Changes in translatable poly(A) RNA from differentiated potato tissues transformed with shoot-inducing Ti T1-DNA of Agrobacterium tumefaciens

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Summary

Two dimensional gel electrophoresis was used to examine differences in steady state total poly(A) RNA from untransformed potato (Solanum tuberosum cv. Maris Bard) and potato transformed with shoot-inducing T1-DNA from A. tumefaciens. RNA was compared from phenotypically very distinct in vitro cultured shoots, more similar grafted plants and tubers. In each case between 200-400 translation products were identified representing the more abundant poly(A) mRNA's. In general, poly(A) RNA from the transformed tissues gave more high molecular weight products. This increase was most evident in poly(A) RNA from shoot cultures. Depending on the tissue examined, 1–5% of the translation products with a molecular weight < 43 KD were observed to increase or decrease in abundance. The influence of T-DNA on cellular gene expression in the different transformed potato tissues is discussed in relation to previously determined changes in T-DNA gene expression (particularly of the T-DNA cytokinin gene) and the corresponding changes in endogenous hormone concentrations. It is concluded that some of the specific changes in low molecular weight products are either directly caused by the increased cytokinin levels or are indirectly involved in maintaining the transformed phenotype.

Introduction

It is clear that gene expression in plants can be altered by exogenous application of hormones. Rapid changes in mRNA populations (within 20 min) occurred after auxin was applied to tissues such as hypocotyls of soyabean, maize and pea (5, 12, 14, 18, 31, 33) and some of these changes may be direct effects of the hormone on transcription. However, little information is available for cytokinin mediated changes. Excised pumpkin cotyledons cultured for 4 days on benzyladenine when analysed by two dimensional electrophoresis showed both increases and decreases in proteins (9). Tobacco tissues cultured for 3 days on a medium without cytokinins (but with auxins) synthesised a new 33 KD translation product (or family of products) (10). It is not known whether these changes are induced rapidly by a direct effect of cytokinins on gene expression or the result of secondary events. However, there is some evidence that cytokinins might affect DNA transcription (11). Whether changes are direct or indirect alterations in gene expression are likely to be part of a cascade of reactions involving specific mRNA's in any or all of the three mRNA abundance classes (13).

An example of good progress in unravelling a similar cascade of reactions controlling cell growth has been made with mouse cells transformed with SV40 where the large T antigen is mainly responsible for induced changes. Hybridisation experiments showed that expression of 3–5% of the cellular poly(A) RNA had changed significantly (30). Subsequent application of cDNA cloning techniques and enrichment hybridisation with cytoplasmic poly(A) RNA from transformed and untransformed mouse cells led to the isolation of clones of

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Agrobacterium transformed plants provide an analogous situation. Strains of *A. tumefaciens* that carry a Ti-plasmid cause the formation of crown galls when a segment of plasmid DNA is integrated into the plant genome (6, 15, 21, 28). For octopine type plasmids the transferred DNA (T-DNA) contains 8 genes, one of which (for transcript 4) stimulates cytokinin biosynthesis (2, 4, 26) and two (for transcripts 1 and 2) which cause auxin biosynthesis (16, 17, 29, 32). Infection of tobacco induces undifferentiated growth when both types of genes are active but when only the auxin loci are active the induced galls produce roots and when only the cytokinin locus is active shoots are produced (1, 3, 24).

To advance our understanding of how cytokinins alter gene expression we have used the potato line Mbi501B, a derivative of Maris Bard transformed with a shoot inducing strain of *A. tumefaciens*. This strain, LBA1501, is inactivated at one of the auxin loci. The expressed cytokinin gene (T-cyt) enhances the first committed step in cytokinin biosynthesis (2, 4, 19) and as a result transformed potato shoots contain high levels of cytokinin (26). When Mbi501B shoots are grafted onto untransformed potato rootstock the phenotype becomes more normal, tubers develop and there is a reduction in T-cyt expression and cytokinin content (8, 26). Therefore Mbi501B provides a situation where the introduction of a gene affecting plant growth and development can be used to study and unravel the cascade of reactions mentioned above. The results reported in this paper are the first steps in analysing the changes in expression of poly(A) RNA that occur after transformation and alteration of cytokinin content.

**Materials and methods**

**Plant material and extraction of RNA**

Potato lines analysed were cv. Maris Bard, its transformed derivative Mbi501B (26), King Edward and its transformed derivative containing T-DNA from the shoot inducing nopaline type *A. tumefaciens* strain T37. The lines were propagated in vitro as shoot cultures on a Murashige and Skoog medium supplemented with 2% sucrose. The grafted transformed and grafted control plants, both on normal untransformed potato cv. Desiree root stock, were grown in soil in a growth chamber (25).

Total poly(A) RNA was extracted in a Tris:SDS buffer, purified by phenol chloroform extraction and the poly(A) RNA recovered by chromatography on oligo dT$_{12-18}$ cellulose (8). Duplicate RNA extracts were prepared for all tissues except transformed tubers.

**In vitro translation and electrophoresis**

*In vitro* translation of total poly(A) RNA used a rabbit reticulocyte system (BRL Gibco) with [35S] methionine to label the protein. A standard reaction volume (25 µl) contained 500 ng RNA which did not saturate translation. A wheat germ preparation (27) was also tested for *in vitro* translation but was rejected for further studies because it was less efficient at stimulating protein synthesis and gave mainly low molecular weight (MW) products many of which could not be clearly separated by electrophoresis. Aliquots (1 × 10$^6$ dpm) of *in vitro* translation products prepared from RNA extracts which showed comparable translation efficiency were analysed at least in duplicate. Analysis was by gel electrophoresis according to O'Farrell (23) with the following modifications. The isoelectric focussing gel contained in a final volume of 10 ml 5.5 g urea, 1.33 ml 30% acrylamide (28.4% acrylamide, 1.6% bis acrylamide), 1.6% pH 5.7 ampholine, 0.4% pH 3–10 ampholine, 0.034 M Tris pH 6.8, 4.2 M urea and frozen in liquid nitrogen. On thawing the IEF gel was laid on the second dimension gel (14% acrylamide) according to O'Farrell (23). Radioactivity was detected by fluorography (7).

**Results**

**Translation of RNA from in vitro shoot cultures**

Plant material of Maris Bard and Mbi501B was