Mitochondrial import of cyclophilin-D

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Mammalian mitochondria contain an inner membrane pore which opens following an increase in matrix calcium. Pore opening has been implicated in tissue damage following ischaemic injury. The pore is reversibly blocked by cyclosporin A [1] suggesting a role for cyclophilin in pore control. A mitochondrial isoform of cyclophilin (CyP-D) [2,3,4,5] has now been identified. Analysis of the N-terminus of the sequence suggests that it could form an amphipathic helix, typical of many presequences [6]. Cleavage of the precursor is believed to occur between Thr29 and Cys30, to yield mature cyclophilin.

The primer pair CypD1 and CypD2 was used to amplify the entire cyclophilin coding sequence from a cloned CyP-D sequence [S. Virji, J.M. Ward and M. Crompton, unpublished data] introducing an upstream T7 promoter. The product of this amplification was introduced directly into a coupled in vitro transcription/translation reaction (Fig. 1.). Full length precursor CyP-D (pCyP-D) was expressed as a 23kDa radiolabelled protein by the addition of [35S]methionine to the translation reaction.

Figure 1. Schematic representation of the amplification and expression of precursor cyclophilin D (pCyP-D).

Figure 2. Import of precursor cyclophilin. Radiolabelled pCyP-D was incubated with 200µg of rat heart mitochondria for 30 minutes at the indicated temperatures. Half of each sample was treated with proteinase K. The mitochondria were re-isolated by centrifugation. Samples were separated by SDS-PAGE and visualized by autoradiography.

Import studies of radiolabelled pCyP-D using freshly prepared rat heart mitochondria indicated that cyclophilin binds to the outer membrane of mitochondria under all conditions (Fig. 2.). When the import was conducted at 3°C a smaller radiolabelled protein was observed with a molecular weight of approximately 20kDa (track 5). Subsequent protease treatment revealed that this protein is resistant to proteinase K (track 6) indicating that import and presequence cleavage had occurred. At low temperatures or in the presence of the uncoupling agent carbonyl cyanide m-chlorophenylhydrazone (CCCP) no truncated form of CyP-D was identified and all externally bound protein was digested following proteinase K treatment.

This study confirms that nuclear encoded CyP-D is imported as a 23kDa protein and cleaved to a 20kDa mature form. A smaller isoform has been observed in two previous studies [2,4] but not in the current study. Expression of precursor CyP-D was low compared to its mature form (data not shown). Import also proved inefficient with less than 10% of bound precursor being imported and cleaved to its mature form. Nevertheless, import of radiolabelled CyP-D may provide a useful system for future biochemical studies of CyP-D interaction with inner membrane pore structures.

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