Mechanism of mitochondrial fission mediated by the coordinated action of Atg44 and Dnm1

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Research objective

Mitochondria undergo fission and fusion; this morphological control is crucial for mitochondrial homeostasis. The balance between fission and fusion is regulated by diverse GTPase families, and the fission mechanism mediated by Dnm1, in particular, has been studied for many years. During our research on mitophagy in yeast, we identified a novel fission factor, Atg44/mitofissin, which functions independently of Dnm1[1,2]. In the present study, we aimed to elucidate the cooperative mechanism between Atg44 and Dnm1 and to establish a new concept for biological membrane fission.

Methods

Yeast mitochondria exhibit a tubular morphology in fermentation growth media containing glucose, but undergo fragmentation upon shifting to respiratory growth media containing lactate through Atg44- and Dnm1-dependent fission. To investigate the relationship between Atg44 and Dnm1 under these conditions, we performed the following: (1) analysis of Dnm1 behavior in *atg44*Δ cells; (2) analysis of Dnm1-dependent mitochondrial constriction sites; (3) analysis of the outer membrane, inner membrane, and matrix at mitochondrial constriction sites; and (4) analysis of Atg44 localization at mitochondrial constriction sites.

Results

This study yielded the following results. (1) Dnm1 accumulated and constricted mitochondria independently of Atg44; however, Atg44 was essential for fission (Fig. 1). (2) Dnm1-dependent constriction sites in *atg44*Δ cells were extremely narrow (Fig. 2). (3) At constricted mitochondrial sites, no cleavage of the outer or inner membranes occurred, and matrix components were detected only at low levels (Fig. 3). (4) Atg44 accumulation was observed at constricted mitochondrial sites (Fig. 4).

Conclusion

Dnm1 has long been considered to be responsible for mitochondrial constriction and fission.

However, our results show that Dnm1 is solely involved in mitochondrial constriction, whereas Atg44 is the true executor of mitochondrial fission[3].

References

1) Yamashita, SI. *et al*. (2016) Mitochondrial division occurs concurrently with autophagosome formation but independently of Drp1 during mitophagy. *J. Cell Biol*. **215:** 649-665.
2) Fukuda, T., Furukawa, K. (co-first) *et al*. (2023) The mitochondrial intermembrane space protein mitofissin drives mitochondrial fission required for mitophagy. *Mol. Cell* **83:** 2045-2058.e9.
3) Furukawa, K. *et al*. (2024) Atg44/Mdi1/mitofissin facilitates Dnm1-mediated mitochondrial fission. *Autophagy* **20:** 2314-2322.

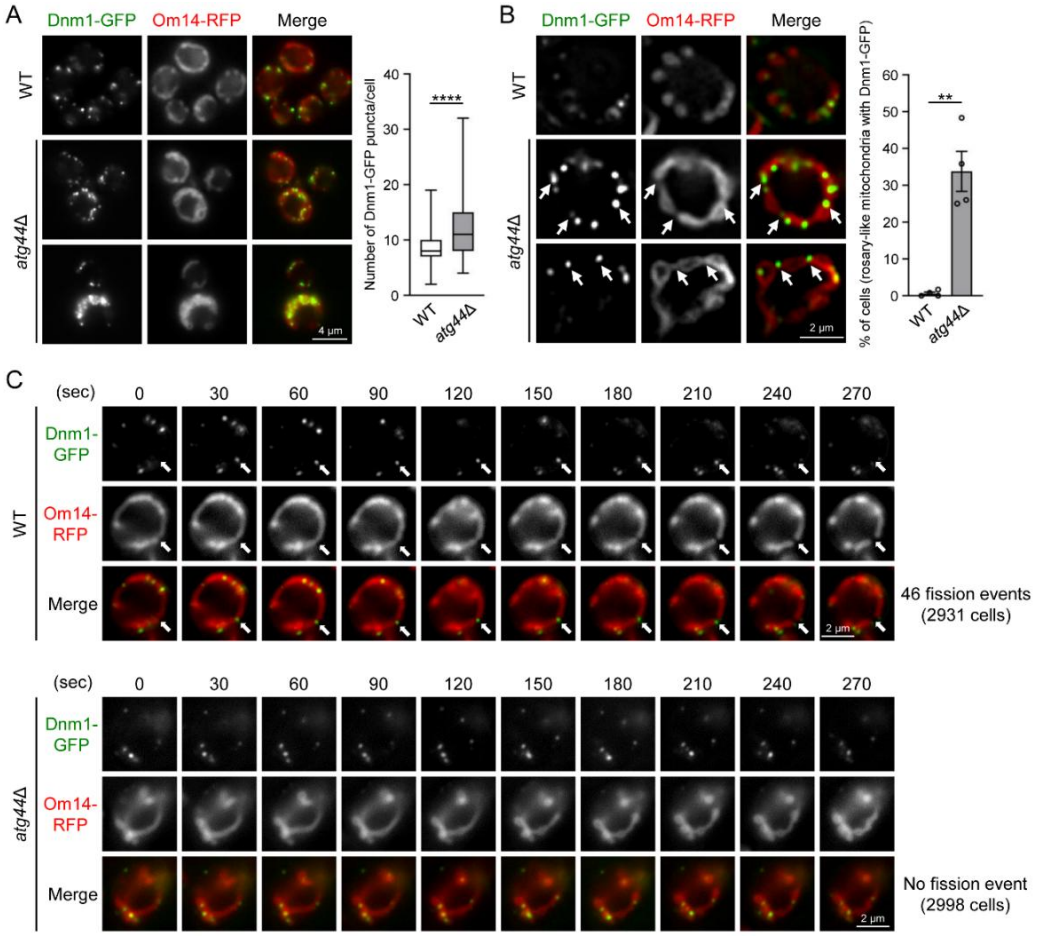

Figure 1: In *atg44*Δ cells, Dnm1 accumulates at mitochondria but cannot complete fission
(A) In *atg44*Δ cells, numerous Dnm1-GFP puncta accumulate at enlarged mitochondria. (B) Fragmented mitochondria are observed in wild-type cells, while strong constrictions are observed at Dnm1-GFP accumulation sites in *atg44*Δ cells. (C) Time-lapse analysis of mitochondrial fission revealed no Dnm1-dependent fission in *atg44*Δ cells.

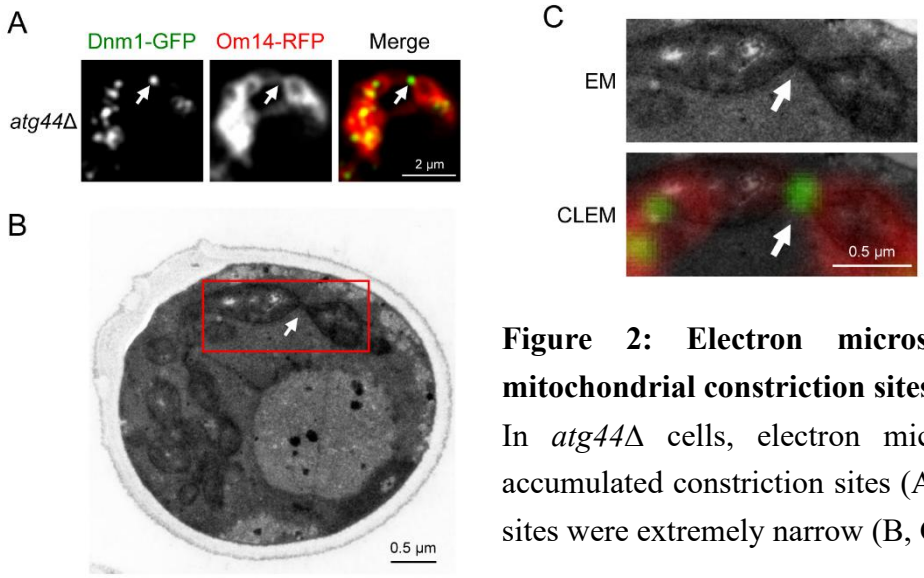

Figure 2: Electron microscopy analysis of mitochondrial constriction sites

In *atg44*Δ cells, electron microscopy of Dnm1-accumulated constriction sites (A) revealed that these sites were extremely narrow (B, C).

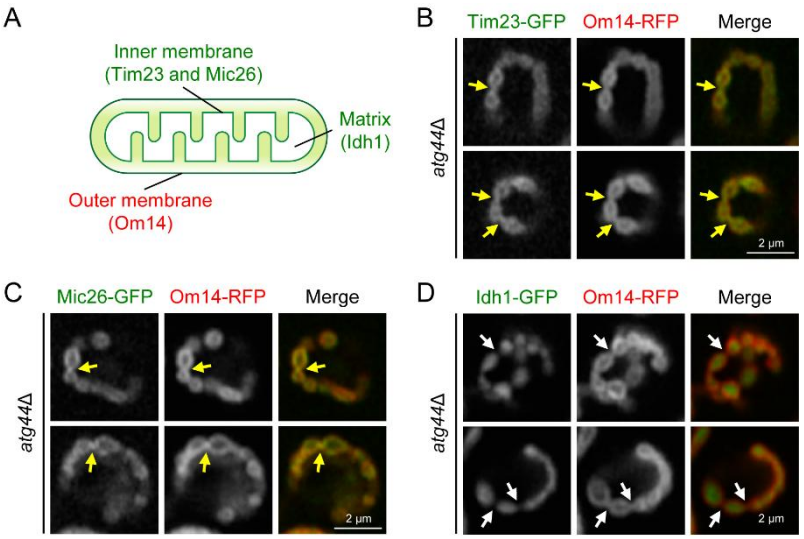

Figure 3: Fluorescence microscopy analysis of mitochondrial constriction sites

The outer membrane, inner membrane, and matrix proteins (A) were labeled with GFP or RFP to observe the constriction sites. No inner membrane cleavage was observed (B, C), and the matrix narrowed to a level where it was barely detectable (D).

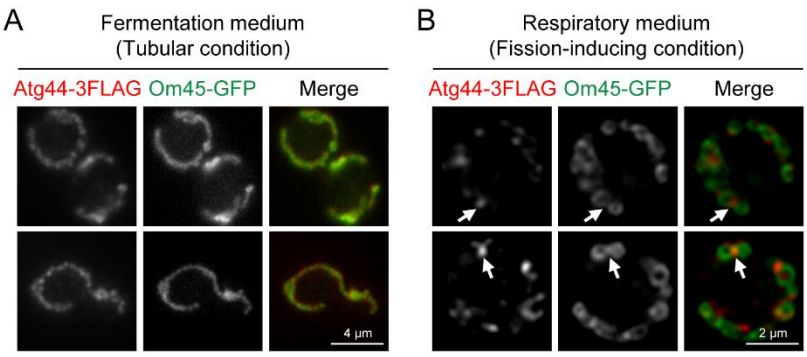

Figure 4: Atg44 accumulation at mitochondrial constriction sites

Immunofluorescence staining revealed that Atg44 was uniformly localized within the mitochondria during fermentation growth (A), whereas Atg44 accumulated at mitochondrial constriction sites during respiratory growth (B).