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Data Article

Data on the role of iba57p in free Fe^{2+} release and $O_2^{\bullet -}$ generation in *Saccharomyces cerevisiae*

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ABSTRACT

The related study has confirmed that in *Saccharomyces cerevisiae*, iba57 protein participates in maturation of the [2Fe–2S] cluster into the Rieske protein, which plays important roles in the conformation and functionality of mitochondrial supercomplexes III/IV in the electron transport chain (Sánchez et al., 2018) [1]. We determined in *S. cerevisiae* the effects of mutation in the *IBA57* gene on reactive oxygen species (ROS) and iron homeostasis. Flow cytometry and confocal microscopy analyses showed an increased generation of ROS, correlated with free Fe^{2+} release in the *IBA57* mutant yeast. Data obtained support that a dysfunction in the Rieske protein has close relationship between ROS generation and free Fe^{2+} content, and which is possible that free Fe^{2+} release mainly proceeds from [Fe–S] cluster-containing proteins.

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Specifications Table

Subject area	*Biology*
More specific subject area	*Cell biology*
Type of data	*Graphs, figures*
How data was acquired	*ROS and Fe^{2+} determination by flow cytometry using a BD Accuri C6 Flow Cytometer (BD Biosciences) and observation by using a confocal microscope (Olympus FV1000).*
Data format	*Analyzed and images*
Experimental factors	*ROS and Fe^{2+} determination in S. cerevisiae cells using fluorescent probes.*
Experimental features	*Real-time quantification of ROS and Fe^{2+} in S. cerevisiae cells suspensions were determined by flow cytometry and cellular structures were co-localized by confocal microscopy.*
Data source location	*Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México.*
Data accessibility	*Data are provided with this article.*

Value of the data

- There is an established relation between *IBA57* mutation and the Rieske protein maturation in *S. cerevisiae*, which affects the electron transport chain functionality.
- *IBA57* mutation in *S. cerevisiae* is correlated with ROS generation and loss of iron homeostasis.
- This dataset provides new insights into the mechanism of ROS generation in *S. cerevisiae*, dependent of the ETC functionality.

1. Data

Treatments with 80 μM menadione in the *Saccharomyces cerevisiae iba57Δ* mutant caused significant impairment in its growth rate (Fig. 1a–b). The levels of free Fe^{2+} even without oxidant were significantly incremented in a time-dependent fashion in cell suspensions of the *iba57Δ* mutant yeast (Fig. 1c). The *iba57Δ* mutant displayed a significant increment of superoxide radical ($O_2^{\bullet -}$) generation with a dose-dependent of Fe^{2+}, determined by flow cytometry (Fig. 1d).

The western blot assays showed that the Rieske protein (Rip1p) was absent in the *rip1Δ* mutant, and decreased expression level was found in the *iba57Δ* mutant (Fig. 1e). When extracts from cultures grown on YPD plus high Fe^{2+} concentration (20 μM) or menadione as ROS-inducer were used, the Rip1p expression increased significantly in the WT, but not in the *iba57Δ* mutant.

Microscopy analysis shows an increment in ROS generation, associated with release of free Fe^{2+} in the *iba57Δ* mutant (Fig. 2). Interestingly, the high-intensity fluorescence observed in the *iba57Δ* mutant, which exhibited a full dissipation of mitochondrial membrane potential was associated with loss of iron homeostasis in the yeast cells.

2. Materials and methods

2.1. Yeast strains and growth conditions

Mutant strains *iba57*Δ, *rip1*Δ, and *grx5*Δ correspond to the haploid *S. cerevisiae* BY4741 (Mat a, *his3*Δ, *leu2*Δ0, *met15*Δ0, *ura3*Δ0) and its *KanMX4* interruption gene (Open Biosystems). Growth tests were carried out as described [1].

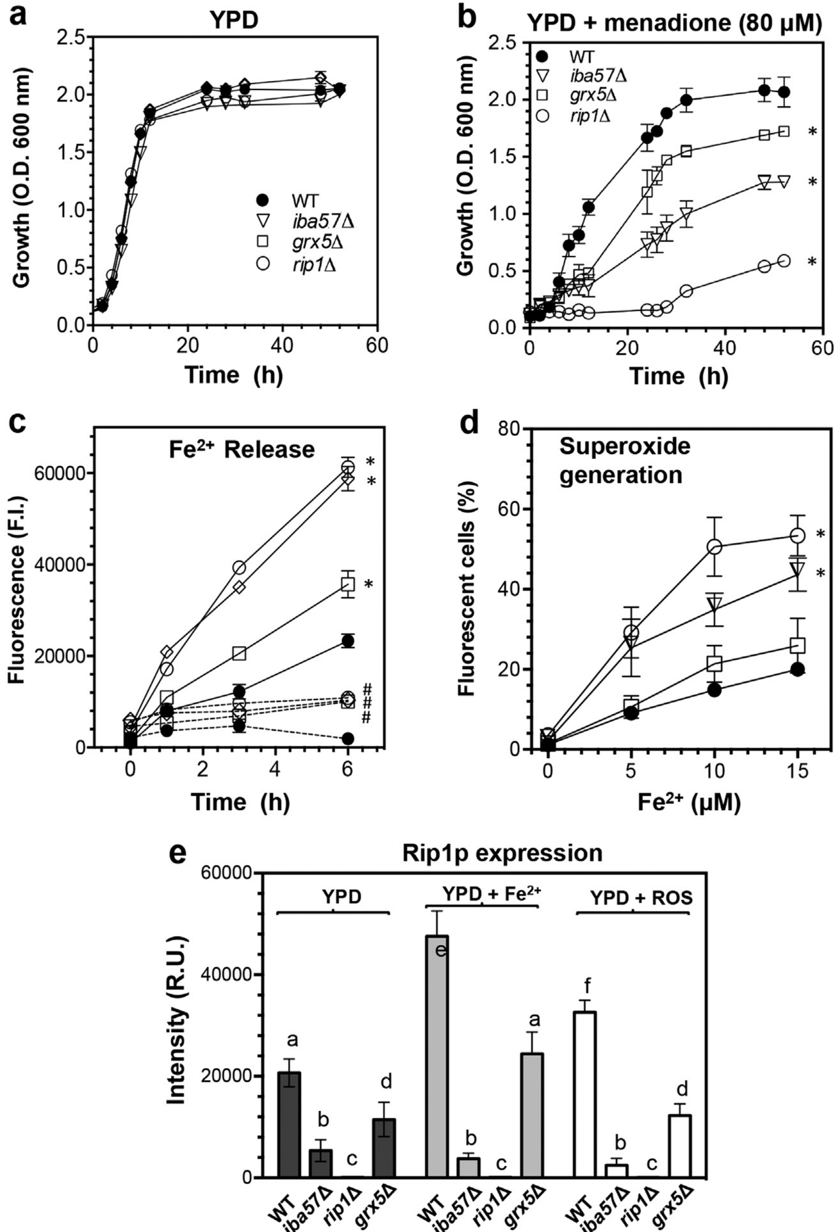

Fig. 1. Effect of the *IBA57* deletion over the growth of *Saccharomyces cerevisiae*, iron release, superoxide generation and Rip1 protein expression. a–b) Growth kinetics of *S. cerevisiae* strains grown without and in the presence of menadione 80 μM as ROS-inducer. **c**) Kinetics of Fe^{2+} release. Treatments without menadione (dashed lines) and with 80 μM menadione (continuous lines). d) $O_2^{\bullet-}$ generation in yeast suspensions treated with different concentrations of Fe^{2+} [$FeSO_4(NH_4)$]. a–d) Values are the mean of three independent experiments. **e**) Densitometry analysis of cellular extracts free-cells immunoblotted for Rip1p expression; yeast extract of cultures grown on: YPD (glucose), YPD with Fe^{2+} [$FeSO_4(NH_4)$] 20 μM, and YPD with menadione 80 μM. Means and SE are indicated as bars ($n = 3$). ANOVA was used to compare treatments. Significant differences ($p < 0.05$) are indicated as symbols (*, #) or with different lowercase letters.

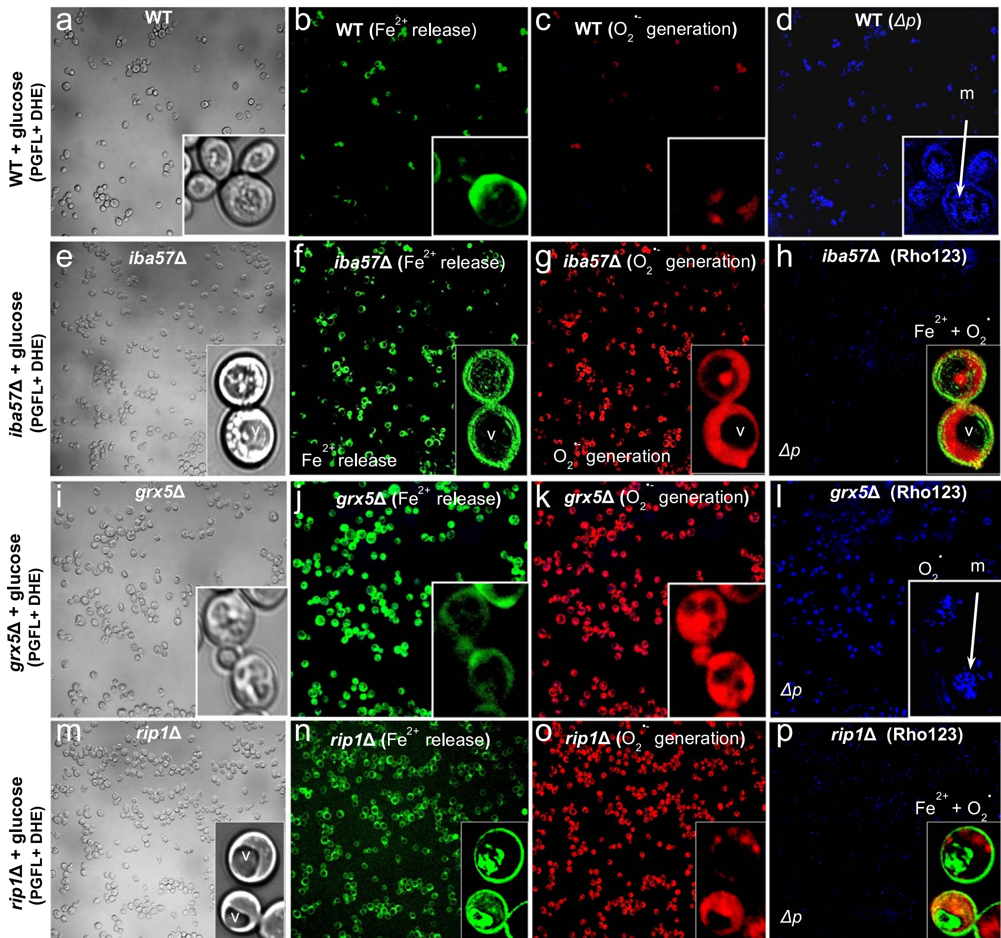

Fig. 2. Microscopy images of *Saccharomyces cerevisiae* cells for co-localization of free Fe^{2+} and superoxide in intracellular compartments. YPD-grown yeast cultures were loaded with the fluorescent probes PGFL and DHE for determination of free Fe^{2+} and $O_2^{\bullet -}$, respectively; incubated for 30 min at 30 °C and co-loaded with Rhodamine 123 for membrane potential (Δp) detection as a mitochondrial co-localization marker, and observed using a confocal microscope. a–d) Wild type (WT) yeast; e-h) *iba57*Δ mutant; i-l) *grx5*Δ mutant; and m-p) *rip1*Δ mutant. Cells are shown in which mitochondria and vacuoles are indicated by (m) and (v), respectively. Free Fe^{2+} accumulation is shown as green cells and green granules within the cells, $O_2^{\bullet -}$ generation areas are shown as red granules within the cells, and mitochondrial structures (Δp) are shown as cyan granules within the cells, using the Rho123 probe. Images of the cells were taken at 10× to 60× magnifications using a confocal microscope (Olympus FV1000).

2.2. Real-time quantification of ROS and Fe^{2+} content in S. cerevisiae cultures

Intracellular ROS and Fe^{2+} in cell suspensions were determined using cell-permeant fluorescent probes quantified by flow cytometry [1–3]. For superoxide ($O_2^{\bullet -}$) determination, yeast were incubated with 5 μg/mL dihydroethidium (DHE, Molecular Probes, Invitrogen); while as for free Fe^{2+} was used the indicator for heavy metals Phen green FL 5 μg/mL (PGFL; Molecular Probes, Invitrogen) in presence of 1 mM of the chelator 1,10-Phenanthroline (Sigma). DHE- and PGFL-fluorescence was quantified by flow cytometry monitoring the emission fluorescence at 587/40 nm and 533/30 nm, respectively; using a BD AccuriC6 Flow Cytometer (BD Biosciences).

2.3. Determination of Rip1p expression by Western blot in S. cerevisiae

Mitochondrial protein extracts 50 μg were separated by electrophoresis on SDS-PAGE gels, membranes for Western blot procedure were treated as described [1–3]. Bands intensity in films were quantified using the Image J software and data graphed as Rip1p expression intensity.

2.4. Confocal microscopy of yeast suspensions

S. cerevisiae YPD-grown cultures were loaded with the fluorescent probes DHE or PGFL and Rhodamine 123 as detailed [1–3], treated with menadione (80 μM) and mitochondrial co-localization was analyzed using a confocal microscope (Olympus FV1000). The emission signal of fluorescence was monitored at 560–580 nm for DHE, 405–505 nm for PGFL, and 533–563 nm for Rhodamine 123.

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Transparency document. Supplementary material

Transparency document associated with this article can be found in the online version at https://doi.org/10.1016/j.dib.2018.03.023.

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