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Glutathione-induced growth of embryogenic tissue of white spruce correlates with changes in pyrimidine nucleotide metabolism

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Abstract

Exogenous applications of reduced glutathione (GSH) and oxidized glutathione (GSSG) promote growth of embryogenic tissue of white spruce during a 7-day subculture period. Compared to control tissue, a statistically significant increase in fresh weight, as well as RNA and DNA content, was observed in the presence of GSH and GSSG during the last days in culture. The effects of these two metabolites on pyrimidine nucleotide metabolism was investigated by following the metabolic fate of ^{14}C-orotic acid, a precursor of the de novo synthesis, and ^{14}C-uridine and ^{14}C-uracil, intermediates of the respective salvage and degradation pathways. Compared to control embryos, GSH-treated tissue was able to utilize a larger fraction of supplied orotic acid for UMP production, possibly due to the increased activity of orotate phosphoribosyltransferase (OPRT). The activity of this enzyme increased markedly in tissue cultured with GSH during the last days in culture. Salvage of uridine for nucleotide and nucleic acid synthesis was observed in all treatments, especially in GSSG-treated tissue at day 7. The increased salvage activity in this tissue correlated with the increase in activities of the two uridine salvage enzymes, uridine kinase (URK) and nucleoside phosphotransferase measured with uridine (NPT(uridine)). Compared to control tissue, tissue treated with either GSH or GSSG was able to utilize a large fraction of uracil for nucleotide synthesis, denoting a better ability to divert this precursor from degradation. Nucleotide and nucleic acid analyses revealed that in both GSH and GSSG-treated tissue, the endogenous levels of UTP, CTP, as well as those of RNA and DNA, were increased compared to those of control tissue. Overall, the results from this study suggest that GSH and GSSG can induce growth of embryogenic tissue of white spruce through distinct metabolic changes of pyrimidine nucleotides.

Keywords: Embryogenic tissue proliferation; Glutathione; *Picea glauca*; Pyrimidine metabolism

1. Introduction

Somatic embryogenesis, the production of embryos in culture, can only be successfully achieved under appropriate culture conditions which allow proliferation of somatic cells and subsequent development into mature embryos. This is especially true for conifer species, including spruce, which compared to flowering plants, are more difficult to propagate in culture. The initial step of the overall embryogenic process is the generation of embryogenic tissue and its maintenance in proliferation medium. Depending on genotypes and explant types, i.e. mature or immature zygotic embryos, the rate of proliferation can vary a lot and affect the embryogenic developmental program [1]. Low proliferation of the embryogenic tissue can be a serious problem if somatic embryogenesis has to be used for propagation purposes where a large quantity of somatic embryos must be generated in a short period of time.

Although somatic embryogenesis in spruce has been the subject of many structural, physiological, and molecular investigations, most of them deal with the developmental and maturation phase of the embryogenic process [1–3].

Abbreviations: GSSG, oxidized glutathione; GSH, reduced glutathione; NPT(uridine), nucleoside phosphotransferase measured with uridine; OPRT, orotate phosphoribosyltransferase; UDPG, UDP-glucose; URK, uridine kinase; UPRT, uracil phosphoribosyltransferase

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One of the few studies dealing with embryogenic tissue proliferation, examines the role of glutathione and its redox state [4]. Reduced glutathione (GSH) and its oxidized form (GSSG) have been shown to participate in a variety of diverse physiological events in both animals and plants [5–7]. Alterations in the ratio of the two forms within the overall glutathione pool regulate gene expression [8–10] and affect cell division and differentiation [3]. In particular, GSH may have a direct role in cell division processes, as high concentrations of this metabolite are found in rapidly growing cells [11]. In agreement with this observation, applications of GSH in the maintenance medium have been found to increase the rate of embryogenic tissue proliferation of white spruce. In this study, it was also demonstrated that the GSH-induced cell division may be the result of profound changes in the pattern of synthesis and utilization of purine nucleotides [4]. In particular, high levels of both adenine and adenosine utilization for nucleoside monophosphate and diphosphate synthesis were measured in GSH-treated tissue, suggesting that this metabolite may induce cell division by affecting energetic processes [4]. However, so far there is no information on the effect of glutathione on pyrimidine metabolism. This is important since pyrimidine nucleotides, like their purine counterparts, are precursors of nucleic acid biosynthesis and are major metabolites involved in bio-energetic processes and in several metabolic cycles. Comprehensive studies on pyrimidine biosynthesis in several plant species have revealed the presence of two independent pathways for pyrimidine nucleotide synthesis [12–14]. Pyrimidine nucleotides can be synthesized de novo from small precursor molecules [18] or via salvage mechanisms which utilizes bases and nucleosides [19]. A pathway for the degradation of pyrimidine nucleotides has also been reported (reviewed in [20]).

As an extension of previous work [4] and to gain a better understanding on the effects of glutathione on nucleotide metabolism, it is the objective of the present study to analyze the changes in pyrimidine nucleotide synthesis and utilization in embryogenic tissue of spruce treated with GSH or GSSG. This was achieved by following the metabolic fate of radiolabeled orotic acid, precursor of the de novo pathway and uridine and uracil, respective intermediates of the salvage and degradation pathways of pyrimidine metabolism (Fig. 1). In addition, the specific activity of key enzymes involved in these pathways was also measured.

2. Material and method

2.1. Plant material and glutathione application

Maintenance of white spruce (*Picea glauca*) embryogenic tissue and exogenous applications of GSH or GSSG

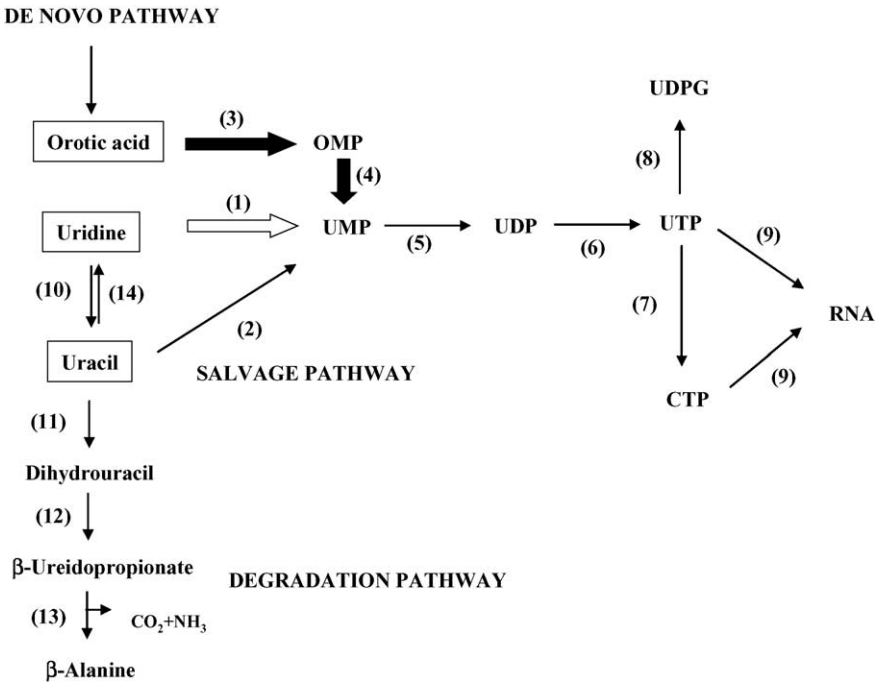

Fig. 1. Possible metabolic fate of exogenously supplied orotic acid, uridine, and uracil in white spruce embryogenic tissue. Enzymes: (1) uridine kinase and/or nucleoside phosphotransferase; (2) uracil phosphoribosyltransferase; (3) orotate phosphoribosyltransferase; (4) orotidine-5′-monophosphate decarboxylase; (5) nucleoside monophosphate kinase; (6) nucleoside diphosphate kinase; (7) CTP synthase; (8) UDP-glucose pyrophosphorylase; (9) RNA polymerase; (10) uridine nucleosidase; (11) uracil reductase and/or dihydrouracil dehydrogenase; (12) dihydropyriminase; (13) ureidopropionase; (14) uridine phosphorylase. Solid bold arrows indicate GSH-induced up-regulation of the de novo pathway. Transparent bold arrow illustrates increased activity of the salvage pathway in the presence of GSSG.