CYTOKININS AND THE DIVISION OR EXPANSION OF PLANT CELLS

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Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, May, 1988.

PREAMBLE

"Cytokinins: Plant Hormones in Search of a Role"

This title of a book recently published by the British Plant Growth Regulator Group is an apt description of what is probably the least understood of the major hormones of the higher plants — the cytokinins.

The cytokinins participate in almost all of a plant's growth responses involving cell division, cell expansion and differentiation. However, virtually every aspect of the cytokinins — biosynthesis, mechanism of action and metabolism — is poorly understood.

The slow progress in understanding the mechanism of action of the cytokinins partly stems from the lack of a simple plant system in which the hormone can be studied away from the influences of the other hormones and in which only one of the many responses induced by the cytokinins is found.

The purpose of this thesis was to search for such a system and when found it was hoped that some progress could then be made toward an understanding of the mechanism of action of the cytokinins. For various technical reasons this was not achieved, however, some of the technical difficulties were at least partially overcome.

ABSTRACT

The effect of cytokinins was studied in three systems: the alga *Chlorella*, callus cultures and etiolated cucumber cotyledons.

In *Chlorella* cultures:

1) A range of concentrations of 6BA and IP had no effect on growth;

2) Low concentrations of an anticytokinin had no effect on growth, whereas higher concentrations appeared to be inhibitory.

3) Characterisation of the *Chlorella* species suggested that it was surrounded by an impermeable sporopollenin layer which hindered the uptake of cytokinin.

4) The uptake of radioactive adenine occurred readily, whereas the uptake of radioactive 6BA was very slow in both growing and saturated cultures of *Chlorella*.

5) Extracts isolated from *Chlorella* and the medium in which *Chlorella* was growing contained cytokinin-like activity in two bioassays.

6) HPLC analyses of these extracts showed that there were fractions which eluted at the positions of IP and IPA.

In callus cultures:

A.1) A carrot callus was grown from the secondary phloem of the storage root of carrot.

2) This callus, which was grown on 2,4-D and kinetin, produced roots and shoots when subcultured onto IAA and kinetin.

3) Growth on 2,4-D alone was independent of the presence of kinetin.

4) Growth was inhibited in the presence of an anticytokinin, suggesting that the callus produced a cytokinin.

B.1) A tobacco callus was grown from a young leaf of tobacco.

2) This callus habituated to cytokinin independence following subculture onto lower concentrations of kinetin.

3) Subculture of the habituated callus onto a higher concentration of kinetin resulted in the production of roots and shoots.

C.1) Cytokinin-dependent soybean and tobacco callus cultures were obtained from the Botany Department, University of Otago.

2) Analysis of the total proteins from suspension and callus cultures of soybean by 1-D polyacrylamide gel electrophoresis showed one small 6BA-induced change in the proteins from the suspension cultures.

In etiolated cucumber cotyledons:

1) 6BA caused the expansion of excised etiolated cucumber cotyledons after a 24-hour incubation in the dark in a solution containing 6BA, in comparison to cotyledons incubated in water only.

2) The cotyledons curved upwards and in the light microscope the cells of the vascular bundles and the lower epidermis exhibited greater expansion than the upper epidermis.

3) Electron microscopic examination showed that the central vacuole of palisade cells from cotyledons treated with 6BA had expanded and that the cytoplasm had probably lost water and was compressed by the vacuole against the cell wall.

4) In contrast to other research, there was no apparent increase in polysome formation in 6BA-treated cotyledons in comparison to untreated cotyledons examined in the electron microscope.

5) A number of protein extraction methods were tried before a method was found which produced a protein extract suitable for both 1-D and 2-D polyacrylamide gel electrophoresis analyses.

6) 1-D and 2-D polyacrylamide gel electrophoresis showed that a number of proteins either increased or decreased following the treatment of cotyledons with 6BA.

7) A number of RNA extraction methods were tried, to obtain RNA suitable for translation *in vitro*. Only one method produced RNA which appeared to be free of contaminating substances. Weak translation of this RNA was obtained *in vitro* and it might be possible to develop conditions for optimal translation of the RNA given an adequate supply of an *in vitro* translation system.

ACKNOWLEDGEMENTS

I thank my supervisor Professor R. Ralph for his help during the time of this degree and for editing this manuscript. Thanks are also extended to Dr W. Judd for his advice in regards to experimental problems encountered with polyacrylamide gel electrophoresis and staining and for the use of some of his chemicals and equipment. Dr H. Nonhebel, of the Biochemistry Department, made available her time, chemicals and equipment for the HPLC and GC analyses, all of which I thank her for.

T. Cooney, PhD student, Biochemistry Department, was very helpful with HPLC and GC analyses, which was very much appreciated. P. Lawson helped with the HPLC analyses, for which I thank her. I thank John White for assisting with the thin sectioning. He and Terry Gruijters were always ready to help and advise on electron microscopic and photographic problems and, therefore, I extend my appreciation to them.

Special thanks go to E. Podivinsky (PhD student, this department) for moral support and with whom many discussions were held on the problems of RNA isolation from cucumber cotyledons and other tissues, and how to solve these problems.

I would also like to express my thanks to the other members of the Department of Molecular and Cellular Biology, in particular those of the Molecular Biology Laboratory, who gave assistance and encouragement.

Finally, the most important thanks of all go to my husband, John, for all his encouragement and for spending many hours typing this thesis and assisting with the printing of the photographs.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

ABBREVIATIONS

1-D	one-dimensional
2-D	two-dimensional
6BA	6-(benzylamino)purine
2,4-D	2,4-dichlorophenoxyacetic acid
ABA	abscisic acid
AC	anticytokinin
ACC	1-amino-2-ethylcyclopropane-1-carboxylic acid
BSTFA	bis(trimethylsilyl)trifluoroacetamide
cDNA	complementary DNA
CGF	*Chlorella* growth factor
cpm	counts per minute
CTAB	cetyltrimethylammonium bromide
d.d.	double-distilled
DMSO	dimethylsulphoxide
dpm	disintegrations per minute
DTT	dithiothreitol
EDTA	ethylenediaminetetra-acetic acid
ER	endoplasmic reticulum
GA	gibberellin
GC	gas chromatography
GC-MS	gas chromatography-mass spectroscopy
GF	growth factor
HPLC	high performance liquid chromatography
IAA	indole-3-acetic acid
IEF	isoelectric focusing
IP	isopentenyladenine
IPA	isopentenyladenosine

mAmps	milliamperes
MS	Murashige and Skoog
M W	molecular weight
NAA	naphthaleneacetic acid
NEPHGE	non-equilibrium pH gel electrophoresis
n m	nanometre
NP40	Nonidet P-40
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PMSF	phenylmethylsulphonyl fluoride
psi	pounds per square inch
PVP	polyvinylpyrrolidone
rpm	revolutions per minute
RuBPCase	ribulose 1,6-bisphosphate carboxylase
SAM	S-adenosylmethionine
SDS	sodium dodecyl sulphate
TCA	trichloroacetic acid
TMS	trimethylsilyl
Tris	2-amino-2-hydroxyl-methylpropane-1,3-diol
uv	ultraviolet
v/v	volume for volume
w/v	weight for volume
wgt	weight
xs	times

CHAPTER ONE