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PREVIEW

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**CHANGES IN RNA AND PROTEIN METABOLISM DURING AGING AND
AFTER WOUNDING AND AUXIN TREATMENT OF PEA EPICOTYLS**

The University of Nebraska - Lincoln

PH.D. 1982

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PREVIEW

CHANGES IN RNA AND PROTEIN METABOLISM DURING AGING AND AFTER
WOUNDING AND AUXIN TREATMENT OF PEA EPICOTYLS

by

Anne M. Schuster

A DISSERTATION

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Life Sciences

Under the Supervision of Professor Eric Davies

Lincoln, Nebraska

September, 1982

TITLE

CHANGES IN RNA AND PROTEIN METABOLISM DURING AGING AND AFTER

WOUNDING AND AUXIN TREATMENT OF PEA EPICOTYLS

BY

Anne M. Schuster

APPROVED

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ACKNOWLEDGMENTS

I would especially like to thank Eric Davies for his continued patience, advice, guidance and friendship. I am also appreciative of the help and advice given by my supervisory committee members-- Steve Schwartzbach, David Galbraith, Jim Van Etten and Les Lane. I am also grateful for the time spent in other labs learning valuable techniques with the aid of several committee members, as well as Shelby Freer, Antonio Monroy, Carl Pederson and Brian Larkins.

I also thank Dan Kuczmariski, Mark Horrum, Bill Hays and Sue McCarthy for their conversation, help and friendship. I would like to thank Russ Meints for ongoing technical and theoretical aid and for his encouragement and friendship. Finally I am grateful to my parents for always supporting me and particularly to my father for stimulating my interest in plants and biology.

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ABBREVIATIONS

NH₄Ac:	ammonium acetate
EDTA:	ethylenediaminetetraacetate, di-sodium salt
GPS:	glycine phosphate salt buffer
poly(A):	poly(adenylic acid)
poly(U):	poly(uridylic acid)

PREVIEW

LITERATURE REVIEW

Aging of Plant Tissues

"Aging" is a term used in several contexts. It can refer to the activation of metabolic events after excision and aerobic incubation of storage tissues; senescence; or the maturation of tissue during development. The use of the term here is different from those listed above. Briefly, aging is defined as a decline in metabolic events following the removal of the apical meristematic region (and therefore the endogenous source of auxin) from etiolated pea plants, resulting in a cessation of growth. However, this process is reversible and the tissue is easily rejuvenated following wounding or auxin treatments. This definition of aging differs from the first three for the following reasons.

First, "aging", or activation of plant storage organs, is nearly opposite to the process described here. This activation begins when tissue from any of several plant species (e.g., Jerusalem artichoke, carrot, potato, beet) is sliced and incubated in solution. Most of the events which occur during "aging" are reviewed by Kahl (1978), and they include: increased respiration rates; formation of poly-somes; enhanced RNA and protein synthesis; activation, degradation or de novo synthesis of many enzymes (e.g., RNase, peroxidase, phenyl-alanine ammonia lyase); ethylene formation; and a variety of responses to phyto-hormones. Although this activation process is

unrelated to the aging process studied here, it is relevant to wounding studies and will be discussed later in that context.

Second, Beevers (1976) recognized the ambiguity in the term "aging" and suggested that it is different from senescence. He proposed defining senescence as "the deteriorative events which precede the death of a mature cell". Adoption of this definition would therefore eliminate the aging process here as a senescence phenomenon, primarily because the aged pea stem tissue, which assuredly has deteriorated compared to the unaged tissue, is far from dying. It is also easily reactivated metabolically whereas tissue which has senesced beyond a certain point cannot be rejuvenated.

Third, the aging process here is most similar to events which occur during the maturation of certain tissues. Pea stem tissue, for example, becomes more metabolically inactive the further it is from the apical meristem (Davies and Larkins, 1973). In this sense, the basal tissue of an unaged, actively-growing pea plant resembles the apical tissue of an aged pea plant. There have been several reports which show the protein synthetic activity declines during maturation (Lin et al., 1973 and references therein). Some of the work comparing basal and elongating regions of soybean hypocotyl tissue is also relevant. Travis et al. (1973) found that the two tissues responded quite differently. The elongating tissue showed little or no response to auxin treatment or to excision and incubation (i.e., wounding) in terms of polysome formation whereas the basal tissue exhibited increased polysome formation and protein synthetic activity

following both auxin treatment and excision. Using more sensitive techniques, Zurfluh and Guilfoyle (1980, 1982a,b) showed that the elongating tissue does respond to both excision and auxin treatment as evidenced by alterations in the 2-D gel patterns of proteins labeled in vivo or polypeptides synthesized in vitro. The basal tissue was shown to exhibit even greater differences.

Since the aged peas are metabolically inactive they have been used as controls to study the effects of auxin (Davies and Maclachlan, 1968; Davies and Larkins, 1973; Davies, 1975) and wounding (Davies and Schuster, 1981). During the course of these and other studies, some of the events occurring during the aging process of etiolated peas have been revealed. Davies and Maclachlan (1968) showed there is an increase in the β -1,3-glucanase activity while the levels of protein and nucleic acids decrease. Cellulase activity was also seen to decline (Datko and Maclachlan, 1968) while a disaggregation of free polysomes (Larkins and Davies, 1973) occurred.

Wounding of Plant Tissues and Wound Signals

Plants are often wounded, whether experimentally or naturally. Excised tissues are commonly used for experimental research because of the ease of manipulation. Recently researchers have realized that the act of excision may have as much or more influence on the plant's response than the actual experimental treatment (e.g., hormone) being studied (Hanson and Trewavas, 1982). Naturally-occurring wounds are inflicted by plant pathogens or by unfavorable environmental factors such as wind and hail.

We have shown that aged pea plants respond to wounding by increased polyribosome formation and protein synthesis, measured both in vivo and in vitro (Davies and Schuster, 1981). These responses are seen in tissues adjacent to and distant from the wound site, which suggests that a signal is being generated and transmitted throughout the plant stem. Although the identity of the signal is not yet known, we have postulated several potential candidates that might be involved, such as Ricca's factor (Van Sambeek et al., 1976), traumatin (Bonner and English, 1937), the proteinase-inhibitor-inducing-factor (PIIF) (Green and Ryan, 1972), ethylene (Salveit and Dilley, 1978), an action potential (Pickard, 1973) or a change in ion fluxes (Gronewald and Hanson, 1980). Some of these, however, do not strictly fit the criteria required for the signal here and can thus be eliminated. Ricca's factor (Van Sambeek et al., 1976) is transmitted unidirectionally through the plant whereas the signal here evokes a response bi-directionally (Davies and Schuster, 1981). The role of traumatin (Bonner and English, 1937) as a specific wound hormone has been questioned (Rosenstock and Kahl, 1978). PIIF, an oligosaccharide released from the cell wall after wounding (Bishop et al., 1981) has been shown to be transmitted throughout tomato plants. This causes the vacuolar accumulation of two protein inhibitors of chymotrypsin (Gustafson and Ryan, 1976). This response has been shown to be light-dependent (Bryant et al., 1976) whereas the wound response in

aged pea plants occurs in the dark, and the PIIF response is typically measured in hours whereas we detect polysome formation within 15 minutes after wounding (Davies and Schuster, 1981).

Wound-induced ethylene formation was shown to occur in excised pea stem segments after a lag period of 26 minutes (Salveit and Dilley, 1978). The duration of the lag period implies that ethylene cannot be the wound signal stimulating polysome formation (Davies and Schuster, 1981). Wound-induced ethylene biosynthesis in certain plants is impaired in the presence of cycloheximide (Salveit and Dilley, 1979; Yu and Yang, 1980). Yu and Yang (1980) showed the de novo synthesis of 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase), the enzyme which regulates ethylene biosynthesis, was required for wound-induced ethylene formation. Thus, ethylene is most likely produced in the aged pea tissue following excision, and may be involved in some later events, but ethylene probably is not the signal itself. In fact, the signal which stimulates polysome formation in aged peas may be the same signal which induces ethylene formation observed by others.

At present, the most likely candidates for the wound signal involves a change in ion flux (Gronewald and Hanson, 1980) or an action potential (Pickard, 1973). Wounding of corn roots rapidly reduces the influx of K^+ and efflux of H^+ and causes a hyperpolarization of membrane potential (Gronewald and Hanson, 1980). Gronewald and Hanson (1980) suggest this is the result of an alteration in the plasma membrane H^+/K^+ ATPase, which becomes partially disabled after

excision. Hanson and Trewavas (1982) claim that an initial wound response is the recovery of the ATPase (and the ion flux) during a washing period of the excised tissue. This recovery is affected by cycloheximide in several plant tissues (Hanson and Trewavas, 1982 and references therein) which suggests a connection between new protein synthesis and the wound response. Thus, these data suggest that an initial response to excision is the immediate disablement of the plasma membrane ATPase resulting in impaired ion flux, and that recovery from this wound-induced situation involves protein synthesis. This agrees well with the timing of the wound response in aged peas (Davies and Schuster, 1981).

An action potential has also been implicated as the signal since it has been shown to be generated bidirectionally throughout the pea plants at rates rapid enough to precede polysome formation (Davies and Schuster, 1981a). However, this action potential has not yet been strictly correlated with the wound response.

Wounding of Plant Tissues and Protein Synthesis

More is known about several of the wound responses themselves, than about the signal that evokes them. Much work has been done to characterize wound responses in plant storage tissue, although these were originally attributed to "aging" rather than wounding (Kahl, 1978). The work ranges from cytological to detailed biochemical studies (Kahl, 1978), but only some of those events related to protein synthesis will be considered here.

Studies using Jerusalem artichoke have shown that a series of events occur after wounding (Setterfield et al., 1978). Initially, monosomes are recruited into polysomes followed by the synthesis of new messenger RNA. There is a turnover of the rRNA somewhat later. Membrane-bound polysomes increase, although only a low percentage of the polypeptides made in vitro from these polysomes are sequestered within endoplasmic reticulum membranes.

Kahl (1971a,b; 1973; 1974) has also shown that polysome formation and increased protein synthesis occurs after wounding of potato tuber tissue. More recently, Kahl (1978a) has reviewed the regulation of activity for many enzymes after wounding of different plant storage organs. Several of these enzymes are synthesized de novo.

Cherry (1978) has shown increases in RNA and DNA polymerase activities after wounding of sugar beet storage tissue, and this response could be a result of either activation or de novo synthesis of the enzymes. This tissue also exhibited rapid polysome formation, presumably as a result of recruitment of monosomes onto pre-existing message (Wasilewska and Cherry, 1974).

Ishizuka et al. (1981) also found rapid polysome formation occurring after excision of potato tuber tissue. In contrast to some other studies they attribute the increased protein synthetic rate to be a result of new mRNA synthesis and claim that the pre-existing message contributes very little in this wound response.

Only a few reports are available on the affect of wounding on protein synthesis in non-storage plant tissues. Travis et al. (1973)