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Ribosomal RNA synthesis: A possible regulatory mechanism

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Biochimica comparata. — *Ribosomal* RNA *synthesis: A possible regulatory mechanism* (*). Nota (**) di FILIPPA A.M. ALBERGHINA, presentata dal Corrisp. E. MARRÈ.

RIASSUNTO. — Viene discusso un meccanismo di regolazione della sintesi di rRNA in *Escherichia coli*, che è suggerito dalle indicazioni derivate da un modello dinamico della crescita cellulare precedentemente discusso. Si suggerisce che la velocità di sintesi di rRNA sia modulata dai nutrienti i quali modificherebbero l'equilibrio della reazione che dà origine al fattore di trascrizione ψ, la cui disponibilità condiziona la velocità di sintesi di rRNA. Il fattore ψ sarebbe in equilibrio con la forma libera del fattore di allungamento della sintesi proteica TuTs, la cui concentrazione è funzione inversa del livello cellulare di ribosomi. Questo meccanismo consentirebbe alle cellule di modulare la velocità di sintesi di rRNA secondo il nutriente disponibile e l'attuale livello cellulare di ribosomi. Le cinetiche sperimentali per la sintesi di rRNA durante le transizioni nutrizionali vengono giustificate da questo meccanismo.

In some previous papers I have proposed a model for the regulation of cellular growth, in which the three major macromolecular syntheses occurring in a cell are considered: those of DNA, rRNA and protein, and is suggested the way in which they are interlocked and reciprocally controlled [1, 2, 3]. This model has been shown to be able to predict with accuracy the growth kinetics of cells of various organisms in different growth conditions [1, 2, 3, 4]. Of course these results are not the proof that the model indicates the actual regulatory mechanisms of the macromolecular syntheses. A critical test in this sense would be to identify at the molecular level the transducers, the signals and the comparators predicted by the model.

For example the synthesis of rRNA is indicated by the model to be under a negative feed-back control, in which the reference input is a function of the nutrient available to the cell and for which more ribosomes are allowed to be made only when the actual level of ribosomes in the cell is below the value set by the reference input. Thus the molecular mechanism which regulates the synthesis of rRNA should be able to adjust the rate of rRNA synthesis to the nutrients and to " count " the ribosomes already present in the cell.

As will be discussed in this paper, at least in the case of fairly simple cells (bacteria), there is enough information to make a hypothesis on a possible molecular mechanism for the control of rRNA synthesis.

E. coli cells in each steady state of balanced exponential growth have a characteristic rate of rRNA synthesis [5]. Except for minor adjustments this rate is a function of the ability of the RNA polymerase to initiate tran-

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scription of the rDNA genes [5], and there being only one kind of RNA polymerase present in bacteria [6], this ability depends upon the amount of a transcription factor (ψ) which makes the RNA polymerase molecules able to transcribe rDNA. The ability of the RNA polymerase of *E. coli* to shift from making mRNA to make rRNA has been put in evidence [7]. The protein elongation factor TuTs has been shown to substitute ψ, and to stimulate preferentially *in vitro* the synthesis of rRNA [8], [9].

A SUGGESTED REGULATORY MECHANISM

The rate of rRNA synthesis in *E. coli* could be adjusted to both the nutrients available to the cell and to the actual level of ribosomes (R), if one hypothesizes the following reactions:

$$(1) \qquad \text{TuTs (bound to R)} \underset{a}{\rightleftarrows} \text{TuTs} \underset{b}{\rightleftarrows} \text{TuTs}^* \ (= \psi) .$$

Assuming the amount of the total TuTs per genome to be fairly constant at different rates of growth, as indicated by a revaluation of the data of Gordon [10], the amount of free TuTs decreases by increasing the R level (reaction *a*), offering therefore a simple way of " counting " the ribosomes present in the cell.

On the other hand each nutritional condition will set a specific equilibrium value for reaction (*b*), the value of [TuTs*]/[TuTs] being higher for higher growth rates. The elongation factor in the TuTs* form corresponds to ψ.

To have an initial evaluation of the validity of the hypothesis given in equation [1], let us consider the kinetics of rRNA synthesis observed during nutrient transitions.

In shift-down the rate of rRNA synthesis just following the exhaustion of the rich medium is lower than that specific for the new nutrient [11]. If the poor nutrient sets a lower value for the (*b*) equilibrium, just at the beginning of the shift-down, while the (R) level will be higher than that characteristic for the new nutrient, this would reduce the rate of rRNA synthesis. In fact the higher (R) level will trap in reaction (a) more TuTs than it should under the new steady state condition and it will follow that less TuTs* would be present under the equilibrium condition proper for the new medium. So the rate of rRNA synthesis will be lower than that expected for the growth in the new medium.

The reverse (increase of the (b) equilibrium following the addition of a rich nutrient, while a larger amount of TuTs is free, the (R) level being lower than that characteristic for the new medium, due to the previous growth in the poor medium) will account for the rate of rRNA synthesis enhanced over that of the new medium as observed in shift-up [5].

Finally the following circumstantial evidence seems to support the indicated regulatory mechanism.

E. coli cells growing at low concentrations of fusidic acid in a given medium have a lower growth rate but a higher RNA content and faster synthesis of stable RNA species than the control cells [12]. Fusidic acid prevents aminoacyl–tRNA–EF·Tu binding the ribosomes [13, 14, 15]. These findings may be interpreted as follows: fusidic acid interfears with reaction (*a*) leaving more TuTs free than in the corresponding normal growth. The equilibrium value of reaction (*b*) is set by the nutrient; in the presence of fusidic acid, the concentration of TuTs being higher, a larger amount of TuTs* will be made than in the control cells, therefore explaining the higher stable (ribosomal) RNA content and the rate of synthesis of the treated cells.

Experiments aimed at testing the validity of the hypothesis are now under way in our laboratory.

REFERENCES

[1] F. A. M. ALBERGHINA (1974) – « Rend. Acc. Naz. Lincei », *56*, 971–975.
[2] F. A. M. ALBERGHINA (1975) – « BioSystems », *7*, 183–188.
[3] F. A. M. ALBERGHINA (1975) – « J. Theoret. Biol. », *55*, 533–545.
[4] F. A. M. ALBERGHINA and E. MARTEGANI (1975) – Manuscript in preparation.
[5] N. O. KJELDGAARD and K. GAUSING (1974) – In the Ribosomes, editors M. Nomura, A. Tissières and P. Lengyel, Cold Spring Harbor Laboratory.
[6] R. R. BURGESS (1971) – « Ann. Rev. Biochem. », *40*, 711–740.
[7] P. P. DENNIS and H. BREMER (1974) – « J. Mol. Biol. », *89*, 233–239.
[8] A. TRAVERS (1973) – « Nature », *224*, 15–18.
[9] A. TRAVERS (1974) – In the Ribosomes, editors M. Nomura, A. Tissières and P. Lengyel, Cold Spring Harbor Laboratory.
[10] J. GORDON (1970) – « Biochemistry », *9*, 912–917.
[11] R. B. HARSHAM and H. YAMARAKY (1971) – « Biochemistry », *10*, 3980–3982.
[12] P. M. BENNET and O. MAALØE (1974) – « J. Mol. Biol. », *90*, 541–561.
[13] N. RICHMAN and J. W. BODLEY (1972) – « Proc. Nat. Acad. Sci. USA », *69*, 733–736.
[15] E. CUHDLIFFE (1972) – « Bioch. Biophys, Res. Comm. », *46*, 1794–1801.