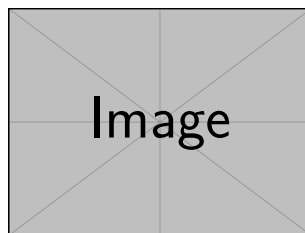


Firstname LASTNAME

Characterisation of a Novel Protein Complex Involved in Post-Transcriptional Regulation During Cellular Stress Response



Laboratory:
Supervisor:
Internship dates:

Laboratory of RNA Biology, Montpellier, France
Dr. Jane Smith
02 February 2025 – 30 June 2025

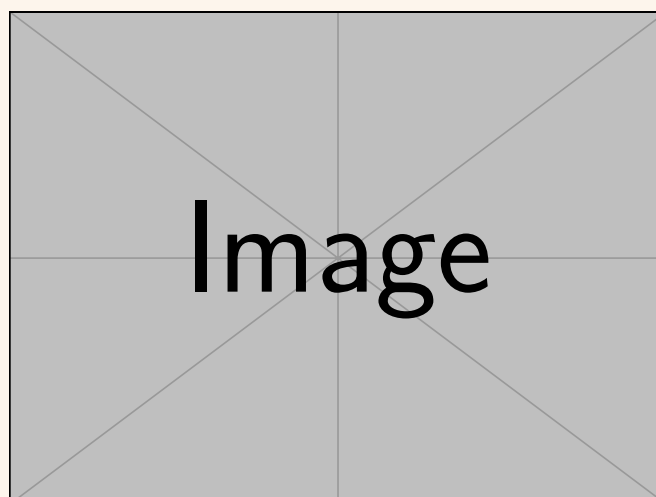
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Previous internships

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L3	Lab name, City, Country	Dr. Firstname LASTNAME	Biological topic
M1	Lab name, City, Country	Dr. Firstname LASTNAME	Biological topic
M2			

Abstract

Write your abstract here. Single paragraph, 150–200 words, English. No literature references permitted. Include: (1) brief biological background and question addressed, (2) description of main results without extensive experimental detail, (3) summary of significance. Keep language accessible to non-specialists. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna. Donec vehicula augue eu neque.



Keywords: *RNA-binding proteins, stress granules, post-transcriptional regulation, proteomics, yeast two-hybrid*

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1. Introduction

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2. Materials and Methods

Cell culture and strains. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna.

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3. Results

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3.1 Identification of interaction partners

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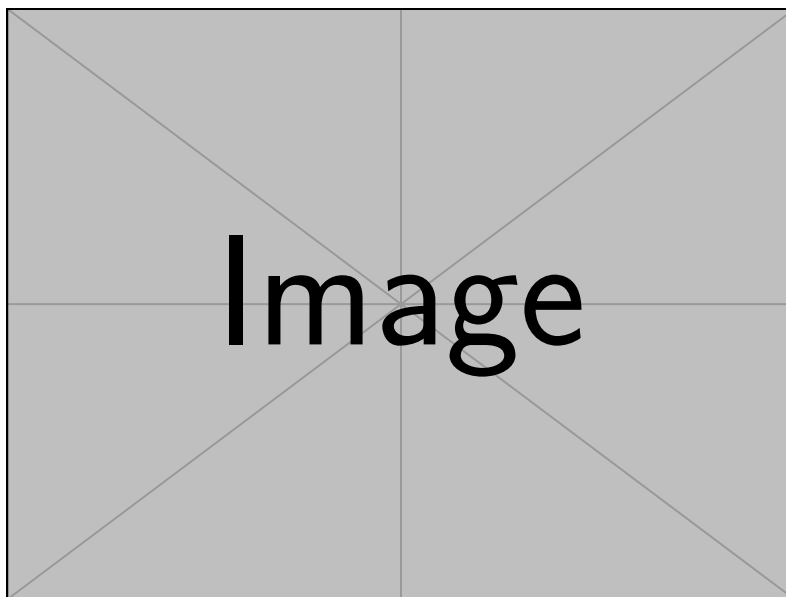


Figure 1. Identification of proteins co-purifying with the bait. (A) Schematic of the pull-down strategy. (B) Volcano plot ($n = 3$ biological replicates); dashed lines: $p < 0.05$ and fold-change ≥ 2 ($\log_2$ scale). (C) Validation by co-IP; Input (I), flow-through (FT), eluate (E). Scale bar = $10 \mu\text{m}$. Data are mean $\pm$ SD. See also Fig. 4.

3.2 Functional validation

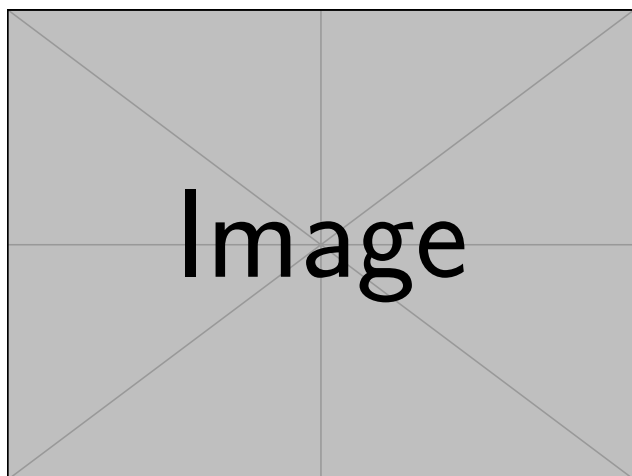


Figure 2. Co-immunoprecipitation confirms the interaction *in vivo*. Input (I), flow-through (FT) and eluate (E) are shown. Molecular weight markers (kDa) on the left. Three independent experiments gave similar results^[1].

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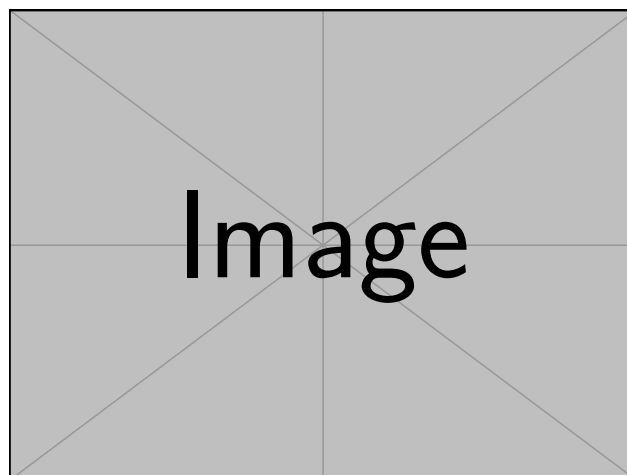


Figure 3. Stress-induced relocation of the complex. Confocal images of GFP-tagged bait under basal conditions (left) and after 30 min treatment with $1 \text{ mM H}_2\text{O}_2$ (right). Scale bar = $5 \mu\text{m}$. $n > 100$ cells per condition, $p < 0.001$, Mann-Whitney U -test^[2].

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Table 1. Summary of identified interaction partners. Proteins co-purifying with the bait identified by mass spectrometry ($n = 3$ biological replicates). Enrichment is expressed as $\log_2$ fold-change (FC) over the control. p -values corrected by Benjamini–Hochberg. nd, not detected in control.

Protein	Function	$\log_2$ FC	Adj. p -value	Validation
ProteinA	RNA-binding	4.2	1.3×10^{-4}	co-IP, Y2H
ProteinB	Stress granule	3.8	2.1×10^{-4}	co-IP
ProteinC	Translation factor	2.9	8.3×10^{-3}	Y2H
ProteinD	mRNA decay	2.4	1.2×10^{-2}	—
ProteinE	Scaffold/unknown	1.8	4.5×10^{-2}	—

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3.3 Structural characterisation

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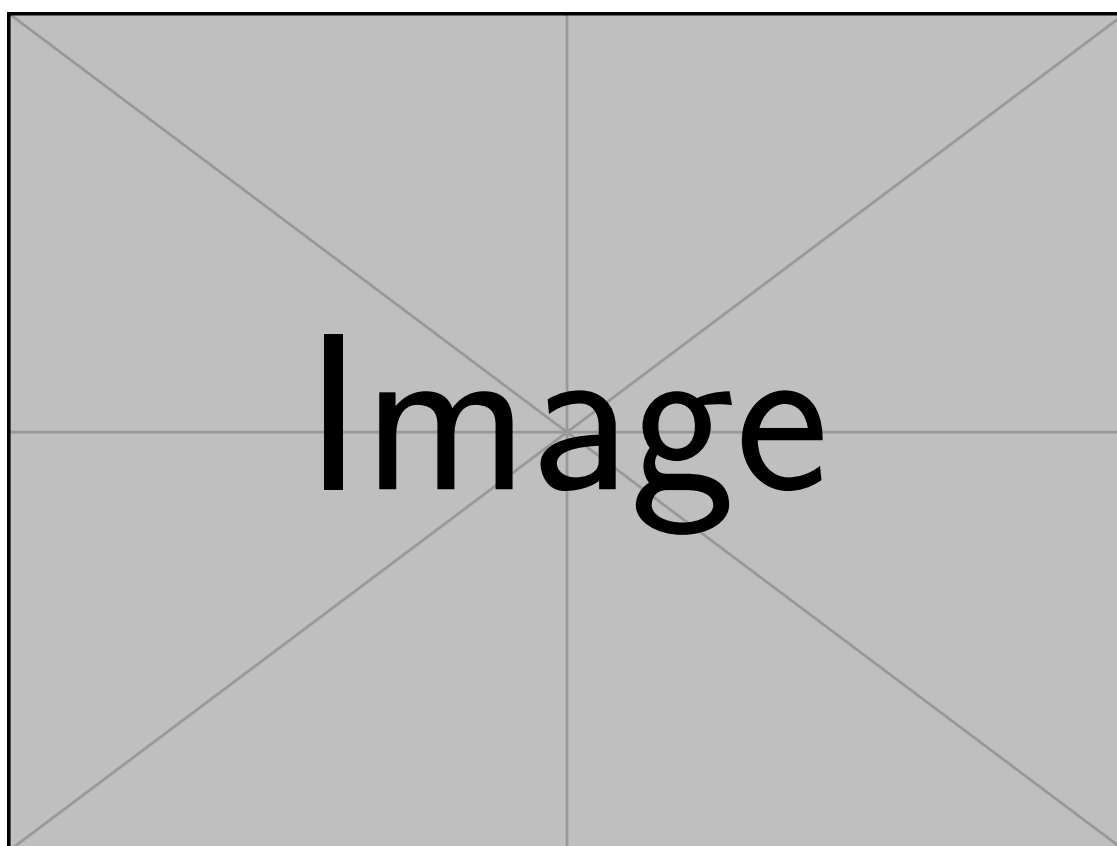


Figure 4. Interaction network of the identified complex. Nodes: proteins coloured by functional category. Edge thickness: interaction score. Outlined nodes: validated by ≥ 2 independent methods. $K_d = 8.5 \times 10^{-9}$ M (SPR, $n = 3$)^[3]. Visualised with Cytoscape 3.9.

4. Discussion

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Table 2. Strains used in this study. All strains are *S. cerevisiae* BY4741 background unless otherwise stated.

Name	Genotype	Source
BY4741	<i>MATa his3Δ1 leu2Δ0</i>	Nguyen et al., 2020
yFL001	BY4741 <i>protA-GFP::HIS3</i>	This study
yFL002	BY4741 <i>ΔprotB::KanMX</i>	This study

[TODO: Discuss limitations and propose follow-up experiments.]

LaTeX reference examples (delete before submission)

Below are live examples of every useful construct for a biology report. Delete this entire subsection before submitting.

Units (siunitx). Speed: $30\text{ cm} \cdot \text{s}^{-1}$ or $30\text{ cm} \cdot \text{s}^{-1}$
Cell diameter: $10\text{ }\mu\text{m}$ (or $10\text{ }\mu\text{m}$).
Protein concentration: $200\text{ }\mu\text{g} \cdot \text{mL}^{-1}$.
Reaction temperature: $37\text{ }^{\circ}\text{C}$ (or $37\text{ }^{\circ}\text{C}$).

Centrifugation: 1500 rpm for 10 min at $4\text{ }^{\circ}\text{C}$.
Wavelength: 280 nm or 280 nm.
Molarity: 5 mM NaCl, 2 μM inhibitor (μM).
Range: 50 μL to 100 μL .

Scientific notation. Avogadro: 6.022×10^{23} .
Boltzmann: $1.38 \times 10^{-23}\text{ J}\cdot\text{K}^{-1}$.
Dissociation constant: $K_d = 8.5 \times 10^{-9}\text{ M}$.
Fold enrichment: 3.2-fold increase.

Chemical formulae (mhchem).

- Hydrogen peroxide: H_2O_2
- Energy currency: $\text{ATP} \longrightarrow \text{ADP} + \text{P}_i$
- Redox: $\text{NAD}^+ + \text{H}^+ + 2\text{e}^- \longrightarrow \text{NADH}$
- Ion: Ca^{2+} , Mg^{2+}
- Radioisotope label: ^{32}P
- Buffer: 50 mM NaH_2PO_4 , pH 7.4

Statistics.

- All data are mean $\pm$ SD ($n = 3$ biological replicates) unless stated otherwise. Unpaired two-tailed Student’s t -test: $p = 0.021$.
- One-way ANOVA: $F(2, 12) = 8.4$, $p = 0.005$, Tukey post-hoc.
- Mann–Whitney U -test: $p < 0.001$.
- Pearson correlation: $r = 0.95$, $p < 0.001$.
- Effect size: ≥ 2 -fold ($\log_2$ scale).
- $n > 100$ cells per condition.

Gene and protein names.

Gene (yeast, italics) *cdc28*, *act1*.
Protein (yeast, roman) Cdc28, Act1.
Gene (human, italics caps) *BRCA1*, *TP53*.
Protein (human, roman caps) BRCA1, p53.
Mutant allele *ade2* Δ .
Species *S. cerevisiae*, *E. coli*, *C. elegans*.

Equations. Michaelis–Menten:

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

(1)

where $V_{\max}$ is the maximal velocity and K_m the substrate concentration at half-maximal velocity.
Henderson–Hasselbalch:

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

Beer–Lambert:

$$A = \varepsilon \cdot l \cdot c$$

Hemoglobin Binding Model (cf. Eq. 1):

$$\theta = \frac{[L]^n}{K_d^n + [L]^n} = \frac{1}{1 + \left(\frac{K_d}{[L]}\right)^n} \quad (2)$$

$$\frac{d\theta}{dt} = k_{on}[L]^n(1 - \theta) - k_{off}\theta \quad (3)$$

Cross-references. See Fig. 1 for the pull-down results, Fig. 2A,B for the co-IP validation, and Fig. 4 for the full interaction network. Statistical methods are described in Section 2.

See Figs. 1 and 2 for the pull-down and co-IP data.

Bibliography citations (superscript coloured [n]). As shown previously^[1], the complex assembles under stress. These results are consistent with independent reports^[2,3]. For reviews, see^[4–6].

Typography notes. *In vivo*, *in vitro*, *in situ* — always italics.

Multiplication sign: 5×10^6 cells (never letter x).

Plus-minus: 12.3 ± 0.4 kDa.

Approximately: ≈ 300 nm.

Non-breaking space before units and references: $10 \mu\text{m}$ (use $\sim$).

En-dash for ranges: 10–20 μm (two hyphens in source).

Author Contributions

F.L. designed and performed all experiments, analysed the data, and wrote the manuscript.

J.S. conceived the project, provided reagents and supervised the internship.

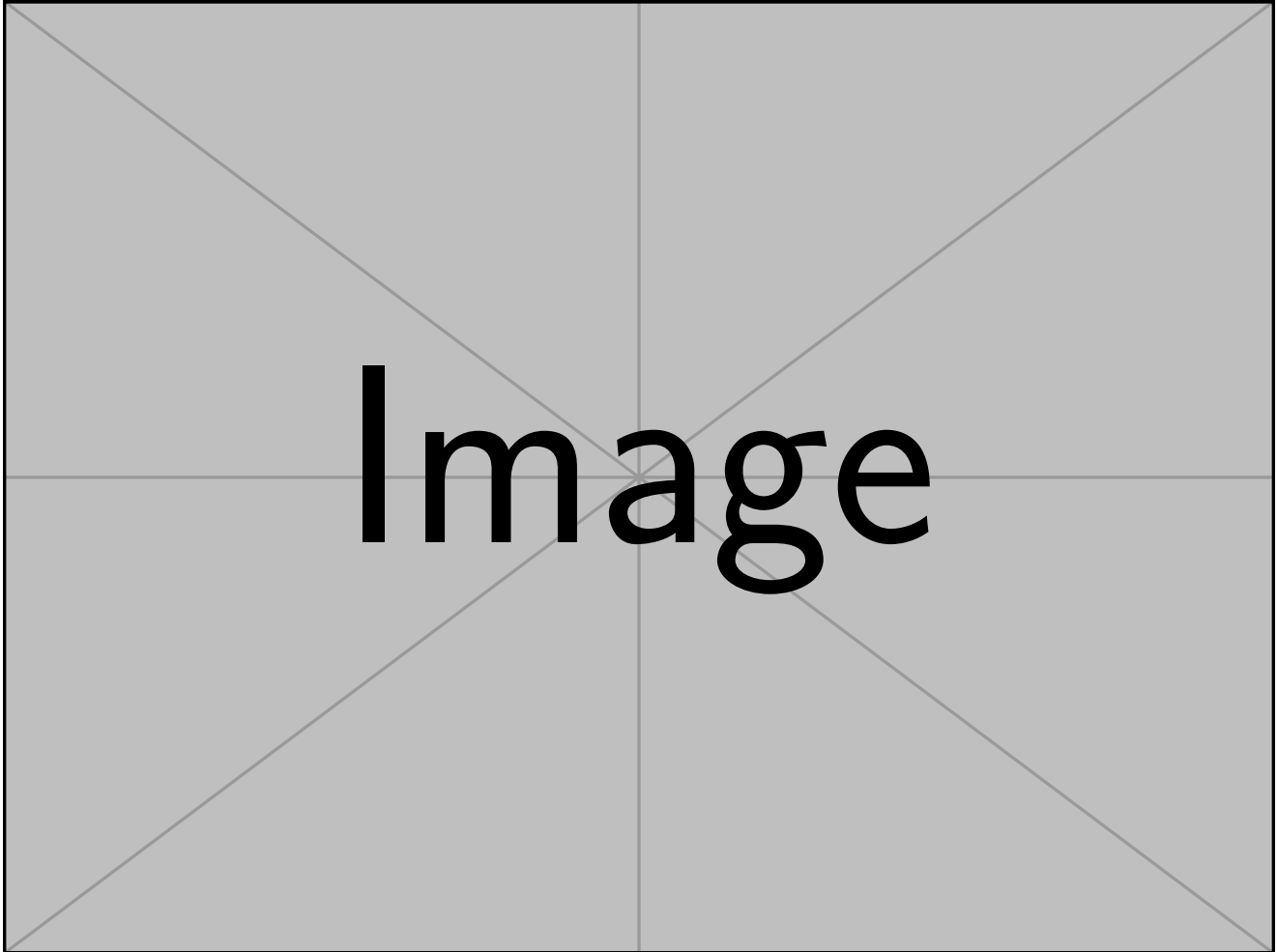
J.D. provided academic supervision and critically revised the manuscript.

Acknowledgements

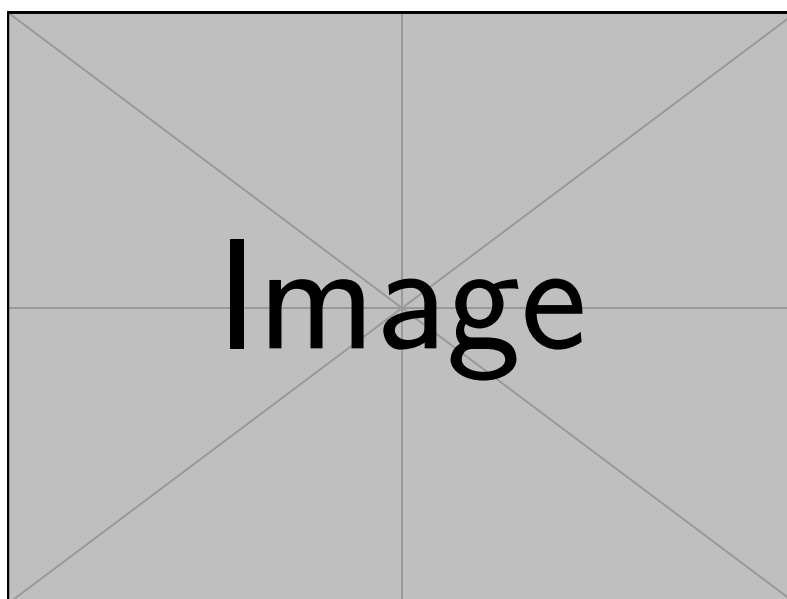
This report was typeset using a custom L^AT_EX class developed for ENS de Lyon Biosciences internship reports.

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- [1] M. Nguyen, H. Deng, D. K. Bhatt, M. Bhatt, and S. Vincent. “The Drosophila R-Spondin ortholog Dorsocross interacts with Wingless to pattern the embryonic epidermis”. In: *Development* 147.3 (2020), dev183699.
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Supplemental Figure S1. Representative confocal micrograph of GFP-tagged Ssa1 in control cells. Cells expressing Ssa1-GFP were imaged by confocal microscopy (63× oil, 0.13 μm /pixel). Scale bar: 5 μm . ($n = 50$ cells from three independent experiments.)



Supplemental Figure S2. Western blot confirming equal loading. Total protein extract (20 μg per lane) was separated by SDS-PAGE and probed with anti-PGK1 as a loading control. Molecular weight markers (kDa) are indicated on the left.