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IMPORT OF THE MAMMALIAN CYTOCHROME P450 (scc) PRECURSOR INTO PLANT MITOCHONDRIA

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According to previous reports (Matocha M. F. and Waterman M. R. (1984) J. Biol. Chem. 259, 8672-8678 and (1986) Arch. Biochem. Biophys. 250, 456-460) import of the cytochrome P450 (scc) precursor into mitochondria is tissue-specific. The present paper shows that *in vitro* synthesized bovine cytochrome P450 (scc) precursor can be imported into isolated soybean cotyledon mitochondria and processed therein to the mature size product. This shows that heterologous import of the cytochrome P450 (scc) precursor is possible and that import into mitochondria of this precursor is not restricted to steroidogenic tissues.

The mechanisms of intracellular protein transport and specific protein targeting into correct subcellular compartment as well as protein processing and modification events inside the organelle have been intensively studied during the last decades. A lot of information has accumulated and great progress has been achieved in the studies of import of nuclear-encoded organellar proteins into mitochondria (1, 2). A number of publications testify that the mechanisms of import and processing events are universal, i. e. they are free of tissue and species-specific limitations (3-6). Moreover, in many cases fusion proteins, composed of a non-mitochondrial reporter protein coupled to the N-terminal targeting presequence of a mitochondrial protein, were imported into mitochondria and processed therein (7-10). On the basis of all these data it might be concluded that the only feature required of a polypeptide chain to be imported into mitochondria is that it must contain the mitochondrial targeting presequence.

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However, in some cases heterologous protein import into miochondria does not occur (3, 5, 11, 12). This is best demonstrated by cytochrome P450 (scc) and cytochrome P450 (11β) (5, 11, 12). The former is found in the mitochondria of all steroidogenic tissues (i. e. adrenal cortex, testis, ovary and placenta) while the only location of the latter is adrenal cortex mitochondria. These cytochromes were suggested to be imported and processed only into steroidogenic mitochondria (5, 12). i. e. in those mitochondria which are actively involved in sterol transformations. This suggestion was based on the observation that import-competent heart mitochondria, although capable of binding the precursors of P450 (scc) and P450 (11β), were incapable of importing and processing these precursors (5, 11 12). These studies were interpreted to indicate that the binding of the precursors to heart mitochondria was not specific and that the tertiary structure of the cytochromes may not be recognized by receptor sites on the surface of heart mitochondria but is recognized on the surface of adrenocortical mitochondria. The existance of tissue-specific protein import is important in both fundamental and applied terms. First, it can serve as a model for better understanding of the mechanism of protein import. Second, it is important to gain better insight into heterologous expression, import and processing of cytochromes P450 (scc) and P450 (11β) to construct transgenic organisms (plants, fungi and yeast) with modified sterol metabolism.

The aim of the present work was to investigate whether *in vitro* import and processing of the *in vitro* synthesized precursor of bovine cytochrome P450 (scc) into isolated plant mitochondria is possible.

MATERIALS AND METHODS

The expriments were carried out with plasmid pTUT (13) containing the full-length bovine cytochrome P450 (scc) cDNA inserted at the *Sma* I site under the control of a T7 promotor. This plasmid was linearized with the *Kpn* I restiction endonuclease and expressed in the presence of [^{35}S]-methionine using TNT T7 Coupled Reticulocyte Lysate System from Promega according to the manufacturers instructions. Soybean cotyledon mitochondria were isolated from 7 day old green cotyledons according to (14). *In vitro* import into isolated soybean cotyledon mitochondria was carried out according to (15), with the modifications that no GTP was added in the import mixture and that the samples were continously shaken for 15 min at 23 °C.

RESULTS AND DISCUSSION

Fig. 1 shows that several polypeptides were synthesized in the transcription/translation system of the cDNA clone of the cytochrome P450 (scc) (lane 1). The upper band corresponded to the cytochrome P450 (scc) precursor as

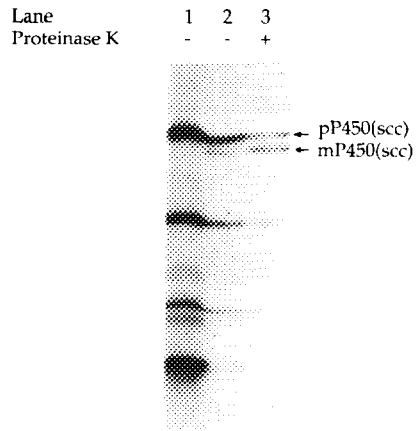

Fig. 1. Synthesis and import of the bovine cytochrome P450 (scc) precursor into isolated soybean cotyledon mitochondria. Lane 1, translation of mRNA for cytochrome P450 (scc) in a rabbit reticulocyte lysate cell-free system (the upper band corresponds to the cytochrome P450 (scc) precursor (p) with a molecular weight of 52 kDa). Lane 2, import mixture with soybean cotyledon mitochondria. Lane 3, as lane 2 with proteinase K added showing that both precursor (52 kDa) and mature (m) (49 kDa) forms were insensitive to the added proteinase.

judged by molecular mass estimation (52 kDa) and immunological cross-reactivity studies with antibodies against the cytochrome P450 (scc) (not shown). The other bands found in the transcription/translation mixture were either non-complete translation products resulting from the translational pausing or initiation from internal methionines or they could be products of proteolytic degradation of the precursor. Three polypeptides from the translation mixture were predominantly bound to soybean mitochondria (lane 2), however only a portion of the cytochrome P450 (scc) precursor of 52 kDa and a newly produced protein of 49 kDa corresponding to the mature cytochrome P450 (scc) became insensitive to exogenously added proteinase K (lane 3). These results show that the precursor of cytochrome P450 (scc) could be bound and imported into plant mitochondria and that a fraction of the precursor was processed to a mature size product of 49 kDa. Presence of the non-cleaved precursor inside mitochondria is not unusual with plant mitochondria using a heterologous import system (15).

These experiments are the first to reveal that mammalian cytochrome P450 (scc) precursor can be imported into heterologous mitochondria and processed in these mitochondria to the normal size mature form. The above results may indicate that import and processing of the mammalian cytochrome P450 (scc) precursor is not necessarily a tissue-specific event or/and that plant mitochondria are either normally involved in steroid metabolism or contain

import machinery similar to that of steroidogenic tissues. The latter possibility suggests the presence of a unique receptor for this class of proteins in plant mitochondria or the presence of specific lipids involved in the binding of this precursor. Though it is known that some plants contain pregnenolon (for review see ref. 16), a plant analogue of mammalian P450 (scc) has not been found so far. Our investigation may constitute a preliminary step for the incorporation of sterol metabolism in plants by genetic engeneering techniques.

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