Generation of active oxygen in elicited cells of *Arabidopsis thaliana* is mediated by a NADPH oxidase-like enzyme

Radhika Desikan[a], John T. Hancock[a], Marcus J. Coffey[b], Steven J. Neill[a,*]

[a] *Department of Biological Sciences, University of the West of England, Coldharbour Lane, Bristol BS16 1QY, UK*
[b] *Department of Biochemistry, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK*

Received 4 December 1995; revised version received 7 February 1996

Abstract Suspension-cultured cells of *Arabidopsis thaliana* generated active oxygen species (AOS) (measured by luminol-dependent chemiluminescence) following challenge with the bacterial protein elicitor harpin or the protein kinase activator phorbol 12-myristate 13-acetate. These responses were blocked by inhibitors of superoxide dismutase (SOD), NADPH oxidase and protein kinase. Harpin treatment also resulted in an increase in cell death, a response reduced by inhibitors of AOS generation or AOS scavengers. Extracellular SOD activity was found to be present in cell culture medium. Immunoblotting of *Arabidopsis* extracts revealed the presence of proteins immunologically related to the human neutrophil NADPH oxidase complex, and cell-free reconstitution assays showed that human neutrophil cytosol combined with *Arabidopsis* membranes could initiate superoxide generation. These data suggest that the enzyme catalysing the generation of superoxide in elicited *Arabidopsis* cells is similar to the mammalian NADPH oxidase and that a signalling cascade leading to AOS generation involves protein phosphorylation.

Key words: Active oxygen; *Arabidopsis thaliana*; Elicitor; NADPH oxidase; Phosphorylation; Reconstitution; Superoxide dismutase

1. Introduction

The hypersensitive response (HR) is a characteristic phenomenon exhibited by plants in response to pathogen challenge, leading to tissue necrosis at the site of infection. It is now apparent that one of the earliest events characteristic of HR is a rapid oxidative burst, i.e. the release of active oxygen species (AOS) by elicited plant cells. These AOS include superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$) [1,2]. The significance of AOS acting as a signal towards host defense in plant-pathogen interactions has been demonstrated by H_2O_2-mediated oxidative cross-linking of the plant cell wall during HR [3,4], phytoalexin formation in cultured cells [5–7] and induction of cellular protection genes [4].

A defensive mechanism of mammalian phagocytes in response to invading organisms involves the oxidative burst, releasing AOS which are ultimately toxic to the pathogen. The key enzyme of this respiratory burst is NADPH oxidase, catalysing the reaction:

$$2\,O_2 + NADPH \rightarrow 2\,O_2^{\cdot-} + NADP^+ + H^+$$

This plasma membrane-bound haem and flavin containing enzyme complex consists of at least five components originating from both the membrane and cytosol [8,9]. The membrane associated cytochrome complex consists of two components, a 91 kDa glycosylated transmembrane protein ($gp91^{phox}$) and a 22 kDa non-glycosylated subunit ($p22^{phox}$). Following cellular activation, the cytosolic polypeptides of 40, 47 and 67 kDa, termed $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ respectively, translocate to the plasma membrane to be associated with the cytochrome complex.

The exact enzymatic processes mediating the release of AOS in plants remain to be elucidated, but a number of lines of research point to similarities with the mammalian system. A series of investigations by Doke et al. [10–13] provides evidence for a plasma membrane-bound NADPH oxidase in *Phytophthora*-treated potato. Evidence that the primary AOS is superoxide has been presented for cultured spruce [14] and tomato [15] cells and inhibitor studies with elicited rose cells have demonstrated striking parallels with the mammalian NADPH oxidase [16]. Furthermore, soybean proteins immunologically related to components of the mammalian complex have been reported recently [17].

Here, we show for the first time that cell suspension cultures of *Arabidopsis thaliana* generate AOS following challenge with the bacterial protein elicitor harpin and the protein kinase agonist phorbol 12-myristate 13-acetate (PMA). Using a combination of approaches, we provide evidence that this AOS generation is mediated by an enzyme system similar to the NADPH oxidase of mammalian phagocytes and that the signalling steps leading to AOS generation involve protein phosphorylation.

2. Materials and methods

2.1. *Arabidopsis thaliana* cell cultures

Cell suspension cultures of *A. thaliana* were initially obtained from Dr. M. May, University of Oxford, UK [18]. Suspension cultures were subcultured every 7 days by transferring 10 ml of a 7 day old culture into 100 ml of fresh AT3 medium (Murashige and Skoog medium supplemented with 3% (w/v) sucrose, 0.5 mg/l naphthalene acetic acid, 0.05 mg/l kinetin, pH 5.5). Cell cultures were incubated in a controlled growth chamber at 24°C, maintained in the dark on a rotary shaker at 110 rpm. Cell viability was assessed using the vital stain Evans Blue. Aliquots were removed from each of three replica treatments and the percentage viability of the cells determined microscopically.

2.2. *Protein extraction from cell suspension cultures*

Filtered cultures were ground in a prechilled mortar and pestle at 4°C in extraction buffer (1 ml: 3 g fresh tissue; 50 mM HEPES-KOH, pH 7.8, 250 mM sucrose, 0.1 mM EDTA, 2-mercaptoethanol, 1:1000 of 14 M stock). The homogenate was then sequentially centrifuged at

*Corresponding author. Fax: (44) (117) 976 3871.

Abbreviations: AOS, active oxygen species: CL, chemiluminescence; DDC, *N,N*-diethyldithiocarbamate; DPI, diphenylene iodonium; HR, hypersensitive response; PMA, phorbol 12-myristate 13-acetate; phox, phagocytic oxidase; SOD, superoxide dismutase

SSDI S0014-5793(96)00177-9

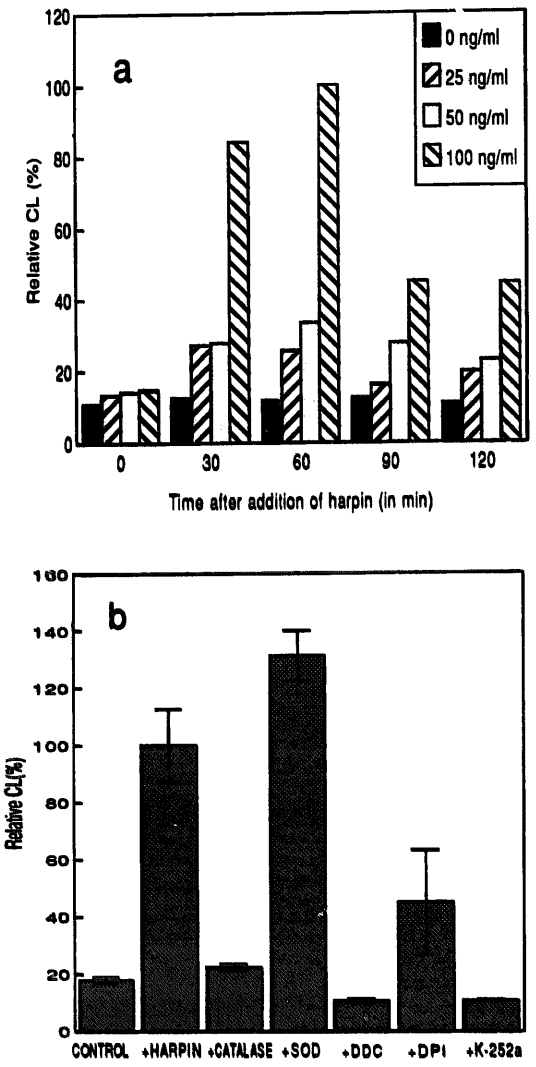

Fig. 1. Harpin-induced generation of AOS by cell cultures of *Arabidopsis*. Data are presented as relative chemiluminescence (CL) with 100% representing CL measured 60 min after the highest concentration of harpin added (1.34×10^5 cpm). (a) Dose response for harpin. Data from a single representative experiment. (b) Effect of catalase (5 μg/ml), SOD (2.6 μg/ml), DDC (1 mM), DPI (10 μM) and K-252a (2 μM) on CL in cells stimulated with harpin (100 ng/ml). Data represent mean±S.E. of three individual experiments.

5000×*g*, 10 min, 20000×*g*, 20 min, and 140000×*g*, 120 min, to obtain cytosolic and microsomal fractions. Protein concentrations were then determined [19].

2.3. Electrophoresis and immunoblotting

Protein samples extracted and quantified as described above were electrophoresed on 12% SDS-polyacrylamide gels. Human neutrophil samples were prepared as described in [20]. Protein fractions were electroblotted onto Immobilon-P membrane for 30 min at 250 mA using 10 mM CAPS (3-[cyclohexylamino]-1-propanesulfonic acid), 10% methanol transfer buffer. Probing and detection of Western blots were performed as described in the ECL Western Blotting detection kit (Amersham). Primary antibodies were diluted 1:1000 (anti-$p47^{phox}$) and 1:2000 (anti-$p67^{phox}$) in TBS-T (Tris-buffered saline-Tween; 20 mM Tris base, 137 mM NaCl, 0.1% Tween 20, pH 7.6). Antibodies were obtained from Prof. O.T.G. Jones, University of Bristol, and Prof. A.W. Segal, UCL, London.

2.4. Elicitor treatment of cells

For elicitor treatment, dark cultured cells were washed by successive centrifugation (3 times at 400×*g*, 10 min, in sterile distilled water), and resuspended in fresh AT3 medium. Cells were allowed to equilibrate by leaving on the shaker for 4 h before treatment with elicitors. Harpin (obtained from Dr. S.Y. He, University of Kentucky, USA) and PMA (in DMSO, Sigma) were used at the indicated concentrations. Aliquots of cell suspensions were removed at specific time intervals, spun down in a microcentrifuge and the supernatant used in the chemiluminescence assay. Catalase (from bovine liver, Sigma), superoxide dismutase (SOD, Cu/Zn isoform, from bovine erythrocytes, Sigma), diethyldithiocarbamate (DDC, sodium salt, Sigma), diphenylene iodonium (DPI, a gift from Prof. O.T.G. Jones, University of Bristol), and K-252a (in DMSO, from Calbiochem) were added to the cells at the indicated concentrations 10 min before addition of elicitors.

2.5. Chemiluminescence assay for detection of active oxygen species

The chemiluminescence (CL) assay used was dependent on the presence of peroxidase to catalyse the oxidation of luminol. The assay was conducted in a total volume of 5 ml, comprising 3.35 ml of potassium phosphate buffer (50 mM, pH 7.9), and 150 μl horse radish peroxidase (at 1 mg/ml) placed in a plastic scintillation vial. Elicited or control cell supernatant (500 μl) was added to the vial and 1 ml of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma; 3.5 mg/ml in DMSO, 100 μl of which was diluted in 5 ml assay buffer) was added just before placing the vial in the scintillation counter. Counting was determined in the out-of-coincidence mode on an LKB Scintillation counter; counts were reported every 15 s for 1 min and the last two values averaged [16].

2.6. Cell-free reconstitution assay

Reconstitution experiments and subsequent assay of superoxide generation were carried out as described in [20].

2.7. Superoxide dismutase assay

Superoxide dismutase activity present in *Arabidopsis* culture medium was assayed using the xanthine/xanthine oxidase assay (Sigma) or as the ability to inhibit cytochrome *c* reduction by PMA-stimulated neutrophils [21].

3. Results

Harpin elicited the production of AOS in a dose-dependent manner in cell suspension cultures of *Arabidopsis* (Fig. 1a). We found that washing and equilibration of cells were essential to reduce high levels of AOS in non-elicited cells. This has been observed with other species [14,16,22], possibly due to substances that interfere with CL. Moreover, mechanical agitation of cells may also induce AOS production [23,24]. The harpin response was transient, with maximal CL being observed after 60 min. To probe the nature of the AOS-generating system, harpin-challenged cells were treated in various ways. Chemiluminescence was reduced by catalase, and enhanced by SOD (Fig. 1b). Inhibitors of both SOD (DDC) and NADPH oxidase (DPI) reduced the CL response. Similarly, the protein kinase inhibitor K-252a prevented harpin-induced CL. Treatment with harpin significantly reduced the viability of *Arabidopsis* cell cultures, from 85 ± 3.5% to 56 ± 1% 3 h after the addition of harpin. Pre-treatment of the cells with DDC, DPI and catalase at the indicated concentrations used in AOS generation largely prevented harpin-induced cell death, so that the resulting viability data were 66 ± 2%, 74 ± 5% and 73 ± 4% respectively. Pre-treatment with SOD did not appear to affect the viability of the cells (55 ± 14%).

To investigate further the requirement for protein phosphorylation, cells were treated with the protein kinase agonist PMA. Such treatment stimulated AOS production by *Arabi-*

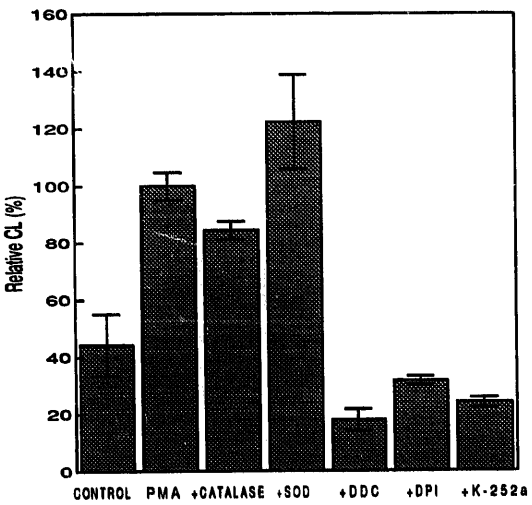

Fig. 2. PMA-induced generation of AOS. Effect of catalase (50 μg/ml), SOD (2.6 μg/ml), DDC (1 mM), DPI (10 μM) and K-252a (2 μM) on CL in cells stimulated with PMA (1 μg/ml). Data represent mean ± S.E. of three individual experiments. Values are represented as relative CL with 100% representing CL measured 30 min after the addition of PMA (1×10^6 cpm).

dopsis cells (Fig. 2), although the response was more rapid than with harpin, peaking at ca. 30 min. This is similar to the PMA response we observed with porcine whole blood (data not shown). The addition of catalase resulted in only a slightly reduced response, for reasons unknown, while SOD caused an increase in CL. DDC, DPI and K-252a all significantly reduced PMA-stimulated AOS production by *Arabidopsis* cells.

The presence of SOD activity in *Arabidopsis* culture medium was assayed using two methods. In the first, culture medium added to PMA-stimulated neutrophils that were generating superoxide, was able to mimic commercial SOD in inhibiting the cytochrome *c* reducing activity of the neutrophils. In the second method, culture medium was assayed in the xanthine/xanthine oxidase assay and was again found to reproduce the effects of commercial SOD. In both, the activity of *Arabidopsis* culture medium could be overcome by the addition of DDC (data not shown).

To determine if *Arabidopsis* cells contain proteins with structural similarities to the mammalian NADPH oxidase, extracts were probed with anti-NADPH oxidase antibodies. Polyclonal antiserum raised against a recombinant human $p47^{phox}$ protein recognised a strong 47 kDa band in cytosol preparations from *Arabidopsis* (Fig. 3a). Similarly, anti-peptide polyclonal antiserum raised against a human $p47^{phox}$ NADPH oxidase sequence also recognised the 47 kDa band (Fig. 3b). A number of other bands were also detected, although some of these were similarly present in human neutrophil extracts (Fig. 3a,b). Interestingly, a strong band at ca. 95 kDa was also recognised by both antibodies. Anti-peptide polyclonal antiserum to a $p67^{phox}$ sequence of the human neutrophil NADPH oxidase also recognised an *Arabidopsis* polypeptide at 67 kDa in addition to a strong band at ca. 45 kDa (Fig. 3c).

To establish if *Arabidopsis* cells contain proteins with functional similarity to the mammalian NADPH oxidase components, membrane and cytosol fractions from human neutrophils and *Arabidopsis* cells were reconstituted in a superoxide generating assay as described in [20]. Superoxide generation could be initiated by the reconstitution of *Arabidopsis* membranes with neutrophil cytosol (Table 1), although the combination of *Arabidopsis* cytosol with neutrophil membranes did not reconstitute activity. Control experiments with membrane and cytosol fractions alone yielded no superoxide production.

4. Discussion

Suspension cultures of *Arabidopsis* represent an attractive system as *Arabidopsis* is a model species for a diversity of topics in plant molecular biology [25], including several aspects of plant-pathogen interactions [26]. Previously, elicitor-induced defence gene expression has been characterised in suspension cultures of *Arabidopsis* [27]. Here, we present the first evidence that elicitor-induced AOS generation in *Arabidopsis* is mediated by a NADPH oxidase-like enzyme and that the signal transduction process involves protein phosphorylation.

Harpin elicited AOS generation in a dose-dependent manner (Fig. 1a). Harpin is a heat-stable protein isolated from several species of gram negative plant pathogenic bacteria; the genes required for its production (*hrp*) are conserved amongst these bacteria [28]. Harpin elicits HR in non-host plants and confers pathogenicity towards susceptible plants [29–32]. We have clearly demonstrated for the first time, that harpin elicits AOS generation in *Arabidopsis*, suggesting that these cells are capable of recognising harpin and transducing the signal into a transient generation of AOS. A similar transient phase of AOS generation has been reported for other systems [1]. We also observed that harpin treatment led to significant cell death, implying some similarity with the HR seen in planta [30,31]. Such cell death was counteracted by treatments that reduced the level of H_2O_2, suggesting that it was the H_2O_2, or a subsequent reaction product, that initiated cell death.

Chemiluminescence provides an easy and rapid method for the detection of AOS in elicited plant cells [33]. However, the ambiguity in the luminol-based assay is that the AOS detected cannot be precisely attributed to $O_2^{\cdot-}$, H_2O_2 or both [23]. Nevertheless, our data are consistent with the primary AOS being $O_2^{\cdot-}$ which dismutates to give rise to H_2O_2, which is then detected in the CL assay. The CL reaction was inhibited by DDC, a chelator of metal ions which inhibits Cu/Zn SOD [34] and prevents AOS generation in rose cells [16], and by catalase, which would be expected to remove H_2O_2 (Fig. 1b).

Table 1
Cell-free reconstitution of superoxide-generating activity using cytosol (C) and membrane (M) fractions of human neutrophils (N) and *Arabidopsis* (A) cells

Homologous reconstitution: 10 μg NM +200 μg NC	0.5 ± 0.1
Heterologous reconstitution: 10 μg AM +200 μg NC	0.04 ± 0.01
10 μg AC +200 μg NM	0
Controls: NM; NC; AM; AC	0

The rate of superoxide production is expressed as μmol/min/mg membrane protein. Results are expressed as mean ± S.E. of four assays.

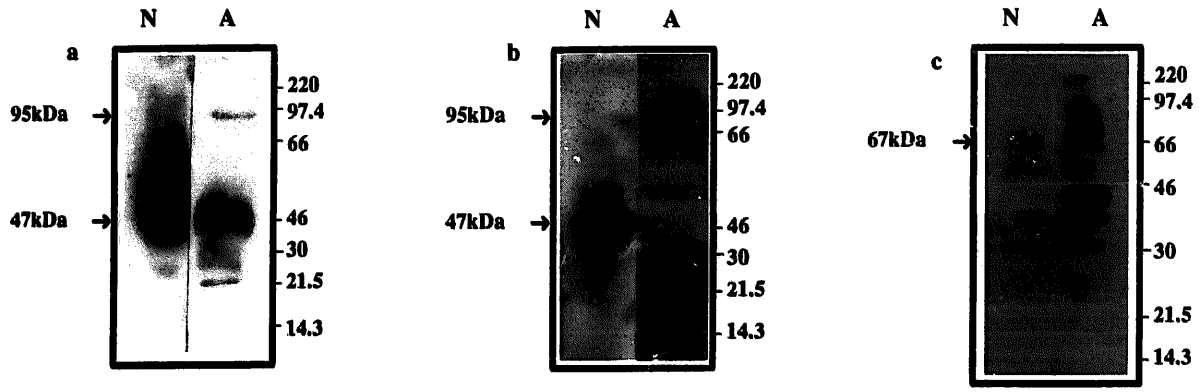

Fig. 3. Western blots for detection of NADPH oxidase proteins. N: human neutrophil cytosol; A: *Arabidopsis* cytosol. (a) Detection using anti-recombinant $p47^{phox}$ antiserum; (b) detection using anti-peptide anti-$p47^{phox}$ antiserum; (c) detection using anti-peptide anti-$p67^{phox}$ antiserum.

Superoxide is most readily measured in neutrophil systems using the cytochrome *c* reduction assay [21]. Although this assay has been used to detect and quantify $O_2^{\cdot-}$ in plant systems [13,15] it is subject to considerable variation. Even so, we were able to measure an increase in SOD-inhibitable cytochrome *c* reducing activity following stimulation of *Arabidopsis* cells with PMA (data not shown), further suggesting that superoxide is the primary AOS generated. $O_2^{\cdot-}$ does not readily transverse membranes; if it is generated extracellularly then dismutation to H_2O_2 must also occur extracellularly. Exogenous SOD caused a slight increase in the CL response (Fig. 1b) implying that this extra $O_2^{\cdot-}$ dismutation enhanced that already occurring. Chemiluminescence was inhibited by DDC and we did detect DDC-sensitive SOD activity in *Arabidopsis* culture medium using two different assays. Furthermore, a recent report has demonstrated extracellular SOD activity in pine needles [35]. However, it must be recognised that $O_2^{\cdot-}$ will dismutate even in the absence of SOD, and that DDC may react with proteins other than Cu/Zn SOD. There is also the possibility that the SOD activity in the culture medium originated from broken cells.

Reversible protein phosphorylation is a core component of eukaryotic cell signalling [36], with plant-pathogen interaction being no exception [37]. Inhibition of protein phosphorylation by the kinase inhibitor K-252a has been shown to prevent AOS release in soybean [4,38], parsley [22] and harpin-elicited tobacco cell suspensions [32]. Here we have shown that it also prevents harpin-induced AOS generation in *Arabidopsis* cell cultures. Furthermore, AOS generation could also be stimulated by the phorbol ester PMA, a potent activator of protein kinase C and the respiratory burst in neutrophils [8]. To our knowledge, PMA has so far only been observed to promote AOS generation in soybean (A. Levine, unpublished data, from [17]). Since it is known that PMA stimulates NADPH oxidase activity in neutrophils via phosphorylation of its cytosolic component [8,9] it is possible that a protein kinase C-like enzyme might be involved in AOS generation in *Arabidopsis*. As yet, no known protein kinase C genes have been cloned from plants [39], but recently a gene encoding a phospholipase C enzyme, potentially generating activators of protein kinase C, has been cloned from *Arabidopsis* [40].

To investigate if the enzyme responsible for superoxide generation in *Arabidopsis* is similar to the mammalian NADPH oxidase, we used a number of approaches. AOS generation was inhibited by DPI, an inhibitor of the neutrophil NADPH oxidase [41]. However, DPI is not specific to NADPH oxidase and may also inhibit other flavin containing enzymes. With this caveat in mind, further experiments were used to address this question. Using antibodies raised against components of human NADPH oxidase, we have identified immunologically related proteins in *Arabidopsis* (Fig. 3). The antibodies were not purified before use and did also recognise a number of other bands including some in neutrophil extracts, a problem encountered by other workers [20]. However, they have been used previously to identify NADPH oxidase components in non-neutrophil cells [20,42] and did recognise proteins at the appropriate molecular weight in both neutrophil and *Arabidopsis* extracts. Furthermore, a 47 kDa protein was recognised by two independent anti-$p47^{phox}$ antibodies. In addition, an *Arabidopsis* protein of ca. 95 kDa was also recognised by both these antibodies, suggesting the possibility that the p47 component may exist as a dimer, or that NADPH oxidase domains are present on a larger protein. A strong band was also detected by the anti-$p67^{phox}$ antibody at ca. 45 kDa, but the identity of this protein remains, as yet, unknown.

We have also shown that *Arabidopsis* cells contain components functionally related to the human NADPH oxidase (Table 1). Clearly, human neutrophil cytosol components were able to co-operate with *Arabidopsis* membranes to generate superoxide. Conversely, the combination of *Arabidopsis* cytosol and neutrophil membranes was not active. The reasons for this are not known, but could be because of a low abundance of *Arabidopsis* cytosol factors that may be rate-limiting for activity or due to the presence of inhibitory factors.

In summary, we have used a combination of experimental approaches including the use of inhibitors, immunological and reconstitution experiments, to provide evidence that elicitor-induced generation of superoxide in *Arabidopsis* cells involves a NADPH oxidase-like enzyme, and that this signal transduction process involves protein phosphorylation. We are currently extending our work to probe the involvement of various signalling molecules in this response, and using reverse transcription PCR and Northern hybridisation to identify gene products encoding the various components of the enzyme complex in *Arabidopsis*.

Acknowledgements: This work was supported by a research grant from HENCE. We would like to thank Dr. M. May for the *Arabi-*

dopsis cells, Dr. S.Y. He for the harpin and Professors A.W. Segal and O.T.G. Jones for providing the antibodies.

References

[1] Mehdy, M.C. (1994) Plant Physiol. 105, 467–472.
[2] Sutherland, M.W. (1991) Physiol. Mol. Plant Pathol. 39, 79–93.
[3] Brisson, L.F., Tenhaken, R. and Lamb, C. (1994) Plant Cell 6, 1703–1712.
[4] Levine, A., Tenhaken, R., Dixon, R. and Lamb, C. (1994) Cell 79, 583–593.
[5] Apostol, I., Heinstein, P.F. and Low, P.S. (1989) Plant Physiol. 90, 109–116.
[6] Devlin, W.S. and Gustine, D.L. (1992) Plant Physiol. 100, 1189–1195.
[7] Kondo, Y., Hanawa, F., Miyazawa, T. and Mizutani, J. (1993) in: Mechanisms of Plant Defense Responses (Fritig, B. and Legrand, M. eds) pp. 148–151, Kluwer Academic Publishers, Netherlands.
[8] Babior, B.M. (1992) Adv. Enzymol. Relat. Areas Mol. Biol. 65, 49–95.
[9] Jones, O.T.G., Jones, S.A., Hancock, J.T. and Topley, N. (1993) Biochem. Soc. Trans. 21, 343–346.
[10] Doke, N. (1985) Physiol. Plant Pathol. 27, 311–322.
[11] Doke, N. (1985) Physiol. Plant Pathol. 27, 323–334.
[12] Doke, N., Miura, Y., Sanchez, L.M. and Kawakita, K. (1994) in: Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants (Foyer, C.H. and Mullineaux, P.M. Eds.) pp. 178–197, CRC Press, London.
[13] Doke, N. and Miura, Y. (1995) Physiol. Mol. Plant Pathol. 46, 17–28.
[14] Schwacke, R. and Hager, A. (1992) Planta 187, 136–141.
[15] Vera-Estrella, R., Blumwald, E. and Higgins, V.J. (1992) Plant Physiol. 99, 1208–1215.
[16] Auh, C.-K. and Murphy, T.M. (1995) Plant Physiol. 107, 1241–1247.
[17] Tenhaken, R., Levine, A., Brisson, L.F., Dixon, R.A. and Lamb, C. (1995) Proc. Natl. Acad. Sci. USA 92, 4158–4163.
[18] May, M.J. and Leaver, C.J. (1993) Plant Physiol. 103, 621–627.
[19] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[20] Jones, S.A., Wood, J.D., Coffey, M.J. and Jones, O.T.G. (1994) FEBS Lett. 355, 178–182.
[21] Jones, O.T.G. and Hancock, J.T. (1994) Methods Enzymol. 233, 222–229.
[22] Kauss, H. and Jeblick, W. (1995) Plant Physiol. 108, 1171–1178.
[23] Glazener, J.A., Orlandi, E.W., Harmon, G.L. and Baker, C.J. (1991) Physiol. Mol. Plant Pathol. 39, 123–133.
[24] Legendre, L., Rueter, S., Heinstein, P.F. and Low, P.S. (1993) Plant Physiol. 102, 233–240.
[25] Meyerowitz, E.M. and Somerville, C.R. (1994) *Arabidopsis*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[26] Dangl, J.L. (1993) Int. Rev. Cytol. 144, 53–83.
[27] Davis, K.R. and Ausubel, F.M. (1989) Mol. Plant-Microbe Interact. 2, 363–368.
[28] Willis, D.K., Rich, J.J. and Hrabak, E.M. (1991) Mol. Plant-Microbe Interact. 4, 132–138.
[29] He, S.Y., Bauer, D.W., Collmer, A. and Beer, S.V. (1994) Mol. Plant-Microbe Interact. 7,289–292.
[30] He, S.Y., Huang, H.-C. and Collmer, A. (1993) Cell 73, 1255–1266.
[31] Wei, Z-M., Laby, R.J., Zumoff, C.H., Bauer, D.W., He, S.Y., Collmer, A. and Beer, S.V. (1992) Science 257, 85–88.
[32] Baker, C.J., Orlandi, E.W. and Mock, N.M. (1993) Plant Physiol. 102, 1341–1344.
[33] Baker, C.J., Harmon, G.L., Glazener, J.A. and Orlandi, E.W. (1995) Plant Physiol. 108, 353–359.
[34] Misra, H.P. (1979) J. Biol. Chem. 254, 11623–11628.
[35] Streller, S. and Wingsle, G. (1994) Planta 192, 195–201.
[36] Hunter, T. (1995) Cell 80, 225–236.
[37] Chasan, R. (1995) Plant Cell. 7, 495–497.
[38] Chandra, S. and Low, P.S. (1995) Proc. Natl. Acad. Sci. USA 92, 4120–4123.
[39] Stone, J.M. and Walker, J.C. (1995) Plant Physiol. 108, 451–457.
[40] Hirayama, T., Ohto, C., Mizoguchi, T. and Shinozaki, K. (1995) Proc. Natl. Acad. Sci. USA 92, 3903–3907.
[41] O'Donnell, V.B., Tew, D.G., Jones, O.T.G. and England, P.J. (1993) Biochem. J. 290, 41–49.
[42] Kummer, W. and Acker, H. (1995) J. Appl. Physiol. 78, 1904–1909.