CHIRALITY AND STEREOCHEMICAL RECOGNITION IN DNA-PHYTOHORMONE INTERACTIONS: A MODEL APPROACH

FRANCIS H. WITHAM

Dept. of Biology, 202 Buckhout Laboratory

LAWRENCE B. HENDRY

Dept. of Chemistry, 152 Davey Laboratory,
The Pennsylvania State University, University Park, Pa. 16802

and

ORVILLE L. CHAPMAN

Dept. of Chemistry, University of California, Los Angeles, Calif. 90024

Abstract. Space-filling molecular models of selected phytohormones and DNA, employed as described herein, illustrate possible *in vivo* stereochemical recognition between nucleic acids and intercalated phytohormones. In this regard, the absolute chirality of certain phytohormones, and that of DNA may be essential for the recognition process. It is speculated further that the specific interactions shown by molecular models have significance in the evolution of plant regulatory mechanisms.

1. Introduction

Chemically heterogeneous phytohormones (synthetic and naturally occurring substances) are involved in the regulation of a broad spectrum of responses during plant morphogenesis. Many of these responses cannot be attributed always to the action of one substance or class of phytohormone. Indeed, depending upon their endogeneous levels, the plant tissue and experimental conditions, the auxins, gibberellins and cytokinins influence many of the same responses e.g. cell division, enlargement, etc. Therefore, it is reasonable to expect similarities in the mechanism(s) by which this array of molecular species influence physiological processes in plants.

Interestingly, cellular receptors of phytohormones have not been isolated and characterized (Kende and Gardner, 1976; Jacobsen, 1977) although there is some evidence that specific membrane sites (Hertel *et al.*, 1972; Batt and Venis, 1976; Batt *et al.*, 1976), macromolecular receptors (Matthysse and Phillips, 1969; Mondal *et al.*, 1972; Biswas *et al.*, 1975) and mediators (Venis, 1971) particularly for the auxins do exist. In this regard, the mechanism(s) of cellular perception of phytohormonal information and its subsequent biochemical conversion into a measurable response(s) remains enigmatic. Nevertheless, it is becoming increasingly clear from existing information that at least some of the auxins, gibberellins and cytokinins affect the physical properties of DNA (Jacobsen, 1977). For examples, indole-3-acetic acid (IAA), gibberellic acid (GA$_3$) and 6-furfuryl aminopurine (kinetin), as inferred from melting studies, hydrogen-bond to DNA, influence *in vitro* the coiling of the DNA helix and reduce chromatin-protein

binding (Fellenberg, 1969a; Fellenberg, 1969b; Kessler and Snir, 1969; Bamberger, 1971). Thus, it is possible that *in vivo* phytohormones interact with nucleic acids in the genome or other plant cellular organelles.

Following an approach outlined previously (Hendry *et al.*, 1977), we will illustrate with appropriate space filling Corey-Pauling-Koltum (CPK) molecular models the feasibility of stereochemical recognition between double-stranded DNA and phytohormones. This approach although based exclusively on models may provide insights as to the specific interactions between phytohormones and DNA that may be required in the cellular perception and reception of phytohormonal information.

2. Related Structural Aspects of Phytohormones and DNA

The structures of some compounds which exhibit activity as phytohormones are illustrated in Figure 1. Many of the structures as written have much in common with the
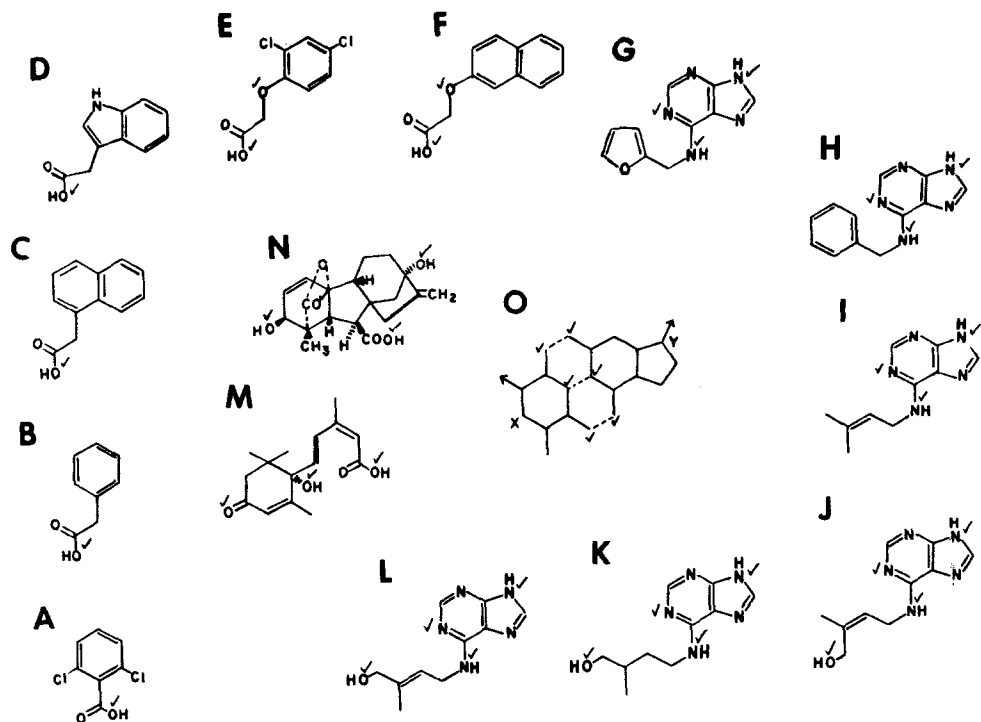

Fig. 1.   Structures of selected phytohormones and the general DNA base-pair skeleton. The structures are: (A) 2,6-Dichlorobenzoic acid; (B) Phenylacetic acid; (C) α-Naphthaleneacetic acid (NAA); (D) 3-Indoleacetic acid (IAA); (E) 2,4-Dichlorophenoxyacetic acid (2,4-D); (F) β-Napththoxyacetic acid (BNOA); (G) 6-Furfurylaminopurine (kinetin); (H) 6-Benzylaminopurine (6-BAP); (I) 6-(3-Methyl-2-butenylamino)purine (i[6] Ade); (J) 6-(4-Hydroxy-3-methyl-cis-2-butenyl-amino)purine (cis-zeatin, c-io[6] Ade); (K) 6-(4-Hydroxy-3-methylbutylamino)purine (dihydrozeatin, hio[6] Ade); (L) 6-(4-Hydroxy-3-methyl-trans-2-butenylamino)purine (zeatin, io[6] Ade); (M) Abscisic acid (ABA); (N) Gibberellic acid (GA₃); (O) Base pair template.

general shape of the DNA-base pair skeleton. For example, the cytokinins (G-L) especially complement considerable portions of the base pair structure (O) and hence contain stereochemical features requisite for interaction with DNA. Thus, during the presumed interaction of certain phytohormones with DNA, stereochemical recognition between complementary binding functional groups is likely to occur especially if the hormones are intercalated between base pairs. Although some of the compounds presented, do not conform completely to the base pair template, all contain at least one heteroatom available for hydrogen bonding which appears in a common position(s) indicated by checks (√) in each structure. Several of these positions correspond to locations in the base pair skeleton (O) where hydrogen bonds occur. The exact location and pattern of heteroatoms could be highly significant with respect to the formation of hydrogen bonds between DNA base pairs and an intercalated phytohormone. In many compounds the heteroatoms (most often hydroxyl groups) are separated by an internuclear distance (corresponding to the approximate distance between positions 3 and 17 of steroids or x and y of the base pair skeleton) previously described as sufficient to allow binding across the phosphate oxygen of the DNA backbone (Hendry *et al.*, 1977). Most, if not all of the chemicals presented (Figure 1) under appropriate conditions of pH, hydration and ionic strength can bind to at least one phosphate oxygen of DNA. Hence, the positioning of heteroatoms in the helix via hydrogen bonding coupled with the stereochemistry dictated by adjacent base pairs could aid in specifically regulating the intercalation of phytohormones into DNA.

3. Space Filling Molecular Models of Phytohormones and DNA

Three dimensional CPK-space-filling models of the auxins, IAA, α-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) and their placement suitably hydrogen bonded in DNA (between base pairs) are illustrated in Figure 2. Although the dissimilar chemical and physical features of the three phytohormones are quite evident (Figure 2A), when viewed as shown (Figure 2B) from above the aromatic rings and substituents, the structures are remarkably similar. This portion of the molecules when positioned similarly in DNA (Figure 2C–E) may represent important complementary recognition features for alignment in the helix. The three molecules depicted in this manner and the majority of compounds exhibiting auxin activity contain one hydroxyl group which under the appropriate conditions may bind to a phosphate oxygen between base pairs (Figure 2C–E) (although we have bound the three models between adenine-thymine (A-T) and cytosine-guanine (C-G), it is not the only suitable fit among the 16 possible base pair cavities (Hendry *et al.*, 1977)).

With respect to the cytokinin models, it is not surprising that the adenine nucleus complements the base pairs when intercalated between adenine-thymine (A-T) and A-T (Figure 3). Both kinetin (Figure 3A–B) and 6-(3-methyl-2-butenylamino)purine (Figure 3E–F) are bound to the phosphate backbone via hydrogen bonding through the 9-position of the purine ring. Zeatin, on the other hand, is bound to the two phosphate
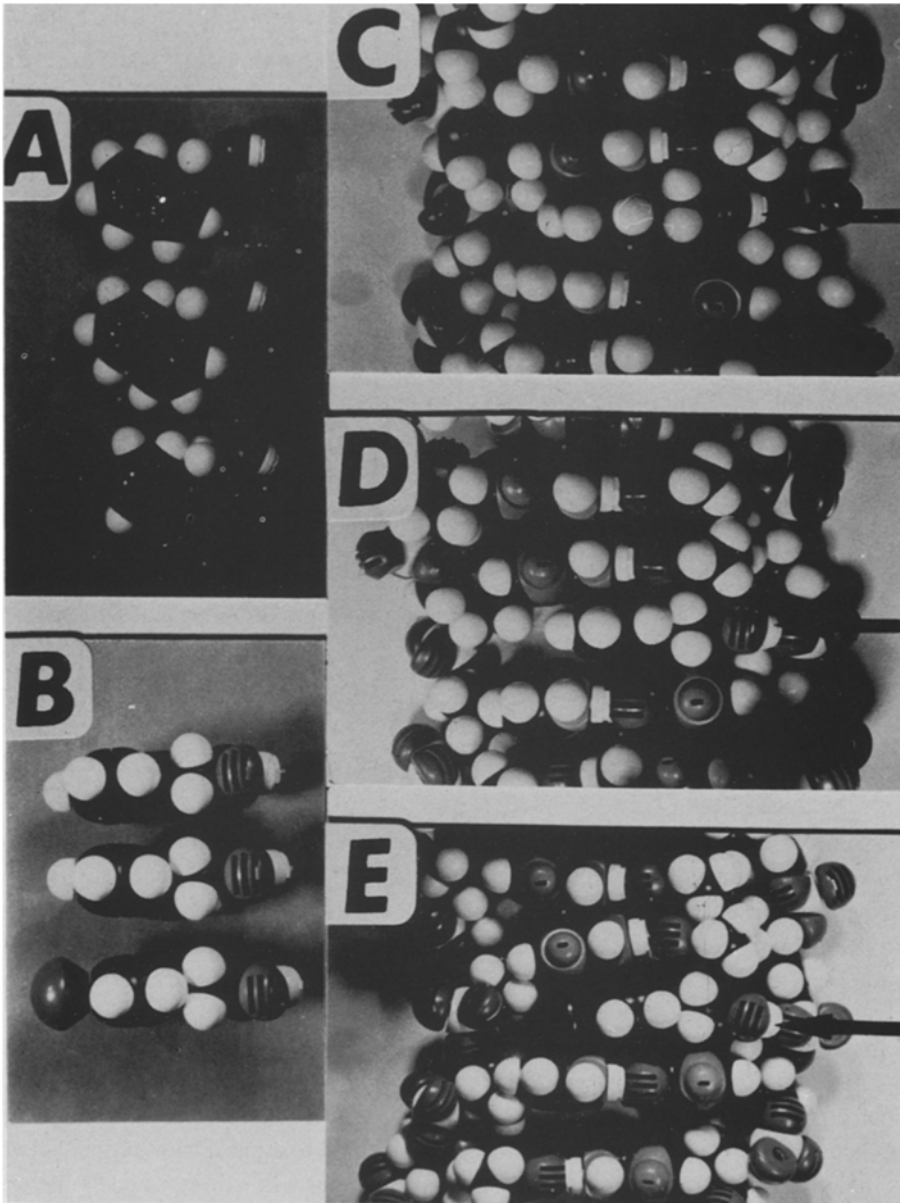
Fig. 2. CPK space-filling models of proposed DNA-auxin interactions (arrows indicate hydrogen bonding recognition sites. (A) Top to Bottom: IAA, NAA, 2,4-D; (B) Top to Bottom: IAA, NAA, 2,4-D; (C) IAA interaction with right-handed DNA helix between A-T and C-G base pairs; (D) NAA interaction with right-handed DNA helix between A-T and C-G base pairs; (E) 2,4-D interaction with right-handed DNA helix between A-T and C-G base pairs. The helices are oriented with the phosphate backbones 5′ to 3′ on the left and 3′ to 5′ on the right. Here, we refer to the backbone orientation (eg. 5′ → 3′) from top to bottom whereas previously (Hendry *et. al.,* 1977) we used the somewhat unconventional method (eg. 5′ → 3′) from bottom to top.

oxygens of adjacent DNA strands through the hydroxyl group of the side chain as well as through the 9-position of the adenine ring (Figure 3C–D).

The binding characteristics of the relatively bulky and chiral compound GA_3 to DNA are particularly interesting when GA_3 (Figure 4B) is intercalated between T-A and A-T of the right-handed helix (Figure 4C). In this configuration there is a possible four-point
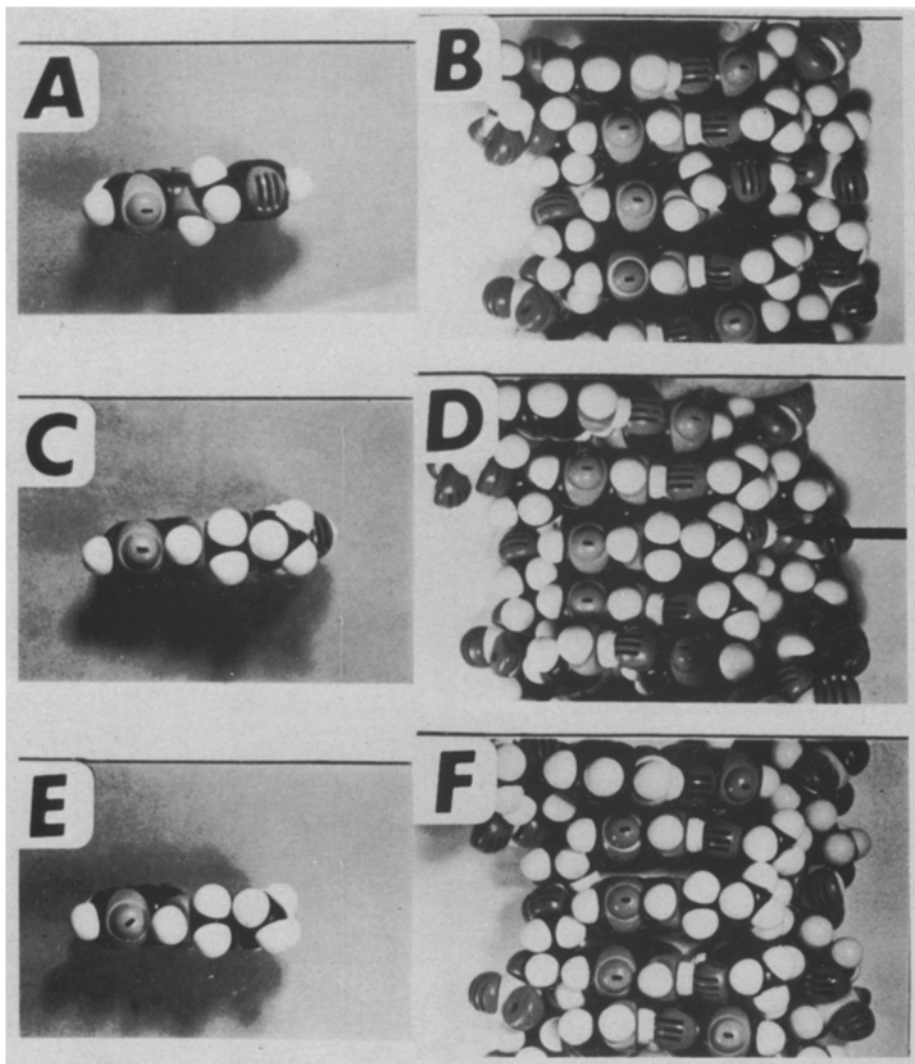

Fig. 3. CPK space-filling models of proposed DNA-cytokinin interactions (arrow indicates hydrogen bonding recognition site). (A) kinetin; (B) Kinetin interaction with right-handed DNA helix between A-T and A-T base pairs; (C) Zeatin; (D) Zeatin interaction with right-handed DNA helix between A-T and A-T base pairs; (E) i^6 Ade; (F) i^6 Ade interaction with right-handed DNA helix between A-T and A-T base pairs. The helices are oriented with the phosphate backbones 5' to 3' on the left and 3' to 5' on the right.
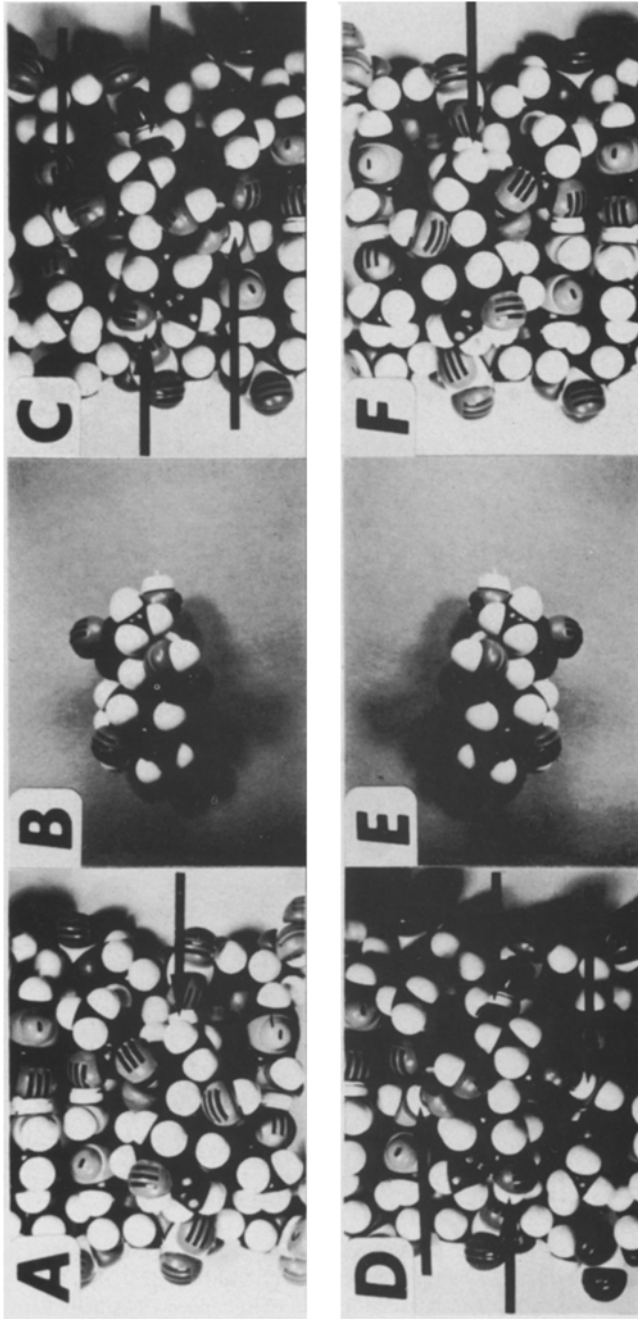
Fig. 4. CPK space-filling models of proposed DNA-Gibberellin interactions (arrows indicate hydrogen bonding recognition sites): (A) Natural GA$_3$ interaction with the left-handed DNA helix between A-T and T-A base pairs; (B) Natural GA$_3$; (C) Natural GA$_3$ interaction with the right-handed DNA helix between T-A and A-T base pairs; (D) Unnatural enantiomer of GA$_3$ interaction with left-handed DNA helix between A-T and T-A base pairs; (E) unnatural enantiomer of GA$_3$; (F) Unnatural Enantiomer of GA$_3$ interaction with right-handed DNA between T-A and A-T base pairs. The right-handed helices are oriented with the phosphate backbones 5' to 3' on the left and 3' to 5' on the right. The left-handed helices are oriented with the phosphate backbones 3' to 5' on the left and 5' to 3' on the right.

hydrogen bond attachment. This placement of GA$_3$, as determined empirically with models, is of further interest in that the closely related GA$_7$ has been shown experimentally to bind highly specifically to AT-rich DNAs (Kessler, 1971a) and in combination with a DNA ligase, induces the formation of circle-like loops in nuclear DNA (Kessler, 1971b). Further, the synergism and antagonism observed between ethidium bromide, a known intercalating agent, and GA$_3$ in promoting cucumber hypocotyl growth (Snir and Kessler, 1975) indicates that gibberellins may interact with DNA by intercalation. Interestingly, a model of abscisic acid, the well known antagonist of the gibberellins, although not illustrated here intercalates similarly between base pairs containing A-T.

The biologically unknown enantiomer of GA$_3$ (Figure 4E) does not exhibit the same alignment in the helix as the natural enantiomer when placed into the DNA model (Figure 4F). It does not complement the bases (T-A and A-T) nor does it conform to the topography of the helix when we attempted to align it in the same fashion as the natural enantiomer. The binding of GA$_3$ to the phosphate backbone and corresponding lack of binding of the unnatural enantiomer depends upon the stereochemical positions of the phosphate oxygens in the helix which are dictated by the chirality of the ribose moieties. We should point out, however, that the model of the unnatural enantiomer in orientations other than that illustrated will fit into the helix but will not conform to the topography and coiling of the helix and exhibits poor binding and alignment with the bases as compared with that of the natural enantiomer. Thus, the accommodation of the absolute stereochemistry of natural GA$_3$ in the right-handed DNA-helix is directed by the absolute chirality of this phytohormone.

Further evidence of the importance of chirality in DNA-phytohormonal interactions can be observed in an examination of the stereochemistry of a model of the biologically unknown left-handed DNA-helix (Figure 4A, 4D). The naturally occurring GA$_3$ does not intercalate completely into the left-handed helix and does not align with both of the appropriate phosphate backbone oxygens (Figure 4A). The nonnaturally occurring enantiomer of GA$_3$ does intercalate completely into the left-handed helix (Figure 4D). Thus, the chirality of the helix may be important for the recognition of certain phytohormones and as suggested previously for other chiral biologically active substances (Hendry *et al.*, 1977).

4. Conclusions and Speculations

In view of the current information concerning the interactions between phytohormones and chromatin components coupled with the phytohormonal alterations in the physical properties of DNA, it is tempting to speculate that the chemical information inherent in the structure of a given phytohormone intercalated into DNA produces template modifications important in the control of numerous morphogenetic processes. The CPK-molecular model studies indicate that the interaction of phytohormones with DNA by intercalation and stereochemical recognition is plausible. Further we believe that the

specificity of stereochemical recognition observed during the intercalation of models of chiral biologically active molecules, such as GA_3, into DNA is not coincidental (Figure 4).

The template modifications presumably occurring from intercalated phytohormones may be particularly important to the mechanics of transcription and/or translation. Whether the transcribed or translated information ultimately takes the form of response effectors, receptors, derepressors or enzymes involved in stereospecific biosynthetic reactions, etc. could depend entirely upon the location of intercalation site(s), the nature of the stereochemistry of a given DNA cavity(ies) and that phytohormone(s) which it accommodates. In this regard, the influence of various metallic ions, chromosomal protein, pH, ionic strength of the DNA microenvironment and role of receptors on phytohormonal interaction with DNA must be considered.

Our observations and descriptions of stereochemical binding of molecules to DNA are based upon that fit which in our estimation best conforms to and complements the general topography of the helix in both uncoiled and partially coiled conformations. Further refinements concerning the details of the actual intercalation sites, specific recognition features and type of alignment require considerably study. Admittedly, the proposed interactions of a given unmodified phytohormone with DNA *in vivo* may be erroneous in view of the known interconversion and metabolic modifications of the auxins (Fawcett, 1961; Shantz, 1966) and the occurrence of cytokinins as ribonucleosides (Miller, 1965; Hall *et al.*, 1966; Hecht *et al.*, 1969; Krasnuk *et al.*, 1971) etc. Also, the ideas presented here do not account for the so-called 'rapid' phytohormone-mediated responses, the action of ethylene and numerous other details related to the action of phytohormones. Similarly, the interaction of some phytohormones with DNA (or RNA) as described with the models may not be the primary event in their mode of action during ontogenesis. However, even if some phytohormones do not affect biological responses by direct interaction with DNA in 'real time', it is difficult to conceive that stereochemical recognition between active molecules and DNA did not take place during the evolution of primative cells. We therefore postulate that stereochemical recognition between intercalated phytohormones and DNA (and possibly RNA) as illustrated herein is significant to plant life.

References

Bamberger, E. S.: 1971, *Phytochemistry* 10, 957.

Batt, S. and Venis, M. A.: 1976, *Planta* 130, 15.

Batt, S., Wilkins, M. B., and Venis, M. A.: 1976, *Planta* 130, 7.

Biswas, B. B., Ganguly, A., Das, A., and Roy, P.: 1975, in G. P. Telwar (ed.), *Regulation of Growth and Differential Function in Eukaryote Cells*, Raven Press, New York, p. 461.

Fawcett, C. H.: 1961, *Ann. Rev. Plant Physiol.* 12, 345.

Fellenberg, G.: 1969a, *Planta Berl.* 84, 324.

Fellenberg, G.: 1969b, *A. Pflanzenphysiol.* 60, 457.

Hall, R. H., Robins, M. J., Stasiuk, L., and Thedford, R.: 1966, *J. Amer. Chem. Soc.* 88, 2614.

Hecht, S. M., Leonard, N. J., Burrows, W. J., Armstrong, D. J., Skoog, F., and Occolowitz, J.: 1969, *Science* 166, 1272.

Hendry, L. B., Witham, F. H., and Chapman, O. L.: 1977, *Perspect. Biol. and Med.* 21, 120.

Hertel, R., Thomson, K. St., and Russo, V. E. A.: 1972, *Planta* **107**, 325.

Jacobsen, J. V.: 1977, *Ann. Rev. Plant Physiol.* **28**, 537.

Kende, H. and Gardner, G.: 1976, *Ann. Rev. Plant Physiol.* **27**, 267.

Kessler, B.: 1971a, *Biochim. Biophys. Acta* **232**, 611.

Kessler, B.: 1971b, *Biochim. Biophys. Acta* **240**, 330.

Kessler, B. and Snir, I.: 1969, *Biochim. Biophys. Acta* **195**, 207.

Krasnuk, M., Witham, F. H., and Tegley, J. R.: 1971, *Plant Physiol.* **48**, 320.

Matthysse, A. G. and Phillips, C.: 1969, *Proc. Nat. Acad. Sci. (U.S.A.)* **63**, 897.

Miller, C. O.: 1965, *Proc. Nat. Acad. Sci. (U.S.A.)* **54**, 1052.

Mondal, H., Mondal, R. K., and Biswas, B. B.: 1972, *Nature New Biol.* **240**, 111.
      B. B.: 1972, *Nature New Biol.* **240**, 111.

Shantz, E. M.: 1966, *Ann. Rev. Plant Physiol.* **17**, 409.

Snir, I. and Kessler, B.: 1975, *Physiol. Plant* **35**, 191.

Venis, M. A.: 1971, *Proc. Nat. Acad. Sci. (U.S.A.)* **68**, 1824.