Study of purinosome assembly in cell-based model systems with de novo purine synthesis and salvage pathway deficiencies

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Abstract

The purinosome has been observed in a broad spectrum of cells, but some studies claim that it is an artefact of the constructs used for visualization or stress granules resulting from the exposure of cells to nutrient-reduced growth media. Both may be true depending on the method of observation. To clarify this point, we combined two previously used methods, transfection and immunofluorescence, to detect purinosomes in purinosome-free cells deficient in particular DNPS steps (CR-DNPS cells) and in cells deficient in the salvage pathway, which resulted in construction of the purinosome regardless of purine level (CR-HGPRT cells).

To restore or disrupt purinosome formation, we transiently transfected CR-DNPS and CR-HGPRT cells with vectors encoding BFP-labelled wild-type (wt) proteins and observed the normalization of purinosome formation. The CR-DNPS cell line transfected with a DNA plasmid encoding an enzyme with zero activity served as a negative control for purinosome formation.

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Protocol

Step 1.