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Proliferating cell nuclear antigen (PCNA) interacts with a meiosis-specific RecA homologues, Lim15/Dmc1, but does not stimulate its strand transfer activity

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Abstract

PCNA is a multi-functional protein that is involved in various nuclear events. Here we show that PCNA participates in events occurring during early meiotic prophase. Analysis of protein–protein interactions using surface plasmon resonance indicates that *Coprinus cinereus* PCNA (CoPCNA) specifically interacts with a meiotic specific RecA-like factor, *C. cinereus* Lim15/Dmc1 (CoLim15) *in vitro*. The binding efficiency increases with addition of Mg^{2+} ions, while ATP inhibits the interaction. Co-immunoprecipitation experiments indicate that the CoLim15 protein interacts with the CoPCNA protein *in vitro* and in the cell extracts. Despite the interaction between these two factors, no enhancement of CoLim15-dependent strand transfer activity by CoPCNA was found *in vitro*. We propose that the interaction between Lim15/Dmc1 and PCNA mediates the recombination-associated DNA synthesis during meiosis.

Keywords: Meiosis; Recombination; DNA replication; Strand invasion

Proliferating cell nuclear antigen (PCNA) was originally identified as a DNA sliding clamp for DNA polymerases and was an essential components of replication machinery [1–3]. PCNA is also a multifunctional protein that is involved in various nuclear events such as nucleotide excision repair, mismatch repair, base excision repair, and maintenance of DNA methylation [4]. These activities are mediated through interaction with various factors such as DNA polymerases δ [5], ε [6], and DNA methyltransferase 1 (Dnmt1) [4]. Also, factors with different biological activity, such as nuclease FEN-1, a cell cycle inhibitor p21 [7], and DNA polymerase δ [8], competitively interact with the same domain of the PCNA protein. These observations suggest that PCNA is a key regulator of various reaction types and may function as a bridge between distinct nuclear events. Despite the demonstrated multi-functionality of PCNA, it is undetermined whether or not PCNA is involved in DNA recombination-related process.

During meiotic prophase, homologous chromosomes are recombined. Meiotic recombination is composed of several steps. First, meiosis-specific double-strand breaks appear to be introduced and this is followed by formation of single-stranded DNA. The single-stranded ends then invade regions of homology in the other allele. After ensuing strand invasion and initial repair synthesis, the crossover and the non-crossover pathways diverge [9]. These reactions are mediated by the coordinated activity of vari-

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ous proteins including RecA-like protein, an ATPase playing a central role in the strand transfer reaction [9,10]. In eukaryotes, Lim15/Dmc1 and Rad51 have been identified as RecA homologues.

While Rad51 is expressed in both meiotic and somatic cells and functions in DNA repair reactions, Lim15/Dmc1 expression is restricted to meiotic cells [10]. Recent studies revealed that Lim15/Dmc1 interacts with various nuclear proteins during meiosis. Tid1 interacts with both Rad51 and Lim15/Dmc1 [11]. Meiosis specific proteins Mei5 and Sae3 form a ternary complex with Lim15/Dmc1 [12,13]. Recently, Hop2–Mnd1 complex [14] and DNA topoisomerase II [15] were reported as novel binding partners for Lim15/Dmc1. Furthermore, we identified that Ubc9 interacts with Lim15/Dmc1 and mediates sumoylation of Lim15/Dmc1 [16]. These studies indicate that Lim15 has a pivotal role during meiosis through the interaction between multiple binding partners.

From the basidiomycete, *Coprinus cinereus*, we previously cloned the genes for Lim15/Dmc1 (CoLim15) and PCNA (CoPCNA) [17,18]. We have found that both CoPCNA and CoLim15 are expressed abundantly in the zygotene stage of meiotic prophase [17–19], prompting us to study the potential interactions between the CoPCNA and CoLim15 proteins. Here we demonstrate that CoLim15 interacts with CoPCNA both *in vitro* and in the cell extracts. Despite the interaction between the two factors, when using naked DNA template as a substrate, no enhancement of CoLim15-dependent strand transfer reaction by CoPCNA was observed. We discuss the possible role of PCNA in meiotic recombination-related events via Lim15.

Materials and methods

Culture of C. cinereus and collection of the fruiting bodies. The basidiomycete *C. cinereus* (ATCC #56838) was used in this study. The culture methods used were as described previously [18].

Recombinant proteins and antibodies. Recombinant his-tagged CoLim15 (His-CoLim15), N-terminal region of CoLim15 (His-CoLim15N), C-terminal region of CoLim15 (His-CoLim15C), his-tagged CoRad51 (His-CoRad51) and his-tagged CoPCNA (His-CoPCNA), and each antibody were as described previously [18,19]. We found two differently sized transcripts from a single PCNA gene, CoPCNA-α and CoPCNA-β, respectively [18]. The focus of this report is CoPCNA-α.

Surface plasmon resonance (SPR). His-CoLim15 and His-CoPCNA binding analyses were performed using a BIAcore Biosensor instrument (Supplementary information).

Co-immunoprecipitation. The rabbit anti-CoPCNA polyclonal antibody, the rabbit anti-CoLim15 polyclonal antibody or control rabbit IgG was coupled with CNBr-activated Sepharose beads according to the manufacturer's instructions (Amersham Bio). The mixture of 60 pg His-CoPCNA and 60 pg His-CoLim15 (*in vitro* interaction), or aliquots of 20 mg of crude extract from early meiotic prophase tissues (1–4 h after karyogamy; cell extracts) were prepared in buffer A (50 mM Tris–HCl, pH 7.5, 1 mM EDTA, and 10% glycerol containing 0.15 M NaCl, 5 mM 2-mercaptoethanol, and protease inhibitors (1 mM PMSF, 1 μM leupeptin, and 1 μM pepstatin A)). The input was incubated with 100 μl of primary antibody or the control rabbit IgG-conjugated beads for 1 h at 4 °C. The beads were isolated by centrifugation at 2000 rpm for 30 s and resuspending with 200 μl of buffer A. The washing step was repeated by 5–10 times. At the final washing step, the supernatant was removed by centrifugation at 15,000 rpm for 2 min completely. The bound materials were eluted from the beads with 30 μl of buffer B (50 mM glycine–HCl, pH 2.5, and 0.01% Triton X-100). After neutralization of the pH by adding 5 μl of 1 M Tris–HCl, pH 7.5, the bound material was analyzed by immunoblotting with the rat anti-CoPCNA antibody and the rabbit anti-CoLim15 antibody.

GST-pull-down assay. Lysates for the GST-pull-down assay were prepared as described [20] with minor modifications. Glutathione beads (30 μl) were equilibrated with buffer A. Two hundred microliters of *Escherichia coli* extracts containing approximately the same quantity of GST or GST-CoPCNA (GST-CoPCNA$_{1–368}$, GST-CoPCNA$_{1–184}$, GST-CoPCNA$_{1–260}$, and GST-CoPCNA$_{185–368}$) fusion proteins was mixed with the beads. After washing the beads with 200 μl of buffer A five times, we added a 100 μl of *E. coli* extracts containing the His-CoLim15 recombinant proteins in buffer A and incubated the mixture for 1 h at 4 °C. After washing the beads with 200 μl of buffer A five times, we eluted the bound materials by incubating with 20 μl of buffer A supplemented with 5 mM reduced glutathione for 15 min at 4 °C. Protein samples were analyzed by Western blotting using the rabbit anti-CoLim15 antibody. The GST-pull-down assay of GST-CoLim15 proteins was performed as described elsewhere [16], and analyzed by Western blotting using the rabbit anti-CoPCNA antibody.

Strand transfer assay. Strand transfer assays were performed as described previously [21].

Results

CoLim15 interacts with CoPCNA

Previously, we observed that both CoPCNA and CoLim15 are highly expressed in the zygotene stage of *C. cinereus* cells [17,18,21]. Due to the multiple functions of the PCNA protein, we wished to test the possibility that CoPCNA is involved in meiotic recombination via CoLim15, which plays a central role in this process. We utilized the BIAcore system to test a direct protein–protein interaction between the two factors. The BIAcore system is an instrument based on surface plasmon resonance (SPR) which measures real-time interaction between a ligand coupled to a detection surface (sensor chip) and a ligand that is injected. The SPR signal reflects changes in refractive index at the sensor surface upon protein–protein interaction: the refractive index changes as a consequence of binding events close to the sensor surface and is related to the increase of mass on the surface [22].

The His-CoPCNA protein (6600 RU) was conjugated to the CM 5 sensor chip (CoPCNA-chip). Then purified His-CoLim15 or His-CoRad51 protein was injected onto the CoPCNA-chip at two different concentrations. When 150 or 750 nM His-CoLim15 was injected into the system, we detected 473 or 1738 RU of His-CoLim15 bound to the CoPCNA-chip, respectively. Interestingly, CoPCNA did not significantly interact with another RecA-like protein, CoRad51, which has significant homology with CoLim15. In the presence of 150 or 750 nM His-CoRad51, we detected only 38 or 44 RU of His-CoRad51 bound to the CoPCNA-chip, respectively (Fig. 1A). Furthermore, the binding efficiency increased with higher concentration of His-CoLim15, but not His-Rad51 (data not