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An image based high throughput screen to identify regulators of Imp containing RNP granules

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I. Context

In vitro, RNAs and proteins are frequently packaged into diverse dynamic macromolecular structures named RNA granules. These assemblies form upon phase separation of individual RNA and protein components, a process involving the establishment of multivalent weak interactions and their regulations via post-translational modifications. Defects in their properties have been associated with several human pathologies. However, our knowledge of these dynamic structures relies essentially on the study of P bodies and stress granules.

We are interested in the highly conserved RNA binding protein Imp whose mammalian counterpart’s overexpression correlates with poor prognosis in several cancers. In vitro, Imp is present in cytoplasmic RNP granules, distinct from P bodies and visible both in neuronal cell bodies and axons. They are also detected in Drosophila S2R+ cultured cells.

Taking advantage of this cellular model, we have undertaken a genome-wide RNAi-based visual screen to identify factors that regulate the formation of Imp-containing granules. This requires combining high throughput microscopy with the development of a computational pipeline for automatic image analysis. This pipeline first segments and discriminates healthy from dead nuclei, storing this information in an interactive SQLite database that enables experimental quality control. Then, GFP–Imp granules are detected using the SPADe algorithm in the cytoplasts of healthy cells. From the pilot screen we have performed to validate the experimental design and develop our pipeline for data mining are presented.

II. Pilot screen

Two sublibraries were screened:

- A: from DRSC (Harvard, USA) 1:5 084 MIP images to cell masks
- B: high throughput RNAi pilot screen

Plate map 488 nm : GFP signal segmentation and analysis

405 nm : DAPI signal

99.32 % of the images are enhanced (B), shown here, the mushroom body neurons.

(a) Overlay GFP and DAPI signals

(b) Image-based high throughput screen to identify regulators of Imp containing RNP granules

488 X-918 pixels, 10 bit 125M

CellProfiler

CellProfiler Analyst

CellProfiler Supervised Phenotype Classification

CellProfiler Database

- 2,4 084 MIP images from 24 plates*240 wells*16 views*17 stacks*2 channels => 3.10^6 images !

SPADe

Defects and clustering of positive hits

Specification of healthy nuclei outlines

- Identification of cell death inducing conditions

Specification of cell outlines and structures

- Visual inspection of raw data indicators

1. The cell population inside each well is heterogeneous

2. In transfected cells, expression levels of the GFP-Imp molecule are variable: low, medium or high. Only cell expressing GFP-Imp at a low level are interesting.

3. Depending on the cells, the RNP-Imp granules may be more or less numerous, larger or smaller; they may accumulate locally or be distributed homogeneously throughout the cytoplasm.

4. Inactivation of most of the genes tested does not induce a detectable phenotype by eye

5. Hits with more granules detected (about 1%)

V. Semi-Automatic Cell Segmentation:

- GFP MIP images to cell masks

GFP MIP images (A) are first locally-normalized to reduce the effect on the non-uniform illumination. Then, a watershed-based segmentation produces the corresponding over-segmented (superpixel) image (B). Finally, the interface allows to manually select the superpixels to segment the cells of interest by creating masks (B). In the future, an automatic process for superpixels merging will be developed.

VI. SPADE:

- an algorithm for small particle detection

The mask generated by the supervised segmentation algorithm (A) is used to extract from the original MIP image processed for enhanced contrast normalization (B), the GFP positive cytoplasts of healthy cells whose intensity ranges from 100 to 1000 (C). This letter is analyzed by SPADE that computes the joint-point process concept of minimizing an energy function with the use of a pre-defined definition of shapes, allowing the detection of small objects, ranging from 4 to 25 pixels in a homogeneous context (D) (1). http://spade.gforge.inria.fr/  2. https://www.genes-fct.org

VII. Preliminary data:

- Images from different control wells (LacZ and water) of one or two independent plates as well as images from 2 possible hits were analyzed as described before (IV to VI). Randomization of the controls. For quality control, we use median normalization per plate (excluding controls).

Collaborators

- DRSC (Harvard, USA) - High throughput RNAi screen

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