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SYNTHESIS OF CELL WALL COMPONENTS: ASPECTS OF CONTROL

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Abstract—The dynamic qualities of the plant cell wall are explored. Recent developments in the understanding of how membrane systems operate in the synthesis of wall components is discussed. Particular attention is paid to the synthesis of polysaccharides and wall-bound phenolics, while the identification of some wall bound proteins and the subsequent study of their synthesis and processing is also described. Probable molecular controls, both qualitative and quantitative, governing the appearance of newly synthesized components within the wall are discussed The conditions under which sets of components are modified specifically, by the accumulation of newly synthesized material or extracellular enzyme action, are outlined Some deficiencies in understanding how these aspects may be interrelated, especially with regard to mechanisms of signal transduction are highlighted However the potentially rapid capacity for implementation of changes in the extracellular matrix of the cell, probably commencing with processes of activation of specific subsets of genes in response to particular signals, is striking

INTRODUCTION

The purpose of this review is to attempt to bring together diverse observations on the dynamic qualities of the plant cell wall In general, the view of the cell wall is moving away from that of a passive extracellular matrix concerned with maintenance of shape and rigidity to that of a much more dynamic structure Thus, the plant cell wall undergoes profound changes during growth and development and in response to stress and pathogenesis, and may, as a source of information, be intimately involved in self and non-self recognition processes. The latter aspects are perhaps not surprising considering the existence of a complex laminated structure forming a series of potentially selective surfaces This review considers those underlying synthetic and modificational events initiated in response to a variety of signals which are fundamental to the expression of changes in this laminated matrix at the cellular level. There is a large body of studies appertaining to these areas but a considerable lack of understanding of the assembly of such a complex structure remains, although an attempt is made here to outline where the impact of newer types of approaches might bring forth advances. Nevertheless, the problems involved in understanding the myriad of molecular recognition events involved in the synthesis and assembly of a 'secondary' structure such as the plant cell wall seem particularly daunting when one considers the astonishing potential complexity of 'primary' gene expression [1], without subsequent changes.

Cell wall structure

Although the detailed analysis of cell wall structure is beyond the scope of this review which is primarily concerned with synthesis of components, some comments about subsequent assembly and interrelationships are necessary. Considerable advances of course have taken place in the analysis of structural components generated by chemical or enzymatic means of extraction [2–8]. These detailed studies have been largely confined to simpler primary walls and have served to classify the range of non-cellulosic polysaccharides. In one case, the cell wall of the cultured sycamore cell, this has been accomplished to an extraordinarily sophisticated level. These methodologies have revealed the range of linkages and in some cases partial sequences in the primary structures, branched and unbranched, in the more complex polysaccharides. These are now accepted for the major components. However, there is much controversy about the nature of the interactions and associations of polysaccharides and proteinaceous components in the non-lignified wall which in part resides in an adherence to traditional classifications based on methodologies of extraction It is important to ascertain whether the

Abbreviations. CAD, cinnamoyl alcohol dehydrogenase; CA4H, cinnamic acid 4-hydroxylase, 4CL, 4-hydroxycinnamic acid-CoA ligase, CHI, chalcone isomerase; CHS, chalcone synthase; COMT, caffeic acid *O*-methyl transferase; HRGP, hydroxyproline-rich glycoprotein, IAA, indoleacetic acid, NAA, naphthylacetic acid, PAGE, polyacrylamide gel electrophoresis; PAL, phenylalanine ammonia-lyase

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heterogeneities observed in the analysis of isolated material within the broad types of polysaccharide and their associated molecules is indicative of the true range found *in vivo* It may be that traditional analytical procedures are inadequate to answer these questions completely and that complementary studies of the biosynthetic machinery, as regards the full range of enzymatic specificity, however redundant or spatially or temporarily separated it may be, may hold many of the answers At least, studies at this biosynthetic level, which may include polysaccharides in the process of secretion, do not have the added complexities due to associations generated by the formation of additional bonds extracellularly or even during isolation Many of these extracellular cross-linkages between individual components have been examined using partial enzyme degradations [6–9] Nevertheless, although early theoretical attempts incorporated the observed range of secondary linkages, models for the associations of individual components with known primary structures have met with limited success Newer elegant studies using ultrastructural localization with lectins and enzymes [10–12] and polyclonal and monoclonal antibodies [13–15] should bring about rapid improvements of our understanding in this area When used in combination, such as in utilizing different-sized colloidal gold particles to localize separate components distinguished by species-specific antibodies or other interacting proteins, important information on the associations found within the matrix and during wall assembly should become apparent and complement traditional histochemical, autoradiographic and electronmicroscopic methods [16–19] The exciting prospect remains for the development of probes for the synthetic components (cDNAs and antibodies) and for their cell wall products (antibodies and histochemical agents) to visualize changes at the level of single cells using *in situ* hybridization and cytochemical methodologies

These temporal interrelationships of the component structures are even less studied since much of the detailed information has been derived from primary walls The potential complexity of these processes is highlighted when one considers that even within a single cell such as a germinating *Fucus* zygote, distinct polysaccharide moieties are directed to different areas of the cell during rhizoid development [20, 21] However, such directed deposition is beginning to be understood, as is subsequent assembly, with the recent demonstration of the reassembly of *Chlamydomonas* glycoproteinaceous wall components *in vitro* [22] Although similar proteins exist in higher plant cell walls, such *in vitro* studies must be a long way off although initial attempts at reconstitutions are being made [23]

Faced with such complexity, understanding the synthesis and assembly of such a structure and its subsequent modification during growth and differentiation or in response to environmental factors, would seem daunting However some considerable progress has been made, particularly in plant phenolic metabolism This review deals with recent advances in understanding the synthesis of wall components, polysaccharides, proteins and phenolics and initial attempts to define the temporal and spatial controls operating With the input of immunological and molecular biological techniques some of these changes in components can now be followed and thus open up the possibility of beginning to understand the molecular nature of the control systems

Synthesis of components

Glycosylation Unlike glycosyl transferases of animal cell endoplasmic reticulum and Golgi body [24–27], no plant glycosyl transferase of polysaccharide synthase has been fully characterized at the molecular level. The reason partly resides in the difficulty experienced in solubilizing and reconstituting the individual enzyme systems that have been characterised so far Thus a number of alternative approaches have been designed for indirect identification of these elusive enzymes.

Understandably most interest has been directed towards those systems synthesizing homopolymers or those containing simple repeated units For the more complex polysaccharides, the possibilities of complex precursors, block synthesis or partially redundant enzyme specificity has to be taken into account when examining the interaction of the synthetic systems However some progress has been made in developing conditions which lead to solubilization of polysaccharide synthases as a prerequisite for purification and molecular identification Interestingly Triton X-100 has been found to be most efficient at solubilizing activity although recoveries are invariably low Other detergents, often considered to represent milder condition have not been employed to such success and it is interesting to speculate whether the greater micelle size generated by Triton X-100 may have a significant effect in incorporating additional membrane bound factors The polysaccharide synthases appear to have K_ms in the μM range and similar requirements for Mg^{2+} and/or Mn^{2+}, while it is thought intermediate transfers involving lipid glycosides are not involved [28–30] This is in contrast to the situation involved in protein glycosylations, some of the products of which are targetted into the wall, where in common with similar glycosylations in animal cells lipid intermediates are involved [31–33] In particular the following glycosyl transferases have been described

Polysaccharides.

Glucans The identity of cellulose synthase has been a matter of considerable debate for some time Partly contributing is the failure to achieve accurate and substantial synthesis of $\beta(1\rightarrow4)$ linkages from UDP- or GDP-glucose precursors with membrane or partially reconstituted preparations as a prerequisite for purification This is exemplified by one report [34] of rates of cellulose synthesis *in vitro* approaching that found *in vivo* that was later qualified The problems experienced in achieving $\beta(1\rightarrow4)$ glucan synthesis reflect the difficulty in preparing, for possible reasons discussed below, higher plant plasmalemma, which are uncontaminated with endomembranes capable of other glycosylations Perturbation of membrane integrity also appears to activate the formation of $\beta(1\rightarrow3)$ linkages which may be catalysed by the same enzyme or part of the cellulose synthase complex [35–38] Since it is anticipated that a molecular identification of $\beta(1\rightarrow3)$ glucan synthase or callose synthase will be achieved soon [39–41] it may indirectly lead to the characterisation of cellulose synthase. The theory behind the equivalence of these activities has been expounded most eloquently [42] and is based on a number of previous observations [35–38, 43, 44] The identity will probably be confirmed by those attempts to identify cellulose synthase by indirect methods so far employed including electrophoretic methods [45–47], active-site-

directed labelling [45, 48, 49] and radiation bombardment inactivation [50]

Polypeptides so far identified as good candidates are an M_r 68 000 polypeptide from red beet [47, 50]; or three of M_r 18 000, 43 000 or 73 000 from mung bean [45, 48] although the 73 000 has recently been thought to be involved in transport and the 43 000 may represent a glycoprotein since the label appears to be covalently bound [Read, S. M., personal communication] The 18 000 polypeptide may possibly have some regulatory function. At present, without identification of the enzyme, it is not known whether the changes in enzyme activity that have been observed in several systems [51–54] have their underlying basis in the amount of enzyme present and ultimately gene regulation

Synthases involved in hemicellulose synthesis Among those already described in which the product has been identified to an appreciable extent include synthases involved in xylan, xyloglucan, glucuronoxylan and glucomannan synthesis. Xylan synthases catalysing homopolymer β (1→4) xylose linkages have been described from corn [55], mung bean [56], sycamore [57] and French bean [58] and appear similar as regards cofactor requirements. Attempts to solubilize these enzymes lead to low recovery for the sycamore enzyme [57] and for the bean enzyme (Bolwell, G. P., unpublished results) making further purification difficult. Xyloglucan synthesis by membrane preparations from pea [59–62], suspension cultured soybean cells [63–67] and bean cells [68] has been described although the mechanism of the concerted enzyme activities is unclear [69] The question as to whether side chain residues are added to preformed backbone or during growth of the heteropolymer chain is also unresolved for the interaction of glucuronyl- and xylosyl-transferases involved in glucuronoxylan synthesis catalysed by membrane preparations from pea epicotyls [69, 70] Similar questions also arise during glucomannan synthesis which has been described in membrane preparations from *Phaseolus aureus*, pea and *Pinus sylvestris* [71–78]. For the heteropolysaccharides, available evidence points towards a concerted synthesis at the site of the growing chain perhaps by multienzyme complexes. The fact that side chain synthesis cannot be brought about by the addition of primers makes an alternative mechanism of addition to a preformed backbone unlikely, while despite extensive searches for intermediates, the demonstration of block synthesis *via* perhaps lipid-linked precursors has been largely ruled out [28–30, 69] Complex oligosaccharides linked to lipid donors have been demonstrated but do not have the relevant block structure; these appear to be involved in protein glycosylations and are discussed below.

Synthases involved in pectic polysaccharide synthesis. Homogalacturonon synthesis has been studied in mung bean [79] sycamore [80] and pea [81] No information is available on rhamnogalacturonan synthesis or the dauntingly complex minor substituted rhamnogalacturonan-based wall components whose structure has been worked out so elegantly Arabinan synthesis has been described for membranes from mung [56] and French bean [58] In the latter case use of a monoclonal antibody lead to a preliminary identification of the arabinan synthase as an M_r 70 000 polypeptide [82]

Other transferases have been described that are involved in either backbone or side chain elaboration. Some of these are involved in protein glycosylation but might also be involved in polysaccharide synthesis. These include galactosyl transferases [83–85] and fucosyltransferases [86–88] However, the fucosyl-transferase from *Fucus* is known to be involved in homopolymer fucan synthesis, while another described in pea is involved in fucosylation of a preformed xyloglucan nonasaccharide [89]

Glycosyl transferases in protein glycosylations. Considerable progress is now being made in understanding protein glycosylation some of the products of which will be targetted into the wall. Following the pioneering work in non-animal systems especially yeast [32, 90, 91] the basic gluNAc_2-man_9glc_3 core oligosaccharide *N*-linked to peptide-asparagine so elegantly worked out in animal cells [24, 25] has been demonstrated in plants [92, 93]. This type of glycosylation and subsequent processing, where this is permissible depending on conformational constraints [94], is now well understood for membrane preparations from French bean [33, 95–99], mung or soybean [100–103]. Although there is some debate about a role for glycosylated domains, possible involvement in sorting of newly synthesized proteins for secretion into the wall is an attractive possibility. However glycosylation is not a requirement for the transport of phytohaemagglutinin and β-fructosidase [104, 105]. It therefore still remains a challenge to understand how *N*-linked glycoproteins such as phaseollin and phytohaemogglutinin are targeted into protein bodies while peroxidases, for example, are directed to the wall Molecular investigations currently implicate multiple recognition sites based on secondary and tertiary structures at the *N*- and perhaps *C*-termini Monoclonal antibodies to glycosylated domains may enable further investigations, a multiantigenic oligosaccharide site on secreted proteins has been identified [MacManus, M., personal communication] which may represent a particular processed oligosaccharide motif

O-linked glycosylations are less understood. Evidence for the involvement of pentosyl-lipid intermediates has been obtained [106, 107] but these may not be obligatory [108–110] *O*-linked glycosylations are also found in secreted products, e.g. threonine- and serine-linked oligosaccharides of maize-root slime [111, 112] or serine- and hydroxyproline-linked oligosaccharides of wall-bound proteins Membrane preparations capable of carrying out such glycosylations have been described [106–112] for a number of sugar precursors

Fine control of polysaccharide synthesis. As discussed below evidence suggests the major controlling factor in the qualitative production of polysaccharides resides in the complement of synthases found at any one time in the endomembrane system. However the availability of nucleoside diphosphate sugars appears to be under some fine control through a feed-back inhibition of UDP-glucose dehydrogenase by the level of UDP-xylose [113] This mechanism probably acts to restrict the accumulation of sugar nucleotides although it can be bypassed by the inositol pathway [114] There is little information on the respective regulation of these alternative pathways. The levels of nucleoside diphosphate sugars appear not to be limiting to any great extent [113, 115, 116] probably since the levels of enzymes involved in the interconversions, UDP-glucose dehydrogenase and decarboxylase and the epimerases, appear to be maintained [113, 117, 118] There is little or no direct evidence for fine control mechanisms at the level of the synthases

except for a modulation of a xylan synthase by nucleoside mono- and diphosphates [113] and the complex and as yet not unequivocably demonstrated modulation of cellulose synthase [39, 42]. The latter may be directly related to the regulation of callose synthesis discussed in another context below [41, 119]

Proteins

Structural proteins Although there have been considerable advances in the identification and structural characterization of hydroxyproline-rich glycoproteins, other aspects, including their biological role, remain obscure Nevertheless, there is ample circumstantial evidence for a fundamental role in cell wall structure and disease resistance although confirmation awaits more direct evidence, perhaps employing transformation systems and antisense-RNA technology This is now possible with the identification and cloning of genes encoding proline-rich precursors from a number of sources [120–122] Sequences currently available encode 'extensin' HRGPs but it has become apparent that a number of other precursors undergo peptide-proline hydroxylation and glycosylation before secretion into the wall These include arabinogalactans [122 125], solanaceous lectins [122, 125, 126] and others yet to be classified [107, 127, 128] The full range of HRGPs has yet to be demonstrated and characterisation at the protein level has been difficult due to insolubilization of HRGP precursors in the wall [129, 130] Some progress has been made by isolating precursors by salt elution from the wall [131–133] or at their site of synthesis within the endomembrane system [128, 134] As a result a number of HRGPs have been described These vary in M_r of the deglycosylated monomer, being in the range 30 000–55 000, and with a hydroxyproline content of between 6–50% Genes from a number of sources encoding extensin-like variants have been identified and sequences are available [122] These are characterized by repeated amino acid sequences encoded by codons of restricted redundancy Comparative homologies of HRGPs that are not of the extensin family are awaited, which will incidentally give an indication of the range of amino acid sequences subjected to proline hydroxylation

Recently, another class of proteins, probably wall-bound, which are rich in glycine have been inferred both from amino acid analyses of plant cell walls and from the sequence of a *Petunia* protein, has been described [135, 136] These may replace HRGPs in some species It is interesting to speculate whether both might have evolved from some of the common G/C rich portions of the genome where the codon frequencies CCX (pro) and GGX (gly) would be prevalent

The range of proline residues capable of being hydroxylated is dependent on the specificity of the prolyl hydroxylase (EC 1 14 11 2) This is now a well characterized enzyme from a number of sources [137–140] The enzyme is tetrameric having two active subunits with M_r of 65 000 (which may dissociate into two polypeptides of M_r 32 500 in the *Vinca* enzyme [141]) and two inactive subunits of M_r 60 000 which do not copurify on affinity chromatography in the presence of dithiothreitol [Robinson, D., personal communication]. In contrast, another report of the purification of the enzyme from *Chlamydomonas* identifies an M_r 40 000 protein [142] At this time the discrepancy over the *Chlamydomonas* enzyme is unresolved [140, 142] An antibody raised against the M_r 65 000 subunit from bean also cross-reacts with a putative M_r 60 000 subunit [143] and also recognises an M_r 32 000 polypeptide from potato in Western blots [Bolwell, G P., unpublished results] The plant prolyl hydroxylase has very similar properties to the mammalian enzyme but differs in being capable of the hydroxylation of polyproline *in vitro* The vertebrate enzyme [144] consists of two active α subunits and two β subunits whose cDNA sequence has close homology with a protein disulphide isomerase [145] suggesting a complex remarkably adapted to post translational modifications It is not known whether the 60 000 subunit of the plant enzyme has a similar function Alternatively, the 60 000 isomerase may represent a separate protein (Hanauske-Abel, H., personal communication) The plant enzyme is associated with the endoplasmic reticulum, but whether smooth or rough requires absolute confirmation [137–143], although being a luminal protein [146] it could possibly be found in both

Hydroxyproline residues are also arabinosylated in extensin and *solanaceous* lectins [112, 122, 125] while in the arabinogalactans the side chains are more complex [123, 124] Oligosaccharide lipids possibly involved in intermediate transfers have been described [84, 106, 107] and transfer to endogenous polypeptides has been demonstrated The serine residues of HRGPs are galactosylated, a transferase which may catalyse this post translational modification has been described [83, 147]

Enzymes A great number of enzymes thought to be wall-bound have been described of which having particular relevance to this review are the hydrolases and peroxidases although other groups of enzymes include proteases and phosphatases A number of these activities, due to technical difficulties in wall isolation, require absolute confirmation of a wall localization, probably through immunological techniques

Peroxidases Peroxidases have long been familiar components of plant membranes and walls and have long been recognised as capable of bringing about intermolecular cross-linking of phenolic residues in the presence of hydrogen peroxide Total cell peroxidase activity is a product of a range of differentially induced and localized isoenzymes [148 154] some of which may be wall-bound Peroxidases are also secreted from suspension cultured cells [155–158] They form a very heterogenous group when analysed on the basis of pI and M_r, usually by histochemical staining following gel electrophoresis. Correlating such bands on gels to activity *in vivo* should be undertaken with caution as other haeme proteins, such as cytochrome P450, and Cu^{2+} phenolases can exhibit peroxidase activity under certain circumstances The complete characterisation of a wall-bound peroxidase at the molecular level is anticipated soon [cf 159–162]

Hydrolases Plant cells can exhibit a large range of hydrolase activities which are dependent on the tissue or mode of induction Thus the glycosidases involved in seed germination form an extensively characterised group [163, 164] which have some relevance since some storage polysaccharides are wall-bound and resemble the non-cellulosic polysaccharides of primary and secondary cell-walls of vegetative tissues Thus mobilisation of these storage materials, e.g xyloglucans of the Leguminosae, or of wall-material during fruit ripening or abscission may represent enzymatic mechanisms which may be responsible for cell-wall turnover at a much lower rate in vegetative tissues, itself a controversial issue until re-

cently Since the specific expression of genes encoding glycosidases occurs at a high level in some tissues this has attracted some interest recently leading to the characterisation of cellulases [165, 166], β [1–3] glucanase [167–169], chitinase [170–173], exo β [1–4] glucanase [61, 174] and detection of activities of α and β glycanases specific for the whole range of residues found in wall polysaccharides [175–179] However, at the protein level it has often not been established which of the hydrolases identified are actually secreted into the wall, how active they are *in vivo*, or whether the products of their activity are mobilized to any great extent in intact vegetative tissues Specific products of the hydrolysis of wall polysaccharides can be readily demonstrated in suspension cultured cells however [180, 181]. Breaking of such covalent bonds may have important consequences for growth through wall loosening mechanisms Additionally, production of specific oligosaccharide fragments may of course have important consequences for cellular control mechanisms [182–186]. Similarly, proteases may be important in wall loosening Wall-bound protease activity has been demonstrated [187, 188]

Lignin and phenolics

Enzymology and control of phenolic metabolism The pathway from L-phenylalanine through hydroxycinnamoyl intermediates provides substrates which converted to their coenzyme A thiol esters, serve as precursors which are channelled into branch pathways leading to the biosynthesis of a number of classes of secondary products [189–191] Many of the enzymes of the central phenylpropanoid pathway and the lignin and flavonoid branch pathways are now well characterized and their properties have been reviewed [192–194] With the increasing use of immunological and molecular biological approaches there have been considerable advances in our understanding recently

Phenylalanine ammonia-lyase (PAL, E.C. 4 3.1 5.), the first enzyme of the phenylpropanoid pathway leading to the formation of lignin, is probably the most extensively studied enzyme of secondary metabolism and has been the subject of many reviews including one in this series [195] PAL from most sources appears to be a tetrameric enzyme possessing two dehydroalanine-containing active sites per molecule The subunit M_r appears dependent on the source and mode of purification but is usually in the range 77 000–83 000 for most species [196–199]. If analysed by chromatofocussing, the enzyme from bean [200] or potato [Gilliatt and Northcote, personal communication] can be resolved into at least four forms of differing pI and kinetic properties This suggests the negatively cooperative kinetics previously reported for PAL [201] may reflect combined activities of the different forms with differing Michaelis–Menton properties. Molecular cloning of cDNA encoding PAL has been described from parsley [202] and bean [203] and has lead to the isolation and characterisation of three PAL genes from bean [204] With the wide availability of the cDNA clone it is anticipated that PAL genes from a number of species will soon be characterized. However questions remain as to the association of the subunits and the true nature of the active site.

Cinnamic acid 4-hydroxylase (CA4H, E.C.1. 14 13 11), the second enzyme of the central phenylropanoid pathway is a cytochrome P450-dependent mixed function oxygenase [205, 206] Other reactions in phenolic metabolism thought to be catalysed by cytochrome P450s include the 5'-hydroxylation of ferulic acid [207] in phenylpropanoid metabolism and a number in isoflavonoid phytoalexin biosynthesis [208, 209]. As yet none of the proteins responsible for these activities has been identified unequivocably at the molecular level due to difficulties in purification and reconstitution. However a P450 of M_r 56 000 capable of some reconstituted CA4H activity was isolated from Jerusalem artichoke [210] Another P450, possibly capable of CA4H activity, of M_r 52 000 had been identified in tulip using homologous antibodies which did not cross-react with antigens from any other species [210]. Similarly, heterologous antibodies to bacterial P450 did not cross-react with antigens from plant species [212] while a report of the identification of a M_r 48 000 polypeptide as a possible CA4H [144] using a cross reactive anti-rat P450 monoclonal antibody may have been premature due to the semi-specific behaviour of the antibody [Boobis, A. R. and Sesardic, T., personal communication] Plant P450s thus appear much more heterologous than animal P450s where antigenic homologies [213, 214] have aided the identification of many P450s and are reflected in the sequences of the P450 gene superfamily where cDNA and/or amino acid sequences of over 60 gene products are now known [215].

Subsequent hydroxylation of *p*-coumaric acid to caffeic acid has often been thought to be catalysed by a phenolase of low specificity. However phenolase isoforms have been implicated as specifically catalysing this activity [216–219]. Alternatively, other mixed function oxidases have been implicated and recently an FMN-dependent microsomal activity has been described from potato [220] The coumarate 3-hydroxylase has therefore not yet been identified [221]. The *S*-adenosylmethione-dependent caffeic acid *O*-methyltransferase (COMT E C 2 1 1.-), in contrast, has been isolated from a number of tissues [222] and can be distinguished from those involved in methylation of flavonoids [223, 224]. Further hydroxylation to 5'-hydroxyferulic acid by a cytochrome P450 [207] precedes methylation to sinapic acid [224]

At least two types of 4-coumarate: CoA ligases (4CL E C. 6 2.1 12) with differing substrate specificities have been described [226–229] Thus, type I initially purified from soybean has highest affinities for 4-coumarate, ferulate and sinapate, the CoA esters of which are substrates for the lignin pathway; a second isoenzyme, type II, also purified from parsley cells as well as soybean has highest affinities for 4-coumarate and caffeate, the CoA esters of which appear to be the sole substrates for the first enzyme of the flavonoid/isoflavonoid pathway of species dependent subunit M_r 42 000–46 000 chalcone synthase (CHS) [230–234] Subsequent methylations of the flavonoid B-ring appears to take place after the formation of the ring structure by condensation with malonyl CoA [222, 223]. The CHS, of relevance to this review both as a control point of the relative flux through the pathway and in responses to pathogenesis, also appears to have isoforms [235–237] that are the product of a multiple gene family that have now been identified and sequenced [238–242]. Similarly cDNA has been obtained for the M_r 60 000 parsley 4CL and has lead to the identification and sequencing of the two genes encoding this particular isoenzyme [202, 243, 244].

The synthesis of all these enzymes appears to be subject to induction and repression by a large number of factors and is discussed below. In addition, the flux through the pathway appears to be under stringent fine

control particularly at the level of PAL where differential appearance of isoforms [200] has now been observed in parsley [245] A number of studies have provided evidence which suggests that intra-cellular levels of *trans*-cinnamic acid or some metabolite of it may act as a signal for the regulation of the flux through PAL The effects of exogenous additions of cinnamic acid, which supresses PAL extractable activity *in vivo* [246–249] and L-α-aminooxy-β-phenylpropionic acid (AOPP) which acts as a powerful competitive inhibitor of PAL resulting in the superinduction of PAL activity [250–253] as measured *in vitro*, can be interpreted in terms of the involvement of cinnamic acid in a dual control mechanism involving inhibition of the rate of PAL synthesis and stimulation of the rate of PAL removal [254–257] A more recent study using antibody and cDNA probes has demonstrated that cinnamic acid added exogenously to induced bean cells does not affect the rate of turnover of PAL subunits *in vivo*, but rather mediates irreversible inactivation of the enzyme [257, 258] A non-dialysable factor from cinnamate-treated bean cells stimulates removal of PAL activity from enzyme extracts *in vitro*, this effect being dependent upon the presence of cinnamic acid and is accompanied by an apparent loss or reduction of the dehydroalanine residue at the PAL active site (as detected by titration of the active site by tritiation or with affinity probes) in the absence of an accompanying loss in the levels of immunodetectable enzyme subunits Such a mechanism accompanied by product inhibition of PAL catalytic activity by cinnamic acid especially in view of the apparent kinetic properties of the enzyme would be consistent with PAL acting as a self regulating valve controlling the entry of phenylalanine into secondary pathways from which fixed carbon may not be readily retrievable *trans*-Cinnamic acid may also have a feed-forward effect on the operation of the flavonoid pathway since it activates chalcone isomerase (CHI E C 5 5 1.6) the second enzyme of the branch pathway [259] Exogenously added cinnamic acid is readily fed through the phenylpropanoid pathway, however, leading to increased accumulation of intermediates and wall phenolics so may not modulate these later enzymes [255, 257, 260] It does however also have profound affects in switching off the synthesis of PAL and the enzymes CHS and CHI situated at the start of the flavonoid branch pathway [258, 261] All these effects are probably dependent on the state of the cells and it remains to be proven whether they truly represent mechanisms involved in down-regulation of phenolic metabolism *in vivo* These studies will probably require the production of regulatory mutants either produced by selection, as has already been achieved [261a], or DNA technology In relation to the accumulation of wall bound phenolics the 4CL enzyme also represents a fine control point, for apart from considerable differences in specificity the activity of both ligases is modulated by the relative levels of AMP and ATP [193] Study of such control mechanisms as a model system has important consequences for the operation of secondary pathways when biotechnological applications are desired [261, 262]

Enzymes of lignin formation The two enzymes responsible for the reduction of CoA esters to the corresponding alcohols, cinnamoyl-CoA NADPH oxidoreductase (E.C 1 2 1 44.) and a cinnamoyl alcohol NADP oxidoreductase (CAD E.C.1 1 1 -) [263–267] show substrate preferences for 4-coumaroyl-, feruloyl- and sinapoyl-CoAs A cDNA has recently been obtained encoding the M_r 40 000 CAD from bean [268] using the heterologous anti (poplar CAD) antibody which has allowed investigation of the induction kinetics of a enzyme specific to the lignin pathway for the first time The CoA reductase has also been purified and has a subunit M_r of 38 000 from poplar [266] A cDNA coding for the CoA reductase has recently been obtained (Grand, C., personal communication).

Polymerisation Many types of crosslinkages both covalent and noncovalent occur in the wall, of which hydrogen bonding, dependent on the water content, must be the main cohesive force, especially between the polysaccharides. In the primary wall other non-covalent bonds, ionic-bonds and Ca^{2+} bridges, may also contribute substantially [9] Covalent cross-linkages such as diferulolyl linkages, isodityrosine linkages, which are found in primary and secondary walls, and in lignin of secondary walls, profoundly effect the extensibility, digestibility and adherence of cell walls [9] Following lignification the water present in the growing wall is excluded and the matrix in which the cellulose microfibrils are embedded becomes hydrophobic producing a composite which is rigid [269]. Most of these crosslinkages are thought to be synthesized extracellularly under the action of peroxidase and controlled by the availability of phenyl-linked wall polymers which may be preformed to some extent within the endomembrane system [270] and the supply of hydrogen peroxide Although the activity of peroxidase in the wall is dependent on specific isoenzymes and may vary it does not however appear to ever be rate limiting There is also some evidence that the availability of ascorbate, possibly dependent on wall-bound ascorbate oxidase, may also be a regulatory factor consistent with its familiar role in the chloroplast [271] Thus polymerization of lignin within the wall takes place after oxidation of the hydroxycinnamyl alcohols to mesomeric phenoxy-radicals The half-life of these free radicals is very short before they react together to give lignin and to form linkages between lignin and the polysaccharides of the wall [9, 272–274] Some of these features can be demonstrated *in vitro*

In contrast to the highly heterogeneous mixture of C–C and C–O bonds generated in lignin formation, other phenolic linkages found in primary and secondary walls appear to be more specific The isodityrosine ether- and diferulate biphenyl- linkages, identified by controlled digestion of walls [9, 275, 276] occur within and between specific macromolecules. Isodityrosine linkages have been identified intramolecularly in peptides whose sequence is homologous to extensin [122, 129, 130, 132, 277] Since soluble HRGPs are rapidly insolubilised in the wall it is thought that similar linkages may be generated intermolecularly *in vivo* [278]. However these have not yet been identified. Attempts to generate such linkages *in vitro* with salt extracted HRGP precursor and peroxidase lead to polymerisation of extensin precursors to dimers at least and insolubilization [130, 133, 279–281] but most of the linkages appear to be due to dityrosine formation Diferulate linkages appear to be mainly found between pectic fragments [9, 275] Esterification of pectic arabinose residues by feruloyl-CoA appears to take place intracellularly [133, 282] and are subsequently polymerised in the wall Although these types of crosslinkages, which for diferulate occur about once in every 100 sugar residues, are found within the

growing primary wall the main cohesive force must still be the hydrogen bonding allowing a flexibility to the structure of the wall which depends on its most variable feature, the water content.

In addition to lignin other hydrophobic encrustations are found. Suberin is a complex polymer of long chain fatty acids into which cinnamic acids such as ferulic and *p*-coumaric acids are incorporated by covalent linkages. The phenylpropanoids of suberin are less methoxylated and more highly condensed than in lignin Polymerisation of these aromatic components appears to be catalysed by a wall bound anionic isoperoxidase [154, 283, 284]. The aliphatic component contains substantial proportions of dicarboxylic acids but very little midchain oxygenated monomers [285, 286] This differs from cutin which originates as C_{16} and C_{18} ω-hydroxy fatty acids which are modified to 9, 10, 16, or 18 hydroxy- or epoxy-derivatives. The aliphatic precursors of both polymers are generated by analogous reactions [285, 286]. The reactions specific to the hydroxylation of cutin include those catalysed by cytochrome P450s for the 10-hydroxylation, 9, 10 epoxide formation and the ω-hydroxylation [287–289]. Formation of the dicarboxylic acids of suberin are catalysed by a specific dehydrogenase [290]

Mineralization such as silicification is also found, particularly amongst the grasses [291]. Although, deposition of silicic acid or of a tropolone complex [292] is believed to be non-enzymatic, it appears at the onset of secondary wall formation [293] or in response to infection [294] and is therefore under developmental controls. Initial investigations into these controls have been carried out during the development of grass hairs [295] and infection of bean [296].

Supramolecular aspects

It will be seen that the characteristic wall structure is dependent to a large extent on the type of matrix in which the cellulose microfibrils are embedded. In contrast to cellulose, which is synthesized extracellularly [297, 298], the matrix precursor are synthesized, transported and directed intracellularly [299–301] to be assembled extracellularly. Therefore sites of synthesis and mechanisms of transport constitute essential control points. Precursors and matrix polymers are synthesized within the endomembrane system, the endoplasmic reticulum and Golgi apparatus and transported to the wall. In general, the capacity of plant cells for synthesis and secretion of extracellular components is more rapid than is often appreciated having a $t_{1/2}$ for transit of between four and ten min [128, 300, 302] Indeed, recent structural studies suggest that the P face of the plasmalemma can be in an extreme state of dynamic flux with what are probably both exocytosing vesicles, as products of endomembrane, and apparently endocytosing vesicles, as products of the plasmalemma, plainly discernible [303–305, Hawes, C. R., personal communication]. It is only recently that the heterogeneity of such vesicles is beginning to be understood with, for example, the unequivocable demonstration of chathrin coated vesicles characteristic of endocytosis in plant cells [306–309]. The capacity for endocytosis has been consistently unrecognised in plant cells and the discovery of endocytotic vesicles may have important consequences for understanding cell signalling as well as optimisation of the uptake of desired macromolecules introduced artificially [305–311]. Similarly, the number of exocytosing vesicles at any one time will have importance for the rapidity of cellular responses to stimuli so that the composition and amount of wall material deposited may be dependent on the capacity for vesicle fusion. Also the directed deposition of such vesicles, as demonstrated by a number of electronmicroscopic and inhibition studies, is apparently dependent on the operation of the cytoskeleton, especially the microtubles, as is the alignment of the cellulose microfibrils [312–318].

It has often been assumed that the sites of glycosylation and the operation of the endomembrane system is similar to that extensively studied in animal systems [146, 319, 320, 321] although some aspects of transport and secretion have been challenged [322] Many aspects of similar processes in plant cells are still to be resolved [321] but it is assumed that deployment of newer techniques such as immunogold localization will answer many questions Thus, the localization of synthetic enzymes, the site of synthesis and compartmentation within the endoplasmic reticulum and individual Golgi cisternae and the nature of the different types of transport vesicles may be better understood soon

Most membrane-bound and probably all secreted proteins are probably synthesised on membrane-bound ribosomes and subjected to the constraints of molecular recognition as to their subsequent fate and destination. Some of the vast problems in understanding these processes may become clearer as the sequences of more of these types of plant proteins become known In this way, some of the leader sequences are now recognised for secreted plant proteins of known and unknown function [121, 122, 135, 179, 323–325] Knowledge of such sequences may have important consequences for genetic engineering where gene products introduced into transgenic plants are required to be directed into the apoplast; for example chitinase or toxins [326] Recognition and secretary mechanisms are generally presumed to be similar to secreted products in animal cells but at the moment little information is available on the processing of wall proteins unlike those transported into protein bodies and other sites [90–104, 327] These latter glycoproteins are also the best studied examples of the sites of localization of specific glycosylations

Rather less sophisticated studies have investigated the sites of glycosylation of both wall-bound glycoproteins and polysaccharides by the apparent localization of enzymes activities assayed in isolated membrane fractions *in vitro* and in membrane fractions radioactively labelled *in vivo* Thus although activity can be detected for polysaccharide syntheses in both endoplasmic reticulum and Golgi apparatus, the bulk of the activity is confined to the Golgi *in vivo* [58, 328]. Other similar studies on enzyme activities involved in glycosylation of wall proteins in isolated membranc fractions have localized a number of activities [139, 329, 330]. However, it is anticipated that use of antibodies specific for particular glycosylated epitopes for immunocytochemistry will resolve many of these questions unequivocably since endomembrane structures are defined by their appearance in the electron microscope Development of such probes will incidentally aid specific identification of isolated membrane fractions when they have lost much of their characteristic morphology.

The site of synthesis of lignin precursors has long been controversial since the idea of a membrane bound multienzyme complex was first suggested [331]. Part of the

problem resides in the fact that although the oxygenases are microsomal, the first enzyme of the pathway PAL is usually isolated as a soluble enzyme [191–199] However PAL may be associated with the endoplasmic reticulum [332, 333] and at least 10% of immunoprecipitated PAL subunits are of microsomal origin in bean (Bolwell, G P, unpublished results) PAL has multiple forms, some of which may be compartmentalized [332–334] for function in different pathways The synthesis of lignin precursors can be associated with isolated microsomal vesicles [335] which may be derived from those actively involved in transport to the wall by subsequent fusion with the plasmalemma in the intact cell Polymerisation of lignin subsequently occurs in the wall Vacuolar phenolics may also be transported from the E.R [336] Furthermore the endomembrane may be the site of synthesis and the cell wall site of deposition of non-glycosylated methylated flavonoids [337].

One enzymatic activity definitely associated with the plasmalemma appears to be the synthesis of cellulose [338, 339] In this context, the underlying reasons for the difficulties experienced in the isolation of plant plasmalemma, which cannot be achieved in anything like the purity of animal cell plasmalemma [340], resides, in the author's opinion, in the rapid state of dynamic flux of plant plasmalemma described above. Besides bringing about difficulties in investigating the synthesis of cellulose this property also poses problems in the investigation of putative cell surface receptors involved in various recognition phenomena Cellulose microfibrils appear to be spun out from the plasmalemma into the matrix, the intimate involvement of the membrane being graphically illustrated by the profound effect of perturbations such as plasmolysis which lead to a switch over to callose synthesis [42] Through the observed changing orientation of the microfibrils synthesis is thought to be directed by a moving enzyme complex, which may be represented by the characteristic arrays of particles observed in freeze-fractured plasmalemma and which itself may be directed in some way by the orientation of the microtubules This process has considerable importance since it has been estimated that it is the inner 10% layer of the cell wall that is most important for the physical properties of the cell wall [341, Fry, S C, personal communication] With the capacity for rapid vesicle transfer this layer immediately overlying the plasmalemma has increased significance both for the exocytosing wall modifying components and for a possible internalization of putative signal molecules

Thus, vesicle fusion remains an important cellular event. It was studies on wall regeneration in protoplasts and in plasmolysed cells together with observations of the early fusion events in cell plate formation that has shown that matrix polymers are deposited in the wall as a result of vesicle fusion. As with animal systems [342] the processes involved require both Ca^{2+} and specific proteins in initial binding and subsequent fusion [321, 343–345] As yet there have been few direct studies in plant cells on the stages involved although putative fusigenic epitopes have been identified The involvement of Ca^{2+} is another example of the ubiquitous importance of this ion [346]. Recently there has been intense interest in Ca^{2+} mobilization during cell signalling part of which may be involved in the mediation of fusigenic events Other processes related to Ca^{2+} mobilization may involve other components of cell signalling such as the inositol phosphate pathways and protein kinases, though studies of such signal transuction pathways, so elegantly worked out in animal systems, are still very much in their infancy in plants It remains to be demonstrated if plants do indeed adopt similar mechanisms but as yet there is little convincing evidence that all these pathways lead to gene activation in plant cells It may be that the plant cell wall may hold the key to many aspects of signal transduction which would be unique to plant cells

Controls

Both endogenous and external stimuli can modulate profoundly cell wall structure These include such stimuli as endogenous or externally applied plant growth regulators, mechanical stresses, electromagnetic radiation, gravity, pathogenesis and the various biotic and abiotic elicitor molecules some of which are probably involved in recognition processes Many of these stimuli have target sites in common and may involve common pathways of information transduction so it is useful to draw comparisons of their effects at the molecular level However it is important to note that where gene polymorphisms exist each individual gene may be placed in a different regulatory circuit so that although the same modified functional entity is produced it may represent a differentially synthesised isoform

A picture is emerging of a relative if not unidentical basis to many of the changes observed in response to differing stimuli that is now beginning to be rationalized With a few notable exceptions the underlying basis to most of these changes resides in processes of gene activation *de novo* The recognition of gene expression as the basis to these types of responses in plant cells has come from the deployment of 2D gel analysis and recombinant DNA techniques to identify newly synthesized mRNAs coding for both known and as yet unidentified gene products

Growth and plant growth regulator action The relationship of auxin action to the induction of growth and its accompanying changes in the primary cell wall has long been a subject of intense study and led for example to the 'acid growth' theory, and its subsequent modification [325, 347] A wide range of earlier studies including measurement of changes in the total RNA population and inhibitor studies indicated that cell elongation may be mediated by auxin-regulated gene expression [325] Studies deploying the newer technologies [325, 349–353] have shown that a number of RNA species accumulate, some after only 5–30 min, after auxin application to a variety of tissues A number of these genes have now been sequenced but as yet no function has been ascribed However, two auxin regulated genes from soybean, which appear to be related, contain repeated PPVYK sequences [325] which whilst differing from extensin are similar to a related transcript identified earlier [120] It has therefore been speculated whether these proteins are destined for the cell wall as post translationally modified HRGPs, which are functional during later cell differentiation and maturation Although premature, it may well be that alterations in the expression of genes encoding cell wall proteins by auxin is important in these transitions

A number of enzyme activities related to cell wall modification have been shown to be modulated by auxin action In many different tissues the composition of the

pectin changes during development and this can be modified, particularly the synthesis of arabinan, by the application of auxins [353–357]. But it is in the hemicellulose composition of the primary cell wall that profound and perhaps most significant changes as regards aspects of control take place Thus during auxin-induced cell elongation in pea increased xyloglucan and β (1→4) glucan synthase activities occur while endoβ (1→4)glucanase activity increases in the wall [61, 62, 358] It is not yet known whether the basis of the changes in activity reflect protein synthesis *de novo* The major target site for the glucanase activity is thought to be the xyloglucan and thus promotes wall loosening leading to growth under turgor and also to the production of xyloglucan nonasaccharide As far as the regulatory action of oligosaccharides is concerned, the case for the nonasaccharide acting as antiauxin is extremely strong [182–185] so that a self-regulating system of auxin-induced cell elongation being counteracted by the subsequent production of the antiauxin from the cell wall may be constituted [181].

There is little information of the effects of gibberellins on individual wall components [359, Graebe, J., personal communication] although they too promote cell expansion expressed in terms of wall extensibility [360, 361]. However, gibberellic acid supressed peroxidase secretion and may aid cell expansion through prevention of cross-linkages in the wall [9, 180, 362]. Gibberellic acid-treated cells may also secrete pectins [180] so it will be interesting to determine whether target molecules differ substantially from those in auxin-treated cells undergoing cell expansion.

As yet there is little understanding of the signal transduction processes involved in the action of these growth regulators although putative receptors have been identified and localized At the moment, the evidence for these is largely circumstantial It is a major challenge to elucidate the intervening processes leading to gene activation some of which, such as the proven targets for auxin action, are involved in wall modification. Development of *Agrobacterium* vectors leading to constitutive production of auxin or cytokinin with the subsequent activation in transgenics offer an exciting approach to identifying these genes [Schilperoort, R and Grand, C, personal communications]

Differentiation Similar considerations of the nature of the control mechanisms apply to the changes in response to differing stimuli which are described subsequently. Thus, understanding the molecular events involved in plant cell differentiation is a major goal of modern biology Changes in the components of the cell wall are a feature of this differentiation. During the transition from primary to secondary wall synthesis there is a marked shift in the synthesis of the differing species of polysaccharide broadly characterized by the cessation of pectin deposition and the enhanced deposition of hemicellulose and α-cellulose together with the onset of lignification [269] The type of pectin, hemicellulose and lignin monomers accumulated is species-specific and within a species, of course, the range of cell types, often classified on the basis of wall morphology, is considerable [69]. Many differentiated cell types will contain characteristic combinations of these species-specific wall polymers Faced with this immense complexity of cell types the most accessible differentiating tissue system is probably that associated with vascular differentiation and most notably the process of xylogenesis which has been the subject of previous monographs [363, 364]. Some of these studies have documented the changes in enzyme activities associated with the synthesis of wall components in differentiating cambial tissues. In sycamore, it has been shown that as the cells differentiate there are large increases in levels of PAL and xylan synthase activities accompanied by losses in polygalacturonan synthase activity [57, 80] While in *Pinus sylvestris*, where the major hemicellulose is a glucomannan, large increases associated with cambial differentiation are observed for the glucomannan synthase system [78]. It is thought that these increases are brought about by enhanced gene expression in response to growth regulator action Similar studies on differentiating tissues such as hypocotyls have shown increases in activities of enzymes involved in hemicellulose synthesis accumulated during stages of differentiation of vascular tissue along the growth axis [60, 61, 70].

Valuable insights into the interaction of plant growth regulators resulting in the characteristic changes in wall composition, especially with regard to lignification, have come from studies of differentiation in tissue-cultured cells often as part of studies of morphogenesis in general. Reproducibly high levels of cytodifferentiation have been achieved for a variety of systems such as isolated cells, especially from *Zinnia*, [365–369], explants [370–373], callus cultures of both dicotyledons and monocotyledons [374–376], and suspension cultured cells [377–380] Tissue cultures have proven good model systems especially if induced changes still occur with the same timing and magnitude as in developing intact tissues then there is no compelling reason to suppose that the basic controls differ between the two systems. Although the combination of plant growth regulators used for the induction of differentiation is arrived at empirically, as a general rule it is brought about by a depletion of auxin in the medium which is often supplemented with a relative increase in exogeneous cytokinin and the provision of a carbohydrate source usually sucrose The auxin most commonly used to induce meristematic, activity is 2,4-D which is more slowly metabolized than IAA or NAA and is therefore more active in maintaining the undifferentiated state. A number of studies have demonstrated loss of polypeptide species as analysed on 2-D gels and these may be associated with regeneration potential. In any case, depletion of endogenous 2,4-D on transfer to a 2,4-D-free medium is probably an absolute prerequisite for morphogenesis which is inhibited by retention of this growth regulator The action of cytokinin is also modified in long-term cultures which may become habituated [381] leading to a loss of morphogenic potential [382] A deeper understanding of these phenomena is of great importance in elucidating the control of gene expression during differentiation.

Molecular studies have concentrated on the synthesis of specific RNA and protein species associated with differentiation. At present a number of specific proteins that are dependent on growth regulator treatment may be resolved by two-dimensional PAGE [347, 348, 383] Such studies illustrate the subtlety of the differentiation process since no extreme shifts in patterns of synthesis tend to be observed unlike for instance during pathogenesis (see below). The study of selective gene expression is probably best served by looking at specific markers. Many of these markers for differentiation remain func-

tionally unidentified and there have been few developments of probes (antibodies and cDNAs) with respect to differentially regulated proteins of known function

In contrast, the characteristic changes in enzyme activities associated with growth and vascular differentiation in tissue cultures have been documented. Thus increases in enzyme activities associated with pectin synthesis are observed during the periods of growth and cell division during the cell cycle [354, 379] In nondifferentiating cultures the characteristic changes observed in relation to the cell cycle have been accurately mapped in a synchronous culture. Here most of the accumulation of glucan polymers occurs during G1 preceded by the necessary metabolic activity [54, 118, 135, 384, 385] Transfer to morphogenic media induces xylan synthase activity associated with secondary wall formation during xylogenesis [375, 379] Although results of preliminary experiments suggests these changes may be due to *de novo* synthesis and possibly reflect gene expression this has not yet been demonstrated rigorously Unlike matrix polymers no information is yet available on the control of cellulose synthesis during differentiation In contrast to polysaccharide synthesis, more is known about the induction of lignification where increases in the individual activities of the pathway from shikimic acid to lignin and specific isoenzymes of peroxidase have been documented to varying degrees in many different systems Although perhaps not as rigorously demonstrated as in the responses to other stimuli, increases in activities of PAL and isoenzymes of 4-coumarate ligase during differentiation probably reflect increased gene expression [374, 375, 377–380]

These aspects of differential gene expression are crucial to the development and maintenance of specific cell types The transcriptional control mechanisms involved in establishing these developmental states still remain elusive as regards the interplay of plant growth regulator action that promotes them One way in which the cell wall might be intimately involved in control of morphogenesis might be in the provision of oligosaccharins that regulate morphogenesis Thus, an oligogalacturonide isolated from primary walls of suspension cultured sycamore cells can bring about modulation of apparent auxin and cytokinin action during tobacco callus morphogenesis [186] These fragments like the xyloglucan nonasaccharide may hold the key to many aspects of plant cell signalling if they could be demonstrated *in vivo*

Stress and ethylene Environmental factors such as light, temperature, water relations or mechanical constraints can also have considerable influence on wall components The effect of light on etiolated seedlings is an obvious example but the changes in the wall components that ensue are, of course, part of the general developmental programme of growth and differentiation triggered during photomorphogenesis, a subject too complex to be treated here The induction of the phenylpropanoid pathway by light is of course very well documented and under light stress conditions (high or low intensity white light or UV light) the products tend to be channelled into the formation of protective flavonoid compounds [193] Similarly, although many of the effects of temperature shock are beginning to be elucidated at the molecular level [386], none has yet been shown to be related to the synthesis of wall components Although water deficits have profound effects of cell wall synthesis and cell enlargements and also on the levels of lignin precursors and other secondary compounds no clear relationships have been established and most studies relating to the wall have been physiological [387] Also, for osmotic stress extensive investigations have been directed to parameters such as respiration, accumulation of low M_r compounds and lipid metabolism and other than treatments leading to plasmolysis, few studies have determined the direct effects of incipient water stress on wall metabolism Under extreme conditions of plasmolysis incorporation of sugars other than arabinose into newly synthesised polysaccharides is decreased [388] A protein which by virtue of its hydroxyproline content may be related to wall-bound proteins accumulates in salt stressed cells [127] In contrast, the effect of mechanical constraints as regards the synthesis of cell wall components have been well characterized at the biochemical level Thus a constant mechanical perturbation stimulates the directed deposition of callose, the thigmotropic response [389] Callose synthesis is also a feature of wounding [41] and since wounding has many features in common with pathogenesis, which also presents additional aspects of control, both are discussed in more detail below The whole subject of the effects of wounding on plant cells has received some attention and much of the biochemistry of the responses is now well understood [390–392] However, here too it is the mechanisms of signal transduction that lead to increased gene expression remain elusive, other than to believe they may be related to depolarization phenomena exhibited by damaged cells [393–396] In contrast, signal transduction at the molecular level in response to wounding monolayers of animal cells is beginning to be understood [397, 398, Jones, D, personal communication]

Besides the stimulation of callose synthesis, wounding also effects the synthesis of other wall components and these are classically associated with the production of ethylene [399–402] A large number of metabolic activities are stimulated in response to wounding these include the general gross responses of regeneration of membranes, enhanced respiration, protein synthesis, stimulation of secondary pathways and changes in growth regulator activity and in some cases cell division [390–392] Cell walls are regenerated at the wound site with enhanced deposition of lignin- or suberin- like materials and HRGPs The underlying basis of these changes, with the exception of the synthesis of wound callose, appears to reside in *de novo* gene expression However, once again it is important to emphasise that differential gene expression mediated by different regulatory circuits in response to each stimulus where gene polymorphisms occur may well be operating Thus stimulation of the transcription of PAL, for example, by wounding or elicitor action may involve differential expression of the different genes especially in relation to the timing of the responses

Although a direct role for ethylene in disease resistance is controversial there is abundant evidence for a role in the transmission of the wounding response Ethylene stimulates general mRNA and protein synthesis and modulates the level of specific transcripts [403, 404] Thus similar transcripts of specific HRGPs can be induced by wounding and ethylene, translated and processed, although subtle differences in the range produced may be observed [120 122, 405] Ethylene also brings about a cessation in xyloglucan deposition through loss in β-glucan synthase and xyloglucan synthase activities though unlike auxin treatments no enhanced endo β [1→4]glucanase activity is observed [61, 62] Other hydrolases are synthesized however Thus the differential

synthesis of specific hydrolases occurs either in wounding or under conditions of terminal differentiation such as abscission or fruit ripening. Ethylene generally stimulates transcription of chitinase probably as a defense response which interestingly may also be observed at abscission zones [170–173, 406]. Enhanced transcription of polygalacturonase genes is particularly well documented in ripening tissues [164, 179] and β [1–3] glucanases show enhancement by ethylene action [166, 407]. This may involve transcription of specific isoforms of a variety of hydrolases at abscission zones [cf. 408]. Although ethylene stimulates the accumulation of many specific mRNAs there is as yet no clear picture of the mechanisms involved, the putative receptor and the subsequent signal transduction mechanism The mechanism of response coupling of the initial stimulus to ethylene production also remains elusive but is crucial for understanding such processes of commercial importances as ripening, senescence of flowers and leaves and abscission.

Pathogenesis and elicitor action. Although the involvement of the plant cell wall in adherence is a matter of conjecture [409], following binding of a pathogen to the plant cell surface, a series of characteristic changes in the host cell wall appear immediately underlying the infection site. A number of electron microscopic and cytochemical studies have revealed the similarity of these papilla-like and other structures below the site of spore or penetrating hyphae in many diverse families of dicotyledons and in monocotyledons, particularly the Gramineae [410–413] Similar encrustations of wall materials have been observed in vascular tissues where these are target sites for pathogens. These studies suggest a callose-like polysaccharide material is usually deposited which becomes progressively encrusted with hydrophic or lypophilic material which in general appears to be both glycoproteinaceous and lignin-like. In some species, especially those of the Solanaceae the complex lipid-phenolic, suberin is deposited at the site [285] These changes, although more localized, resemble those found as a general wounding response but other components, intimately linked to disease resistance, must occur in addition to the recognition phenomenon. Understanding the highly localized wall reaction, probably linked to the hypersensitive response following the initial recognition event, indicates that synthesis of wall components may be of fundamental importance to disease resistance. Although patently a component of plant disease resistance many of these characteristic wall changes would not be expected to represent direct products of resistance genes *per se* since these changes are represented in both resistant and susceptible interactions where the timing of their appearance differs.

The deposition of callose is an early event in these interactions, The phenomenon is widespread, being observed not only in microbial attack but also, as described above, during mechanical stimulation, wounding or disruption of the plasmalemma-wall interface, which can also occur during normal cell development such as in sieve plate formation in phloem. Callose formation can in fact precede the characteristic ethylene production seen in some of these phenomena. While the evidence accumulating points to many responses involving changes in the patterns of the synthesis of wall components having an underlying basis in gene activation, with possibly some element of post-translational modification, deposition of callose appears to be an exception. Thus callose formation in suspension cultured cells of soybean, in response to membrane perturbations brought about by elicitor action, is due to stimulation of $\beta(1\rightarrow3)$glucan synthase activity which possibly represents a modulated activity of the cellulose synthase system, and may be coupled to an influx of Ca^{2+} ions across the plasmamembrane [41, 119, 414, 415]. Wall-bound enzymes such as acid phosphatases can also be activated by Ca^{2+} ions [416] and secretion of peroxidases [417] or of pectic fragments by plasmolysed cells [418] are Ca^{2+} dependent so that Ca^{2+} fluxes may therefore play an essential part in establishment of changes in wall structure stimulated by pathogenesis. Whether such ion-fluxes can be coupled to some mechanism leading to gene activation, perhaps as a second messenger, requires further examination.

In addition to these activation mechanisms the infection of hypocotyls of cultivars of *Phaseolus vulgaris* with conidia of different races of *Colletotrichum lindemuthianum*, for example, causes marked coordinated increases in the rates of synthesis of PAL and other enzymes of phytoalexin production as measured by *in vitro* translation and for PAL and HRGPs as measured by blot analysis [203, 237, 419, 420]. Incompatible responses are characterized by early accumulation of these mRNAs and a hypersensitive response; during compatible interactions there is no early response but rather a later accumulation of mRNAs associated with phytoalexin production during attempted lesion limitation. These types of study indicates that specific accumulation of defence related mRNAs is an early component during race-cultivar specific interactions and that signal transmission occurs to pre-activate defence genes in hitherto uninfected cells. Expression of these genes leads subsequently to the accumulation of HRGPs and lignin-like phenols at the infection site. In many ways the modified nature of these materials compared to the related structures usually accumulated resembles the transition from net cellulose to callose synthesis occuring during the perturbation of the plasmamembrane. The modified nature of phenolic material might be explained perhaps by premature exposure of phenylpropanoid precursors to phenolases and peroxidases as a result of membrane disruption leading to free radical formation of species other than lignin alcohols. These might react with other polymers before deposition. It is apparent that during the hypersensitive reaction target cells experience highly disturbed metabolism leading to the production of numerous highly reactive free radicals including superoxide anion [421–423] Similarly abnormal metabolites produced by wounded cells can also act as attractants for pathogens and inducers of virulence genes [409].

Use of tissue cultures of, for example, parsley, soybean or French bean treated with elicitor molecules, derived from pathogens, also exhibit many of these changes in wall components found in the intact interaction, though accumulation of components would not be expected to show the localized deposition found in the intact interaction. Mechanisms of gene activation have been indicated to varying degrees of technical sophistication for increases in activity of PAL [202, 203, 419, 424–429], probably cinnamate-4-hydroxylase, 4-coumarate ligase [202, 428, 429], cinnamyl alcohol dehydrogenase [268] in lignin-like synthesis, and prolyl hydroxylase [143], probably arabinosyl transferase [107], and various forms of proline-rich precursors [128, 419, 420, 430, 431] in HRGP synthesis. The hydrolyases, chitinase and β [1→3]glucanase [326, 426] are also induced but it is not yet known whether they are targeted into the wall These

increases occur alongside species-specific increased gene activation involved in phytoalexin accumulation [419, 424–429], pathogenesis related proteins [432, 433] some of which are now known to be isoforms of chitinase [Scheel, D., personal communication], and in the intact situation, protease inhibitors [434] and ethylene production [434, 435] Detailed studies of the timing of such responses have been made to attempt to discover putative causal links in order to understand the basis of disease resistance Although transcript run-off experiments have established that gene activation can occur within 30 min of addition of elicitor to suspension cultured cells it is now generally considered that genes conferring true resistance encode products involved in initial recognition events Attempts have been made to isolate specific cell surface receptors that bind elicitor molecules and one such putative interaction has been described [436] Elicitor molecules may be hydrolytic products of fungal or host cell wall [410, 437, 438] The variety of elicitor molecules is ever increasing and includes examples of oligosaccharides, lipids and proteins or glycoproteins However, it seems difficult to envisage how, faced with such inherent variety, resistance and susceptibility could arise from coevolved receptor-ligand interactions as has been postulated With such a diversity of stimuli giving rise to similar changes in wall components which can be highly localized, a perturbation of an endogenous self-recognition process in the form of continual interplay between wall and protoplast could be an alternative basis of some of these responses Certainly the action of these molecules in bringing about rapid membrane depolarisation events based upon multiple ion-fluxes [439–441] as seen in both the cell culture and in infection sites could be symptomatic of a general membrane response involving many parameters rather than due to a particular target receptor Such a perturbation would be immediately fed back through the endomembrane system rapidly generating a number of novel compounds or leading to an abnormal accumulation of material which could act as signals The response coupling mechanisms leading to gene activation remain obscure, however, but are currently an area under intensive investigation These may be related to the types of signalling mechanisms known to lead to gene activation in animal cells, such as in responses to growth factors or during the Ca^{2+} ion influx stimulated activation of *C fos* oncogenes On the other hand, as yet unidentified modes of inter- and intracellular communication may be indicated One study for example implicates direct nuclear targetting of fungal-derived elicitor [427] Such a mechanism has become more feasible with the establishment of a rapid endocytotic pathway in plant cells Armed with probes to study early events in gene activation and with the acquision of sequences for *cis*-acting controlling sequences functional analysis at the gene level becomes feasible, possibly leading to the identification of the putative *trans*-acting factors Signal transduction mechanisms may then become less intractable, but will probably require absolute identification of the elicitor molecules actually acting *in vivo*. Some of these molecules are thought to be products of the host cell wall [409, 437, 438] Thus although oligogalacturonide fragments produced from isolated walls were first identified as inducers of protease inhibitors or phytoalexins they are also capable of eliciting changes in host wall components (Murphy, D., personal communication). Although one difficulty in explaining their action in the plant has been noted [442] at the cellular level they are certainly effective in a number of systems. Their mode of action can be synergistic with other elicitors [443] and a number of studies point out differences, possibly temporal, in elicitor action in the differential activation of related genes The action of microbial hydrolases specific for pectin degradation is also thought to bring about resistant responses through the production of pectic fragments [444] Application of these enzymes brings about rapid ion fluxes [445] The similarity of the range of responses in the type of product molecule induced to all these many different primary stimuli as regards synthesis of wall components is of course striking However, since the responses are not identical in timing and site of deposition it must mean other controls must operate in some extremely subtle way and the very essence of non-self recognition still remains obscure

CONCLUSIONS

The whole area of plant cell wall metabolism offers an exciting challenge since it requires an understanding of molecular events at all levels of organisation Thus, increases in our knowledge are unlikely to be brought about by application of single technologies due to the complex modifications and interactions that contrive to assemble and change the extracellular matrix Yet it is now realised that these dynamic properties are fundamental to the adaptability of the individual cell and to the exchange of information with the environment, in addition to the structural role played by the plant cell wall Thus these properties deserve to be explored, particularly as plant science moves into a new age In the area of molecular biology, where the search for regulated gene products accelerates, it is becoming apparent that many of the earliest genes induced in response to a wide variety of stimuli are involved in the modification of existing wall structures Although the mechanisms of signal transduction remain obscure, wall products can act as signal molecules Thus it is obvious that the cell wall is intimately involved as part of the 'sensing' of the environment and in 'responding' through the ensuing changes It is striking how many of these changes, both during normal development and under conditions of stress, have many similarities However the subtle differences observed suggest additional controls operate both at the molecular and supermolecular levels Identification of the signal transduction pathways and mechanisms of gene switching together with the post-translational aspects of protein function, including the synthesis of polysaccharides, remain the greatest challenges If some genes involved in cell wall synthesis join the ranks of those suitable for transformation studies as a view to possible genetic engineering, for example, of disease resistance, then all the aspects of subsequent processing should be recognised

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