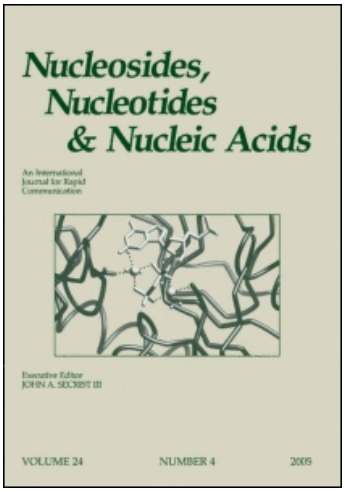


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# Synthesis and Transport of a Specific Premessenger RNP Particle

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# SYNTHESIS AND TRANSPORT OF A SPECIFIC PREMESSENGER RNP PARTICLE.

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Abstract. The three dimensional structure of a specific premessenger RNP particle has been studied using electron microscope tomography. The structure of the globular particle in the nuclear sap can be described as a bent ribbbon with the ends in close proximity to each other. During translocation the ribbon opens up and elongates as it passes through the nuclear pore.



The biochemical study of specific premessenger RNP particles has for a long time proven difficult. This is due to instability of the RNA in conjunction with difficulties in separating one RNP species from the total RNP population. However, secretory proteins synthesized in the salivary glands of Chironomus tentans in its larval stage derive from conspicuously large transcripts on the Balbiani Ring (BR) genes. The chromosomes in these cells are polytene, carrying thousands of identical parallell chromatids. The BR genes are intensely transcribed with the DNA looping out from the giant chromosome in big puffs[1,2]. Immediately upon synthesis, the transcript is complexed with RNA packaging proteins to form a large globular premessenger RNP particle with a diameter of about 50 nm. It has a skewed doughnut shape with a hole in the centre. Due to their size, abundancy and characteristics, the RNP particles can be studied both biochemically and ultrastructurally[3]. They can be readily identified in the electron microscope, not only during synthesis in the puffs, but also during transport in the nucleus and during translocation to the cytoplasm[4,5].

The three dimensional (3D) structure of the BR RNP particles at different stages in the cell has been studied with a recently developed

technique for electron microscope tomography (EMT)[6,7,8]. EMT is a general procedure for 3D reconstruction of single asymmetric macromolecular objects. The plastic embedded specimen is cut into ultrathin sections and colloidal gold is deposited on top. An area of interest in a single section is photographed in the electron microscope from different angles of incidence. Typically 25 pictures are taken while the specimen is tilted inside the microscope from -60 to +60 degrees. The photographs are digitized and stored in a computer. The collodial gold particles serve as reference markers to align the photographs to each other. A transmission electron microscope picture of a biological specimen can be regarded as a 2D projection of a 3D structure at the moderate resolution possible with imaging of heavyatom stained plastic sections. Several such projections taken a different angles carry sufficient information to accomplish a 3D reconstruction of the structure[9,10,11,12]. The 3D reconstruction can thus be calculated according to the filtered backprojection principles as a series of 2D reconstruction maps along the tiltaxis. The 3D reconstruction can be displayed and analyzed on a modern computer controlled vector/raster display[13].



The EMT technique has been used to reconstruct the BR RNP particle in three dimensions during its synthesis, transport in the nucleus and the translocation through the nuclear pore complex. Several individual RNP particles in the nuclear sap were reconstructed, and four that were found to be completely within the plastic section were further analyzed. They showed great similarities to each other regardless of their original orientation within the section. The high correlation coefficient (0.8) granted the calculation of an average structure at a resolution of 8.5 nm[8]. The large globular particle could now be described as a rather thick, bent and slightly skewed ribbon with its ends close to each other. The bent ribbon could be divided into four separate domains, each with its own structural characteristics regarding shape and dimensions. The asymmetry of the particle allowed a comparison of the reconstruction to the stages

of synthesis of the BR RNP and thus the direction of synthesis of the bent ribbon could be assessed.

During translocation through the nuclear pore complex the structure of the RNP particle is dramatically changed[4]. The globular particle becomes extended and elongated with a length of 135 nm. 3D reconstructions of early stages of translocation has been studied. During this stage the particle gradually unfolds, keeping the not yet unfolded domains of the structure in a state that can be very well compared to the corresponding domains in the nuclear sap particle (to be published). The structural changes of the particle can therefore be followed and the domain that first enters the pore identified. Because the structure is extended and less compact, it also gives more information about the internal structure of the ribbon. The nuclear sap particle has been studied at higher resolution (5 nm) and the constituent fibre[3] making up the bent ribbon is observed. A tracing of the folding of this thin fibre is currently under way by supplementing the higher resolution structure with the information from the extended parts of the structure during translocation.

Isolation and initial biochemical characterization of the BR RNP particle has recently been accomplished[14]. Electronmicroscope studies of the isolated glutaraldehyde fixed particles indicate a well preserved overall 3D structure. Protein analysis and production of antibodies in conjunction with the EMT method will allow mapping of the constituent components of the BR RNP particle in three dimensions.

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