5 March 1971, Volume 171, Number 3974 SCIENCE

Cytokinins: Permissive Role in Seed Germination

With other plant hormones, cytokinins regulate germination and dormancy by a novel mechanism.

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Virtually all processes connected with growth, development, and metabolism in plants and animals are governed in one way or another by hormones. Recent studies make it increasingly evident that hormones, including phytohormones, may act in concert in determining a physiological response. In many studies physiological responses, such as tropism, flowering, lateral bud growth, stem elongation, cell division, and growth of cells and organs in culture have been tested by more than one hormone. Information from such studies has enabled plant physiologists to control and predict responses of a tissue, an organ, or an intact seedling (*1*, *2*). However, until recently, relatively few studies on hormonal actions or interactions in the area of seed dormancy and germination have been attempted. The reason for this may have been a preoccupation of plant physiologists with studies on auxins, gibberellins, and cytokinins on plant systems seemingly more attractive than seeds. It may, on the other hand, have been due to a lack of a real incentive. Two recent discoveries have contributed significantly to the resurgence of interest in the area of hormonal actions and interactions in seed processes. First is the demonstration that gibberellin controls the production of hydrolytic enzymes in barley grain (*3*, *4*), and the other, the finding that abscisic acid (*5*), a natural inhibitor, can act as a dormancy-inducing agent in buds of woody plants (*6*). These discoveries give insights into the mechanisms by which hormones might control seed germination and dormancy. The regulation of seed dormancy and germination by the cytokinins, however, remains largely unknown. This can be attributed to the fact that cytokinins, by themselves, do not have any profound effect on seed germination (*7*).

This article deals with studies on hormonal interactions, primarily those involving cytokinins (*2*, *8*), in the regulation of dormancy and germination in seeds. These studies have led to the discovery of a novel mechanism of cytokinin action in the regulation of germination. Molecular effects of cytokinin will be examined with a hope of providing further insight into the modes of action of these hormones in germinative processes.

Cytokinin-Inhibitor Antagonism

Our initial experiments on germination were designed around the notion that hormones might oppose (or modify) each other's effects on germination. It is well known from a number of in vitro studies with plant systems that certain hormones do, in fact, oppose each other's effects. Thus, for example, in lateral bud development and elongation of stem and hypocotyl segments, cytokinins oppose auxin effects (*9*). Germination inhibitors are known to occur in seeds (*10*). A buildup of growth promoters (*11*) and a decrease in the amount of inhibitors (*12*) occurs in seeds and buds during emergence from dormancy. These studies suggest that germination of a seed could perhaps be inhibited and that the inhibition could perhaps be reversed by applying "physiological" concentrations of exogenous inhibitors and promoters. Indoleacetic acid (IAA), one of the promoting hormones, has little or no promotive effect on germination. It inhibits germination at high concentrations (*13*). Indoleacetic acid and its derivatives completely inhibit the germination of Grand Rapids lettuce seeds at relatively low concentrations (*14*). Kinetin (a cytokinin) sensitizes the light-requiring Grand Rapids lettuce seeds so that they germinate with a smaller dose (a lower intensity) of light than is normally required for their germination (*15*). This hormone promotes germination in the dark only slightly (*7*). Of the hormones available, only gibberellin and inhibitors are positively implicated in germinative processes. With this background we set out to determine which of the promoting hormones would oppose the effect of inhibiting hormones in the germination of lettuce seeds (varieties Grand Rapids and White Paris). The germination of both varieties of lettuce seed is completely inhibited by the naturally occurring inhibitors, coumarin (*16*), xanthatin (*17*), and a partially purified inhibitor from immature wheat hulls (*18*, *19*). The inhibition in each case can be reversed by kinetin, but not by IAA or gibberellin (*18*). In the photosensitive variety (Grand Rapids) reversal of inhibition is achieved only in light (or red light, 660 nanometers), whereas in the nonphotosensitive variety (White Paris), kinetin reverses the inhibition in light as well as in dark. These results, in addition to showing an antagonism between inhibitors and a cyto-

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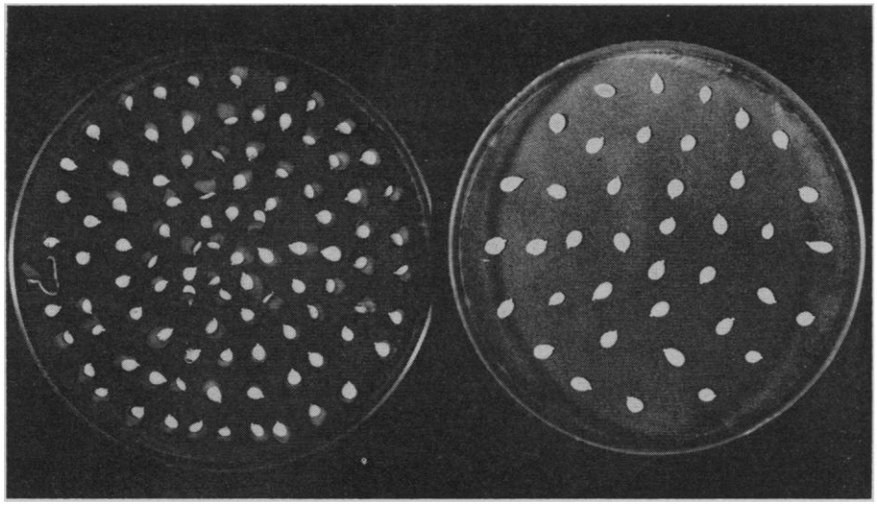

Fig. 1. Growth of dormant (unchilled) excised pear embryos on blotters soaked in water. (Right) Freshly excised dormant embryos. (Left) After 2 weeks the cotyledon of the embryo in contact with the moist surface has grown considerably and turned green while the upper cotyledon has not grown and remained white. The photographs are in different scales. Courtesy of C. E. Heit.

kinin in germination, indicate a close relationship between hormonal interactions and the phytochrome system, which is characteristic of photosensitive lettuce seeds and many other photoreactive plant systems (*20*).

With the synthesis (*21*) and availability of abscisic acid, the most potent naturally occurring inhibitor known, we extended our studies to include this inhibitor. Inhibition of germination in the seeds of lettuce, barley, *Xanthium*, and pear induced by abscisic acid was reversed by one or the other of the cytokinins (kinetin, benzyladenine, and zeatin) used (*22–25*). Zeatin (*2, 26*), the naturally occurring cytokinin, isolated from corn kernels, was slightly more active than kinetin in reversing the inhibition of lettuce seed germination by coumarin or abscisic acid (*22*). Our findings were confirmed by a number of workers who showed similar antagonism between inhibitors and cytokinins in seed germination of lettuce (*27, 28*), *Brassica oleracea* (*29*), *Fraxinus ornus* (*30*), and *Setaria lutescens* (*30a*). However, in some seeds or embryos abscisic acid or coumarin inhibition is reversed by gibberellin as well as by cytokinins (*25, 27, 29, 30*). In other cases gibberellin is nearly ineffective and the inhibition of germination by abscisic acid is reversed only by cytokinins (*28, 31, 32*). Cytokinin-inhibitor antagonism is not limited to seed germination but is also found in seedling growth (*23, 28, 32*), growth of culture of *Lemna minor* (*33*), and in radish leaf senescence (*27*). From these studies it appears that cytokinins alone or in combination with gibberellin might exert a regulatory control in the germination of seeds. These results also suggest that cytokinins can be effective agents for releasing buds or seed from dormancy. Dormancy was increasingly regarded as due to the presence of inhibitors.

Cytokinins, Inhibitors, and Seed Dormancy

Many correlations described in earlier works led to the hypothesis that endogenous inhibitors are involved in bud and seed dormancy (*34*). One of the most notable instances of seed dormancy is that of the small, dormant "upper" seed in the fruit (bur) of *Xanthium pensylvanicum* (cocklebur) (*35*). Dormancy in this seed is due to two water-soluble inhibitors present in the embryo (*36*). In a number of buds, release of dormancy after the winter season is associated with the decrease in the amount of inhibitors (*12, 37*). Extracts of dormant buds of *Betula pubescens* inhibit the growing buds of the same species (*38*). Presence of inhibitors is also indicated in dormant seed or embryos as leaching results in better germination (*36, 39, 40*). An interesting example of loss of inhibitor by leaching is provided by rosaceous embryos. The two cotyledons of the excised dormant embryo grow at different rates when placed on a moist surface. The cotyledon in contact with the surface grows rather rapidly and turns green while the other cotyledon shows little or no growth and remains white. This results in considerable difference in size of the two cotyledons of the growing embryo (Fig. 1). This phenomenon was first observed in *Sorbus aucuparia* (*41*) and also occurs in *Pyrus, Malus, Chaenomeles*, and other rosaceous genera (*42*). It was shown that inhibitor or inhibitors are released adjacent to the cotyledon in contact with the moist surface (*42a*).

Cold treatment which breaks the dormancy of certain seeds increases the amount of growth promoters, notably gibberellin or gibberellin-like substances (*11, 39, 43*). Although evidence of a direct interaction between inhibitors and promoters of dormancy release was lacking it was generally believed that such might be the case. In one instance partial release of dormancy by gibberellin as well as cytokinin was obtained in excised embryos of seeds with chilling requirement (*44*). Extracts of nondormant (chilled) *Fraxinus excelsior* embryos could make the dormant (unchilled) embryos grow (*39*). In view of the fact that cytokinins oppose the effect of inhibitors in germination, we examined the effect of these hormones on the dormant upper seed of *Xanthium*. It was no surprise that kinetin broke the dormancy of this seed (*45*). The cytokinin probably penetrates the testa and "neutralizes" the inhibitors present in the embryo, thus enabling the embryo to rupture the seed coats. As noted above the two cotyledons of the dormant embryo of rosaceous species grow at different rates due presumably to more rapid leaching of the inhibitor from the cotyledon in contact with the germination medium. When 2 micrograms of kinetin in a 10-microliter agar droplet was applied on the cotyledon (upper) which was not growing, it too started to grow and turn green and in some cases overgrew the cotyledon (lower) in contact with the moist surface (*42*). Such antagonism between cytokinin and the endogenous inhibitors occurs in the excised embryos of a number of rosaceous species.

As abscisic acid became available tests were carried out to determine whether this inhibitor had the same physiological function, namely that of keeping the seed dormant, as that shown for dormancy inhibitors. A relationship between inhibitor and bud dormancy was clearly established when application of abscisic acid to leaves induced dormancy in nondormant buds

(*46*). This inhibitor now appears to be the cause of dormancy in *Rosa arvensis* (*47*), *Fraxinus americana* (*48*), *Prunus persica* (*49*), *Persea grantissima* (*50*), *Gossypium hirsutum* (*51*), and perhaps in many other seeds.

The effect of growth promoters on abscisic acid–induced dormancy is carried out in various places. Abscisic acid increases the "depth" of dormancy in the small, upper seed of *Xanthium* (*24*). This is indicated by the fact that either a higher concentration of kinetin or longer duration is required to release the dormancy. In the case of *Fraxinus ornus* embryos, gibberellin as well as cytokinin causes partial release of dormancy induced by this inhibitor (*30*). Abscisic acid–induced dormancy in the fronds of *Lemna minor* is broken only by benzyladenine; gibberellin and IAA are ineffective (*33*). In the case of chilled pear embryos, abscisic acid–induced dormancy is overcome to some extent by gibberellin as well as by kinetin (*25*). A combination of these promoting hormones is more effective than either of them alone. Thus, seed dormancy can result from the presence of inhibitors and growth promoters such as cytokinins and gibberellins can play important roles in determining the state of dormancy of a seed. The results also indicated that other hormones or other factors might be required for complete release of dormancy, at least in some seeds.

Cytokinins and the Permissive Role

It was well known that gibberellin more than any other hormone is involved in the initiation of germination and germinative processes. Recent studies further stress its primary role in germination (*3, 4, 31, 32, 52, 53*). Cytokinins, on the other hand, by themselves have little or no effect on germination (*7, 31, 32, 54*). However, the evidence that cytokinins may have a unique role in the control of dormancy and germination came from several studies with barley and lettuce seed. In the case of barley, gibberellin-mediated production of α-amylase in excised aleurone layers is easily blocked by low concentrations of abscisic acid. This inhibition is overcome only slightly by excess gibberellin (*53*). Likewise, production of α-amylase as well as germination (coleoptile growth) in the intact grains of the same variety (Himalaya) of barley are inhibited by abscisic acid and coumarin and are recovered only slightly by the addition of excess gibberellin. However, when a cytokinin, such as kinetin or benzyladenine, is added to the inhibited system the enzyme production as well as germination are almost fully recovered (*23, 32*) (Fig. 2). The small increases in growth and enzyme activity induced by exogenous gibberellin in presence of the inhibitors (abscisic acid and coumarin) probably reflect the promotive effects of gibberellin, independent of the sphere of inhibitor "influence." This is indicated by the fact that the degree of promotion is nearly the same by gibberellin (over water control) as that obtained by gibberellin plus inhibitor (over inhibitor) (Fig. 2A). The parallel effects of gibberellin in presence or absence of an inhibitor suggest a lack of an interaction between the two hormones (*32*). Thus, the apparent small reversal by gibberellin of abscisic acid– or coumarin-inhibited processes described above should not be regarded as a true reversal in absence of proper controls. On the other hand, other results (Fig. 2, B and C) strongly suggest that cytokinins and inhibitors interact with each other (*23, 32*). These results further indicate that an interplay of several hormones may be required at times for the completion of germinative processes. Thus, phytohormones might have designated functions in the control of germination and dormancy with gibberellin assuming the primary role and inhibitors and cytokinins assuming the "preventive" and "permissive" roles, respectively (*32*). Although it may be that the term "permissive" is used for the first time to designate an action of a phytohormone, this term has been used to

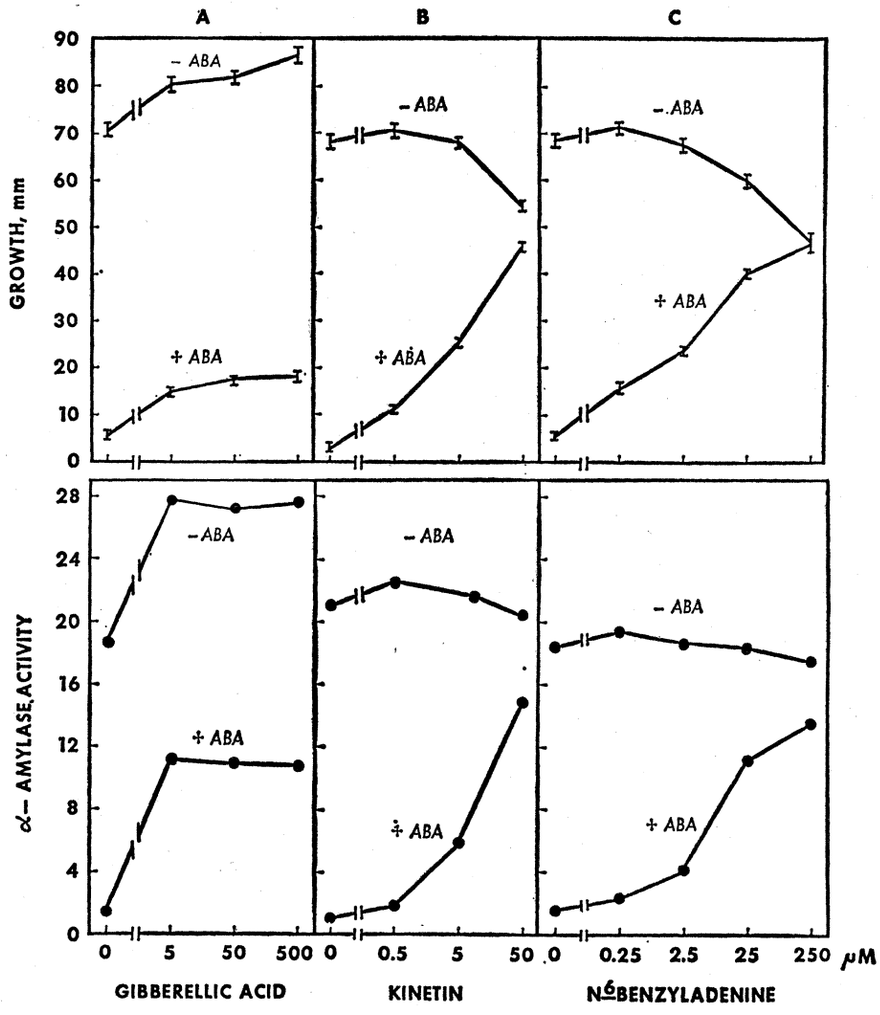

Fig. 2. Hormonal control of α-amylase and coleoptile growth in Himalaya barley. The effects of increasing concentrations of gibberellic acid (A) and cytokinins, kinetin (B), and benzyladenine (C) in presence (+ABA) and absence (–ABA) of abscisic acid on growth (above) and α-amylase activity (below) are shown. The seeds were grown for 4 days in the dark prior to measurements. Abscisic acid concentration, 6 μM. Data from Khan (*32*).

describe hormonal interplay in animal tissues (*55*). A notable example is that of insulin, the presence of which is essential for estrogens and prolactin to promote growth of the uterus and mammary gland (*55*). Insulin, by itself, appears to have no effect on these processes. Likewise, cytokinins, although not affecting germination directly, appear to be essential for completion of gibberellin-induced germinative processes when these processes are blocked by inhibitors.

Further evidence for the permissive role of a cytokinin in germination comes from studies with Grand Rapids lettuce seeds which show a highly specific gibberellin-mediated germination response (*56*). These seeds germinate in the dark only in presence of gibberellin. Abscisic acid completely inhibits the hormone-induced germination. This inhibition (Fig. 3) is reversed by a cytokinin but not by an excess of gibberellin (*31*). Little or no germination is obtained by cytokinins in the dark, nor are these hormones able to reverse the inhibition by abscisic acid in the dark in absence of gibberellin. However, cytokinins counteract the effect of inhibitors in the same seed in light (*18, 22*). Presumably, then, gibberellin or gibberellin-like compounds are produced in the light. Nonphotosensitive lettuce seed, such as White Paris (which germinates equally well in light or in dark), or other seeds, which do not need either light or exogenous gibberellin for germination, presumably contain enough endogenous gibberellin to mediate germination (*13, 57*). These data again indicate that an interplay of several hormones may control germination with each hormone having a designated function.

Cytokinins and Inhibitors May Be Dispensable

From the studies described above it appears that hormones have selective functions in the control of germination and that all hormones may not always be essential for the manifestation of germination and dormancy. Gibberellin appears to be indispensable, however. Cytokinins are required to remove the block to germination imposed by inhibitors. In absence of such a block (inhibitor), a cytokinin is not required for germination and thus is dispensable. An attempt was made to find a seed in which this is the case. Grains of cereals,

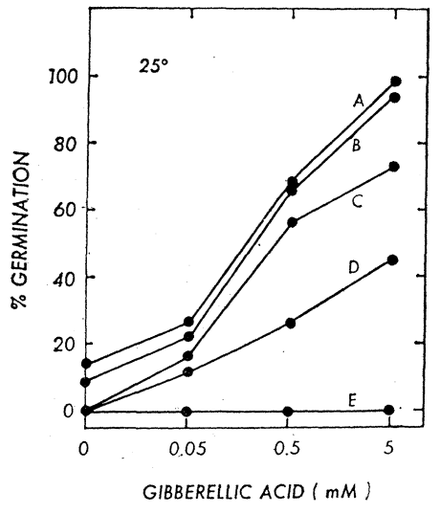

Fig. 3. Hormonal control of germination in Grand Rapids lettuce seeds grown in total darkness for 48 hours. (*A*) Kinetin (0.05 m*M*); (*B*) water; (*C*) kinetin (0.05 m*M*) plus abscisic acid (0.04 m*M*); (*D*) kinetin (0.5 m*M*) plus abscisic acid (0.04 m*M*); (*E*) abscisic acid (0.04 m*M*). Data from Khan (*31*).

when freshly harvested, are dormant and must be stored dry from a few days to several months in order to break their dormancy (*58*). Dormancy of these grains after harvest can also be broken by a few days of cold treatment (*59*) or by gibberellin (*60*). The dormancy of the barley varieties Catskill and Erie is similarly broken by dry storage or by treatment with gibberellin (*61*). Both of these treatments result in an increase in the capacity of the grain to produce α-amylase which suggests that dry storage leads to increased production of gibberellin. "After-ripening" in dry storage results in an increased capacity of the *Avena* embryo to produce gibberellin (*62*). Unlike gibberellin, kinetin fails to break the dormancy or to cause an increase in α-amylase production in grains of Erie barley (*61*). Gibberellin-induced release from dormancy as well as enzyme production in these grains is easily blocked by low amounts of abscisic acid. That a cytokinin is capable of exercising its permissive effect on gibberellin-induced processes in these systems is shown by the fact that kinetin easily removes the block to the release of dormancy (coleoptile growth) and enzyme production imposed by abscisic acid (*61*). These results suggest that dormancy in these grains is probably not due to a block of gibberellin-mediated processes by a natural inhibitor. If that were the case, kinetin would have broken the dormancy of these seeds in the absence of the exogenous gibberellin as well. Thus, dormancy in freshly harvested barley, and perhaps other cereals, may be due to a lack of gibberellin, a hormone perhaps essential for the initiation of germinative processes. An important corollary from this work is that dormancy can result not only from an excess of an inhibitor in the seed, as was generally believed, but it can also result from a lack of a gibberellin (in absence of an inhibitor) or a cytokinin (in presence of an inhibitor).

These results score the importance of external environments in determining hormonal balance and thereby germinability of a grain or a seed. It can be concluded that barley grains, or perhaps most seeds, have built-in capabilities for switching from the dormant to the germinative state and vice versa. In nature these capabilities are geared to environmental conditions. In spite of hormonal or other structural means for adapting to environmental changes, seeds too are "fooled" at times. Premature germination, presumably due to hormonal imbalance in cereals and other seeds, is not uncommon.

A Working Hypothesis

Based on the results of studies reported above, as well as others, a working hypothesis for the hormonal control of dormancy and germination has been presented (*61*). It has been assumed that gibberellins, cytokinins, and inhibitors are necessary regulators of dormancy and germination in seeds. The hypothesis, schematically presented in Fig. 4, shows eight different sets of hormonal or physiological situations likely to be encountered in seeds. The presence or absence of any one of the three classes of hormones (shown as gibberellin, cytokinin, and inhibitor), at physiologically active concentrations, might dictate whether the seed will remain dormant or will germinate. On the basis of this scheme the seed is dormant in the absence of gibberellin (situations 5–8), whether cytokinin or inhibitor is present or not; or in its presence when the inhibitor is also present but cytokinin is absent (situation 3). Germination occurs in presence of gibberellin and absence of inhibitor (situations 2 and 4) whether cytokinin is present (situation 2) or not (situation 4); or in presence of inhibitor with cytokinin to oppose its effect (situation 1).

This scheme clearly gives gibberellins the primary role in the control of germination. The roles of inhibitors and cytokinins are secondary and essentially preventive and permissive, respectively. One extremely important feature of this hypothesis is that dormancy results not only from the presence of inhibitors, as is generally regarded, but also from absence of gibberellins or cytokinins. It is not likely, however, that germination in nature is governed by absolute presence or absence of a hormone. Perhaps it is more appropriate to say that individual hormones in a seed, at any one time, are at a physiologically effective or a physiologically ineffective concentration. The effective or ineffective concentrations of hormones in a biological system, such as a seed, in turn, must depend on many metabolic and environmental factors. Thus, there could be more physiological situations governing germination than those envisaged here. However, we believe this is an organized attempt at predicting seed dormancy and germination on the basis of hormonal balance and interplay. It is not known how universal this scheme is. We do not preclude the occurrence of other hormones in seeds or embryos or their participation in the control of germination. However, as we saw before, there exists a very strict specificity with respect to the particular hormone or hormones to which a seed will respond.

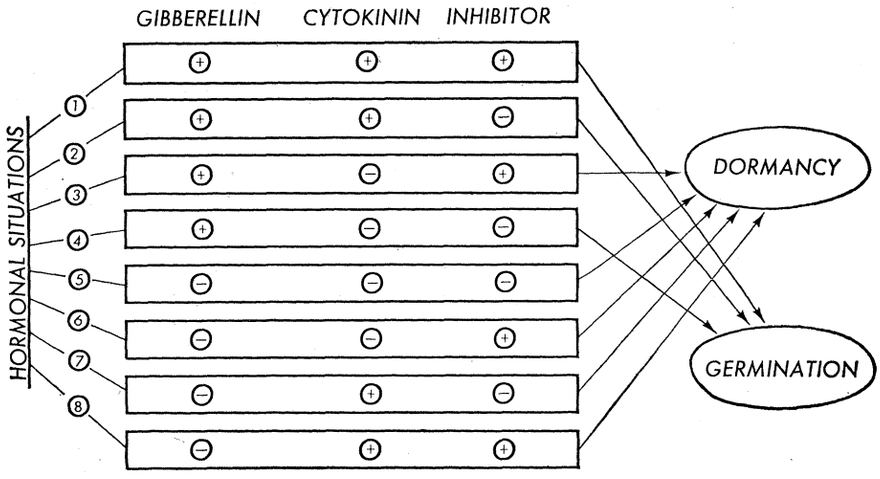

Fig. 4. A model for the hormonal mechanisms of seed dormancy and germination using gibberellin, cytokinin, and inhibitor. It shows eight hormonal or physiological situations likely to be found in seeds. Presence of any one type of hormone at physiologically active concentrations is designated by the plus sign and its absence by the minus sign. See text for details. Adapted from table 3 in Khan and Waters (*61*).

Selective Molecular Action of Cytokinins

In view of the unique role of cytokinins in dormancy and germination described above we attempted to determine the molecular effects of these hormones. Although the modes of action of phytohormones, including cytokinins, are far from known, several studies indicate that the actions of cytokinins were distinctly different from those of other phytohormones. Kinetin-induced release of *Xanthium* seed from dormancy is inhibited by actinomycin D. This suggests that kinetin's action may require DNA-dependent RNA synthesis (*45*). Gibberellin and IAA are ineffective in releasing dormancy of these seeds. As in the case of *Xanthium* seed (*24, 45*), dormancy can be induced by abscisic acid in *Lemna minor* and it can be broken by a cytokinin (*33*). Induction of dormancy results in the inhibition of synthesis of nucleic acid, and treatment with cytokinin results in more rapid synthesis of DNA than that of RNA. Dormancy is not broken by IAA and gibberellin (*33*). Similarly, apical dominance that results in bud dormancy in tobacco is relieved by benzyladenine and dormancy release in buds is accompanied by DNA synthesis (*63*). These results suggest that DNA synthesis might be involved in the action of cytokinin.

A comparative study of the effect of phytohormones on RNA metabolism was undertaken in excised tissues, isolated nuclei, and in chromatin preparations. In excised pear embryos abscisic acid inhibition of the incorporation of radioactive phosphorus (^{32}P) into various RNA species is selectively reversed by kinetin and gibberellin. Although kinetin reverses the inhibition of incorporation of labeled precursors into ribosomal RNA, gibberellin is more active than kinetin in reversing the inhibition of labeling in the DNA-associated RNA fraction (*25*). The pattern of ^{32}P-incorporation into various nucleic acids during reversal of abscisic acid inhibition by a combination of kinetin and gibberellin is similar to that obtained after dormancy release by cold treatment (*25, 64*). This suggests a hormonal interplay during dormancy release by cold treatment. Cytokinin induces enhanced synthesis of RNA in isolated nuclei. This effect is inhibited by actinomycin D but is reversed by high concentrations of the cytokinin (*65*). This suggests that cytokinin or a cytokinin-mediator complex might bind to DNA and thus compete with repressors for operator sites or might bring about derepression by other mechanism. Unlike IAA and gibberellin, the increase in rate of RNA synthesis caused by kinetin does not depend on the presence of the hormone during isolation of nuclei (*66*). Furthermore, cytokinin elicits increased RNA synthesis from chromatin as well as from DNA templates in presence of a protein mediator, a feature not yet known for other phytohormones. Specificity of cytokinin with respect to DNA suggests that the hormone recognizes some aspect of DNA (*66*). Abscisic acid induces increases in the uridine monophosphate content and decreases in the guanosine monophosphate content of the rapidly labeled RNA species of excised pear embryos (*67*) and of excised lentil root tips (*68*) and this effect is reversed by kinetin (*68*). Kinetin as well as gibberellin induces increases in activity of chromatin-bound RNA polymerase in dormant pear embryos (*69*). Although kinetin-induced increase in the activity of RNA polymerase is reversed by abscisic acid, that caused by gibberellin is not. These results suggest that hormones act differently on transcription of DNA to RNA. The action of kinetin appears to be closely associated with the readout pattern of the genome. Furthermore, it appears that hormonal interactions during transcription might cause quantitative and qualitative changes in the RNA transcribed. How these results can be translated to the functional aspects of the primary permissive and preventive roles of gibberellin, cytokinin, and inhibitor, respectively, is not known.

Some actions of cytokinins on pro-

tein synthesis also indicate that they might be acting on the gene. Cytokinins reverse the inhibition by abscisic acid of α-amylase production in the germinating barley seeds (*32*). Exogenously applied gibberellin is incapable of reversing this inhibition. In embryoless barley seed halves, however, cytokinin fails to reverse the inhibition by abscisic acid of gibberellin-induced α-amylase production (*32*). This suggests that some factor associated with the embryo is required for cytokinin reversal of inhibition caused by abscisic acid. In the production of isocitrate lyase (*70*) and proteinase (*71*) in the cotyledons of germinating squash seed, cytokinins partly substitute for the embryonic axis, normally required for maximum development of enzyme activities. Gibberellin and IAA fail to replace the stimulus. An antagonism between kinetin and abscisic acid in the production of nucleases in barley chromatin preparations has been reported (*72*). Cytokinin-induced formation of tyramine methylpherase in roots of germinating barley appears to be a result of de novo synthesis (*73*).

Cytokinins occur as bases in the soluble RNA's of a variety of organisms. The occurrence of the *cis* isomer of zeatin, 6-(3-methylbut-2-enylamino)-purine, in plant tissues (*74*), its location adjacent to the anticodon of the yeast transfer RNA's, and the ease with which it can be modified chemically, open up the possibility that cytokinins perhaps play a unique role in protein assembly. How the occurrence of cytokinins in soluble RNA's is related to their physiological function, however, is not known. The studies reported here emphasize that our understanding of the molecular action of cytokinins, as well as other hormones, is quite fragmentary. However, these results do indicate that hormones may act selectively in altering or modifying pathways of metabolism.

Conclusion

A seed represents a phase in the life cycle of a flowering plant. More than any other phase in a plant's life cycle, it is equipped to face the rigors of environment. Besides having highly elaborate protective features, it must also have specialized and highly versatile metabolic means for the control of germination and dormancy. Environmental factors, notably light, temperature, and moisture, profoundly influence the type and amount of individual hormones and, thereby, hormonal balance. These factors rarely have identical effects on metabolism, transport, or breakdown of various hormones or other metabolites controlling the production of hormones. Perhaps the best known example of hormonal balance and interplay, determining plant responses, is provided by tissue culture studies. It is known that a high concentration of IAA promotes root growth, whereas a high concentration of kinetin determines shoot growth. When both hormones are in high concentration, callus tissues show undifferentiated growth (*75*). Similarly depending on the relative concentrations of effective hormones as well as presence or absence of a hormone, a seed must exhibit different physiological and metabolic responses. Furthermore, hormones clearly have designated functions in germination. For instance, the permissive action of cytokinins is based on concrete evidence, even though exogenous hormones were used to show this novel action of cytokinins. An immediate benefit from this finding is that it gives insight into many new ways in which hormones might possibly regulate dormancy and germination in seeds.

The control of seed germination and dormancy by external application of hormones (gibberellin, cytokinins, inhibitors) probably reflects the natural control of dormancy and germination. This conclusion seems to be justified in view of the following facts: (i) Physiological or low concentrations of hormones control seed processes. (ii) Hormones act selectively in germination. (iii) There exists a very strict specificity with regard to the particular hormone or hormones to which the seed will respond. (iv) Gibberellic acid substitutes for endogenous gibberellin or gibberellins in the production of hydrolytic enzymes in embryoless barley seed halves (endosperm) (embryo is the source of natural gibberellin). (v) Gibberellins, cytokinins, and inhibitors are all known to occur in seeds.

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76. I thank the American Seed Research Foundation for support of most of the works originating from this laboratory. The earlier works of the author were supported by a grant from the Herman Frasch Foundation to N. E. Tolbert at Michigan State University where he was a postdoctoral research associate.

Vitamin B_{12}

Biochemical studies elucidate the role of this complex molecule in diverse metabolic processes.

Thressa C. Stadtman

In 1948 the isolation of crystalline vitamin B_{12} was announced simultaneously by research teams working at two of the world's large pharmaceutical concerns, Merck in the United States and Glaxo in England (*1*). Elucidation of the complete structure of this red, cobalt-containing substance culminated seven more years of intensive work which included the brilliant x-ray analysis of the crystalline vitamin by Hodgkin and associates (*2*), as well as the efforts of many others, on the chemical characterization and biological assay of numerous fragments of the complicated molecule. Among the many excellent accounts of this phase of investigations on the chemistry and nutritional aspects of the vitamin B_{12} class of compounds is the series of monographs entitled *Vitamin* B_{12} by E. Lester Smith, in particular the third revision (*3*).

The next dramatic development in vitamin B_{12} research, from the standpoint of the biologist and chemist alike, was the discovery by Barker and coworkers (*4*, *5*) of the biologically active forms (coenzyme forms) of the B_{12} vitamins. Discovery of the coenzyme derivatives was an outgrowth of Barker's effort to elucidate the mechanism by which a little known anaerobic bacterium, *Clostridium tetanomorphum*, was able to ferment glutamate. He demonstrated that the first step in this fermentation involved cleavage of the α,β-carbon-carbon bond of glutamate and rearrangement of the carbon skeleton to form the branched chain isomer β-methylaspartic acid. This led ultimately to the discovery that the isomerization reaction is catalyzed by a specific mutase and that a light sensitive derivative of vitamin B_{12}, coenzyme B_{12}, is an obligatory cocatalyst in the reaction. The circumstances that led to the discovery of B_{12} coenzyme thus illustrate how a biochemical problem, initiated from one standpoint, may take an unexpected direction of even more general and perhaps greater significance.

Although the coenzyme derivatives are, in fact, the more abundant naturally occurring forms of B_{12} in most organisms, their existence was overlooked in the earlier investigations because of the rapidity with which they are decomposed by visible light. Treatment with either acid or cyanide ion also increases their rate of decomposition; both were used in most of the earlier procedures devised for the isolation of vitamin B_{12} from natural sources. In particular, cyanide was widely used because the cyano derivative of the vitamins proved to be much more stable (*6*).

What particularly excited the chemist as the structure of the light-labile B_{12} coenzymes (Fig. 1) was unraveled was the finding that these substances contain a deoxyadenosine moiety covalently linked, through the 5′-carbon atom, to the cobalt in the corrin ring of the vitamin (*7*). This finding represented the first known naturally occurring substance containing carbon covalently bonded to cobalt; and moreover, the existence of a stable alkylcobalt compound of any kind was demonstrated for the first time.

It is not within the scope of the following discussion to consider in detail the chemistry of the vitamin B_{12}

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