FAPS a new FACS based method for the purification of membrane-less organelles such as P-bodies

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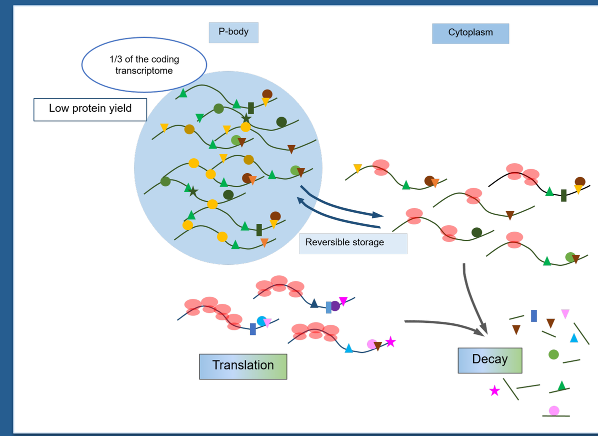
WHAT ARE THE DYNAMICS OF RNA STORAGE P-BODIES ACROSS THE CELL CYCLE ?

INTRODUCTION

P-bodies (Processing-bodies), are spherical cytoplasmic ribonucleoprotein granules, of all eukaryotic, animal and plant cells. They contain 1/3 of the coding transcriptome. Which have a 500 nm diameter and rarity less than a dozen per cell. The mRNA they store include those encoding proteins involved in cell cycle.

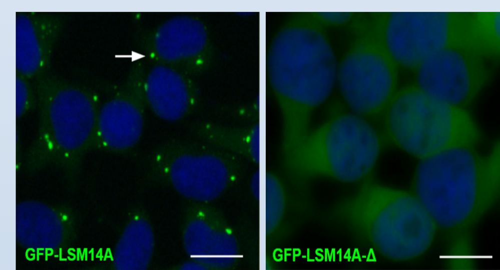


ABSTRACT

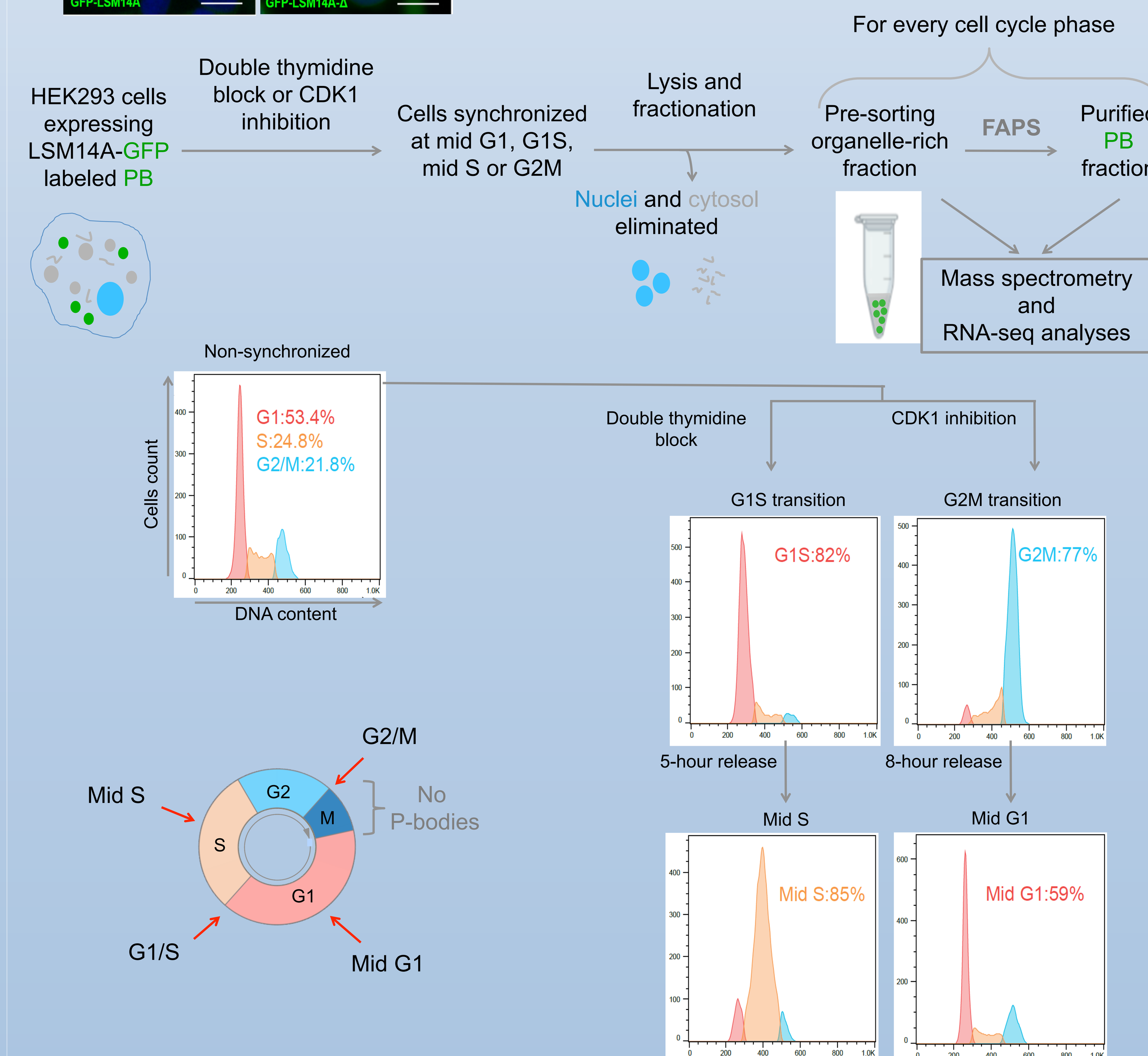
Subcellular RNA localization is a fundamental post-transcriptional process that can fine-tune gene expression. One aspect of RNA trafficking that has been receiving increasing attention is targeting into phase-separated ribonucleoprotein (RNP) granules, which include processing bodies (p-bodies). P-bodies contain RNA as well as proteins involved in various aspects of RNA metabolism such as translational repression. Although they are conserved in eukaryotes and constitutively expressed in mammalian cells, their exact cellular functions remain unknown. This is partly owed to the difficulty in purifying them due to their lack of membrane, small size, and the absence of molecular markers unique to them. Here, we describe Fluorescence Activated Particle Sorting (FAPS), a flow cytometry-based method to purify membrane-less organelles such as p-bodies.

AIM AND METHOD

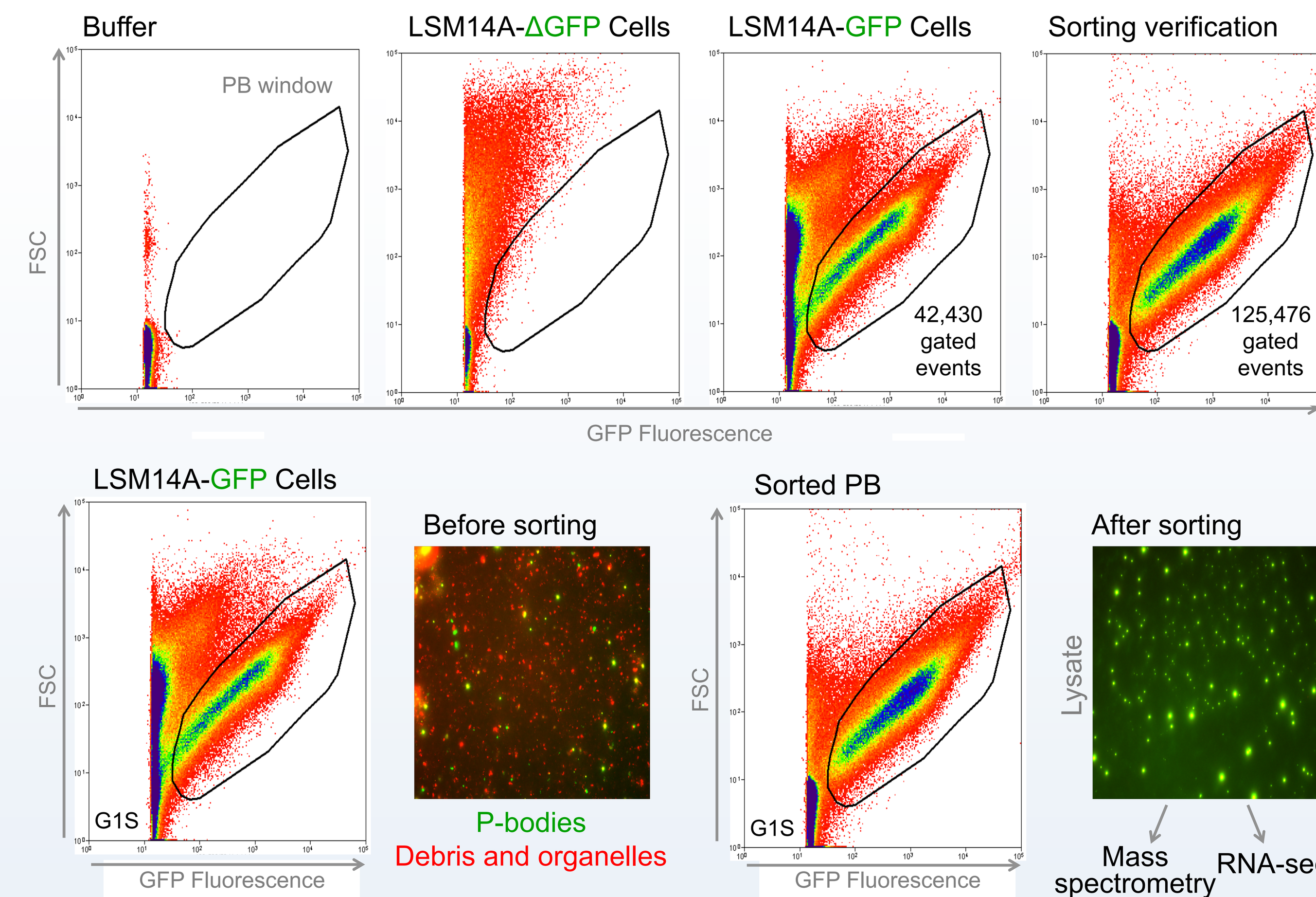
FAPS uses GFP-tagged P-body fluorescence and particle size to detect and sort granules from a cytoplasmic cellular lysate.

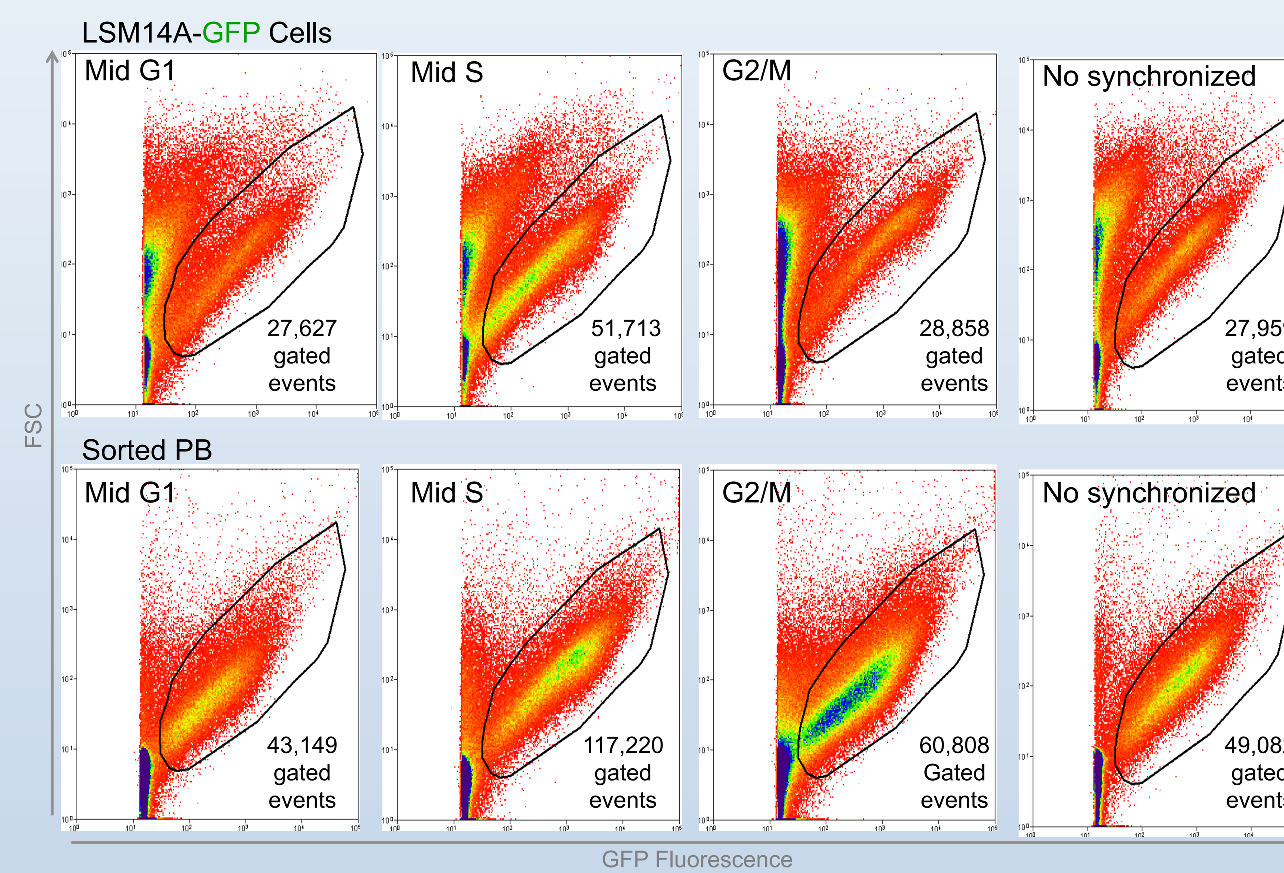


- P-bodies labeled through LSM14A-GFP in HEK cells.
- Truncated protein not in P-bodies as a negative control (diffuse GFP).

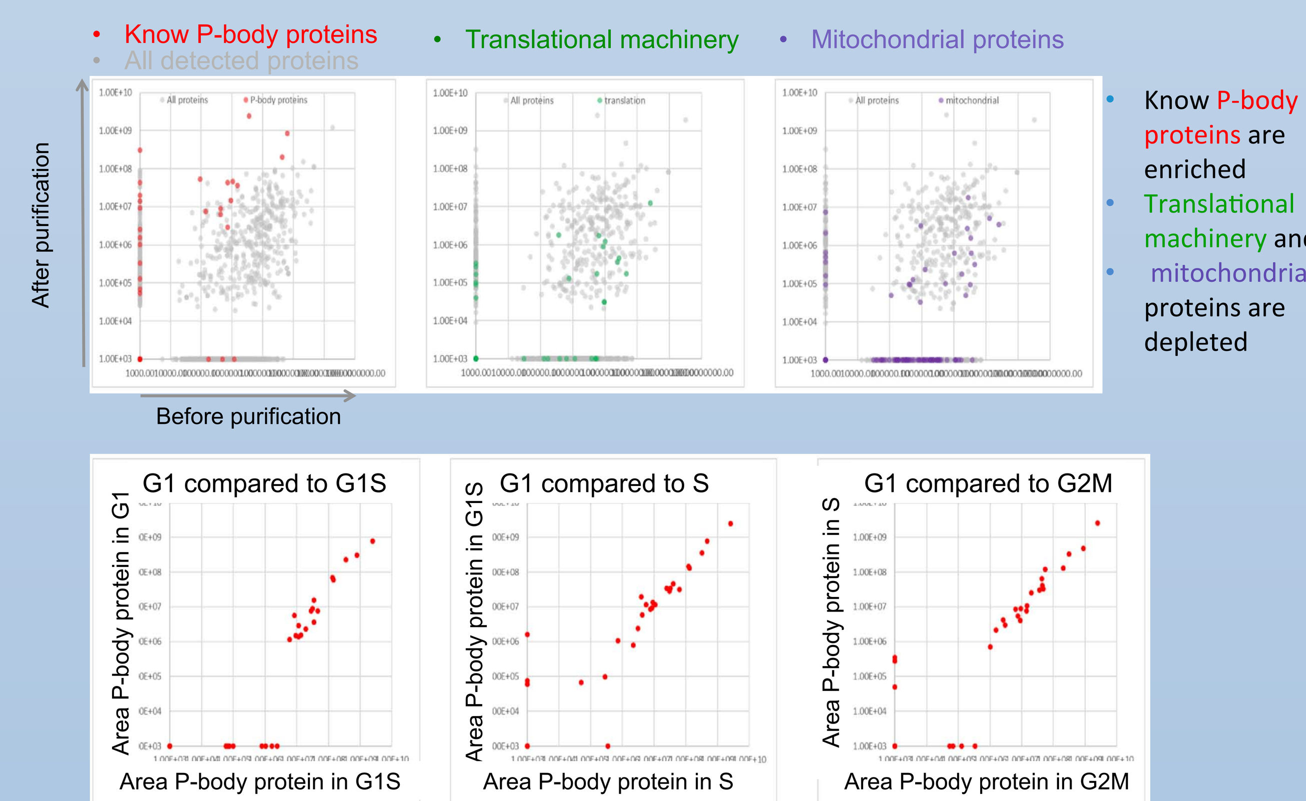


SORTING





A control consisting of a lysate-free buffer as well as cells expressing diffuse cytoplasmic non-granule GFP is used to determine the sorting window. Sorted events are collected in a stabilizing buffer and a fraction of them is re-injected in the cell sorter for validation (MoFlow Astrios EQ, Beckman-Coulter).



RESULTS

P-body protein content across the cell cycle

- Know P-body proteins
- All detected proteins
- Translational machinery
- Mitochondrial proteins

After purification / Before purification

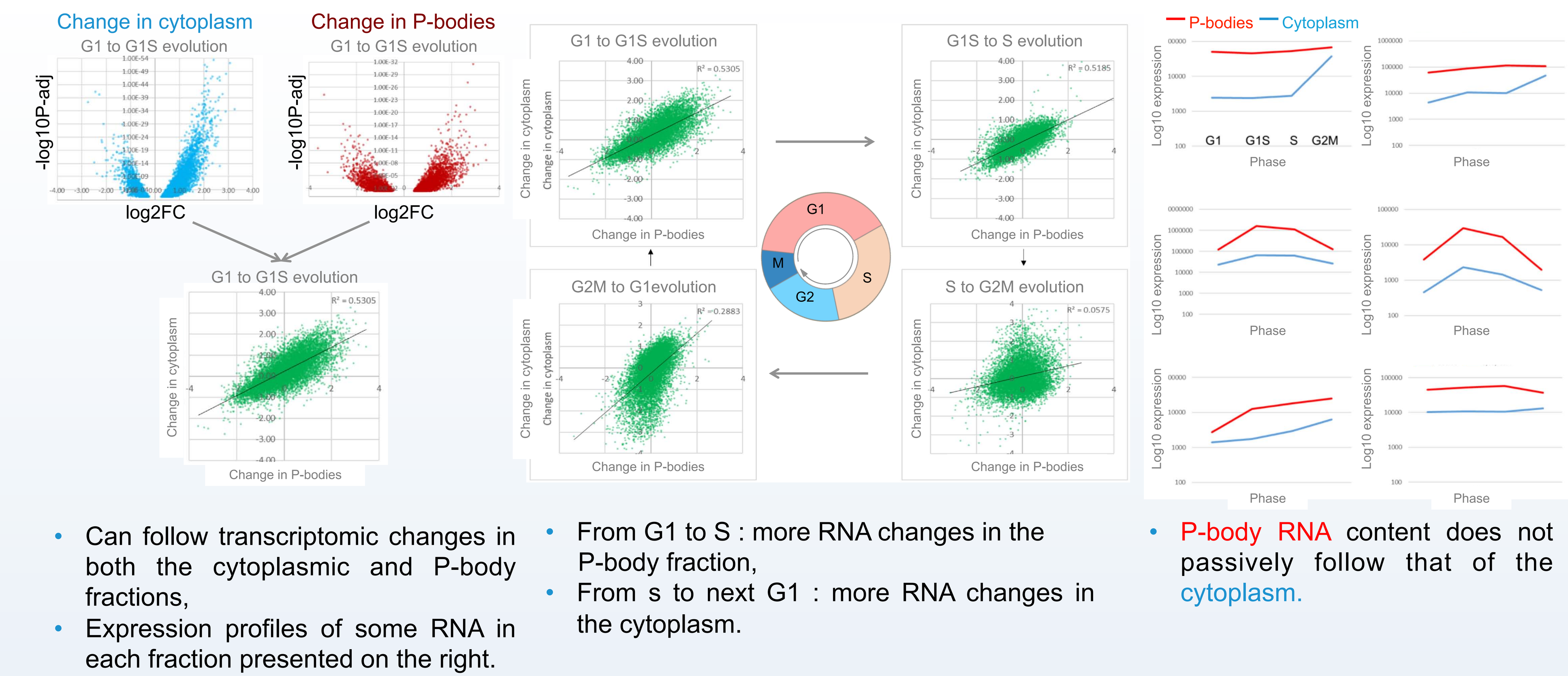
- Know P-body proteins are enriched
- Translational machinery and mitochondrial proteins are depleted

G1 compared to G1S — Area P-body protein in G1 / Area P-body protein in G1S
G1 compared to S — Area P-body protein in G1S / Area P-body protein in S
G1 compared to G2M — Area P-body protein in S / Area P-body protein in G2M

The FAPS-purified fraction corresponds to P-bodies, main P-body proteins do not significantly vary in purified P-bodies across the cell cycle.

RESULTS

P-body RNA content across the cell cycle

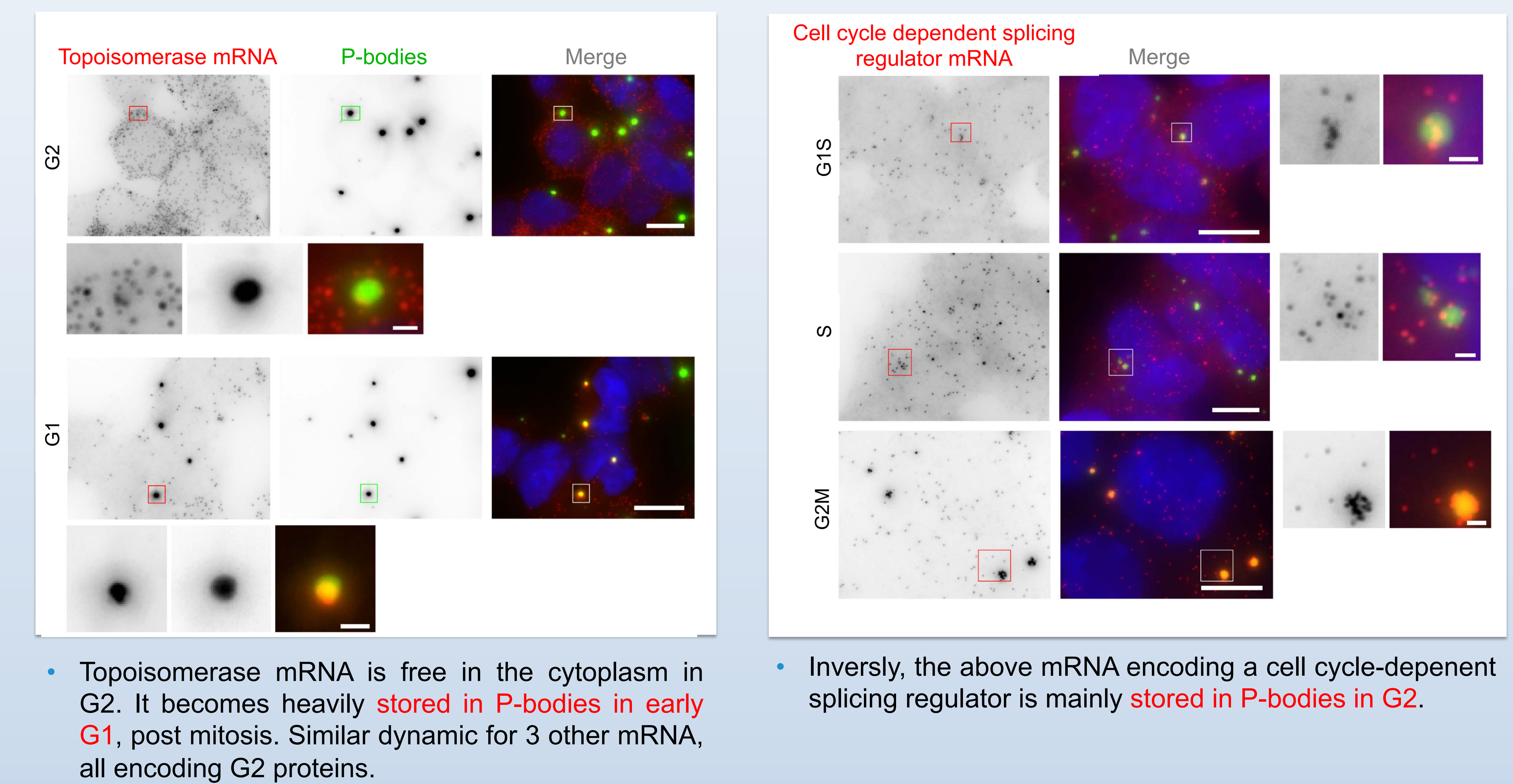


- Can follow transcriptomic changes in both the cytoplasmic and P-body fractions,
- Expression profiles of some RNA in each fraction presented on the right.

- From G1 to S : more RNA changes in the P-body fraction,
- From s to next G1 : more RNA changes in the cytoplasm.

- P-body RNA content does not passively follow that of the cytoplasm.

Validation by single molecule FISH



- Topoisomerase mRNA is free in the cytoplasm in G2. It becomes heavily stored in P-bodies in early G1, post mitosis. Similar dynamic for 3 other mRNA, all encoding G2 proteins.
- Inversly, the above mRNA encoding a cell cycle-depenent splicing regulator is mainly stored in P-bodies in G2.

Cyclin mRNA — Merge (G1S, S, G2M)

APC regulator mRNA — Merge (G1S, S, G2M)

- An mRNA encoding a cyclin is mainly stored in P-bodies in G1S.
- mRNA encoding an APC regulator is significantly stored in cell cycle phases. Storage peaks in S phase, ~ 60% of mRNA in P-bodies.

CONCLUSION

P-bodies are dynamic sites of RNA storage : they undergo more transcriptomic changes in the 1st half of the cell cycle, from G1 to S. These changes are often not proportional to cytoplasmic expression levels : no evidence of a P-body buffering function.
Differential mRNA storage in P-bodies can be quantitively significant depending on the cell cycle phase.

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