CP60 and BEAF-32 proteins are sumoylated in vivo

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Post-translational modifications of substrate proteins by proteins of the SUMO family are widespread among eukaryotes. Using a model organism *D. melanogaster*, we study the effect of sumoylation on the properties of transcription factors CP60 and BEAF-32. Using the yeast two-hybrid system (Y2H), we showed that the CP60 and BEAF proteins directly interact with the Ubc9 protein, which is a SUMO E2 ligase. The BEAF protein interacts with Ubc9 through the C-terminal sequence of 200-280 aa. The CP60 protein interacts with Ubc9 through the C-terminal sequence 420-440 aa. Using bioinformatics, canonical sumoylation sites of CP60 and BEAF were predicted. We made point substitutions at the CP60 and BEAF predicted sites. The Ubc9 protein did not interact with either mutant forms of CP60 or BEAF in Y2H assay. Therefore, the tested CP60 and BEAF sumoylation sites are involved in sumoylation. In addition, sumoylation of CP60 and BEAF was tested in IP experiments on S2 cells. As a result of immunostaining with antibodies to the tested proteins, we observed 2 bands. The lower one corresponded to the unmodified forms of CP60 and BEAF proteins, and the upper one corresponded to SUMO modifications. The upper band was detected by antibodies to the dSmt3 protein. The BiFC method was used to study the interaction between CP60, BEAF, and dSmt3 proteins. Constructs expressing the full-length CP60 and BEAF proteins, labeled with the fluorescent half of Venus, and the dSmt3 protein, labeled with the fluorescent half of the CFP, were created. On S2 cell culture, it was demonstrated that the CP60 and BEAF proteins are sumoylated in vivo. We have found that CP60 and BEAF form discrete speckles within the nucleus. Such speckles are partially colocalized with the CP190 protein, marking “insulator bodies”. It is possible that, like the proteins of the Su(Hw) insulator, CP60 and BEAF are involved in regulatory complexes by sumoylation.
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