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Changes of purine and pyrimidine nucleotide biosynthesis during shoot initiation from epicotyl explants of white spruce (*Picea glauca*)

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Abstract

Nucleotide metabolism was investigated during white spruce organogenesis by following the metabolic fate of ^{14}C-labeled adenine, adenosine and inosine, as purine precursors, and orotic acid, uridine, and uracil, as pyrimidine intermediates. Key enzymes of purine and pyrimidine metabolism were also assayed during the organogenic process. White spruce epicotyl explants cultured on shoot-forming (SF) medium had a better ability to utilize adenine and adenosine for nucleotide and nucleic acid synthesis, compared to tissue cultured on non-shoot forming (NSF) medium. High levels of salvage products were observed in SF tissue after 10 days in culture, when shoot formation was initiated along the epicotyl axis of the explants. Such a differential utilization of purine precursors was mainly due to the higher specific activity of the two adenine and adenosine salvage enzymes, adenine phosphoribosyltransferase (APRT) and adenosine kinase (AK), measured in SF tissue. Similar catabolism of inosine was observed in both SF and NSF conditions during the 30 days of culture. For pyrimidines, the higher activities of the de novo, salvage, and degradation pathways observed in SF tissue, compared to NSF tissue throughout the course of the experiment, clearly denote a faster turnover of pyrimidine nucleotides in the former. Taken together, these results suggest that a better utilization of purine bases and nucleosides for nucleotide and nucleic acid synthesis, as well as a more rapid turnover of pyrimidine nucleotides, represent a physiological switch, which occurs during the initiation and continuation of the organogenic process in white spruce.

Keywords: De novo organogenesis; Nucleic acid synthesis; Purine bases and nucleosides; Purine salvage enzymes; Pyrimidine nucleotide turnover; Salvage products

1. Introduction

In the last few years, an increasing effort has been directed towards the in vitro propagation of white spruce (*Picea glauca*), which represents an economically important species in North America [1]. Although somatic embryogenesis of white spruce is preferentially utilized for large scale propagation, as well as a model system for physiological and biochemical studies during embryogenesis of conifers [2,3], shoot induction via organogenesis represents an alternative in vitro propagation technique for this species [4,5]. In response to cytokinin, in fact, a large number of new shoot primordia are formed from excised epicotyls of white spruce, without intervening callus formation [4,6]. As such, this system can be of valuable use for exploring aspects of primary metabolism occurring during direct shoot initiation.

Metabolic studies conducted during organogenesis of tobacco and *Pinus radiata* have revealed that the cytokinin-induced switch in development, leading to shoot formation, reflects

Abbreviations: ARN, adenosine nucleosidase; APRT, adenine phosphoribosyltransferase; AK, adenosine kinase; IK, inosine kinase; NPT, nucleoside phosphotransferase; NSF, non-shoot forming; OPRT, orotate phosphoribosyltransferase; SF, shoot forming; UPRT, uracil phopshoribosyltransferase; UK, uridine kinase

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alterations in macromolecule synthesis and accumulation, as well as changes in activity of many enzymes [7,8]. Differential accumulation and utilization of starch, carbon and nitrogen under shoot forming (SF) and non-shoot-forming (NSF) conditions have been documented. Changes in nucleic acid synthesis is also an early event during the organogenic process, as increasing staining for both DNA and RNA, as well as increased incorporation of labeled thymidine and uridine into these fractions was reported in *P. radiata* [9]. Such changes clearly suggest that alterations in the synthesis of purine and pyrimidine nucleotides must occur during the early events of shoot initiation, in order to support the increased nucleic acid synthesis.

As reviewed previously [10,11], purine and pyrimidine nucleotides are important metabolites involved in a variety of cellular processes, including synthesis of nucleic acids, sucrose, polysaccharides, phospholipids, and other secondary products. In plants, synthesis of nucleotides can occur de novo, from amino acids and other small molecules, or through a salvage mechanism which utilizes preformed bases and nucleosides [11–13]. In addition, independent degradation pathways are responsible for nucleotide catabolism [14,15]. Changes of these pathways often relate to differentiation and development [16,17]. Extensive studies conducted during somatic embryogenesis of white spruce, for example, have revealed that major alterations of de novo, salvage, and degradation pathways occur during the process [18–26]. Specifically, an increased contribution of the de novo and salvage pathways to nucleotide synthesis, as well as a reduction of the activity of the degradation pathway, demarcate important developmental events, such as somatic embryo germination [22–24]. These findings, also substantiated by investigations conducted on in vivo systems [27,28], clearly demonstrate the importance of nucleotide metabolism for normal growth and development.

As a part of our comparative study between somatic embryogenesis and organogenesis of white spruce, it was our objective to investigate whether alterations in synthesis and utilization of purine and pyrimidine nucleotides represent a switch needed for shoot initiation in white spruce. This was achieved by following the metabolic fate of exogenously supplied bases and nucleosides in white spruce epicotyls cultured under shoot-forming (SF) and non-shoot-forming (NSF) conditions. For purines, ^{14}C-labeled adenine and adenosine were utilized as markers for the salvage pathway, and inosine as intermediate of the degradation pathway (Fig. 1). For pyrimidines, ^{14}C-labeled orotic acid, uridine, and uracil were used for investigating the rate of the de novo, salvage, and degradation pathway, respectively (Fig. 2). Furthermore, the key enzymes of both purine and pyrimidine metabolism were measured.

2. Materials and methods

2.1. Plant material and culture conditions

White spruce seeds (lot # 7431580.1; germinability 92%) were obtained from the National Tree Seed Center, Fredericton, N.B., Canada. After sterilization in 25% Javex bleach for

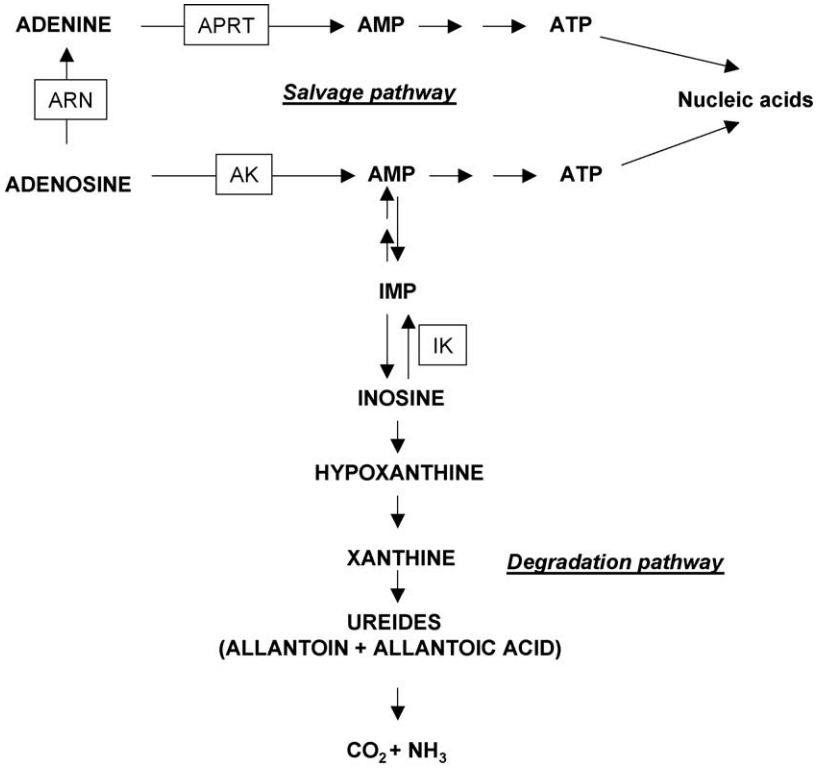

Fig. 1. Possible metabolic fate of labeled adenine, adenosine, and inosine in epicotyls of white spruce embryos. Enzymes measured are enclosed in boxes. APRT, adenine phosphoribosyltransferase; ARN, adenosine nucleosidase; AK, adenosine kinase; IK, inosine kinase; IMP, inosine monophosphate.