PREVIOUS WORK

Brief History

The function of the aleurone layers of cereal grains as a secretory tissue during seed germination was first recognized by Haberlandt as early as 1890. He observed that diastase enzyme activity, responsible for starch digestion, was present in seed coats and aleurone layers of rye grains, and also demonstrated that the growing embryo was necessary for the production of diastase by the aleurone cells. Brown and Escombe (1898) found that amylase caused the liquefaction of the starchy endosperm of barley seeds. Later, this was extensively investigated and confirmed by several workers (MacLeod and Miller, 1962; Briggs, 1963; Yomo and Inuma, 1964; Varner and Chandra, 1964). It was shown that the signals originating from the embryo (Haberlandt, 1890; Yomo, 1958) which trigger the enzyme production by the aleurone, were gibberellins (Yomo, 1960a) and that these factors could, in fact, be replaced by gibberellic acid (Yomo, 1960b; Paleg, 1960).

The isolated aleurone layers or embryoless half seeds of barley respond to added gibberellic acid (GA) by producing and releasing several enzymes (Pollard, 1969) including amylase (Varner, 1964), protease (Jacobsen and Varner, 1967), ribonuclease (Chrispeels and Varner, 1967a), glucanase (Jones, 1971) and phosphatase (Bailey
et al., 1976; Obata and Suzuki, 1976). The response is highly variable with variety and even with different harvests of the same variety of barley, therefore, in many laboratories seeds of a single variety and harvest are used. Other important factors on which the response is dependent are the preincubation conditions. The term "preincubation" was introduced by Varner (1964) to cover the period of metabolism prior to GA addition, during which the half seeds were preincubated on moist sand for 3 to 5 days at 17°C to 30°C. He found that within the limits indicated, there was no detectable influence of variations of time and temperature on the capacity of the half seeds to respond to GA added after preincubation. Dry, nonpreincubated seeds treated with GA gave inconsistent results because of overlapping of responses due to imbibition and those due to GA. A variety of preincubation conditions have been used by other workers, ranging from 2 to 8 days imbibition in sterile distilled water at 30°C or at room temperature (Pollard, 1969; Obata and Suzuki, 1976).

GA-induced Enzyme Release

It has been well established that amylase and protease are synthesized in and released from the aleurone cells, in response to GA, after a lag period of 8 to 10 hours (Jacobsen and Varner, 1967). Although it has been demonstrated that the synthesis of amylase and protease
are GA controlled processes, the role of the hormone in
the stimulation of enzyme release is not very clear
(Chrispeels and Varner, 1967a; Jacobsen and Varner,
1967). The study of enzyme release from aleurone cells
has been difficult because the processes of enzyme
synthesis and release cannot be clearly separated experi-
mentally (Chrispeels and Varner, 1967a).

Another group of hydrolases including glucanase,
ribonuclease and acid phosphatase do not show a similar
response to GA. There is a marked increase in their
activities during imbition of seeds, but very little
increase in the total amount of enzyme activity occurs on
addition of GA (Chrispeels and Varner, 1967a; Jones, 1971;
Ashford and Jacobsen, 1974). However, release of these
enzymes into the incubation medium is dependent on GA,
and these enzyme systems have been employed to study the
GA-controlled enzyme release (Chrispeels and Varner,
1967a; Taiz and Jones, 1970; Jones, 1971; Ashford and
Jacobsen, 1974; Bailey et al., 1976).

(i) Glucanase - Enzyme glucanase was released into
the incubation medium after a lag period of 4 hours and
the rate of glucanase release, which is a GA-dependent
process, was proportional to the concentration of the
hormone (Taiz and Jones, 1970; Jones, 1971). Unlike
amylase, the presence of GA was required only during the
lag phase, as removal of GA at the end of the lag period did not affect the subsequent enzyme release (Jones, 1971). Although GA enhanced glucanase release, it did not significantly affect the total amount of glucanase formed in aleurone layers as compared to the water controls (Taiz and Jones, 1970; Jones, 1971).

Inhibitors of RNA synthesis inhibited both the formation and release of glucanase (Jones, 1971). 6-methylpurine was the most effective inhibitor of RNA synthesis, as noted in the case of amylase (Chrispeels and Varner, 1967b). Actinomycin D and 8-azaguanine were almost ineffective in inhibiting glucanase production and release if applied eight hours after the addition of GA. In contrast, cycloheximide, an inhibitor of protein synthesis, brought about a rapid decrease in enzyme release, even when added at this stage (Jones, 1971). The inhibitory effect of cycloheximide on glucanase release was quite different from its lack of effect on amylase release (Varner and Mense, 1972). The release of glucanase was an active process, having a $Q_{10}$ greater than 3.0 and was inhibited by respiratory inhibitors (Jones, 1971).

(ii) Ribonuclease - Time course studies of ribonuclease release showed a lag of about 24 hours after GA application (Chrispeels and Varner, 1967a). The synthesis
of ribonuclease, which like glucanase started during imbibition on water (Taiz and Jones, 1970; Jones, 1971), continued in the presence of GA for 48 hours (Chrispeels and Varner, 1967a).

The inhibitors of both protein and RNA synthesis prevented the development of release capacity for ribonuclease enzyme, if added in early stages, but had no effect on the rate of ribonuclease release, once the release process was developed. This indicates that these inhibitors do not interfere with the release process itself but with the development of release capacity (Chrispeels and Varner, 1967a).

(iii) Acid Phosphatase - Phosphatase enzyme was detected in dry barley seeds but its activity increased during imbibition of embryoless half seeds (Bailey et al., 1976). The release of phosphatase was greatly stimulated by GA and occurred after a 12 hour lag period (Bailey et al., 1976). The duration of the lag phase was extended, if the period of preincubation of half seeds was increased (Obata and Suzuki, 1976). Whereas, Bailey et al. (1976) found that the total phosphatase and the extractable enzyme activities were also enhanced in the presence of GA, Obata and Suzuki (1976) noted that the intracellular activity of phosphatase was inversely related to the increase in the extracellular enzyme activity.
The increase in activity of phosphatase during and after imbibition was due to activation as well as de novo synthesis which occurred both in the presence and absence of GA (Bailey et al., 1976). They suggested that although the hormone does not control the de novo synthesis, it is responsible for the activation of the enzyme.

(iv) Peroxidase - Very little work has been done to study the effect of GA on peroxidase enzyme in embryoless halves of barley. GA induced the production and release of peroxidase enzyme in barley half seeds (Harmey and Murray, 1968). The release of peroxidase in presence of GA was detected after a lag period of 12 hours (Harmey and Murray, 1968; Pollard, 1969). The GA-induced increase in the level of peroxidase present in the half seeds preceded the GA-enhanced enzyme release (Harmey and Murray, 1968).

GA-induced Enzyme Synthesis

Aleurone cells incubated in the presence of radioactive amino acids and GA produced labelled amylase (Varner and Chandra, 1964). Later, it was rigorously demonstrated that the enzymes amylase (Filner and Varner, 1967) and protease (Jacobsen and Varner, 1967) are synthesized de novo in the aleurone cells in response to
GA. Acid phosphatase (Ashford and Jacobsen, 1974; Bailey et al., 1976), ribonuclease and glucanase (Bennett and Chrispeels, 1972), however, fall into a category of GA-promoted enzymes in aleurone which increase during imbibition in the absence of GA. Although the synthesis of ribonuclease and glucanase is de novo, it is not under GA control (Bennett and Chrispeels, 1972). However, the release of these two enzymes and acid phosphatase is GA-dependent (Chrispeels and Varner, 1967a; Taiz and Jones, 1970; Jones, 1971; Bennett and Chrispeels, 1972; Ashford and Jacobsen, 1974; Bailey et al., 1976; Obata and Suzuki, 1976).

The synthesis of amylase by aleurone layers can be substantially reduced and ultimately stopped by the removal of GA midway during the course of incubation. Readdition of GA causes immediate resumption of amylase synthesis at the control rate without a new lag period (Chrispeels and Varner, 1967b). Thus, the presence of the hormone is required during the lag period as well as during the period of enzyme synthesis.

Dinitrophenol, p-fluorophenylalanine and cycloheximide inhibit synthesis of amylase and protease indicating that phosphorylative energy and protein synthesis are required for the production of these hydrolases (Varner, 1964;
Varner et al., 1965).

**Effect of Different Factors** - The response of aleurone tissue to GA has a lag period varying from 4-20 hours after the addition of the hormone. Among the early responses is the appearance of glucanase activity, which occurs within 4 hours of GA application (Taiz and Jones, 1970). Synthesis and release of amylase and protease occur after a lag of 8 to 10 hours (Jacobsen and Varner, 1967). Acid phosphatase (Bailey et al., 1976) and peroxidase (Harmey and Murray, 1968) are released following 12 hours of GA treatment. Ribonuclease, on the other hand, is released still later and does not appear in the incubation medium until about 20 hours after GA addition, although its synthesis starts during imbibition, even before the addition of GA (Chrispeels and Varner, 1967a).

The nature of the lag period in the induction of amylase synthesis by gibberellic acid was investigated and several factors were shown to modulate the duration of the lag phase:

1. **Effect of temperature** - The lag phase between the application of GA to barley aleurone layers and the beginning of amylase synthesis is minimum at 30°C (Paleg, 1961; Goodwin and Carr, 1972a). Incubation temperature hardly affected the background activity in the absence of
added hormone. When incubation temperature was main-
tained at 30°C during the first 2 hours after GA
application and subsequently lowered to 25°C (called
"temperature step down incubation"), the duration of
the lag period was reduced to that as the total incuba-
tion at 30°C. There are thus two rate limiting reactions
during the lag phase, the first which is highly tempera-
ture sensitive, and the second which is relatively
insensitive to temperature (Goodwin and Carr, 1972a).
Whereas, the temperature step down incubation has a
sharp pH optimum of 5.00 to 5.05 and requires the
presence of ferric ions in the medium, the incubation
at the optimum temperature i.e. 30°C is insensitive to
either pH or iron concentration (Goodwin and Carr,
1972a).

(ii) Ferric ion requirement - As already mentioned,
incubation of aleurone layers at 30°C did not require
the presence of ferric ions in the medium. However,
ferric ions supplied during the preincubation period,
increased the amount of amylase synthesized subsequently
in the presence of GA (Goodwin and Carr, 1970). Further-
more, it was shown that ferric ions are required at the
time of initiation of amylase synthesis, and although
they increased the total amount of amylase produced in
the presence of GA, they did not affect release of the
enzyme (Goodwin and Carr, 1970). The GA-enhanced amylase synthesis was inhibited by the iron chelators bathophenanthroline sulphate and O-phenanthroline. This inhibition could be reversed by ferric chloride. Ethylene diamine tetra-acetic acid did not cause any such inhibition (Goodwin and Carr, 1970).

(iii) Calcium ion requirement - Calcium ions were required for maximum accumulation of amylase as they stabilized the enzyme against degradation by the protease (Chrispeels and Varner, 1967a). Jacobsen et al. (1970) observed that the action of calcium ions was principally on the synthesis and stability of amylase isozymes 3 and 4 and had no effect on amylase isozymes 1 and 2 and the synthesis of protease enzyme. A requirement for externally added calcium ions, during the second part of the lag phase (i.e. 3 to 4 hours after GA application), was shown by Goodwin and Carr (1972b).

(iv) Effect of inhibitors - Amylase synthesis was inhibited by a number of inhibitors of RNA synthesis (Briggs, 1963; Varner and Chandra, 1964; Chrispeels and Varner, 1967a, b). The inhibitors like 6-methylpurine, 8-azaguanine and abscisic acid were effective if supplied during either the lag phase or the enzyme synthesis period. However, actinomycin D, while effective if
supplied along with the hormone, was almost ineffective when added at the end of the lag period (Varner and Chandra, 1964; Chrispeels and Varner, 1967b). Goodwin and Carr (1972c) showed that the induction of amylase synthesis by GA was most sensitive to actinomycin D over a short period late in the lag phase. The latter part of the lag phase was earlier found to be less sensitive to temperature (Goodwin and Carr, 1972a). Actinomycin D did not inhibit any metabolic processes like respiration, leucine, uridine or uracil uptake, leucine incorporation or leucine pool size and as it only inhibited uracil and uridine incorporation into RNA, it was suggested that it acted by inhibiting RNA synthesis (Goodwin and Carr, 1972c).

**Mechanism of GA-induced Enzyme Release and Synthesis**

1) Early Responses of Aleurone Cells to GA -

(i) Structural modifications - The gibberellin-enhanced production and secretion of hydrolases which occurs after a lag phase is preceded by several cytological changes, similar to those occurring in the aleurone layers during germination (Van der Eb and Nieuwdorp, 1967; Jones, 1969c). Most prominent changes are the extensive proliferation of rough endoplasmic reticulum (Jones, 1969c; Vigil and Ruddat, 1973) and increased number of polyribosomes (Evins,
(ii) Changes in phospholipid metabolism — The incorporation of $^{14}$C-choline into a fraction containing endoplasmic reticulum (Evins and Varner, 1971) and incorporation of $^{32}$P$_1$ into phospholipids (Koehler and Varner, 1973) are also greatly increased by GA. This enhancement begins about 4 hours after GA application and is prevented by abscisic acid, cycloheximide and 6-methylpurine (Koehler and Varner, 1973). Within two hours of GA addition, increases in the activities of two membrane bound enzymes of the cytidine diphosphate choline pathway of lecithin biosynthesis are also observed. The two enzymes are phosphorylcholine-cytidyl transferase and phosphorylcholine-glyceride transferase, and their activities are partially inhibited by abscisic acid, cycloheximide and actinomycin D (Johnson and Kende, 1971). However, high concentration of mannitol (0.6M) which prevents the increase in the incorporation of $^{32}$P$_1$ into phospholipids, did not inhibit the activities of these enzymes (Koehler et al., 1972).

(iii) Hormonal control of membrane modification: a model — The fact that these events precede GA-evoked formation and secretion of hydrolases and are sensitive
to the same treatments (i.e. abscisic acid, cycloheximide) which inhibit GA-enhanced hydrolase synthesis has led Johnson and Kende (1971) to put forward a post-transcriptional model based on the necessity of membrane proliferation for the synthesis and secretion of the hydrolases. They suggested that GA induces the structural and functional reorganisation of the endomembrane system of aleurone cells for the synthesis and secretion of the hydrolytic enzymes. The ability of GA to modify the already existing membranes is suggested by the observations that it enhances the release of oligosaccharides (Pollard and Singh, 1968), inorganic ions (Eastwood and Laidman, 1971) and sucrose (Chrispeels et al., 1973). Another such membrane modification by GA is the inhibition of the activity of membrane bound arabinosyl transferase, an enzyme involved in cell wall pentosan biosynthesis (Johnson and Chrispeels, 1973).

2) **Lysosome Concept** - The secretion of enzymes from several animal and plant cells is known to occur through small membrane bound vesicles called lysosomes. These enzymes are apparently synthesized on the rough endoplasmic reticulum (RER), which in such cells is abundant. The newly synthesized enzyme moves into the lumen of RER and gets enclosed in a membranous vesicle for transport through the cytoplasm to the plasmalemma.
(Campbell, 1970). In aleurone cells of cereals, there is massive synthesis and secretion of hydrolases in the presence of GA, suggesting that enzyme release may be through vesicles. Electron microscopic studies done by Vigil and Ruddat (1973) demonstrated the induction by GA of RER formation and budding off of vesicles from the reticulum. Attempts to locate hydrolase activity in vesicles yielded conflicting results. Gibson and Pales (1972) observed that most of the amylase and protease activity, after differential centrifugation of an homogenate of wheat aleurone cells, was present in a particulate fraction and suggested that these hydrolases were lysosomal. However, Jones (1969a, b, 1972) found no evidence that distinct secretory vesicles are involved in the transport of amylase and glucanase in barley aleurone cells and suggested that these are soluble enzymes which are released directly from the cytoplasm across the plasmalemma. Autoradiographic studies of barley aleurone cells, pulse or pulse chased with radioactive leucine (Chen and Jones, 1974a, b) also favoured the idea that after synthesis on the RER, the hydrolytic enzymes pass to the exterior of the cells without the participation of vesicles. On the other hand, results based on gel filtration studies, a technique which separates the particulate and soluble enzyme activities, are
consistent with the hypothesis that amylase is secreted via membrane bound vesicles (Firn, 1975).

3) **The Pathway of GA-induced Release** — Release of enzymes from aleurone layers into the starchy endosperm occurs in two phases namely (1) active secretion of the enzymes across the plasmalemma out of aleurone cells, and (2) release of the enzymes, presumably by diffusion through the walls and thus out of the aleurone layer (Varner and Mense, 1972). The limiting step in the release of these hydrolases from the aleurone layers could be either their secretion through the plasma membrane or their release through the walls. Varner and Mense (1972) showed that diffusion through the cell walls is the rate limiting step in GA-stimulated enzyme release and that secretion is not under the direct control of the hormone.

(1) Cytochemical evidences — Cytochemical investigations of barley aleurone layers revealed that following GA treatment regions of aleurone cell walls become digested (Taiz and Jones, 1970; Ashford and Jacobsen, 1974). Taiz and Jones (1970) suggested that glucanase which is produced by aleurone cells in response to GA is responsible for the digestion of the cell walls. Ashford and Jacobsen (1974) concluded
that in the presence of GA, acid phosphatase enzyme is secreted into the walls, presumably through the plasmodesmata (Jones, 1972), from where it is released predominantly through the digested wall areas out of the aleurone layer. In the absence of GA, though some enzyme is secreted into the walls it is not released, as no digested wall channels are formed. Such enzyme is measured biochemically as being within the aleurone layer (Ashford and Jacobsen, 1974).

These evidences confirmed the earlier ultrastructural observations that GA brings about a dissolution of the aleurone cell wall (Van der Eb and Nieuwdorp, 1967; Jones, 1969b), and also indicated that the cell wall and not the plasma membrane is responsible for limiting the movements of the hydrolases from the aleurone cells to the endosperm, as was first proposed by Varner and Mense (1972).

(ii) Biochemical evidences - A major fraction of the hemicelluloses of the cell walls of cereal endosperm (Mares and Stone, 1973) and of monocotyledons in general (Burke et al., 1974) is arabinoxylan. Taiz and Honigman (1976) found that several enzymes which participate in the degradation of arabinoxylan increase
in activity during germination of intact barley seeds and their activity is enhanced by GA in isolated aleurone layers. These include endo-β-1,4-xylanase and two glycosidases: β-xylopyranosidase and α-arabinofuranosidase. With the help of scanning electron microscope, they demonstrated that the increase in the activities of these enzymes during germination is accompanied by a progressive and parallel degradation of the aleurone cell wall. Dashek and Chrispeels (1977) confirmed that GA stimulates the release of an enzyme (or enzymes) with xylanase activity in aleurone layers. In addition they demonstrated that GA induces the breakdown of barley aleurone cell walls. This was determined by measuring the total pentose residues in the wall and in the incubation medium. About two thirds of the total cell wall pentose was released into the medium during 60 hours of incubation with GA.

4) GA Control of mRNA Synthesis - Inhibitors of RNA synthesis like actinomycin D and 6-methylpurine block the hormone-controlled synthesis of hydrolases (Chrispeels and Varner, 1967b). But, as already mentioned, actinomycin D is effective only if added during the lag phase (Goodwin and Carr, 1972c) and at high concentrations (Chrispeels and Varner, 1967b), whereas
6-methylpurine is inhibitory even when added as late as 11 hours after the addition of GA (i.e. after the lag period). Besides inhibiting amylase synthesis, 6-methylpurine was also found to substantially reduce the incorporation of \(^{14}\)C-uridine by the aleurone layers, though hardly affecting incorporation of \(^{14}\)C-leucine (Chrispeels and Varner, 1967b). These observations are consistent with the hypothesis that certain kind of RNA, specific for amylase synthesis, might be produced in response to the hormone (Varner, 1964; Varner and Chandra, 1964). Jacobsen and Zwar (1974a, b) demonstrated that the incorporation of labelled ribonucleosides into poly(A) containing RNA was increased in the presence of GA in barley aleurone layers. This was confirmed by Ho and Varner (1974). They also reported that amylase synthesis was insensitive to cordycepin if supplied after 12 hours of GA treatment, suggesting that amylase is translated from a stable messenger RNA. Later, Higgins et al. (1976) found that the level of translatable mRNA for amylase increased in the hormone treated barley aleurone layers in parallel with the enhanced rate of enzyme synthesis. This was done by immunoprecipitation of the products of cell free translation system by amylase. They could not, however, distinguish the mechanism by which the level of translatable mRNA might be increased. It
could be by: (1) a decreased rate of mRNA degradation; (2) an enhancement of the translational capacity of mRNA for amylase or (3) synthesis of new mRNA molecules. Their results taken together with the fact that GA enhances the synthesis of poly (A)-containing RNA (Jacobsen and Zwar, 1974a, b; Ho and Varner, 1974), although not eliminating an effect on degradation, provide correlative evidence that GA stimulates the synthesis of mRNA for amylase.

5) **Cyclic AMP Mediation of GA Action** - Cyclic 3', 5'-adenosine monophosphate (cyclic AMP) is known to be involved in the modulation of hormonal effects in animals. Many hormones operate by catalyzing the conversion of ATP to cyclic AMP, which then acts as a second messenger to elicit a response in the target tissue (Sutherland, 1972). In plants, the existence of cyclic AMP is controversial (Amrhein, 1977). However, exogenously supplied cyclic AMP can mimic the actions of some phytohormones like auxins (Salomon and Mascarenhas, 1971, 1972), gibberellins (Duffus and Duffus, 1969; Galsky and Lippincott, 1969; Pollard, 1970; Nickells et al., 1971; Barle and Galsky, 1971) and cytokinin-like hormones (Wood et al., 1972). However, there is a controversy regarding the stimulation of amylase synthesis by cyclic AMP in barley.
aleurone cells (Duffus and Duffus, 1969; Galasky and Lippincott, 1969). Keates (1973) found no increase in the incorporation of $^{14}$C-adenosine into cyclic AMP by barley aleurone layers in response to GA. Further, there was no enhancement in the recovery of $^{14}$C with $^3$H-cyclic AMP barley aleurone, incubated with $^{14}$C-adenosine, in the presence of papaverine, theophylline or caffeine. These three are inhibitors of mammalian cyclic nucleotide phosphodiesterase enzyme and are used to increase endogenous levels of cyclic AMP, though there is as yet no evidence that these compounds inhibit plant phosphodiesterase (Lin and Varner, 1972). Keates (1973), on the basis of above observations and other evidences, suggested that cyclic AMP does not mediate the effect of GA in barley aleurone cells.

Kessler and Kaplan (1972) proposed that unlike in animal systems, cyclic AMP acts as the first messenger, namely the inducer of the hormone in barley aleurone. Their hypothesis, based on data on time kinetics, inhibitor effects and endogenous gibberellin levels is that cyclic purine nucleotides act at the genome level to induce gibberellin biosynthesis, which in turn stimulates the formation of amylase.
6) **Control through Phytochrome?** - Enzyme induction by phytochrome has been reported by several workers (Durst and Mohr, 1966; Attridge and Smith, 1967; Mohr, 1970). It has been suggested that phytochrome exerts its action through the mediation of hormones (Galston and Davies, 1969). In fact, phytochrome dependent increase in the level of extractable gibberellin-like substances has been detected following red light irradiation of etiolated barley or wheat leaves (Reid *et al.*, 1968; Loveys and Wareing, 1971), in homogenates of etiolated barley and wheat leaves (Reid *et al.*, 1972; Cooke and Saunders, 1975) and in suspensions of intact etioplasts from wheat (Cooke *et al.*, 1975). This increase is thought to be due to the stimulation of *de novo* GA synthesis and to the release of active GAs from a "bound" form (Reid and Clements, 1968; Loveys and Wareing, 1971). However, Drumm *et al.* (1971) found that GA could not substitute for phytochrome in amylase induction in mustard seedlings, while in embryoless half seeds of barley, which produce amylase in response to GA, there was no phytochrome-controlled production of amylase (Drumm *et al.*, 1971).

**Effect of Abscisic Acid on Aleurone Cells**

Abscisic acid (ABA), a plant hormone, has been shown
to counteract the effect of GA in many systems, including the response of barley aleurone layers to GA. All the GA effects, which occur prior to or during the synthesis and release of amylase are prevented or reversed by ABA. The GA-enhanced events inhibited by ABA also included: Phospholipid synthesis and rough endoplasmic reticulum proliferation (Evins and Varner, 1971; Koehler and Varner, 1973), the appearance of two transferases involved in lecithin biosynthesis (Johnson and Kende, 1971; Ben-Tal and Varner, 1974) synthesis of membrane bound polyribosomes (Evins and Varner, 1972) and poly(A) RNA synthesis (Ho and Varner, 1974; Jacobsen and Zwar, 1974).

The mode of action of ABA is not clear but it is known that neither does it inhibit GA synthesis (Thomas et al., 1965; Smith and Sadari, 1970), nor is it a competitive inhibitor of GA, as high concentrations of GA cannot completely overcome the inhibition by ABA (Chrispeels and Varner, 1966; Jacobsen, 1973). But a combination of GA and ethylene can completely overcome the ABA effect (Jacobsen, 1970). ABA does not alter the rate of respiration or phosphorylation and does not inhibit the incorporation of radioactive precursors into RNA and protein (Chrispeels and Varner, 1966).
Although the synthesis of amylase after 12 hours of GA treatment is no longer under transcriptional control (Ho and Varner, 1974), it has been observed that the inhibition of amylase formation by ABA at this stage is dependent on the continuous synthesis of short lived RNA (Ho and Varner, 1974). These workers found that inhibition of amylase synthesis which occurs even when ABA is added after 12 hours of GA treatment, can be overcome by the simultaneous addition of cordycepin (3'-deoxyadenosine), indicating that ABA does not affect the stability of amylase mRNA.

**Peroxidase**

**General Characteristics**

Peroxidases (donor: \( \text{H}_2\text{O}_2 \) oxidoreductase; E.C. 1.11.1.7) are widely distributed among higher plants and exist in multiple molecular forms (Scandalios, 1974). These are enzymes which in presence of hydrogen peroxide can oxidise a wide range of hydrogen donors such as phenolic substances, ascorbic acid and aliphatic and aromatic amines (Saunders et al., 1964), NADH and NADPH (Akazawa and Conn, 1958), oxalate and oxaloacetate (Keten and Mann, 1953), dihydroxyfumarate (Chance, 1952), cytochrome C (Chance, 1954) and many other compounds (Burris, 1960).
Although peroxidases have been studied extensively both in whole plants, in plant parts, and to some extent in plant cell cultures, their physiological functions remain unclear. Peroxidases have been implicated in and associated with the formation of lignin and lignin-like substances (Lipetz and Garro, 1965; Brown, 1966; Kepler et al., 1972), cell wall extensibility (Ridge and Osborne, 1970b), ion transport (DeJong, 1966), response to injury and disease resistance (Kosuge, 1969; Rousseaux et al., 1971; Severs et al., 1971; Matsuno and Uritani, 1972; Sako and Stahmann, 1972; Birecka et al., 1973; Birecka and Miller, 1974), fruit ripening (Frenkel, 1972; Haard, 1973), the biosynthesis of ethylene (Yang, 1968; Mapson and Wardale, 1972) and control of indole-3-acetic acid (IAA) level in plants (Galston et al., 1953). In fact, one of the more significant roles ascribed to peroxidase is the regulation of auxin metabolism and hence a possible involvement in many developmental phenomena (Galston and Davies, 1969).

Evidence offered in support of the idea that peroxidase can act as an IAA oxidase, is mainly that both types of enzyme activities remain together through various stages of purification during membrane ultrafiltration, gel filtration and ion exchange chromatography (Hoyle, 1972; Darbyshire, 1973). Further, many investigators...
have reported that dual catalysis is present in all isozymes of peroxidase (Kay et al., 1967; McCune, 1961; Macnicol, 1966, 1973; Retig and Rudich, 1972; Frenkel and Hess, 1974; Gove and Hoyle, 1975).

Localization

Peroxidase activity is found in most plant tissues, but generally the highest activity is detected in roots. Plant peroxidase has a broad subcellular distribution. It is found not only in the cytoplasm (Plesnicar et al., 1967), but also associated with cell walls (De Jong, 1967; Ridge and Osborne, 1970b; Meudt and Stecher, 1972; Lee, 1974), membranes (Mast, 1970; Parish, 1972; Lee 1974), nuclei and nucleolus (Bae, 1973), mitochondria (Ivanova et al., 1966; Penon et al., 1970; Parish, 1972; Darimont and Baxter, 1973; Lee 1974), ribosomes (Gasper 1970; Penon et al., 1970; Darimont and Gaspar, 1971; Darimont and Baxter, 1973; Lee, 1974) and chloroplasts (Ivanova et al., 1970; Jain et al., 1978). Majority of the peroxidase present in the cell is soluble (Plesnicar et al., 1967). About 10-20% of the total peroxidase activity is sedimentable (Parish, 1972, 1975). Most of the cell wall associated peroxidase can be freed by disruption of membranes by detergents or by sonication (Plesnicar et al.,
1967; Liu and Lamport, 1974; Parish, 1975). A small percentage (only about 1.4%) of the peroxidase in cell homogenates is consistently sedimentable and firmly bound to the cell wall (Parish, 1972, 1975; Liu and Lamport, 1974). Histochemically, peroxidase has been shown to be associated with cell walls (Parish, 1975; Oostrom et al., 1975) and with plant vacoules (Poux, 1969; Hall and Sexton, 1970; Parish, 1975).

**Regulation**

Peroxidase enzyme activity is influenced by an interaction of genetic, developmental, hormonal and environmental factors (Galston and Davies, 1969). The peroxidase activity is dependent on the genotype of the plant, genetically dwarf plants show very high peroxidase activity (Evans and Alldridge, 1965). GA, which promote growth in these dwarfs, evokes a decrease in the peroxidase activity (McCune and Galston, 1959) or a change in peroxidase isozyme pattern (McCune 1961; Galston and McCune 1961). Brewbaker and Hasegawa (1975) detected thirteen isozymes in maize with tissue specific variations. Certain isozymes were present or prominent only during specific phases in the life cycle, indicating specialized roles. They identified genetic loci governing polymorphism of nine of these isozymes, havin
26) **Hormones** - Regulation of peroxidase activity in plants by hormones has been well investigated. Most of the plant growth hormones are known to modify peroxidase activity. An interaction of various hormones in the regulation of the activity of peroxidase isozymes in lentil embryonic axis was shown by Gaspar *et al.* (1973).

a) Indoleacetic acid - Peroxidase has long been known to catalyze the oxidation of IAA, which in turn affects the enzyme activity in different ways. IAA is reported to repress certain isoperoxidases in dwarf pea stem sections (Ockerse *et al.*, 1966), tobacco and *Pelargonium* pith tissues (Galston *et al.*, 1968; Lavee and Galston, 1967, 1968), oat coleoptile sections (Stuber and Levings, 1969) and wheat coleoptile tissue (Whitmore, 1971). At the same time, promotion of other isoperoxidases by IAA was observed in tobacco and *Pelargonium* pith tissues (Galston *et al.*, 1968; Lavee and Galston, 1967, 1968). Treatment of lentil roots with high concentrations of IAA induced the synthesis of two anionic peroxidases but had no effect on the cationic ones (Penton *et al.*, 1970). An enhancement of peroxidase activity by IAA was also seen in the cell walls of
Nicotiana leaves and spicas (Meudt and Stecher, 1972).

Ethylene – A modification in peroxidase activity following ethylene application to excised as well as intact plants has been reported by many workers. Ethylene stimulated peroxidase activity in petiole, stem and leaf blade tissue of cotton plants (Herrero and Hall, 1960; Morgan and Fowler, 1972), in etiolated pea tissue (Ridge and Osborne, 1970a, b), in tobacco flower pedicels (Henry et al., 1974), in sweet potato root discs (Imazeki et al., 1968; Gahagan et al., 1968; Shannon et al., 1971) and in intact tobacco plants (Adams and Galston, 1974). Hardly any change in peroxidase activity was seen after ethylene treatment in excised root tissues from carrot, turnip and radish (Stahmann et al., 1966), in excised root tissue from a sweet potato variety (Gahagan et al., 1968) and in situ cotton cotyledonary blades (Morgan and Fowler, 1972). Decreases in peroxidase activity of sweet potato by ethylene have also been noted (Stahmann et al., 1966).

Cytokinins – Cytokinins are also known to influence peroxidase activity in diverse ways. Kinetin (6-furfurylaminopurine) was reported to increase the peroxidase activity in barley seedlings (Gaspar et al., 1969) and lentil roots (Gaspar and Xhaufflai, 1967).
but lowered the enzyme activity in dwarf beans (Jain et al., 1969). Galston et al. (1968) reported an interaction of kinetin and IAA in the modification of peroxidase activity in tobacco pith tissue, where the enhancement of enzyme activity was dependent on the concentration of IAA.

Gibberellic acid — Application of GA caused an increase in peroxidase activity in rice leaves (Hayashi et al., 1956) and in the oxidation of IAA in roots of a number of plants (Fang et al., 1960). McCune (1961) and Galston and McCune (1961) reported that the distribution of peroxidase isozymes in dwarf corn was modified by GA and the resulting isoperoxidase pattern resembled that found in normal corn plants. Similarly, Birecka and Galston (1970) found that GA increased the activity of a dominant isoperoxidase but decreased that of other isozymes in dwarf pea internodes. An enhancement of IAA oxidase activity following GA treatment was observed in corn leaves, and at the same time, the endogenous level of IAA in the GA treated plant was lower as compared to the control (Gaspar and Bouillenne-Walrand, 1966). Pilet (1957) reported a protection in vitro by GA on the degradation of IAA with carrot tissue extract, whereas Kögl and Elema (1960) showed that GA had no effect on the in vitro oxidation
rate of IAA with extracts from *Pisum*. GA increased peroxidase activity in tobacco pith cultures (Galston *et al.*., 1968) and in soybean seeds (Seth and Pillay, 1971). The intensity of peroxidase isozymes in aleurone layers of wheat grains was enhanced in the presence of GA after 24 hours of soaking, but decreased after 72 hours (Tao and Khan, 1975).

2) Light - Although a lot of information is available on the control of peroxidase activity by hormones, not enough literature is available on the effects of light on peroxidase activity. Graham *et al.* (1970) reported a light mediated increase in peroxidase activity of detached maize leaves. It has been shown in our laboratory (Jain *et al.*, 1978) that white light increased the level of peroxidase during greening of maize shoots. This peroxidase activity was associated with chloroplasts. Red light treatment enhanced the level of peroxidase in dwarf pea seedlings (*Anetis et al.*, 1975). Penel and Greppin (1975) reported red/far red reversible effect on the peroxidase activity in spinach. Acetylcholine could mimic the effect of red light on peroxidase activity. The modification of the enzyme activity by red and far red light was instantaneous (Penel and Greppin, 1973). Changes in the
isoperoxidase pattern were recorded, on transferring spinach plants from continuous light to short day conditions and from short day conditions to continuous light (Penel and Greppin, 1975). Sharma et al. (1976) demonstrated that phytochrome controls peroxidase activity in maize in a manner characteristic of photomodulation. Peroxidase activity associated with membranous vesicles in *Cucurbita pepo* was rapidly modified in the presence of phytochrome (Penel et al., 1976). Repression of some anionic peroxidases by light was observed in tobacco leaves (DeJong, 1973). On the other hand, Siegel and Galston (1967) found no qualitative differences in the isoperoxidase composition of pea grown in darkness and in light, though, there were some quantitative differences.