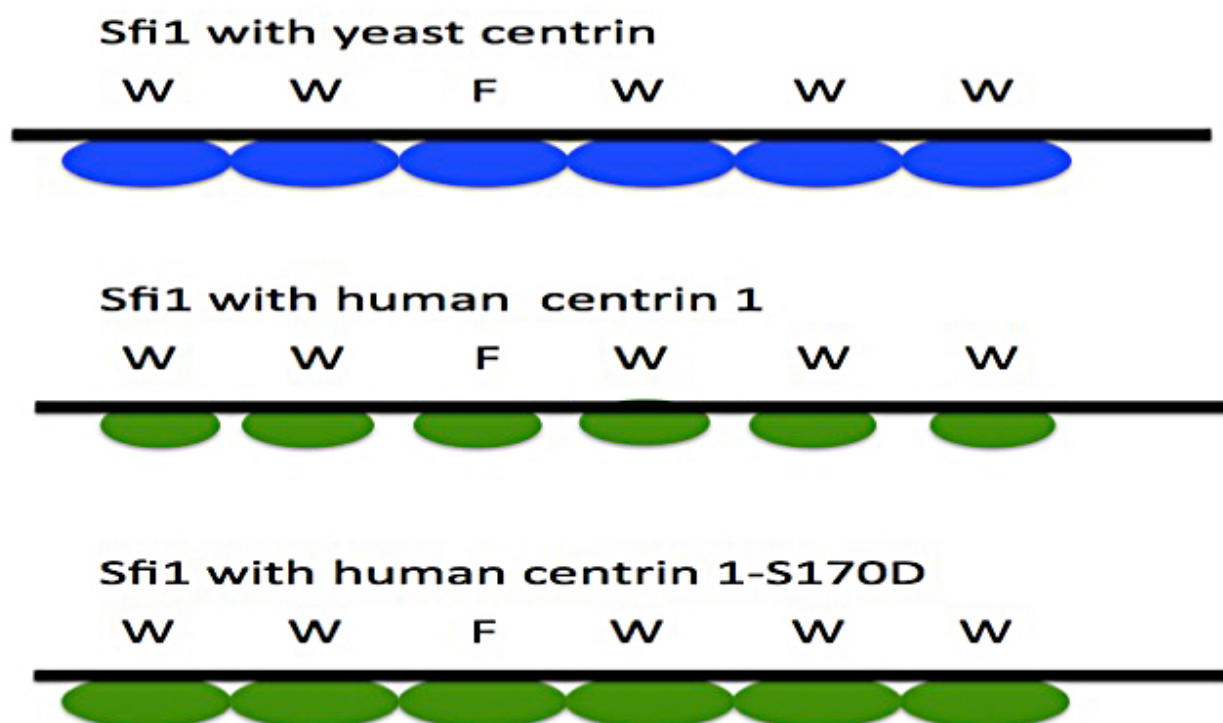


New insights into the interaction of centrin with Sfi1

The Sfi1 protein is localized to the centrosome that is also called the spindle pole body in yeast. Thus, Sfi1 is involved in the centrosome behaviour. As a consequence of the DNA replication, the centrosome is also duplicated and each one of the two copies migrates at the two opposite poles of the cell and gives rise to the achromatic spindle that allows the segregation of chromosomes toward the two new sister cells. Deletion of Sfi1 causes cell division arrest in yeast demonstrating that Sfi1 is involved in cell division (P.J. Ma, et al., 1999). The Sfi1 protein is composed of about twenty motifs that bind a small acidic protein called centrin. Centrin is a calcium-binding protein that binds also other protein targets in cell.



Sfi1 (black) in complex with yeast centrin (blue) or human centrin (green). Sfi1 is composed of six centrin-binding sites. Tryptophan (W) and phenylalanine (F) are the major amino acids that bind centrin. Centrin 1-S170D is a mutant that mimics the phosphorylation at residue Serine 170.

We attempted to elucidate the mechanism by which Sfi1 in association with centrin, participates in centrosome duplication. Specifically, we hypothesised that the regulation of the biological activity of the Sfi1-centrin complex could be modulated through the binding of centrin to Sfi1 in concert with centrin-centrin interactions. The amino acids composition of the centrin-binding motif of Sfi1 is important for the complex formation and for its stability. The amino acids involved in interaction are

arranged in a hydrophobic motif (Leucine-X-X-X-Leucine-X-X-Tryptophane). To understand what is important for the protein interaction, we modified this composition and we furthermore analysed the affinity of centrin for the modified Sfi1. The structure of the yeast truncated Sfi1 (containing three centrin-binding motifs) in complex with centrin has been already published (S. Li, et al., 2006). It shows that centrin-centrin interactions as well as centrin-Sfi1 interactions exist the complex. In case of human, only a structure of a complex composed of a peptide corresponding to the centrin-binding of Sfi1 and centrin has been solved and analysed (J. Martinez-Sanz, et al., 2010). However, our experiments analysed the thermal stability of Sfi1 (containing six centrin-binding motifs) and human centrin. The centrin composing the complex was either from yeast or from human. We modified the amino acid sequence of the centrin-binding motifs especially we replaced the functional residue Leucine Leucine Tryptophane that make contacts with centrin by a non functional residue such as Alanine that cannot make contacts with centrin. We did this replacement either in two adjacent or in two non-adjacent centrin-binding motifs. Next we measured the thermal stability of the Sfi1-centrin complexes. The level of the thermal stability brought information about contacts that occurred between Sfi1 and centrin as well as between the six centrin molecules that bind the six centrin-binding sites composing the Sfi1 fragment. We observed that calcium binding to centrin stabilised the complexes Sfi1-centrin. The role of calcium binding is to expose the centrin interface for the best binding to target. We also observed that centrin-centrin interactions exist for complexes with yeast centrin but not with human centrin. Mutated yeast centrin (Glutamine⁹⁷Glycine, Alanine¹⁰⁹Threonine, Phenylalanine¹⁰⁵Leucine) lead to the arrest of the cell division (P.J. Ma, et al., 1999). We constructed homologous mutations in human centrin (Glutamine¹⁰⁵Lysine, Alanine¹⁰⁹Threonine, Phenylalanine¹¹³Leucine) and we observed that the thermal stability of Sfi1-centrin complexes was reduced suggesting that these centrin mutations affect the role of Sfi1 in cell.

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