DINAMICS OF RIBOSOME BIOGENESIS DURING THE POLLEN DEVELOPMENTAL PROGRAM BY *IN SITU* LOCALIZATION OF NUCLEOLAR TARGETS

Pilar S. TESTILLANO[2], Pablo GONZÁLEZ-MELENDI[1], Paricherer AHMADIAN[1], Guadalupe PRÉSTAMO[1], Concepción G. MENA[1], Mª del Carmen RISUEÑO[1]

[1] *Centro de Investigaciones Biológicas, CSIC, Velazquez 144, 28006 Madrid.Spain. Fax: 34-1-562.75.18*

[2] *Centro de Biología Molecular Severo Ochoa , CSIC/UAM, Cantoblanco, Madrid*

The nucleolus, is a highly dynamic organelle whose structural components and funtional organization vary in relation to the ribosomal synthesis rate. During microsporogenesis, the nucleolus progressively reactivates and the molecular machinery of ribosomal transcription and processing is reorganized. The *in situ* techniques based on the use of molecular probes to localize specific targes makes it possible to localize molecules and functions in specific subcellular structures, and in cells at precise developmental stages. In this paper, the immunolocalization of various nucleolar antigens and the *in situ* hibridization for localizing rRNA have been used at light (LM) and transmission electron (TEM) microscopy levels to study the ribosomal transcription and processing in relation to nucleolar architecture during the *in vivo* pollen developmental process, in *Capsicum annuum* L., pepper.

Cryotechniques were used to process the plant material for LM and TEM and to preserve the antigenicity and accesibiliy of antigens and transcripts to antibodies and probes. Anthers were either cryofixed in liquid propane at -160°C and cryosectioned, or fixed in paraformaldehyde, dehydrated by PLT and cryoembedded in Lowicryl K4M. In most of samples, the methilation-acetylation (MA) method, an ultrastructural cytochemical technique for nucleic acid-containing structures, was performed to improve the ultrastructural visualization of the nuclear structures in combination with the immunogold labelling. Immunofluorescence and immunogold labelling were done using antibodies recognizing molecules involved in ribosomal transcription and processing: anti-NOR 90 serum against the Upstream Binding Factor (UBF) associated to the RNA pol I; anti-fibrillarin, nucleolar protein involved in early events of pre-rRNA processing (Fig. 1), anti-DNA/RNA hybrid antibodies to map transcription sites; and anti-RNA antibodies (Figs. 2, 3). Digoxigenin labelled rRNA probes encoding the 18 S and 25 S fragments from *Arabidopsis* were used for *in situ* hybridization.

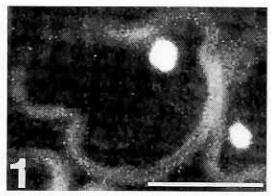
The distribution of the ribosomal transcripts was studied during this developmental process. The 18S and 25S rRNA species were localized by ultrastructural RNA/RNA *in situ* hybridization (ISH) on the microspore and pollen grain nucleoli during their development. In all developmental stages studied the nucleolus and ribosome-rich areas of the cytoplasm were labelled, and a lower signal was also observed in the nucleoplasm. ISH results clearly indicated an heterogeneous distribution of ribosomal transcripts in the nucleolar components (Figs. 5, 6) and variations with nucleolar activity and developmental stage. In vacuolate microspores (Fig. 4), nucleoli showed abundant ISH signal on the GC which ocupies the nucleolar periphery and large areas of its interior; a moderate signal was observed on the DFC. The nucleoplasm labelling coming from the pre-ribosomes in transport to the cytoplasm, showed important variations at specific developmental stages, being specially abundant in mature vegetative nucleus. The nucleolar vacuole of activity appearing at specific developmental stages with high transcriptional activity also showed ISH signal..

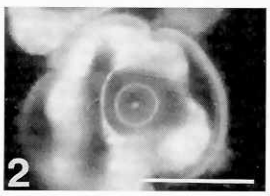
The immunolocalization assays localized several antigens belonging to the rRNA synthesis and processing machinery during pollen development, specifically on microspore and bicellular pollen grain nucleoli. Anti-DNA/RNA hybrid antibodies revealed sites of transcription at specific regions of the nucleolar DFC, labelling being higher in vacuolate microspore and vegetative nucleoli. The anti-UBF antibodies labelled the DFC but showed a more spread distribution than the hybrids. Anti-DNA/RNA hybrid and anti-UBF double labelling assays revealed sites of colocalization at specific regions of the nucleolar DFC of the developing pollen grain. Fibrillarin was also immunolocalized on the microspore (Fig. 1) and bicelular pollen grain nucleoli, immunogold labelling was found on the DFC, the labelling was different in vacuolate, generative and vegetative nucleoli, gold particles following the distribution of the DFC which varies its location in those nucleoli at different developmental stages. These results clearly indicate the compartmentalization of functions in the nucleolar structural components, the fibrillar ones being involved in transcription and early processing. The anti-RNA immunofluorescence showed a heterogeneous brightness in the nucleoli of vacuolate microspore and bicellular pollen grains, together with a high positive signal in the cytoplasm (Figs. 2, 3). Differences in the immunofluorescence signal between both the vegetative and generative nucleoli were observed. The nucleolar components displayed a heterogeneous anti-RNA gold labelling density, the GC showing abundant labelling, whereas the DFC apeared moderately labelled. This fact resulted in differences in density and heterogenecity of the nucleolar labelling at specific developmental stages during microsporogenesis and maturation of the pollen grain.

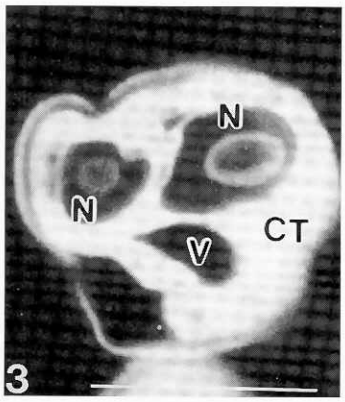
The localization patterns of ribosomal transcripts and several nucleolar antigens constitute useful cellular images of the state of ribosomal biogenesis activity in the pollen grain nucleus during its process of development, and reveal differences in such activity at specific developmental periods.

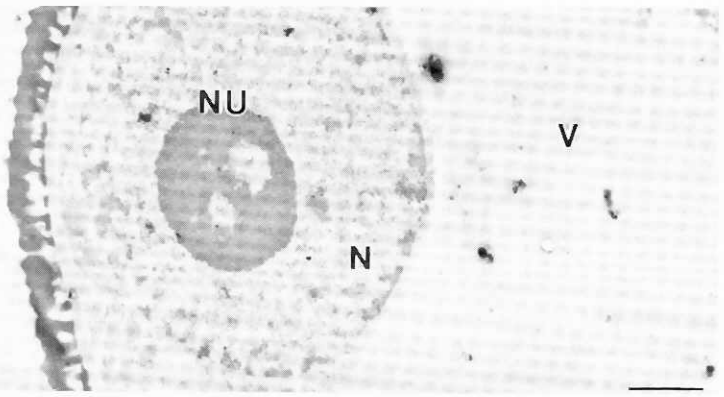
References

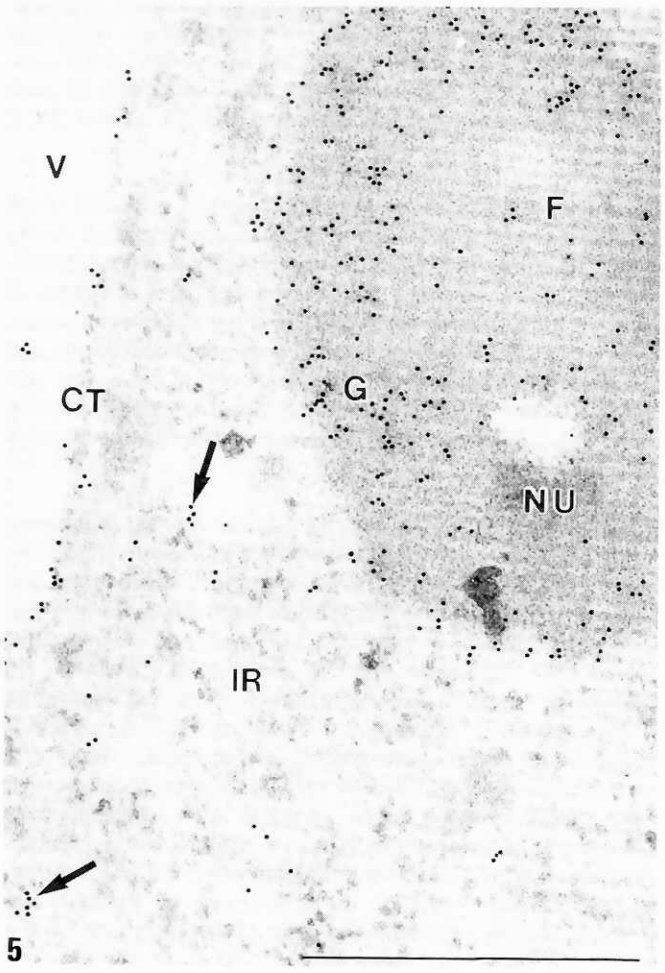
Testillano, P.S., González-Melendi, P., Ahmadian, P., and Risueño, M.C. (1995). The immunolocalization of nuclear antigens to study the pollen embryogenesis. Exp. Cell Res.221: 41-54.
González-Melendi, P., Testillano, P.S., Ahmadian, P., Fadón, B. and Risueño, M.C. (1996). New *in situ* aproaches to study the induction of pollen embryogenesis in *Capsicum annuum* L. Eur. J. Cell Biol. 69: 373-386.
Risueño, M.C. and Testillano, P.S. (1994) Cytochemistry and immunocytochemistry of nucleolar chroamtin in plants. Micron 25: 331-360.
Mena, C.G., Testillano, P.S., González-Melendi, P., Gorab, E. and Risueño, M.C. (1994) Immunoelectron microscopy of RNA combined with nucleic acdi cytochemistry in plant nucleoli. Exp. Cell Res. 212: 393-408.

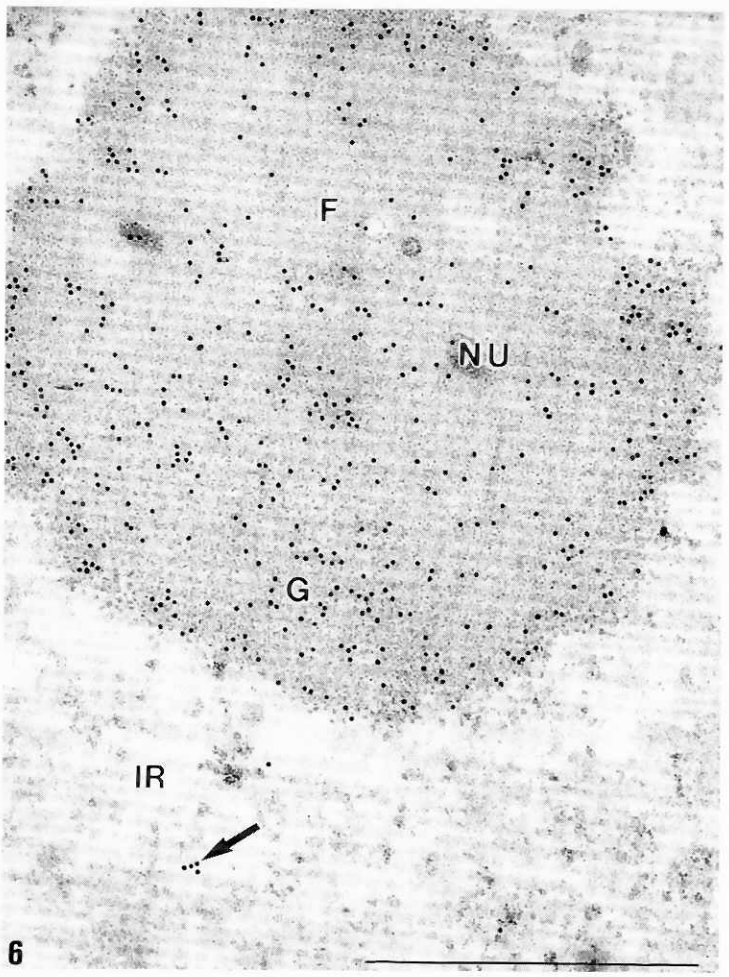












Figs 1 to 3: Immunofluorescence on pollen cryosections. Figs. 1, 2: Vacuolate microspore. Fig 1: Anti-fibrillarin. Fig 2: Anti-RNA. Fig. 3: Bicellular pollen grain, anti-RNA. Figs. 4 to 6: Vacuolate microspores, 25 S rRNA *in situ* hybridization (Figs 5 and 6). Heterogeneous labelling is found on the granular (G) and dense fibrillar (F) components of the nucleolus (Nu), some gold particles (arrows) appear in the interchromatin region (IR). Cytoplasm, CT; cytoplasmic vacuole, V; Nucleus, N. Bars in LM, 10 μm; in EM, 1 μm.