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Progress in Characterising PKR, a Plant-Encoded and Double-Stranded RNA-Activated Protein Kinase

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During the infection of an RNA virus, replication occurs via a double-stranded (ds) RNA intermediate. As dsRNA is rare in a cell, these necessary replication intermediates of RNA viruses alert the host cell of a virus invasion. In turn, a signal cascade of defence mechanisms is activated, of which a sentinel is the dsRNA-binding protein, protein kinase R (PKR). In mammals, the expression and activity of this ~68 kDa protein has been extensively studied and it is induced to high levels by interferon treatment. Upon binding to dsRNA, PKR autophosphorylates and phosphorylates the protein translation initiator eIF2 α . This renders eIF2 α inactive, leading to the loss of protein translation and an inhibition of virus protein expression.

Recently, these hallmark activities of mammalian PKR have also been detected in plants. Furthermore, a ~68 kDa protein is immunoreactive with anti-mammalian PKR antibodies and when probed with mammalian PKR cDNA, a mRNA similar to the size of the human transcript has been detected in wheat and tobacco. These compelling results indicate the presence of PKR in plants. However, attempts so far have not been successful in determining the gene sequence.

Our aim is to identify plant PKR, initially by protein purification and then to the gene sequence. Using dsRNA affinity protein purification techniques on *Arabidopsis* extracts we detect a ~68 kDa band that is phosphorylated while bound to dsRNA and is immunoreactive with the anti-human PKR antibody. By protein sequence analysis using trypsin digestion and mass spectrometry of proteins of ~68 kDa, we aim to identify potential candidates for plant PKR. Currently, we are developing the approach to express plant PKR candidate proteins using an *E.coli*-based translation system. Subsequently, the candidate proteins will be tested for similarities to the characteristics of mammalian PKR. Our progress to date in characterising plant-encoded PKR will be presented.