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Structure and Function of the PP2A-Shugoshin Interaction

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SUMMARY

Accurate chromosome segregation during mitosis and meiosis depends on shugoshin proteins that prevent precocious dissociation of cohesin from centromeres. Shugoshins associate with PP2A, which is thought to dephosphorylate cohesin and thereby prevent cleavage by separase during meiosis I. A crystal structure of a complex between a fragment of human Sgo1 and an AB′C PP2A holoenzyme reveals that Sgo1 forms a homodimeric parallel coiled coil that docks simultaneously onto PP2A's C and B′ subunits. Sgo1 homodimerization is a prerequisite for PP2A binding. While hSgo1 interacts only with the AB′C holoenzymes, its relative, Sgo2, interacts with all PP2A forms and may thus lead to dephosphorylation of distinct substrates. Mutant shugoshin proteins defective in the binding of PP2A cannot protect centromeric cohesin from separase during meiosis I or support the spindle assembly checkpoint in yeast. Finally, we provide evidence that PP2A's recruitment to chromosomes may be sufficient to protect cohesin from separase in mammalian oocytes.

INTRODUCTION

The chromatids of bivalent chromosomes, like their mitotic counterparts, are held together by a multisubunit complex called cohesin, whose α-kleisin (Rec8), Smc1, and Smc3 subunits form a tripartite ring within which sister DNAs are thought to be trapped (Haering et al., 2008; Nasmyth and Haering, 2005). The first meiotic division is triggered by destruction of sister chromatid cohesion by a thiol protease called separase, which opens the cohesin ring by cleaving its α-kleisin subunit (Buonomo et al., 2000). This splits the bivalent into a pair of dyad chromosomes that are segregated to opposite poles at the first meiotic division. Crucially, the two chromatids of each dyad remain associated with each other both during and after anaphase I, because cohesin holding sister centromeres together is protected from cleavage by separase by a class of proteins called shugoshins (Goldstein, 1980; Kerrebrock et al., 1995; Kitajima et al., 2004; Rabitsch et al., 2004). The persistence of centromeric cohesion is essential to ensure that sister kinetochores and hence individual chromatids are pulled to opposite poles at the second meiotic division. A failure to protect centromeric cohesin from separase might contribute to the human aneuploidy caused by chromosome missegregation during meiosis I in oocytes, especially in older women (Vogt et al., 2008).

How do shugoshins protect sister chromatid cohesion at centromeres? Recent work indicates that shugoshins protect centromeric cohesin by interacting with protein phosphatase 2A (PP2A) (Kitajima et al., 2006; Riedel et al., 2006). PP2A holoenzymes are composed of a catalytic C subunit, a structural or scaffold A subunit, and a regulatory B subunit. The A and C subunits bind directly to each other, forming a core enzyme. The B subunits, in contrast, are much more varied, and mammals possess at least 18 types, which belong to four subfamilies: PR55/B, PR61/B′, PR72/B″, and PR110/B‴ (Janssens and Goris, 2001; Lechward et al., 2001; Sontag, 2001; Yu, 2007). During meiosis in both fission and budding yeast, a shugoshin, namely Sgo1, is found stably associated with AB′C PP2A holoenzyme (Riedel et al., 2006). This led to the suggestion that phosphorylation of cohesin's Rec8 subunit may be required for its cleavage by separase (Brar et al., 2006; Riedel et al., 2006), as is at least partly the case for its Scc1 mitotic counterpart (Alexandru et al., 2001), and that by recruiting PP2A, Sgo1 prevents cleavage of centromeric Rec8 by inducing its dephosphorylation. Consistent with this hypothesis, yeast mutants lacking PP2A's B′ subunits fail to prevent Rec8's removal from centromeres at the first meiotic division, which is accompanied by their precocious disjunction soon after the first meiotic division (Riedel et al., 2006).

However, inactivation of PP2A causes highly pleiotropic phenotypes, because the enzyme has a wide variety of functions and substrates, and the effect on chromosome segregation of mutating PP2A could conceivably be due to indirect effects. To address whether recruitment of PP2A really is a crucial part of the mechanism by which shugoshins protect centromeric cohesin, it is necessary to understand how PP2A actually binds to shugoshin and to use this information to investigate the phenotype of mutant proteins that are defective in their ability to bind

the phosphatase. This sort of approach is equally important for disentangling the role of PP2A-shugoshin interactions during mitotic chromosome segregation in mammals, where hSgo1 has a crucial role in preventing dissociation of cohesin from centromeres (Kitajima et al., 2005; McGuinness et al., 2005; Salic et al., 2004), and it is unclear whether Sgo1's role is to recruit PP2A to centromeres or the converse (Kitajima et al., 2006; Tang et al., 2006).

PP2A binds to an hSgo1 fragment containing its N-terminal 176 amino acids, and the interaction is abolished by mutation of a highly conserved asparagine (N61I) within a region predicted to form a coiled coil (Tang et al., 1998, 2006). The N61I mutation abolishes persistence of sister centromere cohesion during meiosis in *Drosophila* (Kerrebrock et al., 1995), but it might have rather pleiotropic consequences, as it supposedly also affects the ability of hSgo1/ MEI-S332 to bind chromosomes (Tang et al., 1998, 2006) and possibly disrupts Sgo1's putative coiled coil.

A crystal structure of a complex formed between a fragment of hSgo1 and an AαB56γCα PP2A holoenzyme (from now on referred to as AB′C PP2A or PP2A) reveals that Sgo1 forms a parallel coiled coil whose N- and C-terminal ends bind to C and B′ PP2A subunits, respectively. Analysis of phenotypes of shugoshin mutants defective in PP2A binding demonstrates that recruitment of PP2A by Sgo1 is essential for the protection of sister chromatid cohesion at centromeres at meiosis I and for the spindle assembly checkpoint (SAC) during mitosis in yeast and that recruitment of PP2A to chromosome arms may be sufficient to block the resolution of chiasmata in mouse oocytes. Another important implication of our findings is that PP2A's specificity is not determined solely by its regulatory B subunits.

RESULTS

Shugoshin's Putative Coiled Coil Is Responsible for PP2A Binding

Recombinant hSgo1 fragments containing hSgo1's N-terminal 176 residues (Figure 1A) aggregate, but MBP-Sgo1 fusion proteins are soluble and interact with PP2A. Binding studies with a variety of MBP-Sgo1 fusion proteins reveal that the putative coiled coil (residues 47–105) confers interaction with AB′C PP2A holoenzyme (Figure 1B). A slightly shorter fragment (51–96) also confers binding, but with much lower affinity (Figures 1B and S1).

Two Shugoshin Molecules Interact with a Single PP2A Holoenzyme in Solution

Because the coiled-coil region is predicted to form a homodimer, we determined the molecular stoichiometry of the PP2A-Sgo1 complex. Both size-exclusion chromatography and dynamic light scattering indicate that the complex formed between PP2A and MBP-Sgo1(51–105) has a molecular weight of ~250 kDa, while that formed with Sgo1(51–105) after MBP had been removed by cleavage has a molecular weight of ~148–160 kDa (Figures 1C and S1). Because the molecular weights of the AB′C PP2A holoenzyme, Sgo1(51–105), and MBP are 146 kDa, 6 kDa, and 45 kDa, respectively, the molecular stoichiometry of the PP2A-Sgo1 complex in solution is likely 1:2. This molar ratio is consistent with MBP-Sgo1 bands having twice the intensity of PP2A subunits with an equivalent molecular weight in Coomassie blue-stained SDS gels of purified PP2A-Sgo1 complexes purified using either the GST tag on the PP2A A subunit or the MBP moiety of MBP-Sgo1 and/or by gel filtration (Figures 1B, 1D, 1E, and S1A). To show that there is only a single PP2A holoenzyme in the complex, we incubated GST-tagged PP2A A subunit (GST-A) with excessive untagged A, B, and C subunits and MBP-Sgo1. If there were two or more PP2A holoenzymes, then GST-A should pull down untagged PP2A A subunits, which we do not observe (Figure 1D). The complexes contain at least two Sgo1 molecules, because after mixing Sgo1 fragments with different lengths and tags (one was fused to MBP and the other to MBP and a FLAG epitope), addition of PP2A holoenzyme enabled FLAG affinity beads to pull down both MBP-Sgo1 proteins (Figure 1E). Dimerization of Sgo1 fragments within PP2A complexes is not caused by their fusion to MBP, because copurification occurs even when the latter is removed (Figure 1F, Supplemental Data).

Crystal Structure of the PP2A-Sgo1 Complex: Overall Architecture

We failed to obtain useful crystals with complexes containing Sgo1(51–105) or longer fragments, but eventually obtained a crystal structure with Sgo1(51–96), which lacks a complete coiled-coil region (Figures S1B and S1C). Despite not forming stable homodimers in solution (data not shown), MBP-Sgo1(51–96) does pull down PP2A holoenzyme (Figure 1B), though the complex dissociates partially during gel filtration, producing a peak fraction containing a mixture of the PP2A-Sgo1 complex and unbound PP2A holoenzyme (Figures S1B and S1C). It is this mixture that forms useful crystals, presumably because "free" PP2A holoenzymes shield the hydrophobic surface of Sgo1's coiled coil not covered by the binding of the first PP2A holoenzyme (see below).

The crystal structure was determined at 2.7 Å resolution (Table 1). Each asymmetric unit within the crystal lattice contains one human PP2A AB′C holoenzyme, one Sgo1(51–96) peptide, and one microcystin PP2A inhibitor molecule. The Sgo1(51–96) peptide forms a single long helix. Two Sgo1 peptides, related by a two-fold crystallographic symmetry, form a parallel coiled-coil homodimer and interact with both B and C subunits of two PP2A holoenzymes (Figure S2). In this way, two symmetry-related PP2A holoenzymes interact with symmetrical surfaces of the Sgo1 coiled-coil dimer. Because only a single PP2A holoenzyme interacts with the Sgo1 dimer in solution, the two surfaces of the Sgo1 coiled-coil homodimer must be slightly different, though this is not apparent in the crystal structure. We imagine that the two surfaces of Sgo1's coiled coil have different affinities for PP2A, with the lower affinity surface binding PP2A only under crystallization conditions, when protein concentrations are much higher. Structure-based mutagenesis (see below) confirmed that the PP2A-Sgo1 interface observed in our crystal structure is physiologically relevant. For the rest of this work, we will discuss the interaction of one PP2A AB′C complex with two Sgo1(51–96) peptides and, for simplicity, refer to them as Sgo1a and Sgo1b, respectively (Figure 2).