Mitochondrial events during development of thermogenesis in *Sauromatum guttatum* (Schott)

Thomas E. Elthon*, Roxy L. Nickels, and Lee McIntosh**
MSU-DOE Plant Research Laboratory and Biochemistry Department, Michigan State University, East Lansing, MI 48824-1312, USA

Abstract. Changes in the mitochondrial electron-transport chain were followed in the thermogenic inflorescence of *Sauromatum guttatum* Schott from 5 d before thermogenesis to 3 d thereafter. The capacities of the alternative and cytochrome pathways of mitochondrial electron transport were found to be developmentally coordinated to contribute to the thermogenic events in the appendix and the sterile floral regions. Electron flow through the alternative pathway is believed primarily responsible for heat production, and this pathway was expressed to the highest degree in both tissues during thermogenesis. In the appendix, the cytochrome chain was shut down considerably during thermogenesis, forcing electron flow through the alternative pathway and thus yielding maximum heat production. The shut-down of the cytochrome chain does not occur in the sterile floral region which may explain why this region is not as thermogenic as the appendix. Cytochrome-oxidase difference spectra indicated that the cytochrome oxidase of appendix mitochondria was not capable of accepting electrons on the day of thermogenesis, and that this capacity was partially restored by the following day even though the tissue was senescing at this time point. Relative levels of messenger RNAs for cytochrome-oxidase subunits I and II were found to decrease the day before thermogenesis, which could result in lower levels of these proteins in appendix mitochondria on the day of thermogenesis.

The capacity for overall mitochondrial protein synthesis was also investigated and was found to drop continuously from 5 d before thermogenesis to 3 d thereafter, even though the capacities of the electron-transport chain were changing dramatically. The levels of mitochondrial ribosomal RNA levels decreased during development, which could explain the overall drop in mitochondrial translational efficiency. Experiments concerning the synthesis of the alternative-oxidase proteins indicated that they were most likely nuclearly encoded, and that their expression could be induced by salicylic acid.

Key words: Alternative pathway (respiration) – Cytochrome oxidase – Mitochondrion – Respiration, alternative – Salicylic acid – *Sauromatum* – Thermogenesis

Introduction

Thermogenic respiration in plants has fascinated biologists since its discovery by Lamarck in 1778 (see Meeuse 1966). Although thermogenicity has been found in many plant species, only a few have been readily available for biochemical investigations. These include the Aroids *Arum italicum* Mill. and *Arum maculatum* L. which are native to Europe, *Symlocarpus foetidus* L. (eastern skunk cabbage) which is plentiful in northeastern North America, and the tropical Aroid *Sauromatum guttatum* Schott (voodoo lily) of which a population has been established by Bastiaan Meeuse (Botany Department, University of Washington, Seattle, USA). The inflorescence of these species consist of a spadix surrounded by a leaf-like spathe. In *Arum* and *Sauromatum*, the sterile appendix region of the spadix becomes thermogenic for a short peri-
od of time (about 7 h) during development, effect-
ing volatilization of amines to attract insects in a 2-d cross-pollination scheme (Meeuse 1966). Heat production in all of these spadices is believed to result primarily from electron flow through the alternative pathway of the mitochondrial electron-transport chain (Day et al. 1980).

We have previously solubilized and characterized the alternative oxidase of *Sauromatum guttatum* (Elthon and McIntosh 1986). This made it possible to purify the alternative oxidase and to identify the oxidase as a cluster of proteins with apparent molecular weights of 37, 36, and 35 kilodaltons (kDa) (Elthon and McIntosh 1987). Polyclonal and monoclonal antibodies produced to the alternative oxidase of *Sauromatum* were found to cross-react with *Arum* and *Sympllocarpus* alternative-oxidase proteins, as well as those of a number of other species (Elthon and McIntosh 1987; Elthon et al. 1989). Thus, these antibodies will be useful for investigating the alternative pathway in other systems. In this paper we report the results of further studies of the changes that occur in mito-
chondria of the spadix of *Sauromatum* during its development. We have found that these mito-
chondria are continuously modified during spadix de-
velopment, with these changes contributing greatly to the physiology that underlies the thermogenic event.

**Material and methods**

*Plant material and mitochondria isolation. Sauromatum guttatum* Schott (voodoo lilies) were maintained in a greenhouse at 27 ± 2°C as previously described (Elthon and McIntosh 1986). Washed mitochondria from various regions of the inflorescence were isolated by the method of Schwitzguebel and Siegenthaler (1984), and purified on sucrose gradients according to Douce et al. (1972). Purified mitochondria were resuspended in reac-
tion media consisting of 250 mM sucrose and 30 mM 3-(N-morpholino)propanesulfonic acid (Mops) (pH 6.8). Total mitochondrial protein was estimated using a modified Lowry assay (Larson et al. 1986).

**Determination of electron-transport-chain capacities.** Mitochondrial activities and electron-transport-pathway capacities were determined as in Elthon and McIntosh (1986) using 1 mM NADH as the substrate. After an initial rate of NADH oxida-
tion was established, the mitochondria were uncoupled through addition of 0.5 μM p-trifluoromethoxycarbonyl cyanide (FCCP) before estimating the electron-transport-pathway capacities. The capacity of the alternative pathway was taken as that oxy-
gen uptake in the presence of 1 mM KCN that was sensitive to 1 mM salicylhydroxamic acid (SHAM), and the cytochrome pathway as that sensitive to KCN in the presence of SHAM. Oxygen-uptake assays were performed at 25°C in 1 ml of reaction medium using a Rank Brothers (Cambridge, UK) electrode. The oxygen content of air-saturated water was estimated according to Estabrook (1967).

**In-organelle translations.** Mitochondrial protein synthesis was studied with isolated mitochondria essentially as described by Leaver et al. (1983). Mitochondria were isolated from tissue employing sterile buffers and in a sterile hood, and were incubated for 90 min in a translation mixture (see above) containing in part 35S]methionine and an unlabeled mixture of the other amino acids. During this incubation, mRNAs present in the mitochondria are translated, resulting in radiolabelling of the mitochondrially encoded proteins. The energy mixture used for the mitochondrial translations consisted of creatine phosphatase (25 μg per reaction) plus 8 mM creatine phosphate and 6 mM ATP. Separation of mitochondrial proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transfer of these proteins to nitrocellulose, and antibody probing were carried out as before (Elthon and McIntosh 1987). Autoradiography of labelled mitochondrial proteins was done using X-OMAT AR film (Eastman-Kodak, Rochester, N.Y., USA) after their transfer to nitrocellulose. To determine the amount of radioactivity present in a protein band, that region of the nitrocellulose was excised, placed in a scintillation vial, and solubilized with aceton (1 ml). After addition of 9 ml of scintillation solution (Ready-Solv CP; Beckman, Palo Alto, Cal., USA), the radioactivity present was determined by liquid scintillation counting.

**Isolation of mitochondrial nucleic acids.** Mitochondria used for isolation of total RNA were isolated and purified in media treated with diethylpyrocarbonate to inactivate RNases (Blumberg 1987). The purified mitochondria were solubilized in a solubilization buffer consisting of diethylpyrocarbonate-treated ENT buffer (15 mM ethylenediaminetetraacetic acid (EDTA), 150 mM NaCl, and 10 mM 2-amino-2-hydroxyethyl)-1,3-propanediol (Tris), pH 7.6) plus 1% (w/v) N-lauroylsarcosine. Solubilization was for 10 min on ice with occasional stirring. The nucleic acids were partially purified through three phenol extractions, one chloroform: phenol:isoamyl alcohol (25:24:1, by vol.) extraction, and one chloroform:isoamyl alcohol (24:1, w/v) extraction. All extraction solutions were saturated with diethylpyrocarbonate-treated ENT buffer. The nucleic acids were then further purified by two ethanol precipitations and a 70% ethanol wash. Finally, the resulting mitochondrial nucle-
acid precipitates were solubilized in diethylpyrocarbonate-
treated water, quantitated spectrophotometrically (Maniatis et al. 1982), and stored at −80°C. The protocols of Selden (1987) were used for formaldehyde-agarose gels of RNA, Northern transfers, and nucleic-acid hybridizations. Nucleic-
acid probes were prepared by nick translation (Maniatis et al. 1982).

**Results and discussion**

**Heat production and mitochondrial electron-transport-chain capacities during development of the appendix and sterile floral regions of the Sauromatum spadix.** The alternative pathway is highly expressed in the mitochondria of a distinctive yellow cortex tissue that is present in the spadix of *Sauromatum* (Elthon and McIntosh 1986). This cortex tissue is most prevalent in the appendix region, but it is also found in the sterile floral region of the spadix (refer to Fig. 1 of Elthon nd McIntosh 1986 for a diagram of the floral anatomy of the voodoo lily). Both of these regions display thermogenesis,