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RCC1 Uses a Conformationally Diverse Loop Region to Interact with the Nucleosome: A Model for the RCC1–Nucleosome Complex

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The binding of RCC1 (regulator of chromosome condensation 1) to chromatin is critical for cellular processes such as mitosis, nucleocytoplasmic transport, and nuclear envelope formation because RCC1 recruits the small GTPase Ran (*Ra*s-related *n*uclear protein) to chromatin and sets up a Ran-GTP gradient around the chromosomes. However, the molecular mechanism by which RCC1 binds to nucleosomes, the repeating unit of chromatin, is not known. We have used biochemical approaches to test structural models for how the RCC1 β-propeller protein could bind to the nucleosome. In contrast to the prevailing model, RCC1 does not appear to use the β-propeller face opposite to its Ran-binding face to interact with nucleosomes. Instead, we find that RCC1 uses a conformationally flexible loop region we have termed the switchback loop in addition to its N-terminal tail to bind to the nucleosome. The juxtaposition of the RCC1 switchback loop to its Ran binding surface suggests a novel mechanism for how nucleosome-bound RCC1 recruits Ran to chromatin. Furthermore, this model accounts for previously unexplained observations for how Ran can interact with the nucleosome both dependent and independent of RCC1 and how binding of the nucleosome can enhance RCC1's Ran nucleotide exchange activity.

Keywords: chromatin biology; nucleosome recognition; guanine nucleotide exchange; mitosis; protein–protein interactions

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Introduction

Mitosis, nucleocytoplasmic transport, and nuclear envelope dynamics all rely on spatial coordination within the eukaryotic cell mediated by the small GTPase Ran (*Ra*s-related *n*uclear) protein and its guanine-exchange factor (GEF), RCC1 (regulator of chromosome condensation 1).[1–6] RCC1 associates with chromatin and both recruits and activates Ran to create a concentration gradient of RanGTP (Ran in its GTP-bound state) around the chromosomes. This RanGTP gradient is a key positioning signal within the cell for the aforementioned cellular processes and depends fundamentally on the binding of RCC1 to nucleosomes.

Previous biochemical and structural studies have determined the structures of RCC1 and Ran and how they interact with each other. Human RCC1 is a 421-amino-acid protein with an N-terminal tail of approximately 20 residues and a C-terminal seven-bladed β-propeller domain with strong structural similarity to WD40 repeat proteins such as the β-subunit of signal transduction G proteins (Fig. 1a).[7] Each blade in the β-propeller structure contains four antiparallel strands with loops between each strand. The equivalent loops that decorate the two faces of the β-propeller structure in the WD40 β-propeller cousin proteins frequently mediate protein–protein interactions with interacting proteins.[8–16] In fact, the crystal structure of RCC1 bound to Ran shows that loops from all seven blades on one side of the RCC1

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Abbreviations used: RCC1, regulator of chromosome condensation 1; Ran, Ras-related nuclear; GEF, guanine-exchange factor; GST, glutathione *S*-transferase; GSH, glutathione; LANA, latency-associated nuclear antigen; DHFR, dihydrofolate reductase; TEV, tobacco etch virus; BSA, bovine serum albumin; PDB, Protein Data Bank.

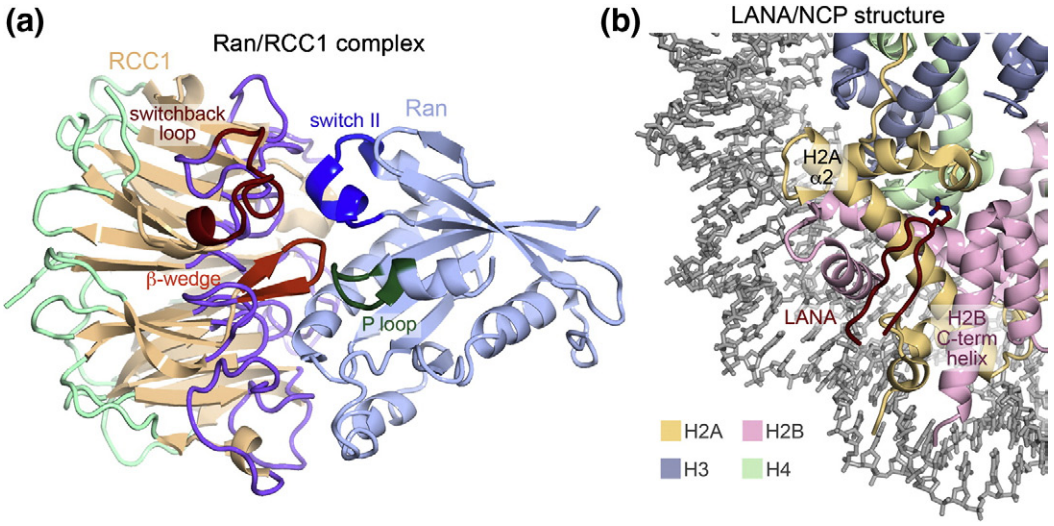

Fig. 1. Ran/RCC1 and LANA/nucleosome core particle structures. (a) Ribbon representation of Ran/RCC1 crystal structure [Protein Data Bank (PDB) ID 1I2M]. The RCC1 β-strands are shown in beige, the loops on the Ran-interacting side are in cornflower blue, the loops on the non-Ran-interacting side are in light green, the conformationally diverse switchback loop is in red, and the β-wedge is in orange. The Ran protein is shown in light blue with its nucleotide interacting regions switch II in blue and the P-loop in green. (b) Ribbon and stick representation of the LANA/nucleosome core particle crystal structure (PDB ID 1ZLA). The histone proteins H2A, H2B, H3, and H4 are shown in yellow, pink, blue, and green, respectively, while the nucleosomal DNA is colored gray. The backbone of the LANA 14 amino acids visible in the structure is shown in red, as is the Arg9 that makes key interactions with the histone dimer.

β-propeller interact with Ran in the complex (Fig. 1a).[17] A β-hairpin extension of RCC1 repeat 3, termed the β-wedge, mediates interactions with key regions of Ran such as the Ran nucleotide-binding P-loop and the GTPase switch II region.[17] These interactions explain how RCC1 promotes the otherwise intrinsically slow exchange of guanine nucleotides in Ran, thus creating the RanGTP gradient around the chromosomes through the combination of this guanine-exchange activity and RCC1's association with the nucleosome.

In contrast, we have relatively little structural information regarding how RCC1 binds to chromatin despite the importance of this interaction for the formation of the RanGTP gradient. Two different mechanisms have been proposed for how RCC1 binds to the nucleosome. Based on initial observations that RCC1 has DNA-binding activity as judged by binding to DNA-cellulose resin, RCC1 was proposed to bind to chromatin via contacts with DNA.[18] Furthermore, an unusual posttranslational modification of RCC1, N-terminal α-methylation, has been found to affect RCC1–chromatin association *in vivo* and to confer a twofold increase in double-stranded DNA binding activity *in vitro*.[19] Yet another study has shown that RCC1's N-terminal tail with its DNA-binding activity is not required for chromatin binding and may instead function as a nuclear translocation signal for RCC1.[20] A second mechanism for RCC1–chromatin interactions emphasizes protein–protein interactions instead of protein–DNA interactions. Glutathione *S*-transferase (GST) pull-down assays showed that RCC1 associates directly with nucleosomes via the histone H2A/H2B dimer and that the RCC1 β-propeller domain (i.e., without the N-terminal tail) was sufficient for this interaction.[21] Since removing the histone tails did not affect RCC1/nucleosome interactions, it appears that the surface-exposed globular regions of the histone H2A/H2B mediate binding of RCC1 to the nucleosome. However, the molecular mechanism for this interaction is not known. Neither is it understood why binding of RCC1 to the nucleosome enhances RCC1's GEF activity on Ran.[21]

As the fundamental repeating unit of chromatin, the nucleosome has been the subject of intensive investigation in gene regulation and chromatin biology.[22,23] Although once considered to be a repressive structure that occludes nuclear factors from interacting with DNA in a eukaryotic cell, the nucleosome is now understood to be an active participant in gene regulatory processes and that a multitude of chromatin modification, remodeling, and transcription factors function on a nucleosome template.[24–26] However, we still lack a detailed understanding of how such chromatin factors and enzymes actually interact with their nucleosome substrate. The crystal structure of the 23-amino-acid Kaposi's sarcoma herpesvirus LANA (latency-associated nuclear antigen) peptide bound to the nucleosome core particle currently offers the only atomic view of a chromatin factor interacting with the nucleosome.[27] The crystal structure (Fig. 1b) and corroborating biochemical data indicate that the LANA peptide interacts with a solvent-accessible acidic patch created by the nucleosome H2A α-helix α2 and the H2B C-terminal helix.

In this study, we analyze how RCC1 binds to the nucleosome by employing biochemical methods to test structural models. We find that RCC1 targets a region of the nucleosomal H2A/H2B dimer