**PIEF-GA-2010-252927 RoxNP, redox regulation of nuclear proteins**

***1. Summary report***

Cell proliferation (mitosis) controls growth in all organisms. Loss of mitosis control results in the development of cancer in animals, whereas exposure to stress causes an arrest of the cell cycle. When cells perceive stress, specific signals block mitosis but the mechanisms that underpin this control are poorly described and understood. The tripeptide glutathione (GSH) is not only a major antioxidant in all higher eukaryotes but the pool of GSH in the nucleus is important in cell cycle control. This innovative project seeks to characterise nuclear GSH, including transport and protein regulation functions to elucidate how it controls cell proliferation. The results obtained in this project concerning the characterisation of the functions of GSH in the model plant *Arabidopsis thaliana* lead to the following conclusions:

1. *GSH depletion significantly increases in the redox states of the nucleus and cytosol, and causes arrest of the cell cycle in roots but not shoots, with accompanying transcript changes linked to altered hormone responses but not oxidative stress.*

This conclusion is based on the following findings:

* Severe GSH depletion specifically inhibited root meristem development, while low root GSH levels decreased lateral root densities.
* The redox potential of the nucleus and cytosol of *Arabidopsis thaliana* roots determined using roGFP probes was between -300 to -320mV.
* Growth in the presence of the GSH synthesis inhibitor buthionine sulfoximine (BSO) increased the nuclear and cytosolic redox potentials to approximately 260mV.
* GSH-responsive genes including transcription factors (SPATULA, MYB15, MYB75), proteins involved in cell division, redox regulation (glutaredoxinS17, thioredoxins, ACHT5 and TH8) and auxin signalling (HECTATE1), were identified in the GSH-deficient *root meristemless 1-1* (*rml1-1*) mutant, and in other GSH synthesis mutants (*rax1-1*, *cad2-1*, *pad2-1*) as well as in the wild type following the addition of BSO.
* Inhibition of auxin transport had no effect on organ glutathione levels but exogenous auxin decreased the root glutathione pool.

1. *Glutathione has important functions in the nucleus*

* The roGFP probes detected GSH in the nucleus of root cells.
* The specific fluorescent probe 5-chloromethylfluorescein diacetate (CMFDA) was also used to estimate the intracellular distribution of GSH between the nucleus and cytosol. The CMFDA stain was visible in the cytosol and nucleus of root cells but migrated within 30 minutes to the vacuole.
* Hydroxyurea was used to arrest the cell cycle at the G1/S transition.
* Nuclei with intact nuclear envelopes were purfied using the INTACT system. However, the yield of intact nuclei was very low. Alternative methods were therefore developed to purify specific nuclear proteins, particicularly histones and PARP. The analysis of redox modification of plant histones (carbonylation and poly-ADP ribosylation) was optimized in collaboration with the group of Federico Pallardo (University of Valencia, Spain). These techniques are currently being used to characterise carbonylation and poly-ADP ribosylation of histone proteins during the cell cycle.

1. *Glutathione recruitment into the nucleus* may involve *Bcl-2 associated athanogene* (*BAG*) proteins and the ALADIN protein

The roles of *Bcl-2 associated athanogene* (*BAG*) proteins and the nuclear pore complex (NPC) protein ALADIN in transporting GSH into the nucleus were characterised in *Arabidopsis*.

* Growth rates, tissue glutathione contents and the subcellular distribution of GSH were similar in the shoots and roots of the single *bag* mutants and the wild type plants.
* To invesigate further, the single mutants were crossed and double and triple mutants for the *BAG* genes were isolated.
* Growth rates, tissue glutathione contents and the subcellular distribution of GSH were similar in the shoots and roots of the double mutants.
* The *bag1bag2bag3*, triple mutants had an increased number of lateral roots relative to the wild-type.
* The *bag1bag2bag3*, accumulated less GSH in the roots and leaves than the wild-type.
* The *aladin* mutant shows slow growth of shoots and roots. However, GSH, ascorbate and pyridine nucleotide (NAD(P)(H)) levels were similar in the mutant and wild-type shoots and roots.
* The growth of the *aladin* roots was increased by GSH treatment.
* Root growth in the *aladin* mutants was more sensitive to GSH depletion induced by BSO than the wild-type.
* Photosynthesis was impaired in the *aladin* mutants relative to the wild-type.

*Socio economic impact*

These studies have greatly inreased our current understanding of how the antioxidant GSH regulates plant growth and development, particularly in relation to GSH funtions in the nucleus and GSH-dependent regulation of gene expression. The plant cell paradigm is a useful a model for the study of nuclear GSH and its functions in other organisms. For example, the characterisation of Arabidopsis mutants lacking the ALADIN protein has implications for studies on this protein in relation to human disaease.