Age-dependent Variations in Transcriptional Response to Wounding and Gibberellic Acid in a Higher Plant

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Abstract. The reaction of potato tuber tissue upon wounding and gibberellic acid (GA₃) treatment is strictly dependent on the tuber age. Young, rapidly growing tubers decline both chromatin-bound, DNA-dependent RNA polymerase (EC 2.7.7.6) activity and template availability as a consequence of wounding and are not responsive toward GA₃. At the onset of dormancy, ripened tubers do not at all respond to wounding or hormones, but later on develop the ability to increase their transcriptional rate and template accessibility, both after injury and treatment with 10⁻⁷ mol l⁻¹ GA₃. The size of the nucleolus and the rRNA content of the ribosomal population follows the same pattern.

Key words: Gibberellic acid – RNA polymerase – rRNA synthesis – Solanum – Wounding.

Introduction

The wounding of plant tissues is a necessary prerequisite for tumor induction by Agrobacterium tumefaciens (Lippincott et al., 1975). Whereas the causative agent has been identified as part of a large plasmid of the bacterium (Van Larebeke et al., 1974) which is transferred to the host cell and stably integrated into the host cells' genome (Chilton et al., 1977), the importance of wounding is by no means clear. It seems that specific attachment sites for the bacteria are exposed through wounding and, moreover, the reaction of the cell upon wounding (conditioning) and the role of plant hormones in this reaction are important elements for tumor manifestation. As has been known for a long time, the age of the host plant determines the extent of competence toward tumor formation. This is also the case with white potato tuber tissue, an ideal substrate for tumor induction. During the life cycle of that organ phases of non-competence and competence exist. Since transcription of the oncogenes is an important step in tumor establishment (Drummond et al., 1977), age-dependent fluctuation of transcription and its changes after wounding and hormone-treatment is of central importance to our understanding of plant tumorigenesis.

Materials and Methods

Plant Material and Treatment

Potato tubers (Solanum tuberosum L., cv. “Saskia”) of selected quality were planted in April. Swollen stolons, small tubers of 3–5 cm diameter, large tubers with a diameter of 6–7 cm, and big tubers of more than 7 cm in diameter were harvested in July and August. If not immediately used in experiments, the big tubers were kept at +7°C. This temperature was raised to 20°C two days before use.

Wounding of the tuber tissues from different developmental stages was brought about by a simple slicing of them into small pieces which were extensively rinsed in sterilized distilled water and incubated for various periods of time in 6 mmol l⁻¹ phosphate buffer (pH 6.5) containing 10⁻⁵ mol l⁻¹ streptomycin sulfate and 10⁻⁵ mol l⁻¹ penicillin G in Erlenmayer flasks under constant aeration in the dark. The incubation mixture for the hormone-treated samples contained additionally 10⁻⁷ mol l⁻¹ GA₃. After the incubation period, the tissue fragments were rinsed with sterile water, blotted dry and immediately homogenized.

Chromatin Isolation and Purification

Chromatin was isolated and purified essentially as described in a preceding paper (Wechselberger et al., 1979). Chromatin-associated DNA-dependent RNA polymerase activity and template availability was determined according to the standard methods detailed in that very paper. DNA was estimated according to Burton (1956), with calf thymus DNA as standard.

Ribosomal RNA Content and Nucleolar Size

The methods for extraction and estimation of rRNA from purified ribosomes and subunits, as well as the determination of nucleolar areas, has been presented in detail (Wechselberger et al., 1979).
Results and Discussion

Chromatin-bound DNA-dependent RNA Polymerase Activity in intact Potato Tubers, Wounded and Hormone-treated Tuber Tissues as a Function of Age

The formation of potato tubers starts with the enlargement of the pith tissue cells of stolons near the stolon tip and coincides with the flowering of the mother plant. At the same time, the activity of chromatin-associated RNA polymerase activity rises considerably, but decreases again when the still-growing tuber has reached a size of between 3 and 5 cm in diameter. The subsequent period of dormancy causally seems to be the dormancy of genetic activity: only very low endogenous RNA polymerase activity can be detected with isolated chromatin. At the onset of sprouting, there is a marked increase in enzyme activity (Fig. 1). If young, actively growing tubers, which are still swollen stolons, are wounded or wounded and concomitantly treated with gibberellic acid (GA₃), then an inhibition of RNA polymerase ensues. In mid-August, at the time of cessation of growth and ripening of the tubers, both treatments have virtually no effect on endogenous RNA polymerase. However, if fully grown tubers of a diameter of more than 7 cm are used for these experiments, then wounding and GA₃-treatment triggers a striking increase in RNA polymerase activity (Fig. 1). This tendency holds true for the whole period of dormancy: low endogenous RNA polymerase activity, wound-induced increases, further increment in the presence of 10⁻⁷ mol l⁻¹ GA₃. At the onset of sprouting, especially pronounced at the end of January, wounding may cause a stimulation of transcription to about 200% and GA₃ as much as 600% over the control tissue. The end of sprouting, which begins in mid-March, again makes the tissue non-responsive toward wounding and hormone-treatment (Fig. 1).

These tissue- and age-dependent phenomena are detailed in Figure 2. Swollen stolons respond to wounding and concomitant GA₃ application with a drastic decline in RNA polymerase activity so that after about 30 h of woundhealing almost no activity can be detected with isolated chromatin (A). Some weeks later, with the tubers being the size of a pea, there is no visible response at all, whereas, fully ripened organs increase their RNA polymerase activity after wounding, even though no effect of GA₃ can be seen (B). Dormant tubers react upon injury with an enhancement of their transcriptive activity, at which time the tissue becomes competent for GA₃: application of this hormone generally leads to a further stimulation of transcription. The same holds true for sprouting tubers (C and D).

Progressing differentiation within the tuber causes the general decline in metabolic activity, especially in the activity of RNA polymerase action (Fig. 1). The end of the growth phase marks the beginning of dormancy. Dormant tissues, if wounded, increase their transcriptive activity in order to repair the dam-