Intimate Contacts of Mitochondria with Nuclear Envelope as a Potential Energy Gateway for Nucleo-Cytoplasmic mRNA Transport

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Abstract. The aim of the present study was to show that close contacts of mitochondria with nuclear envelope need not be just an accidental situation in the cell, but that such contacts could serve for flow of energy from a place of origin directly to a place of consumption.

Mitochondria in close proximity to the nuclear envelope can be found virtually in all metabolically active cells. We used transmission electron microscopy to demonstrate this entity in different leukemia cells of human origin (patient's blood) and in mouse leukemia cell line. At high resolution, not only close proximity but even fusion of mitochondrial and nuclear membranes can be seen. Based on available data about mRNA transport through the nuclear pore complex and observed contacts of mitochondria with nuclei, we hypothesize that such contacts can provide a gateway for energy delivery to power mRNA export from the nucleus to the cytoplasm. Hence the lumen of the nuclear envelope can serve for transport or even storage of macroergic molecules in a manner similar to sarcoplasmic reticulum in fast-twitch skeletal muscles.

Key words: Mitochondria — Nuclear envelope — Source of energy — mRNA transport

Introduction

It is generally accepted that in interphase cells the entire macromolecular transport between the nucleus and the cytoplasm goes through the nuclear pore complexes (NPCs). Recently, proposed models of translocation of receptor-cargo complexes through the NPC attribute the major role as to directionality and source of energy to the small GTPase Ran. The energy requirement is to create a gradient of small GTPase Ran in different nucleotide states across the nuclear envelope (reviewed in Macara 2002). GTPase Ran is undoubtedly engaged in protein import and export,

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but no similar data have been obtained as to mRNA export (reviewed in Weis 2003). Export of most mRNAs is independent of the Ran system and karyopherins (Clouse et al. 2001). Different mechanisms of determining directionality and source of energy have yet to be found. As has been published elsewhere, the pathway of most mRNA export is different from that of proteins (Iborra et al. 2000, 2002; Prachař 2002). Based on the model of mRNA export and observation of mitochondria in the close proximity to the nuclear envelope, we hypothesize that the energy source for mRNA export may be localized in the lumen of the nuclear envelope and may be continuously refilled from mitochondria.

Materials and Methods

Cell culture

Mouse leukemia cell line L1210 (purchased from the American Type Culture Collection, Rockville, MD, USA) was maintained in RPMI 1640 medium supplemented with 10 % fetal bovine serum. The cells were fixed in exponential phase of growth. These cells were chosen because they produce retroviral particles and this sign of metabolic activity can be directly observed in an electron microscope on a single cell.

Cultured thymocytes originated from thymus obtained from a child who underwent heart operation. The child was stated healthy except for a congenital heart disease. The cultured thymocytes can thus be considered healthy cells.

White blood cells from two leukemia patients were also examined for the presence of mitochondrial contacts with the nuclear envelope. The first patient was a new case (first diagnosis before therapy) of acute myeloid leukemia. The second case was a relapsed plasma cell leukemia patient. These patients were arbitrarily chosen to check that the situation *in vivo* is similar to that in the cells cultured *in vitro*.

Preparation of samples for transmission electron microscopy

Cultured L1210 cells were quickly pelletted (2 min) and immediately fixed without any washing. Fixation, contrasting, embedding and staining of sections were done by conventional procedures except that a iodination step was added. Briefly, the procedure was as follows: fixation with 3 % glutaraldehyde in PBS for 1 h, 1 % OsO_4 for 1 h, iodination (see bellow), washing 3 times with cold deionized water, next again 1 % OsO_4 for 1 h, contrasting with saturated solution of uranyl acetate in deionized water – overnight. For washing, cells were resuspended in water and shortly centrifuged 3 times between all steps. Subsequently, the cells were resuspended in low melting point agarose at 37 °C, pelletted and kept in ice bath for short time – embedment in agarose. The following step was dehydration in 70 % and 100 % ethyl alcohol. Embedment was done in the Poly/Bed 812 epoxy resin (Polysciences). The epoxy resin was kept at 37 °C for 48 h to harden and at 60 °C for the next 24 h. Ultrathin sections were collected on copper grids without any membrane. Section thickness was 50–60 nm. Staining of the ultrathin sections was done on a drop of saturated solution of uranyl acetate in water at 37 °C (2 h) and

the last step was contrasting on a drop of Reynolds solution at laboratory temperature (5 min). Unless otherwise stated, all procedures were carried out at laboratory temperature.

Iodination of samples enhances contrast of various cellular structures. Iodination was done according to the conventional procedure of protein radioactive labeling by the chloramine T method except for slight modifications as follows. Common nonradioactive iodine was used instead of radioactive iodine. Iodination was done in ice bath and at very low ionic strength. Introduction of iodine into protein and other molecules by itself enhances contrast inconsiderably. The effect takes place in the last step, when lead probably binds to places where iodine is present. The method is in development and yet not optimized. In this work, the same material (blocks) was used for observation as previously (Prachař 2002).

Cells obtained from the leukemia patients and cultured human thymocytes were contrasted by a conventional contrasting method (OsO_4, uranyl acetate, lead citrate).

Images were collected using JEOL JEM-1200 EX transmission electron microscope at 80 kV.

Results

During investigation of nuclear pores, we have noticed that mitochondria can very often be observed in close proximity or even touching the nuclear envelope in the cells of the L1210 mouse leukemia cell line. (Figures 1, 2 and 5). Another examples of mitochondria in close proximity to the nuclear envelope are shown in Figures 3 and 4. The L1210 cell line is characteristic by a very rapid cell growth and its activity is demonstrated also by frequent occurrence of mitosis (cross sectioned chromosomes) and, indirectly, by budding retroviral particles.

We have also noticed that in some cases, where mitochondria are in intimate contact with the nuclear envelope, their outer membranes are fused with outer nuclear membrane (Fig. 5). A contact of membranes can sometimes be found even with mitochondria being rather distant from the nuclear membrane (Fig. 2). The situation where membranes of mitochondria and nuclear envelope are fused is very often found in the rapidly growing L1210 cell line in contrast to the cells from leukemia patients or in normal cultured thymocytes. Fused membranes can only exceptionally be found in patient cells but this can be due to a lower incidence of this phenomenon in the cell and hence a lower probability to find it in sections. Moreover, the cells from leukemia patients were fixed up to one hour after blood collection, thus metabolic activity of those cells can be much lower.

The hole observed in mitochondrial membranes evidently represents one type of mitochondrial transport channels or "filters" (Fig. 5b). A fusion of the outer and inner membrane creates this hole. The diameter of the channel is about 12 nm. There is a darker material inside the hole – perhaps a proteinous transport complex which is in fact also a stopper against free mixing of two different environments

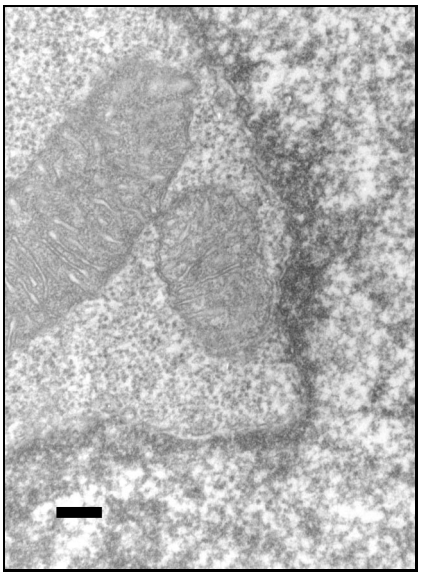


Figure 1. Example of mitochondria in close proximity to or in contact with the nuclear envelope in cells of the L1210 mouse leukemia cell line. Bars represent 200 nm.

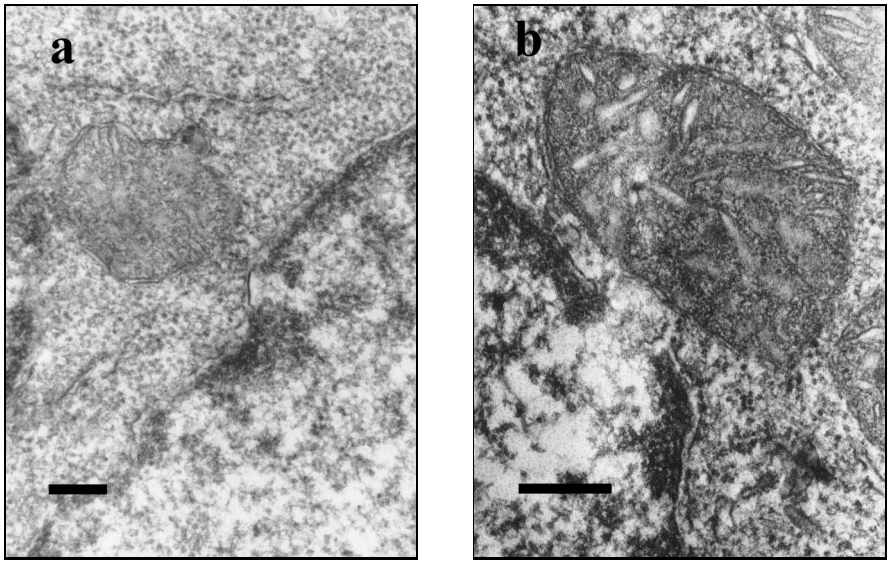


Figure 2. Contact of mitochondria with the nuclear envelope and probably also with a nuclear pore over a "longer" distance (L1210 cell line). Bars represent 200 nm (figures a,b).

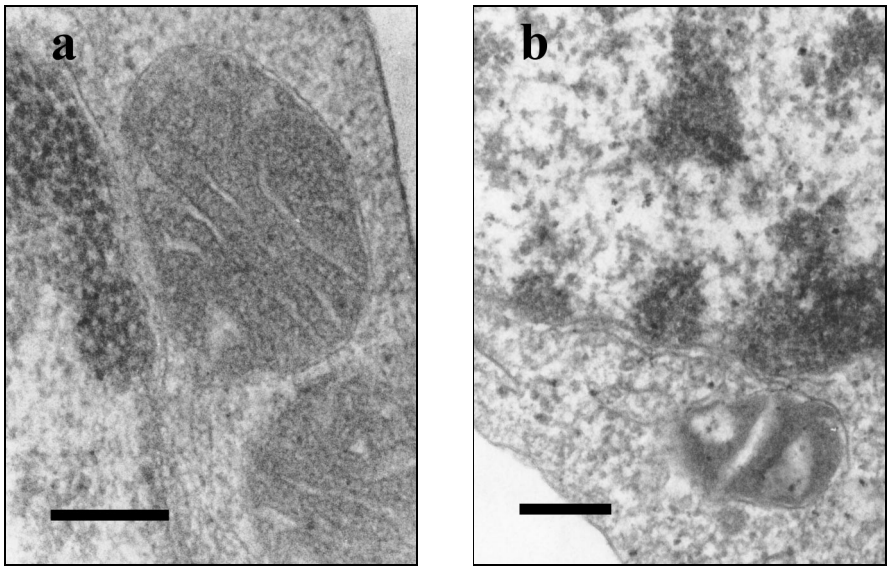


Figure 3. Examples of mitochondria contacts with the nuclear envelope in leukemia patient cells. **a)** The cell originates from the patient with acute myeloid leukemia. **b)** The cell from the patient with plasma cell leukemia. Bars represent 200 nm.

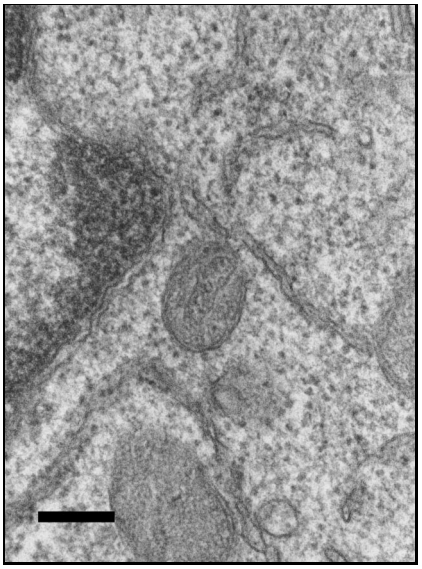
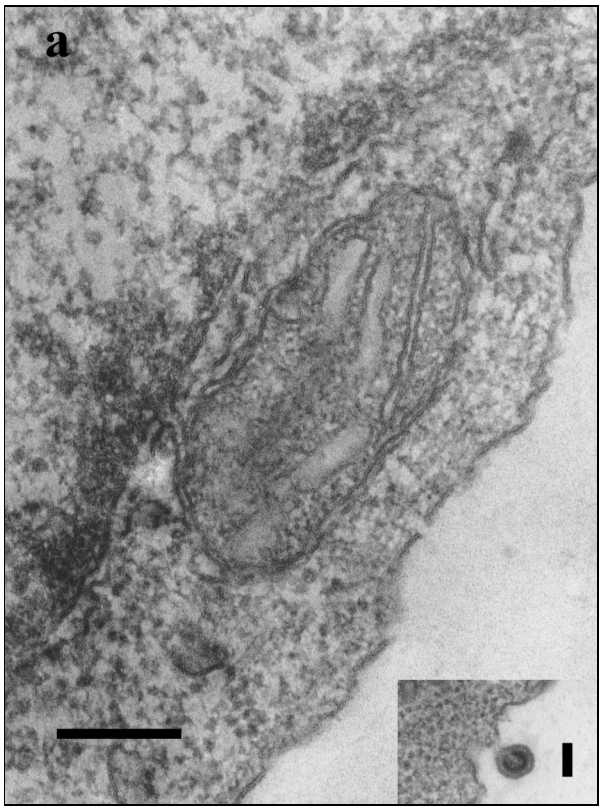
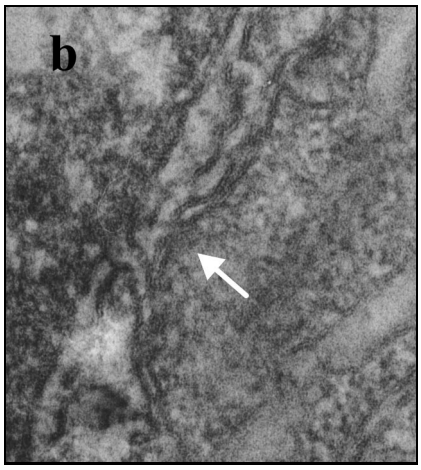
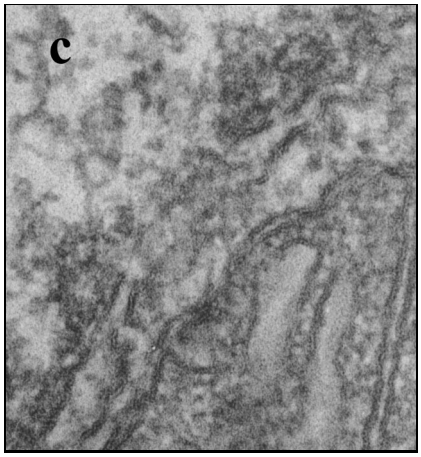


Figure 4. Close proximity of mitochondria to the nuclear envelope and contacts and connection of mitochondria to the endoplasmic reticulum. The cells were cultured normal human thymocytes. Bar represents 200 nm.







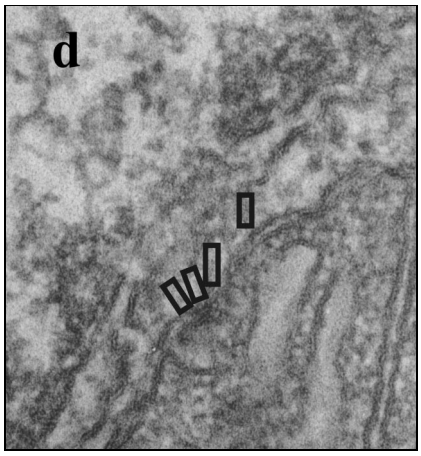


Figure 5. a) Connection of mitochondria with the nuclear envelope. Fusion of outer nuclear and outer mitochondrial membranes. Bar represents 200 nm. Insert shows a budding retroviral particle from a different place of the same cell. Budding retroviral particles confirm metabolic activity of the L1210 cells. Bar in the insert represents 100 nm. **b)** Detail of Fig. 5a showing a transport channel in mitochondrial membranes with darker material (arrow) which probably represents a transport protein complex. **c)** Detail of Fig. 5a showing the place where a mitochondrion covers the region of nuclear pores. This can also shows a transport between the mitochondrion and the nucleus (see Fig. 5d). **d)** The same region as in Fig. 5c with presumable material transported between the mitochondrion and the nucleus (faint shadows in Fig. 5c) highlighted with black rectangles.

– in this case, the lumen of the nuclear envelope and the mitochondrial matrix (Fig. 5b).

In case of cells with rich endoplasmic reticulum, we can easily find mitochondria in the close proximity to the nuclear envelope, but also in contact with the endoplasmic reticulum. This case is demonstrated in Fig. 4. that represents the situation in cultured human thymocytes.

In case of small mitochondria, the contact of mitochondria with the nuclear envelope occurs at places where heterochromatin is present from the nuclear side. Larger mitochondria also cover the nuclear pores. Apparently, there also exists some communication between the mitochondria and the nuclear pores. Faint shadows can be seen in the direction between the mitochondrion and the nuclear pore. This case is demonstrated in Fig. 5a, and Figs. 5c and d, which are details of Fig. 5a. This communication evidently represents some transport, but it is unclear of what and in which direction. Such communication (oriented shadows) can be recognized, albeit not easily, even in the case that mitochondria are present at larger distance from the nuclear envelope (not shown).

Discussion

With the exception of the cultured thymocytes, we demonstrate contacts of mitochondria with the nuclear envelope only in malignant cells (leukemia cell line, leukemia patients). Contacts of mitochondria with nuclear envelope in normal cells can be found easily on micrographs made for different purposes in many textbooks (cytomorphology, ultrastructure of the cell etc.). This phenomenon is so common that we suppose mitochondria to be in contact with the nuclear envelope (or the endoplasmatic reticulum) in every metabolically active cell. The reason may be the flow of energy from mitochondria to the lumen of the nuclear envelope.

From sections observation it is not easy to quantify the frequency of close contacts of mitochondria with the nuclear envelope. Assuming that every tenth observed cell shows an actual contact of a mitochondrion with the nucleus and that each nucleus is sectioned more than 50 times, it is clear that more than one mitochondrion is in contact with the nucleus. Observation of whole cells or thicker sections (thicker than those for transmission electron microscopy) with antibody labelled nuclei and mitochondria by light or fluorescent microscope can provide more exact quantitative results. Cells of different metabolic activity could be used in such experiments (for example, cells cultured at lower concentration of serum in medium or starving for serum).

The proximity of mitochondria to a potential energy consumer seems to be an economical and logical solution – the cell need not to transport energy over long distances through different environments. Fusion of the mitochondrial and outer nuclear membrane can be considered an event provoked by an intensive need of energy by a rapidly metabolizing cell. The close proximity of mitochondria to the nuclear envelope or the endoplasmic reticulum indicates that the lumen of

these cisterns could be used as an energy reservoir. If so the question arises of the processes that this energy could be used for.

The nucleocytoplasmic protein transport through the nuclear pore complexes is powered by the energy from GTP hydrolysis, with the small GTPase Ran being the key player in this process (for a review, see Weis 2003 and references therein). It follows from the results published earlier that the mechanism of mRNA transport is in most cases different from that of proteins (Iborra et al. 2000; Clouse et al. 2001; Iborra 2002; Prachař 2002; Walther et al. 2002; Lei et al. 2003). So far, no role of the GTPase Ran has been found in mRNA export. The protein NTF2, which serves as an importer for the GTPase Ran is not transported through the pores that just export mRNA (Iborra et al. 2000). Hence the small GTPase Ran cannot be directly engaged in mRNA export.

The data published elsewhere (Prachař 2002) and our model of mRNA export (Fig. 6), indicate that mRNA passes through the long tunnels in the nuclear pore complex. Such travel necessarily requires energy. It may be important that the peripheral channels are in contact with the nuclear membrane at the point where inner and outer nuclear membranes are joined. We have shown earlier (Prachař 2002) that there are several hydrophilic (most probably protein) groups that compose the wall of the peripheral channel at that place.

The so called anchor is localized in the lumen of the nuclear envelope at the place where the peripheral channel touches the point of the fused inner and outer membranes. The anchor protrudes about 24 nm into the lumen of the nuclear envelope (in case of human leukemia cells). This region contains two large proteins – POM121 and, mainly, gp210. Following mitosis, NPC proteins are recruited to the reforming nuclear envelope in a sequential order (Bodoor et al. 1999). The integral membrane protein POM121 is recruited early; however, gp210 is recruited fairly late in the nuclear envelope reformation. The early recruitment of POM121 suggests this protein may play a role in membrane fusion to create the membrane pore and initialize the assembly of NPC intermediates. Nevertheless, there is no need to protrude by such a rather long distance into the space of the nuclear envelope lumen to accomplish the above mentioned function or to anchor any structure on the opposite membrane side. Thus the big molecules of proteins may also have a different function, along with the initiation of NPC assembly intermediates and/or anchorage. The NPC is in fact anchored in the nuclear lamina, which is more rigid structure. Except that, the so-called anchor is located in the lumen of the nuclear envelope and can be associated with additional proteins which need not be necessarily isolated together with the NPC proteins.

We hypothesize that the place where the peripheral channel touches the fusion place of inner and outer nuclear membrane might contain the motor for mRNA movement. The energy source for the motor might be localized in the lumen of the nuclear envelope (on the network of lumenal or integral membrane proteins), and energy can be continuously refilled from the mitochondria that are in contact with the nuclear membrane. There exists a well described analogy of a direct energy cross talk between organelles – mitochondria and sarcoplasmic reticulum in fast-twitch

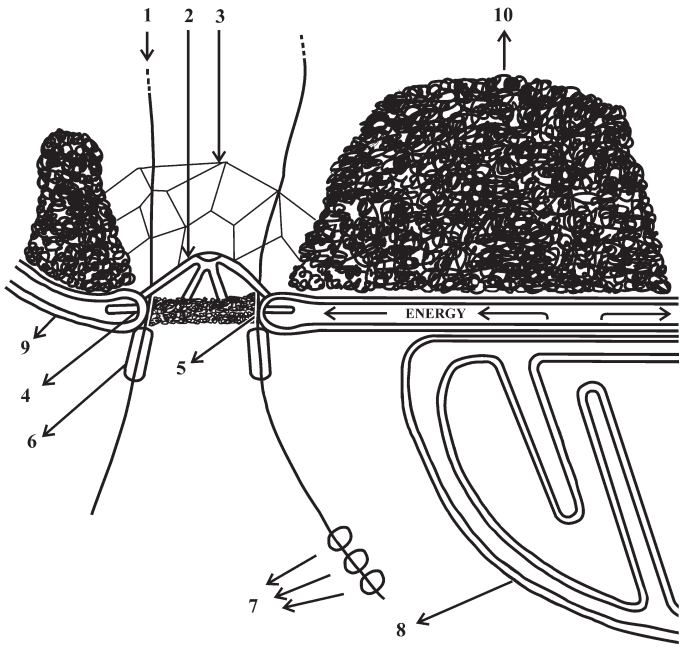


Figure 6. Model of mRNA export through the nuclear pore complex showing a potential energy source for the motor of this process. The model assumes that RNA export uses a different and independent path through the pore complex than that used for protein transport (Prachař 2002) The suggested path goes through a peripheral channel and next through a hollow cytoplasmic rod, a subunit of the cytoplasmic ring.
1, mRNA; 2, basket of the nuclear pore complex; 3, the nuclear pore complex associated cage composed of nuclear filaments; 4, anchor located in the lumen of the nuclear envelope and starting at the place where the outer and inner nuclear membranes are joined. At this place from the side of the nuclear pore complex, the peripheral channel touches or even copies the shape of the nuclear membrane. The mRNA moving motor might be located at this place; 5, peripheral channel; 6, hollow cytoplasmic rod – a subunit of the cytoplasmic ring; 7, ribosomes (polyribosome); 8, mitochondrion in close proximity to the outer nuclear membrane; 9, nuclear envelope; 10, heterochromatin. Energy flow in the lumen of the nuclear envelope can be realized by a different mechanism than a passive diffusion of ATP. Therefore the designation "ENERGY" (rather than ATP) was used in the figure.

skeletal muscles of mice suggesting a direct ATP/ADP channeling between site of energy production (mitochondria) and energy utilization (Kaasik et al. 2003; Weiss and Korge 2001). Cross talk between mitochondria and endoplasmic reticulum was also described (Rizzuto et al. 1998; Rutter and Rizzuto 2000).

There is as yet no evidence in support of our speculation but these logical considerations can provoke a search for an mRNA export motor at the proper place. Proteomic analysis might bring an answer to this point.

Once the motor moving mRNA through the NPC tunnels is located, the mechanism, ensuring proper navigation of mRNA ribonucleoprotein from the place of synthesis (transcription factory) towards the nuclear pore complex will still have to be identified.

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Final version accepted: October 20, 2003