Structural Interaction between the Nuclear Pore Complex and a Specific Translocating RNP Particle

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Abstract. The transport of Balbiani ring (BR) premessenger RNP particles in the larval salivary gland cells of the dipteran *Chironomus tentans* can be followed using electron microscopy. A BR RNP particle consists of an RNP ribbon bent into a ringlike structure. Upon translocation through the nuclear pore complex (NPC), the ribbon is straightened and enters the central channel of the NPC with the 5′ end of the transcript in the lead. The translocating ribbon is likely to interact with the central channel but, in addition, the remaining portion of the ribbon ring makes contact with the periphery of the NPC. To determine the nature of this latter interaction, we have now studied the connections between the RNP particle and the border of the NPC during different stages of translocation using electron microscope tomography. It was observed that the 3′ terminal domain of the ribbon always touches the nuclear ring of the NPC, but the precise area of contact is variable. Sometimes also a region on the opposite side of the ribbon ring reaches the nuclear ring. The pattern of contacts could be correlated to the stage of translocation, and it was concluded that the particle-nuclear ring interactions reflect a rotation of the ribbon ring in front of the central channel, the rotation being secondary to the successive translocation of the ribbon through the channel. The particle's mode of interaction with the NPC suggests that the initial contact between the 5′ end domain of the ribbon and the entrance to the central channel is probably crucial to accomplish the ordered translocation of the premessenger RNP particle through the NPC.

THE active transport of macromolecules between the nucleus and the cytoplasm occurs through the nuclear pore complex (NPC)[1] (for recent reviews about nucleo-cytoplasmic transport, see Gerace, 1992; Newmeyer, 1993; Dingwall and Laskey, 1992; Izaurralde and Mattaj, 1992; Panté and Aebi, 1993). The NPC is tripartite and contains a central spoke-plug assembly surrounded by two coaxial rings, the nuclear ring facing the nucleus and the cytoplasmic ring the cytoplasm (Franke and Scheer, 1974; Maul, 1977; Hinshaw et al., 1992; Akey and Radermacher, 1993). The basic framework of the NPC is symmetric with an eightfold symmetry seen in the direction of transport (face-on direction) and a twofold symmetry between the two halves facing the nucleus and cytoplasm (edge-on direction). There are also peripheral structures making the NPC as a whole asymmetric (for discussion, see Panté and Aebi, 1993; Stewart, 1992). Notably, on both sides there are fibers connected to the rings and extending out into the surrounding medium for putative binding of components to be transported (Franke and Scheer, 1974; Maul, 1977; Scheer et al., 1988). The cytoplasmic fibers are rather short and contain at least two specific components, nup180 (Wilken et al., 1993) and nup214 (Kraemer et al., 1994). On the nucleoplasmic side, long fibers are organized into a basket-like structure (Ris, 1991; Jarnik and Aebi, 1991; Goldberg and Allen, 1992); again a specific protein, nup153, has been identified (Sukegawa and Blobel, 1993; Cordes et al., 1993).

RNA is transported through the NPC as ribonucleoprotein (RNP) complexes (tRNA possibly being an exception) (Dreyfuss et al., 1993; Izaurralde and Mattaj, 1992). The translocation of RNP from the nucleus to the cytoplasm takes place through a gated channel in the center of the NPC. The passage of endogenous premessenger RNP particles (Stevens and Swift, 1966; Mehlin et al., 1991) as well as putative nucleolar particles (Franke and Scheer, 1974) through the central channel has been directly visualized in the electron microscope. Furthermore, tRNA and 5S rRNA coupled to colloidal gold particles have been observed passing through the central channel (Dworetzky and Feldherr, 1988). In this latter study, it was also shown that gold particles as large as ~25 nm in diameter can translocate through the channel, which agrees with the maximal diameter of the endogenous RNP in transit. Apart from the passage of macromolecules through the

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1. *Abbreviations used in this paper*: BR, Balbiani ring; NPC, nuclear pore complex; RNP, ribonucleoprotein.

gated channel, the NPC is known to allow diffusion of smaller molecules through a 9-nm channel (Feldherr, 1975; Paine et al., 1975; Lang et al., 1986). Whether this passive transport takes place through the central channel or through more peripheral channels within the NPC is still unknown.

When the RNP complexes reach the nuclear envelope, they are probably first bound to the nucleoplasmic fibers (Mehlin et al., 1992) and subsequently transferred to the entrance of the central channel and translocated through the channel. This mechanism could be in analogy with the protein import process which has been shown to comprise an energy-independent initial binding to the cytoplasmic fibers and a subsequent energy-requiring translocation step (Newmeyer and Forbes, 1988; Richardson et al., 1988). Both RNA export and protein import are blocked by anti-nucleoporin antibodies and the lectin WGA, suggesting similarities between the two pathways and emphasizing the involvement of nucleoporins in the process (for review see Newmeyer, 1993). Whether the nucleoporins, which are NPC components, function as receptors for the entities to be translocated or are parts of the translocation machinery, or both, has not been settled.

The RNA transport is a signal-dependent, receptor-mediated process, that requires energy (Zasloff, 1983; Khanna-Gupta and Ware, 1989; Bataillé et al., 1990; Dargemont and Kühn, 1992; for review see Dingwall and Laskey, 1992). The current research is focused on defining the signals and receptors involved in the transport of the various RNA species. Early work on tRNA showed that its transport is dependent on intact D and T stem loops; single mutations in these loops affected the transport (Tobian et al., 1985). It is not clear whether the mutations prevent the formation of a transport complex or whether mutated tRNA is retained within the nucleus by association with a nuclear structure. The transport of ribosomal 5S RNA is known to be dependent on the interaction between 5S RNA and either the transcription factor TFIIIA or the ribosomal protein L5 (Guddat et al., 1990). U snRNAs require a monomethyl-inverted guanosine cap structure to be exported (Hamm and Mattaj, 1990; Jarmolowski et al., 1994). A monomethyl cap is also important for the transport of messenger RNA (Hamm and Mattaj, 1990; Dargemont and Kühn, 1992), although the absence of a cap structure does not preclude but rather reduce transport (Jarmolowski et al., 1994). Several nuclear cap-binding proteins have been identified (Patzelt et al., 1983; Rozen and Sonenberg, 1987; Ohno et al., 1990; Izaurralde et al., 1992) and could represent critical components in the transport process. The RNA export signals for the various species are evidently different, and competition experiments have shown that there are likely to be different pathways to reach and pass through the nuclear pore (Jarmolowski et al., 1994). It is still too early to state whether the signal components that have been identified represent the complete signal or whether additional components are involved. Furthermore, it is not clear whether the signals are relevant for the putative RNP-NPC interactions, or whether they are involved in the transfer of the RNP complex from the gene to the NPC, which could comprise a series of events (for discussion, see Izaurralde and Mattaj, 1992).

As transport of all the RNA species are blocked with anti-nucleoporin antibodies (Fetherstone et al., 1988) and WGA (Neuman de Vegvar and Dahlberg, 1990; Bataillé et al., 1990; Dargemont and Kühn, 1992), it seems likely that the pathways converge to a common translocation pathway through the nuclear pore. Even if there is a nucleoporin receptor in the nucleoplasmic fibers, there could well be additional receptors in, e.g., the center of the NPC (cf. Akey and Goldfarb, 1989) as the RNP particles are probably following a pathway within the NPC. In addition, interactions between the RNP particle and the translocation machinery have to take place, irrespective whether a gate opening-diffusion mechanism or a gate opening-mechanochemical mechanism are advocated (e.g., Stewart, 1992). Evidently, in order to understand the transport of RNP through the nuclear pores, we need more information on the signal in various RNP complexes, the receptors within the NPC and the transfer of the RNP within and through the NPC.

In our own laboratory, we have chosen an ultrastructural approach to directly visualize the transport of a specific premessenger RNP particle through the NPC in order to establish the recognition of the particle at the NPC and the translocation of the particle through the NPC (Mehlin et al., 1992). The RNP particles, named Balbiani ring (BR) granules, are present in the salivary glands of the larvae of the dipteran *Chironomus tentans* and are suitable for electron microscopy due to their exceptional size and abundance. The BR-genes, 35–40 kb in length, encode information for secretory polypeptides with molecular mass around 10^6 D (for review see Wieslander, 1994). The 75S RNA molecule is packed with proteins immediately upon synthesis into a 50-nm RNP particle (Andersson et al., 1980; Olins et al., 1980; Skoglund et al., 1983). The completed and released RNP particle can be described as an RNP ribbon, 30–60-nm broad and 10–15-nm thick, bent into a ringlike conformation (Skoglund et al., 1986). The ribbon can be divided into four structural domains, domain 1 through 4. By comparisons between the growing particle on the gene and the completed particle in the nucleoplasm, it could be concluded that the 5′ end of the transcript is located in domain 1 and the 3′ end in domain 4 (Skoglund et al., 1986).

During translocation through the NPC, the structure of the BR particle is drastically changed (Stevens and Swift, 1966; Mehlin et al., 1991). The ringlike particle is positioned in front of the NPC, and upon translocation the bent ribbon becomes extended and pass through the nuclear pore with the 5′ end of the transcript in the lead (Mehlin et al., 1992). Apart from the interaction between the translocating ribbon and the center of the NPC, we also observed a structural connection between the 3′ end domain and the periphery of the NPC (Mehlin et al., 1992). To study the nature of this latter contact, we have now carried out three-dimensional reconstructions of BR RNP particles during different stages of translocation. We observed that the bent ribbon makes contact with the nuclear ring of the NPC. The interaction was found to be variable but could be correlated to the stage of translocation and most likely reflects a rotation of the ribbon ring in front of the NPC. Since the 5′ end of the ribbon interacts initially with the central part of the NPC and the position

of the other contact regions are variable, this study indicates that it is the 5′ end with its cap structure that is important for the recognition of the particle at the NPC.

Materials and Methods

Specimen Preparation

We have followed the method earlier used to characterize the nucleoplasmic BR RNP particles (Skoglund et al., 1986). For this purpose, salivary glands were prepared from fourth instar larvae of *Chironomus tentans*, fixed in 2% glutaraldehyde for 2 h at 4°C and 1% osmium tetroxide for 30 min at 4°C, dehydrated, and embedded in Epon. Thin sections (60–75 nm) were cut in an ultramicrotome and put on 200 mesh copper grids. The sections were stained with lead citrate and uranyl acetate. A droplet with 10-nm colloidal gold particles was put on top of the sections and the liquid surplus was removed after 1 min.

Electron Microscopy

Electron micrographs were recorded in a JEOL TEM-SCAN 100CX at 33,000× magnification. Tilt series comprising 25 images from −60° to +60° were photographed. Micrographs were recorded at 0° tilt angle before and after the tilt series in order to test for specimen damage. Tilt series showing any visual sign of specimen damage were discarded. Approved micrographs were digitized and compared using power spectrum, R-value, phase residual, and Fourier shell correlation (Radermacher, 1988; Frank et al., 1981). The difference in resolution between the images taken before and after the recording of a tilt series, was mainly due to improper focus adjustment and uncertainties in the positioning of the grid (a backlash in the goniometer may cause an error in the tilt angle readout when the images are not taken in a consecutive series, as is the case when the 0° tilt view is recorded before and after the tilt series). The plastic sections were also tested for shrinkage during the tilt series by the use of colloidal gold particles on both sides of the section. No shrinkage could be observed during the recording of the tilt series. However, there exists an initial shrinkage of the section on the order of 30% (unpublished data from reconstructions of TBSV virus), but since the sections are stabilized before the recording of the images this does not influence the quality of the reconstructions.

Three-Dimensional Reconstruction

The micrographs were digitized with an Optronics P-1000 drum scanner with a raster size of 25 μm (gives a pixel size of 0.758 nm at 33,000 × mag-

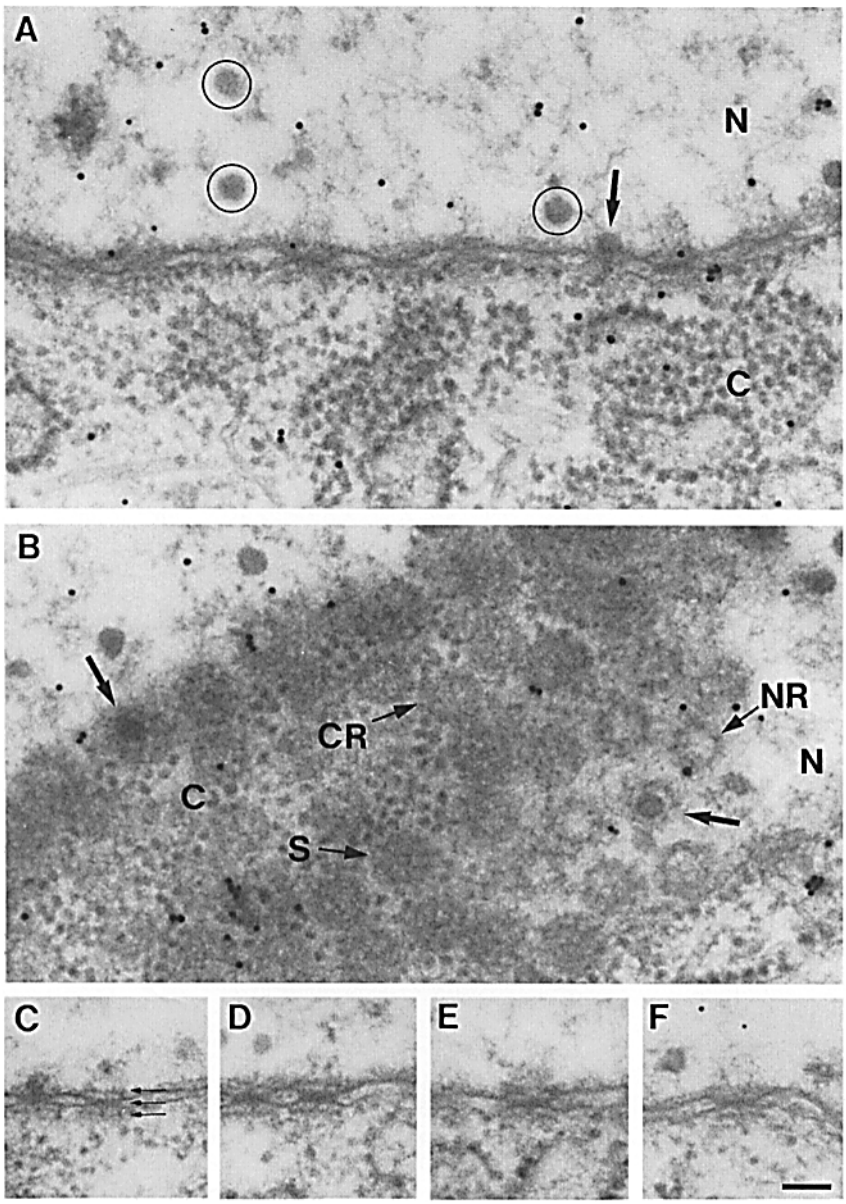

Figure 1. The structure of the NPC in the salivary gland cells of *Chironomus tentans*. *A* shows a section perpendicular to the nuclear envelope; the nucleus is at the top and the cytoplasm at the bottom. The nuclear and outer membranes of the nuclear envelope can be seen and are fused at the NPCs. Ribosomes are attached to the outer membrane. Three BR RNP particles can be recognized in the nucleus (*encircled*), while one is translocating through a pore (*thick arrow*). Examples of NPCs viewed edge-on are displayed in *C–F*. Note that the tripartite structure of the NPC is clearly visible: the nuclear ring (*upper arrow in C*), the spoke assembly (*middle arrow*) and the cytoplasmic ring (*lower arrow*). *B* shows a portion of the nuclear envelope that is sectioned almost parallel with the envelope. Nucleoplasm (*N*) appears to the left and to the right, while a streak of cytoplasm (*C*) can be seen in between (*lower left corner*). Since the plastic section is thinner than the NPC, the components may be tentatively identified also in this face-on view. The nuclear ring (*NR*) is visible close to the nucleoplasm, the cytoplasmic ring (*CR*) close to the cytoplasm, and the spoke assembly (*S*) in between. Some RNP particles are caught during transport (*thick arrows*). The gold markers used for alignment in the EMT method (see Materials and Methods) are visible as black dots. Bar, 100 nm.

nification). Image processing was initially performed with a VAX 11/750 and later with a Convex C210 computer. Alignment of the images were achieved with an iterative least-squares refinement procedure using the colloidal gold particles as reference markers. The three-dimensional reconstruction was calculated with radially weighted back projection and low-pass filtered to a homogeneous resolution of 5 nm. The reconstructions were contoured as wire frame models and processed using interactive computer graphics programs (TRACER and TV) on an Evans and Sutherland PS390 and a Silicon Graphics 210VGX display.

The reproducibility of the tomographic method has been evaluated in Skoglund et al. (1986) and in Öfverstedt et al. (1994). Comparisons between projections of a reconstruction and the corresponding electron micrographs demonstrated the high fidelity of the reconstruction procedure (Schmekel et al., 1993).

Results

The Nuclear Pore Complex in Chironomus tentans

The NPC in the salivary gland cells from *Chironomus tentans* can be recognized at low magnification in sections both perpendicular (Fig. 1 *A*) and parallel (Fig. 1 *B*) to the nuclear envelope.

In the edge-on view, the spoke assembly and the surrounding nuclear and cytoplasmic rings can be identified in Fig. 1 *A* and more clearly seen in Fig. 1, *C–F*. In Fig. 1 *C*, the central, densely stained spoke assembly and the nuclear and cytoplasmic rings have been indicated by three arrows.

The recognition of the tripartite structure in the edge-on view depends on the direction of sectioning. This can be shown by tilting the section around an axis parallel to the nuclear envelope; at a certain viewing angle the three NPC layers are clearly visible, but as the specimen is tilted away from this angle they appear less well defined and finally disappear. The three components are, therefore, not always visible in the electron micrographs. The three-dimensional reconstructions may, on the other hand, be rotated freely, and in our reconstructions it is always possible to find an angle of view where the tripartite structure appears.

In the face-on view (Fig. 1 *B*), the ring-like structure and the eightfold symmetry of the NPC can be discerned (e.g., at *S*). It is possible to recognize the tripartite structure of the NPC also in the face-on view. This is due to the fact that the section in Fig. 1 *B* is thinner than an NPC and, therefore, only a portion of each NPC is represented in the image. Furthermore, since the NPCs in Fig. 1 *B* are sectioned at different levels, all the three major NPC components can be tentatively identified: the spoke assembly (marked *S*), the nuclear ring (*NR*) on the nucleoplasmic side, and the cytoplasmic ring (*CR*) on the cytoplasmic side.

BR RNP Particles during Translocation

BR RNP particles appear in the nucleoplasm close to the nuclear envelope (encircled in Fig. 1 *A*). An unfolding RNP particle can be seen passing through the central channel of NPC in an edge-on view of the complex (arrow in Fig. 1 *A*). Also in the face-on view, the penetrating, heavily stained RNP particles can be recognized in the complex (thick arrows in Fig. 1 *B*). In both views it is evident that the BR RNP particles are translocated through the center of the NPC (Fig. 1, *A* and *B*).

At an early stage of translocation, most of the RNP ribbon is still bent into a ring while a minor part is fed into the NPC, giving the particle a hook-like contour (Fig. 2, *A* and *B*). Later, the ribbon unrolls more and more until it is completely extended (Fig. 2 *C*). The process is ordered in the sense that the particle is always translocated with the 5′ end of the transcript in the lead (Mehlin et al., 1992).

In images of translocating RNP particles, it is also possible to identify the tripartite structure of the NPC and roughly relate the position of the particle to the NPC components (Fig. 2). The spoke assembly (at arrow) in the

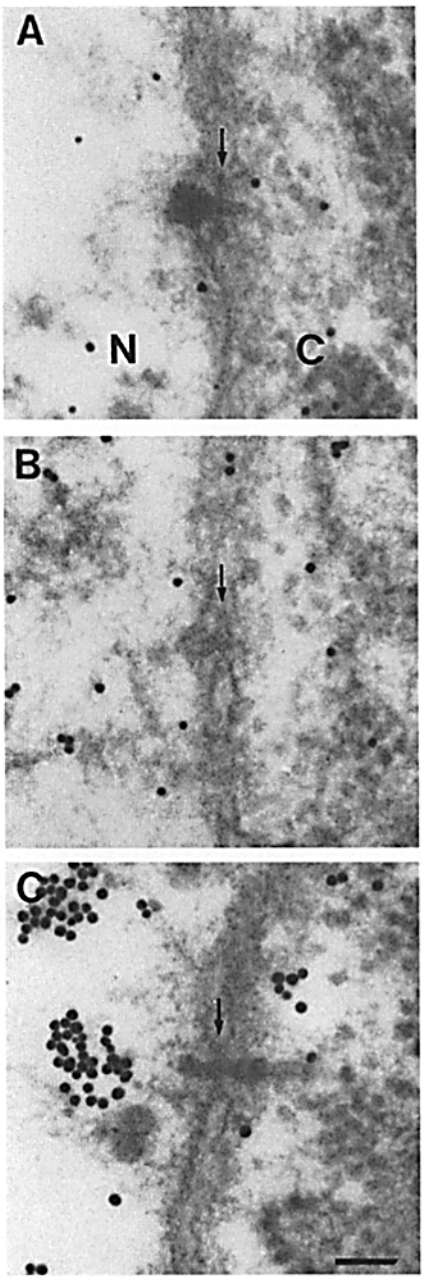

Figure 2. Three RNP particles in different stages of translocation through the NPC. Nucleoplasm is seen to the left (*N*) and cytoplasm (*C*) to the right. The tripartite structure of the NPC can be discerned, and the spoke assembly level has been indicated by arrow. Gold markers appear as black dots. Bar, 100 nm.

middle of the NPC embraces the extended ribbon of the RNP particle, which has a width of 25 nm. On the nucleoplasmic side, the nuclear ring can be seen surrounding the globular portion of the RNP particle (e.g., Fig. 2 *A*).

Tomographic Technique

In an earlier three-dimensional reconstruction study, we established that the RNP particle does touch the border of the NPC concomitant with the passage of the unfolded ribbon through the central channel (Mehlin et al., 1992). To further investigate the nature of this peripheral contact, we have analyzed six RNP particles at different stages of translocation with electron microscope tomography (Skoglund et al., 1986) (for technical information, see Materials and Methods). At the earliest stage of translocation, the RNP particle had only started to change shape, while at the latest the entire particle consisted of a straightened ribbon.

The reconstructions of the translocating RNP particles were based on tilt series, each encompasing 25 images from −60° to +60°. One example of such a tilt series is presented in Fig. 3 (only every 15° tilt image is included in the figure). To test for beam damage during the recording of the tilt series, the 0° tilt was always photographed before and subsequent to the tilt series proper (Fig. 3). Structural details of the pore complex (e.g., the nucleoplasmic filaments) were scrutinized, and the series was discarded if deterioration of the structure could be detected. The micrographs were also tested for global changes by computational procedures (power spectrum, R-value, phase residual, and Fourier shell correlation; see Radermacher, 1988; Frank et al., 1981). The micrographs were found to be similar to a resolution between 4.5 and 3.5 nm, and therefore showed no sign of specimen damage affecting the data at the resolution studied (5 nm).

The RNP particles were reconstructed in three dimensions along with surrounding material. The thickness of the sections (60–75 nm) had been chosen to obtain high resolution of the RNP particles. However, the sections were too thin to get a complete view of the NPCs. Therefore, no attempts were made to carry out a detailed analysis of the NPCs in our reconstructions; we only identified the major components such as the spoke assembly, the nuclear ring, and the cytoplasmic ring.

The NPCs including the fibers attached to the nuclear and cytoplasmic rings, were often less stained than the RNP particles. To identify the connections between a given RNP particle and the NPC, it was therefore often necessary to work at a lower contouring level than normally used for the visualization of the RNP particle. This low contouring level was chosen so that the NPC components identified in the micrographs could be discerned in the reconstruction. Several different contouring levels were tested in order to investigate how the choice of level influenced the interpretation. At too high a contouring level (with only the darkest stained material included), the NPC material could not be seen. At too low a contouring level, too much background material was included. No additional and reliable interactions between the RNP parti-

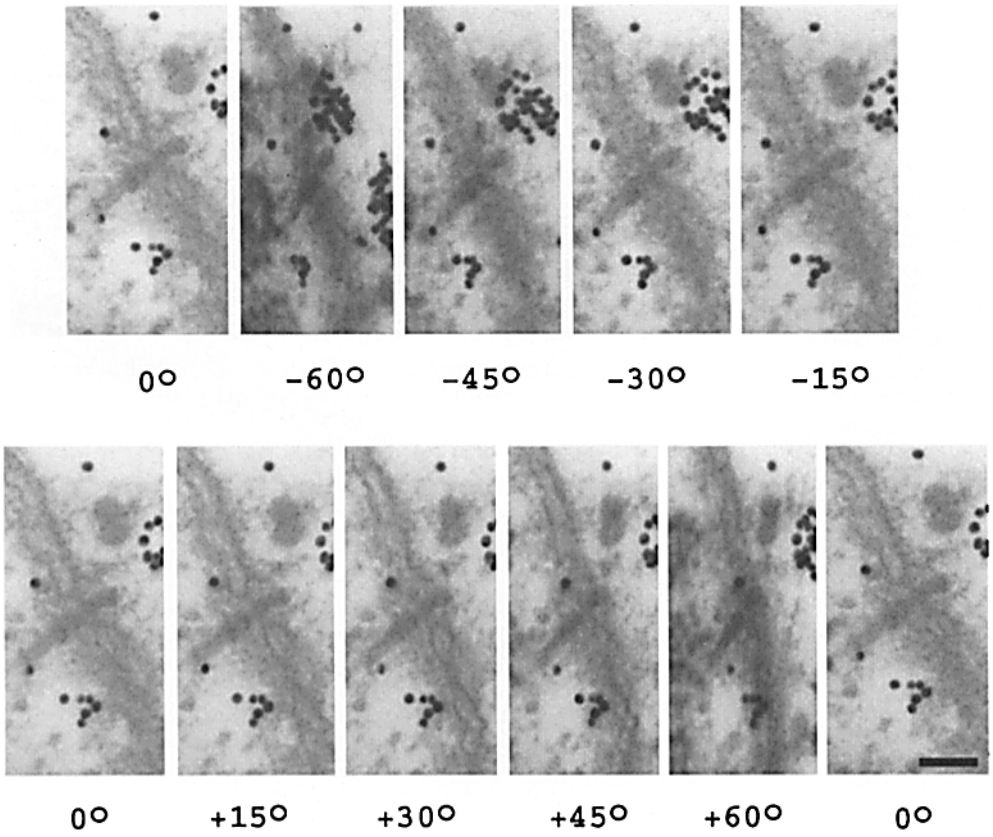

Figure 3. Tilt series of a translocating RNP particle. The series comprises 25 images from +60° to −60°; only the images corresponding to every 15° are presented in the figure. The 0° tilt is recorded before and after the tilt series proper for evaluation of possible beam damage. The nucleus is to the right and cytoplasm to the left. The tilt axis is essentially vertical. Gold particles added to the specimen serve as alignment markers. Bar, 100 nm.

cle and the NPC could be observed at this low contouring level.

Interactions between Translocating RNP Particles and the NPC

Three reconstructions of RNP particles in different stages of translocation are shown in Fig. 4 (*right*) along with nucleoplasmic reference particles (*left*). The three particles reconstructed are the same as those presented in Fig. 2 as electron micrographs (the same order). In Fig. 4, in each case the total reconstruction, comprising the RNP particle, the NPC as well as other surrounding material, is presented in white. The translocating particle has been specifically demarcated and is shown in blue. By comparing the reconstruction with the corresponding micrographs (Fig. 2), it is possible to identify the material belonging to the spokes (marked *S*) and the nuclear ring (marked *NR*).

The reference RNP particle is presented in Fig. 4 as an average structure at 8.5 nm resolution (*yellow* and *brown*) (Skoglund et al., 1986; average of four reconstructions). It has been aligned to the globular portion of the translocating RNP particle, which was accomplished by a comparison of the major features of the two particles (slit, contour, shape of the cavity, etc.; the slicing technique presented below proved helpful; for the alignment procedure, see further Mehlin et al., 1992). The brown part of the reference particle represents the portion of the translocating RNP particle that has started to unfold and enter the NPC, while the yellow part corresponds to the remaining globular portion of the particle.

The three particles represent three different stages of translocation, the earliest one in Fig. 4 *A* and the latest one in Fig. 4 *C*. The nuclear envelope is vertical in the reconstruction and the unfolding RNP particle moves through the NPC towards right. It can then be seen that the reference particle (*yellow* and *brown*) is rotating to match the translocating particle as described above. The brown part grows larger from *A* to *C* as the translocating particle is being unrolled and transported through the NPC.

To identify the exact regions of contact between the translocating RNP particles and the NPC, we cut each reconstruction into a number of thin slices. This approach was found to be necessary to demonstrate that genuine contacts do exist and to decide where they appear. As examples, we show below two of the sliced reconstructions, one of an early RNP particle and one of a late particle.

The reconstruction of the early RNP particle exhibited in Fig. 4 *A* is visualized with the slicing technique in Fig. 5. Six consecutive slices through the translocating particle are shown to the right in *A–F*; the color code is the same as in Fig. 4. The position of the slice is indicated on a top view of the particle presented to the left; the slice is demarcated by two white lines. A connection between the RNP particle and the NPC can be seen in Fig. 5, *C* and *D* (*red arrows*). The contact is located at domain 4 of the RNP particle. A comparison between Fig. 4 and Fig. 2 *A* shows that the NPC material that makes this contact belongs to the nuclear ring. On the opposite side of the particle, there is no connection between the RNP particle and the NPC (not even at a lower contouring level). The region of the RNP particle interacting with the NPC is mapped on the reference particle in Fig. 4 (white patch at red arrow).

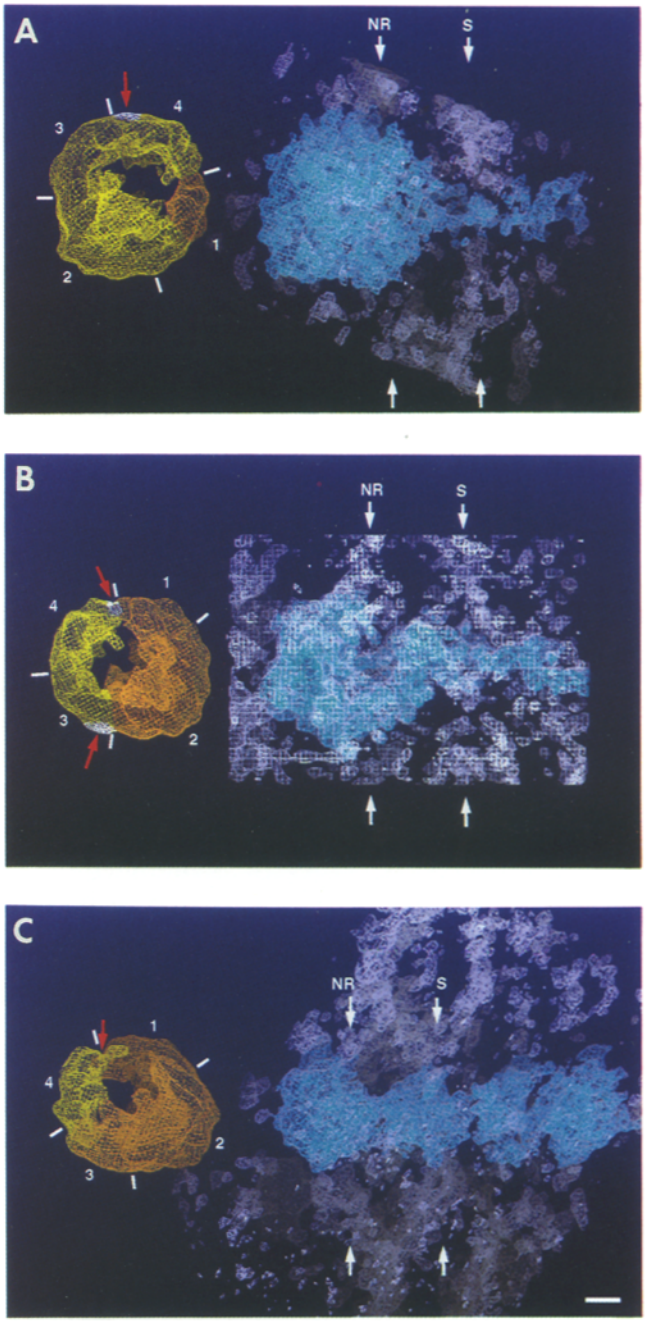

Figure 4. Three-dimensional reconstructions of RNP particles in consecutive stages of translocation. The electron micrographs of the corresponding particles at 0° tilt angle are shown in Fig. 2, *A–C*. In each reconstruction, the translocating particle (*blue*) is compared with a nucleoplasmic reference particle (*yellow* and *brown*), which is aligned to the intranuclear portion of the translocating particle. The surrounding material, including the NPC, is seen in white. The positions of the nuclear ring (marked *NR*) and the spokes (marked *S*) are indicated. The brown part of the reference particle corresponds to the portion of the translocating RNP particle that has unrolled and entered NPC. The domains (*1–4*) are indicated on the reference particle with white boundary lines and figures. The position of the contact region between the translocating particle and the nuclear ring of the NPC is indicated on the reference particle as a white patch (*red arrow*). The RNP particle (*blue*) and the reconstruction as a whole (*white*) have been contoured at the same cut-off level. Bar, 10 nm.

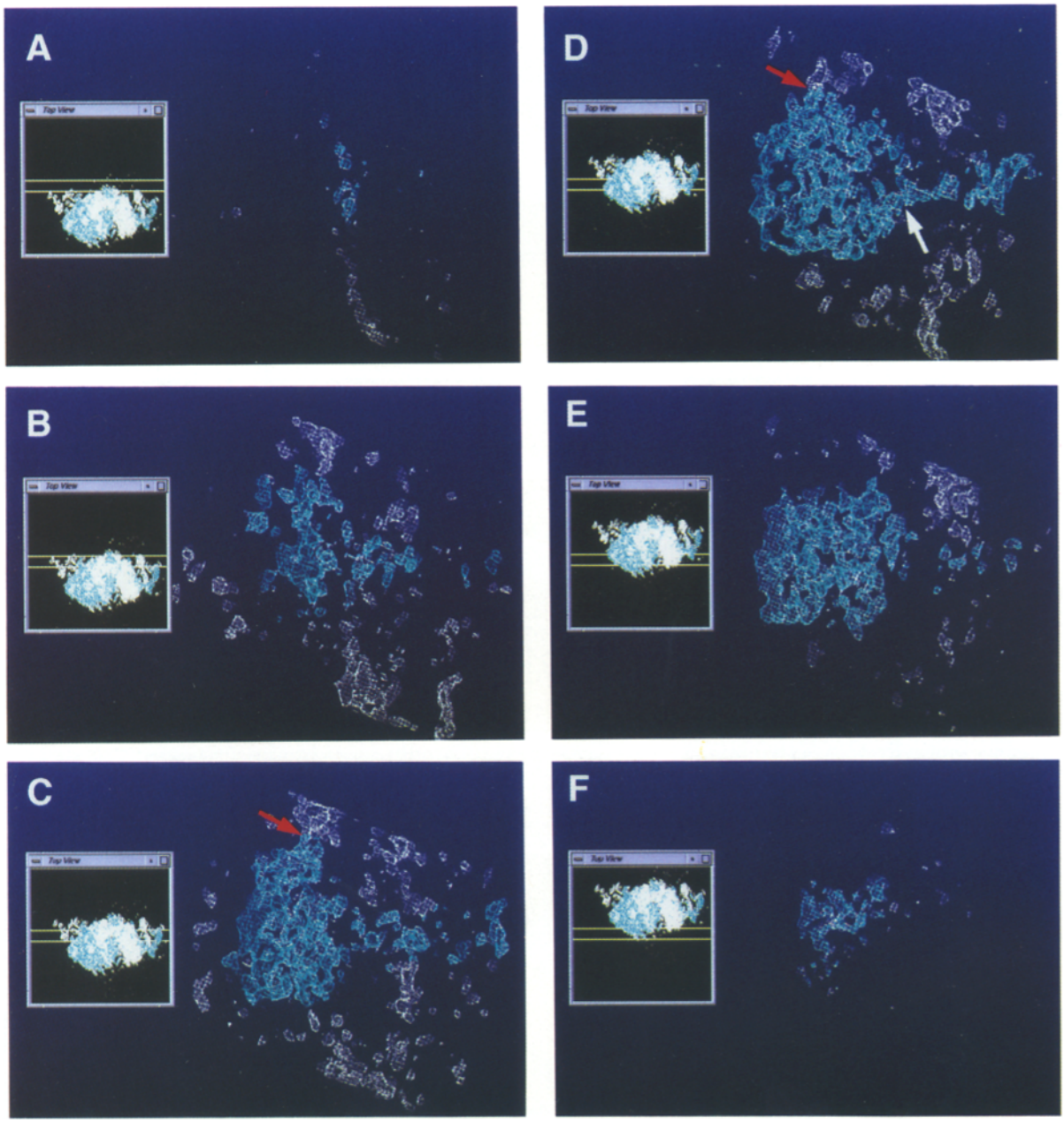

Figure 5. Consecutive slices through the reconstruction of the early translocating particle presented in Fig. 4 *A*. The slices are shown to the right in *A–F*. The position of the slices in the reconstruction is demonstrated to the left in a top view of the translocating particle; the slice is demarcated by two white lines. The structural connection between the NPC and domain 4 of the RNP particle is indicated (*red arrows*). There is no contact to the NPC on the other side of the RNP particle. The transition, where the RNP particle changes shape from a globular structure to an extended ribbon, is abrupt and marked with a white arrow.

The reconstruction of the late RNP particle in Fig. 4 *C* is presented with the slicing technique in Fig. 6. Also in this case only one connection can be seen: between the particle's domain 4 and the NPC (red arrows in Fig. 6). A comparison between Fig. 4 *C* and Fig. 2 *C* shows that the material, that is contiguous with the RNP particle, belongs to the nuclear ring also in this case. The contact region is marked on the reference particle in Fig. 4 *C* (*red arrow*). On the opposite side of the particle, the NPC is close, but an interaction could not be convincingly established (Fig. 6). In other reconstructions, however, such a second contract was demonstrated (see below).

All together six translocating RNP particles were investigated in the same manner as the two particles considered above. For all six, connections between the particle and the periphery of NPC could be demonstrated. All the particles showed a region of contact in domain 4. Even the particle with the ribbon fully extended maintained this connection, suggesting that this connection is kept as long as any part of the RNP particle remains on the nucleoplasmic side of the NPC. Three of the six particles exhibited an additional connection on the opposite side of the RNP particle. It was possible to identify the tripartite structure of the NPC in all six reconstructions, and again the con-

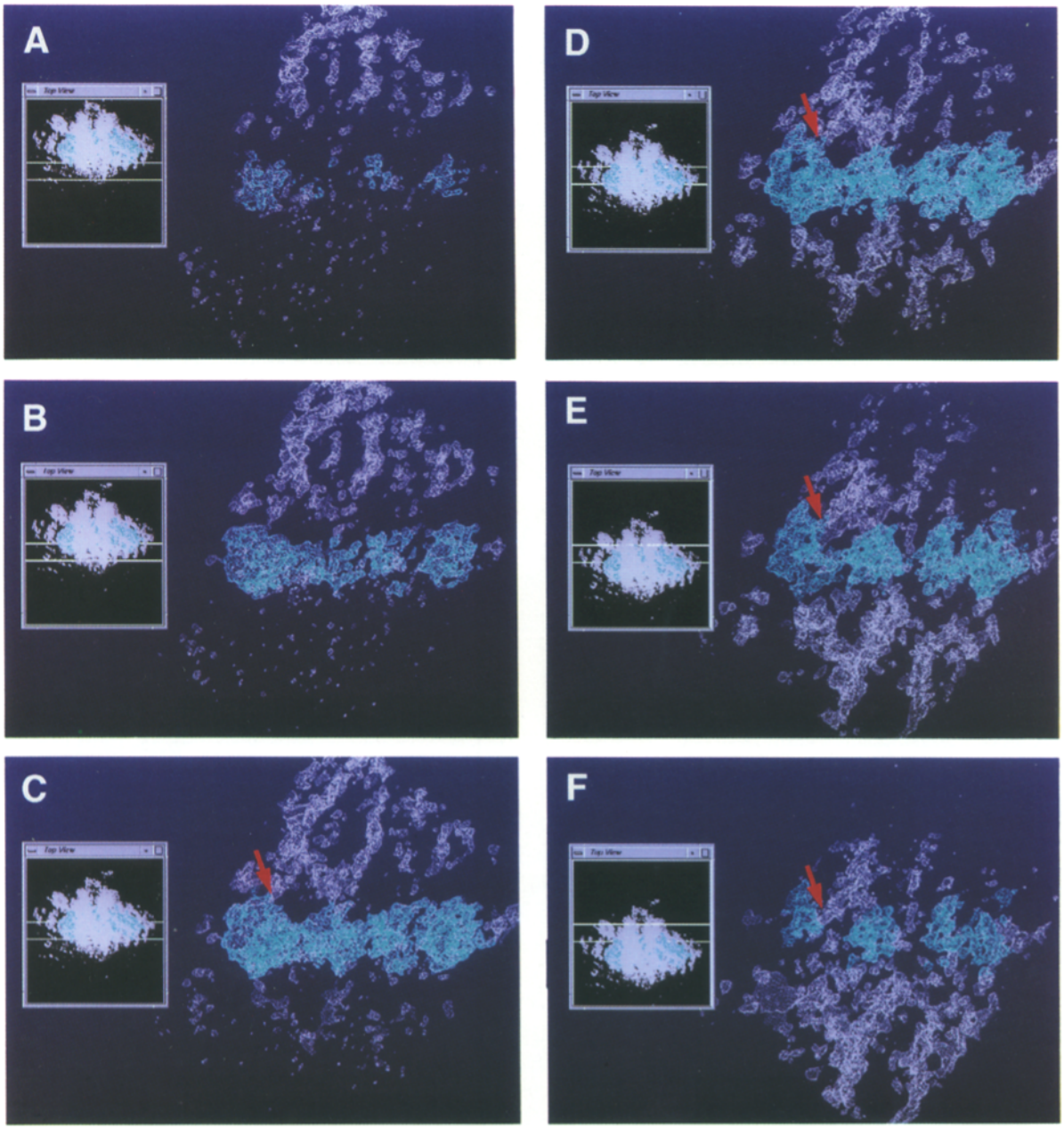

Figure 6. Consecutive slices through the late reconstruction of the translocating RNP particle presented in Fig. 4 *C*. The organization of the figure and the symbols are the same as in Fig. 5. The connection between the nuclear ring of the NPC and the RNP particle is indicated with red arrows. Although tentative, a second interaction between the NPC and the opposite side of the particle could not be convincingly demonstrated.

nections were always between the particle and the nuclear ring of the NPC.

All the nine established connections between the globular part of the RNP particle and the nuclear ring were marked on one and the same reference particle (Fig. 7). Even if all the particles do have a connection to domain 4, the interaction does not occur at a specific region within the domain. Instead the contact regions are distributed from one end to the other of domain 4. When a second connection was present, it always appeared on the opposite side of the particle. The three connections were mapped to domains 2 and 3 as seen in Fig. 7.

To analyze the significance of the observed contact pattern, we investigated how far the translocation had progressed in the six cases studied, i.e., how large a portion of the ribbon that had been translocated through the NPC. The earliest stage of translocation was designated 1 and the latest, the fully extended ribbon, 6. The figures were inserted into the corresponding contact regions in Fig. 7. Evidently, the position of the contact regions within domain 4 is related to the stage of translocation. At an early stage the contact region is close to domain 3 while at a late stage of translocation it is at the end of domain 4 in the particle. When the particle passes through the pore, the

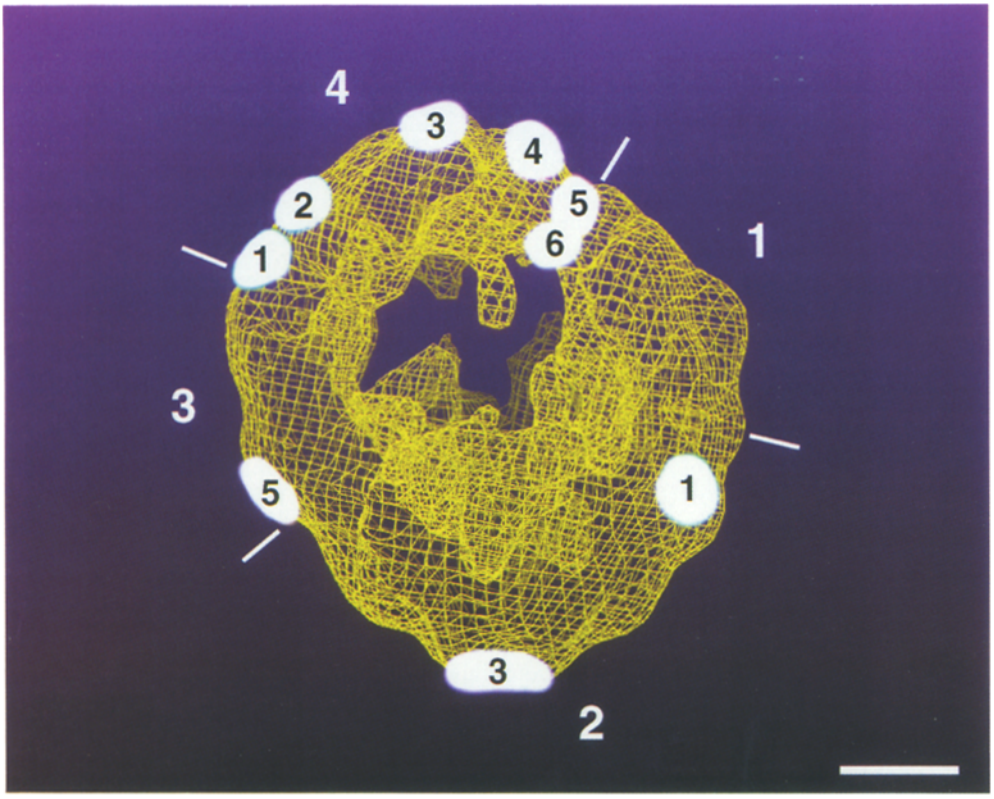

Figure 7. Regions on the RNP particle interacting with the nuclear ring of the NPC. The four structural domains of the particle (*1–4*) have been indicated with white figures. Six RNP particles have been studied and ordered according to their stage of translocation (the earliest named *1*, and the latest *6*). The contact regions have been mapped on the reference particle (*white patches*), and the corresponding particle designations have been inserted (*1–6* in *black*). A correlation between the position of the contact regions and the stage of translocation can be seen both within domain 4 and within domains 2 and 3. Bar, 10 nm.

contact seems to shift gradually along domain 4. On the opposite side of the particle, the three contact regions in domains 2 and 3 also show a similar correlation between their position and the stage of translocation (clock-wise order). Again, it is indicated that the contact moves gradually along the surface of domains 2 and 3 concomitant with the translocation process.

The pattern of contact regions can be understood when the exact position of the particle in relation to the NPC is considered, and if it is assumed that the ribbon ring rotates in front of the pore. At the entrance to the NPC, the ribbon ring attains a position perpendicular to the nuclear envelope, and consequently also to the plane of the nuclear ring of the NPC (see Fig. 4). When the extended ribbon moves through the pore, the remaining ribbon ring rotates around an axis through the center of the ring. The periphery of the ribbon ring touches the nuclear ring of the NPC with one contact in domain 4 and occasionally also one on the opposite side of the ring in domains 2 or 3. When the ribbon ring rotates in its position perpendicular to the nuclear ring, the two contacts between the ribbon and the nuclear ring of the NPC gradually shift along the periphery of the particle after the pattern shown in Fig. 7. Due to the upright position of the ribbon ring, there are no contact regions on the lateral sides of the ring (Fig. 7) (cf. the top views of the particle in Figs. 5 and 6). The rotational movement can best be illustrated by comparing the position of the reference particles in Fig. 4: the three translocating particles represent consecutive stages during translocation, and the reference particles aligned to the remaining intranuclear portion of the translocating particles can be seen rotated in relation to each other. As assumed above, the rotation seems to occur more or less around a fixed axis, which can be demonstrated in Fig. 7. If the contact regions 1, 3, and 5 in domain 4 and the corresponding regions in domains 2 and 3 are interconnected with lines, these will cross each other in the center of the RNP particle. Thus, the rotational movement of the particle seems to occur around an axis passing approximately through this cross point.

The Transition between the Bent and Extended RNP Ribbon

As shown above, the bent ribbon of the particle is being straightened when entering the central channel. It was observed that there is a sharp transition, a kink, between the bent and extended ribbon (white arrow in Fig. 5 *D*; see also Fig. 4 *B*). The position of this transition point appears remarkably constant with respect to the NPC: the distance between the transition point and the center of the NPC, the spoke assembly plane, was measured to be ~23 nm. This structural observation is compatible with the presence of a central channel rather than a pore and indicates the position of the entrance to this channel within NPC.

Discussion

The present study has been focused on the transport of a large premessenger RNP particle through the nuclear pore complex. The conformational changes of the particle upon translocation as well as the contacts of the particle with the pore complex have been established. The results are summed up in Fig. 8. In our discussion we will consider three specific aspects of the transport process: the recognition of the RNP particle at the entrance of the NPC, the

translocation of the particle through the central channel, and the rotation of the trailing portion of the particle and its interaction with the nuclear ring. Smaller RNP granules and fibers have earlier been observed within the NPC (Franke and Scheer, 1974), but unfortunately, the information on the behavior of these RNP particles is still too limited to justify more detailed comparisons with the present data.

Recognition of the BR RNP Particle at the NPC

The BR particles are first seen attached to the nucleoplasmic fibers that are anchored to the nuclear ring of the NPC (Mehlin et al., 1992). It is not clear how a BR particle subsequently moves towards the center of the NPC, but it has to finally dock in a specific manner in front of the entrance of the central channel in order to start to translocate with the 5′ end of the transcript in the lead. The earliest stage of translocation studied here should approximately reflect the position of such a docked particle (Fig. 8 *A*). The ring-like particle attains a position perpendicular to the nuclear envelope. The 5′ end of the particle is deep into the NPC and contiguous with the entrance to the pore. There is at least one additional contact between the particle and the NPC: the border between the particle's domains 3 and 4 and the nuclear ring.

This putative docking position with the particle's 5′ end in front of the pore requires a specific recognition between at least one part of the particle and the NPC. It is not excluded, but seems less plausible to us, that the initial interaction between the particle and the nucleoplasmic fibers directly results in a precise positioning of the particle at the entrance of the central channel. Nor do we believe that the peripheral particle-nuclear ring contact is instrumental in bringing the particle into its precise docking position, as this interaction is likely to be secondary to the translocating event (see below). We rather favor the alternative that the 5′ end of the transcript with its cap structure and/or cap-binding proteins recognizes the entrance to the channel and thereby positions the particle before translocation. Such a conclusion is in good agreement with recent biochemical data suggesting that the cap structure is necessary for the transport of snRNA (Hamm and Mattaj, 1990; Jarmolowski et al., 1994), and it facilitates the transport of premessenger RNA (Jarmolowski et al., 1994; see also Dargemont and Kühn, 1992). An interaction between the 5′ end of the transcript and structures at the entrance of the channel could conceivably trigger processes such as gate opening and possibly also initiate a mechanochemical machinery, if present.

Translocation of the RNP Particle through the Central Channel

Evidently, the central channel cannot accommodate the particle with its diameter of 50 nm, but the extended ribbon is let through end on. The ribbon passes through the central channel maintaining its width of ~25 nm, which probably corresponds to the maximal width of the channel (Dworetzky and Feldherr, 1988). It should be noted that the zig-zag pattern of the elementary RNP fiber within the ribbon is essentially maintained during the translocation stage (cf. Fig. 4).

When passing through the pore, the ribbon is remarkably straight both in front of and behind the level of the spoke assembly (Fig. 8, *B–F*) (see also e.g., Stevens and Swift, 1966; Mehlin et al., 1991). One possible explanation would be that the ribbon is guided through a channel rather than through a pore. A similar conclusion has been drawn from analysis of the transport of nucleolar material in amphibian oocytes (Franke and Scheer, 1974). Massive amounts of particulate material could be seen passing through the NPC in a dumb bell configuration indicating the presence of a central channel (see e.g., Fig. 25, *D–E* in Franke and Scheer, 1974).

The position of the entrance to such a channel could be tentatively established in the present study from the position of the sharp transition between the leading translocating ribbon and the bent ribbon in the trailing globular portion of the particle (Fig. 8, *B–E*). The kinked appearance of the ribbon is difficult to explain if there is no structure guiding the ribbon. The transition point is well defined and located 23 nm in front of the central spoke assembly plane (Figs. 4 *B* and 5 *D*). As indicated in e.g., Fig. 8 *B*, the tran-

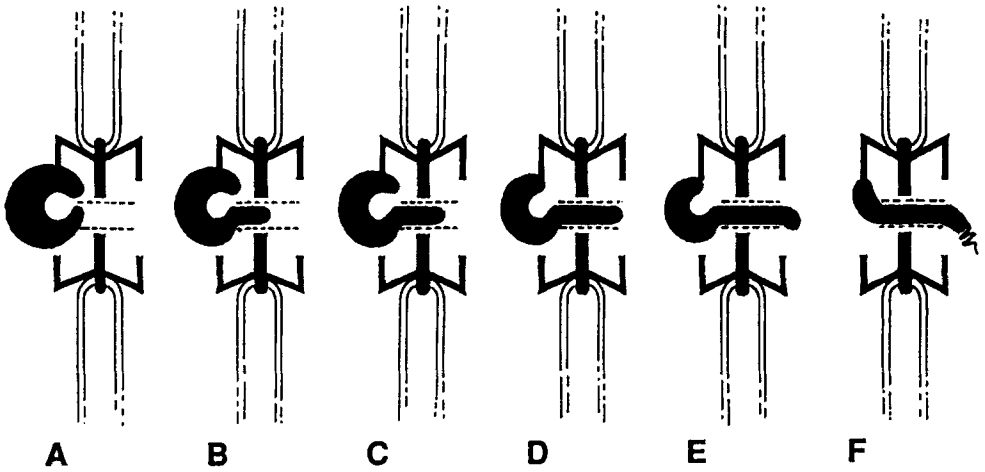

Figure 8. Schematic presentation of the translocation of a BR RNP particle through an NPC and the interaction between the particle and the tripartite complex. Upon translocation, the bent ribbon is straightened and passes through a central channel (*marked by dots*). The transition between the bent and extended ribbon is abrupt and indicates the position of the entrance to the channel. The trailing ribbon ring rotates in front of the entrance, and a sliding contact between the particle and the nuclear ring is maintained during the translocation. Note that this contact is kept also after the cessation of the rotation. On the cytoplasmic side of the NPC, the elementary RNP fiber in the ribbon starts uncoiling.

sition point is located between the plane of the nuclear ring and that of the spoke assembly (cf. Figs. 2 *A* and 5 *D*) and should indicate the position of the entrance to the central channel. The other end of the channel is more difficult to demarcate. The ribbon is maintained straight until the level of the cytoplasmic ring but then often deviates, and the elementary thin RNP fiber starts uncoiling (e.g., Fig. 1 *A*; see also Fig. 4 in Mehlin et al., 1991) (Fig. 8 *F*). We, therefore, suggest that the exit of the channel is located at about the plane of the cytoplasmic ring. The position of the putative channel, ~50 nm in length, has been indicated by dotted lines in Fig. 8.

As a central channel must be contained within a physical body, an NPC component of the corresponding dimensions has to be predicted placed in the center of the spoke assembly. Classical electron microscopic studies have demonstrated that there is a central plug at this position (Franke and Scheer, 1974; Maul, 1977). More recently, Akey and Radermacher (1993) have described an hour glass shaped cylinder, designated transporter, attaining the same central position in NPC. The problem has been to demonstrate that this plug/transporter does represent a genuine NPC component and not simply material in transit. The translocating behavior of the BR RNP particle and the amphibian nucleolar material does indicate that there is a specific NPC component in the center of the spoke assembly, that accommodates a central channel.

Rotation of the Trailing Portion of the RNP Particle and Contacts between the Particle and the Nuclear Ring

Our analysis of the contacts between the trailing globular portion of the RNP particle and the nuclear ring of the NPC shows that the particle rotates more or less around an axis passing through the center of the ribbon ring and being parallel with the nuclear envelope (Fig. 8, *B–E*). The rotation seems secondary to the passage of the extended ribbon through the central channel. The ribbon ring, perpendicular to the nuclear ring, touches this structure initially at its border between domains 3 and 4 (Fig. 8 *B*), and then the contact moves along domain 4 (Fig. 8, *C–D*) to reach the end of domain 4 (Fig. 8 *E*). Occasionally, there is also a second connection between the particle and the nuclear ring on the opposite side of the particle, and again the pattern of contacts suggests that the contact slides along domains 2 and 3. The reason for the fact that there is always an interaction between domain 4 and the nuclear ring could be that such a tilted position of the ribbon ring facilitates the translocation of the ribbon through the central channel (minimal kink at the entrance to the NPC) (Fig. 8 *B*). Whether or not the particle then also touches the nuclear ring on the opposite side could be dependent on a slight flexibility in the exact positioning of the particle within the NPC or on dynamic changes within the particle and/or the nuclear ring.

It is striking that the particle maintains contact with the nuclear ring also very late in the translocation process when the ribbon is more or less extended (Fig. 8 *F*). Whether or not this connection is of different nature than the preceding, seemingly unspecific interactions between the particle and the nuclear ring during the rotational movement, remains to be established. It should be recalled that the poly(A) tail has been invoked in the regulation of RNA export (e.g., Wickens and Gurdon, 1983). Furthermore, it has been claimed that phosphorylation/dephosphorylation of the poly(A)-binding protein, known to be associated with the nuclear envelope, affects the binding of the protein to poly(A) as well as the export rate of messenger RNA (Schröder et al., 1987). A speculative hypothesis would then be that the final ribbon-nuclear ring contact can control whether or not the particle does leave the nucleus.

Conclusions

The BR RNP particle docks in front of the entrance to the central channel: the ribbon ring attains a vertical position with its 5′ end domain at the very entrance. Upon translocation the ribbon extends and moves through the channel, and the remaining portion of the ribbon ring rotates. The ribbon ring seems to slide against the nuclear ring of the NPC with one contact region in domain 4 and occasionally also an additional one on the opposite side in domains 2 and 3. The initial contact between the 5′ end of the ribbon and the central channel is probably crucial to accomplish proper docking and the ordered translocation with the 5′ end of the transcript in the lead. The peripheral interactions between the trailing portion of the particle and the nuclear ring are likely to be secondary to the translocation of the ribbon through the central channel. It is noted that the connection between the 3′ end domain of the ribbon and the nuclear ring is maintained late during the translocation. The behavior of the ribbon during translocation indicates that the ribbon passes through a 50-nm-long and 25-nm-wide central channel supporting the concept of a specific NPC component in the center of the spoke assembly accommodating such a central channel.

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