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## Data Article

## Data on intracellular localization of RPSA upon alteration of its redox state

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## ABSTRACT

Ribosomal Protein SA (RPSA), a component of the 40S ribosomal subunit, was identified as a H<sub>2</sub>O<sub>2</sub> target in HeLa cells [1]. In order to analyze the intracellular localization of RPSA in different redox states we overexpressed wild-type RPSA (RPSAwt) or RPSA containing two cysteine to serine residue substitutions at positions 148 and 163 (RPSAmut) in HeLa cells. The transfected cells were exposed to H<sub>2</sub>O<sub>2</sub> or N-acetylcysteine (NAC) and RPSA subcellular localization was assessed by immunofluorescence in permeabilized cells. In addition, co-immunofluorescence for RPSA and Ribosomal Protein S6 (RPS6) was performed in cells overexpressing RPSAwt or RPSAmut. Finally, the ribosomal expression of endogenous RPSA in the presence or absence of H<sub>2</sub>O<sub>2</sub> was analyzed by Western blot.

The data presented in this work is related to the research article entitled “Hydrogen peroxide regulates cell adhesion through the redox sensor RPSA” [1].

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## Specifications Table

Subject area	Biology
More specific subject area	Redox signaling
Type of data	Images (microscopy and immunoblots), graph

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How data was acquired	<i>Immunofluorescence and image acquisition (Leica DMI 4000B inverted microscope with a spectral Leica TCS SPE confocal (Leica)), Western-blot and image acquisition (ImageQuant LAS 500 (GE Healthcare))</i>
Data format	<i>Figures containing confocal microscope processed images and analysis of Western blot by densitometry</i>
Experimental factors	<i>HeLa cells were cultured and transfected with pCIC-RPSAwt or pCIC-RPSAmut.</i>
Experimental features	<i>Transfected HeLa cells were incubated in control, oxidizing (with H<sub>2</sub>O<sub>2</sub>) or reducing (with N-acetylcysteine (NAC)) conditions. Untransfected cells were used to assess the expression of endogenous RPSA in ribosomes in oxidizing conditions.</i>
Data source location	<i>n.a.</i>
Data accessibility	<i>Data are supplied in this article</i>

### Value of the data

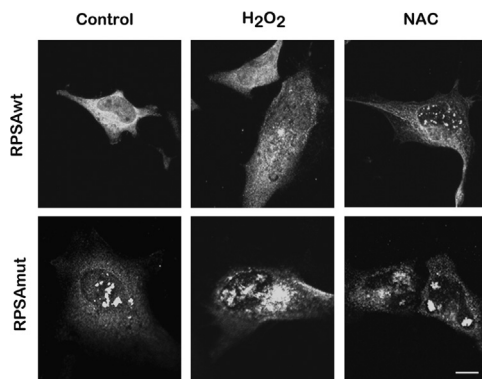
- Ribosomal proteins are redox regulated.
- The redox state of ribosomal proteins might be relevant for their subcellular localization.
- The understanding of RPSA redox regulation could provide insights about new redox signaling mechanisms.
- This data can be used to study the role of H<sub>2</sub>O<sub>2</sub> in RPSA-dependent translation.

## 1. Data

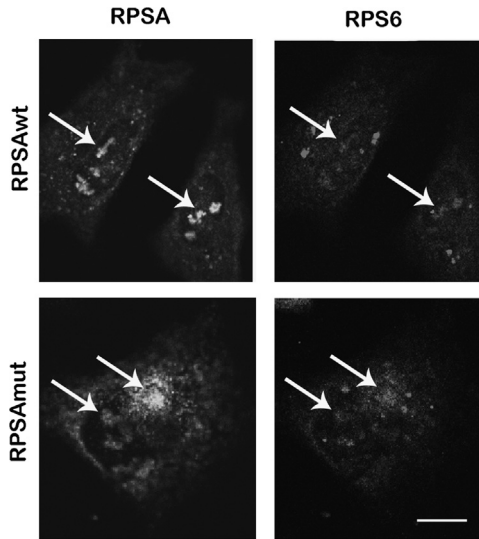
HeLa cells were transfected with pCIC-RPSAwt or pCIC-RPSAmut and incubated in control, oxidizing (with H<sub>2</sub>O<sub>2</sub>) or reducing (with NAC) conditions. RPSA expression was then analyzed by immunofluorescence. The expression was cytoplasmic and nuclear (Fig. 1).

Overlap of RPSA and the 40S ribosomal subunit RPS6 was assessed by co-immunofluorescence in HeLa cells overexpressing RPSAwt or RPSAmut (Fig. 2).

Western blot analysis of endogenous RPSA expression in ribosomal extracts was performed in HeLa cells in control or oxidizing (with H<sub>2</sub>O<sub>2</sub>) conditions (Fig. 3). H<sub>2</sub>O<sub>2</sub> treatment decreased RPSA ribosomal expression.



**Fig. 1.** RPSAwt or RPSAmut intracellular localization in different redox conditions. HeLa cells were transfected with pCIC-RPSAwt or pCIC-RPSAmut and incubated in control, oxidizing (with H<sub>2</sub>O<sub>2</sub>) or reducing (with NAC) conditions. Maximum intensity projections of confocal images of immunofluorescence for RPSA in cells fixed with PFA and permeabilized with Triton X-100. Scale bar = 10 μm.



**Fig. 2.** Comparison of RPSAwt or RPSAmut intracellular expression with that of RPS6. HeLa cells were transfected with pCIC-RPSAwt or pCIC-RPSAmut. Single optical sections from confocal images of co-immunofluorescence for RPSA and the 40S ribosomal subunit RPS6 in cells fixed with methanol and permeabilized with Triton X-100. Arrows indicate regions of overlap. Scale bar = 10  $\mu$ m.

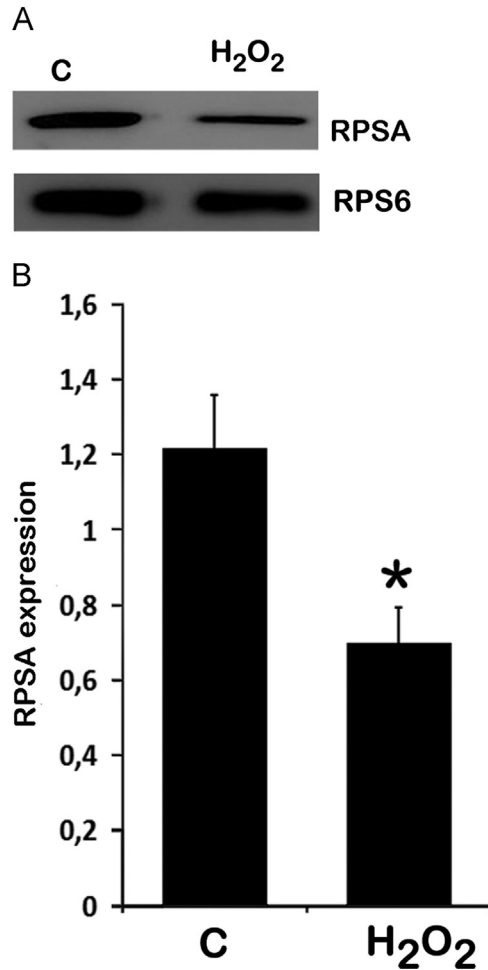
## 2. Experimental design, materials and methods

### 2.1. Cell line, plasmids and cell transfection

HeLa cervical carcinoma cells were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in DMEM (Hyclone, Thermo Scientific) supplemented with 10% (*v/v*) fetal bovine serum (FBS, Sigma-Aldrich), penicillin-streptomycin solution (Hyclone, Thermo Scientific) and 2 mM L-glutamine (Hyclone, Thermo Scientific) in uncoated or 10  $\mu$ g/ml laminin-coated plates (laminin, L2020, was purchased from Sigma-Aldrich). pCIC-RPSAwt (with the coding region of human RPSA [Uniprot:P08865]) and pCIC-RPSAmut (with the coding region of human RPSA containing two cysteine to serine residue substitutions at positions 148 and 163) are described in [1]. These plasmids were transfected into HeLa cells through lipofection with Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. The presence of IRES:mCherry-NLS [2] in all plasmids allowed the identification of transfected cells.

### 2.2. Immunofluorescence and imaging

HeLa cells were plated on laminin-coated glass coverslips and left to adhere for 24 h, after which they were transfected with either pCIC-RPSA or pCIC-RPSAmut and incubated overnight. Cells were then incubated with 20–40  $\mu$ M H<sub>2</sub>O<sub>2</sub> by the steady state delivery method [3] for 30 min, after which they were fixed in 4% (w/v) PFA in PBS for 15–20 min at room temperature or in 100% methanol for 15 min at  $-20^{\circ}$  C. After fixation, cells were permeabilized with 0.5% (v/v) Triton X-100 in PBS for 15 min. Primary antibodies were then incubated overnight at 4  $^{\circ}$ C after blocking with 3% (v/v) FBS in PBS. Secondary antibodies were incubated for 2 h at room temperature. Immunofluorescence to detect wild-type or mutant RPSA was performed on fixed HeLa cells using the H-141 primary rabbit polyclonal antibody (1:100, sc-20979, Santa Cruz Biotechnology), or the F-18 primary goat polyclonal antibody (1:50, sc-21534, Santa Cruz Biotechnology) in the co-immunofluorescence experiment with the rabbit polyclonal  $\alpha$ -S6 Ribosomal Protein (1:50, 2212, Cell Signaling). Secondary antibodies used were goat  $\alpha$ -rabbit Alexa 488 (1:1000, Molecular Probes, Invitrogen); donkey  $\alpha$ -goat Alexa 488



**Fig. 3.** Endogenous RPSA ribosomal expression in different redox conditions. (A) Representative Western blot for endogenous RPSA expression in ribosomal extracts from HeLa cells, in control or oxidizing (with H<sub>2</sub>O<sub>2</sub>) conditions. RPS6 expression was used as loading control. (B) Quantification of Western blots (values show the mean and SEM; two-tailed Student's *t* test, \**p* < 0.02; *N*=3).

(1:1000, Molecular Probes, Invitrogen); goat  $\alpha$ -rabbit Alexa 568 (1:1000, Molecular Probes, Invitrogen). Immunofluorescence images were obtained on a Leica DMI 4000B inverted microscope using a spectral Leica TCS SPE confocal. For this, we took Z-stacks for each of at least 5 independent fields per coverslip with a Leica ACS APO 63x/1.15 water objective, a step number of 5–20 and step size of 0.5–1  $\mu$ m. Wavelength selection bandwidths were 500–560 nm for the 488 nm laser and 585–650 nm for the 532 nm laser.

### 2.3. Extraction of ribosomal proteins and Western blot

HeLa cells were plated in uncoated plates and incubated with a sublethal concentration of 20–50  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 30 min using the steady state delivery method [3]. After H<sub>2</sub>O<sub>2</sub> incubation, cells were harvested and ribosomal protein extraction was performed as described previously [4]. Briefly, cells were washed twice with ice-cold PBS and scraped into ice-cold PBS pH 7.4 and centrifuged for 5 min at 500 g. The pellet was resuspended in buffer A (250 mM sucrose, 250 mM KCl, 5 mM MgCl<sub>2</sub>, 50 mM

Tris-HCl pH 7.4) followed by NP40 addition for a final concentration of 0.7% (v/v). Cellular suspension was incubated on ice for 10 min and centrifuged for 10 min at 750 g. The supernatant corresponding to the cytoplasmic fraction was further centrifuged at 12500 g for 10 min to eliminate the mitochondria present in the pellet. KCl was added to the supernatant to a final concentration of 0.5 M and centrifuged over a sucrose cushion (1 M sucrose, 0.5 M KCl, 5 mM MgCl<sub>2</sub>, 50 mM Tris-HCl pH 7.4) for 2 h at 250,000 g at 4 °C. The pellet containing the ribosomes was washed twice with ice-cold water and resuspended in a buffer containing 25 mM KCl, 0.5 mM MgCl<sub>2</sub> and 50 mM Tris-HCl pH 7.4.

Protein loading buffer was added to each sample and protein content was analyzed by SDS-PAGE using 8% (w/v) polyacrylamide gels, after which it was transferred to a nitrocellulose membrane. Primary antibodies were diluted in blocking solution (PBS, 0.1% (v/v) Tween-20, 3% (w/v) milk) and added to the membrane which was incubated overnight at 4 °C. Primary antibodies used were goat polyclonal  $\alpha$ -RPSA (F-18) (1:200, sc-21534, Santa Cruz Biotechnology) and rabbit polyclonal  $\alpha$ -S6 Ribosomal Protein (1:200, 2212, Cell Signaling). Membranes were then washed and incubated with species-matched peroxidase-conjugated secondary antibody diluted in blocking solution (1:5000, sc-2020 and sc-2004, respectively, Santa Cruz Biotechnology). Labeled bands from washed membranes were detected by ECL (Millipore), images were acquired with ImageQuant LAS 500 (GE Healthcare) and protein expression was quantified with Fiji [5]. RPSA ribosomal expression was normalized by total protein loaded (Ponceau staining) (3 independent experiments).

## Conflict of interest

none

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