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The role of the ASPL-TFE3 fusion protein in Alveolar Soft Part Sarcoma

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The AAA+ ATPase Valosin-Containing Protein VCP (also known as Cdc48/p97) is a central player in maintaining protein homeostasis. VCP is best described for its role in unfolding ubiquitinated proteins prior to their degradation. Like many other AAA+ ATPases, VCP functions as a homo-hexameric complex and is regulated by interactions with many binding partners [1-4]. One such binding partner is alveolar soft part locus (ASPL), which was shown to destabilize VCP hexamers into VCP-ASPL heterotetramers [5, 6]. Interestingly, a chromosomal translocation mutation in ASPL causes alveolar soft part sarcoma (ASPS), a rare soft tissue cancer with poor prognosis. The non-reciprocal chromosomal translocation t(X;17)(p11.2;q25) leads to the formation of a fusion protein between the N-terminal region of the ASPL gene with the C-terminal region of transcription factor E3 (TFE3) [7]. The ASPL-TFE3 fusion protein (hereafter AT3) retains the VCP binding domain of ASPL and the DNA binding, activation domain, and nuclear localization signal domain of TFE3[7-9]. Several studies have shown that AT3 up-regulates various transcripts that are essential for cell proliferation [4,10-11]. However, the relation between VCP and the AT3 fusion protein is poorly understood. The premise of this work is to investigate the structure and function relationship between AT3 and VCP.

The destabilization of VCP by ASPL is caused by C-terminal motifs within ASPL, including an α-helical lariat and cis-Pro touch-turn (residues 313-553) [6]. Interestingly, the ASPL portion within the AT3 fusion protein (residues 1-311) lacks these destabilization motifs. Based on these observations, we investigated whether AT3 interacts with intact or destabilized VCP complexes. Co-immunoprecipitation (co-IP) experiments from HEK293T cells transfected with AT3 indicate that AT3 forms stable complexes with VCP hexamers (Fig. 1). Negative stain EM analysis of purified VCP-AT3 particles indicate an enrichment of VCP hexamers, which is in contrast with the loss of hexamers from co-IPs of native ASPL (Figure 1). In order to investigate whether AT3 is sufficient to interact with VCP, we generated an in vitro reconstitution system using recombinantly purified His-tagged VCP, AT3 and ASPL. The components were combined and analyzed by size exclusion chromatography, which demonstrated that VCP forms a large complex in the presence of AT3 but not ASPL. Purified complexes were imaged by cryo-EM and subsequent reconstructions reveal novel densities that are putatively ascribed to AT3 (Figure 2). Ongoing efforts are underway to determine higher-resolution structures, which will be critical to resolve how AT3 drives transcription mis-regulation and provide a structural framework for ASPS treatment.

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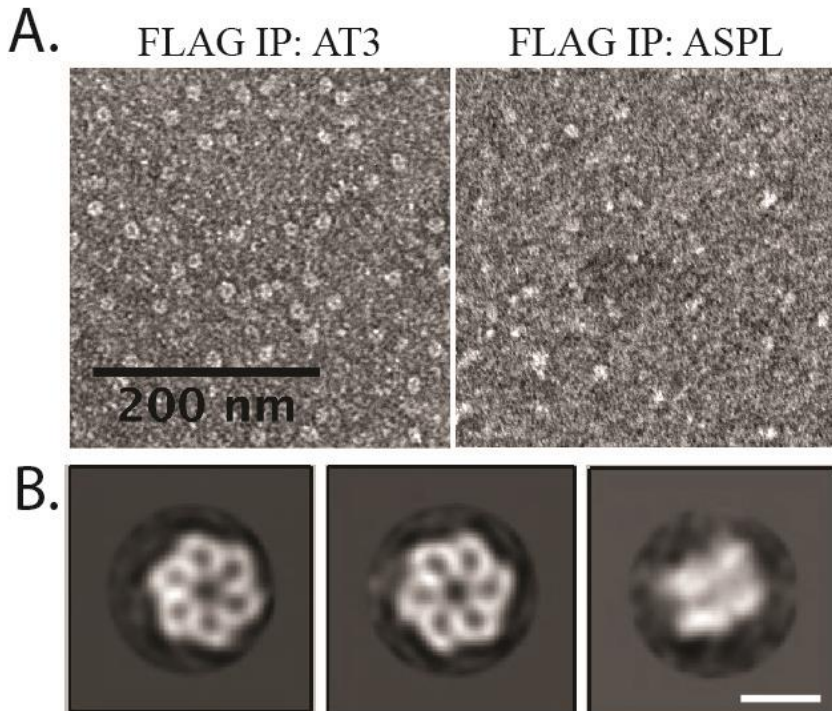

Figure 1. (A): Negative stain TEM micrographs of FLAG-IP eluates after AT3 or ASPSCR1 overexpression in HEK293T cells (scale bars = 200nm). (B): Reference-free 2D class averages of negatively stained particles recovered from FLAG-AT3 co-IP reveal intact VCP hexameric assemblies. Left and middle, VCP top views (3620 and 2774 particles in each class); Right, VCP side view (182 particles). Scale bar = 20 nm.

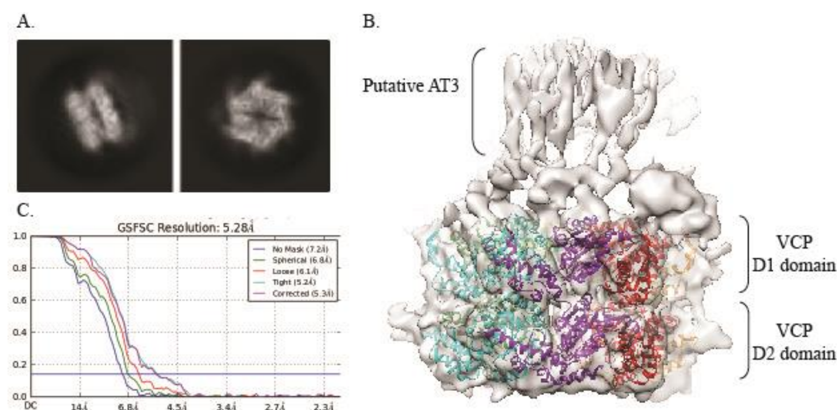

Figure 2. (A) Subset of representative, reference-free 2D class averages. (B) Reconstruction of recombinant AT3/VCP complex. (C) Gold-standard FSC plots.

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