COLD INDUCED CHANGES IN PROTEIN SYNTHESIS IN RAPESEED

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INTRODUCTION.

For many plants tolerance to freezing conditions can be induced by a cold, but not chilling treatment (GRAHAM and PATTERSON, 1982). This mechanism holds true for rapeseed (KACPERSKA-PALACZ et al., 1977, SIKORSKA et al., 1979). These two groups have pointed to the role of changes in proteins and phospholipids during acclimation to frost. We have examined the changes in protein synthesis in young seedlings from a winter rapeseed following a 48 hours treatment at 2°C. These results have been recently published elsewhere (MEZA-BASSO et al., 1986).

MATERIALS AND METHODS.

Rapeseed (Jet neuf) seeds were germinated in the dark at 18°C on wet filter papers for 48 hrs. At that time the seed coats are removed and half of the seeds were transferred at 0-2°C while the remaining is kept at 18°C as control.

in vivo labelling are carried out with 20 seedlings incubated in the presence of 500 µl ^{35}S-Methionine (175 µCi/ml, 1470 Ci/mmol) for the appropriate period. Proteins were extracted and analyzed by two dimensional O'FARRELL gel electrophoresis (MEZA-BASSO et al., 1986). mRNA was prepared and purified as previously described (LAROCHE-RAYNAL et al., 1984). It was translated *in vitro* in a rabbit reticulocyte cell-free system and translation products were analyzed by 1 or 2-D gel electrophoresis.

Immunoprecipitation was carried out according to MARTIN and NORTHCOTE.

1982, using an anti radish rubisco serum prepared by FOURCROY et al., 1985.

RESULTS.

Changes in *in vivo* protein synthesis 2D patterns.

Cold treatment induces a number of changes the most striking being a considerable slowering of hypocotyl elongation. During the 48 hours at 0°C the fresh weight remains stable while it almost doubles in the control sample. We first analyzed the changes in protein contents: a slight decrease ($\simeq$ 25%) is noted at 18°C due to hydrolysis of storage proteins but in contrast the protein amount is stable at 0°C. These results suggest that there is much less protein degradation at 0°C than in the control samples. A further step was to study the changes in protein synthesis. ^{35}S-methionine uptake is not significantly different at 0 and 18°C but final incorporation into proteins is less efficient at 0°C. Nevertheless it is clear that cold treated seedlings actively incorporate radioactivity into their proteins. Figure 1 shows the results of an analysis

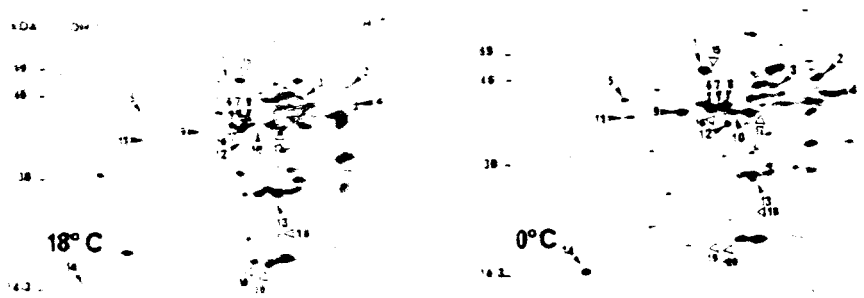

Fig. 1.- 2-D patterns of *in vivo* protein synthesis at 18°C or 0°C. Spots 1 to 14 (black arrows) correspond to polypeptides the relative synthesis of which increases at 0°C. Spots 16 to 20 (open triangles) are those which decrease.

of the protein synthesized in control and cold treated seedlings during the last 6 hours

of the cold treatment. A number of remarkable changes are obvious. Spots numbered 1 to 14 correspond to polypeptides barely detectable in control seedlings and which become prominent in the cold-treated sample. One can conclude that the synthesis of these polypeptides is induced or stimulated relatively to the other proteins. On the other hand, spots numbered 15-20 correspond to proteins the synthesis of which is repressed at 0°C.

Changes in mRNA populations.

We tried to compare these *in vivo* protein synthesis patterns with patterns obtained after *in vitro* translation of corresponding mRNA. This is shown in figure 2.

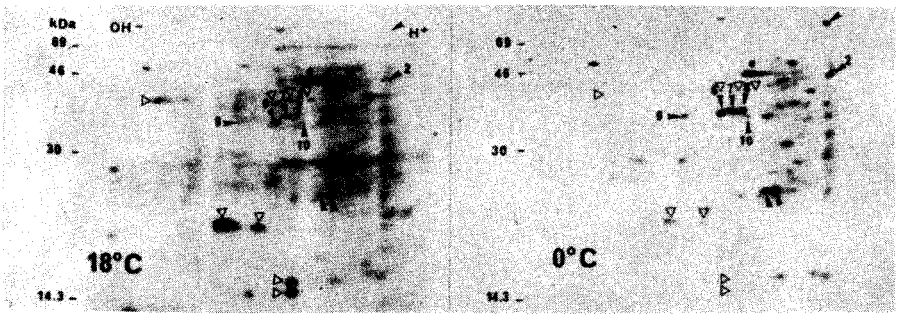

Fig. 2.- 2-D patterns of *in vitro* protein synthesis at 18°C and 0°C. Polyadenylated mRNA (0.3 µg) were translated in the presence of 10 µl of rabbit reticulocyte lysate. Black arrows denote spots with increased intensity at 0°C and open triangles spots which decrease. Numbers correspond to those in figure 1.

Again we observed striking differences between the two samples with some polypeptides being relatively stimulated and others repressed. Such changes indeed reflects changes in the relative proportion of the various mRNA. By co-migration, we have been able to establish that spots numbered in the figure 2 pattern correspond to spots with the same number in *in vivo* protein synthesis pattern (figure 1). The other unidentified spots in figure 2 might correspond to primary translation products which need further *in vivo* processing. They can be indentified only using specific probes or antibodies. Alternatively they could be uncomplete translation products.

Identification of a protein repressed at 0°C.

As shown in figure 3 we attempted to identify some of the polypeptides with available antibodies. The major polypeptide repressed at 0°C between 22-23 kDa was identified as the precursor of small subunit of rubisco (FOURCROY et al., 1985). It corresponds to spots 19 and 20 in figure 2 which are repressed *in vivo* . We also suspect that spot 2, which is increased at 0°C both *in vivo* and *in vitro* corresponds to phenylalanin ammonialyase but this remains to be proven.

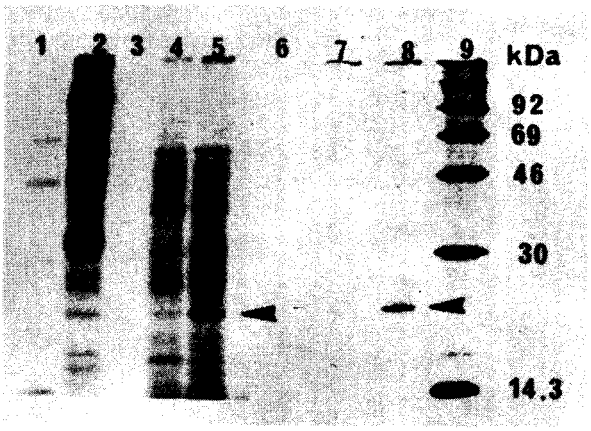

Fig. 3.- Identification of the mRNA for the small subunit of rubisco as a mRNA repressed by cold treatment. Polypeptides synthesized *in vitro* were separated by SDS-PAGE and the gel was fluorographed. Lanes 1 and 9: molecular weight markers, lane 3 standard TMV RNA, lanes 3 and 6: no mRNA, lanes 4 and 7: mRNA from 0°C seedlings, lanes 5 and 8: mRNA from 18°C control seedlings, lanes 6, 7 and 8 products immunoprecipitated with rubisco small subunit antiserum from samples identical to those in lanes 3,4 and 5.

DISCUSSION

These results clearly establish that during cold acclimation remarkable changes

occur in gene expression. The relative intensity of the synthesis of some proteins is increased at 0°C whereas that of others is decreased. Similar increases and decreases are observed at the level of the amount of mRNA. The mechanisms regulating these changes are not clear at that time. They could reflect a regulation at the transcription level with specific genes being turned on or off. Alternatively some mRNA can be simply preferentially stabilized while others are selectively degraded. Elucidating these mechanisms will require the isolation of specific cloned probes for each group of mRNA.

Independantly of elucidating these mechanisms one can probably use these patterns to predict the ability of a line to resist to cold conditions because we observed some interesting differences in analyzing the response of a spring rapeseed cultivar which is less tolerant to cold conditions.

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