Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Filippa A. M. Alberghina, Enzo Martegani

Macromolecular synthesis at low temperature in Neurospora crassa

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **55** (1973), n.5, p. 546–553.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1973_8_55_5_546_0>

Biochimica comparata. — *Macromolecular synthesis at low temperature in* Neurospora crassa. Nota di FILIPPA A. M. ALBERGHINA e ENZO MARTEGANI (*), presentata (**) dal Corrisp. E. MARRÈ.

RIASSUNTO — Temperature superiori a 40° C ed inferiori a 20° C inibiscono nettamente la crescita di miceli di *Neurospora crassa* in saccarosio minimo o supplementato con casaminoacidi. Il grafico di Arrhenius della costante della velocità di crescita in funzione della temperatura suggerisce che fenomeni di denaturazione di macromolecole avvengano a temperature inferiori a 20° C. L'aggiunta di casaminoacidi stimola la velocità di crescita solo a temperature superiori a 25° C.

Per studiare quali variazioni avvengono nelle sintesi macromolecolari a bassa temperatura miceli di *Neurospora crassa* in crescita esponenziale a 30° C sono stati rapidamente (in meno di 2 minuti) portati a 8° C.

Le sintesi nette di RNA e di proteine risultano inibite del 90% in modo coordinato.

Questi risultati suggeriscono che l'abbassamento della velocità di crescita non è correlato all'inibizione preferenziale di una delle due sintesi macromolecolari.

INTRODUCTION

A low temperature deviation of the Arrhenius plot of the growth rate is generally observed in microorganisms (fig. 1). The sharp decrease of the growth rates shown in fig. 1 for temperatures below 12° C for *E. coli* and *Pseudomonas aeruginosa* and below 2° C for a psycrophylic strain of *Pseudomonas fluorescens* are currently interpreted as caused by cold denaturation of macromolecular component(s) essential for growth.

The cold denaturation of proteins has been studied in several model systems and the important role of the collapse of the clathrate structure of water has been underlined [1]. Several thermodynamical equations have been developed which account for the cold denaturation of a protein as result of the differential effects of the temperature change on the conformation of the macromolecule in itself and the change of conformation due to interaction with the solvent [2].

More unsettled is the question of which macromolecules essential for growth are affected by the low temperature: changes in membrane conformation [3], inhibition of the initiation reaction of protein synthesis [4] or of the processing of ribosomal RNA [5] have been suggested as possible effects of low temperature.

(*) Centro di Studio del CNR per la Biologia Cellulare e Molecolare delle piante. Istituto di Scienze Botaniche – Università di Milano – 20133 Milano.

(*) Nella seduta del 26 novembre 1973.

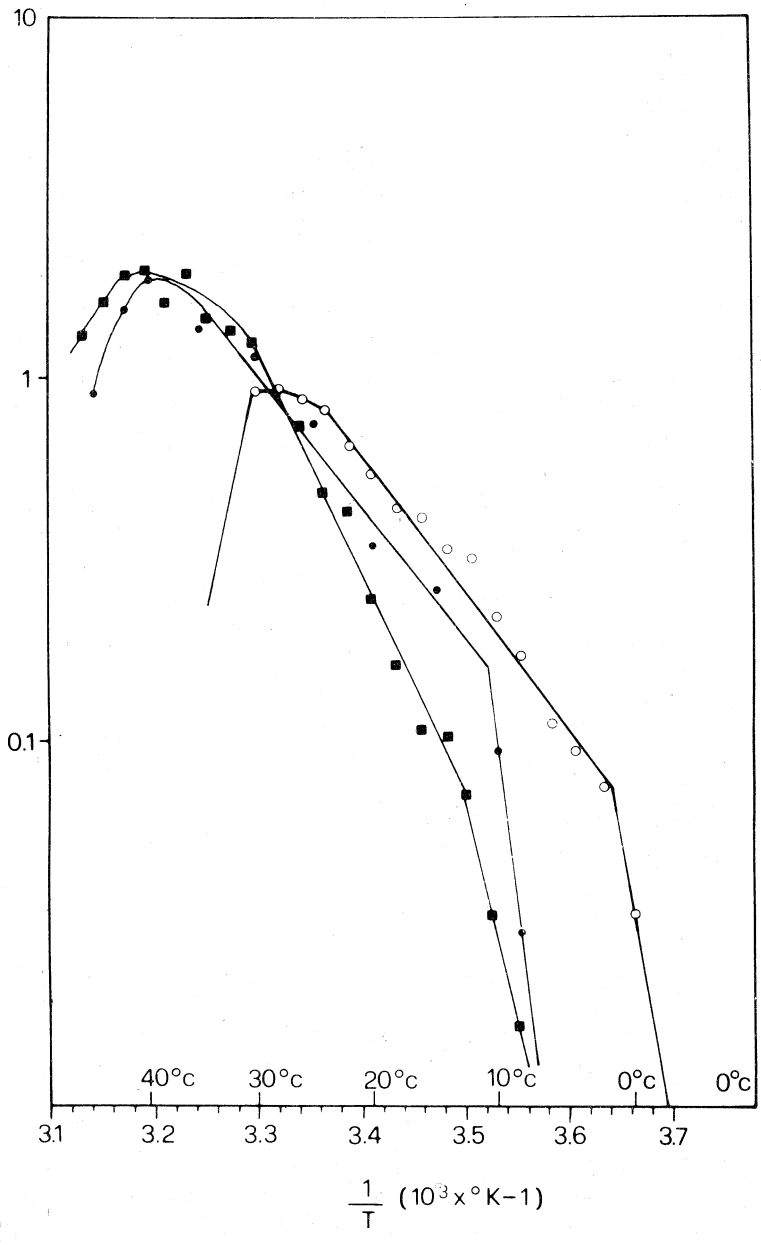

Fig. 1. – Arrhenius plot of the rates of growth of *E. coli* (■), *Pseudomonas aeruginosa* (●) and *Pseudomonas fluorescens* 21°–3° C, a psycrophylic strain (○).

The constant of the rate of growth at the different temperatures given by Ingraham [12] are presented in a semilogarithmic plot showing the deviations at low temperatures.

In the present paper we present data on the effects of temperature on growth and macromolecular synthesis in *Neurospora crassa*. A coordinate inhibition of RNA and protein net synthesis is shown.

Materials and methods

Organism 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 [6]. Vogel's mineral medium was supplemented with 2% (*w/v*) sucrose and when indicated with 1% (*w/v*) casein hydrolyzate. For the experiments reported in fig. 2 700 ml-flasks containing

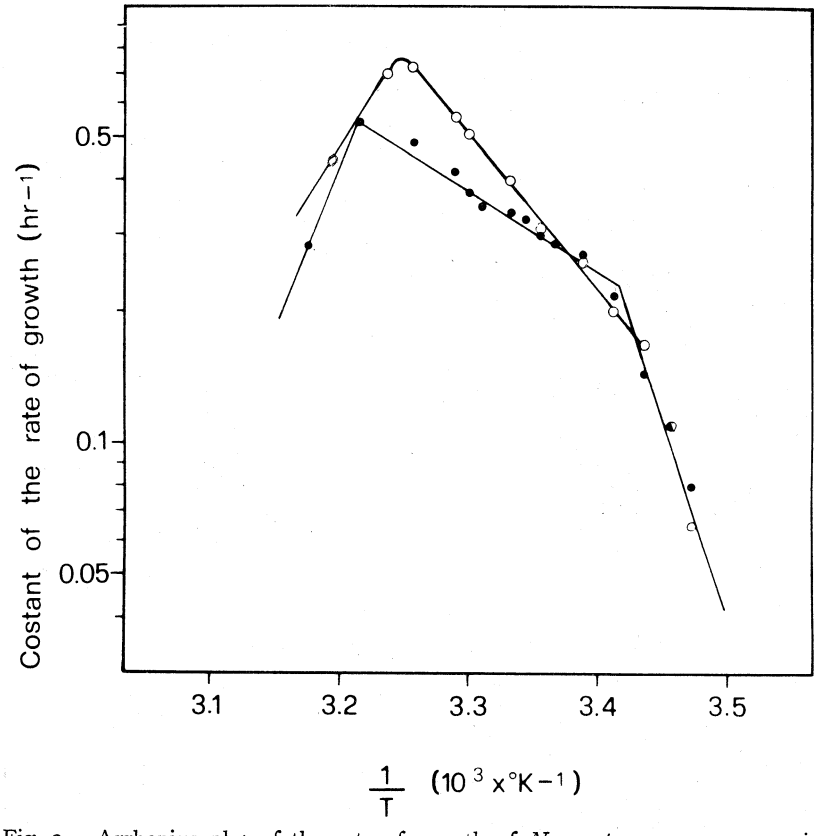

Fig. 2. – Arrhenius plot of the rate of growth of *Neurospora crassa* grown in sucrose minimal (●) or in sucrose plus casaminoacids medium (○).

The constants of the rate of growth measured at different temperatures as A 450 nm change as well as dry weight change are reported.

200 ml of medium were inoculated with $2 \cdot 10^7 - 3 \cdot 10^7$ conidia, then incubated in a Dubnoff water bath at the required temperature. The growth was followed as increase of the absorbance at 450 nm (A 450 nm) or of dry weight and the constant of the rate of growth, K (hr^{-1}) was determined as previously reported [6].

For the experiments reported in fig. 3 and fig. 4, the cultures were placed in water-jacketed flasks connected to the water circulation of a KB 300 thermostat (Fryka Kältetechnik) for the experiments below 20° C or to the water circulation of a BT 60/100 thermostat (Fryka Kältetechnik) for the experi-

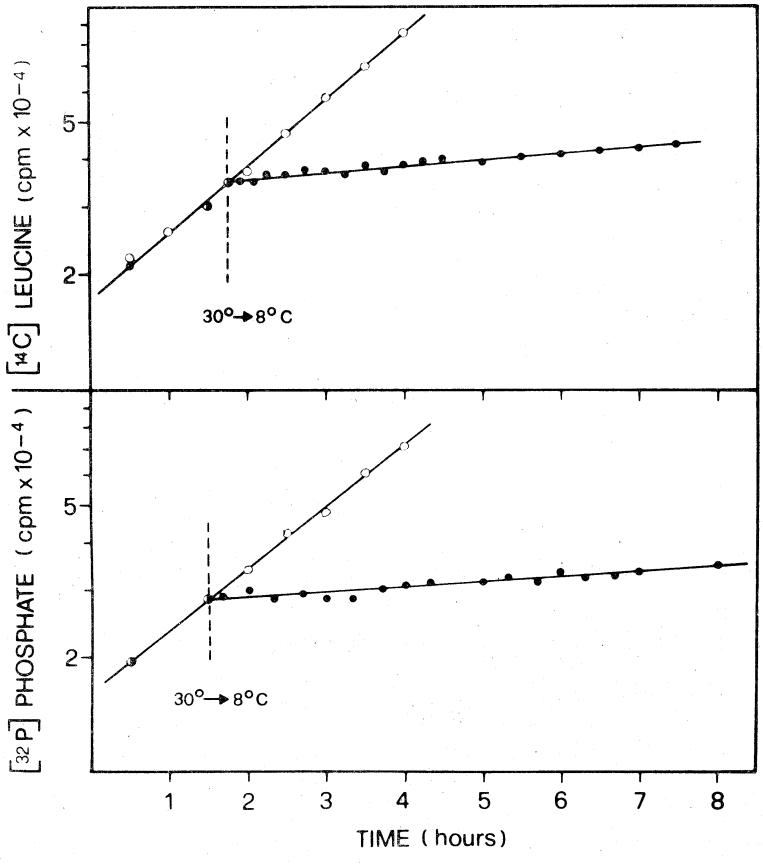

Fig. 3. – Net RNA and protein synthesis in *Neurospora crassa* during temperature shift from 30° C to 8° C.

Net RNA synthesis at 30°C (○) and at 8°C (●) was determined by measuring ^{32}P incorporation. Net protein synthesis at 30°C (○) and at 8°C (●) was monitored by measuring the incorporation of L– carboxyl–^{14}C leucine. Both ^{32}P ortophosphate (2 mM final concentration of phosphate) and carboxyl–^{14}C leucine (0.5 mM) were added to the culture medium before the inoculation of conidia, to ensure pool equilibration before the beginning of the sampling. Note that the ^{32}P and ^{14}C incorporation in the culture at 30° C are linear on the semilogarithmic plot and have the same slope (K=0.38), therefore indicating that the cells which were subjected to the temperature shift were in balanced exponential growth. At 8° C, the K of net protein synthesis is 0.035, that of net RNA synthesis is 0.031 as calculated from the slopes of the straight lines. The temperature transition was achieved for an aliquot of the culture (80 ml) when indicated in 2 min. using the apparatus described in the methods. For another aliquot of the culture (80 ml) the incubation at 30° C was continued.

ments at higher temperatures. The temperature transitions were achieved by transferring the culture (80 ml) through a heat exchanger whose temperature was set 4° C below the desired temperature: in these conditions the temperature transition was completed in 90–120 sec. The apparatus employed is going to be described in detail elsewhere [7].

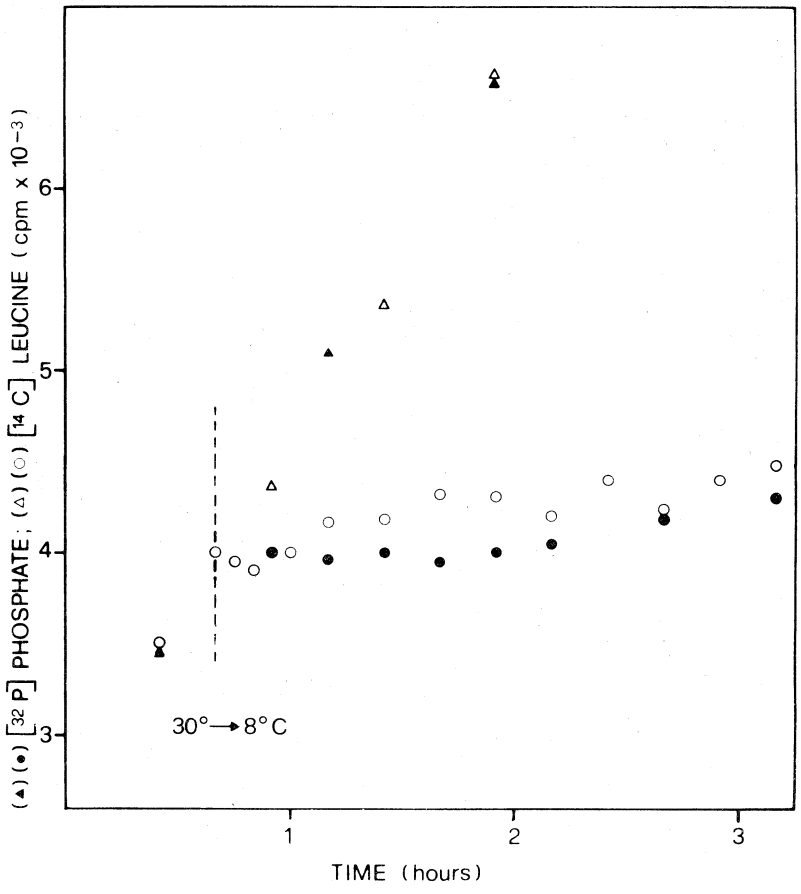

Fig. 4. – Net RNA and protein synthesis in *Neurospora crassa* after a temperature transition from 30° C to 8° C.

Net RNA synthesis at 30° C (▲) and at 8° C (●); and net protein synthesis at 30° C (△) and at 8° C (○) were determined as indicated under fig. 3. The results are reported on a linear graph.

Incorporation of radioactive precursors into nucleic acids and proteins.

When RNA accumulation was followed by measuring ^{32}P incorporation, 10 μCi of ^{32}P– ortophosphate (carrier-free) obtained from Radiochemical Center (Amersham) were added to 100 ml culture immediately after the inoculation with the conidia. The concentration of KH_2PO_4 in Vogel's mineral medium was lowered to 2 mM. It has been previously shown [6] that this concentration of phosphate does not modify the initial rate of growth of *Neurospora crassa* mycelia and it does not limit growth under our experimental conditions.

At the times indicated in the figs. 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% trichloroacetic acid. At the same time, 2 ml aliquots

were added to 2 ml cold 20% trichloroacetic 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 from that of the hot trichloroacetic acid-precipitable fraction, represents the amount of radioactive ^{32}P incorporated into nucleic acid [8]. Due to the very low DNA content of *Neurospora* cells, ^{32}P incorporation detects essentially RNA accumulation [9].

When protein accumulation was determined 0,05 mmoles L– carboxyl ^{14}C leucine (0.1 Ci/mole) obtained from New England Nuclear Corporation, were added to 100 ml culture (whose phosphate concentration was 2 mM).

Aliquots of 2 ml were withdrawn as indicated previously and the radioactivity incorporated in the hot trichloroacetic acid-precipitable material was determined.

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

RESULTS

In *Neurospora crassa* mycelia growing in a minimal sucrose or in sucrose plus casaminoacids the rate of exponential growth increases as the temperature increases and it reaches its maximum at 36°–38° C (fig. 2). The Arrhenius plots of the constant of growth rates in the two media at different temperatures show a sharp change of slope at 20° C (fig. 2).

A quite interesting point is that at temperatures below 25 °C the addition of casaminoacids does not stimulate the rate of growth, while it does so in a significant manner at temperatures above 25° C. The activation energy (E) calculated from the slope in the low temperature range is the same (25 K cal/mole) for the growth in both media, while in the high temperature range (20°–37° C) it is higher (15,8 K cal/mole) for the growth rate in sucrose plus casaminoacids than for growth on minimal sucrose (7.8 K cal/mole).

Activation energy values of the order of 10 K cal/mole are normal for enzymatic catalysis, while values of the order of 25–100 K cal/mole are indicative of denaturation process [10].

To obtain some information on whether a macromolecular synthesis becomes rate limiting at low temperatures, we have determined how a temperature shift (from 30° C to 8° C) affects the kinetics of net synthesis of RNA and protein.

Cultures of *Neurospora crassa* were grown in minimal sucrose at 30° C in presence of ^{32}P ortophosphate or of leucine–carboxyl– ^{14}C. The radioactivity incorporated into nucleic acids, essentially stable RNA [9], or into proteins was determined in cultures growing at 30° C. As fig. 3 shows, the rates of precursors incorporation at 30° C are exponential functions of time and the constant of exponential rate (K) calculated for both RNA and protein accumulation is 0.38. To study the effects of low temperature an aliquot of the culture in exponential balanced growth at 30° C was transferred at the time

indicated to 8° C (the temperature transition was complete in 2 min). The rates of RNA and protein net synthesis were both severely reduced by the temperature drop. The constant of exponential rate (K) calculated from the data presented in fig. 3 is 0.035 for protein net synthesis and 0.031 for RNA net synthesis.

That the transition from 30° C to 8° C inhibits to a comparable extent both RNA and protein accumulation is further confirmed by the results shown in fig. 4, where a short time kinetics of net macromolecular synthesis is shown.

The coordinate inhibition of RNA and protein accumulation has been observed in all the other temperature transitions we have studied: 30° to 18°C; 30° to 15° C; 30° to 12° C (results not shown).

Discussion

Temperature as well as the nutrients deeply affects the rate of growth of *Neurospora crassa* mycelia [6]. While a decrease of the rate of growth obtained by changing the type of nutrient available to the cells (i.e. a shift-down transition) causes the dissociation of the net synthesis of DNA, RNA and protein with a specific block of ribosomal RNA synthesis [9], there is no dissociation of RNA and protein net synthesis when the rate of growth is changed by lowering the temperature.

Therefore the hypothesis that the low temperature induces the cold denaturation of an enzyme required for a specific macromolecular synthesis (for instance causing the inhibition of protein synthesis by a block of the initiation) appears too simple to account for the experimental findings. In fact if the hypothesis were correct a dissociation of the two macromolecular syntheses would seem more likely than their coordinate inhibition.

Another experimental approach (the determination of the levels of DNA, RNA and proteins) could be used to find out whether growth at low temperature is characterized by rates of macromolecular synthesis which maintain between them the same ratios which are determined at 30° C [11]. The measurements of these levels in fact would give a first indication whether the balanced state maintained at 8° C is the same as that maintained at 30° C. A more adequate description of the effects of the transitions to low temperature on the macromolecular syntheses would require an analysis of the extent of degradation processes during the same period, because the net synthesis (or accumulation) of a macromolecule is the balance between the synthesis and the degradation of the same molecule.

Finally it is interesting to discuss the observation reported in fig. 2: the addition of casaminoacids to the medium stimulates the rate of growth only at temperatures above 25°C. At 30°C cells growing in sucrose plus casaminoacids have a higher ribosome content than cells growing in minimal sucrose [11]. It would be interesting to find out whether this effect is lost at temperatures below 25° C where the rates of growth are the same irrespective of the addition of aminoacids.

Experiments along these lines are in progress.

REFERENCES

[1] G. N. LING, in: *Thermobiology*. A. H. Rose ed. Academic Press, London (1967).
[2] J. F. BRANDTS, in: *Thermobiology*. A. H. Rose ed. Academic Press, London (1967).
[3] J. FAWELL and A. ROSE, « Ann. Rev. Microbiol », *21*, 101 (1967).
[4] H. FRIEDMAN, P. LU and A. RICH, « J. Molec. Biol. », *61*, 105 (1971).
[5] Y. KURIYAMA and J. L. LUCK, « J. Molec. Biol. », *73*, 425 (1973).
[6] F. A. M. ALBERGHINA, « Arch. Mikrobiol » *89*, 83 (1973).
[7] F. A. M. ALBERGHINA and E. MARTEGANI (manuscript in preparation).
[8] D. B. ROODYN and H. G. MANDEL, « Biochim. Biophys. Acta », *41*, 80 (1960).
[9] E. STURANI, F. MAGNANI and F. A. M. ALBERGHINA, « Biochim. Biophys. Acta », *319*, 153 (1973).
[10] F. H. JOHNSON, H. EYRING and M. J. POLISSAR, « The Kinetic basis of molecular biology », Wiley, New York (1954).
[11] F. A. M. ALBERGHINA, E. STURANI and J. R. GOHLKE (Manuscript in preparation).
[12] A. H. INGRAHAM, « J. Bacteriol. », *76*, 75 (1958).