Proteomic analysis of the *Arabidopsis* nucleolus

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Plant and cell growth and development depend on the regulation of expression of thousands of genes. Regulation occurs at a number of different levels, such as transcription, RNA processing and translation. The cell must produce all of the machinery required to switch genes on and off, to process and transport RNAs, to translate mRNAs into proteins and to produce protein or protein-RNA complexes. Transcription and processing occur in the nucleus and the wide range of different activities which underpin gene expression involve many sub-compartments or bodies within the nucleus, and dynamic interactions of different components and machineries among these sub-nuclear bodies. The most prominent nuclear sub-compartment is the nucleolus. The nucleolus is traditionally recognised as the site of ribosome production involving ribosomal DNA transcription, ribosomal RNA processing and ribosomal subunit assembly. However, over the last five years it has become apparent that the nucleolus is involved in a much wider range of activities and processes. For example, it is involved in aspects of processing or export of some mRNAs and tRNAs, the processing and assembly of RNA-protein particles (RNPs) such as telomerase RNP and the signal recognition particle (SRP), and potentially in RNA turnover and nuclear translation.

In plants, the only nuclear bodies identified so far are the nucleolus, and Cajal bodies, which function in the maturation of small nuclear and nucleolar RNPs. To understand the complex events underpinning gene expression, we have carried out a proteomic analysis of nucleoli purified from protoplasts of *Arabidopsis*. This work has been carried out in collaboration with the research groups of Prof. Peter Shaw, John Innes Institute, Norwich, and Prof. Angus Lamond, University of Dundee (responsible for characterisation of the nucleolar proteome of humans). The analysis of the *Arabidopsis* nucleolar proteome allows a comparative proteomic analysis of these two widely separated higher eukaryotes to identify common and species-specific nucleolar components. We have so far identified around 200 different proteins, including ribosomal proteins, known nucleolar and RNA-binding proteins, putative DNA/chromatin binding proteins, histone acetylases/deacetylases, DEAD box helicases, splicing and translation factors, putative RNA transport factors and snoRNP core proteins. In addition, we have identified proteins which are specific to the plant nucleolus, with no homologies to human proteins, and proteins of unknown function which occur in both the plant and human nucleolar proteome, suggesting a conserved function. We are currently fusing available full-length cDNAs for the identified proteins to the green fluorescent protein (GFP) to examine the localisation of the proteins in *Arabidopsis* cells (Figure 1). To date, the high percentage of GFP fusions showing nucleolar labelling highlights the quality of the plant nucleolar preparation. Detailed analysis of their sub-nucleolar location will provide clues about potential functions of the proteins and the different processes in which they are involved.

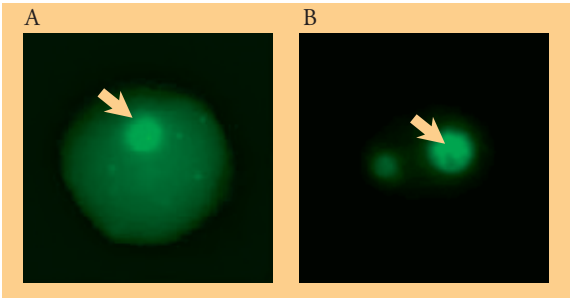

Figure 1 Nucleolar localisation of proteins of unknown function which are plant-specific (A) or found in both plant and human nucleolar proteomes (B). Arrows indicate nucleoli.