culture medium were inoculated with $1-2 \times 10^5$ conidia/ml then incubated in a Dubnoff water bath at 30 °C. After a convenient period of growth in the poor medium 100 ml of a richer medium were added. The growth was followed as increase of the absorbance at 450 nm (A_{450nm}) and the constant of the rate of growth, K (hr^{-1}) was determined as previously reported [4].

Incorporation of radioactive precursor into nucleic acids.

In the experiments in which the ^{32}P incorporation into RNA was measured, 10 μCi (in the case of growth in glucose, sodium acetate or nutrient broth) or 40 μCi (in the case of growth in glycerol) of ^{32}P-orthoposphate (carrier free) obtained from Radiochemical Center (Amersham) were added to 100 ml,

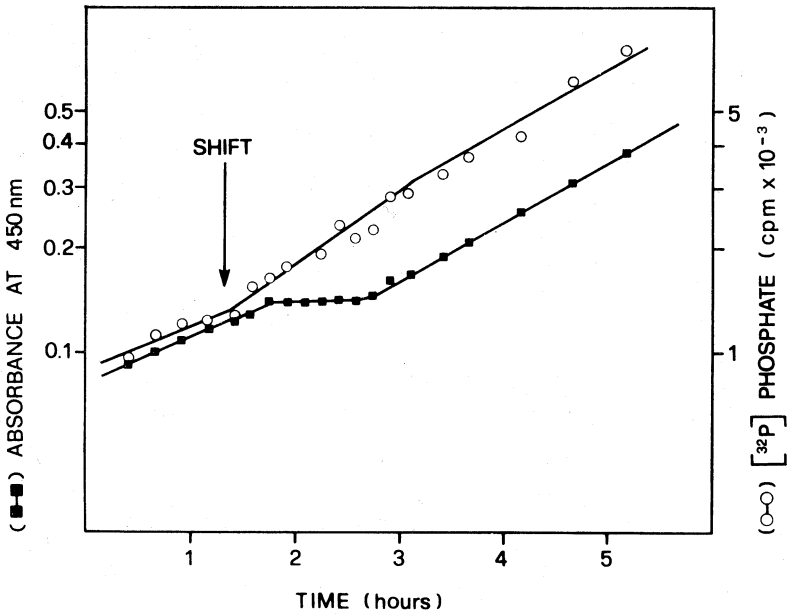

Fig. 1. – Growth and RNA accumulation in *Neurospora crassa* during a shift-up transition of growth from acetate to nutrient broth. The cells were growth in minimal acetate (40 mM); at the time indicated by the arrow 100 ml of the culture growing in minimal acetate were added to 100 ml of the fresh medium containing 0.75 % yeast extract, 0.75 % nutrient broth, 2 % sucrose. Growth was monitored as change in A_{450nm} (▲—▲); RNA accumulation was determined by measuring the incorporation of ^{32}P into the cold trichloroacetic acid precipitable fraction, subtracted by the hot precipitable fraction (○—○).

culture immediately before the inoculation with the conidia. In this experiments the concentration of KH_2PO_4 in Vogel's mineral medium was lowered to 2 mM, as it has been previously shown [4] that this concentration of phosphate neither modifie the rate of growth of *Neurospora crassa* mycelia nor limits growth under our experimental conditions. At the times indicated in the figs. 1 and 2, 2 ml aliquots of culture were withdrawn and added to 2 ml cold 20 % trichloroacetic acid. After 20 min. in ice, the suspension was filtered through a Millipore HA (0.45 μ) filter and extensively washed with cold 5 %

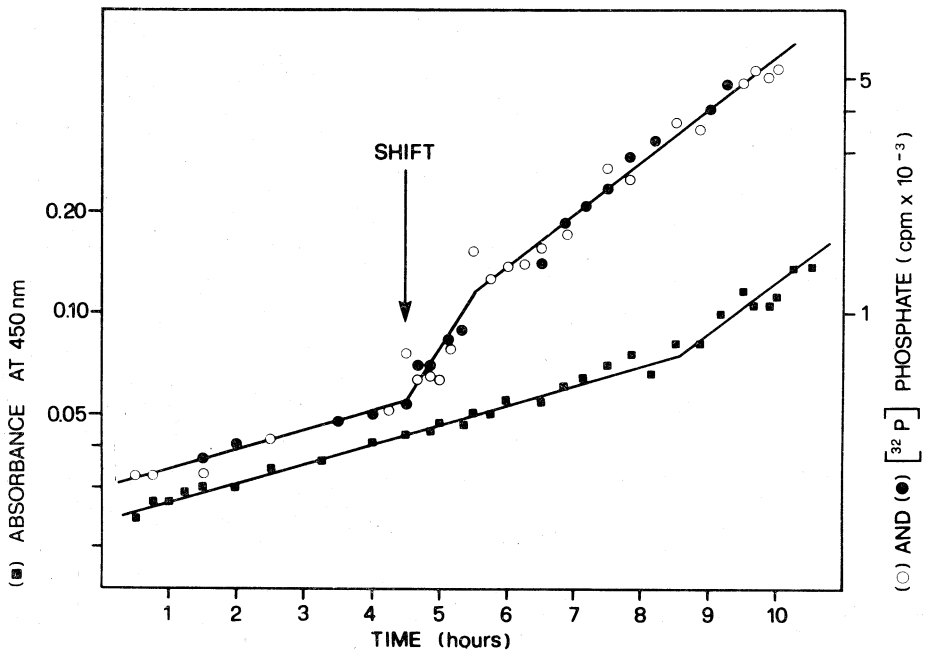

Fig. 2. – Growth and RNA accumulation in *Neurospora crassa* during a shift–up transition of growth from glycerol to glucose. The cells were growth in glycerol (100 μg/ml); at the time indicated by the arrow 100 ml of the culture growing in glycerol were added to 100 ml of the fresh medium containing 2% glucose. Growth and RNA accumulation were determined as indicated in the legend of fig. 1.

trichloroacetic acid. At the same time, 2 ml aliquots were added to 2 ml 20 % thichloroacetic acid and heated at 90 °C for 30 min. The suspension was filtered and washed as indicated above. The amount of radioactivity incorporated into the cold trichloroacetic acid-precipitable fraction, subtracted by that of the hot trichloroacetic acid-precipitable fraction, represents the radioactivity incorporated into nucleic acids [5]. Due the very low DNA content of *Neurospora crassa* cells, ^{32}P incorporation detects essentially RNA accumulation [4].

The dried Millipore filters were counted in a Packard Liquid Scintillation Counter (model 3320) according to standard procedures.

Results and discussion

Neurospora crassa mycelia growing exponentially in different culture media show different rates of growth: in Table I the constants of the rate of growth (K, hr^{-1}) of *Neurospora* mycelia growing in Vogel's minimal medium supplied with the following carbon and nitrogen sources: sucrose plus nutrient broth, glucose, acetate and glycerol, are reported.

The RNA level (expressed as μg RNA/genome) of these culture was found to increase proportionally with the rate of growth as reported in the second column of Table I. Therefore during the shift from a poor to a richer medium, we should expect that the RNA content of the culture increases so to reach the level reported for the second condition.

The kinetics of this transition has been studied in the following experiments.

The accumulation of RNA was studied by measuring the incorporation of ^{32}P-orthophosphate which, in *Neurospora crassa*, detects almost exclusively RNA formation [5]. The radioactive precursor was added to the medium at the moment of the inoculation of conidia so to ensure pool equilibration before the beginning of the sampling.

In fig. 1 the shift-up from acetate to nutrient broth is shown. During the growth in acetate the curves of A_{450nm} and of ^{32}P incorporation were parallel with the same rate constant (K = 0.30) indicating that the cells were growing exponentially on acetate. When the nutrient broth was added to the culture medium the increase of A_{450nm} is blocked and at the same time the net synthesis of RNA is increased very markedly (K = 0.50). After about 90 min the growth as A_{450nm} resumes (K = 0.38) and the rate of RNA accumulation slows down reaching apparently a new condition of balanced exponential growth, about 3 hours after the shift (K = 0.38). It has to be noted that the rate of growth achieved after the shift to nutrient broth is muchslower than that measured in nutrient broth medium itself (K = 0.63) reported in Table I.

Then the shift from glycerol to glucose was studied in cells in exponential balanced growth (K = 0.31). The same increase of A_{450nm} continues at the previous rate (K = 0.13) for about 4 hours after the shift-up. The rate of accumulation of RNA is greatly stimulated after the shift. The constant of the initial rate being K = 0.73; then it slows down considerably (K = 0.36) and, apparently a new condition of balanced exponential growth is achieved. In this case the rate of growth measured after the shift is that typical of growth on the new medium (K = 0.35).

In conclusion the results which have been shown indicate that a rapid and sizable change in the rate of the net synthesis of RNA is produced by nutritional shift-ups.

These phisiological conditions are therefore convenient for studies on the regulation of RNA synthesis and considering that about 80 % of total RNA is rRNA more specifically for the studies on ribosomal RNA synthesis.

The transition for nutritional shift-up gives a situation opposite to that of the transition for nutritional shift–down which is referred in the Introduction causes a specific restriction of rRNA synthesis. Thus it seems that the integrated study of the two conditions should be useful in order to understand the mechanisms of the regulation of the synthesis of rRNA in eukaryotic cells.

TABLE I.

Rates of growth and RNA levels of Neurospora crassa *cells in exponential growth on different media.*

Growth medium	Rate of growth (K, hr^{-1})	RNA level (μg per genome)
NUTRIENT BROTH	0.63	2.44×10^{-6}
GLUCOSE	0.35	1.34×10^{-6}
ACETATE	0.28	1.11×10^{-6}
GLYCEROL	0.18	0.71×10^{-6}

The situation in eukaryotic microorganism can be different from that described in prokaryotes [9], as different RNA polymerase molecules [10] catalyze the synthesis of ribosomal and of messenger RNA so that it is not possible to hypothize [9], as suggested for *E. coli*, that a number of RNA polymerase molecules shift from the unstable to the stable RNA geens so to yield the rapid response of the stimulation of the synthesis of rRNA.

Due to the multiplicity of the RNA polymerase it is likely that, following a nutritional shift-up, molecules of RNA polymerase I which were not functionally active become able to bound to chromatine [11] or that more RNA polymerase I molecules are made. It is to be recalled in fact that in yeast [12] the level of the nucleolar RNA polymerase is higher at higher growth rates, so that a new synthesis of RNA polymerase I can be expected to occur during a nutritional shift-up. Experiments along these lines are in progress.

REFERENCES

[1] O. MAALØE and N. O. KJELGAARD (1967) – *Control of Macromolecular Synthesis*, New York and Amsterdam, W. A. Benjamin.
[2] O. MAALØE (1969) – « Dev. Biol. Suppl. », *3*, 33–40.
[3] F. A. M. ALBERGHINA, E. STURANI and J. GOHLKE – « J. Biol. Chem. », in press.
[4] F. A. M. ALBERGHINA (1973) – « Arch. Mikrobiol. », *89*, 83–94.
[5] E. STURANI, F. MAGNANI and F. A. M. ALBERGHINA (1973) – « Biochim. Biophis. Acta », *319*, 153–164.
[6] M. CASHEL and J. GALLANT (1969) – « Nature », *221*, 838–841.

[7] R. B. HARSHAM and H. YAMAZAKI (1971) – « Biochemistry », *10*, 3980–3982.
[8] F. A. M. ALBERGHINA, L. SCHIAFFONATI, L. ZARDI and E. STURANI (1973) – « Biochim. Biophis. Acta », *312*, 435–439.
[9] P. P. DENNIS and H. BREMER (1973) – « J. Mol. Biol. », *75*, 145–159.
[10] R. G. ROEDER and W. J. RUTTER (1969) – « Nature », *224*, 234–237.
[11] FU-LI YU (1974) – « Nature », *251*, 344–346.
[12] J. SEBASTIAN, F. MIAN, H. O. HALVORSON (1973) – « FEBS Letters », *34*, 159–162.
[13] H. J. VOGEL (1956) – « Mikrobiol. Genet. Bull. », *13*, 42–50.