

## **The use of *in situ* hybridisation and immunocytochemistry to characterise coiled bodies in plant meiocytes**

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Coiled bodies are ubiquitous nuclear organelles that seem to be related to RNA processing, as is suggested by the presence in these bodies of essential components of the pre-mRNA splicing machinery as well as some elements of rRNA maturation (1). These processes have been widely studied through the use of molecular biology techniques, although the use of *in situ* localisation in order to transpose this knowledge to the cellular level is becoming more and more important.

In the present study, we have investigated nuclear bodies morphologically similar to coiled bodies for the presence of key components during the meiotic prophase of olive pollen. Anthers of *Olea europaea* were fixed in 4% paraformaldehyde and 1% glutaraldehyde and embedded in Unicryl. A variety of techniques to detect nucleic acids by TEM have been used. The EDTA regressive staining method, preferential for ribonucleoproteins, showed these nuclear bodies well contrasted in comparison to chromatin masses which appeared bleached by this method. Monoclonal anti-DNA antibodies failed to detect DNA in these structures while intense labelling was observed in the chromatin masses. 18S rRNA was detected by *in situ* hybridisation using a digoxigenin labelled antisense RNA probe from *Raphanus* (2). The probe detected rRNA at different locations in the nucleolus and ribosomal rich areas of the cytoplasm, but did not label the coiled bodies (Fig. 1). Finally, we have optimised the conditions for hybridisation using digoxigenin-labelled antisense (or sense) oligo probes synthesised from mouse and *X. laevis* U3 RNA consensus sequence (3). U3 is a small nuclear RNA (snRNA) which has been shown to play an essential role in processing vertebrate and yeast rRNA (4). The U3 snRNA was detected in these structures as well as in some areas of the nucleolus (Fig.2).

Our results prove that these bodies are not directly involved in rRNA synthesis nor maturation, but suggest their relation with the rRNA maturation machinery as they contain U3 snRNA.

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### **REFERENCES**

1. Ochs RL, Stein TW, Tan EM (1994) *J Cell Sci* 107, 385-399
2. Delseny D, Cooke R, Penon P (1983) *Plant Sci Lett.* 30, 107-119
3. Reddy R and Gupta S (1990) *Nucleic acid Res* 18, 2231-2235.

4. Fournier MJ and Maxwell ES (1993) *Trends Biochem Sci* 18, 131

**Figures 1 and 2: Localisation of rRNA and U3 snRNA by *in situ* hybridisation to Unicryl sections of olive meiocytes.** Bar = 1 $\mu$ m. **Figure 1:** 18S rRNA antisense probe from *Raphanus*. The hybridisation signal is found in the nucleolus (N) and ribosome-rich areas of the cytoplasm (cyt). Coiled bodies show no labelling (star). **Figure 2:** U3 snRNA oligo probe. Gold particles are present on certain areas of the nucleolus as well as on coiled bodies (star).

