

## Low temperature-induced changes in the relative amounts of ribosomal RNAs in potato mitochondria

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### Abstract

Field-harvested tubers of the potato cultivars Russet Burbank, Kennebec and ND 651-9, were stored at low (3°C) or at higher (18°C) temperature for three weeks. Intact mitochondria were isolated from the stored tubers and were purified by Percoll gradient centrifugation. Mitochondrial RNA was isolated from the purified mitochondria in triaurincarboxylic acid- and laurylsarkosine-containing media and resolved by electrophoresis in formaldehyde-containing agarose gels. The ribosomal RNA (rRNA) bands were visualized by ethidium bromide staining and quantified by densitometric scanning of the agarose gel. Storage at low temperature resulted in the appearance of a large size precursor rRNA and in decline in the relative amounts of fragments derived from the 26S rRNA in all cultivars and selections. The results indicate a possible effect of low temperatures on proper mitochondrial ribosomal RNA processing and its significance to mitochondrial activity is discussed.

**Key words:** Cold treatment, mitochondria, potato, ribosomal RNA.

### Introduction

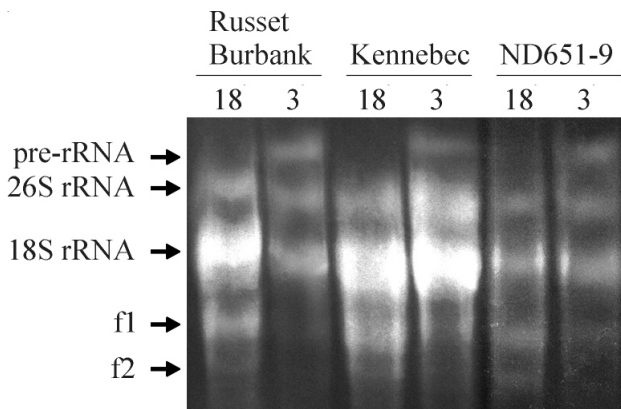
Low temperature has a profound effect on mitochondria. It causes a gradual change in the lipid desaturation pattern and alterations in the relative percentages of the various classes of membrane lipids<sup>1,2,3</sup>. In addition, cold treatment causes lower cytochrome c oxidase and respiration activity<sup>4,5,6,7,3</sup>. Mitochondrial ATPase activity is inhibited by cold and a cyanide-insensitive respiration appears<sup>8,9</sup>. There is also a previous report<sup>10</sup> of cold-mediated inhibition of proper splicing of the potato invertase gene and even more importantly an inhibition of human mitochondrial rRNA processing<sup>11</sup>. If low temperature, indeed, inhibits rRNA processing and maturation of functional potato mitoribosomes, then a low temperature-induced decline in the amounts of mitochondrially coded cytochromes and other energy related enzymes would impair respiration. Like other plant tissues<sup>12</sup>, potato tubers exposed to low temperatures (0 to 6°C) gradually accumulate sugars<sup>13</sup>. Reaction between accumulated reducing sugars and free amino-compounds during processing results in undesirable darkening of the various potato products<sup>14,15</sup>. The hypothesis that mitochondria might be involved in cold-induced sweetening is supported by results of crosses between cultivars of different sugar accumulating ability in which an element of maternal inheritance is suggested<sup>16</sup>. Previous studies<sup>17</sup> demonstrated that the mitochondrial genome of high sugar accumulating potato cultivars differs from that of low sugar accumulating genotypes. It has also been shown<sup>18,19</sup> that a 26-kDa mitochondrially-coded polypeptide, different than the cyanide-resistant oxidase, can be detected among the mitochondrial proteins of sugar accumulating potato cultivars exposed to low temperatures. It has been hypothesized before<sup>17,19,20</sup> that mitochondrial dysfunction could contribute to sugar accumulation. In this work, an effect of low temperatures on potato mitochondrial ribosomal RNAs is presented as an additional evidence, supportive of the proposed theory<sup>20</sup> for a causative role of inhibition of energy-producing processes in sugar accumulation in plant tissues.

### Materials and Methods

Field-harvested potato tubers of cultivars Russet Burbank, Kennebec and of the selection ND651-9 were stored at 3°C and at 18°C for three weeks after harvest and before mitochondria isolation. Tuber mitochondria were isolated and purified by Percoll gradient centrifugation as described previously<sup>18</sup>. The mitochondria were stored as a pellet at -80°C until use. All solutions and glassware used in RNA analysis were treated with diethyl pyrocarbonate (DEP), with the exception of the microfuge tubes that were used directly from a freshly opened container. The mitochondrial pellet obtained from 200 g fresh weight potato tubers was resuspended in 500 mL of 0.1M MOPS, 1mM triaurincarboxylic acid, 1% w/v sodium lauroylsarkosine, 40 mM sodium acetate, 5 mM EDTA, pH 7.0 buffer, and extracted four times with phenol/chloroform. The RNA was precipitated overnight at 4°C after addition of an equal volume of 4M LiCl, recovered by a 30min microcentrifugation, and resuspended in 10µL of DEP-treated autoclaved distilled water. The RNA was denatured by addition of an equal volume (10 µL) of denaturing buffer (200 mM MOPS, 5 mM EDTA, 12% v/v formaldehyde, 50% v/v deionized formamide) to each sample, incubation at 60°C for 20 min, and immediate chilling in ice. Five µl of loading buffer (100mM MOPS, pH 7.0, 50% v/v glycerol, 0.2% w/v bromophenol blue) were added to each sample before they were analyzed by electrophoresis at 60 V in a 2% w/v agarose gel containing 200mM MOPS, pH 7.0, 7% v/v formaldehyde (17 mL of 37% v/v formaldehyde in 100mL agarose gel). After ethidium bromide staining of the gel, the rRNA bands were visualized under UV light and photographed using a Kodak Polaroid 55 positive/negative film at exposure time 45 s and lens at maximum aperture. The percentage of each ribosomal RNA band was estimated by scanning the lanes in the developed negatives, by employing a Biorad Model 620 densitometer operating in the light transmission mode, and processing the data in a Gaussian format peak analysis using the provided data processing software.

## Results

In this work, the 26S and 18S rRNA bands, of approximate size 3.3 and 2.3 kb respectively, were easily detected in mitochondrial RNA extracts from tubers of all cultivars and at both temperature treatments (Fig. 1). Smaller size bands of approximately 1.4 and 1.1kb (f1 and f2 in Fig.1), were also detected. Like in cytoplasmic rRNA processing, mitochondrial rRNAs are first synthesized as a precursor transcript of about 4 kb, that is then processed into the individual mature rRNA species<sup>21</sup>.



**Figure 1.** Ethidium bromide-stained agarose gels of mitochondrial rRNA bands from tubers of various potato cultivars. The tubers were stored for three months to two different temperatures (3°C and 18°C) before mitochondria isolation. The total amount of RNA per lane was as follows. Russet Burbank 18°C: 20 mg; Russet Burbank 3°C: 10 mg; Kennebec 18°C: 30 mg; Kennebec 3°C: 30 mg; ND651-9 18°C: 10 mg; ND651-9 3°C: 10 mg. Low temperature (3°C) storage induced the appearance precursor rRNA (pre-rRNA) of about 4 kb. It also caused the relative decline of fragments f1 and f2 derived from splitting of the 26S rRNA. In this work, such a precursor molecule (pre-rRNA) was detected only in mitochondria from low temperature-treated (4°C) potato tubers. Densitometric scanning of the agarose gels has shown that low temperature treatment also causes a decline in the relative amounts of the 1.4 and 1.1 kb fragments (Table 1).

**Table 1.** Relative amounts (%) of potato mitochondrial rRNA bands resolved by electrophoresis on agarose gels, stained with ethidium bromide and scanned densitometrically. Mitochondria were isolated from potato tubers stored at two different temperatures (3°C and 18°C).

| rRNA band | 3°C  |      | 18°C |      |
|-----------|------|------|------|------|
|           | %    | s.d. | %    | s.d. |
| pre-rRNA  | 15.6 | 3.3  | 20.4 | 2.2  |
| 26S rRNA  | 22.7 | 5.7  | 45.3 | 4.2  |
| 18S rRNA  | 42.8 | 3.9  | 27.3 | 8.9  |
| f1        | 14.5 | 5.7  | 7.1  | 5.8  |
| f2        | 4.4  | 2.3  |      |      |

f1, f2: fragments derived from splitting of the 26S rRNA.

## Discussion

Two major bands of size 3.3 and 2.3 kb, identified as 26S and 18S rRNA respectively, and smaller rRNA fragments, considered to result from fractionation of the 26S rRNA molecule, are detected when mitochondrial RNA is analyzed by agarose gel electrophore-

sis<sup>22,23,24</sup>. Splitting of the large ribosomal subunit rRNA to produce at least three fragments is also occurring with the 23S chloroplastic rRNA, and is attributed to the omission of Mg<sup>++</sup> and Ca<sup>++</sup> in EDTA-containing RNA isolation media<sup>25,26</sup>. Mg<sup>++</sup> and also Ca<sup>++</sup> seem to stabilize the rRNA structure and to inhibit nicking. Divalent cations were omitted from the RNA isolation media used in this work. No splitting of the small ribosomal subunit chloroplastic or mitochondrial rRNA has ever been observed in any of the examined organisms. The cytoplasmic rRNAs of all examined plants or animals are also stable, with the exception of the large ribosomal subunit rRNAs of *Amoeba* and *Euglena*. The unprocessed pre-rRNA band becomes visible after cold treatment and can reach the very significant relative amounts of over 15%. The usually observed nicking of the 26S rRNA to produce fragments f1 and f2 is also relatively less effective as it can be inferred from the lower percentages of these bands in cold-treated tubers. The overall picture is one of inhibition of rRNA processing by low temperatures. Cold-mediated inhibition of mitochondrial rRNA processing has been detected before in humans<sup>11</sup>. In this work, this has been confirmed for potato mitochondria as well. Protracted for a long time, it would be expected to result in translationally less active mitochondria with a much-reduced complement of mitochondrially-coded proteins. In fact, reduced amounts of cytochromes of the oxidative phosphorylation electron transport chain in cold-treated tissues have been reported before<sup>4,5</sup>. It has been proposed<sup>20</sup> that an inhibited electron flow and a failing ATP synthesis would lead to calcium and phosphate ion redistribution between the cytoplasm, cell exterior and cell organelles, that in turn would result in mobilization of starch and increased intracellular reducing sugar levels. The data obtained in this work are presented as additional supporting evidence for the involvement of mitochondria in cold-inducible sugar accumulation in plant tissues. Nevertheless, as it has been already pointed out<sup>20</sup>, this by no means implies that mitochondrial respiration is the sole participant in sugar accumulation but only one of several involved energy-generating processes.

## Conclusions

This is another evidence for an effect of low temperatures on mitochondrial function. Considered together with a number of other published work on this subject, as discussed in the introduction and discussion sections, it strengthens the idea of an involvement of mitochondria in sugar accumulation in plant tissues through a cold-inducible inhibition of their energy-producing activity.

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