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β Clamp Directs Localization of Mismatch Repair in *Bacillus subtilis*

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SUMMARY

MutS homologs function in several cellular pathways including mismatch repair (MMR), the process by which mismatches introduced during DNA replication are corrected. We demonstrate that the C terminus of *Bacillus subtilis* MutS is necessary for an interaction with β clamp. This interaction is required for MutS-GFP focus formation in response to mismatches. Reciprocally, we show that a mutant of the β clamp causes elevated mutation frequencies and is reduced for MutS-GFP focus formation. MutS mutants defective for interaction with β clamp failed to support the next step of MMR, MutL-GFP focus formation. We conclude that the interaction between MutS and β is the major molecular interaction facilitating focus formation and that β clamp aids in the stabilization of MutS at a mismatch in vivo. The striking ability of the MutS C terminus to direct focus formation at replisomes by itself, suggests that it is mismatch recognition that licenses MutS's interaction with β clamp.

INTRODUCTION

Replicative DNA polymerases are responsible for duplicating entire genomes with high fidelity (for review see Johnson and O'Donnell, 2005). Despite the high fidelity of this process (e.g., Kool, 2002), replication errors still occur. Correction of these errors requires the enlistment of mismatch repair (MMR) proteins to restore the proper coding sequence (for review see Kunkel and Erie, 2005). MMR is an important, highly conserved repair pathway that is found in both prokaryotes and eukaryotes (Culligan et al., 2000).

In bacteria, deletion of MMR genes results in a several hundred-fold increase in mutation frequency (Cox et al., 1972). In humans, several primary tumors from cancers including hereditary nonpolyposis colorectal carcinoma (HNPCC) and Turcot syndrome (Fishel et al., 1993; Hamilton et al., 1995) have MMR defects, suggesting that increased mutation frequency could contribute to the development of these cancers. The function of MMR proteins is not limited to their clearly defined roles in MMR. For example, in bacteria MMR proteins can also participate in an antirecombination mechanism preventing recombination between nonidentical DNA sequences and thereby serving to preserve species barriers at a molecular level (Rayssiguier et al., 1989). In eukaryotes, MMR proteins have been shown to function in meiosis (Hunter and Borts, 1997; Williamson et al., 1985) and are important for maintaining repetitive DNA sequences (microsatellites) (Orth et al., 1994). Moreover, MMR proteins contribute to several other cellular processes including apoptosis (Hickman and Samson, 2004) and DNA damage checkpoint activation (Yoshioka et al., 2006).

The central and most intensively studied role for MMR is in the repair of mismatched nucleotides or insertion-deletion loops introduced principally as polymerase errors. In both prokaryotes and eukaryotes, MMR is initiated through the binding of a MutS homolog to mismatched DNA (for review see Schofield and Hsieh, 2003). In the paradigmatic *E. coli* system, MutS recognizes a mismatch followed by the recruitment of MutL (Schofield et al., 2001). MutL coordinates the actions of the nicking endonuclease MutH (e.g., Hall and Matson, 1999) and the loading of UvrD helicase (Hall et al., 1998) at the incised nick to result in the ATP-dependent separation of the DNA strand encoding the mismatch (Oeda et al., 1982). The mismatch-containing strand can then be degraded by one of several exonucleases (Viswanathan et al., 2001). The single-strand region is filled in by DNA Pol III and sealed by ligase to complete the repair process (Lahue et al., 1989).

In eukaryotes, a heterodimer of *MutS h*omologs (*Saccharomyces cerevisiae* MSH2-MSH6 [MutSα] [Prolla et al., 1994b] and hMSH2-hMSH6 in humans [Acharya et al., 1996]) is responsible for the recognition of most mismatches and small insertion or deletion loops (Drummond et al., 1995). These proteins recruit heterodimeric *MutL h*omologs, (*S. cerevisiae* MLH1-PMS1 [MutLα], and human hMLH1-hPMS2 [Prolla et al., 1994a]), which function analogously to *E. coli* MutL and appear important for coordinating the actions of the remaining proteins in the pathway. The purified human proteins have been used to reconstitute MMR in vitro (Dzantiev et al., 2004). These studies have revealed that MMR excision from a 5′ strand break requires MutSα, an exonuclease (EXO1), and single-stranded binding protein (RPA). MMR excision from a strand break 3′ to the mismatch requires the proteins mentioned above in addition to MutLα, the clamp loader (RFC), and the replication processivity clamp (PCNA). These experiments indicate that the protein assemblies and mechanisms required for excision and repair are different depending on the directionality of the strand break relative to the mismatch. Theses studies also demonstrate the dependence on a processivity clamp for 3′ directed repair.

In *B. subtilis*, both MutS and MutL fused to green fluorescent protein (GFP) localize as discrete foci in cells exposed to the

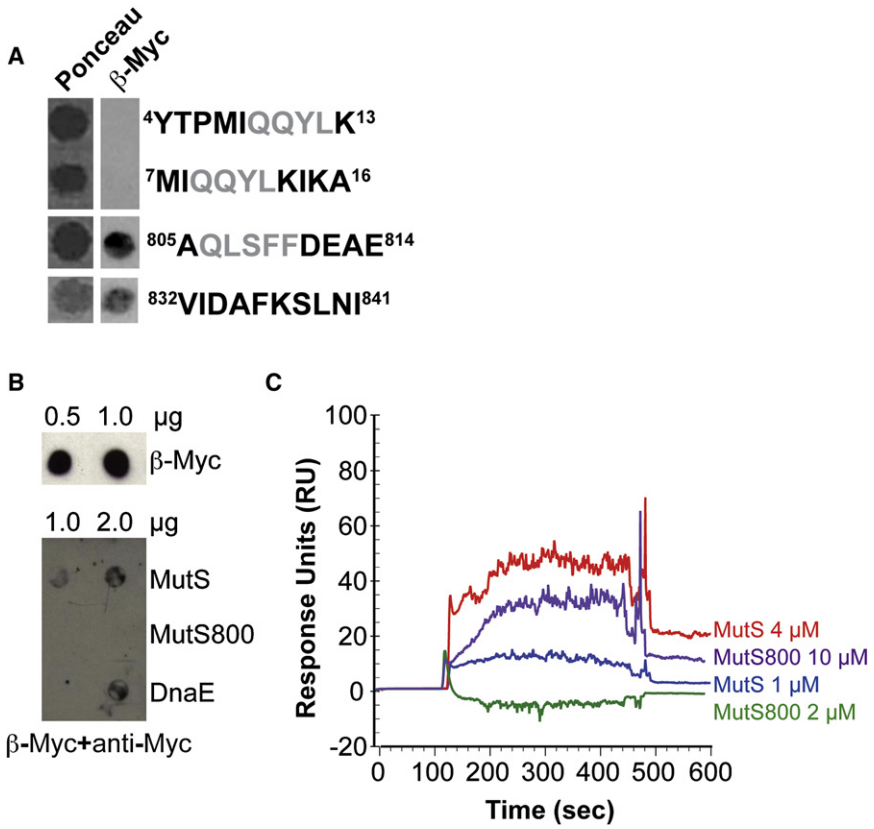

Figure 1. MutS Interacts with the β Clamp through the C-Terminal 58 Amino Acids

The peptide array and far-western blot were probed with β clamp bearing a single Myc tag (β-Myc).

(A) Shown are 10-mer peptides of interest from the MutS N terminus that failed to bind β or the C-terminal 58 amino acids that bound β-Myc. The amino acid sequence is indicated, and the β clamp binding motif or N-terminal motif is highlighted in gray. Ponceau staining of the peptides is shown in the left-most panel.

(B) β-Myc, MutS, MutS800, and His-DnaE probed with β-Myc.

(C) Interaction between MutS or MutS800 with β clamp covalently linked to a sensor chip was completed using surface plasmon resonance as measured with a BIAcore biosensor (Experimental Procedures). Representative SPR traces for MutS 1.0 μM (blue) and 4.0 μM (red), and MutS800 2.0 μM (green) and 10.0 μM (purple) are shown.

mismatch-inducing agent 2-aminopurine (2-AP) (Smith et al., 2001). A new challenge in understanding MMR is to determine the protein interactions that allow for the coordinated assembly of MMR foci in vivo. *B. subtilis* serves as a particularly useful system for addressing this issue because *B. subtilis* is the only bacterium in which MMR proteins have been visualized in vivo. In addition, like eukaryotes and most bacteria, *B. subtilis* uses a DNA methylase-independent mechanism for strand discrimination indicating that *B. subtilis* serves as a valuable model system for understanding this process.

Experiments in several systems have shown that MutS homologs interact with replication processivity clamps (β clamp in bacteria and PCNA in eukaryotes) (Gu et al., 1998; Kleczkowska et al., 2001; Lau and Kolodner, 2003; Lee and Alani, 2006; Lopez de Saro et al., 2006; Umar et al., 1996). Disruption of the interaction between MSH6 and PCNA in *S. cerevisiae* results in mild mutator phenotypes in vivo (for review see Schofield and Hsieh, 2003). The only modifications shown to result in strong mutator phenotypes encompass large deletions of the N-terminal domain that forms a flexible linker to PCNA (Shell et al., 2007). In *E. coli*, internal deletion of a β clamp binding motif renders MutS refractory to β clamp binding in vitro, yet in vivo the corresponding *mutS* allele confers a wild-type MMR phenotype (Lopez de Saro et al., 2006). Collectively, MutS homologs from several organisms have been shown to bind their resident processivity clamps; however, the in vivo significance for this interaction is unclear.

We examined the mechanism that governs the focus formation response of MutS to mismatches in *B. subtilis*. We studied an assortment of MutS variants altered in their C terminus and found that interaction between MutS and β clamp is required for MutS-GFP focus formation. Furthermore, we found that the C-terminal 58 amino acids are not only necessary but also sufficient for focus formation and that these foci exhibit the same subcellular distribution as the replisome. We also found that MutS deleted for this C-terminal region is reduced for binding to β clamp, although this purified mutant protein retains the ability to bind a mismatch in vitro. Taken together, we conclude that interaction between MutS and β is the major molecular interaction that facilitates focus formation and that β clamp aids in the stabilization of MutS at a mismatch in vivo.

RESULTS

The C Terminus of MutS Is Required for Interaction with β Clamp

Several lines of evidence indicate that processivity clamps play an important role in MMR (Kleczkowska et al., 2001; Lopez de Saro et al., 2006; Umar et al., 1996). *B. subtilis* MutS contains a putative β clamp binding motif (^{806}QLSFF^{810}). It has been hypothesized that β clamp binding motifs modulate the interaction between a variety of proteins and the β clamp (Dalrymple et al., 2001). In vitro studies of *E. coli* MutS have shown that both its N- and C-terminal regions interact with β clamp (Lopez de Saro et al., 2006). The N-terminal MutS motif is necessary for MMR in *E. coli*, while the C-terminal MutS motif appears to be dispensable (Lopez de Saro et al., 2006) in vivo.

As a first step toward determining whether β clamp contributes to mismatch-dependent MutS-GFP focus formation in *B. subtilis*, we used a peptide array approach to identify MutS sequences capable of interacting with the β clamp. Unlike *E. coli*, we found that peptides containing the conserved N-terminal motif (^{9}QQYL^{12}) failed to bind β (Figure 1A). Instead, we found that