Mitochondrial fusion intermediates revealed in vitro.

Meeusen S, McCaffery JM, Nunnari J. Science. 2004 Sep 17;305(5691):1747-52.

Presented by Adrienne Kish
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Background

- Previously Known:
  - Mitochondrial fission and fusion events balanced in eukaryotes
  - Inner and outer membranes functionally and structurally distinct
  - 3 required proteins interact *in vivo*:
    - Ugo1 (outer membrane fusion)
    - Mgm1 (GTPase tethered to the inner membrane and localized to the intermembrane space for fission)
    - Fzo1 (integral membrane protein for fusion)
    - *(Dnm1 another GTPase for fission)*
  - Same protein machinery for inner and outer membrane fusion suggested

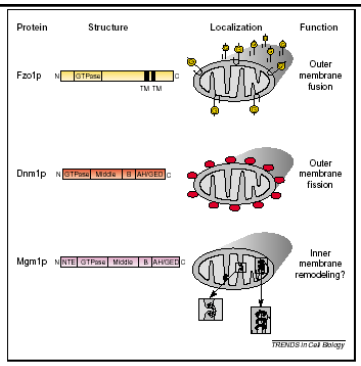
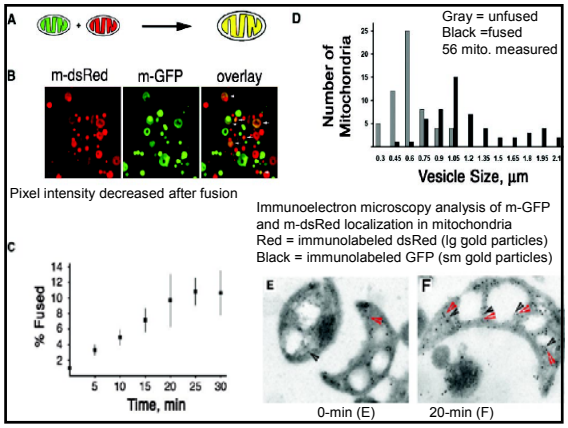
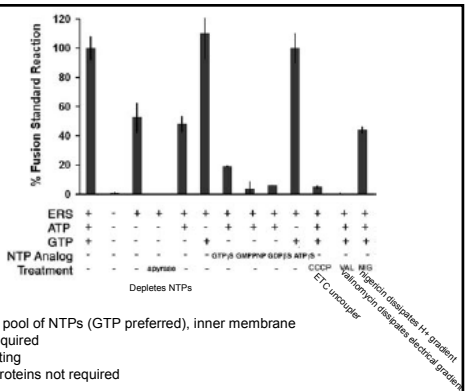


Fig. 2. Three large GTPases control mitochondrial morphology in yeast. The predicted domain structures of Fzo1p, Dnm1p and Mgm1p, and their submitochondrial localization patterns are shown here. The known (Fzo1p, Dnm1p) or postulated (Mgm1p) function of each GTPase in mitochondrial membrane dynamics is indicated. The magnified views below the Mgm1p localization diagram show possible roles of Mgm1p in inner membrane fission (left) or cristae formation (right).

Background

- Unknown:
  - Mechanism of membrane fusion (role of proteins and their interactions)
- Question:
  - What are the discrete steps in mitochondrial membrane fusion in fission yeast and what is the role of the 3 main proteins in those steps?

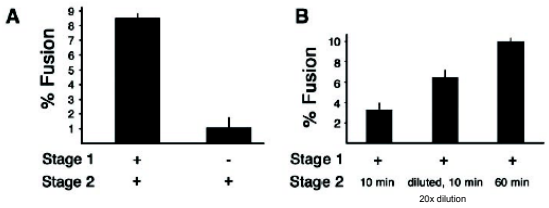




- ERS and a pool of NTPs (GTP preferred), inner membrane potential required
- GTP is limiting
- Cytosolic proteins not required

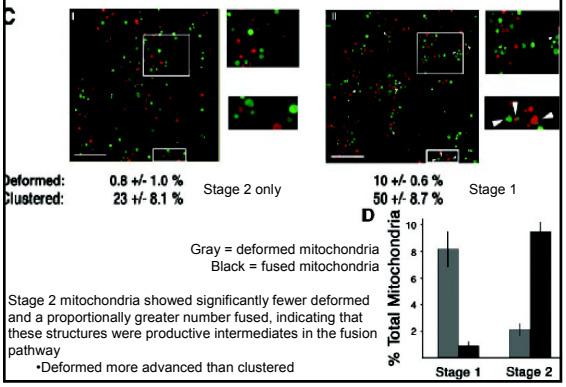
Stage 1 = concentration and incubation

Stage 2 = addition of exogenous NTPs and energy regeneration system



Concentration rate-limiting for fusion, but not required

Clustered and deformed fusion intermediate structures seen in Stage 1 but not Stage 2 (Stage 1 By-Pass)



Gray = deformed mitochondria
Black = fused mitochondria

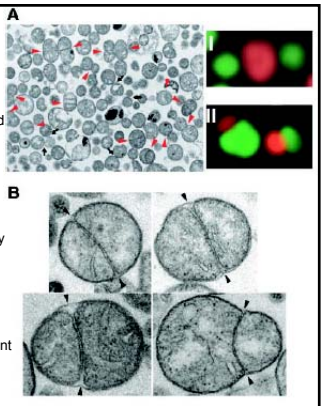
Stage 2 mitochondria showed significantly fewer deformed and a proportionally greater number fused, indicating that these structures were productive intermediates in the fusion pathway

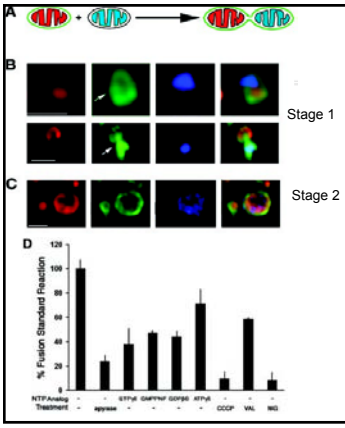
- Deformed more advanced than clustered

(**A**) EM analysis of stage 1 mitochondria (Black = clustered Red = deformed) beside magnified fluorescent images of clustered (I) and deformed (II) mitochondrial structures

(**B**) EM images of representative deformed mitochondrial pairs. Arrowheads indicate regions of continuous outer membrane encapsulating two separate, but tightly aligned and opposing inner membranes.

- Inner membrane fusion independent of outer membrane fusion





Mitochondrial outer membrane fusion *in vitro*

- om-GFP, m-dsRed, m-BFP
- Inner membrane fusion occurs in Stage 2

Energetics of outer membrane fusion

- Endogenous GTP and inner membrane proton gradient required to promote fusion of the outer membrane

EM analysis and fluorescence-based assays for mitochondrial outer and inner membrane fusion

- Fzo1 highly conserved transmembrane GTPase, candidate for mediating outer membrane fusion

Type of mitochondria	Temp. (°C)	Outer membrane fusion		Inner membrane fusion
		Fluorescence (%)	EM (%)	Fluorescence (%)
WT × WT	22°	100 (9.2%, $n > 600$)	100 (6.3%, $n > 300$)	100 (12%, $n > 300$)
WT × WT	37°	100 (8.8%, $n > 400$)	100 (6.4%, $n > 300$)	100 (11.8%, $n > 400$)
fzo1-1 × *fzo1-1*	22°	13 ($n > 600$)	64 ($n > 800$)	88 ($n > 300$)
fzo1-1 × *fzo1-1*	37°	14.5 ($n > 400$)	41 ($n > 600$)	5.5 ($n > 200$)
fzo1-1 × WT	22°	16 ($n > 600$)	60 ($n > 300$)	92 ($n > 200$)
fzo1-1 × WT	37°	16 ($n > 400$)	52 ($n > 300$)	24 ($n > 300$)

- Fzo1-Fzo1 interactions on opposing mitochondrial membranes required to promote outer and inner membrane fusion
- Effects partially suppressed by relatively high NTP concentration present during stage 2 and totally suppressed by wild-type Fzo1

Summary

- Outer and inner membrane fusion events separable and mechanistically distinct
  - Outer membrane fusion required mitochondrial concentration, was driven energetically by a relatively low (endogenous) concentration of GTP and was dependent on the inner membrane proton gradient
  - Inner membrane fusion required the hydrolysis of a relatively high concentration of GTP and the inner membrane electrical gradient
- Model:
  - Outer membrane fusion requires GTP and trans Fzo1 interactions on opposing mitochondria, suggesting that GTP promotes outer membrane fusion by means of Fzo1
  - Ugo1 functions as an adaptor between Fzo1 and Mgm1
  - Fzo1 interactions with inner membrane components may be required in a mechanical manner for the formation of regions of close inner and outer membrane contact within mitochondria or regulatory
- Significance: Dissection of mitochondrial fusion into separate outer and inner membrane fusion events provides an experimental framework for determining the exact functions of the fusion proteins