

SUPPLEMENTARY ONLINE DATA

Mitochondrial carrier protein biogenesis: role of the chaperones Hsc70 and Hsp90

Vincenzo ZARA*¹, Alessandra FERRAMOSCA*, Philippe ROBITAILLE-FOUCHER†, Ferdinando PALMIERI‡ and Jason C. YOUNG†¹

*Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, Via Provinciale Lecce-Monteroni, I-73100 Lecce, Italy, †Department of Biochemistry, McGill University, 3649 Promenade Sir William Osler, Bellini Building, Room 457, Montreal, QC, Canada H3G 0B1, and ‡Dipartimento Farmaco-Biologico, Università di Bari, Via E. Orabona 4, I-70125 Bari, Italy

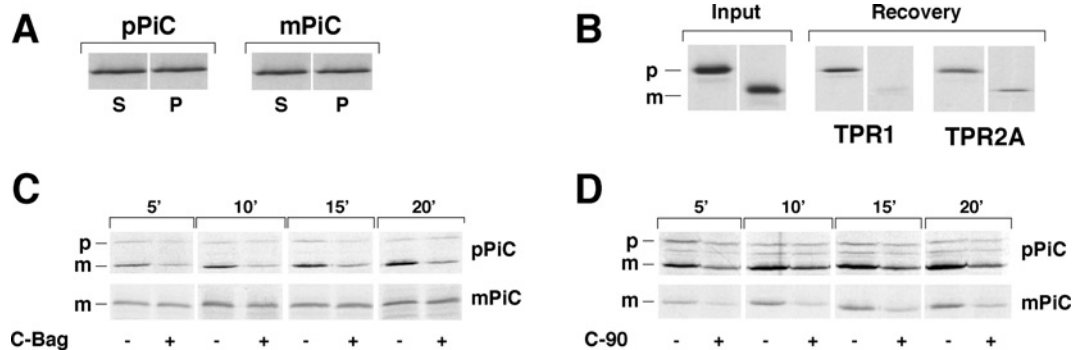


Figure S1 The PiC presequence determines Hsc70 interactions

(A) The reticulocyte lysates, containing radiolabelled pPiC and mPiC, were centrifuged at 40000 rev./min for 45 min at 2 °C using a Ti50 rotor (Beckman). The supernatants (S) and pellets (P) were analysed by SDS/PAGE and fluorography. (B) pPiC and mPiC were radiolabelled by cell-free translation in reticulocyte lysate and incubated with purified His-tagged Hop TPR1 and TPR2A fragments. The protein complexes were recovered by chromatography on Ni-NTA (Ni²⁺-nitrilotriacetate)-agarose. The beads were washed and analysed by SDS/PAGE and fluorography. (C) ³⁵S-labelled pPiC (p) and mPiC (m) were incubated with isolated rat liver mitochondria at 25 °C for the times indicated (5, 10, 15 or 20 min). Reactions contained either no addition (–) or purified C-Bag fragment (+). All samples were subsequently treated with proteinase K and the mitochondria were re-isolated by centrifugation. The proteins were separated by SDS/PAGE and the gels were analysed by fluorography. (D) Import of radiolabelled pPiC and mPiC into rat liver mitochondria was assayed as described in (C) above. Reactions contained either no addition (–) or purified C-90 fragment (+).

Received 24 November 2008/13 January 2009; accepted 14 January 2009
Published as BJ Immediate Publication 14 January 2009, doi:10.1042/BJ20082270

¹ Correspondence may be addressed to either of these authors (email vincenzo.zara@unile.it or jason.young2@mcgill.ca).