Regulation of Normal Mitotic Progression by A-kinase Anchoring Protein 95 (AKAP95)

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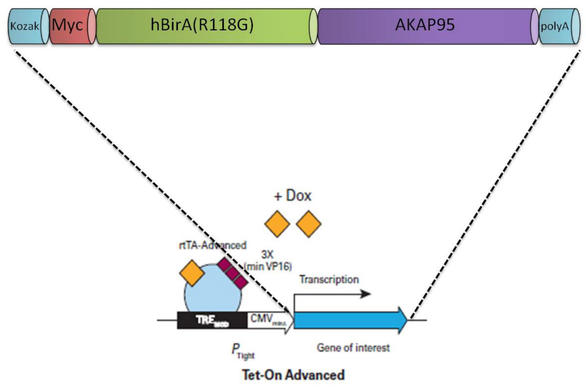
Abstract

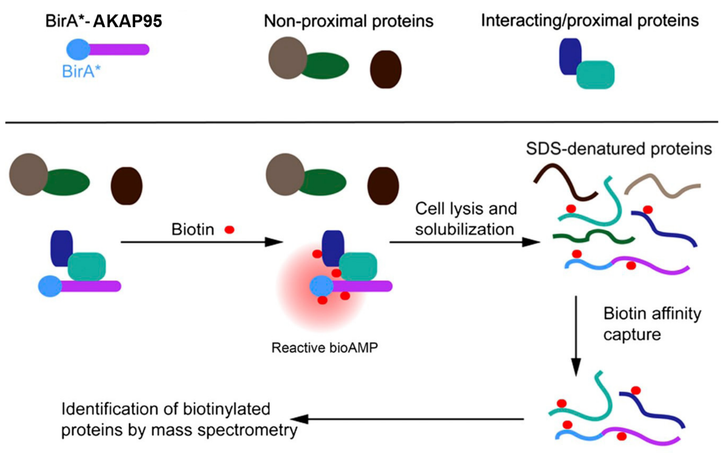
AKAP95 is a DNA-binding protein that in addition to cAMP-dependent protein kinase (PKA), binds other signaling molecules such as protein phosphatase 1. AKAP95 is a nuclear matrix protein and interacts with chromatin modifiers such as histone deacetylase (HDAC) 3, suggesting it may contribute to functionally compartmentalize chromatin or orchestrate fundamental nuclear processes such as replication or transcription. AKAP95 has also been involved in chromosome condensation during mitosis by targeting PKA and the condensin complex to chromatin. We have employed a novel proximity-based protein identification assay (**BioID**), based on the *in vivo* modification of AKAP95 vicinal proteins by a bacterial biotinylating enzyme fused to AKAP95. We identified the nuclear pore complex (NPC) protein Tpr and confirmed interaction by co-immunoprecipitation. AKAP95 siRNA knockdown experiments in HeLa cells lead to an increased number of cells displaying micronuclei and/or abnormal nuclear morphology. Live cell microscopy imaging experiments in HeLa cells constitutively expressing Histone H2B-EGFP to visualize chromatin reveal that these micronuclei result from lagging chromosomes in anaphase that become entrapped at the end of mitosis. Strikingly, a similar chromatin lagging phenotype has previously been reported for Tpr siRNA-depleted cells, suggesting a functional interaction outside of the NPC between AKAP95 and Tpr at the mitotic spindle. We are currently investigating the underlying mechanisms by which AKAP95/Tpr regulate genetic material integrity during chromosome segregation.

1. Identification of *in vivo* binding partners of AKAP95: a BioID approach

BioID: Proximity-based biotinylation assay

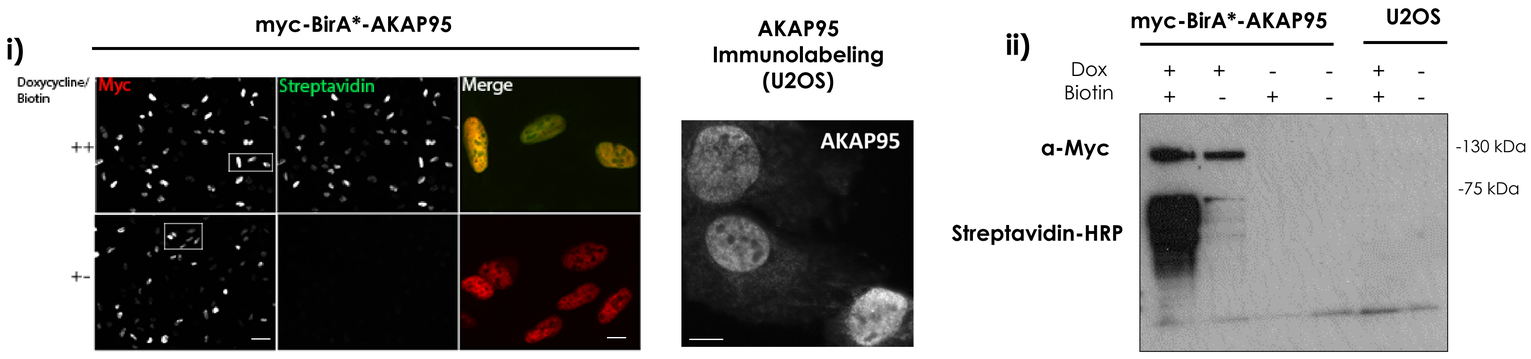


A) Experimental design for BioID protein identification.

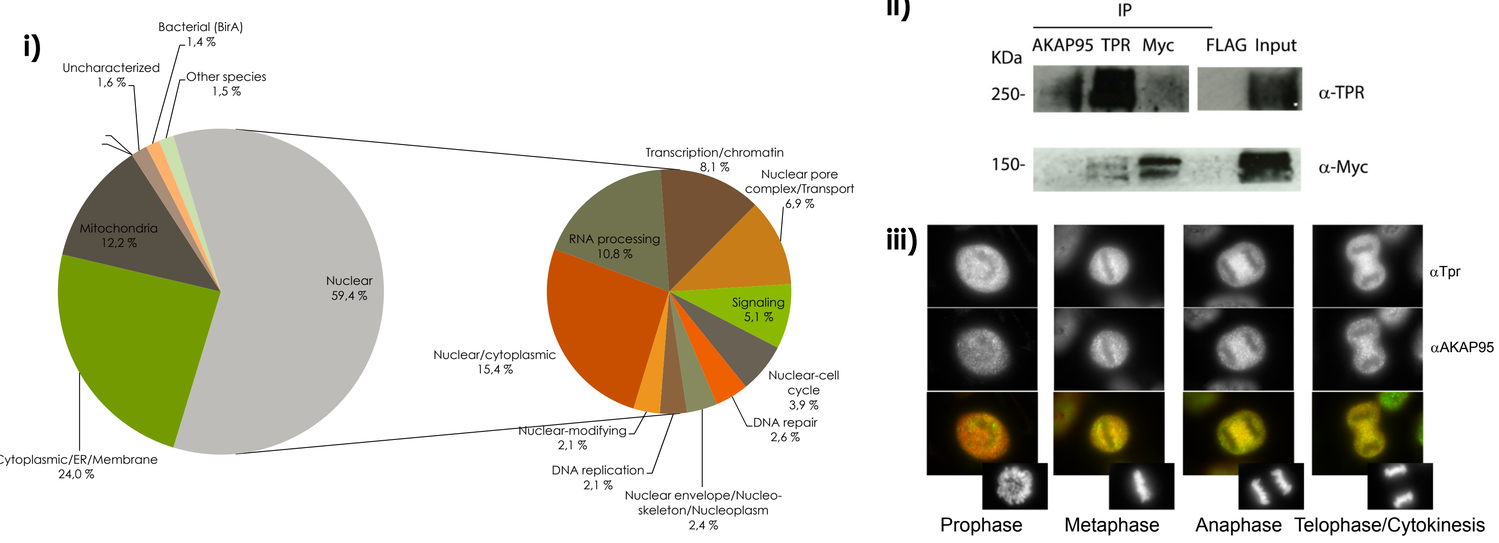


(Roux et al. 2012)

B) U2OS cells stably express myc-BirA*-AKAP95 upon doxycycline (Dox) induction and present the same subcellular localization and pattern as endogenous AKAP95. Biotinylated proteins are detected with fluorescently- (**i**) or HRP-(**ii**) conjugated streptavidin antibodies. Scale bars 25 µm and 5 µm for enlarged

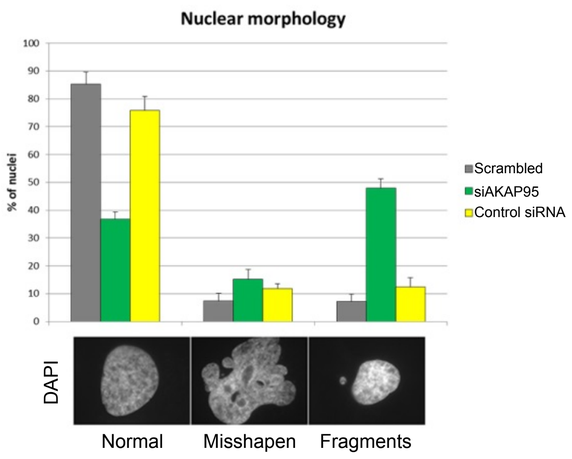


C) AKAP95-BioID screen identifies several DNA metabolism proteins and gene regulators, including Tpr (translocated promoter region) . (**i**) Gene ontology analysis of AKAP95-BioID. (**ii**) Myc-tagged AKAP95 and Tpr co-immunoprecipitate in U2OS cells. (**iii**) AKAP95 and Tpr show colocalization during mitosis.

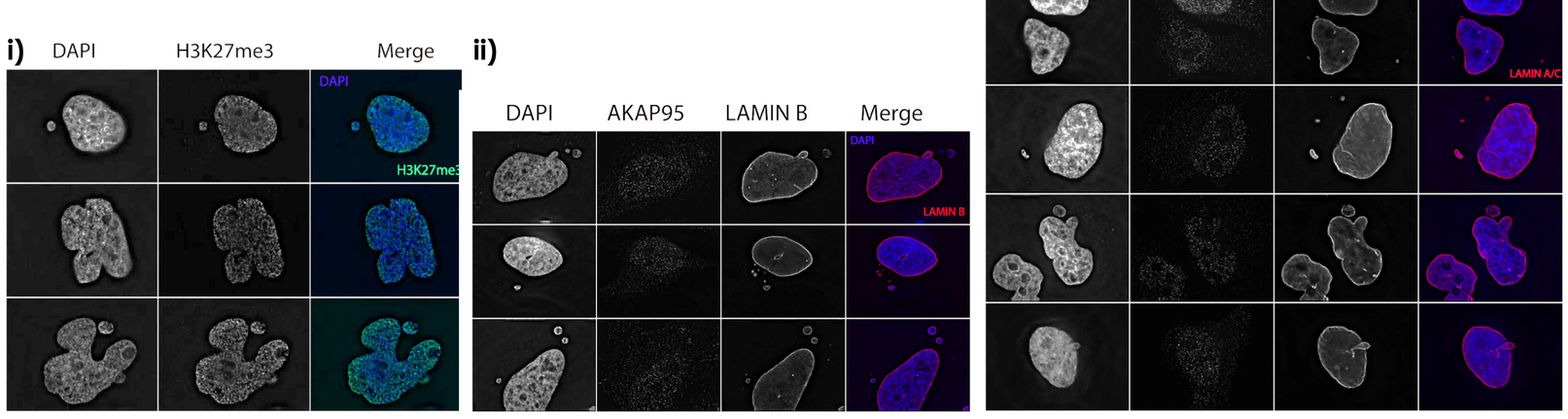


2. Irregular nuclear morphology and chromatin cytoplasmic fragments (CCFs) in AKAP95-depleted cells

A) Loss of nuclear morphology and CCFs appearance upon AKAP95 siRNA-mediated knockdown

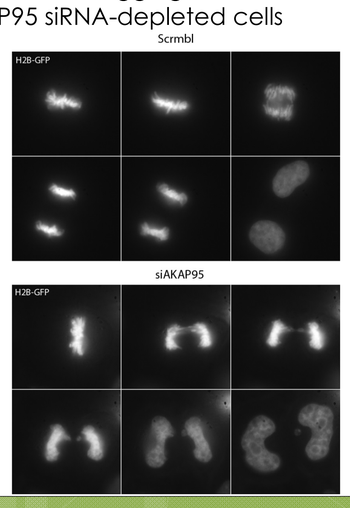


B) CCFs characterization. (**i**) CCFs co-stain with the inactive chromatin mark H3K27me3. (**ii**) Lamin B and (**iii**) A/C are present in CCFs but not all of them contain chromatin.

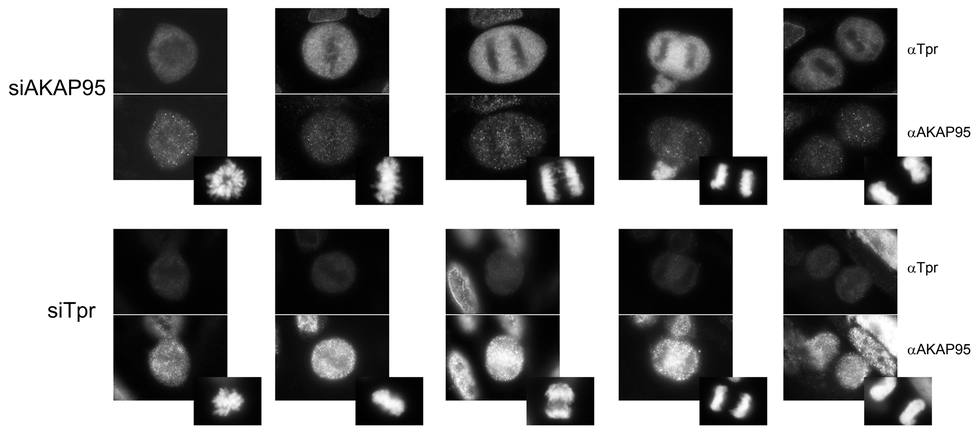


3. AKAP95 depletion leads to lagging chromosomes and Tpr relocalization during mitosis

A) Live cell imaging of H2B-EGFP HeLa cells show lagging chromosomes in AKAP95 siRNA-depleted cells



B) siRNA knockdown of AKAP95 results in redistribution of Tpr during mitosis



Conclusions

¤ AKAP95-BioID identifies novel nuclear binding partners including Tpr, a NPC protein implicated in several oncogenic fusion events.
¤ AKAP95 and Tpr interact during mitosis.
¤ Depletion of AKAP95 results in high proportion of CCFs, which arise from lagging chromosomes during mitotic division.
¤ A fraction of Tpr, for which a similar phenotype has been reported, is anchored by AKAP95 during mitosis.

→ Our results point to a **functional interaction** between **Tpr** and **AKAP95** at the mitotic spindle important for the **regulation of genetic material integrity** during chromosome segregation.

References

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