Identification and Purification of a Soluble Region of BubR1

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The mitotic checkpoint complex (MCC) ensures the fidelity of chromosomal segregation, by delaying the onset of anaphase until all sister chromatids have been properly attached to the mitotic spindle. In essence, this MCC-induced delay is achieved via the inhibition of the anaphase-promoting complex (APC). Among the MCC components, BubR1 plays two major roles in the functions of the mitotic checkpoint. First, BubR1 is able to inhibit APC activity, either by itself or as a component of the MCC. Second, BubR1 activates mitotic checkpoint signaling cascades.

To determine the structure of BubR1, we obtained a soluble BubR1 constructs using a three-step expression strategy. First, we obtained two constructs from BLAST sequence homology searches, both of which were expressed abundantly in the inclusion bodies. Second, we adjusted the lengths of the two constructs by secondary structure prediction, thereby generating partially soluble constructs. Third, we optimized the solubility of the two constructs by modification at the C-terminus. Finally, we obtained a highly soluble BubR1 protein via the *E. coli* expression system.

This report may provide insight into the design of highly soluble constructs of insoluble multi-domain proteins.

Keywords: protein secondary structure analysis, cell-cycle proteins, solubility