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Biochimica. — *Effects of amino acids on the synthesis of stable RNA in* Neurospora crassa. Nota di ENZO MARTEGANI (*), LAURA POPOLO (**), PAOLA GHERSA (**) e RENATA ZIPPEL (**), presentata (***) dal Socio S. TONZIG.

RIASSUNTO. — L'aggiunta di casaminoacidi al mezzo di coltura contenente glucosio come fonte di carbonio, determina un notevole aumento della velocità di crescita e del contenuto di RNA dei miceli di *Neurospora crassa* coltivati a 30 °C.

Tale effetto stimolante risulta essere poco dipendente dalla concentrazione dei casaminoacidi aggiunti e può essere ottenuto ugualmente con una miscela artificiale di soli aminoacidi neutri.

Invece in colture coltivate a 20 °C non si osserva alcun effetto di stimolo in seguito ad aggiunta di casaminoacidi né sulla velocità di crescita né sul contenuto e la velocità di sintesi netta di RNA stabile.

Tale mancanza di stimolo a 20 °C potrebbe essere imputabile ad una rallentata velocità di ingresso degli aminoacidi nella cellula a questa temperatura, tuttavia le misure sia dell'azoto amminico totale che del livello endocellulare di diversi amminoacidi tendono ad escludere questa possibilità. Viene perciò suggerito che a 20 °C si abbia, in *Neurospora crassa* l'inattivazione di qualche componente macromolecolare interessato alla regolazione della sintesi dell'RNA stabile.

In *Neurospora crassa* different rates of exponential growth at 30 °C can be obtained by changing the nutrients available to the cells [1]. Moreover, it has been shown that characteristic relationships exist between the rate of growth and the macromolecular composition of the cells [2] and in particular that the number of ribosomes per genome and the percentage of ribosomal proteins over total protein increase markedly with increasing growth rate [2]. As the control of ribosome synthesis plays a key role in determining the growth rate, it is very interesting to study how nutrients affect the synthesis of ribosomal RNA. During nutritional transition of the carbon source, the primary response is at the level of the rate of the net rRNA synthesis that almost stops during the shift-down transition [3] and is quickly stimulated during a shift-up transition [4]. The molecular mechanisms linking these environmental changes with the cell response are unknown and metabolic "signals" like the guanosine nucleotides, guanosine-3′-diphosphate, 5′-diphosphate and the Phantom Spot, that play a role in the regulation of rRNA synthesis in bacteria [5], have not been found in *Neurospora* cells [6, 7]. Moreover, when the minimal media are supplemented with casaminoacids a marked increase of the rate of exponential growth of *Neurospora* cultures has been shown [1, 2] and,

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in parallel, an increase in the level of ribosomes and the rate of rRNA synthesis is observed [2].

That the aminoacids play an important role in the regulation of the rate of growth and of stable RNA synthesis is shown also from studies in bacteria [8], in lower eukaryotes such as the yeast *Saccharomyces cerevisiae* [9] and in mammalian cells [10]. Maaløe has hypothesized that the size of amino acid pools indirectly controls the activity of RNA polymerase in *E.coli* [11] by increasing the fraction (Ψ_r) of RNA polymerase molecules that transcribe the rRNA genes, although there is already evidence that the extent of tRNA charging, rather than the endocellular level of amino acids, is involved in the regulation of rRNA synthesis [12]. Thus it would appear to be useful to extend the analysis of the effects of changes of nutrients on the regulation of rRNA synthesis and growth in *Neurospora* by studying the effects of availability of amino acids on these processes.

In this paper preliminary results of a study on the effect of casaminoacids on growth and the stable RNA synthesis in *Neurospora crassa* are presented. We show that the stimulatory effect of casaminoacids is due only to the neutral amino acids and that it is temperature dependent as the stimulatory effect has not been observed at 20 °C. This lack of stimulation at 20 °C does not appear to be due to a permeability block and thus it may reflect a real denaturation of molecular component (s) of the RNA synthetizing machinery.

MATERIALS AND METHODS

Organism and growth conditions.

Wild type strain 74 A (St. Laurence) of *Neurospora crassa* was used for the experiments reported. The growth conditions have been indicated in detail elsewhere [2]. Vogel's mineral medium was supplemented with 2 % (*w/v*) glucose and with the indicated concentration of casein hydrolyzate (vitamin free). The growth was followed as increase of the absorbance at 450 nm. ($A_{450\,mn}$) and the exponential growth rate constant (K , h^{-1}) was determined as previously indicated and automatically computed with a Texas Instrument SR 51 calculator.

Incorporation of radioactive precursor into nucleic acids.

RNA accumulation was studied by measuring [^{32}P]-orthophosphate incorporation; 10 μCi of [^{32}P]-ortophosphate (carrier free) obtained from Amersham, Radiochemical Center, were added to 100 ml culture immediately after inoculation with the conidia. The concentration of KH_2PO_4 in Vogel's mineral medium was lowered to 2 mM [1].

At the time indicated 2 ml aliquots of the cultures were withdrawn and the radioactivity retained in the cold-trichloroacetic acid precipitable material was determined as described previously [3].

Chemical determination of RNA and DNA.

200 ml cultures ($A_{450\,mn}$ 0.3–0.5) were collected on fiber glass filters (Whatman GF/A) by suction and the determinations were performed as previously described with the standard orcinol and diphenilamine methods [2].

Determination of total aminic nitrogen pool.

200 ml volumes of culture ($A_{450\,mn}$ about 0.3) were collected on fiber glass filters (Whatman GF/A) by suction, washed with cold distilled water, then mycelial pads were resuspended in 3 ml of cold 5 % TCA and placed in ice. After 30 min. the samples were centrifuged at 3500 rpm on a MSE centrifuge for 10 min. 0.1 ml aliquots of the supernatant were used for the ninhydrin reaction according to the method of Moore and Stein [13]. Pure L-leucine (Sigma) was used to obtain calibration curves for each determination.

Measurement of the intracellular pool of single amino acids.

The intracellular pool sizes of several amino acids were measured by their reaction with [^{3}H]-dansyl chloride (5[methyl-^{3}H]-dimethylamino-1-naphthalen sulphonyl chloride) followed by separation of the dansyl-amino acid derivatives on micropolyamide coated plastic sheets according to the method of Hartley [14]. 30 ml volumes of culture ($A_{450\,nm}$ about 0.4) were collected on fiber glass filters (Whatman GF/A), washed with cold distilled water, and extracted with 4 ml of 75 % (*w*/*w*) ethanol for 30 min in ice. The ethanolic extracts were evaporated to dryness and resuspended with 2 ml of 0.05 M $NaHCO_3$. A 0.4 ml aliquot of the bicarbonate solution was mixed with 0.1 ml of [^{3}H]-dansyl chloride (2 mg/ml, specific activity 100 Ci/mole) in acetone and the amount of each amino acid was determined as described previously [15].

Results and Discussion

The addition of 1 % casaminoacids to the glucose minimal medium causes a marked increase in the rate of exponential growth and the content of stable RNA in *Neurospora* cultures at 30 °C [2–16] (Table I).

The effect is obtained with very low initial concentrations of the casaminoacids: in fact there is no difference in the stimulated growth rate over a large range of concentrations (from 2 to 0.005 % (*w*/*v*)) of casaminoacids added (Table III). By using different mixtures of amino acids in which the proportion of each amino acid is the same as that present in the casein hydrolyzate [17] we have found that only the mixture of neutral amino acids is able to stimulate growth, like the caseine hydrolyzate, while mixtures of acidic or basic amino acids have no effect on the growth rate (Table III).

TABLE I

Medium	Temperature	Growth rate [a] K (h^{-1})	RNA/DNA [b] (w/w)
glucose	30°	0.350 ± 0.05 (35)	35.5 ± 2.5
glucose + casamino acids	30°	0.450 ± 0.02 (12)	44.9 ± 1.9
glucose	20°	0.198 ± 0.06 (8)	36.6 ± 2.3
glucose + casamino acids	20°	0.208 ± 0.08 (8)	36.8 ± 3.4

Constant of the growth rate and RNA/DNA ratios of *Neurospora crassa* cultures growing in glucose and glucose supplemented with 1% of casamino acids at 30 and 20 °C.

(*a*) the average value and standard errors are given. The number of determinations for each growth condition is given in brackets.

(*b*) the average values and standard deviations are given. The values are the average of six determinations.

TABLE II

Concentration of casamino acids (*w/v*)	Growth rate K (h^{-1})
2%	0.450
1%	0.455
0.5%	0.469
0.1%	0.455
0.05%	0.464
0.03%	0.448
0.01%	0.498
0.005%	0.525

Rate of growth of Neurospora crassa cultures in glucose medium supplemented with different concentrations of casamino acids at 30 °C.

TABLE III

medium	Growth rate (K , h^{-1})
glucose	0.36
glucose + casamino acids (0.1%)	0.48
glucose + neutral amino acids [a]	0.47
glucose + basic amino acids [b]	0.37
glucose + acidic amino acids [c]	0.38
glucose + basic and acidic amino acids	0.37

Rate of growth of *Neurospora crassa* cultures in glucose medium supplemented with different mixtures of amino acids at 30 °C.

(*a*) Neutral amino acids: leucine, isoleucine, valine, serine, threonine, glycine, methionine, cysteine, phenylalanine, tyrosine, tryptofane, proline, alanine.

(*b*) Basic amino acids: arginine, lysine, histidine.

(*c*) Acidic amino acids: aspartic acid, glutamic acid.

This shows that not all the twenty amino acids are required for the stimulatory effect in glucose. When cultures of *N. crassa* in glucose plus casaminoacids (1 %) medium are grown at 20 °C, the rate of growth is not significantly different from that of the cultures growing in glucose minimal medium at the same temperature (Fig. 1). Under these conditions the content of stable RNA,

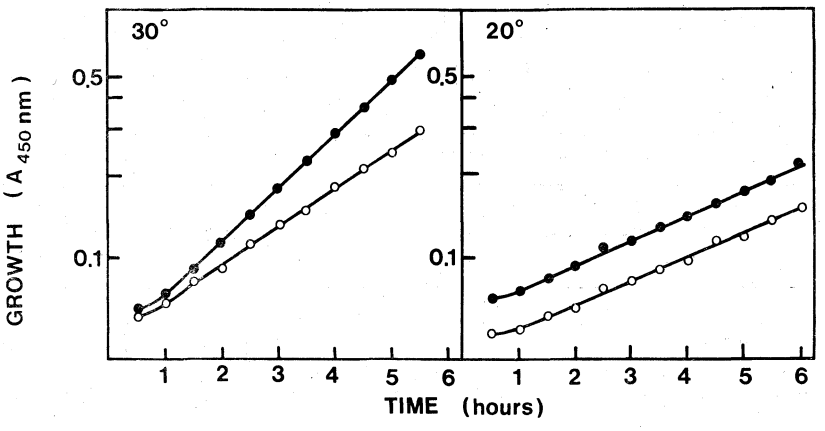

Fig. 1. – Growth of *Neurospora crassa* cultures in glucose minimal medium and in glucose + 1 % casaminoacids at 30 °C and 20 °C.
Absorbance at 450 nm of glucose minimal cultures (○) and of cultures supplemented with 1 % of casamino acids (●).

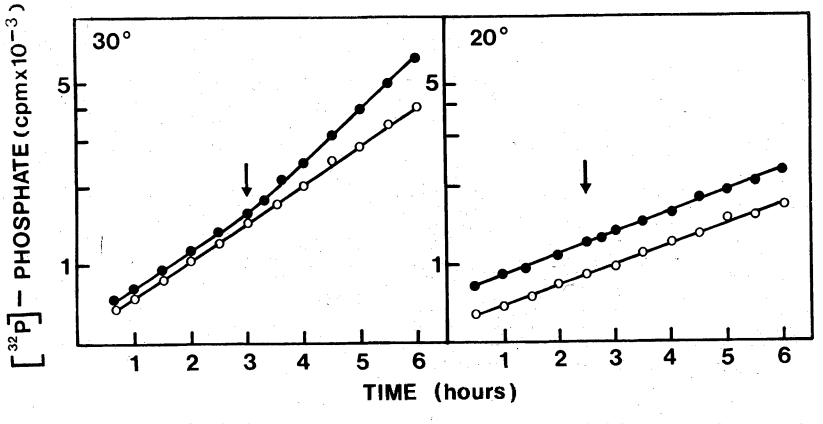

Fig. 2. – Net RNA synthesis in *Neurospora crassa* after the addition of 1 % casamino acids at 30 °C and 20 °C. Net RNA synthesis was determined by measuring ^{32}P incorporation. [^{32}P]–orthophosphate (2 mM final concentrations, specific activity 0.05 Ci/mole) was added to the culture medium before inoculation of conidia. (○) control cultures; (●) cultures supplemented with 1 % of casamino acids at the time indicated by the arrows. The shifts were made by dilution as indicated previously (1).

expressed as RNA/DNA ratios, is not different in the two conditions and it is similar to the level found in the cultures grown in glucose at 30 °C (Table I): the lack of growth stimulation at 20 °C is coupled with a lack of stimulation of stable RNA accumulation and the cell composition is the same with and

without the addition of casaminoacids confirming the strict correlation between growth rate and RNA contents. This aspect is better evidenced by the experiment reported in Fig. 2 in which we measured the accumulation of stable RNA (^{32}P labeled) after the addition of casaminoacids (1 %) to exponentially growing cultures in glucose minimal medium at 30 °C and at 20 °C.

As can be seen a shift-up transition with a marked increase both in the rate of growth and in the rate of RNA accumulation is observed at 30 °C, while no stimulation is shown at 20 °C.

TABLE IV

Growth conditions	amino nitrogen pool $nM/A_{450\,mn}$
glucose at 30 °C	88.6 ± 20
glucose + casamino acids 30 °C	120.8 ± 19
glucose at 20 °C	57.7 ± 16
glucose + casamino acids 20 °C	95.6 ± 16

Total aminic nitrogen pool in *Neurospora crassa* mycelia.

The total aminic nitrogen pool is expressed as nMoles of nitrogen per A_{450} unit of culture. The results are the average of four independent determinations and the standard deviations are given. The concentration of casamino acids was 1 % *w/v*.

This lack of stimulation could be due to a limitation of the uptake of external amino acids at 20 °C. In order to check this hypothesis we measured first the total aminic nitrogen pool with the ninhydrin method (Table IV) and then, in a more accurate way, the pool size of several amino acids, with and without amino acids, both at 30 °C and at 20 °C, as indicated in Table V. While the total aminic nitrogen pool largely swells in the presence of casaminoacids both at 30 °C and at 20 °C, the assay of the pool sizes of amino acids shows only a moderate increase at 30 °C, as well as at 20 °C. The increase of pool size is not even since the level of some amino acids (Leu, Ileu, Val, basic) rises much more than that of others and, for instance, the increase of the valine pool is very marked especially at 30 °C.

These data seem to rule out the possibility that at 20 °C a restriction of amino acid uptake occurs and suggest that the lack of stimulatory effect may be directly due to a reversible denaturation of molecular component (s) acting in the control of the stable (mainly ribosomal) RNA synthesis in *Neurospora*.

Studies are now in progress on the molecular elements involved in this regulation.

TABLE V

Amino acids	Amino acid level (nMole/$A_{450\,nm}$ unit of culture)			Glucose + casamino acids 20 °C
	Glucose 30 °C	Glucose + casamino acids 30 °C	Glucose 20 °C	
Leucine	0.38	1.10	0.27	0.81
Isoleucine	0.24	0.58	0.25	0.23
Phenylalanine	0.28	0.33	0.24	0.28
Valine	0.65	4.37	0.72	1.60
Proline	0.65	0.82	0.43	0.32
Alanine	8.20	8.77	8.50	10.15
Glycine	2.28	2.18	1.71	2.45
Lysine+Arginine+Histidine	5.76	7.73	7.69	7.57

Amino acid levels in *Neurospora crassa* mycelia during exponential growth on glucose and on glucose + 1 % casamino acids at different temperatures.

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