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Auxin Binding Site in Tobacco Cells

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Abstract. A specific, high affinity, IAA binding site was demonstrated in both a cytosolic fraction, and in isolated nuclei, from *Nicotiana tabacum* cv. Wisconsin No. 38 cells grown in suspension culture. The amount of the binding site detected in both these fractions changed during the culture cycle according to a strict pattern. The molecular mass of the binding site was estimated by gel filtration to be approximately 175 000 and it appears to be a protein. When partially purified by affinity chromatography and allowed to pre-incubate with IAA, the site had a significant stimulatory effect on total RNA synthesis, as measured by a cell-free assay system. Unpurified extracts had no such effects. The system behaves rather like the steroid hormone-receptor system in animals.

In order for a phytohormone to exert its specific physiological and biochemical effects it must first be recognized by the responsive cells. This has been taken to imply that recognition and transduction of the chemical (phytohormone) signal can be achieved only if the cell contains at least one constituent that is able to bind the hormone. Phytohormones have characteristic structure/activity relationships (FARRIMOND *et al.* 1981) and they are present and active in the cell at very low concentrations. Proteins are the only cellular constituents which seem likely to be able to satisfy the strict demands of affinity for and specificity towards the phytohormone implied by these observations. The proteins which bind the hormones with high specificity and affinity have been traditionally called receptors and it is assumed that binding of the hormone activates the receptor in a manner such as to transduce the hormonal signal.

LIBBENGA (1978), in reviewing progress in phytohormone receptor research to that date, drew attention to the existence of a type of auxin receptor which was not membrane bound (the soluble receptor) and which might function in a similar manner to certain animal steroid hormone receptors (O'MALLEY and SCHRADER 1976). The Leiden Group's working hypothesis was that auxin molecules bound to a soluble (cytosolic or nucleoplasmic) receptor (LIBBENGA 1978), that the hormone/receptor complex becomes associated with chromosomal acceptors and that this association resulted in enhanced transcription. The approach was directed at:

1. the isolation, purification and characterization of specific, high affinity, low capacity auxin binding proteins;

2. the isolation, purification and characterization of the effector system *i.e.* that system whose activity is modulated by receptor activation;
3. the reconstitution of an auxin-sensitive *in vitro* system by putting receptors and effectors together in an appropriate (artificial) environment.

The progress of the Leiden group's work towards satisfying the objectives has been described in a series of papers published since that review (BOGERS *et al.* 1980, BOUMAN *et al.* 1979, MENNES *et al.* 1978, OOSTROM *et al.* 1980, VAN DER LINDE *et al.* 1984).

The interests of the Leiden and Leicester groups complemented each other precisely and it seemed sensible to set up the collaborative programme which yielded the results outlined in this paper. In an area of endeavour where results described in one laboratory have not always been experimentally confirmed in others, it was also attractive to have the opportunity for independent confirmation of data.

MATERIALS AND METHODS

Cell Cultures

Nicotiana tabacum cv. Wisconsin No. 38 cell suspension cultures were produced from tobacco leaf callus initiated in Leicester. The cell cultures were maintained on LINSMAIER and SKOOG (1965) medium.

In this brief overview it is not appropriate to give full details of the techniques used. These have either been described in the papers cited earlier or by BAILEY (1983). Certain parts of this work will be described in more detail elsewhere.

RESULTS AND DISCUSSION

The soluble binding site isolated from the tobacco cells was found to have very similar characteristics (Table 1) whether it was isolated from the cytosolic fraction or from nuclei. The parameters for the site were calculated in the usual way through a Scatchard analysis (SCATCHARD 1949) and routinely a K_a for IAA of approximately 1×10^8 M^{-1} was found. The number of binding sites varied dramatically according to the culture growth stage. The pH curves for the site showed maximal binding at pH 7.5—8.0 irrespective of location within the cell. The site(s) was sensitive to both trypsin and

TABLE 1

Properties of a non-membrane-bound IAA binding protein from suspension cultured tobacco cells

	Source of binding protein	
	Cytosol	Nuclei
K_a	10^8 M^{-1}	10^8 M^{-1}
No. of binding sites	0—500 fmol mg^{-1} of protein	0—250 fmol mg^{-1} of protein
pH optimum	7.5—8.0	7.5—8.0
Molecular mass	176 000 ± 10 000	176 000 ± 10 000

TABLE 2

Competition assays

Compound	Apparent K_a [M^{-1}] Cytosolic site	Nuclear site
IAA	1×10^8	1×10^8
αNAA	8×10^7	7×10^7
βNAA	9×10^6	
2,4-D	5×10^7	3×10^7
PCIB	7×10^5	*
Indolyl acetonitrile	no competition	no competition
3,5-D	no competition	no competition
D, L-Tryptophan	no competition	*
Indolyl acetaldehyde	no competition	*
Indolyl acetic acid	no competition	*

* signifies not determined.

Competition assays were carried out using radiolabelled IAA (2.5 nmol dm^{-3}) competed with increasing concentrations of unlabelled competitor ($2.5 \times 10^{-9} - 10^{-6}$ mol dm^{-3}). All results were calculated from at least four experiments.

chymotrypsin which completely removed binding activity and thus appeared to be proteinaceous. Estimates of molecular mass based on Sephadex G-200 column chromatography suggested that the sites isolated from cytosol and nuclei were similar also in this respect.

Competition assays (Table 2) revealed that the site(s) was highly specific and again the characteristics of cytosolic and nuclear sites were similar.

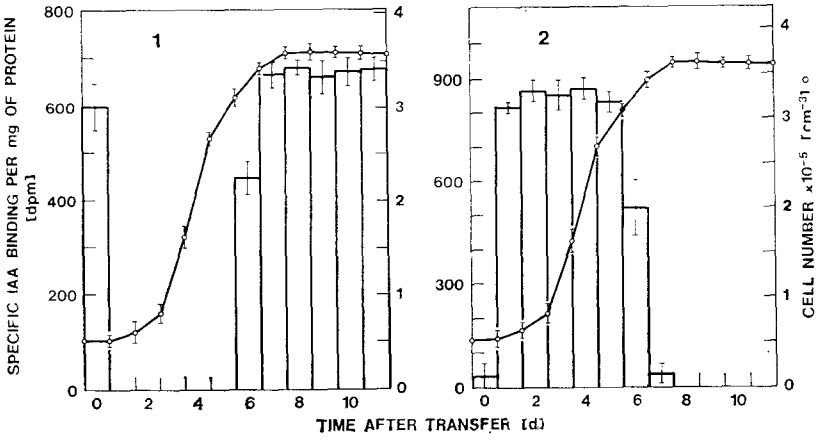

Fig. 1. Modulation of the amount of the cytosolic IAA binding site from *Nicotiana* with time after transfer of the cells to fresh culture medium. Results are the mean of 10 experiments and the error bars show the standard deviation of the mean. Protein 15—20 mg cm^{-3}. Cell numbers are the mean of 10 experiments and the error bars show the standard deviation of the mean.

Fig. 2. Modulation of the amount of the nuclear IAA binding site from *Nicotiana* cells with time after transfer of the cells to fresh culture medium. The results are the mean of 5 experiments and the error bars show the standard deviation of the mean. Protein 10—14 mg cm^{-3}. Cell numbers are the mean of 10 experiments and the error bars show the standard deviation of the mean.

TABLE 3
Influence of the partially purified soluble receptor on UTP incorporation by isolated nuclei

A	B	C	D
Nuclei	Nuclei + IAA	Nuclei + receptor	Nuclei + receptor + IAA
100.0%	99.5%	100.0%	124.3%

The data represent a typical experiment and are presented as percentages of the value for nuclei alone.

The assays of the cytosolic receptor which were carried out first (Fig. 1) provided the somewhat surprising (at the time) information that the site was detectable only during the lag and stationary phases of culture growth. The absence of the site from the cytosol at the time of most active metabolism led naturally to the suspicion that a binding site might be found in the nucleus at this time. Considerable difficulty was, at first, encountered in isolating relatively pure nuclei but it was possible, ultimately, to identify a reproducible pattern of changes in the nuclear site numbers (Fig. 2) which interdigitated almost perfectly with the changes in the cytosol. It was difficult to avoid the conclusion that the site was cycling between the cytosol and the nucleus.

Recently, relatively short response times (15 min to 2 h) have been demonstrated for auxin stimulated RNA transcription (BEVAN and NORTHCOTE 1981, THEOLOGIS and RAY 1982, ZURFLUH and GUILFOYLE 1982). Such rapid responses in gene expression indicate that the responses are closely related to primary auxin effects. Clearly the nuclear binding site identified in the present studies would be ideally situated to act in this way. Affinity column chromatography was used to give a 50 to 60 fold purification of the binding site. The site was eluted from the column by an IAA-containing buffer which was subsequently removed by use of a Sephadex G-50 column; high affinity binding of IAA was demonstrated with this purified material.

The purified (IAA free) binding site had no influence on the *in vitro* incorporation of UTP by nuclei into TCA insoluble material (Table 3) but in the presence of IAA and the receptor incorporation was routinely stimulated.

We believe that this work has gone some way towards satisfying the objectives defined in the Introduction. It is particularly interesting to speculate on the similarities which may exist between the system here defined and the animal steroid/receptor system discussed by O'MALLEY and SCHRADER (1976). One speculates that when the hormone is bound a transformation of the receptor occurs and the complex formed enters the nucleus where it can bind to a chromatin-bound protein and affect RNA synthesis.

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