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Role of RNA and Protein Synthesis in Abscission

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Abstract. **The cell separation aspect of abscission is thought to involve the action of specific cell wall degrading enzymes. Enzymes represent synthesis which in turn is preceded by the synthesis of specific RNA molecules, and it follows that inhibition of either of these processes would also block abscission. Since abscission is a localized phenomenon usually involving 2 or 3 cell layers, RNA and protein synthesis should also be localized. Manipulations of plant material which either accelerate or retard abscission may be due to the regulation of RNA and protein synthesis. This paper is a review of literature concerned with these and related questions.**

For the sake of clarity the terms used in this discussion of abscission are defined as follows. Except for minor shifts in emphasis, the definitions described below were originally proposed by Gawadi and Avery (26).

Abscission. The process of shedding or separating plant parts including leaves, stems, fruits, and flowers.

Separation Layer. The layer of cells involved in the separation of the plant part. Separation is usually achieved by the localized breakdown of intercellular substances.

Cell Division Layer. The layer of cells across an abscission zone that result from cell division. In the species in which it occurs has also been called the abscission or absciss layer.

Protective Layer. The proximal tissue that remains after separation and includes the remaining cells of the separation layer and the cell division layer in those species that possess it. In the case of leaves, this would also be known as the leaf scar.

Abscission Zone. The region at the base of the abscising part through which separation occurs. This zone includes the cell division layer and separation layer.

In this review, the role of RNA and protein synthesis in abscission is discussed in terms of a model system, the idea being to construct a hypothesis and examine the available data to see how well they fit the model.

Von Mohl, in 1860, showed that 2 sets of phenomena are brought into operation in leaf fall. First, the formation of a separation layer in which abscission always occurs, and second the separation of cells from each other with their walls still intact. The xylem cells not included in this separation layer are finally ruptured mechanically. Early theories, of interest only from a historical point of view, have been summarized by Tison (52) and Lee (37).

Auxin-Ethylene Balance (28). IAA or other auxins are instrumental in preventing abscission, and ethylene is capable of inducing abscission. However, the absolute amount of auxin or ethylene *per se* is not the important factor that prevents abscission but rather the amount of auxin in relation to ethylene or relative balance between the 2 factors. One of the effects of artifically supplied ethylene is to inactivate or lower the auxin content of leaves or other organs. With the removal of hormonal inhibition the activity of both hydrolytic and respiratory enzymes becomes accelerated.

Auxin-Ethylene (11). Any cells that are able to separate always tend to do so, but the process is inhibited by the continual supply of growth substances. When this supply falls below some critical level, abscission occurs. The breakdown process depends upon an enzyme that controls pectic breakdown; this system is inhibited by growth substances applied to the distal side while the activator of the system is produced in the general metabolism of the cell, and may be in the nature of ethylene.

Auxin-Gradient (8). Auxin is the principal endogenous regulator of abscission; its gradient across the abscission zone regulates onset and rate of abscission. Abscission does not occur with an auxin gradient characteristic of healthy mature tissues: with high auxin distal to the abscission zone and low auxin proximal to the abscission zone. Abscission occurs after a fall in the ratio of distal to proximal auxin. Abscission is accelerated when the gradient is reversed.

Auxin-Concentration (25). As in the auxin-gradient hypothesis, the principal endogenous regulator of abscission is assumed to be auxin. In distinction to the gradient hypothesis, high concentrations of auxin are thought to inhibit abscission while low concentrations promote abscission. This situation is somewhat analogous to the observation that high concentrations of auxin inhibit growth while low concentrations promote growth.

Auxin-Auxin Balance (32). A given leaf normally has its abscission time controlled by the auxin moving into the petiole from its own leaf blade. Whenever the flow of auxin from the leaf blade

decreases enough (whether from old age or by shading or by deblading), then the weaker abscission-speeding effect of auxin coming from the younger leaves can take effect and the petiole abscises.

Auxin-Senescence Factor (42). Leaf abscission may be controlled not only by endogenous auxins but also by some substance or substances that are produced as the leaf matures, and that may reach a maximum at senescence.

Membrane Integrity (46). The abscission retarding action of auxin may be through its effect on the maintenance of membrane integrity. During senescence, auxin levels drop with a resulting loss of membrane integrity followed by cell separation.

Methionine-Auxin (59). This hypothesis advanced the idea that methionine played a role in abscission because of its role as a methyl donor and that methylation of the carboxyl groups of adjacent pectin molecules may be involved in the splitting of calcium bridges leading to abscission.

Auxin-Gibberellin-Abscission Accelerating Hormone (17). These workers proposed that these 3 hormones interacted in a common mechanism that regulates the process of abscission.

Two Stage Theory (45). Leaf abscission can be divided into 2 stages. The first of these is primarily retarded by auxin; a second later stage is accelerated by auxin.

Endogenous Abscission-Accelerating Substances (9). Abscisic acid (abscisin II, dormin) was thought to be an abscission regulating hormone based on the fact that this compound has been isolated from a large number of plant parts, that the amounts of this hormone increase during periods of aging, and that application of abscisic acid to explants accelerates abscission.

Localized Cellular Senescence (38). The regulation of abscission is composed of 2 stages: first, a metabolic difference develops between cells on the 2 sides of the future separation zone, which establishes the proximal side as having a mobilization advantage over the distal side. After the establishment of this difference, the second stage may follow, including a mobilization of materials out of the distal tissue, a repression of synthetic activities there, and a transition toward the degradation of cell wall components. The actions of the various abscission accelerators and other plant growth substances can be accommodated as preferential effects on these 2 stages of abscission development. The promotive effects of auxin are a consequence of actions on the second stage of abscission development; the promotive effects of ethylene are likewise actions on the second stages of abscission.

The various lines of evidence lead to the concept that abscission may be a case of the precise regulation of cellular senescence by the plant. The regulatory aspect applies not only to the timing of the senescence event, but also to the precise location of the line of cells through which separation will occur.

Aging-Ethylene. For the purposes of this review the following model which is called the aging-ethylene hypothesis of abscission is used as a target for critical experiments aimed at elucidating the mechanism of the abscission process. One of the limitations of this model is that most of the experimental support comes from work with explants and most of these explants consist of 1 species, *Phaseolus vulgaris* L.

Because explants are isolated sections, there are limitations associated with extrapolating results obtained with them to whole plant physiology. However, the advantages of isolating the cells involved in abscission from the rest of the plant and having a large, uniform, and constant supply of experimental material are practical considerations that cannot be ignored.

Abscission is the enzymatic separation of cells involved in maintaining the structural integrity of the explant. The hormone responsible for the induction of these cell wall degrading enzymes is ethylene which normally is continuously produced by the explant. The ability of the hormone to act depends on the sensitivity of the separation layer cells to the gas. These cells remain insensitive to endogenous ethylene levels as long as a supply of juvenility factors or aging retardants (for example auxin and cytokinins) is available from adjacent distal cells. Removal of these juvenility factors by excision of distal leaf cells sets the aging process into motion and the separation layer cells become increasingly sensitive to the ethylene that is already being continuously produced. It follows that once these separation-layer cells pass the threshold from insensitivity to sensitivity to ethylene, any increase in the levels of the gas would result in accelerated abscission. Increased sensitivity of the separation-layer cells to ethylene is only one of the processes set into motion as a result of aging. Other processes include the loss of metabolites such as RNA, chlorophyll, and protein from the distal cells. According to the hypothesis advanced above, the primary role of auxin in abscission is to retard aging. It should be pointed out that this hormone has additional effects that complicate comprehension of its role. Auxin has been shown to stimulate respiration (44), maintain membrane integrity (14, 44, 46), retard formation of the cell division layer (15, 26, 41), and accelerate ethylene production (1, 3, 40). The acceleration of ethylene evolution by other compounds and manipulations (3, 20, 48), tends to complicate proposed roles for these factors in abscission.

According to the aging-ethylene hypothesis, the primary role of ethylene is to accelerate the formation of enzymes responsible for cell separation. However, ethylene, like auxin, has a number of different effects on explants that may or may not contribute directly to abscission. For example, ethylene has been reported to cause petiole swelling (38), stimulate the breakdown of proteins (6, 38, 48), increase the frequency of tyloses in xylem and decrease the callose deposits in phloem (38), accelerate the rate

of IAA inactivation (50), and accelerate the loss of pectin methyl esterase in the abscission zone (42).

The primary purpose in advancing the above hypothesis is to offer a framework for a proposed role for ethylene in abscission, namely that ethylene mediates or initiates the RNA and protein synthesis necessary for cell separation. Before this point is discussed, however, it would be profitable to examine the experimental evidence used to establish this framework. No attempt will be made here to establish the priorities or to cite all the evidence for some of the points but observations will be selected that demonstrate the phenomenon clearly. A thorough discussion of these points have been made elsewhere in this symposium.

The role of juvenility factors is an essential aspect of abscission. The juvenility factor that has been the prime object for study is the growth hormone auxin, although other chemicals such as the cytokinins may also be important. Decrease in the auxin levels available to the separation layer by aging (56) or removal of the leaf blade tissue (56) invariably set abscission into motion. When the leaf blade is replaced by auxin, abscission is inhibited (56). After the supply of juvenility factors has been removed the aging processes are set into motion. The longer the delay before adding auxin after excision the less effective the auxin becomes in inhibiting abscission (45). The promotion of abscission observed after a delayed application of auxin is probably due to the accelerated ethylene production invariably associated with tissue exposed to auxin (1). As the tissue ages, it becomes increasingly sensitive to ethylene. This phenomenon is demonstrated in figure 1. The loss in effectiveness after 2 days is due to a loss in biosynthetic capacity as the tissue ages. As shown by a loss in respiratory activity the physiology of an explant is a downhill affair once it has been excised from a plant. This decrease in respiration is interrupted by a climacteric-like increase with age (12, 16).

Recent rapid advances in knowledge of the role of abscisic acid in abscission suggest that this interpretation may be too simple. Increased levels of abscisic acid are associated with tissue undergoing dormancy (23) and abscission (9). However, direct effects of abscisic acid in accelerating abscission of explants may be due to effects on ethylene production (3).

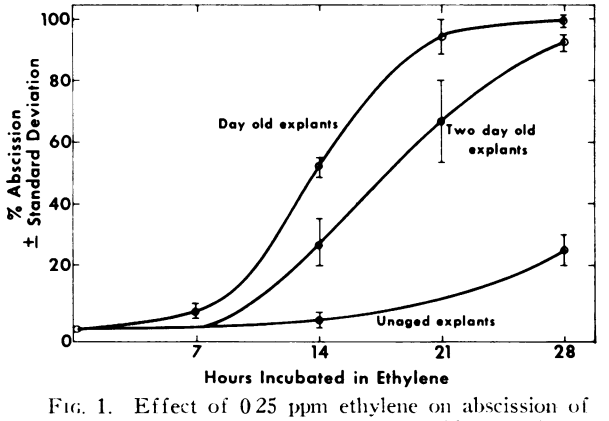


FIG. 1. Effect of 0.25 ppm ethylene on abscission of cotton explants of different ages. From Abeles (3).

The anatomical aspects of the cell separation process have been studied by many workers (see Webster this symposium). Invariably they reported that cell separation was associated with breakdown of the middle lamella and other cell wall material. However, there is no agreement as to the exact nature of the wall fractions involved. A good deal of the problem is due to the lack of precise knowledge of cell wall composition and recent knowledge (10) of wall structure is quite different from the earlier concept of a pectic middle lamella surrounded by a cellulosic primary wall. Except for transitory interest in the idea that wall breakdown was due to acid hydrolysis (24, 47, 57), the current assumption is that cell wall breakdown is due to the action of enzymes.

Even though the identity of these enzymes is only now coming to light, we can assume that the proteins are either synthesized some time prior to abscission or go to work immediately after they have been formed. In the case of preformed enzymes, we have a number of alternatives as to how these proteins come into action; namely, release from lysosomal structures, activation by addition of a prosthetic group or removal of a peptide as in the conversion of trypsinogen into trypsin, or the removal of an inhibitor. Since abscission normally takes place within 2 or 3 layers of cells, we assume for this discussion that these cell-wall degrading enzymes are localized at their site of action and not transported there from some other part of the plant.

The following sequence of events is visualized. Ethylene triggers the synthesis of specific m-RNA and other essential RNA fractions required for protein synthesis such as s-RNA and r-RNA. The assumption that ethylene is the trigger, as opposed to amino acids, auxin, gibberellin, or abscisic acid, is made primarily because ethylene is the common denominator in the action of these and other molecules known to accelerate abscission (3), and no intermediate substance or process has been recognized between ethylene and the initiation of RNA synthesis.

Because of the relative ease of doing inhibitor experiments, the demonstration that actinomycin blocks the action of hormones is a favorite one for investigators exploring the hypothesis that RNA synthesis is essential in the action of hormones. While the action of actinomycin is reasonably specific, that is, it binds with the DNA blocking the transcription process, it has some side effects that can result in ambiguous interpretation. The side effect that concerns us most in the study of abscission is that, by some means, actinomycin increases ethylene production from plant material (5). One

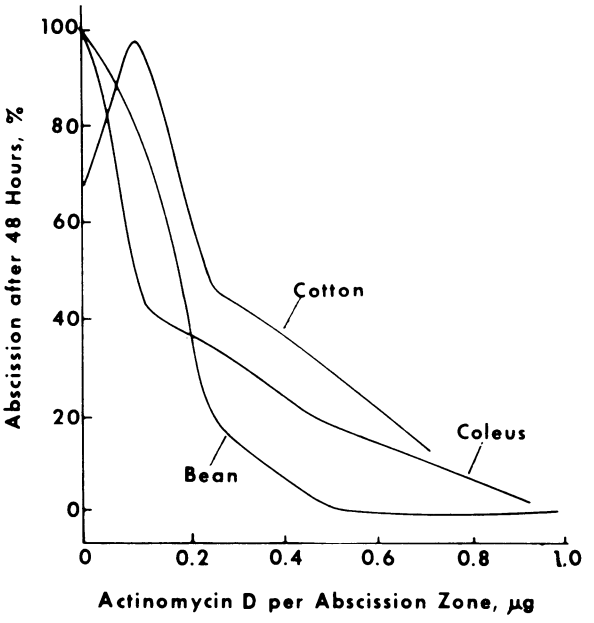


FIG. 2. Inhibition of cotton, *Coleus*, and bean explant abscission by actinomycin. From Abeles and Holm (5).

possible explanation of actinomycin-induced ethylene production is a traumatic effect. Mechanical wounding (58) and X-rays (39) have been shown to accelerate ethylene production from plant material.

Figure 2 presents data showing the inhibition of abscission by actinomycin. One of the problems associated with the use of inhibitors in explants is that the freely diffusable ethylene induced by the inhibitor treatment may mask any effect of the inhibitor if the site of application is removed from the site of action (5). To demonstrate the inhibition of abscission by actinomycin we found that the inhibitor had to be injected next to the separation layer. Application of the compound in agar drops to the cut ends of the explant which represents a 5 mm diffusion barrier, failed to block abscission and in fact accelerated abscission. The combined effects of diffusion barrier, and accelerated ethylene production, may explain the failure of others (35, 53) to observe an inhibition of abscission by RNA and protein synthesis inhibitors.

Another way to demonstrate a requirement for RNA and protein synthesis is shown in figure 3. The explants used in this experiment had been stored under conditions to reduce ethylene levels for 22 hours during which they developed their sensitivity to the gas. Ethylene was then added to the gas phase around the explants, actinomycin and cycloheximide injected into the separation layer, of some of the explants initially, and then into other sets of explants every 2 hours. When abscission was measured 9 hours after the addition of ethylene,

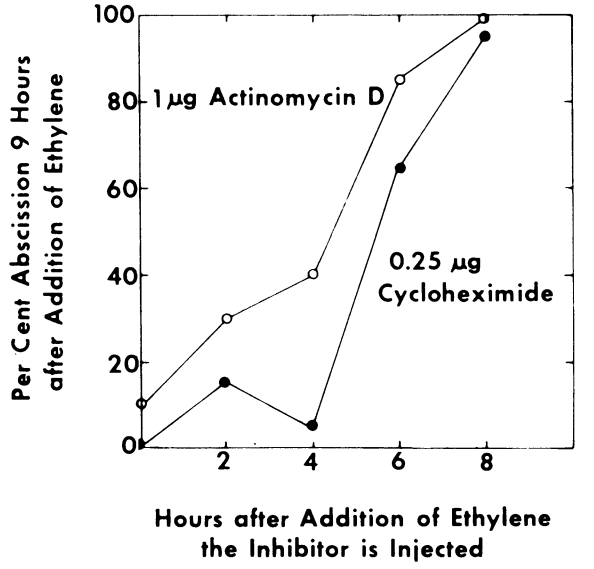


FIG. 3. Effect of actinomycin and cycloheximide on abscission of senescent explants. From Abeles and Holm (4).

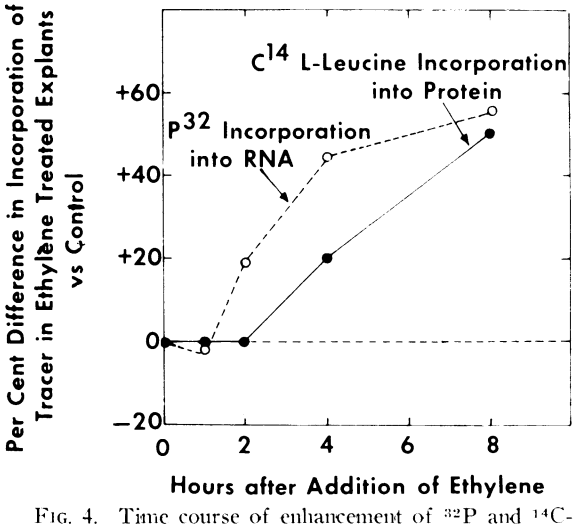


FIG. 4. Time course of enhancement of ^{32}P and ^{14}C-leucine incorporation into RNA and proteins of ethylene-treated *vs.* control bean explants. From Abeles and Holm (4).

cycloheximide was found to inhibit abscission longer than actinomycin. These findings agree with contemporary schemes of protein synthesis in that RNA synthesis is a requirement for protein synthesis. With increasing time after the addition of ethylene, the requisite RNA molecules that are being synthesized accumulate, and the inhibition by actinomycin becomes less effective. It is only after sufficient RNA molecules have been synthesized that the protein synthesis required for cell separation starts, and this is reflected by the longer time that cycloheximide blocks abscission. As these abscission-dependent proteins accumulate, the cycloheximide treatments become less effective.

Table I. *Effect of IAA, Cytokinin SD 8339, and Ethylene on ^{32}P Incorporation Into RNA*[1]

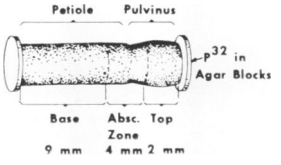
Explants with 10 mm petioles were placed pulvinal end down in either plain agar or agar containing 50 μM IAA or 500 μM SD 8339 for 22 hours. The explants were then placed petiole end down in plain agar and 1.5- by 4-mm agar cylinders containing 2 μc ^{32}P were placed on the pulvinus for 8 hours. Where indicated, ethylene level was 2 ppm. Data shown are the means plus or minus the standard deviation.

Treatment	μg RNA per 10 explants	CPM per μg RNA	Abscission after 30 hr
			%
Control	149 ± 3	2864 ± 7	40
Ethylene	156 ± 5	4156 ± 68	100
IAA	173 ± 5	4611 ± 102	0
IAA + ethylene	151 ± 6	3984 ± 97	10
SD 8339	200 ± 7	4257 ± 81	0
SD 8339 + ethylene	175 ± 4	2843 ± 26	0
			0

[1] From Abeles and Holm (5).

The data on inhibitors and protein synthesis described above only partially support the idea that ethylene is capable of acting as an effector in the abscission process. A more direct approach is to show that ethylene is capable of stimulating RNA synthesis. Figure 4 shows that ethylene was able to increase ^{32}P incorporation into RNA 1 hour after aged explants were treated with 4 ppm ethylene: the enhancement of protein synthesis occurred after a 2 hour lag. The ability of ethylene to increase RNA synthesis depends on an aging process. When explants are treated with IAA or cytokinins to block aging, ethylene has little or no effect on RNA synthesis (table I). Scott (48) also found that ethylene stimulated ^{32}P incorporation into bean explant RNA. However, when abscission was accelerated by senescin which is thought to act by increasing ethylene production, then ^{32}P uptake into RNA was inhibited.

ETHYLENE STIMULATION OF RNA SYNTHESIS IN THE ABSCISSION LAYER OF BEAN EXPLANTS



SECTION	µg RNA CONTROL	µg RNA ETHYLENE	CPM/mg RNA CONTROL	CPM/mg RNA ETHYLENE	% CHANGE
TOP	116	89	592,000	659,000	+11.4
ABSCISSION ZONE	203	204	25,300	39,400	+55.8
BASE	234	221	1,210,000	1,080,000	−11.2

Abscission after 6 hours; controls 24%, 2PPM ethylene 91%.

FIG. 5. Ethylene stimulation of RNA synthesis in the separation layer of bean explants. Bean explants were aged for 24 hours before being placed on their sides, and agar blocks containing 2.5 μc ^{32}P were placed on each end. After 6 hours in the presence or absence of 2 ppm ethylene, agar blocks were removed and the tissue was cut into 3 sections. Abscission after 30 hours; control 24 % ethylene 91 %. From Abeles and Holm (5).

The experiment shown in figure 5 was performed to see if the incorporation of ^{32}P into RNA was a generalized phenomenon or limited to the separation layer. The data indicated that the enhancement occurred mainly in the separation layer and to a lesser extent in the pulvinus.

Radioactive RNA from explants was separated on methylated albumin kieselguhr (MAK) to study rates of incorporation into various fractions or classes of RNA. A comparison of the distribution of radioactivity between control and ethylene-treated explants is shown in figure 6 and table II. Enhancement of ^{32}P incorporation was greatest in the r-RNA fraction. When actinomycin was used to block

Table II. *Effect of Ethylene and Inhibitors on ^{32}P Incorporated into Nucleic Acid Fractionated on MAK Columns*[1]

Explants aged 18 hours at 400 ft-c and 25°. Top 2 mm of pulvinus were cut off and the inhibitors added, 5-FU by agar droplet, actinomycin D by injection. At 22 hours after excision (*i.e.*, 4 hr after inhibitor treatment), ^{32}P agar blocks (8 μc/agar block) were placed on the pulvinal surface and incubated with or without 2 ppm ethylene for 3 to 6 hours. The nucleic acids were extracted by the phenol method and separated on MAK columns with a linear gradient. Abscission: 3 hour control - 0 %, 3 hour ethylene - 17 %, 6 hour control - 17 %, 6 hour ethylene - 80 %, 6 hour ethylene + 5-FU - 80 %, and 6 hour ethylene + actinomycin D - 10 %.

MAK column fraction	C_2H_4 Increased or decreased over control 3 hr	6 hr	Inhibition of C_2H_4 stimulation 6 hr C_2H_4 + 10 mM 5-FU	6 hr C_2H_4 + 1 µg act D
	%	%	%	%
I (s-RNA)	−2	58	49	87
II (DNA-RNA)	62	62	46	95
III (r-RNA)	72	84	53	94
IV (m-RNA)	45	75	35	83
Total Nucleic acids	42	77	51	94

[1] From Holm and Abeles (29).

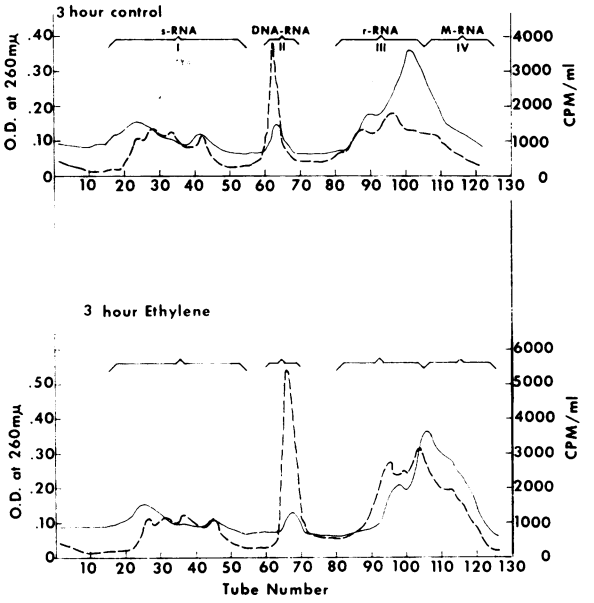


FIG. 6. Distribution of radioactivity in various nucleic acid fractions after 3 hours incubation of aged explants in air or 2 ppm ethylene. The solid line represents the OD at 260 nm and the dashed line represents CPM. From Addicott, Carns, Lyon, Smith, and McMeans (9).

abscission, then the synthesis of all classes of RNA was blocked. On the other hand, 5-fluorouracil added 18 hours after excision inhibited the ethylene enhancement of nucleic acid synthesis 50 % yet did not alter the rate of abscission. The greater inhibition of the synthesis of s-RNA and r-RNA with 5-fluorouracil compared with that of m-RNA is analogous to other plant systems where 5-fluorouracil has been tested (18, 33, 34). This suggests that not all fractions of RNA have to be synthesized for abscission, but that newly synthesized m-RNA is essential.

The enhancement in the DNA-RNA peak was not due to the synthesis of DNA since ethylene had no effect on ^{14}C-thymidine incorporation into DNA (29).

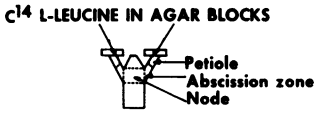
Holm and Abeles (29) further characterized the nucleic acids produced under ethylene stimulation by differential extraction techniques that have been shown in other plant tissues to separate the m-RNA, DNA, and DNA-associated-RNA from the bulk of the tissue RNA (19, 31). This technique involves homogenizing the tissue with buffered phenol and bentonite, centrifuging, and drawing off the aqueous phase (tris-extracted nucleic acid). The interphase and phenol residue are then re-extracted with buffer and sodium lauryl sulphate (called SLS-extracted nucleic acid). The SLS-extracted nucleic acids contained only 24 % of the explant nucleic acids but contained the ethylene stimulated fraction. Separation of the SLS-fraction on MAK columns indicated that the major ethylene enhancement occurred in the messenger and ribosomal regions. These findings agree with other cases in which hormones are known to stimulate the production of enzymes. The general observation is that the hormone responsible for the appearance of the protein also promotes RNA synthesis which generally precedes polypeptide formation. All classes of RNA are synthesized as opposed to the production of only m-RNA (36, 51).

If protein synthesis is required for the cell separation process then it should be localized in the cell separation layer. To test this idea, explant tissue was exposed to ^{14}C-leucine in the presence or absence of ethylene. Abscission occurred in both cases but the explants treated with ethylene abscised more rapidly. Since the assumption was made that protein synthesis was being accelerated by ethylene, it

CPM/μg Protein ± S.D. After 10 Hours

Section No.	1	2	3	4
Treatment				
Control	110±2	9.29±0.19	5.08±0.04	75.0±0.2
4nl Ethylene/ml Gas Phase	112±3	14.8 ±0.1	7.40±0.07	71.3±0.6
% Difference Ethylene vs Control	+1.8	+59	+45	−4.9

FIG. 7. Ethylene stimulation of protein synthesis in the separation layer of bean explants. Agar blocks containing ^{14}C-leucine were placed at the pulvinal and petiole end of bean explants placed on their sides. The explants were subjected to 1 ppm ethylene and control atmospheres for 24 hours. The ethylene treated explants had abscised 92 % after the 24 hours while the control explants showed no abscission. The data represent the mean ± standard deviation. From Abeles and Holm (5).



CPM/μg PROTEIN ± S.D. After 24 Hours

	Petiole	ABS. Zone	Node
Control	1093±42	4.32±0.6	0.75±0.03
1nl Ethylene/ml Gas Phase	911±21	7.98±0.2	0.81±0.06
%Difference Ethylene vs Control	−7.5	+84.5	+8.6

FIG. 8. Ethylene stimulation of protein synthesis in the separation layer of cotton explants. Agar blocks containing ^{14}C-leucine were placed on the petiolar stumps of cotton explants that were subjected to 1 ppm ethylene or control atmospheres for 24 hours. The different sections cut from the cotton explants are as indicated above. The data represent the mean ± standard deviation From Abeles and Holm (5).

should have been possible to observe an enhancement in polypeptide biogenesis in the separation layer of the ethylene-treated explants but not in the surrounding tissues that presumably are not involved in the cell separation process. Such experiments were performed (5) and the results supporting the above hypothesis are shown in figures 7 and 8.

Another demonstration that ethylene action is specifically directed toward ethylene-sensitive tissue is to treat stage I explants with ethylene and to compare the results with stage II explants. If ethylene has no effect on abscission of stage I explants (1, 60) it should also have no effect on protein synthesis. However, in the case of stage II explants ethylene should be able to stimulate protein synthesis. Figure 9 presents data indicating that this view is correct, an ethylene-dependent increase of protein synthesis was absent in stage I explants but readily observable in stage II explants.

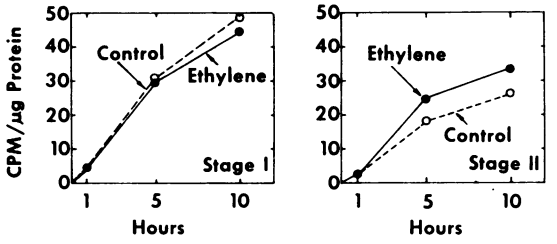


FIG. 9. Effect of ethylene on ^{14}C-leucine incorporation into abscission zone proteins of stage I and stage II explants. From Abeles and Holm (4).

Scott (48) performed what appear to be similar experiments but obtained the opposite results. He found that ethylene promoted ^{14}C-leucine incorporation into separation-layer protein of stage I explants (the treatment having no effect on abscission) but failed to promote ^{14}C-leucine incorporation in the protein of stage II explants (in this case, ethylene promoted abscission). However, when explants were treated with senescin (an abscission-accelerating substance from apples), which was found to accelerate ethylene production from the explants, then both abscission and ^{14}C-leucine incorporation were accelerated. The major difference between these experiments and those reported earlier by Abeles and Holm (4) was the lack of ethylene in the gas phase during the time ^{14}C-leucine incorporation was being measured. It is possible that ethylene must be present in the gas phase during the time of ^{14}C-leucine incorporation was measured. This may explain why ^{14}C-leucine incorporation in the presence of senescin was accelerated since ethylene production from senescin-treated explants was higher than controls.

Valdovinos and Ernest (53) also reported experiments designed to test the idea that protein synthesis is a requirement for abscission. They found that *Coleus* abscission zones isolated from petioles treated with chloramphenicol and D-aspartic acid or stem tissue treated with gibberellic acid and IAA failed to incorporate ^{14}C-leucine faster than controls. The experiments using petiolar applications of chloramphenicol are difficult to interpret because no data from untreated controls were shown. Earlier work has reported data in favor of the view that most if not all substances accelerate abscission because they increase ethylene production (3). If this view is correct then the separation-layer cells in the experiments of Valdovinos and Ernest are really responding to the enhanced ethylene production in the surrounding tissue treated with various abscission accelerators. Excision of the separation layer removes the cells from ethylene, and subsequent incorporation experiments are taking place with tissue that has either already responded to ethylene or is incapable of giving enhanced incorporation data in the absence of ethylene. The abscission-accelerating compounds used in these experiments were applied 2 cm away from the separation layer in the case of petiolar application experiments. It was not shown that chloramphenicol or D-aspartic acid reached the separation layer in sufficient time or in sufficient concentrations to have an effect on protein synthesis in the cell separation process.

Carbon dioxide is known to act as a competitive inhibitor of ethylene and this fact has been used to demonstrate an ethylene requirement in various physiological processes. Since CO_2 inhibits abscission, it should also inhibit the acceleration of protein synthesis by ethylene if the acceleration of protein synthesis by ethylene if the assumption that CO_2 occupies the same site in the cell is valid. The data in table III support this idea since CO_2 inhibited both control and ethylene-induced abscission as well as protein synthesis.

Up to this point we have established that ethylene is capable of increasing RNA and protein synthesis and that based on inhibitor experiments the formation of these biopolymers is essential for the cell separation process. What remains to be demonstrated is the nature of the cell-wall degrading enzymes.

Table III. *Inhibition of Explant Abscission and Protein Synthesis by Carbon Dioxide*[1]

Explants were aged 22 hours at 25° before L-leucine-^{14}C in agar blocks was applied to the pulvinal stump, incubated in the indicated atmosphere for 5 hours.

Treatment	Abscission	CPM/mg protein ± SD
	%	
Control	70	21,100 ± 400
15 % CO_2	0	19,600 ± 700
0.75 ppm Ethylene	100	27,200 ± 100
15 % CO_2 + 0.75 ppm ethylene	80	21,600 ± 300

[1] From Abeles and Holm (4).

Table IV. *Cellulase Activity in Segments Cut From Control Explants and Explants Treated with 2,4,5-Trichlorophenoxyacetic Acid for 24 Hours*[1]

Segment	Cellulase activity[2] Control	2,4,5 T
Pulvinus	19.35 ± 0.05	8.35 ± 4.05
Separation layer	46.55 ± 2.50	6.65 ± 0.15
Petiole	20.10 ± 4.05	6.90 ± 0.20

[1] From Horton and Osborne (30).
[2] Activity after 12 hr incubation, % change in flowtime.

Assuming that the above hypothesis is correct, the cell separating enzymes should have the following characteristics. They should be limited to the cell separation layer, they should appear only after an induction period, ethylene should accelerate their appearance, and inhibitors of aging (auxin, cytokinins), ethylene action (CO_2), and RNA synthesis (actinomycin) and protein synthesis (cycloheximide) should block their appearance. Since both light microscopic and electron microscopic examination (13, 14) have shown that cell wall breakdown occurs during the cell separation process we assume that the enzyme would be capable of altering or breaking down various cell wall components, pectic substances, hemicelluloses, and celluloses.

One of the enzymes that best fits this description is cellulase. Horton and Osborne (30) reported that cellulase was localized in the separation layer (see table IV) and that ethylene increased cellulase activity while 2,4,5-trichlorophenoxyacetic acid decreased it (table V). Increasing cellulase activity has also been shown in ripening tomatoes (22, 27). This observation may have some relevance to the abscission process since ripening and abscission have a number of features in common including sensitivity to ethylene, dependence on aging or maturation of cells, and the occurrence of a climacteric.

Other enzymes have been examined for their role in abscission. Pectin methyl esterase and polygalacturonase (44) are present in significant amounts in the separation layer but are also found in the surrounding tissue and their activity drops rather than increases during abscission. (However, data in favor of a role for pectinase in abscission was discussed by Morre during this symposium).

Table V. *Cellulase Activity in Abscission Zones 24 Hours After Treatment With Abscission-Retarding Concentrations of 2,4,5-Trichlorophenoxyacetic Acid and Abscission Accelerating Concentrations of Ethylene*[1]

Treatment	Cellulase activity[2]
Control	38.8 ± 3.9
2,4,5-T	8.8 ± 7.1
Ethylene	62.6 ± 1.8

[1] From Horton and Osborne (30).
[2] Activity after 22 hr of incubation, % change in flowtime.

Abscission is regulated by a number of hormones, their ultimate effect depending upon the time they are applied to the abscission zone explant. The auxins and also perhaps cytokinins and abscisic acid are involved in the initial steps of the process by regulating aging. It is important to state that aging is not the same as senescence. Aging simply refers to the fact that physiological processes start, change, or stop during the passage of time. Senescence, to this reviewer at least, refers to those processes that inexorably lead to cell death. An explant abscisses before it dies. Both distal and proximal cells respire and synthesize protein after cell separation. The distal cells and some of the surface proximal cells do eventually die, but this clearly takes place after cell separation. Processes that start as a result of aging include the loss of chlorophyll, RNA, and protein from distal tissue (49). The loss of RNA was found to be correlated with the induction of ribonuclease (7). No evidence for the release of RNAase from lysosomal-like structures was obtained. However, when explants were treated with actinomycin or cycloheximide, the loss of chlorophyll, RNA and protein was inhibited. These results suggest that the degradative enzymes associated with senescence may come into play as a result of RNA and protein synthesis.

The auxins have a second effect on explants in that they invariably increase ethylene production. This effect can also be blocked by inhibitors of RNA and protein synthesis (2).

The hormonal control of RNA and protein synthesis and the role of these processes in physiological phenomena are a relatively recent area of botanical research. However, in this reviewer's opinion, the available data suggest that these phenomena are an essential part of abscission. Other examples of similar phenomena in other areas include the induction of α-amylase by gibberellic acid (54) indoleacetylaspartate synthetase (55) and isocitrate lyase (21) by IAA.

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