Membrane protein Oca3 is essential to keep structural integrity of mitochondria and endoplasmic reticulum

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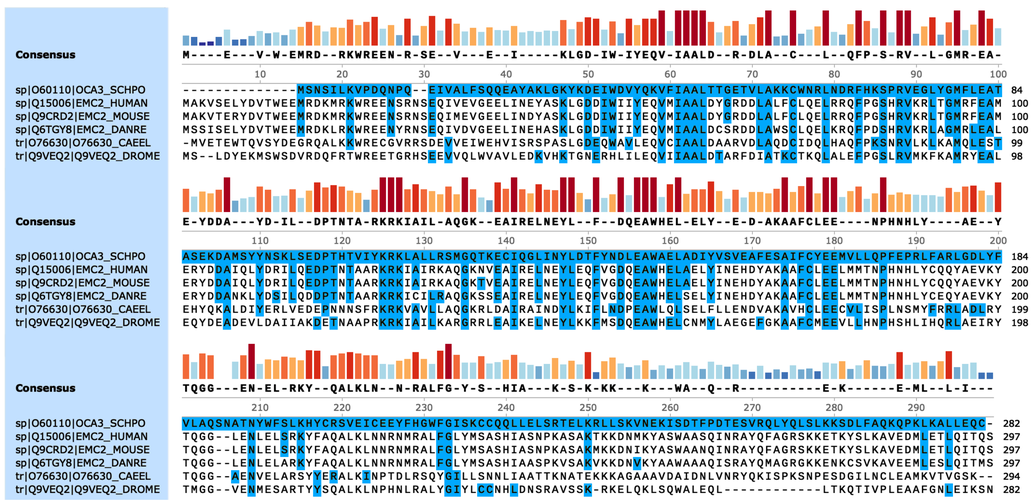
Abstract

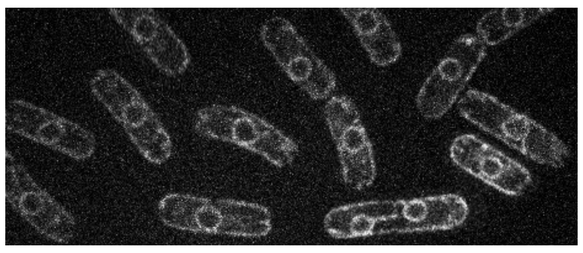
Mitochondrial function is tightly conserved through evolution since it becomes essential for the fitness of any eukaryotic cell. Defective function of this organelle represents the cellular basis of some severe diseases in humans. Thus, the characterization of genes involved in the correct mitochondrial structure and function is critical to understand and treating these diseases. In our laboratory, using the fission yeast as a model, we are characterising the function of *oca3* gene, the ortholog of EMC2 gene in human. This gene is predicted to be a member of the ER membrane protein complex involved in the mitochondrion-endoplasmic reticulum membrane tethering[1]. We find the protein in the non-aqueous phase in cell extracts and Oca3-mCherry tagging actually decorates most cell membranes. Oca3 over-expression cause lethality[2] and the gene deletion becomes cold-sensitive. In both situations aberrant mitochondria aggregations are observed and endoplasmic reticulum seems disorganised. Interestingly, addition of Tween20 restores the viability of *oca3* deletion at low temperature. This result suggests that Oca3 may have a role in membrane fluidity homeostasis.

oca3 is conserved through evolution



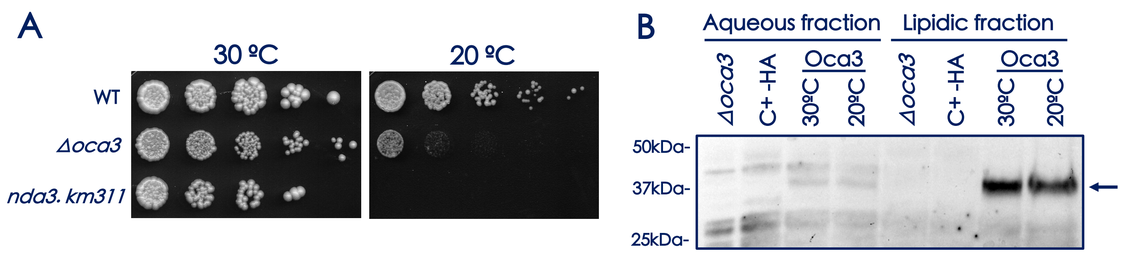
Evolutionary conservation of Oca3/EMC2 protein. A ClustalX alignment was performed between Oca3 and their orthologs in other model organisms, including human. There are two conserved regions corresponding to a tetratricopeptide repeat-containing domain (TPR).

Oca3 decorates most cell membranes



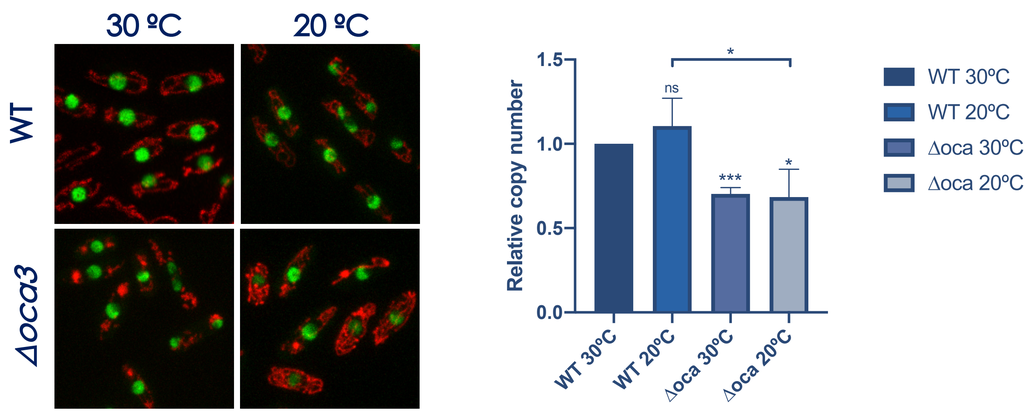
Cellular localization of Oca3. A strain bearing an *oca3-mCherry* was constructed. Oca3 shows cellular localization at most membranes, specially nuclear and cellular membranes.

Δoca3 becomes cold-sensitive



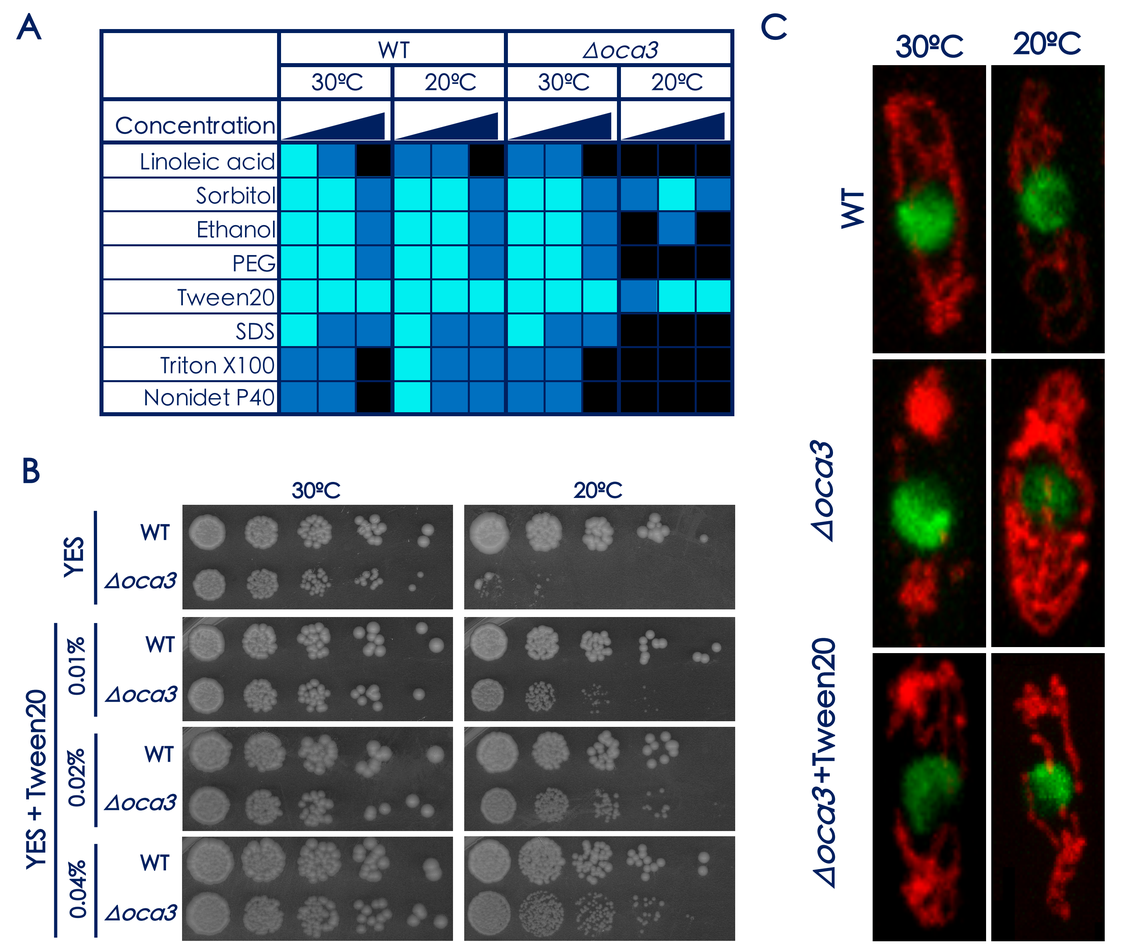
A. Oca3 deletion becomes cold-sensitive. Five fold dilutions were spotted at 30 ºC and 20 ºC respectively. As shown, deletion of oca3 renders lethality at low temperature. **B. Western Blot of Oca3 in different conditions.** It was tested whether the localization of Oca3 was cytosolic or membranous. As expected, Oca3 Protein is present on non-soluble fraction. Expression levels of the protein at different temperatures is also compared, showing no significant differences.

Δoca3 affects mitochondrial distribution and reduces mtDNA



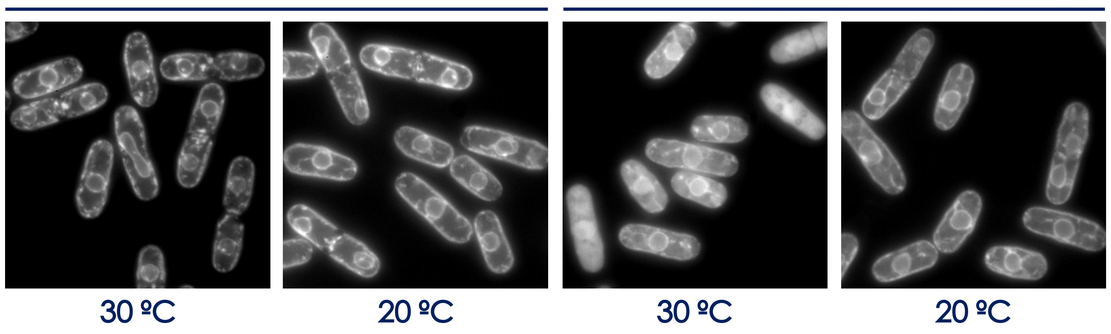
Mitochondrial distribution in wild type vs *oca3* deletion. A *hht2-GFP arg11-mCherry* strain was constructed for chromatin and mitochondrial observation *in vivo* . Deletion produces viable mitochondrial aggregation at 30 ºC but lethal shredding at 20 ºC. **mtDNA copy number is reduced in *Δoca3*.** qPCR results shows a ~0.4 fold reduction of mtDNA copies.

Tween20 restores viability and mitochondrial distribution in *Δoca3*



A. Different substances tested for membrane fluidization. A set of chemicals were tested at increasing concentrations. Viability is color-coded meaning the lighter the more viable where black is lethal. Some compounds are able to restore *Δoca3* phenotype being Tween20 that with the best results. **B. Spot-test of the deletion growing assay with Tween20.** The spot-test shows the ability of Tween20 for suppressing lethality of the deletion at low temperature. **C. Examples of mitochondrial distribution in cells grown in YES+Tween20 0.04% medium.** While deletion growing in normal rich medium (YES) shows the previously mentioned phenotypes, those growing in medium supplemented with Tween20 have more WT-like distribution of mitochondria, suggesting that mitochondrial malfunction could be the causes, or contribute to lethality.

Δoca3 alters normal transport to the endoplasmic reticulum



ER lumen labelling by fluorescence with an ELS. A *pBip1-mCherry-ADEL* strain was constructed for *in vivo* observation of the ER structure. In *oca3* deletion background cytoplasmic signal is elevated at 30 ºC in comparison with 20 ºC or the wild type background.

Conclusions

- Oca3 is a membrane protein evolutionarily conserved. The human ortholog of EMC2[1] has been described as an ER associated protein. Consistently, deletion of Oca3 affects ER integrity in *S. pombe*. However our findings also suggest that Oca3 may have a role in the regulation of membrane fluidity homeostasis, dynamics and interactions of other organelles such as mitochondrial fission and fusion.
- Characterising this novel role could lead to better understanding of some mitochondrial diseases in human.

References

1. Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissman JS, Walter P. An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science. 2009 Jul;325(5939) 477-481. doi:10.1126/science.1175088.
2. Tallada VA, Daga RR, Palomeque C, Garzón A, Jimenez J. Genome-wide search of *Schizosaccharomyces pombe* genes causing overexpression-mediated cell cycle defects. Yeast. 2002 Sep;19(13) 1139-1151. doi:10.1002/yea.902.