Alberghina, L. and E. Martegani.

Modeling Neurospora growth.

Hershko et al. (1971 Nature New Biol. 232:206) proposed the name "pleiotypic response" to indicate the program of events which respond in a coordinate way to environmental changes affecting the rate of growth. The coordination between the various metabolic functions (synthesis and degradation of RNA and of proteins, DNA replication) is hypothesized to be mediated by changes of the levels of small molecules. More recently Tomkins (1975 Science 189:760) suggested that the complex regulatory mechanisms which control cell proliferation may include two components: the metabolic "symbol" and its "domain". The "symbol" is a specific intracellular effector molecule which changes level when the cell is subjected to a modification of environment. The "domain" of a "symbol" is the whole set of metabolic functions controlled by the "symbol". These functions are related by the biological effects they provoke and not by the similarity of their chemical mechanisms. In this way, the cell may behave like a system, i.e. a set of physical components connected by strong interactions which acts as a unit. It may be useful to construct a model that identifies the elements of the system and gives the mathematical relationships between them, thus allowing us to describe known properties, to predict dynamics of the system and eventually to envisage new properties by exploiting the logic intrinsic to the model.

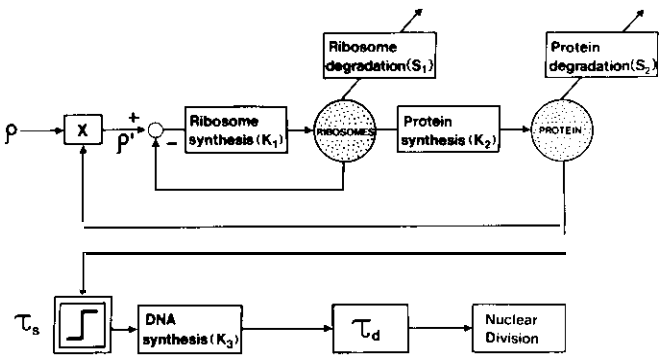
A dynamic model of growth of cell populations has been developed by one of us (Alberghina 1974 Rend. Acc. Naz. Lincei 56:971 and 1975 BioSystems 7:183) taking into consideration the findings on the biochemistry of growth of Neurospora (Alberghina et al. 1975 J. Biol. Chem. 250: 4381; Sturani et al. 1973 Biochim. Biophys. Acta 319:153).

Although very simple in its structure the model has been shown to be able to account for the dynamics of steady states of growth of microbial cells (Alberghina 1974 Rend. Acc. Naz. Lincei 56:971 and 1975 BioSystems 7:183) and, with a minor modification, of transitory states of growth (Alberghina and Martegani 1976 Cybernetica 19:229). The following relationship between various parameters of growth (derived as the analytical solution of the model) has been proposed to hold in steady states of growth:

$$\lambda = K_2 \rho - 1/S_2 \qquad [1]$$

λ being the time constant of exponential growth as $|min^{-1}|$, K_2 the average ribosomal activity, expressed as number of amino acids (polymerized), min^{-1} ribosome^{-1}; ρ the level of ribosomes per unit amount of proteins, expressed as number of ribosomes, amino acids^{-1}; S_2 the time constant of the degradation equation for protein, expressed as |min|.

Figure 1 - The model



The level of ribosomes is controlled by two feedback loops. ρ (the number of ribosomes required by a unit amount of proteins) is a reference input of a sensor which detects also the actual level of proteins in the cell. It yields a second reference input (ρ^1) which indicates the number of ribosomes which should be present, given that the protein level in the cell is that estimated by the sensor. A negative feedback compares ρ^1 with the actual (R) level and acts to increase the (R) level as required. The rate of protein synthesis given by the action (K_2, average ribosome efficiency) of the ribosomes (R) present in the cell. The levels of ribosomes and of proteins may be depleted by the rates of degradation (time constants of the negative exponential equations respectively S_1 for ribosomes and S_2 for proteins). DNA replication starts when the level of proteins becomes larger that τ_s, it proceeds at linear rate (K_3) to completion (K_3^{-1} min later). Nuclear division takes place at the end of a τ_d period which starts at the end of DNA replication.

The model may be used as a tool to better understand the molecular mechanism underlying the control of growth, if it is developed to account for the discrete events in the replication of DNA. Fig.1 presents such a development of the model. The presence of a threshold that controls the replication of DNA divides the system into two subsystems, one the "master", whose state variable are ribosomes and proteins turns on the switch, the second, "the slave", is the subsystem which responds to the switch. The dynamics of the entire system is determined only by that of the "master" subsystem in accord with equation [1] in which only parameters of the first subsystem are present.

In Neurospora the studies on the second subsystem face several problems due to hyphal growth, unusual nuclear division (Weijer et al. 1965 Can.

J. Genet. Cytol. 7:140; Fuller 1976 Int. Rev. Cytol. 45:113) and to the ensuing difficulty to estimate the length of the replicative phase ($1/K_3$ in the model). Instead the first subsystem, the determinant for the dynamics of growth, is easier to describe. We have measured by biochemical methods the values of its parameters in a steady state condition of growth.

Neurospora crassa (74 A) conidia were inoculated in liquid Vogel's medium containing 2% glucose and incubated at 25° C in a reciprocal shaking water bath. The following experiments were done during early exponential growth (A_{450} of the culture 0.3 - 0.4).

For the determination of the level of ribosomes per unit amount of protein (ρ) we determined (a) the amount of RNA and of protein per unit amount of culture (b) the percentage of total RNA that is ribosomal RNA by using techniques previously described (Alberghina et al. 1975 J. Biol. Chem. 250:4381). The value of ρ was calculated by slight modification of the calculation procedure reported in the same paper.

The rate of degradation of protein (S_2) was determined by pulse labelling the culture with L-[^{14}C-carboxyl]-leucine (50 Ci/mole, 10^{-7} M final concentration) for 30 min. When almost all the radioactivity was incorporated into protein a chase was performed with ^{12}C-leucine (final concentration 10^{-4} M). Stability of the proteins was measured by determining the radioactivity remaining as hot TCA precipitable material at various times after the chase.

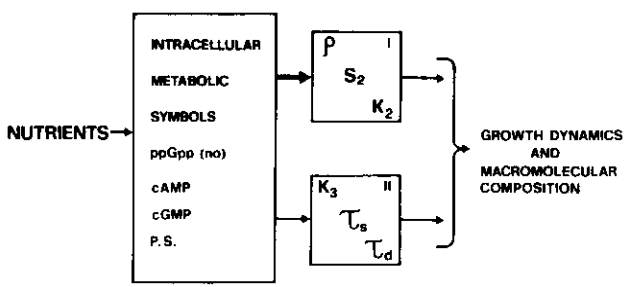
The rate of incorporation of ^{14}C-leucine into protein by a A_{450} unit amount of culture was measured under conditions that were shown to assure rapid equilibration (less than 10 min) of the specific activity of the internal leucine pool and of leucil-tRNA pools with that of the exogenously furnished leucine. From the rate of leucine incorporation and knowing its frequency in Neurospora proteins (Rho and DeBusk 1971 J. Bacteriol. 107: 840) the rate of protein synthesis per unit of culture was calculated. The average ribosomal activity was then estimated by dividing the rate of protein synthesis by the number of ribosomes determined per unit of culture.

The values for the various parameters obtained in this way are: $\rho = 1.48 \times 10^{-5}$; $S_2 = 6000$; $K_2 = 340$. From them a value of 4.86×10^{-3} is calculated for λ in eq. [1] compared with an experimental 4.88×10^{-3}.

The experimental verification of equation [1] we have presented indicates that the model is self-consistent, but of course it does not prove the validity of all the elements or their relationship in the model. Nevertheless we may tentatively assume that in a given nutritional condition the dynamics of growth of Neurospora is determined by the values of the parameters of the first subsystem.

The search may thus begin to identify which "symbol(s)" controls each "domain". Guanosine-5'-diphosphate, 3'-diphosphate (ppGpp) has been suggested as the "symbol" that in bacteria controls the rate of accumulation of rRNA (connected to the ρ parameter of the model). In Neurospora we have shown that rRNA accumulation may be drastically reduced during a shift-down without formation of ppGpp (Alberghina et al. 1973 Biochim. Biophys. Acta 312:435). Recently a novel nucleotide (a modified GTP, called Phantom Spot, Gallant et al. 1976 Cell 7:75) has been implicated in the regulation of rRNA accumulation in E. coli. Studies are now under way in our laboratory to see whether it is present in Neurospora. Cyclic nucleotides (cAMP and cGMP) have been indicated as possible "symbols" (Tomkins 1975). Evidence is available that they occur in Neurospora mycelia and that they change level during the growth of cultures (Pall 1976 Neurospora Newsl. 23:10; Mishra 1976 Naturwissenschaften 63: 485). Preliminary experiments in which we attempted to modify the level of cAMP indicate that the rate of protein degradation (related to the S_2 parameter of the model) is not modified by changes in the level of cAMP. At present our working hypothesis of how nutrients affect growth dynamics of Neurospora is presented in fig. 2. The advantage of presenting a model now is the possibility it offers for interpreting a large number of heretofore unrelated information. Neurospora would appear to be a useful eukaryotic organism with which to investigate the complex regulation of growth.

Figure 2 - Hypothesis for the regulation of growth by nutrients in Neurospora



Nutritional conditions change the intracullular levels of regulatory molecules: the "symbols" which modulate the rates of the various processes of the I and the II subsystem (see text). The dynamics of growth is set by that of the subsystem I of whose parameters nutrients in steady states of growth, primarily affect ρ. The dynamics of the subsystem II relevant for determining macromolecular composition seem to be less affected by nutritional conditions.

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