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Protoemics of integral membrane proteins from developing *Brassica napus*

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As plant seeds develop the accumulation of natural products, starch, oil, and protein undergo dramatic changes. At the early stages of seed filling in oilseeds starch is the principal component. Oil (triacylglycerol) and protein concentrations do not reach a maximum until the later stages of seed development. This metabolic shift within the seed, from production of starch to production of oil and protein, indicates that seed metabolism is regulated temporally. To better understand these metabolic changes it is important to examine the cognate changes in protein expression. Integral membrane proteins represent one class of proteins which are important for inter-organellar metabolic flow. Current two-dimensional electrophoresis techniques are unsuitable for the profiling of hydrophobic membrane proteins. To specifically characterize this class of proteins, a reproducible protocol for membrane protein isolation that can be used with standard sodium dodecyl sulfate polyacrylamide gel electrophoresis needed to be developed. Alkaline sodium carbonate washing of membranes followed by ultracentrifugation appeared to yield washed membrane fractions distinct from total protein fractions. To quantify relative volume and molecular weights of individual bands, Coomassie stained gels were analyzed with ImageQuant software. Identification of these bands was performed by trypsin digesting each protein (in-gel) and obtaining accurate peptide mass 'tags' using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry. Peptide mass fingerprinting resulted in twelve conclusive identifications (out of 25 analyzed). Of these, six proteins were involved in the glucosinolate-myrosinase defense pathway. These proteins are suspected to be membrane associated, and are involved in a defense system that protects plant tissues from herbivory and fungal, viral, and bacterial infections. Other proteins were identified as: the RuBisCO large subunit, histone H3, NADH dehydrogenase subunit, pyruvate dehydrogenase E1 alpha subunit, and two types of cruciferins which are seed storage proteins. Of these, only NADH dehydrogenase is an integral membrane protein. Based on this data, the alkaline sodium carbonate wash method did not effectively enrich for integral membrane proteins. This may be due largely to the fact that certain proteins, especially cruciferin seed storage proteins, RuBisCO and myrosinases, are expressed at much higher levels than integral membrane proteins and are not quantitatively removed from membrane fractions by salt washing alone. Future work will include alternative approaches to membrane protein isolation including organic extraction.