Capacity for RNA Synthesis in 70S Ribosome-deficient Plastids of Heat-bleached Rye Leaves

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Abstract. In the leaves of rye seedlings (Secale cereale L.) grown at an elevated temperature of 32°C the formation of plastidic 70S ribosomes is specifically prevented. The resulting plastid ribosome-deficient leaves, which are chlorotic in light, represent a system for the identification of translation products of the 80S ribosomes among the chloroplastic proteins. Searching for the primary heat-sensitive event causing the 70S ribosome-deficiency, the thermostability of the chloroplastic capacity for RNA synthesis was investigated. The RNA polymerase activity of isolated normal chloroplasts from 22°C rye leaves was not inactivated in vitro at temperatures between 30°C and 40°C. The ribosome-deficient plastids purified from bleached 32°C-grown leaf parts contained significant RNA polymerase activity which was, however, lower than in functional chloroplasts. After application of [3H]uridine to intact leaf tissues [3H]uridine incorporation was found in ribosome-deficient plastids of 32°C-grown leaves. The amount of incorporation was similar to that in the control chloroplasts from 22°C-grown leaves. According to these results, it is unlikely that the non-permissive temperature (32°C) causes a general inactivation of the chloroplastic RNA synthesis in rye leaves.

Key words: Chloroplast biogenesis; RNA) Ribosomes, plastidic – RNA (chloroplastic) – RNA polymerase – Secale – Temperature and RNA polymerase.

Introduction

In several higher plant species the formation of the chloroplast ribosomes exhibits a preferential high-temperature sensitivity (Feierabend and Mikus 1977). At a suitable non-permissive elevated temperature, usually between 32°C and 34°C, the accumulation of 70S ribosomes and of their rRNA in the leaves is prevented, while seedlings of such plants are still able to grow with little impairment (Feierabend and Schrader-Reichhardt 1976; Schäfers and Feierabend 1976; Feierabend 1979). However, defective plastids develop and the 70S ribosome-deficient parts of leaves grown in light are chlorotic (Schäfers and Feierabend 1976). Our investigations with rye seedlings have shown that the non-permissive high temperature appeared to interfere quite specifically with some step in the biosynthesis or assembly of the 70S ribosomes, and its detrimental effects were confined to the development of the plastids (Feierabend and Schrader-Reichhardt 1976; Schäfers and Feierabend 1976; Feierabend 1979). Proteins which have to be synthesized on 70S ribosomes were missing. However, many other chloroplast proteins which are translation products of the cytoplasmic 80S ribosomes were still synthesized and accumulated and, therefore, 70S ribosome-deficient leaves from 32°C-grown rye seedlings serve as a system for studying the role of the cytoplasm in the synthesis, transport, and assembly of chloroplast proteins (Feierabend and Schrader-Reichhardt 1976; Feierabend 1977; Feierabend and Wildner 1978; Feierabend 1979).

According to present knowledge (Bogorad 1975; Boynton et al. 1976; Harris et al. 1977; Kowallik and Herrmann 1977; Bedbrook and Kolodner 1979), the rRNA of the chloroplastic 70S ribosomes and the mRNA for some ribosomal polypeptides have to be transcribed from the chloroplast DNA. Since the chloroplastic rRNA was no longer detectable in 32°C-grown rye leaves, chloroplastic RNA synthesis appeared to be a conceivable candidate for the heat-sensitive step which might be blocked under the non-permissive temperature conditions. In addition, Brandt and Wiessner (1977) have reported that in Euglena, with which a heat-bleaching phenomenon
has been observed for some time (Pringsheim and Pringsheim 1952) and which is also accompanied by a 70S ribosome-deficiency (Cohen and Schiff 1976), the chloroplastic RNA polymerase was inactivated in vitro at the bleaching temperature. Therefore, we have currently investigated whether the RNA synthesis activity of rye chloroplasts is inactivated in vitro or in vivo at the non-permissive high temperature.

Material and Methods

Plant Material and Growing Conditions. Seedlings of winter rye (Secale cereale L) cv. Petkus “Kusto” were grown for 5 or 6 days, as indicated, in the light (5,000±500 lx) in glass-covered plastic boxes on filter paper moistened with either distilled water or nutrient solution (only for material of Fig. 5), at either 22° or 32° C (Feierabend and Schrader-Reichhardt 1976).

Isolation of Plastids. Leaf tips were discarded and the lower parts of the leaf material (completely chlorotic tissue of 32° C-grown leaves) was surface sterilized in a 1% solution of sodium hypochlorite; then they were rinsed several times with sterilized distilled water, blotted dry, and cooled on ice. First the resulting tissue was finely minced with razor blades in 2–3 volumes of ice-cold grinding medium and then briefly and gently ground in a mortar in the presence of a small quantity of sea sand. To measure the RNA-polymerase activity, homogenization was performed in Bottomley’s medium (1970), except that 0.1 M tricine-KOH buffer, pH 8.0, was used. After incorporation of [3H]Uridine into the intact leaves, the tissue was homogenized in the grinding medium described by Feierabend and Bevers (1972), with the following modifications: 4 mM diithreitol was used instead of ascorbate; ficoll was omitted; and 0.01% (w/v) bovine serum albumin was added. The homogenates were pressed through four layers of muslin and four layers of Miracloth. The sediment obtained after 20 s centrifugation at 2,000 g was finely minced with razor blades in 2–3 volumes of ice-cold grinding medium containing 50 μg per ml pancreas RNAase and 8 μg per ml T1-RNAase. Then they were incubated for 20 min at 4° C in order to remove cytoplasmatic RNAs contaminating the plastids at their outer surface. At the end of the ribonuclease treatment the plastids were pelleted (2 min, 4,300 g), resuspended in fresh grinding medium (0.5 ml per g initial fresh weight), layered onto the sucrose gradients, and centrifuged, as described above.

The total content of [3H] and the incorporation of [3H]Uridine into material precipitated by 10% trichloroacetic acid (final concentration) was determined in the various fractions obtained. Per 0.5 ml of the sucrose gradient fractions 0.2 ml of a 0.5% yeast RNA solution was added before precipitation. The precipitates were washed three times with 5% trichloroacetic acid, 80% ethanol, and finally with ether. The washed precipitates were solubilized in hyamine hydroxide or tissue solubilizer TS 1 (Koch-Light Laboratories, Ltd.) and the radioactivity was counted after the addition of Aquasol scintillation fluid. An internal standard was used for the estimation of dps.

Portions of the main plastid-containing gradient fractions were pooled, diluted with an equal volume of 50 mM Tris-HCl, pH 7.6, containing 10 mM MgSO4, after which 0.5 mg ml−1 yeast RNA was added. After lysis by treatment with 0.2 mg ml−1 Proteinase K in the presence of 0.5% sodium dodecylsulfate and extraction with phenol-chresol (Parish and Kirby 1966) the nucleic acids were precipitated with two volumes of ethanol at −20° C. The dried precipitate was dissolved in 50 mM Tris-HCl, pH 7.6, containing 10 mM MgSO4 and incubated with 0.1 mg ml−1 DNAase l (20 min 37° C). Samples of the solution were then dried on filter-paper discs (Whatman 3). The filter discs were washed with 10% trichloroacetic acid, 5% trichloroacetic acid, ethanol; ether (3:1), and ether. They were then dried and counted for radioactivity.

Analytical Methods. Enzyme activities. NADP-dependent glyceraldehydehydrolase dehydogenase (EC 1.2.1.13) and fructose-1,6-bisphosphate aldolase (EC 4.1.2.13) were assayed as described previously (Feierabend and Schrader-Reichhardt 1976). RNA polymerase activity (EC 2.7.7.6) was assayed according to Bottomley et al. (1971) with the following procedural modifications: the assay mixture (0.25 ml) additionally contained chloramphenicol (6.25 μg per 0.25 ml) and calf thymus DNA (33 μg per assay; Bottomley et al. 1972) instead of maize DNA; and the reaction was started by adding 185 KBq 5.62H]UTP (for Figs. 1 and 2: 74 KBq per assay). If not otherwise indicated, the incubation time was 30 min at 37° C. After termination of the reaction 1 ml of a 0.2% yeast RNA solution was added per assay. The acid insoluble precipitates were centrifuged, washed (2:5% trichloroacetic acid+6.0005% UTP, 3:5% trichloroacetic acid in 50 mM sodium pyrophosphate, 1:80% ethanol, 1:ether), and solubilized in hyamine hydroxide or tissue solubilizer TS-1. The samples were added to an Aquasol scintillation fluid and the radioactivity was counted.

Chlorophyll was estimated according to Whatley and Arnon (1963). Sucrose concentrations were determined with a Zeiss Abbe-refractometer, Model A.

Results

RNA Polymerase Activity of Isolated Chloroplasts. Gradient-purified intact chloroplasts obtained from 22° C-grown green rye leaves which were broken by osmotic shock in the assay medium possessed RNA polymerase activity. The in vitro incorporation of [3H]UTP into trichloroacetic acid precipitable material required the presence of the three other nucleotides, ATP, CTP, and GTP, was sensitive to ribonuclease treatment, and, over a wide range, was proportional to the amount of chloroplast material added to the assay (Fig. 1). The incorporation of [3H]UTP was most rapid during the first 20 min and ceased after about 45 min of incubation (Fig. 2). Between 25° C and 42° C the rate of [3H]UTP incorporation increased but had not yet reached its optimum so