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Chapter 5
RuvBL1 and RuvBL2 and Their Complex Proteins Implicated in Many Cellular Pathways

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Abstract RuvBL1 and its homolog RuvBL2 belong to the AAA$^+$ family of ATPases and play important roles in chromatin remodeling, in transcriptional regulation, in DNA repair and in the c-Myc and Wnt signaling pathways. Proteins involved in these pathways are often mutated in human cancers. Both RuvBL proteins form a complex and act alone or together in diverse cellular processes. The three-dimensional structures of human RuvBL1 refined using diffraction data to 2.2 Å resolution and of the human RuvBL1/RuvBL2 complex with a truncated domain II at 3 Å resolution are presented. The dodecameric RuvBL1/RuvBL2 complex structure differs from previously described models. It consists of two heterohexameric rings with alternating RuvBL1 and RuvBL2 monomers that interact with each other via domain II. ATPase and helicase activities of RuvBL1 and RuvBL2 were also tested. Interestingly, truncation of domain II resulted not only in a substantial increase of ATP consumption by the RuvBL proteins, but also in stimulation of helicase activity, which was not observed with the full-length proteins.

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5.1 RuvBL1 and RuvBL2 Are Highly Conserved AAA+ Proteins

RuvBL1 and its homolog RuvBL2 are ubiquitously expressed proteins [1] that belong to the AAA+ family of ATPases (ATPases associated with diverse cellular activities) [2]. This class of ATPases includes nucleic acid processing enzymes, chaperones and proteases. AAA+ proteins often form hexameric ring structures and contain conserved motifs for ATP binding and hydrolysis such as the Walker A and Walker B boxes [3], the Arg-finger and sensor residues. All AAA+ proteins use ATP binding and hydrolysis to exert mechanical forces. ATP hydrolysis is clearly essential for the biological activity of RuvBL1 and RuvBL2 [4–6]. RuvBL1 and RuvBL2 share an homology of *ca.* 30% with the bacterial DNA-dependent ATPase and helicase RuvB [7, 8], which is the motor that drives branch migration of the Holliday junction in the presence of RuvA and RuvC during homologous recombination and recombinational repair of damaged DNA [9]. The ruvA, ruvB and ruvC genes are required for normal levels of cellular resistance to the effects of UV- or ionizing radiation [10]. Helicases, like RuvB, are molecular motor proteins which couple the energy of ATP hydrolysis to unwinding of the energetically stable duplex form of DNA or RNA and translocate along the nucleic acid in an ATP-dependent manner.

5.2 RuvBL1 and RuvBL2 Are Components of Chromatin Remodeling Complexes

RuvBL1 and RuvBL2 were found to be involved in chromatin remodeling by several groups. A fundamental regulatory step in transcription and other DNA-dependent processes in eukaryotes is the control of chromatin structure, which regulates access of proteins to DNA. In the eukaryotic nucleus, DNA is wrapped around an octamer of four core histones in approximately two superhelical turns to form the nucleosome, and arrays of nucleosomes are successively folded into higher-order structures that collectively define chromatin. Packaging of genes into chromatin represses basal transcription and several multisubunit complexes are needed to regulate gene expression by modulating the topology of the nucleosomes in a number of ways.

RuvBL1 and RuvBL2 were found to be part of diverse chromatin remodeling complexes. They are present in two related complexes containing p400, the p400 complex and the TIP60 complex. These complexes perform critical functions in a variety of cellular processes including transcriptional activation, as well as break

repair and apoptosis of double stranded DNA [11–13]. They display ATPase and helicase activities. It was shown that these functions are, at least in part, contributed by RuvBL1 and RuvBL2 [12].

Both proteins are also components of the INO80 complex which exists in yeast and higher eukaryotes. It catalyzes ATP-dependent sliding of nucleosomes along DNA and is involved in the repair of DNA double strand breaks and in transcriptional regulation [5, 14–16]. It was shown that RuvBL1 and RuvBL2 are essential for the structural and functional integrity of the INO80 chromatin remodeling complex [5]. RuvBL1 and RuvBL2 bound to ATP are in the correct conformation to associate with the INO80 complex and initiate the recruitment of the essential actin-like Arp5 subunit assembling the complete functional chromatin remodeling complex.

5.3 RuvBL1 and RuvBL2 Are Involved in Transcription

RuvBL1 and RuvBL2 regulate transcription not only via association with chromatin remodeling complexes, but also through interactions with diverse transcription factors and the RNA polymerase II holoenzyme complex. First, RuvBL1 and RuvBL2 were found to interact with the TATA-binding protein [17, 18] and the large RNA polymerase II holoenzyme complex [19], which contains over 50 components and is responsible for the transcription of protein-encoding genes. Later, RuvBL1/2 were also identified by their physical interaction with the transcription-associated protein β-catenin [1, 20], and with the transcription factors c-Myc [6], E2F1 (only RuvBL1 [21]) and ATF2 (only RuvBL2 [22]). Since then, the mammalian homologs have been implicated in at least two oncogenic pathways, one involving c-Myc and the other β-catenin. Among the transcription factors with oncogenic potential, c-Myc is one of the most frequent sites of mutation in human cancer [23]. The N-terminal portion of c-Myc contains two highly conserved regions, called Myc homology box I (MbI) and Myc homology box II (MbII). The MbII domain is necessary for virtually all c-Myc biological activities, including oncogenic transformation, apoptosis and the ability to block differentiation and stimulate cell proliferation [24–27]. This region was shown to bind to RuvBL1 and RuvBL2 [6]. A missense mutation in the RuvBL1 ATPase motif acts as a dominant inhibitor of c-Myc oncogenic activity but does not inhibit normal cell growth, indicating that functional RuvBL1 is an essential mediator of c-Myc oncogenic transformation [6, 21].

5.4 Crystal Structure and Activities of Human RuvBL1

The crystal structure of RuvBL1 was solved from data at 2.2 Å resolution, showing an overall hexameric molecule with a central channel of approximate diameter of 20 Å, where each monomer is complexed with one ADP molecule [28].

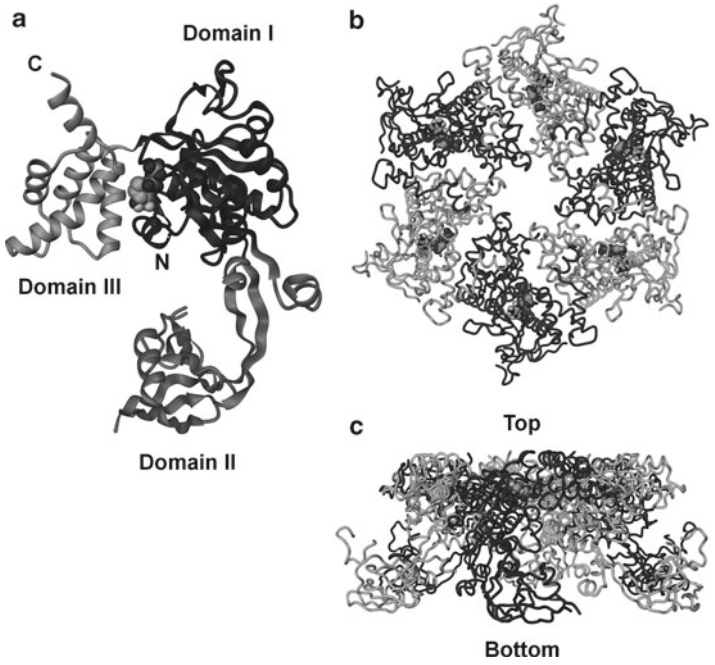

Fig. 5.1 Views of the RuvBL1 3D structure. (**a**) Ribbon diagram highlighting the domain structure; (**b**) Top view of the RuvBL1 hexamer; (**c**) Side view of the RuvBL1 hexamer. In (**b**) and (**c**) the C^{α} chain is represented in tube mode and the monomers are colored *light* and *dark gray* for clarity. In all views, the ADP molecules are drawn in space-filling mode

The monomers contain three domains, of which the first and the third are involved in ATP binding and hydrolysis. Structural analysis allowed the identification of four conserved motifs (Walker A, Walker B, sensor 1 from domain I and sensor 2 from domain III) in RuvBL1, likely to be important in nucleotide-driven conformational changes of the protein structure.

Structural homology and DNA-binding studies suggested that domain II, which is unique among AAA^{+} proteins and not present in the bacterial homolog RuvB, is a novel DNA/RNA-binding domain. The interface between adjacent subunits in the hexamer of RuvBL1 is made up entirely by domain I and domain III. The nucleotide binding pocket is located in this interface, and the hexamerization process seems to block access to this pocket, thus making impossible an exchange from ADP to ATP (Fig. 5.1).

Arg-357 from each monomer is near this interface and sufficiently close to the nucleotide binding site in the adjacent monomer to be able to act as an Arg finger, provided a suitable conformation change takes place.

We were able to demonstrate that RuvBL1 interacted with ssDNA/RNA and dsDNA. Because the central channel seems to be too small to accommodate dsDNA, we assumed that a region outside of the ring makes DNA contacts. Based on this

assumption and the similarity between domain II and DNA binding domains of other proteins we performed electrophoretic mobility shift assays experiments which showed that the new domain II acts as a nucleic acid binding domain.

Although it has been shown that ATPase activity of RuvBL1 is needed for several *in vivo* functions, we could only detect a marginal activity with the purified protein. However, the structure of the RuvBL1-ADP complex suggests that RuvBL1 has all the structural characteristics of a molecular motor, even of an ATP-driven helicase.

5.5 Crystal Structure of the Human RuvBL1/RuvBL2 Complex

The purified wild-type complex of RuvBL1 and RuvBL2 was used for crystallization trials in order to solve its three dimensional structure. Although thousands of conditions were tested, the wild-type complex never crystallized. Thus, deletion mutants of RuvBL1 and RuvBL2 with partially truncated domains II were generated for crystallization purposes [29].

The crystal structure of the RuvBL1ΔDII/RuvBL2ΔDII (R1ΔDII/R2ΔDII) complex was solved from a selenomethionine derivative at 3 Å resolution [30] by a combination of both molecular replacement and MAD methods. It is a double-heterohexameric ring structure with alternating R1ΔDII and R2ΔDII monomers, forming a dodecamer. The central channel (C^{α} – C^{α} distance) in the dodecameric R1ΔDII/R2ΔDII complex structure has an internal diameter similar to that of the RuvBL1 hexamer at both terminals but it is much wider in the central part. Contrary to what might be expected, the two heterohexameric rings in the R1ΔDII/R2ΔDII dodecamer do not interact through the flat ring surfaces, but rather via the retained section of the truncated domains II.

These results differ from those previously reported: our dodecamer is symmetrical as opposed to that proposed for the human [31] and for the yeast complexes [32], even though the hexamer arrangement in the dodecamer is similar. The dodecameric structure of the human RuvBL1/RuvBL2 complex was examined by negative stain electron microscopy at 20 Å resolution, and substantial differences were found between the top and bottom rings [31]. On the other hand, our heterohexameric arrangement of RuvBL1 and RuvBL2 monomers agrees with the yeast Rvb1/Rvb2 complex structure solved by EM [33], but our structure is clearly a dodecamer while they proposed isolated hexamers. In agreement with our results, the dodecameric yeast Rvb1/Rvb2 cryo-EM structure clearly shows that domain II constitutes the interaction site between the two hexameric rings [32]. However, their results suggest that each ring is composed of just one of the proteins, forming homo-oligomeric hexamers, whereas our crystallographic analysis shows two hetero-hexameric rings related by a crystallographic twofold axis forming the dodecamer.

Each of the R1ΔDII and R2ΔDII monomers in the dodecamer is complexed with a mixture of ADP and ATP molecule. The electron density for the the γ-phosphate is clearly weaker than for the other two phosphate groups, suggesting that it may

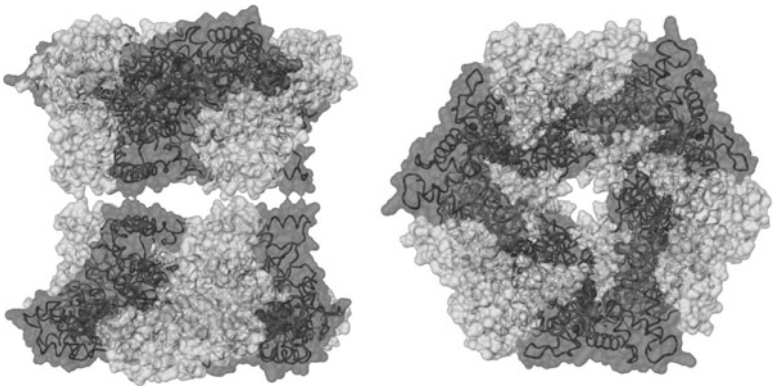

Fig. 5.2 Views of the RuvBL1ΔDII/RuvBL2ΔDII dodecamer – tube C^{α} diagrams with superimposed molecular surface. RuvBL1ΔDII monomers are *light-colored*, RuvBL2ΔDII monomers are *dark-colored*, nucleotide molecules are drawn in space-filling mode

have been partially hydrolized to ADP. For each monomer in the structure, the γ-phosphate is interacting with an aspartate residue in the same monomer located in the Walker B motif, essential for ATP hydrolysis, an arginine residue in the same monomer belonging to the sensor 2 region, and an aspartate from an adjacent monomer which precedes an arginine residue.

Given that both hexameric and dodecameric structures of the RuvBL1/RuvBL2 complex were reported as described above, and that RuvBL1 alone crystallized as a hexamer in its ADP-bound form [28], one can hypothesize that both hexamers and dodecamers of the complex exist *in vivo*. Once it becomes active, this AAA^{+} machine may switch between its hexameric and dodecameric state (Fig. 5.2).

Our crystallographic results suggest that there is a tight interaction between the truncated domains II of RuvBL1 and RuvBL2 forming a dodecameric structure. In general, AAA^{+} proteins undergo conformational changes upon hydrolysis of ATP to ADP. These changes may loosen the interactions between domains II of the two hexamers resulting in either a dodecamer with domains II in a different conformation, or two separate hexameric rings with bound ADP (as seen in the RuvBL1 crystal structure).

Therefore, the change between two different dodecameric conformations or between hexameric and dodecameric structures of the RuvBL1/RuvBL2 complex may be important for activity regulation.

5.6 Activity of the Human RuvBL1/RuvBL2 Complex

We only detected a weak ATPase activity of purified human RuvBL1 and RuvBL2. Important cellular processes of the higher eukaryotes, such as those dealing with DNA metabolism, are often regulated by large multi-protein complexes and it is

therefore likely that strong RuvBL1 or RuvBL2 enzymatic activity can only be seen in such an environment. Importantly, the wild-type RuvBL1/RuvBL2 complex exhibited a threefold to fourfold increase in ATP consumption compared to the single proteins. Surprisingly, the RuvBL1/RuvBL2 complexes with truncated domains II exhibited higher ATPase activity than the wild-type complex. Assembly of RuvBL1 and RuvBL2 into a dodecameric structure may have stimulated the ATPase activity. This synergistic effect on enzymatic activity clearly suggests communication between the two proteins coupled to conformational effects.

These findings, combined with our crystallographic results, indicate that, in vivo, domain II and dodecamerization may play a regulatory role to control ATP consumption. Given that the ATPase activity of RuvBL1 and RuvBL2 is needed for several in vivo functions [4, 6, 34], it can be speculated that cofactors regulate the ADP/ATP exchange by changing the position of domain II, thereby clearing the way to the nucleotide-binding pocket for a more efficient ADP/ATP exchange. As a result, the ATPase activity of RuvBL1 and RuvBL2 could be stimulated.

Even though RuvBL1 and RuvBL2 are the human homologs of the bacterial helicase RuvB, we and other groups did not detect helicase activity for recombinant human RuvBL1 and RuvBL2 [11, 19, 28]. Since the DII-truncated complex of RuvBL1 and RuvBL2 exhibited an unexpected increase in ATPase activity, it was possible that higher ATP consumption might allow the complex to exert helicase activity. Indeed, we observed helicase activity of the truncated constructs R1ΔDII, R2ΔDII, R1wt/R2ΔDII and R1ΔDII/R2ΔDII. Since the wild-type proteins exhibited no helicase activity in vitro, it can be concluded that cofactors binding to RuvBL1 and RuvBL2 in chromatin remodeling or transcription complexes alter the conformation of both proteins, most likely via domain II, and allow them to exert their helicase activity.

Small-angle X-ray scattering studies were performed with the wild-type single proteins and the wild-type and truncated complexes [30]. These studies reveal differences between the oligomerization states of RuvBL1 and RuvBL2 in solution. RuvBL2 forms mainly hexamers and dodecamers independently of its concentration, while RuvBL1 exists as a monomer at lower concentration and forms a hexamer at higher concentration, but is unable to form dodecamers. This diversity in oligomerization characteristics may explain why RuvBL1 and RuvBL2 are recruited into different complexes for their specialized functions. The results also show that truncation of domain II results in a complete dodecamerization of the RuvBL1/RuvBL2 complex, while the wild-type complex consists of a mixture of hexamers and dodecamers in solution, indicating that domain II is involved in dodecamerization and supporting the hypothesis that both oligomers could co-exist *in vivo*.

A detailed description and extensive discussion of the structural results as well as the SAXS studies will be presented elsewhere [30]. Correlations between the truncated dodecameric structure and the wild-type complex will be proposed along with a possible mechanism of how the highly conserved proteins RuvBL1 and RuvBL2 might exert their activities, a matter of long discussion in the literature.

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