Site of Action of Auxin in Adventitious Root Initiation

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INTRODUCTION

It has been shown many times under controlled conditions that auxins are the applied phytohormones which consistently enhance adventitious root production. Indeed, research has shown that division of the root initial cells is dependent upon either applied or endogenous auxin (Hartmann et al., 1990.) However, knowledge of the mechanism of auxin action in adventitious rooting remains an enigma although auxin was identified as a root-forming substance as early as 1934 by Thimann and Went. This paper deals with experiments performed in our laboratory on the regulation of adventitious root initiation in mung bean [*Vigna radiata* (L.)R. Wilcz.]

MATERIALS AND METHODS

Plant Material and General Procedures. Mung bean seeds were surface sterilized in 10% Clorox (v/v) for 10 min and rinsed in tap water. After aeration for 24 h in tap water, they were sown 1 cm deep in plastic trays containing perlite. The growth room was maintained at 26±1°C. A 16-h photoperiod was supplied at a quantum flux density of approximately 205 $\mu E\ m^{-2}\ s^{-1}$.

Uniform cuttings made from 9-day-old seedlings were placed in sterilized distilled water prior to use. Each cutting consisted of a 3-cm hypocotyl, the epicotyl, two primary leaves, and the apical meristem. Ten cuttings were placed in a 19 × 65 mm shell vial containing 1 ml of the treatment solution. After uptake of the various solutions (approx. 2 h), distilled water was added to the cotyledonary node and maintained at this level for the duration of each experiment.

DISCUSSION

Research Suggesting Direct Interaction Between Auxin and DNA During Adventitious Root Initiation in Mung Bean. As mentioned above, auxins appear to be the phytohormone that consistently stimulates rooting (Fig. 1). In mung bean, the synthetic auxins IBA and NAA effectively promote adventitious root formation between a concentration range of 10^{-7} and 10^{-3} molar (Geneve and Heuser, 1982); 2,4-D is less active than the other auxins tested while IAA, the native auxin, is not as active as NAA and IBA possibly because IAA is metabolized (Hess, 1965) or converted to various conjugated forms (Norcini and Heuser, 1985).

In normally-developing, intact, mung bean hypocotyls, the pericycle cells are the site of adventitious root initials. These are the cells (referred to as "rooting-zone parenchyma" [R-ZP]) receptive to the auxin. As a result of cutting, these R-ZP cells are transformed from quiescent parenchyma cells into cells that give rise to the roots. Blazich and Heuser (1979) observed histological changes of the root initial cells during root formation in mung bean cuttings. They noticed that the nuclei and

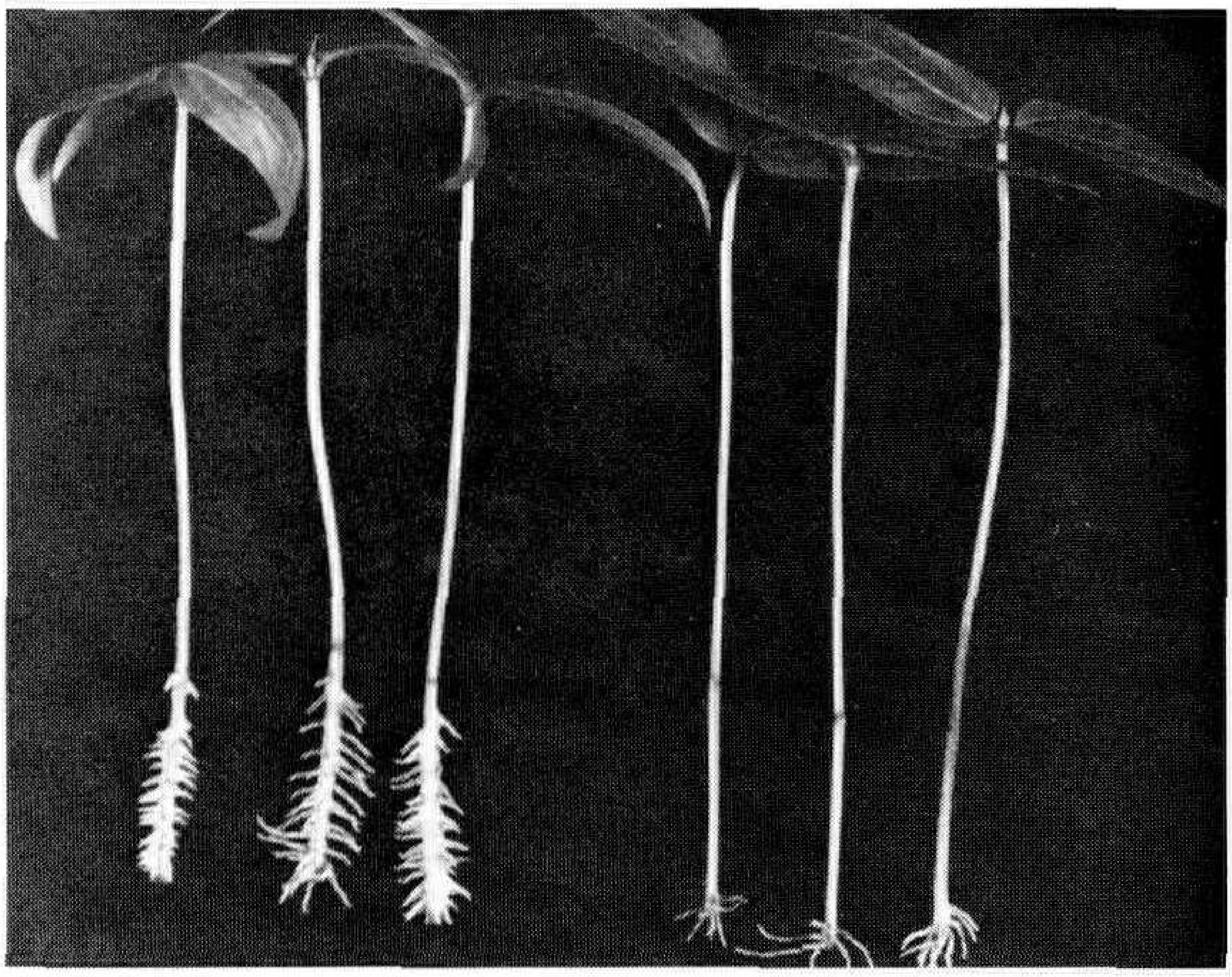

Figure 1. Auxin-induced root initiation and growth of mung bean cuttings. The cuttings on the left were treated with NAA and the cuttings on the right were the water controls. All cuttings were incubated for five days (conditions described in the text)

nucleoli enlarged in these cells approximately 14 h after the cuttings were placed in the rooting solution. Cell division occurred about 10 h later.

The process of root formation involves both cell division and enlargement, and therefore appears to be dependent on synthesis of nucleic acids and proteins. For example, Molnar and La Croix (1972) showed that protein synthesis preceded DNA synthesis and cell division prior to and during rooting in *Hydrangea macrophylla* cuttings. Further, they observed extensive changes in enzymatic activity of the cells responsible for root initiation. Because it is likely that the process of root formation is dependent upon the synthesis of nucleic acids and proteins, chemicals which interfere with or block nucleic acid and/or protein synthesis should inhibit rooting. For example, in mung bean, the exogenous application of 6-methylpurine inhibits root formation presumably due to the production of defective mRNA or due to the inhibition of mRNA synthesis (Blazich and Heuser, 1981).

When mung bean hypocotyls were treated with radioactive uridine,[3H]UR (a precursor of RNA), and thymidine, [3H]TdR (a precursor of DNA), the R-ZP cells showed incorporation of labelled uridine in RNA by 2 h and incorporation of labelled thymidine between 11 and 14 h (Tripepi et al., 1983). This suggests that RNA, protein synthesis, DNA synthesis, and mitosis are important or at least corollary processes of rooting in mung bean cuttings.

Interactions of Auxins with DNA. It is important to understand the mechanism of auxin action for the propagator as well as the basic scientist. The more information known about the action of auxin on root formation, the faster techniques will be developed by growers and propagators to exploit this important phenomenon. Therefore, we are attempting to establish a working hypothesis to explain auxin action relating to root initiation that is based on molecular modeling and subsequent laboratory experimentation.

For a hormone to be active, there must be a receptor(s) even though unequivocal information pertaining to specific receptors in plants is lacking at the present time.

However, in view of existing evidence in the area of molecular biology showing that DNA operates as the template for the transfer of biological information through RNA ultimately resulting in protein synthesis, it is possible that DNA receives the information of many small molecules by interacting with them and, through a modified code, stimulates the production of specific proteins directly involved in the stimulation of a specific response (e.g. root initiation). Certainly, the auxins and other phytohormones are known to affect the physical properties of DNA (Jacobsen, 1977). Witham et al. (1987) have suggested that IAA for example, may intercalate between base pairs and hydrogen bond to DNA. Although the approach of these authors is strictly based on the use of Corey-Pauling-Koltun (CPK) molecular models, it provides insights as to the specific interactions between phytohormones and DNA which may be required for the initiation of varied biological responses, including root formation. Accordingly, they have shown that CPK models of auxins, IAA, α-naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) may be placed suitably hydrogen bonded in DNA between base pairs. It is interesting to note that although dissimilar chemical and physical features of the three auxins are easily observed when the models are viewed as shown in Figure 2A, the structures are remarkably similar when viewed from above the ring structures (Fig. 2B). These similarities, especially the placement of the hydroxyls, provide for the intercalation or binding between base pairs of DNA

On the basis of such modeling, we speculate that the chemical information inherent in a given auxin structure intercalated into DNA may produce modifica-

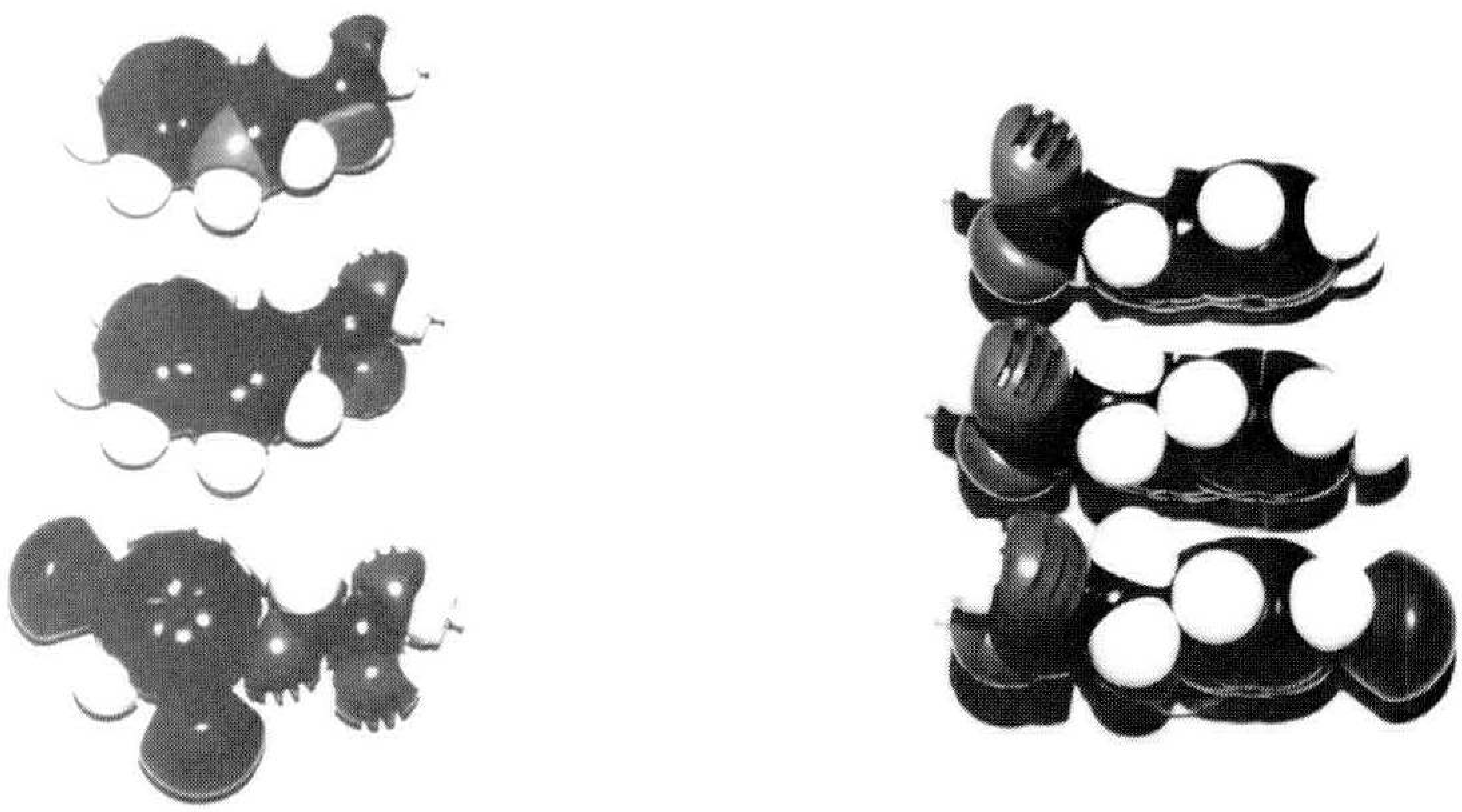

Figure 2. CPK models of representative auxins. (A) Top to bottom: side view of IAA, NAA, and 2,4-D. (B) Top to bottom: IAA, NAA, and 2,4-D CPK models viewed from above the rings.

tion of DNA that leads to the initiation of a response such as rooting or, depending upon the hormone, other important plant growth responses. Laboratory experiments are currently under way to determine whether certain auxins actually intercalate into DNA (as shown with models in Fig. 3) and if such a phenomenon is directly correlated with the stimulation of adventitious root production in appropriately treated mung bean plants.

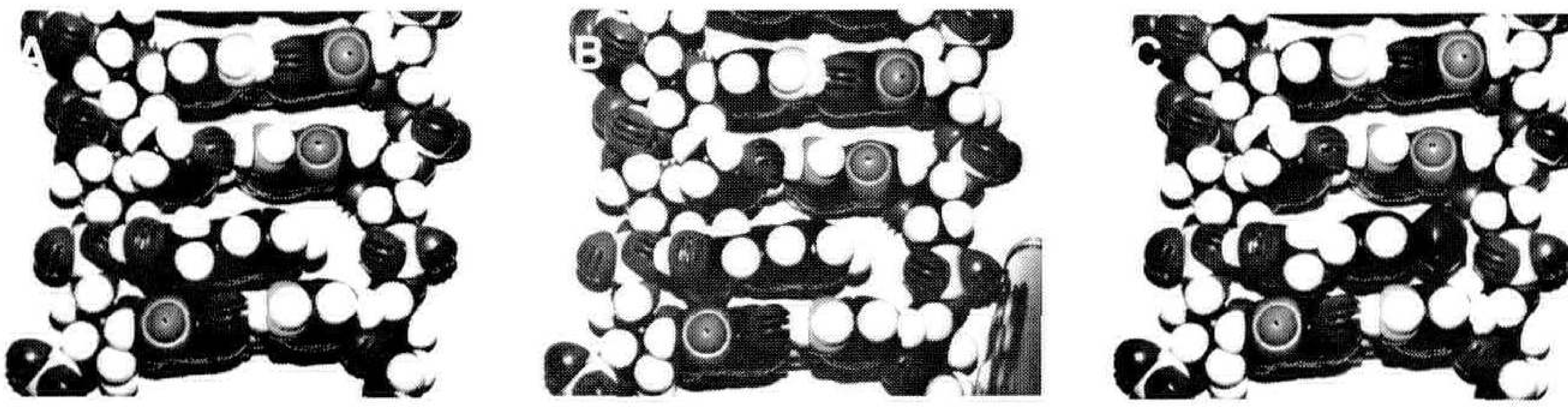

Figure 3. Models of proposed DNA-auxin interactions. (A) IAA interaction with right-handed DNA between thymine-adenine and guanine-cytosine base pairs; (B) NAA interaction with right-handed DNA between thymine-adenine and guanine-cytosine base pairs; and (C) 2,4-D interaction with right-handed DNA between thymine-adenine and guanine-cytosine base pairs.

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