Abstract

Cell division or loss of sister chromatid cohesion prior to achievement of bi-orientation leads to missegregation of chromosomes and causes aneuploidy, which is closely associated with tumorigenesis. To prevent this, the Spindle Assembly Checkpoint (SAC) monitors correct attachment of spindle microtubules to kinetochores (large protein assemblies on centromeric DNA) and coordinates this with cell cycle progression. Bub1 and BubR1, essential SAC components, evolved through several duplication events from an ancestor gene creating two structurally related gene products with highly diversified functions. Previously, BubR1 has been shown to depend on Bub1 for its kinetochore localization, whereas Bub1 localizes to kinetochores independently of BubR1. However, the molecular basis for such differences in recruitment has been unclear, as Bub1 and BubR1 both bind to Bub3, a targeting adaptor for phosphorylated kinetochores. In my PhD project, the basis of the different localization behavior has been identified. I demonstrated that Bub1, but not BubR1, enhances binding of Bub3 to phosphorylated kinetochores via a short motif in its Bub3-binding domain (B3BD) called the "loop". This provided an explanation for why BubR1 relies on an alternative mechanism for kinetochore localization. Consequently, swapping loops created a loss-of-function Bub1, impaired in kinetochore localization, and a gain-of-function BubR1, localizing to kinetochores independently of Bub1. However, the inability of the loop-swap mutant of BubR1 to retain BubR1 SAC function led to the subsequent identification of the specific role of the BubR1-loop. *In vivo* and *in vitro* experiments established that the unique sequence of the BubR1-loop promotes binding of BubR1 to the APC/C. Additionally, this study showed for the first time that kinetochore localization of BubR1 relies on a direct interaction with Bub1. Hetero-dimerization requires structurally equivalent domains in both proteins, the B3BD and a subsequent helical segment. Furthermore, both proteins need to be bound to Bub3 for this interaction to take place. Interestingly, hetero-dimerization of Bub1 and BubR1 did not require kinetochores. Collectively, my PhD work illustrates how gene duplication and subsequent sub-functionalization determine the differences in the behavior of two closely related proteins as part of an essential molecular network providing a further step in our understanding of how the checkpoint signal is generated at the molecular level.