Serological detection of pathogenesis-related proteins

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In double-diffusion tests using an antiserum prepared to PR-1a from *Nicotiana tabacum* cv. Xanthi-nc, PR-1a was shown to be serologically related but not identical to PR-1b and PR-1c. When used in a F(ab')2 ELISA assay, the antiserum could be used to detect as little as 50 pg ml^{-1} of PR-1a.

The ELISA assay detected an increase in what appeared to be serologically related proteins in cowpea and potato following treatment with salicylic acid or virus infection. Salicylic acid treatment of *Gomphrena globosa* induced an increase in a similar protein. Using ELISA no PR-1a-related protein was detected in tomato, cucumber or bean, but its presence was confirmed in potato in double diffusion tests.

This work was done in collaboration with J.F. Antoniw (Biochemistry Department, Rothamsted Experimental Station), D.J. Barbara (Plant Pathology Department, East Malling Research Station, and Patricia Ahl and S. Gianinazzi (Station d'Amélioration des Plantes, Dijon, France).

Translational control of pathogenesis-related-protein synthesis

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The best-studied pathogenesis-related proteins (PRs) of the tobacco mosaic virus (TMV)-localising *Nicotiana tabacum* cultivar Xanthi-nc are PRs-1a (b_1), -1b (b_2) and -1c (b_3). As reported previously (1), it seems likely that the major control point governing synthesis of these proteins is at the translational and not the transcriptional level. Evidence for this hypothesis is summarised below.

Total poly(A)-mRNA was obtained from leaves of healthy and TMV-infected 6-week-old 'Xanthi-nc' plants and used to programme in vitro translation systems. PRs were identified among the translation products of both TMV-infected and healthy leaf mRNA on the basis of charge and size, immune-precipitation and mol. wt values. It was found that each PR protein has its own mRNA and that the size of the mRNA corresponds to the mol. wt of the PR protein coded for, so that neither PRs nor their mRNA's are breakdown products of large precursors.

Supporting evidence for translational control came from the finding that the transcription-blocking antibiotic actinomycin D, without any other inducer present, caused synthesis of PRs when administered to 'Xanthi-nc' leaves (J.P. Carr, J.F. Antoniw and R.F. White, unpublished results).

Most recent experiments have been aimed at the extraction of polyribosomes and messenger-ribonucleoprotein (mRNP) complexes form healthy, TMV-infected and aspirin-treated leaves. If the translational control hypothesis is correct, healthy leaf cells should contain PR-mRNA in a translationally inactive form suquestered by pro-

tein (mRNP complexes) until the correct chemical stimulus or pathogen causes its conversion to a translatable state. The mRNA is then able to direct PR-protein synthesis on the polyribosomes.

Preliminary results indicate that PR-mRNA occurs in polyribosomes from TMV-infected or aspirin-treated leaves but not those from healthy leaves. Furthermore, four completely new polypeptides (MW 34 000, 31 000, 29 000 and 27 000) have been found attached to non-polysomal poly(A)-mRNA from healthy leaves but not in TMV-infected or aspirin-treated leaves. Experiments are in progress to identify the bound mRNA and determine whether the new polypeptides are mRNA 'masking proteins'.

Poly(A)-mRNA, enriched in PR-1a, −1b and −1c mRNA by fractionation on sucrose gradients, has been used for molecular cloning in *E. coli* (J.P. Carr, T.M.A. Wilson, J.F. Antoniw and R.F. White, unpublished results). Hybrid-selected translation procedures are being carried out to identify those clones containing PR-nucleic acid sequences. This PR-cDNA will be used to determine PR-protein sequences, to identify PR genes and to elucidate further the mechanisms which govern PR expression.

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(1) Carr, J.P., Antoniw, J.F., White, R.F. & Wilson, T.M.A., 1982. Latent messenger RNA in tobacco *(Nicotiana tabacum)*. Biochem. Soc. Trans. 10: 353-354.

Pathogenesis-related proteins in crown gall tissue

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Antoniw et al. (1) showed that two pathogenesis-related proteins (PRs), PR-1a and -1b, are produced in large amounts in amorphous callus tissue grown in vitro from leaves of healthy plants of tobacco cv. Xanthi-nc. The PRs are probably induced by the presence in the Murashige and Skoog growth medium, of plant hormones which stimulate growth of the callus and maintain it in an undifferentiated state.

Infection of tobacco tissues by *Agrobacterium tumefaciens* leads to the formation of crown gall tissue; in the process of transformation a segment of DNA (T-DNA) is transferred from the Ti-plasmid into the nuclear genome of the plant cell where it is expressed. Crown gall tissue derived from individual transformed cells normally grows vigorously as an unorganised callus tissue on growth media without added plant hormones. If the T-DNA is modified the crown gall tissue gives rise to shoots without roots and these can be grown into mature plants, by grafting onto normal tobacco rootstocks, and taken through seed to produce transformed progeny. These crown gall-derived plants also do not form roots but can be grown on by grafting.

Large amounts of PRs were observed in unorganised crown gall tissue grown on media without added plant hormones but none were detectable in crown gall-derived plants. This suggests that the production of PRs in transformed plants is associated