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The Macronuclear Envelope of *Tetrahymena Pyriformis GL* in Different Physiological States

V. Nuclear Pore Complexes — A Controlling System in Protein Biosynthesis?

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Summary. A simple formula is derived to calculate the nucleocytoplasmic RNA-Efflux per nuclear Pore complex per min (REP-rate) which is generally applicable both for "growing" and "stationary" eukaryotic cells. In actively growing cells this REP-rate is mainly dependent on the cytoplasmic RNA-pool, the number of RNA-transporting pores, and the growth constant of RNA. These parameters are determined in logarithmic *Tetrahymena pyriformis GL*. In this organism, 45 molecules both of the larger ribosomal RNA (25s rRNA) and of the smaller (17s rRNA) are transported per pore per min from nucleus to cytoplasm. "Pulse-label" experiments with ^{3}H-uridine indicate that the 25s rRNA is obviously transferred more slowly to the cytoplasm than the 17s rRNA. We postulate a "gating hypothesis" on the regulation of the nucleocytoplasmic RNA-efflux by nuclear pore complexes. This gating hypothesis suggests that nucleopores are controlling points of secondary importance in the sequence of gene expression, and do not directly control the cytoplasmic protein synthesis in eukaryotic cells.

In most eukaryotic cells, the transfer of ribonucleoproteins from the main compartment of transcription (nucleus) to the main site of translation (cytoplasm) proceeds principally through the pore complexes of the nuclear envelope (*cf.* reviews: [20, 26, 70]). This RNA-transfer seems to be not only a simple flow but an active transport process [65]. This suggestion is mainly based on three observations: *first*, the nucleocytoplasmic RNA-transfer proceeds against a concentration gradient; *second*, the transfer of at least mRNA is facilitated by ATP [30–32]; and *third*, the nuclear pores are sites of ATPase activity [34, 84, 86]. Moreover, nuclear pore complexes do not constitute open "holes" between nucleus and cytoplasm, but offer an appreciable

electrical resistance [48, 49, 77] and include several characteristic substructures, e.g., the "annulus" and the "centralogranule" (*cf.* reviews: [15, 20, 26, 59, 70]). The nuclear pore complexes are therefore thought to regulate the nucleocytoplasmic RNA-efflux and hence conceivably cytoplasmic protein synthesis (e. g., [19, 56, 65]).

However, this postulated function of nuclear pores has not yet been evaluated quantitatively, nor has anyone proposed a specific concept as to possible regulatory mechanisms in nuclear pores. Some conclusions about such possible control mechanisms might be drawn from comparative, high resolution electron-microscopic studies of nuclear pores in correlation with kinetic analyses of the nucleocytoplasmic RNA-transfer, i.e., how quickly do different RNA-species emerge in the cytoplasm and how many molecules of the various RNA-species are transported per pore per min [80].

We have accordingly performed quantitative electron-microscopic studies of nuclear pore morphology in correlation with quantitative analyses of nucleocytoplasmic RNA-efflux, using the ciliate protozoan *Tetrahymena pyriformis GL* [67, 78–82]. This organism is particularly suited for such studies, since the nuclear envelope does not break down during macronuclear division, the RNA-transfer might proceed through pores exclusively [80], and the RNA-metabolism has already been well investigated in several laboratories (e.g., [6, 7, 35–38, 40–45]).

Our studies, together with work by other authors, have lead us to a working hypothesis which is also in good accordance with several hitherto enigmatic observations in other eukaryotic cells.

Materials and Methods

Tetrahymena pyriformis amicronucleate strain *GL* in the steady state logarithmic growth phase (20,000 to 70,000 cells/ml; generation time = 150 min) were used throughout. The cells were fixed with a mixture of 0.1 % methylgreen and 5 % acetic acid. Then, the diameters of 50 macronuclei were measured at 400-fold magnification by phase-contrast microscopy. Each diameter is an average of two measurements made in two opposite directions at a right angle to each other. The number of nucleopores per μ^2 of nuclear envelope have previously been determined by "freeze-etch" electron microscopy [67]. The percentage of nucleopores containing a centralogranule is a mean value of thin-sectioning and negative-staining data published previously [79, 80, 82]. The cytoplasmics pools of the 25s rRNA and 17s rRNA were determined [80], according to the method of Leick and Andersen [42]. The molecular weights of the 25s rRNA and 17s rRNA were calculated from the respective s-values [57] using Spirin's formula [68].

Results and Discussion

REP-Rates of Different rRNA-Species in Logarithmic Tetrahymena

The nucleocytoplasmic RNA-Efflux per Pore per min (REP-rate) of an average logarithmic *Tetrahymena* cell was calculated according to the following formula, which is generally applicable both for growing and stationary eukaryotic cells:

$$\text{REP-rate} = \frac{C_t}{P_t}(a+k) \qquad (\text{RNA-molecules} \times P_t^{-1} \times \text{min}^{-1}), \qquad (1)$$

where C_t = cytoplasmic pool of RNA at time t, P_t = number of nucleopores transporting RNA at time t, a = logarithmic growth constant of RNA, and k = decay constant of RNA.

These parameters were determined as follows:

C_t: Only the 25s rRNA and 17s rRNA are considered which are the main RNA-constituents of the large and small ribosomal subunits, respectively, in *Tetrahymena pyriformis GL*. The amount of rRNA per average logarithmic cell is 0.24 ng [42], mainly composed of the rRNA-species mentioned above. The cytoplasmic pool of the 25s rRNA and 17s rRNA per average logarithmic *Tetrahymena* cell was determined to be 7.13×10^7 molecules and 7.24×10^7 molecules, respectively [42, 80].

P_t: The mean diameter of *Tetrahymena* macronuclei is 10.9 μm. Assuming a spherical surface, the total nuclear envelope area of an average macronucleus is 370 μ^2. One μ^2 of the nuclear envelope contains 39 ± 9 pores. Therefore, an average macronucleus possesses 14.4×10^3 pores in the steady state logarithmic growth phase of *Tetrahymena*. Though all these pores might be potentially capable of transporting RNA, it seems unlikely that all pores of one nucleus transport RNA effectively at a definite time. However, it is difficult to determine the pores effectively transporting RNA. Thus, we assume that only these pores with a central granule[1] are involved in RNA-transfer though the number of pores effectively transporting RNA must be still lower [80, 82]. This assumption is mainly based on the following: macromolecules, including RNA, are transported from nucleus to cytoplasm through a central channel in the pore (*cf.*, [5, 13, 14, 27, 39, 51, 69, 71, 72, 74, 83, 85]). Therefore, most of the central granule is thought to represent a dynamic structure, i.e., ribonucleoprotein-material at a transitional bound to the permanent pore material during its nucleocytoplasmic passage (*cf.* [1, 2, 19, 61, 64, 75, 79, 80]). Such a dynamic character is further supported by the correlation, in different organisms, between nuclear activity and the number of pores with a centralogranule [10, 19, 55,

1 For definition of central granule *see* ref. [82].

62, 79]. Furthermore, the centralogranule material represents mainly ribonucleoprotein as can be observed by different methods (*cf.* [4, 8, 9, 17, 52, 53, 58]). Since approximately 52% of nucleopores of *Tetrahymena* macronuclei contain a centralogranule, it can be calculated that 7.5×10^3 nucleopores per average macronucleus are involved in RNA-transport in the steady state logarithmic growth phase of *Tetrahymena.*

a: The logarithmic growth constant of RNA was assumed to be $4.62 \times 10^{-3}\ \text{min}^{-1}$.

k: Since $dt = 1$ min, k can be neglected in this actively growing cell system [42].

According to Eq. (1) we calculated that 45 molecules of both 25s rRNA and 17s rRNA are transported per average pore per min during the steady state logarithmic growth of *Tetrahymena.*

These REP-rates are higher than those of other eukaryotes; in HeLa-cells, liver cells and amphibian oocytes about 1 to 3 molecules are transported per pore per min [16, 18]. However, such values might be too low since *all* nuclear pores per nucleus were assumed to participate. Moreover, this number of nucleopores was calculated from isolated, negatively-stained nuclear envelopes; this approach might give too high a pore number because of shrinkage artefacts [67]. Nevertheless, a difference in the REP-rates is expected because of a much higher overall cell activity of *Tetrahymena* compared with that of these other eukaryotes.

Kinetic Analyses of the Nucleocytoplasmic rRNA-Efflux in Logarithmic Tetrahymena

Such analyses were already performed independently by Leick [40, 42, 45] and Kumar [35, 36, 38]. Pulse-label experiments with ^{3}H-uridine and subsequent characterization of the phenol-extracted cytoplasmic RNA on continuous sucrose gradients indicate clearly that the nucleocytoplasmic transfer of the larger 25s rRNA is slower than that of 17s rRNA in *Tetrahymena* cells, as in most other eukaryotes ("All the eukaryotes seem to have in common ... the rapid transport of the smaller ribosomal subunit to the cytoplasm ..."[2]. We have confirmed this in the same *Tetrahymena* cells [80].

Hypothesis

These considerations and observations have led us to postulate the following preliminary gating hypothesis for nuclear pore complexes.

2 Loening, U. G., 1970 [ref. 47]; *cf.* also reviews: [11, 21–23, 50, 60].

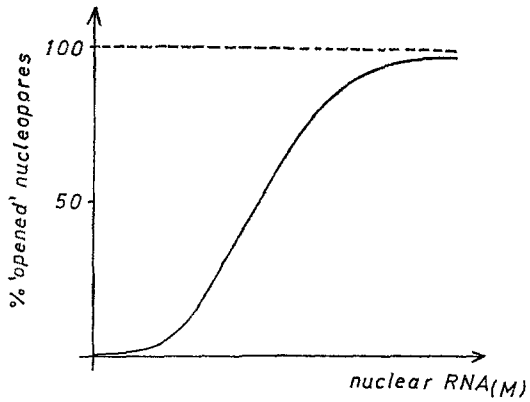

Fig. 1. Diagram of the hypothetical dependence of the percentage of "opened" nucleopores, i.e., pores effectively transporting RNA at a definite time, from the nuclear pool of the respective "cytoplasmic" RNA-species, i.e., nuclear RNA-species to be transported to the cytoplasm

The transport of the different cytoplasmic RNA-species from nucleus to cytoplasm through nuclear pores proceeds nearly in an all-or-none manner; i.e., nuclear pores exhibit different critical thresholds for differently sized RNA-species. At the molecular level this may be interpreted by co-operative effects of the permanent pore material, which seems to be proteins possibly complexed with some lipids. Nucleopores are normally "closed", i.e., the permanent pore material exhibits a "closed" conformation. At such "closed" nucleopores a large amount of large RNA-species and a small amount of smaller RNA-species have to accumulate in order to trigger a specific opening process, i.e., the "closed" conformation of the permanent pore material changes into an "opened" conformation. This permits that a definite and equal amount of the large and small rRNA-species are transported to the cytoplasm per time-unit. In general, the number of opened pores, i.e., pores effectively transporting RNA, per average nucleus is proportional to the nuclear pool of the respective "cytoplasmic" RNA-species, as it is schematically represented in Fig. 1.

In this connection it is interesting to arrive at a rough idea of the amount of RNA which is necessary to trigger the postulated opening process. We have estimated the ratio of the nuclear rRNA-molecules to the nuclear pores with centralogranule in one average macronucleus during the steady state logarithmic growth phase of *Tetrahymena* and compute that about 230 molecules of 25s rRNA and 80 molecules of 17s rRNA are necessary to trigger the opening process for these rRNA's [42, 80].

Since nuclear pores are involved in polysome formation [3, 4, 17, 54, 80, 82] one can assume that different RNA-species are transported through the same pores. Further, assuming that the above computed amount of the two different rRNA-species are accumulated *in* one nucleopore an average opened pore would consequently have at least an equivalent molecular weight of about 350×10^6 D. It is noteworthy that this estimate is in the same range as that found by Du Praw and Bahr [12]; these authors determined the molecular weight of pores of honeybee cell nuclei to be in the range between 110×10^6 D and 530×10^6 D. Moreover, the RNA-content per pore in mature amphibian oocytes was determined to be in the range between 1×10^{-16} g to 5×10^{-16} g [62] corresponding to minimum molecular weights of nucleopores of 60×10^6 D and 300×10^6 D.

If this gating hypothesis is correct, in different physiological states of *Tetrahymena*, showing different overall RNA-efflux-kinetics, there should be different percentages of pores effectively transporting RNA per average macronucleus, but always at about the same REP-rates. Indeed, the proportion of pores containing a centralogranule is reduced in *Tetrahymena* cells of heat-synchronized cultures shortly after the heat treatment and, in comparison, is increased during the first, synchronized division maximum [79, 80, 82]; these physiological states are well known for their reduced and increased rates of RNA-synthesis, respectively (*cf.*, [6, 7, 44]). Despite these different physiological states, about 80 rRNA molecules are always transported per pore per min as calculated preliminary [80]; about the same amount of rRNA is transported in logarithmic growing cells.

Furthermore, it can be predicted that stimulation of RNA-synthesis results in a larger amount of RNA appearing in the cytoplasm per time-unit with more pores effectively participating in RNA-transport. Conversely, a reduction or blockage of RNA-synthesis would delay the overall RNA-efflux since fewer pores transport RNA to the cytoplasm till the nuclear RNA-pool falls below a definite minimum. Then, "opening" is prevented and the RNA remains in the nucleus. Indeed, Leick [40], studying the same *Tetrahymena* cells, found that after application of actinomycin *D* the rRNA-transport to the cytoplasm is first delayed and then abruptly stopped. This is also consonant with the findings that actinomycin *D* not only blocks RNA-synthesis but also hinders nucleocytoplasmic RNA-transfer in other eukaryotic cells (*cf.*, [24, 25, 28, 29, 46, 63, 66, 73, 79]). Furthermore, this can also explain, why, after the application of actinomycin, pores containing a central granule remain. For RNA can still accumulate at these pores but cannot trigger RNA-transport, since the nuclear pool of RNA has fallen below the necessary minimum.

The gating hypothesis sheds some new light on the electrophysiological measurements of Loewenstein [48] and Loewenstein *et al.* [49]. It may be that a stimulation of RNA-synthesis increases the electrical resistance of the whole nuclear envelope since the number of pores occupied by RNA rises per nucleus. This is in accordance with the findings that the hormone ecdysone does not only stimulate chromosomal puffings and herewith increased transcription, but nearly simultaneously raises the electrical resistance of the nuclear envelopes in salivary gland cells of *Drosophila* and *Chironomus* [33, 49, 77].

Finally, this gating hypothesis implies that the nucleocytoplasmic RNA-passage is regulated by conformational changes of the permanent pore material and that these are induced by the accumulating RNA's; nucleocytoplasmic RNA-passage is favored to take place through pores where RNA has been already accumulated. However, since such self-regulation of pores depends on the nuclear RNA-pool, the nuclear pore complexes are controlling points of secondary importance in the sequence of gene expression. Consequently, nuclear pore complexes universally distributed among eukaryotic cells do not directly control cytoplasmic protein synthesis.

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