RELOCALIZATION OF TRANSLATION FACTOR eIF2D TO THE CENTROSOME IN MAMMALIAN CELLS UPON STRESS

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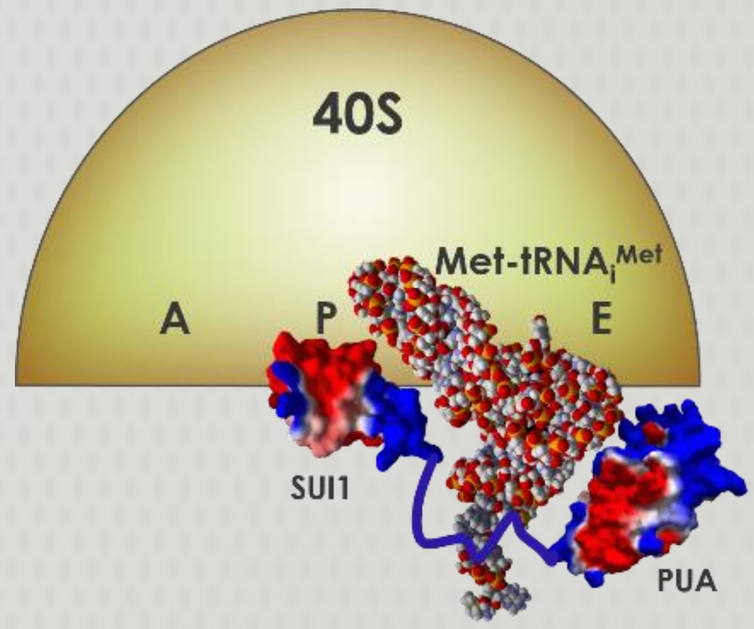
eIF2D – the current state of the art

eIF2D (formerly called ligatin) is a mysterious translation factor that is able to facilitate tRNA binding to the P-site of the ribosome in a GTP-independent manner. Its activity requires an AUG codon of mRNA properly positioned in the P-site before the tRNA binding. In a reconstituted system of eukaryotic translation initiation, eIF2D can replace both eIF2 and eIF5B in reactions of 48S and 80S complex formation, respectively. However, in contrast to eIF2-mediated initiation which is limited to Met-tRNAi, this alternative pathway may operate with elongator tRNAs.

The physiological function of eIF2D is yet unknown. In yeast, eIF2D ortholog TMA64 has genetic interactions and expression profile that predict its involvement in control of the cell cycle and adaptation to stress. In addition to translation-related domains (N-terminal PUA and C-terminal SUI1), eIF2D possesses a much less conserved central part with similarity to Kin17_mid and SWIB/MDM2 domains. This feature suggests that eIF2D may be a component of protein complexes that coordinate cell cycle checkpoints in response to stress. To investigate a putative role of eIF2D in such events, we analyzed its localization within cultured mammalian cells under variety of conditions.



Domain organization of eIF2D

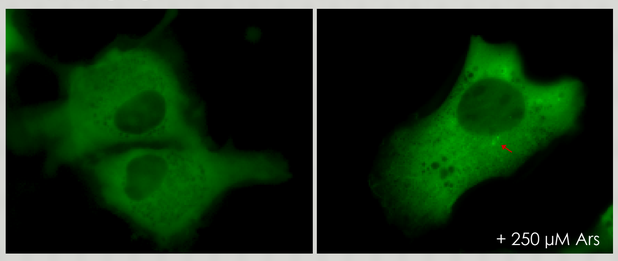
eIF2D domains: *SUI1* – binding to ribosomal P-site
PUA – interaction with tRNA (?)

KIN17+SWIB – what are they for?

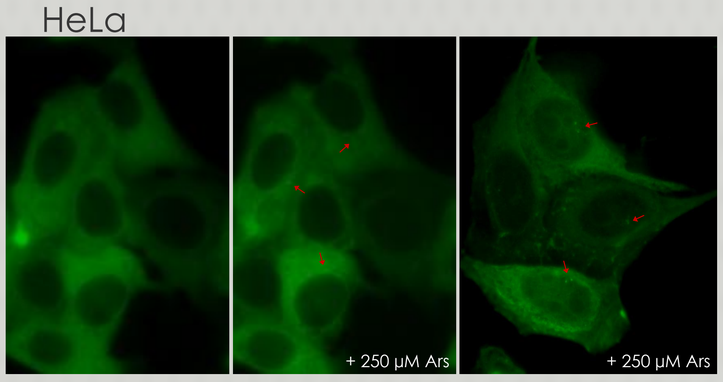
eIF2D on the centrosome

Treatment with 250 μM sodium arsenite within 1 hour leads to occurrence of EGFP-eIF2D foci.

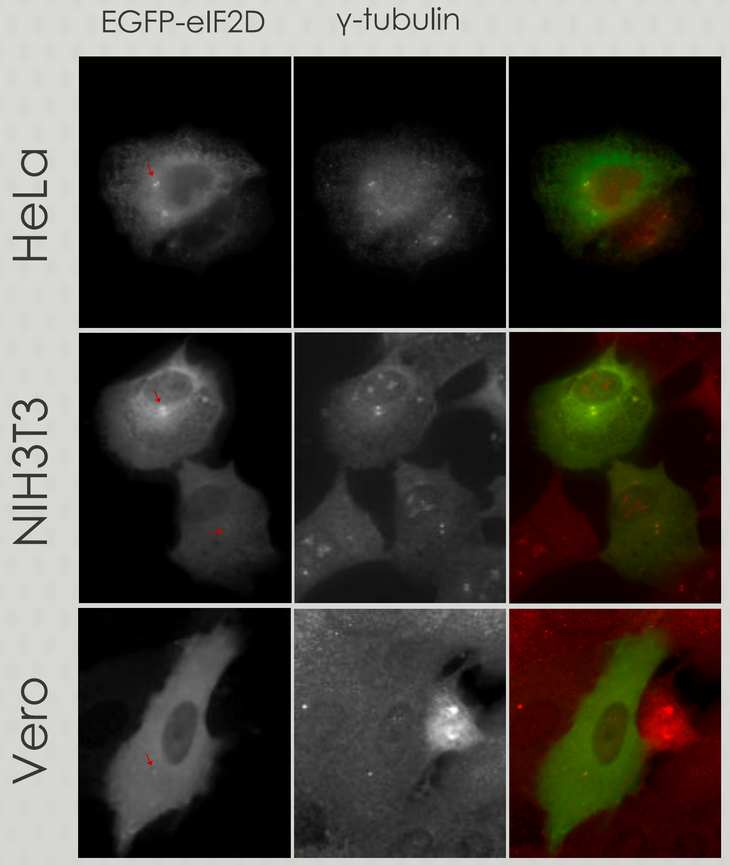
NIH3T3



HeLa



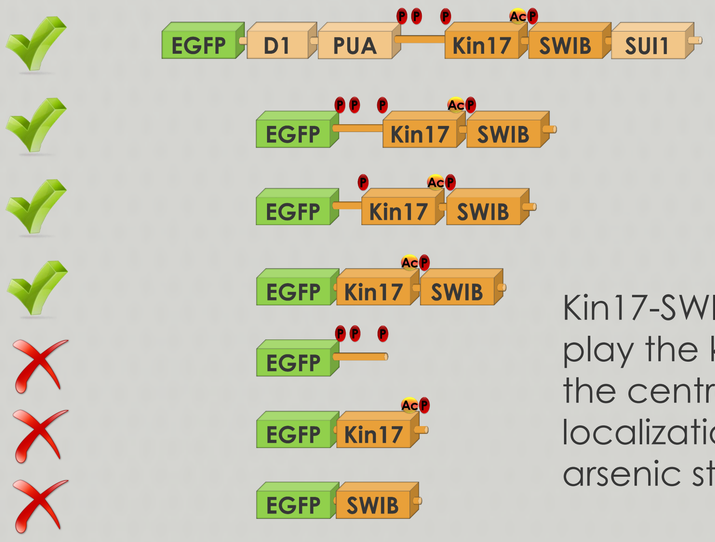
Is the centrosome localization of eIF2D specific?



- Detected foci of EGFP-eIF2D under stress condition were positively stained with antibodies against γ-tubulin (the marker of centrosome) in different cell lines.
- Other stresses (osmotic stress, H_2O_2, DTT, hydroxyurea, UV, etoposide, tunicamycin and other) do not lead to relocalization of eIF2D.
- Treatment with inhibitors of kinases MAPK (SB203580), JNK (SP600125), PLK3 (wortmannin) does not prevent centrosome localization of eIF2D.
- eIF2D localizes to the centrosome in cells with disassembled microtubules. Therefore, the centrosome localization is not a result of non-specific MT-associated transport or putative aggresome formation.

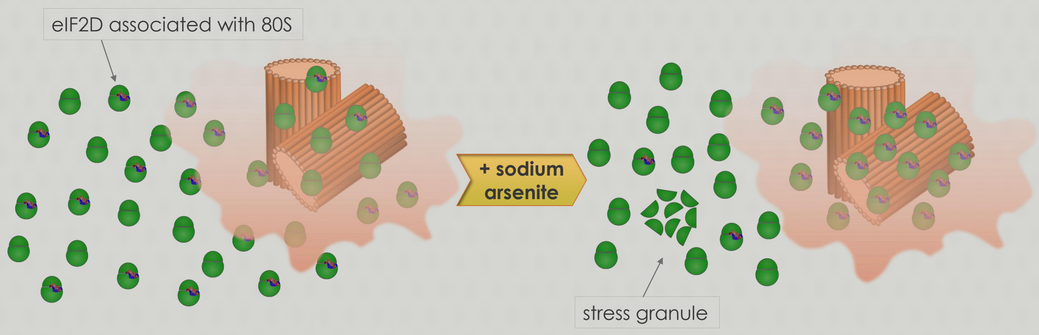
Which part of eIf2D defines centrosome localization?

Centrosome localization | EGFP fusion proteins with different eIF2D fragments



Kin17-SWIB domains play the key role for the centrosome localization under arsenic stress

Conclusions



- Under normal conditions eIF2D is diffusely distributed in the cytoplasm. Upon induction of severe oxidative stress by sodium arsenite, transiently expressed EGFP-eIF2D forms two clearly defined foci.
- They are positively stained with antibodies against γ-tubulin, the marker of centrosome.
- The centrosomal localization of eIF2D is determined by the middle KIN7-SWIB domains of the protein.
- eIF2D is relocated to the centrosome upon induction of stress response and might participate in some specific translation events. This spatially restricted activity of eIF2D may be related to stress response or control of the cell cycle progression.