Scaffold Attachment Factor A (SAF-A) and nuclear actin in chromatin regulation and nuclear architecture

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ABSTRACT

SAF-A (Scaffold Attachment Factor A, also known as hnRNP-U) is a highly abundant multifunctional nuclear protein with functional roles in regulating gene expression through modulating the higher order structure of the genome. It is one of the main scaffold attachment region DNA-binding proteins in human cells and possesses binding domains such as for DNA, RNA and actin. Studies have shown that functionally, SAF-A, has been implicated in the regulation of gene expression, through the interaction with RNA polymerase II and actin [1]. Our group is aiming to better understand how the interaction of SAF-A with nuclear actin affects gene expression through modulating nuclear architecture. Using constructs of SAF-A that lack functional domains and mutant forms of actin, we study their localization and mobility in live human cells.

In the present study, we show that overexpression of C280, a dominant negative construct of SAF-A that includes the RNA and actin binding domains, affects the localization and mobility of overexpressed wtActin and inversely, an effect that is not apparent after expression of full length SAF-A. However, immunofluorescence experiments indicated that expression of SAF-A leads to accumulation of endogenous globular actin in splicing speckles, nuclear domains enriched in pre-mRNA splicing factors and involved in transcription machinery. Furthermore, using a method that we have already established in the laboratory, we are now able to quickly deplete SAF-A from human cells through an auxin-inducible degron (AID) tag [2], and we can investigate possible effects on actin, chromatin remodeling and gene expression. By generating cells stably expressing a validated fluorescent probe exclusively for filamentous nuclear actin (Utr230:GFP[3]), we have seen that depletion of SAF-A leads to accumulation of actin oligomers into distinct nucleoplasmic puncta, with an increasing size and decreasing mobility over time.

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