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Hertel R, Thomson K-S & Russo V E A. *In-vitro* auxin binding to particulate cell fractions from corn coleoptiles. *Planta* 107:325-40, 1972.

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This paper describes a method for *in vitro* binding of auxins to particulate fractions from corn coleoptiles. The binding is auxin-specific: only auxins and auxin analogues that are known to interfere with auxin transport and/or growth specifically bind to the auxin receptors. Saturation kinetics show that the apparent K_D values are between 10^{-6} and 10^{-5} M for auxin and auxin analogues. The particulate fractions that contain the receptors are tentatively identified as plasma membrane. [The SC1® indicates that this paper has been cited in over 165 publications.]

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In spring 1970 I joined Rainer Hertel's group as a graduate student. We were interested in the blue-light photoreceptor of higher plants, and, since it is reasonable to assume that this receptor is located at the plasma membrane, our aim was to isolate and characterize the plasma membrane from corn coleoptiles. At this time, no distinct markers for the plasma membrane of higher plants were available.

Auxin is produced at the tip of the coleoptile and polarly transported from cell to cell. The active transport most likely occurs at the plasma membrane; therefore, we tried to identify auxin-binding sites and use this binding of auxin as a marker for the plasma membrane. Initial binding experiments with radioactively labeled 3-indoleacetic acid and α -naphthaleneacetic acid failed. These negative results shifted our interest to auxin transport inhibitors. Perhaps some of the reported synthetic auxin transport inhibitors

might have better binding constants to the presumed auxin receptors at the plasma membrane.

Luckily, an American postdoc from Michigan State University, James E. Tavares, came to work with Hertel. We obtained phthalic acid anhydride, and Jim synthesized ^3H -1-N-naphthylphthalamic acid (NPA), a compound known to be an inhibitor of auxin transport. We were able to find binding sites for NPA in particulate fractions from corn coleoptiles because the apparent K_D for NPA is 10^{-8} to 10^{-7} M. This "better" binding property was the reason binding assays with labeled NPA worked from the first day on.

With V.E.A. Russo—who received an EMBO fellowship to work on higher plants—we again tried to find auxin binding. With refined methods that we had worked out for the NPA binding assay, we soon were able to identify specific auxin receptors in fractions from corn coleoptiles. Further experiments revealed the existence of two classes of receptors, one for NPA¹ and morphactins² and one for auxins.

I believe the paper has been cited often because the reported auxin receptors have properties that have been generally postulated for animal and plant hormones,³ e.g., binding to the receptor is specific for auxins; saturation range of the receptor occurs at auxin concentrations that are compatible with auxin concentrations known to be active physiologically; and binding to the receptor is reversible. The number of research groups that now work on auxin receptors has increased since 1972 (see reviews³⁻⁵), and a molecular model was proposed by Hertel⁶ that relates auxin transport to auxin action.

1. Thomson K-S, Hertel R, Müller S & Tavares J E. 1-N-naphthylphthalamic acid and 2,3,5-triiodobenzoic acid: *in-vitro* binding to particulate cell fractions and action on auxin transport in corn coleoptiles. *Planta* 109:337-52, 1973. (Cited 60 times.)
2. Thomson K-S & Leopold A C. *In-vitro* binding of morphactins and 1-N-naphthylphthalamic acid in corn coleoptiles and their effect on auxin transport. *Planta* 115:259-70, 1974.
3. Stoddart J L & Venis M A. Molecular and subcellular aspects of hormone action. (MacMillan J, ed.) *Hormonal regulation of development I: molecular aspects of plant hormones*. Berlin: Springer-Verlag, 1980. p. 445-510.
4. Pohl U & Russo V E A. Phototropism. (Colombetti G & Lenci F, eds.) *Membranes and sensory transduction*. New York: Plenum, 1984. p. 231-329.
5. Evans M L. The action of auxin on plant cell elongation. *CRC Crit. Rev. Plant Sci.* 2:311-65, 1985.
6. Hertel R. The mechanism of auxin transport as a model for auxin action. *Z. Pflanzenphysiol.* 112:53-67, 1983.

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