

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gradients, has been used for molecular cloning in *E. coli* (J.P. Carr, T.M.A. Wilson, J.F. Antoniwi and R.F. White, unpublished results).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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Pathogenesis-related proteins in crown gall tissue

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Antoniwi 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

with the physiological state of the cells in a callus which is in turn dependent on the effects of plant hormones. Furthermore, grafted crown gall-derived plants appeared as susceptible to virus infection as untransformed grafted plants. However, crown gall cells in many ways resemble meristematic cells and it is an intriguing possibility that part of the reason why many plants can be freed from virus by culturing apical meristems is due to the induction of the same resistance in these tissues as that associated with the presence of PRs in leaves.

The work described here was done in collaboration with G. Ooms and R.F. White (Biochemistry and Plant Pathology Departments, Rothamsted Experimental Station), and with G.J. Wullems and L. van Vloten-Doting (Department of Biochemistry, University of Leiden, the Netherlands).

- (1) Antoniw, J.F., Kueh, J.S.H., Walkey, D.G.A. & White, R.F., 1981. The presence of pathogenesis-related proteins in callus of Xanthi-nc tobacco. *Phytopath. Z.* 101: 179-184.

Pathogenesis-related-protein synthesis in selected cultivars of beans and cowpeas following leaf damage by carborundum, treatment with aspirin, infection with tobacco mosaic virus, or with the bean or cowpea strain of southern bean mosaic virus

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As an adjunct to a recently initiated, collaborative research project entitled 'The interaction of southern bean mosaic virus (SBMV) with its hosts', we felt it appropriate to examine the possible induction of pathogenesis-related proteins (PRs) in our experimental bean and cowpea cultivars when exposed to a variety of stimuli.

In addition to single-lesion isolates of our first two chosen strains of SBMV [the bean (or type) strain and the cowpea strain], treatment of test plants with the vulgare strain of tobacco mosaic virus (TMV), to which plants are normally considered immune, and spraying with acetylsalicylic acid (1) were thought appropriate stimuli for study.

Plant cultivars chosen were *Phaseolus vulgaris* L. cvs Prince and Pinto, systemic and hypersensitive hosts, respectively, for the type strain of SBMV; and *Vigna sinensis* L. cvs Blackeye and Clay, the respective, corresponding hosts for the cowpea strain of SBMV.

Control plants of each cultivar were either left completely untouched, or were dusted with 300 grit carborundum and gently rubbed with a muslin pad soaked in 50 mM sodium phosphate buffer, pH 7.0, prior to washing in tap water.

After treatment, all experimental plants were grown for 8 days in a roof-top greenhouse with a supplemented 16-h photoperiod at ambient temperatures of 25 ± 5 °C. Batches of leaves were harvested, stored at -80 °C, ground while frozen and weighed. Protein was extracted by thorough grinding for 5-7 min in a chilled mortar and pestle, containing a dusting of 150 grit carborundum and 20 ml MacIlvaine buffer [84 mM citrate, 32 mM Na₂HPO₄, pH 2.8] containing 0.5M sucrose and 42mM (0.3% v/v) 2-mercaptoethanol. Fibre was removed by squeezing through muslin and the resulting extracts (30-35 ml each) were centrifuged for 30 min at 30 000 g at 4 °C. Each