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 RNP 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. Defect 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 RNA-binding visual screen to identify factors that regulate the biogenesis 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 marks, storing this information in an interactive SQL database that enables experimental quality control. Then, GFP-Imp granules are detected using the SPADI algorithm in the cytoplasm of healthy cells. From the pilot screen we have performed to validate the experimental design and develop our pipeline for data mining, we present.

II. Pilot screen

Plate map: 406 genes, 4 plates and H: high level of GFP markers

# Two sublibraries were screened:
- RNA Binding Proteins sublibrary: 406 genes, 4 plates
- Dicer sublibrary: 366 genes, 4 plates

# For well 20-299 cells were randomly transfected (L: low, M: medium and H: high level of GFP-Imp expression).

# Two sublibraries were used:
- Phosphatases – Kinasosublibrary: 366 genes, 4 plates
- RNA Binding Proteins sublibrary: 406 genes, 4 plates

# Experiments done in replicates:
- 24 plates × 200 wells × 16 views × 7 stacks × 5 channels ~ 1.109 images

III. Workflow

High throughput measure based RNA plate screen

- DAPI signal segmentation and analysis
- GFP signal segmentation and analysis
- Step granules detection and analysis
- Statistical analysis
- Genes selected for further studies

IV. Experimental procedure quality check and selection of healthy cells

For each view
- DAPI signal MIP
- 93 008 MIP

CellProfiler
- Object segmentations and Feature extraction

CellProfiler Analyst
- Classification training:
  - 2 sets of 2583 objects
  - 4 classes
  - 25 rules

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.

V. Semi-Automatic Cell Segmentation : from GFP MIP images to cell masks

GFP MIP images (A) are first locally-normalized to reduce the effect on a non-uniform illumination. Then, a watershed-based segmentation produces the corresponding over-segmented (superpixels) image (B). Finally, the interface allows 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. SPADI : an algorithm for small particle detection

The mask generated by the supervised segmentation algorithm (A) is used to extract from the original MIP images the corresponding over-segmentation (B). The refined mask (C) is used to extract the CellProfiler mask (D) and thus, to extract the cellular masks (D).

VII. Preliminary data :

- Plate, Well
- RNAi
- % of GFP per plate

- Average abs of Imp-RNP per unit area (~SEM)

Images from different control wells (LacZ and water) of one or two independent plates as well as images from 3 possible hits were analyzed as described before (IV to VI). Results show robustness of the controls. For quality control, we will use median normalization per plate (excluding controls).

VIII. Perspectives

- Automate the merging process for image treatment
- Develop the statistical framework to detect hits
- In vitro functional analysis of hits
- Improve the experimental roadmap using additional markers (phosphorylated, anti-peace)

Collaborators

- DRSC (Harvard, USA), high throughput RNA screen

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