BIOCHEMICAL AND STRUCTURAL ASPECTS OF NUCLEOCYTOPLASMIC TRANSFER OF RIBONUCLEOPROTEINS AT THE NUCLEAR ENVELOPE LEVEL: FACTS AND THESES

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The eukaryotic cell is defined by the existence of a nuclear envelope, that is a perinuclear cisterna, which separates nucleoplasm and cytoplasm. This intracellular compartmentalization membrane is, however, not absolutely necessary to maintain cell viability as is demonstrated by manifold examples of nuclear envelope disintegration during special phases of cell development (e.g., in various spermiogeneses) and cell cycle (e.g., in prometaphase to telophase of "open" forms of mitoses). In most cells, but apparently not in all, the nuclear envelope contains defined and distinct interruptions, the nuclear pore complexes, which would allow for a direct but dimensionally limited nucleocytoplasmic exchange. These pore complexes are highly organized structures which are unique to the nuclear envelope and the cytoplasmic and nucleoplasmic annulate lamellae. Each pore complex consists of two circles each of eight granular elements (100-250 Å in diameter) arranged in a precise radial symmetry on either edge of the intracisternal fenestra (pore), and eight peripheral granules located in the equatorial plane of the pore which conically project from the pore wall into the pore lumen. In addition, one can find a central dense globular or rod-like element (30-300 Å wide) located in the pore center and a variety of extended or coiled fibrils (25-50 Å in width) associated with these granular constituents (for detailed descriptions of pore complex construction see the recent review articles by Franke,

1970, 1974; Roberts and Northcote, 1970, 1971; Feldherr, 1972; Kessel, 1973; Wischnitzer, 1973; Franke and Scheer, 1974a). The internal membrane-to-membrane diameter of the pore lumen appears to be variable from 600 to 1000 Å but is highly constant in a given nuclear type (Gall, 1967; Franke and Scheer, 1970). The frequency of pore complexes strongly varies among different cell types from 0-3/μ^2 to more than 75/μ^2 and, likewise, the relative nuclear surface area occupied by pores can vary from 0-1 % to 30 %. In many cell developmental processes one notes a correlation of pore frequency and total number of nuclear pores with nuclear transcriptional activity. This, however, is not a general rule since, for example, in many fully mature and inactive oocytes and sperm cells the number and density of pores is only slightly reduced, if at all. Likewise, there is no correlation of the frequency of other pore complex structures such as the central elements with nuclear activity.

According to the general concepts of cell compartmentalization the transcription of the three categories of RNA that are involved in protein synthesis takes place within the nucleus whereas they are functioning in translation only in the cytoplasm. Autoradiographic and biochemical evidence has accumulated which indicates that this translocation is strictly vectorial and one way only: such RNA molecules once being in the cytoplasm never come back, in contrast to the class of RNA that shuttles back and forth between both compartments as discussed by Goldstein and associates (for review see Goldstein, 1974). Thus, it is an obvious function of the nuclear envelope to constitute the interphase nucleus as a zone of exclusion for larger cytoplasmic organelles and particles including those involved in translation (polyribosomes). This principle of exclusion of such cytoplasmic components is illustrated with special clarity with some nuclear types of algae and ciliates, the most prominent example being the giant primary nucleus of the green alga Acetabularia. Here a special cisterna, again fenestrated, is formed around the nucleus and encloses an about 100 nm thick perinuclear layer of a plasmatic phase which neither belongs to the nucleoplasm, because of the absence of

nuclear structures, nor to the cytoplasm, because of the absence of mitochondria, plastids, vesicles, ribosomes et cetera.

While the processes of transcription and translation have been elucidated with some success, there exists still a marked lack of experimental evidence as to the modes and pathways of nucleocytoplasmic translocation of the ribonucleoproteins that contain these RNAs, as well as to the possible means of its regulation. The fate of the RNA- or RNP-structures after detachment from their template is unknown. Close attachments of the lateral fibrils in transcriptionally active cistrons, such as in the peripheral nucleoli, have been occasionally observed. There exists, however, in eukaryotes no direct evidence for a membrane-association of the transcriptional complex and the nascent RNPs as this has been described, for example, for mRNA and rRNA synthesis in the prokaryote, Escherichia coli (for references see Gierer, 1973). At least in the case of rRNA, it has been shown that the newly formed RNP molecules are not existent as distinct isolate entities but rather are integrated into larger structural complexes consisting of particles connected by fine filaments (e.g. Simard et al., 1973). It has been hypothesized by many authors that the nuclear pore complexes provide the major, if not exclusive, gateways for nucleocytoplasmic translocation of large molecules and particles. In particular, this has been suggested for the export of the about 300-700 Å large globular particles derived from defined chromosomal loci (as in the polytene chromosomes of salivary gland cells in Chironomus and Drosophila) or from nucleoli (as in amphibian oocytes). These RNP particles assume a dumbbell shape configuration with a minimal waist width of about 150 Å when migrating through the pore center, thus indicating that only the very center of the pore is penetrable. Micrographs indicative of the existence of alternative nucleocytoplasmic routes such as (i) by delamination of nuclear envelope pockets, (ii) by combined formations of blebs from inner and outer nuclear membrane, or (iii) via large lesions of the nuclear envelope (e.g. Tashiro et al., 1968) have been

shown but these pathways seem to represent only exceptional or cytopathological cases. In this connection it is important to note that isolated nuclear envelopes or nuclear membrane fractions contain a considerable amount of firmly membrane-attached RNA or RNP. The data reported suggest that a large portion of this nuclear membrane RNA is contained in the pore complexes. In the mature oocytes of Xenopus laevis an average pore complex content of 4×10^{-17} g has been determined which in this cell means that about 9% of nuclear RNA is associated with the nuclear envelope. The determinations of considerable RNA contents in nuclear pore complexes correspond with numerous cytochemical demonstrations of RNA with these structures as well as with the demonstrations of direct structural continuity of nuclear pore complex components, via thin fibrils, with identified RNP structures such as nucleolar granules on one side, and polyribosomes on the other side. While most of this membrane-associated RNA is removed along with the accompanying proteins by washing with very low as well as with very high salt concentrations there is some RNA recovered with the membrane lipoprotein material after treatments that, for example, remove ribosomes completely. Analysis of this tightly membrane-linked RNA in nuclear membranes from rat liver and amphibian oocytes by molecular hybridization showed that a considerable part of it still contained rRNA sequences. Ribosomal RNA was noted with nuclear membranes even after natural or drug-induced cessation of nuclear RNA synthesis.

A majority of authors have hitherto favored the notion that processing and assembly of the RNAs potentially active in translation is finished within the confinements of the nucleus. In particular for mRNAs and rRNAs, a series of recent studies, however, has reported, that molecules of the size characteristic for the cytoplasm are not found in significant amounts in the nucleus (Gall, 1966; Rogers, 1968; Ringborg and Rydlander, 1971; Planta et al., 1972; Sillevis Smitt et al., 1972; Scheer, 1973; Scheer et al., 1973; Scherrer, 1973 a,b; Udem and Warner, 1973; Eckert et al., 1974; Franke and Scheer, 1974 a,b; see also the early data of Penman, 1966),

which may indicate that processing is not completed before entering the cytoplasm. We have found that molecules representing later stages of processing of pre-rRNA are enriched in isolated nuclear envelopes. This supports the concept that final processing of these RNAs and their assembly with the protein characteristic for the functional state in translation takes place, but might not even be completed, in the RNP containing structures associated with the membrane material of the nuclear envelope, most likely at the pore complexes. Pulse-chase studies in cultured cells and rat liver using various RNA precursors have revealed a kinetical complexity of the nuclear envelope RNA, indicative of the presence of short-lived RNA which is either (partially) degraded or is in nucleocytoplasmic transit, besides other low turnover RNA moieties. There is also an increasing number of reports demonstrating that some proteins associated with the molecules containing the mRNA and rRNA sequences are different in cytoplasm and nucleoplasm (e.g. Lukanidin et al., 1971, 1974; Georgiev et al., 1972; Kumar and Warner, 1972; Samarina et al., 1973; Soeiro and Basile, 1973; Higashinakagawa and Muramatsu, 1974).

From the mentioned frequent examples of correlation of nuclear activity in transcription, but not in replication, with pore complex numbers it is not too surprising that in a great many different cell types and stages the mean nuclear pore flow rates for RNA molecules, i.e. the mass or number of RNA molecules transferred into the cytoplasm per time unit, is in the same order of magnitude. There are, however, exceptions demonstrating very high as well as very low (even zero) translocation rates. In summary, there is no evidence that the pore complex has any regulatory role in limiting RNA export or protein synthesis.

Although the present data tend to support the vectorial nucleocytoplasmic transfer of RNA via the nuclear pore complexes, it is important to keep in mind that pore complexes and their constituents cannot be regarded as structures that <u>per se</u> are correlated with, or are indicative of, nucleocytoplasmic transport of RNA or any other material. They also occur in the

annulate lamellae, i.e. intranuclear or cytoplasmic cisternae which do not separate different compartments. Moreover, it has to be noted that some of the most conspicuous structures of pore complexes such as the central elements can also be encountered in pore formations of a variety of other membranes such as in endoplasmic reticulum, dictyosomes, and plasma membranes. This indicates that they are characteristic for pore formation processes rather than nucleocytoplasmic transport phenomena.

The literature evaluated in this survey has been collected in recent review articles (Franke, 1974; Franke and Scheer, 1974 a,b).

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