

University of Groningen

Absence of the peroxiredoxin Pmp20 causes peroxisomal protein leakage and necrotic cell death

Aksam, Eda Bener; Jungwirth, Helmut; Kohlwein, Sepp D.; Ring, Julia; Madeo, Frank; Veenhuis, Marten; van der Klei, Ida

Published in:
Free Radical Biology and Medicine

DOI:
[10.1016/j.freeradbiomed.2008.07.010](https://doi.org/10.1016/j.freeradbiomed.2008.07.010)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Aksam, E. B., Jungwirth, H., Kohlwein, S. D., Ring, J., Madeo, F., Veenhuis, M., & van der Klei, I. J. (2008). Absence of the peroxiredoxin Pmp20 causes peroxisomal protein leakage and necrotic cell death. *Free Radical Biology and Medicine*, 45(8), 1115-1124. DOI: 10.1016/j.freeradbiomed.2008.07.010

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Table S1. *Hansenula polymorpha* strains and plasmids used in this study.

Strain	Relevant properties	Reference
WT, <i>leu 1.1 ura3</i>	Wild type NCYC495, <i>leu 1.1 ura 3</i>	[47]
WT, <i>leu 1.1</i>	Wild type NCYC495, <i>leu 1.1 URA3</i>	[47]
WT::P _{AMO} GFP-SKL	Wild type containing a single copy integration of the <i>GFP-SKL</i> gene, behind the P _{AMO} promoter	[20]
<i>pmp20</i>	<i>PMP20</i> disruption strain, <i>leu1.1</i>	This study
<i>pmp20::P_{AMO}GFP-SKL</i>	<i>pmp20</i> containing a single copy integration of the <i>GFP-SKL</i> gene, behind the P _{AMO} promoter	This study
<i>ycal</i>	<i>YCA1</i> disruption strain, <i>leu1.1</i>	This study
<i>pmp20ycal</i>	<i>PMP20</i> and <i>YCA1</i> double deletion strain, <i>leu1.1</i>	This study
Plasmid	Relevant properties	Reference
pHIPX5-GFP-SKL	pHIPX5 containing a gene encoding GFP containing the PTS1 tripeptide –SKL at the extreme C-terminus	[20]
pEBA025	pHIPX5 containing a gene encoding 6 histidine residues N terminally fused to Pmp20	This study
pENTR 221_ura3	Entry plasmid containing the URA gene	This study
pEBA017	Entry plasmid containing 5'-flanking <i>PMP20</i>	This study
pEBA018	Entry plasmid containing 3'-flanking <i>PMP20</i>	This study
pEBA019	Expression plasmid containing the <i>PMP20</i> disruption cassette and uracil gene as marker	This study
pEBA031	PBluescript variant containing the disruption cassette for <i>YCA1</i> and zeocin as marker	This study
pFEM39	pBluescript II KS ⁺ containing the zeocine selection cassette	[48]

Table S2. Oligonucleotides used in this study.

Primer name	Sequence (5'-3')
5'-rev-pmp20	GGGACTGCTTTTTTGTACAAACTTGG <u>TAAG</u> TTGTTAAGAGAGGCGAC*
5'-fwd-pmp20	GGGACAACCTTTGTATAGAAAAGTTGTGCCCTAAATACCGGTTAC
3'-fwd-pmp20	GGGACAACCTTTGTATAGAAAAGTTGTGCCCTAAATACCGGTTAC
3'-rev-pmp	GGGACAACCTTTGTATAATAAAAGTTGCCCTGGAGCGACATGATGCAC
Entr221 -URA F	GGGACAAGTTTGTACAAAAAAGCAGGCTGAGCTTCAACTGATGTTTCAGC
Entr221- URA R	GGGACCACTTTGTACAAGAAAGCTGGGTCTGAAGCACATCAACTGGATCG
cut-fwd-pmp20	ACAGCTTTGCTAGAAGTTTGGACGCC
cut-rev-pmp20	GGAGCGACATGATGCACACAAAAGG
his-pmp20 -fwd	CCAAGGATCCATGCACCACCACCACATCACGTTGTTAAGAGAGGCGACA AATTCC
his-pmp20 -rev	GGTTACAGCTTTGCTAGAAGTTTGGAC
Fw-5'-YCA1	CAATCATCCGCGG <u>TAAT</u> ATCCAGGAAACTCGCACGCCAATTACG*
Rev-5'-YCA1	GGCAGCGCTCTAGAATATCCGTCTTCCTCATCTCCATCCTGGTC
Fw-3'-YCA1	GACTGTCTCGAGCCATTGCCAGCAGGTTGCAGACTCACGTGTATT
Rev-3'-YCA1	CCACTGGTACCGCAACAGCGTGAGATAACTCTGTATAGGGTTCTCC
pcr-yca1-fw	TACCCAGGAAACTCGCACGCCAATTACGG
pcr-yca1-rev	GCAACAGCGTGAGATAACTCTGTATAGGGTTCTCC

* Underlined sequence indicates introduced stop codon.