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Author(s):
Lang, Alexander

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Characterization of the Endosomal Protein Vps13 as a suppressor of the ER-Mitochondria Encounter Structure

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

presented by
ALEXANDER LANG

Diplom-Biologe (t.o.)
University of Stuttgart, Germany

born on 08.01.1982
citizen of Germany

accepted on the recommendation of

Prof. Benoît Kornmann (referee)
Prof. Matthias Peter (co-referee)
Prof. Maya Schuldiner (co-referee)

Summary

The ERMES (Endoplasmic Reticulum-Mitochondria Encounter Structure) complex tethers the endoplasmic reticulum and the mitochondria. It is thought to facilitate interorganelle lipid exchange, and influence mitochondrial dynamics and mitochondrial DNA maintenance. Despite this important role, ERMES is not found in metazoans. Here, we identified single amino acid substitutions in Vps13 (vacuolar protein sorting 13), a large universally-conserved eukaryotic protein, which suppress all measured phenotypic consequences of ERMES deficiency.

Combined loss of *VPS13* and ERMES is lethal, indicating that Vps13 and ERMES function in redundant pathways. Vps13 dynamically localizes to vacuole-mitochondria and to vacuole-nucleus contact sites depending on growth conditions, suggesting that ERMES function can be bypassed by the activity of other contact sites, and that contact sites establish a growth condition- regulated organelle network.

Zusammenfassung

Der ERMES (Endoplasmic Reticulum-Mitochondria Encounter Structure) Komplex verknüpft Endoplasmatisches Retikulum mit Mitochondrien. Es wird angenommen, dass dieser Komplex für Lipid-transfer zwischen diesen Zellorganellen und die Stabilität von mitochondrialer DNA verantwortlich ist, sowie Einfluss auf weitere mitochondriale Vorgänge hat. Trotz dieser bedeutenden Rolle ist ERMES nicht in vielzelligen Organismen zu finden. In dieser Arbeit beschreiben wir die Entdeckung von Einzelnukleotid-Polymorphismen in Vps13 (vacuolar protein sorting 13), einem großen und allgemein konservierten eukaryotischen Protein, welche alle erfassten phänotypischen Konsequenzen von ERMES- Mutanten aufheben.

Der gleichzeitige Verlust von *VPS13* und ERMES ist tödlich für die Zelle und lässt vermuten, dass Vps13 und ERMES in redundanten Prozessen arbeiten. Abhängig von den jeweiligen Wachstumsbedingungen kann Vps13 an Membran-Kontakten zwischen Vakuole und Mitochondrium bzw. zwischen Vakuole und Zellkern gefunden werden. Dies legt ein Modell nahe, in dem verschiedene Membran-Kontakt-Komplexe, in Reaktion auf die jeweiligen Wachstumsbedingungen, als Netzwerk zusammen arbeiten. Das Fehlen von ERMES könnte dabei durch andere Membran-Kontakt-Komplexe ausgeglichen werden.

Statement of Contribution

The work presented is based on the manuscripts of “ER-mitochondrial Junctions can be Bypassed by Dominant Mutations in the Endosomal Protein Vps13” (Lang et al., 2015b) und “ER–mitochondria contact sites in yeast: beyond the myths of ERMES” (Lang et al., 2015a). The title and summary paragraph of this thesis are identical to title and abstract of “ER-mitochondrial Junctions can be Bypassed by Dominant Mutations in the Endosomal Protein Vps13”. Parts of the results and discussion paragraph are similar to the latter mentioned publication. Both manuscripts are attached for comparison (**9**). I performed microscopy, phospholipid isolation and assays, CPY-secretion assays, mitochondrial purification as well as the molecular biological work if not mentioned otherwise below. Next generation sequencing analysis was done together with the Functional Genomics Center Zurich (FGCZ). Analysis of published genetic interaction data, as well as the amino-terminal Uracil tagging of *VPS13* and *VPS13(D716H)* gap-repair cloning from genomic DNA was done by Benoît Kornmann. Genetic analysis and tetrad dissection was done by Benoît Kornmann and me in equal parts. The *VPS13* alleles with GFP integrations after amino-acid residue 446 and 499 were generated together with Arun T. John Peter. The *pVPS13* plasmid (originally named *pSOI1-1*) is a kind gift of Robert S. Fuller. The used *pFA6a_mCherry:KAN* plasmid was a kind gift of Eduardo Cebollero Presmanes. The *GAL-VPS13:TRP1* strain was a kind gift from Jesper Svejstrup. Prof. Benoît Kornmann supervised the project and made significant contributions to writing the published manuscripts.