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REVIEW ARTICLE

RIBONUCLEASES IN VASCULAR PLANTS: CELLULAR DISTRIBUTION AND CHANGES DURING DEVELOPMENT

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INTRODUCTION

PLANT ribonucleases (RNases) have been studied for many years, using a variety of materials. Most workers have studied crude homogenates, making critical analysis of the RNase enzymes difficult, although recent cell localization studies and the use of highly purified preparations from *Zea mays*,[1,2] and *Avena sativa*[3–6] have been helpful in categorizing the enzymes having RNase activity in plant homogenates. In the present review, the classification of Reddi,[7] as extended by Wilson,[1,2] will be used. *RNase I-type* enzymes are RNA specific endonucleases which have a relative purine specificity; most of these liberate purine 3′ nucleotides from cyclic nuleotides, and they are found in the soluble fraction of tissue homogenates, with an acid pH optimum of *ca.* 5. *RNase II* enzymes are also RNA specific endonucleases, with a higher pH optimum than RNase I enzymes, and they are either adsorbed, or form an integral part of ribosomal material in homogenates. *Nuclease I* is an endonuclease which produces 5′ nucleotides from RNA and DNA. *Phosphodiesterases* are included among the enzymes reviewed, since they make up an appreciable portion of the RNase activity present in *Avena* leaf material, and most studies have not distinguished these enzymes from RNA-specific enzymes in the preparations used.[4] For a more comprehensive treatment of the biochemical characteristics of the RNases, the reader is referred to the review by Bernard,[8] and articles by Reddi,[7] Wyen *et al.*,[4–6] Udvardy *et al.*,[3] and Pitt.[9]

[1] WILSON, C. M. (1968) *Plant Physiol.* **43,** 1332.
[2] WILSON, C. M. (1968) *Plant Physiol.* **43,** 1339.
[3] UDVARDY, J. (1969) *Plant Cell Physiol.* **10,** 375.
[4] WYEN, N. V., UDVARDY, J., SOLYMOSY, F., MARRÉ, E. and FARKAS, G. L. (1969) *Biochim. Biophys. Acta* **191,** 588.
[5] WYEN, N. V., ERDEI, S. and FARKAS, G. L. (1971) *Biochim. Biophys. Acta* **232,** 472.
[6] WYEN, N. V., ERDEI, S., UDVARDY, J., BAGI, G. and FARKAS, G. L. (1972) *J. Exp. Botany* **23,** 37.
[7] REDDI, K. K. (1966) in *Procedures in Nucleic Acid Research* (CANTONI, G. L. and DAVIES, R. R., eds.), p. 71, Harper & Row, New York.
[8] BARNARD, E. A. (1969) *Ann. Rev. Biochem.* **38,** 677.
[9] PITT, D. (1971) *Planta* **101,** 333.

RNase DISTRIBUTION AND CHANGES DURING DEVELOPMENT

Subcellular Localization

The greatest RNase activity occurs in the aqueous fraction of cell-free homogenates of plant material. Approximately 75% of the total RNase activity of pea root preparations[10] and 80% of wheat aleurone homogenates[11] is soluble. This activity includes almost all of the neutral and alkaline RNase activity. Most of the soluble acid RNase activity in maize roots is the RNase I type.[2] A soluble RNase enzyme is found in prosphaerosomes and sphaerosomes of tobacco endosperm,[12] and maize seedlings.[13] A RNase of *Ipomoea purpurea* (morning glory) corollas is also membrane-bound.[14] Soluble RNase activity can be demonstrated in the cytoplasm using histological strains.[15] Lysosome-like structures in living hair cells of *Tradescantia virginiana* take up neutral red.[16] The release of RNase from aleurone cells of barley occurs at the time when dictyosome vesicles are proliferating.[17] Matile and Moor[18] have suggested that the enlarging vacuoles of maturing cells are made up of coalescing provacuoles derived from the endoplasmic reticulum. These provacuoles would contain soluble RNases and other hydrolases previously synthesized within the endoplasmic reticulum and encapsulated as the reticulum vesiculated. Material, including RNase, would be incorporated into the central vacuole by encapsulation of larger dictyosome-derived vesicles through invaginations of the tonoplast. Evidence to support these views was obtained using freeze-etched maize root tips,[18] and *Ipomoea purpurea* corolla.[14] Acid RNase has been found in isolated plant vacuoles from maize seedling rootlets,[19] and the autophagic activity of plant vacuoles has been demonstrated.[14,20] This system may have produced the exocellular RNase activity found by Chang and Bandurski in the nutrient medium surrounding maize roots.[21] Lysosome-like structures do not appear to be a source of RNase in bean petiole explants,[22] and the evidence for incorporation of dictyosome vesicles into the central vacuole has been challenged by Jones and Price.[17]

RNase II type enzymes are found in the microsomal fractions of maize root homogenates,[1] maize seedlings,[13] wheat germ,[23] young wheat roots,[24] wheat seedlings,[25] pea seeds,[26] and tobacco leaves,[27] although some of these preparations may also contain a non-specific nuclease, such as Nuclease I.[2] This RNase activity may be removed from the ribosomal material by increasing the pH,[28] or by adding sucrose.[29] Microsomal RNase from maize

[10] LYNDON, R. F. (1966) *Biochim. Biophys. Acta* **113,** 110.
[11] GIBSON, R. A. and PALEG, L. G. (1972) *Biochem. J.* **128,** 367.
[12] MATILE, P. and SPICHIGER, J. (1968) *Z. Pflanzenphysiol* **58,** 277.
[13] SEMADENI, E. G. (1967) *Planta* **72,** 91.
[14] MATILE, P. and WINKENBACH, F. (1971) *J. Exp. Botany* **22,** 759.
[15] WALEK, C. A (1965) *Acta Soc. Bot. Polon.* **34,** 573.
[16] MAHLBERG, P. (1972) *Can. J Botany* **50,** 857.
[17] JONES, R. L. and PRICE, J. M. (1970) *Planta* **94,** 191.
[18] MATILE, P. and MOOR, H. (1968) *Planta* **80,** 159.
[19] MATILE, P (1966) *Z. Naturforsch.* **21b,** 871.
[20] VILLIERS, T. A. (1971) *Nature (New Biology)* **233,** 57.
[21] CHANG, C. W. and BANDURSKI, R. S. (1964) *Plant Physiol.* **39,** 60.
[22] ABELES, F. B., HOLM, R. E and GAHAGEN, H. E. (1968) in *Proceedings 6th International Conference on Plant Growth Substances* (WIGHTMAN, F. and SETTERFIELD, G., eds.), Runge Press, Ottawa.
[23] LANZANI, G. A. and LANZANI, M M. (1967) *Enzymologia* **32,** 236.
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[27] REDDI, K. K. and MAUSER, L. J. (1965) *Proc. Nat. Acad. Sci. U.S.* **53,** 607.
[28] HSIAO, T. (1968) *Plant Physiol* **43,** 1355.
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seedlings is believed by Semadeni[13] to be contained in two types of sphaerosomes, and is associated with fragments of the endoplasmic reticulum. These structures show maximal RNase activities at pH 5·0 and *ca.* 6·7. Balz[30] found a single RNase with optimal activity at a pH of 6·2 in two types of bodies isolated from tobacco seedlings. The pH 6·0 ribosomal RNase activity is localized in the chloroplast ribosomes of spinach leaves.[31] Some of the activity of particle-bound RNase is located in plastids of leaves of rye,[32,33] dodder (*Cuscuta reflexa*, *C. indecora* and *C. campestris*),[34,35] wheat,[36] spinach,[31] and alfalfa.[37] Nuclease activity with an optimum of pH 7·0–7·5 is associated with chromatin material from barley leaves;[38] this association may exist *in vivo*, where it could play an important part in senescence.[39] Lyndon, on the other hand, concluded that the alkaline RNase activity from the nuclear fraction of pea roots is due to cytoplasmic contamination.[10]

Light affects some particle-bound RNases. The activity of one RNase associated with *Lupinus albus* ribosomes is increased in the hypocotyl after receiving a light stimulus.[40] Light and exogenously-applied sucrose inhibit particle-bound RNase activities in excised *Avena* leaves, but stimulate total RNase activity, resulting in an overall increase in RNase activity.[41] The particulate RNase activity was correlated with changes in RNA content. Most of the particulate RNase was associated with the chloroplasts. Light does not affect the overall RNase activity of ageing potato discs, and one enzyme with RNase activity, a non-specific nuclease of the Nuclease I type, is less active when light is present.[43] Both plastid and cytoplasmic polysome formation is stimulated by irradiating etiolated bean leaves with red light, and polysome formation is inhibited by far-red light.[44] These light effects take place without significantly affecting ribosomal RNA synthesis. The ribosomally-bound RNase of *Lupinus albus* seedlings appears to be controlled by phytochrome, with the 'photoconversion of the red absorbing form of phytochrome (P_{fr}) leading to a specific increase in ribosome-bound activity'.[40] This increased RNA degrading activity can be detected within 6–10 hr after transferring 5-day-old dark-grown seedlings to the light; growth of the hypocotyl is dramatically suppressed by the exposure,[45] and there are few polysomes present. The RNase activity associated with spinach leaf chloroplast ribosomes is more pronounced when the ribosomes are in their dissociated state.[31]

Acid RNase activity may be found in the mitochondrial fraction,[10,46] but the RNase activity of the mitochondrial fraction is highly dependent upon the choice of an isolation medium.[46] Sphaerosomes appear to be capable of digesting mitochondria.[47]

[30] Balz, H. P. (1966) *Planta* **70,** 207.
[31] Howe, R. C. and Ursino, D. J. (1972) *Can. J. Botany* **50,** 691.
[32] Szarkowski, J. W., Golaszewski, T. and Ombach, M. (1962) *Naturwissenschaften* **6,** 135.
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Short Term Response to Stress

The acid RNase activity increases following detachment of leaves of tomato,[48–51] *Avena*,[3,6,41,52] barley,[38,53–57] wheat,[54,58,59] tobacco,[56,60] maize coleoptiles,[61] leaf sections of *Rhoeo*,[62] and bean endocarp sections.[63] This increase in activity may be divided into two phases: (a) a rapid increase in RNase activity which is relatively purine-specific, occurring on the first day following detachment; this increase is followed by a decline in activity which begins 6–24 hr after detachment,[5,48,63,64] (b) a long term increase in RNase activity which probably contributes to the death of the isolated plant material.

The short term response is very rapid in tomato leaflets[48] and excised bean endocarp sections,[63] reaching a peak within a few hours, and then declining to control levels within 12 hr following detachment.[48] The response appears to be due either to mechanical damage brought about by the rapid uptake of water, as water under tension in the vascular tissue is released during detachment,[48] or to a direct wounding response.[65,66] Incubation of the leaves in mannitol solution prevents the short-term increase in RNase activity.[48,61] Light stimulates the increased RNase activity in pea internodes,[65] wheat leaves,[59] and *Avena* leaves.[41] Chloramphenicol inhibits the light-stimulated RNase activity;[59] sucrose substitutes for light in this system.[41,59] This increase may also be blocked by the metabolic inhibitors, cycloheximide,[3,63] *p*-chloromercuribenzoate,[66] actinomycin-*D*,[14,67] puromycin,[67] and *p*-fluorophenylalanine.[67] Chloramphenicol is not always an effective inhibitor of increased RNase activity, perhaps due to its slow rate of absorption by some plant tissues; it is effective in tomato leaflets,[50] and tobacco leaves,[67] but not in bean endocarp sections.[63]

Growth regulators are effective preventing rapid changes in the acid RNase activity of leaves. Kinetin and IAA prevent the short term burst of RNase activity when tomato leaflets,[48] and leaves of wheat[66] and *Avena*[41] are detached. The burst of RNase activity in bean endocarp sections is prevented by naphthalene acetic acid[63,68] and is stimulated by abscisic acid (ABA) treatment.[63] The methyl ester of IAA partly suppresses RNase activity in maize coleoptile segments incubated in water.[61]

Wyen *et al.*[6] found that plant growth regulators have a rapid effect on the level of some, but not all nucleolytic enzymes in the *Avena* leaf once it is detached. The target of the hormone appears to be the mechanism which leads to the increase in nuclease level in injured

[48] DOVE, L D (1971) *New Phytologist* **70,** 397.
[49] DOVE, L. D. (1967) *Plant Physiol.* **42,** 1176.
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[63] DELEO, P and SACHER, J. A. (1971) *Plant Cell Physiol* **12,** 791.
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[68] SACHER, J. A. (1969) *Plant Physiol.* **44,** 313.

cells, resulting from detachment. Kinetin and benzyladenine decreased, while ABA and benzimidazole increased the overall nuclease level, beginning as early as 2 hr after treatment. The changes in nuclease activity were accounted for almost entirely by changes in the activity of an acid nuclease of the RNase I type, which produces 2′3′ cyclic nucleotides as breakdown products. Gibberellic acid and IAA had no effect on the system. The principal RNase enzyme active during the first 24 hr, following detachment of the first seedling leaf of *Avena*, is RNase I type; activity of this enzyme levels off after 1 day.[5] Acid phosphodiesterase activity also increases moderately within 8 hr after detachment, but non-specific nuclease activity continues at a very low level.[3] The activity of a non-specific nuclease changes slightly 3 hr after detachment; this enzyme becomes highly active during normal attached leaf senescence.[5] An alkaline phosphodiesterase was unaffected by excision.[6]

Long Term Responses to Stress

Despite the plateau of activity reached after 24 hr,[5] RNase I remains the most active nuclease in *Avena* leaves.[4] Inhibitors of protein synthesis, including chloramphenicol,[48,59,67] *p*-chlormercuribenzoate,[66] puromycin,[67] *p*-fluorophenylalanine,[67] and cycloheximide,[3,62,66] inhibit the increase in RNase activity due to detachment and/or normal leaf senescence. Inhibitors of *m*RNA synthesis, such as Actinomycin-*D*,[13,69] 8-aza-adenine,[67] and 5-fluoro-uridine[62] also inhibit the activity of RNases. Chloromycin A_3 and 5-methylpurine have no effect.[62] *Avena* leaf RNase I is inhibited *in vitro* by its reaction products,[4] and RNase activity is less when tomato leaflets are exposed to uridylic and cytidylic acids.[48]

The activities of one or more RNases increase in a bound fraction of tobacco leaves a few days after detachment; sedimentable protease and esterase also appear. These enzyme activities appear in the soluble fraction soon after their first appearance in the sedimentable fraction.[30] RNase II is usually found bound to a ribosomal particle during isolation, therefore this activity may be due to a RNase II type enzyme.[26] An alkaline nuclease is associated with barley leaf chromatin material.[38,39,53,57,70] This RNase activity increases following leaf detachment, and the increase is prevented by applied kinetin *in vivo*.[38] An ABA-stimulated increase in alkaline RNase activity is also prevented by kinetin.[57] Kinetin suppresses soluble and sphaerosomal RNase activities of tobacco leaves,[30] and it suppresses the RNase activity in detached first seedling leaves of barley,[71] oats,[72] and tobacco leaves.[73] Suppression of RNase activity in tomato leaflets is not due to mobilization of materials from an untreated area of the leaflet.[52]

IAA also suppresses the RNase activity of detached *Rhoeo* leaf sections,[62] *Lens* roots,[74] tomato leaflets,[48] pea internodes,[65,66] wheat coleoptiles,[66] and bean endocarp.[63] ABA-treated maize coleoptile sections show increased soluble RNase activity beginning 8 hr after detachment; ABA inhibits ribosomal precursor synthesis.[75] High concentrations of IAA stimulate high RNase activity when applied below the apex of pea stem tips; the stimulation can be prevented by adding N^6-benzyladenine with the IAA.[29] IAA in lower concentrations can suppress the ABA-stimulated RNase activity of *Rhoeo* leaf sections.[62] Thus, IAA and

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[70] SRIVASTAVA, B. I. S. (1967) *Ann. N.Y. Acad. Sci.* **144**, 260.
[71] SRIVASTAVA, B. I. S. and WARE, G. (1965) *Plant Physiol.* **40**, 62.
[72] DE VECCHI, L. (1971) *Israel J. Botany* **20**, 169.
[73] BAGI, G. and FARKAS, G. L. (1968) *Experientia* **24**, 397.
[74] PILET, P. E. (1970) *J. Exp. Botany* **21**, 446.
[75] BEX, J. H. M. (1972) *Planta* **103**, 1.

ABA may act antagonistically, and IAA and kinetin may also exert opposite effects on RNase activity.

Gibberellins do not change the RNase activity of tomato leaflets,[76] or the microsomal RNase of pea epicotyls,[29] but they stimulate the release of RNase from aleurone cells of barley during the proliferation of dictyosome vesicles in the aleurone layer.[17,77,78] Analysis of the increase by isopycnic centrifugation indicates that the RNase activity is synthesized *de novo* following gibberellin treatment,[79] and gibberellins stimulate the release of RNase by the aleurone cells.[77] Production of active RNase in barley aleurone tissue is inhibited by ABA.[80,81] The acid RNase which is released by gibberellins appears to be lysosomal in nature,[11] although its release lags behind the release of alpha amylase and protease in aleurone tissue.[77] RNase activity in homogenates of dry maize grains is greater than during the imbibition of water; the activity increases later as germination continues.[82] Such an increase is not observed in isolated endosperm. Maximum RNase activity occurs in the embryo, followed by the scutellum, with the lowest activity in the endosperm of the ungerminated maize grain. The activity of nucleases in several times greater in young germinating cereal seeds than in older germinating seeds.[83] The older the seeds, the lower the DNA and RNA contents of the seeds during germination, suggesting that the nucleases may slowly degrade the nucleic acids during storage.

Activity in Senescing Plants

The RNase activities of old leaves of tobacco,[56,84] tomato,[85] *Hedera helix*[86] and *Vicia faba*[87] are higher than young leaves of these plants. The RNase activity is progressively higher as leaves are sampled from the stem apex downward, with a plateau of activity in the oldest leaves.[88] An entirely new RNase activity appears in older *Vicia faba* leaves, while young and older portions of the roots contain the same set of RNase enzymes.[87] Total RNase activity is three times higher in older root sections than in younger root sections. RNase activity associated with apple leaf senescence occurs in the autumn when the photoperiod is less than 12 hr; the RNases reach their highest activity after the first frost.[89]

An inverse relationship between soluble cytoplasmic RNase and RNA exists during senescence of apple leaves[90] and *Ipomoea purpurea* corollas,[14] and particle-bound RNase increases as the total RNA content of apple leaves increases.[90] Bean leaves show an increase in soluble RNase which parallels increases in chlorophyll and RNA, with a peak coinciding with maturation; it declines during senescence, in parallel with the loss of chlorophyll and

[76] DOVE, L. D. unpublished results.
[77] CHRISPEELS, M. J. and VARNER, J. E. (1967) *Plant Physiol.* **42,** 398.
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RNA.[91] The RNase activity of the first seedling leaf of barley declines during development, with an RNA and chlorophyll decline taking place during senescence.[56] Young, detached leaves of bean or radish plants maintain the high RNase and RNA content associated with mature leaves.[92] Dead and dry leaves retain most of the activity they possessed during early senescence, while the protein content declines to less than half the amount present in mature leaves.[93] Ribosomal and *m*RNA are the primary RNA forms degraded during wheat leaf senescence;[36] on the other hand, there are no differences in RNA populations between young and old first seedling leaves of barley.[94]

Increased RNase activity in barley roots and coleoptiles parallels an increase in RNA;[95–98] a similar relationship exists in germinating pea seeds.[99] Large increases in RNA and RNase occur in *Vicia sativa* embryos while the RNA content of the cotyledons falls, and the RNase activity of the cotyledons[100] increases. Increased RNase activity in the cotyledons of peanut seeds occurs during late maturation, when the RNA content declines.[101] Ledoux *et al*[102] have speculated that the germination of barley occurs in three phases: (a) a 3-day period in which translocation of preformed macromolecules from the endosperm to roots and coleoptile predominates; RNA and RNase are among those substances translocated. (b) A second 3-day period in which synthesis predominates in leaves and roots. In these first two periods, RNase and other degradative enzymes may progressively limit the amount of preformed macromolecules transferred from the endosperm to the shoot and roots. Finally, (c) a long etiolation process in the dark is associated with catabolic processes. Pea cotyledons contain a RNase which is not synthetized during germination.[103] Added boron as borate or tetraborate stimulates RNase activity in young shoots and isolated seeds of wheat seedlings;[104] this element is believed to function in translocation.

Soluble RNase may be responsible for the respiratory decline in maize scutellum within 5 days of germination;[105] oxidation and phosphorylation of isolated mitochondria are impaired, but the largely particle-bound RNase is not inhibitory. RNase I is much more active in the endosperm of the opaque-2 mutant of maize during kernal development than in other genotypes.[106,107] The opaque-2 gene is not fully recessive.[108]

While a suppression of RNase activity due to the application of growth regulators has only been demonstrated in leaves attached to the plant in the case of 8-azaguanine application to *Phaseolus radiatus* seedlings,[109] a direct proportionality exists between the auxin

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content of *Lens* roots and the RNase activity of homogenates made from them.[110] RNase activity of roots increases basipetally along roots of *Lens*,[110] *Vicia faba*,[87] and *Allium* cepa.[111] Protoplasts prepared from *Allium cepa* roots maintain the same RNase gradient as the intact root, although the activity is less. A gradient in RNase activity also exists in maize roots, but with a smaller difference in RNase activity, which cannot be detected on a per segment basis.[112] Srivastava has suggested that senescence may be triggered by a hormonal factor in leaves, and the resulting production of chromatin-associated DNase and RNase may kill the cells.[39]

The characteristic RNase changes in senescing plant tissues are sufficiently similar to changes in RNase activity which occur following leaf detachment that it has often been assumed that detachment merely accelerates the normal senescence patterns of attached leaves. Wyen *et al.*[5] found that different RNase patterns are involved in the senescence of attached first leaves of *Avena* plants, than in the destruction of leaves due to leaf detachment. In contrast to the dominant RNase I pattern in detached leaves, a non-specific nuclease was a major component of the RNase activity in attached senescing leaves, especially in the dark. When etiolated *Avena* leaves were illuminated, the activity of this nuclease decreased and the level of a relatively purine-specific RNase increased.

Environmental Influences

Acid RNase activity increases in slowly dehydrated leaves, whether they are detached,[113] or part of a wilting plant.[49,114–116] The increased activity does not occur if the rate of dehydration is rapid;[76,113] the RNase increase may be related to the increase in abscisic acid which occurs during a water stress.[117,118] Drought-hardened maize plants have a lower RNase activity than drought-sensitive controls, and less polysome and *m*RNA degradation occurs in hardened plants. Recovery of polysomes is also more rapid in drought-hardened plants.[119]

Unusually high[76] or low[89] temperatures increase the RNase activity of plant parts. Steam damage increases the RNase activity of attached tomato leaflets.[49] Cold shock increases the RNase activity of *Albizzia julibrissin* leaves.[120] A RNase activity appears in winter wheat seedlings during the first few hours of vernalization.[121] Boron deficiency[122,123] and toxicity[104] also stimulate RNase activity, as does zinc deficiency.[124]

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Toxins, Pathogens and Mechanical Damage

The RNase activity of plant parts increases in response to viral infection.[84,113,125–128] The virus must be active for a maximum response;[113] this response is due to net synthesis of enzyme.[127,128] The RNase increase in infected barley and tobacco leaves is quantitatively similar to the detached leaf response.[54,129] Exposure to the bacterial toxin also stimulates RNase activity.[129] Other bacterial infections,[130] and leaf mite infestations[131] also increase RNase activity. Rusted wheat leaves show increased RNase activity within 24 hr following inoculation.[132] In a recent study of changes in RNase activity following the inoculation of flax cotyledons with flax rust, a new RNase with properties different from the flax rust spore RNase was formed during the infection; it was also different from the RNases of the uninfected flax cotyledons.[133] There was a significant increase in the RNase activity of both resistant and susceptible varieties of flax 3–4 days after inoculation. A second and much greater increase occurred only in the susceptible host at later stages of development.[133] Crown gall tumors have an unusually high RNase activity.[134]

Tobacco leaf discs show increased RNase activity 1–2 hr after rapid infiltration with water, or following damage with carborundum. The rise in RNase activity shows two periods of high activity; the first period occurs soon after detachment and the second usually coincides with, or slightly precedes the onset of visible signs of senescence. The early increase is largely dependent upon wounding and is stimulated or, in some cases, is dependent upon light.[65] The first increase can be prevented by pretreating tomato leaves with kinetin, by treating the leaves with IAA at the time of excision, or by detaching the leaves while they are submerged in a mannitol solution which preserves an osmotic balance in the leaf.[48] Inhibitors of nucleic acid and protein synthesis prevent the short-term increase, and most reduce the RNase activity below the level of attached controls. Less specific inhibitors of protein synthesis and anaerobic conditions partly prevent the increase in RNase activity.[48,67] The RNase activities of excised maize root tips,[135] and γ-irradiated *Coleus* leaves[136] are also increased by X-radiation. The increased activity which results from damage to tobacco[67,137] and maize[127] leaves is apparently due to synthesis of new enzyme, although evidence based entirely on inhibitor studies must be regarded as tentative.

Mechanical damage to the leaves and tubers of *Solanum tuberosum* causes an increase in RNase activity within 2 or 3 hr which continues to increase for the first 15–24 hr following the damage.[64] The major increase is due to an RNA-specific hydrolase, but a minor increase in phosphodiesterase activity also occurs. A small decline in total RNase activity occurs from 18 to 24 hr after damage of one cultivar (Majestic), but the decline does not occur in another cultivar (Orion). A second increase occurs in the first cultivar, with some decline in the activities of the RNases of both cultivars 24 hr after the damage. Isopycnic equilibrium experiments, using deuterium oxide as a density label, provided no evidence that the increased

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enzyme activity following damage was due to synthesis of new enzyme. Purification of an RNA-specific enzyme found in potato tubers, followed by an immunochemical assay for RNase protein showed that its activity is greater than that predicted by the absolute content of RNase protein.[9] Pitt concluded that the enhanced RNase activity following mechanical damage may be due to preformed enzyme. He speculated that the destruction of lysosome particles in the cells may release preformed enzyme into the cytoplasm.

CONCLUSIONS

Studies using crude or partially-purified material have significance in relation to plant development only if the measured changes in RNase activity accurately reflect changes in the plant material from which the homogenates were prepared; definitive work supporting this assumption is lacking. The principal value of studies using highly-purified enzyme preparations is an accurate biochemical description of the enzymes; more of this work is necessary to define their range of metabolic activity. Preparations from damaged plant material have considerably higher soluble RNase activities than undamaged controls. This RNase I type activity may be lysosomal and may have a digestive function. A second RNase activity, of the RNase II type, is associated with the microsomal fraction of plant homogenates. This enzyme may be bound to ribosomes *in vivo* and is affected by light. A non-specific nuclease has been isolated from chromatin material, and if it functions in the plant nucleus, it may regulate transcription. Other enzymes having RNase activity have not been studied in sufficient detail to speculate on their location in plant cells. Highly definitive studies of the role of RNases in plant development must await better cell localization techniques. A RNase-specific stain which can be used with high-resolution light microscopy is badly needed to localize regions of high RNase activity *in vivo*. We may discover that these enzymes help regulate the turnover of *m*RNA which makes cellular differentiation possible. In any case, a great deal of biochemical and cytological work will be needed to clarify the roles these enzymes play in plant development.