

A family of Structural Maintenance of Chromosome (SMC) complexes is essential for key cellular processes ensuring proper cohesion, condensation and replication. They share a common SMC-kleisin architecture allowing them to embrace DNA. In SMC5/6, the NSE1 and NSE3 KITE and NSE4 kleisin subunits form a stable subcomplex, that binds DNA and regulates essential processes. In addition, NSE5 and NSE6 subunits associate with the core SMC5/6 complex and recruit it to DNA repair sites.

The architecture of the SMC5/6 complex is crucial for its proper functioning, and mutations within the human SMC5/6 subunits result in severe syndromes. Therefore, we aimed to analyse interactions within the human SMC5/6 complex and determine its detailed architecture. Firstly, we analysed different parts of SMC5/6 by crosslinking and MS/MS analysis. Our data suggested domain arrangements of hNSE1-hNSE3 and orientation of hNSE4 within the hNSE1-hNSE3-hNSE4 subcomplex. The crosslinking and electron microscopic analysis of the SMC5/6 core complex showed its rod-like architecture with juxtaposed hSMC5-hSMC6 arms. Additionally, we observed fully or partially opened hSMC5-hSMC6 shapes with the hNSE1-hNSE3-hNSE4 trimer localized in the SMC head domains. To complete mapping of the human SMC5/6 complex architecture, we analysed positions of hNSE5-hNSE6 at the hSMC5-hSMC6 arms. We showed that hNSE6 binding to hNSE5 and the coiled-coil arm of hSMC6 is mediated by a conserved FAM178 domain which we therefore renamed CANIN (Coiled-coil SMC6 And NSE5 INteracting) domain. Interestingly, hNSE6 bound both hSMC5 and hSMC6 arms, suggesting that hNSE6 may lock the arms and regulate the dynamics of the human SMC5/6 complex.