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Short communication

HsOrc4-DEPENDENT DNA REMODELING OF THE *ori*-β DHFR REPLICATOR

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Abstract: Replication of DNA in multicellular organisms initiates from origin of replication (*ori*) sequences, which significantly differ in length and complexity. One of the best characterized is hamster dihydrofolate reductase (DHFR), which contains the *ori*-β sequence with several functionally relevant domains, such as an AT-rich region, dinucleotide repeat element (DNR), sequence-induced bend DNA (BEND) and a RIP60 protein-binding site (RIP60). Prior to initiation, *ori* sequences are recognized by origin recognition complex (ORC), which is a hetero hexamer complex that serves as the landing pad for proteins of the pre-replication complex. The function of each ORC subunit is still unclear. In this study, we analyze the function of subunit 4 of the human ORC complex (HsOrc4) in interaction with a plasmid bearing the *ori*-β DHFR sequence. We show that the topologically closed DHFR *ori*-β replicator contains a bubble-like structure within its AT-rich region and that it is reversibly modified in the interaction with HsOrc4. The non-canonical structure of the AT-rich region in the topologically closed *ori* sequence is recognized and changed by HsOrc4 using the energy of supercoiled DNA. These findings could help to further elucidate DNA replication and its possible association with human genetic diseases.

Keywords: HsOrc4, *ori*-β DHFR, Replicator, DNA topology, Topoisomerase I, mung bean nuclease, ORC complex, Supercoiled plasmid

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Abbreviations used: BEND – sequence-induced bend DNA; DHFR – hamster dihydrofolate reductase; DNR – dinucleotide repeat element; ORC – origin recognition complex; *ori* – origin of replication; RIP60 – RIP60 protein-binding site

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INTRODUCTION

Replication is one of the crucial steps in maintaining genome stability [1, 2]. While it is an evolutionary conserved process, the sequences responsible for its initiation, called origins of replication (*ori*), do not share conserved regions in the Metazoa: there are structural and dimension differences from species to species [3]. Despite this sequence diversity, replicators share some common elements, such as an AT-rich region [4], a CpG island [5], matrix attachment sites [6], transcription factor-binding sites [5] and dinucleotide repeats.

The hamster dihydrofolate reductase (DHFR) *ori* sequence is one of the best-characterized metazoan replication origins. It contains three primary initiation sites named *ori*-β, *ori*-β' and *ori*-γ [7]. Mutational analysis of the DHFR *ori*-β replicator reveals several sequence elements necessary for its initiation activity. These include an AT-rich element, a region of intrinsically bent DNA (BEND), a RIP60 protein-binding site (RIP60) and a dinucleotide repeat element (DNR) [4].

One of the protein complexes responsible for detection of *ori* sequences is ORC (origin recognition complex). By binding to the *ori* sequence, this hexamer protein forms a landing pad for other members of the pre-replication complex (pre-RC) [8]. After activation of the pre-RC, ORC subunits dissociate from the complex, preventing reformation of the pre-RC during one cycle of replication. Subunits 2, 3, 4 and 5 form the ORC core complex, while subunits 1 and 6 interact with the core in a dynamic way [9]. The function of each of ORC subunit is not completely understood.

In our previous study, we analyzed the interaction of subunit 4 of human ORC complex (HsOrc4) with linear DNA fragments. HsOrc4 was shown to have preferential binding to triple-stranded over single- or double-stranded DNA [10]. Furthermore, we demonstrated that human ORC4 stimulates the formation of inter- and intramolecular triplexes and creates novel DNA structures, such as homoadenine duplexes [11].

In this study, we analyzed the structures of functionally important regions of the *ori*-β replicator, which was integrated into a plasmid and used as a model to study interaction with HsOrc4. Because plasmids are topologically closed DNA molecules, they are good in vitro models that resembles the topology of local chromosomal DNA domains [12]. Every local structural change of DNA molecule affects the topology of the entire plasmid and its degree of supercoiling. Reversible topological changes to DNA made in interaction with environmental factors (such as proteins) can be "frozen" using topoisomerase I, which catalyzes the relaxation of supercoiled DNA. Therefore, we used plasmid DNA to investigate whether HsOrc4 could induce local changes in DNA structure using the free energy of negative supercoiling.

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MATERIALS AND METHODS

Expression of HsOrc4

Recombinant human Orc4 was expressed in *Escherichia coli* M15 cells and purified over metal affinity resin as described previously [13]. Briefly, protein that contained His-tag was purified under native conditions via affinity chromatography with the commercial medium TALON Metal Affinity Resin (Clontech Laboratories Inc.). To improve the yield of the active protein, the purification procedure also included treatment of bacterial lysates with DNase I. Bound proteins were eluted with elution buffer consisting of 50 mM Na-phosphate buffer (pH 7.0), 300 mM NaCl, 150 mM imidazole and Complete EDTA-free Protease Inhibitors (Roche) for 1 h at 4ºC with constant stirring. Eluate was then passed through Costar columns (Sigma Aldrich Chimie GmbH). To remove insoluble aggregates, the protein was re-purified through glycerol gradient centrifugation. Centrifugation was carried out in 10–30% glycerol gradients prepared in buffer A consisting of 20 mM HEPES (pH 7.9), 30 mM NaCl, 2 mM $ZnCl_2$, 6 mM $MgCl_2$, 0.1 mM ATP, 0.1 mM EDTA, 1 mM DTT and 0.1 mM PMSF. Gradients were centrifuged in an SW 41 Beckman Rotor at 38000 rpm for 20 h at 8ºC. The gradient fractions were collected from the bottom and visualized on SDS-PAGE gel via Commasie staining. Glycerol gradient fractions containing human Orc4 were pooled and kept at −80ºC. Determination of the protein concentration of purified HsOrc4 was done via Bradford assay using a commercial Bradford reagent (Fermentas) according to the manufacturer's instructions.

Plasmids

Plasmids pMCD and pATrep were donated by Mrs. Ellen Fanning [14]. pMCD was made by insertion of *ori*-β into pUC19 and pATrep by swapping the 344bp SphI-EcoRV fragment of pMCD for 297 bp SphI-RvuII fragment from pSV2neo (Clontech Laboratories, Inc.) [4]. Plasmids were isolated with Plasmid Prep (Qiagen), aliquoted, and stored at –20ºC for further use.

Mung bean assay

We used mung bean nuclease (Pharmacia) to detect single-stranded DNA regions. It catalyzes specific degradation of single-stranded nucleic acid fragments producing mono- and oligonucleotides with phosphorylated 5' ends (specifications for mung bean nuclease from Amersham Biosciences). In the standard reaction setup, 240 ng of pMCD plasmid was incubated for 60 min at 37ºC with 0.5 µl mung bean nuclease (45 U/µl) in a 20-µl reaction mixture (pH 7.5). Under these conditions the enzyme predominantly catalyzes plasmid relaxation and generates small amount of linearized DNA, which did not interfere with our analysis. At the end of incubation, reactions were deproteinized using proteinase K (Serva) for 15 min at 37ºC, followed by precipitation.

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The fragment of interest was excised using pair of restriction enzymes EcoNI/SphI (Fermentas) or EcoNI/BglII (Fermentas). The digestion products were deproteined, dephosphorylated using CIAP (BioLabs), and then radioactively labeled using T4 kinase (BioLabs). In order to detect only the sites of mung bean nuclease digestion, the plasmids were radioactively labeled before restriction enzymes were applied (Fig. 1B). The fragments were electrophoretically separated in 6% denaturing acrylamide gel.

Topoisomerase I assay

Topoisomerase I (Topo I, Fermentas) isolated from calf thymus was used for the detection of DNA conformational changes. Topoisomerase I catalyzes the relaxation of positive and negative supercoiled DNA via a temporary interruption of phosphodiesteric bonds in one of the complementary strands. In the standard reaction setup, 400 ng of HsOrc4 was incubated with 500 ng of pMCD in a buffer consisting of 20 mM HEPES (pH 7.9), 30 mM NaCl and 6 mM $MgCl_2$ in a 15-µl reaction mixture. After 30 min of incubation at 37ºC, the mixture was supplemented with 1.5 µl BSA (2 mg/ml; Fermentas), 9.5 µl of buffer for Topo I (2x; Fermentas) and 0.5 µl Topo I (10 U/µl; Fermentas). The relaxation reaction was performed for 30 min at 37ºC. Samples were then treated with proteinase K (Serva), precipitated with ethanol, and diluted in water solution.

Separation of topoisomers

Topoisomers were separated in 0.8% agarose gel. The first dimension electrophoresis was run for 17 h at 3.2 V/cm in TBE buffer consisting of 89 mM Tris base, 89 mM boric acid and 2 mM EDTA (pH 8). Upon completion of electrophoresis, the gel was stained in a 0.1% solution of ethidium bromide in TBE buffer or prepared for the second dimension via incubation for 30 min in 1xTBE buffer with chloroquine (1 µg/ml). Electrophoresis in the second dimension was run for 8 h orthogonally to the first dimension in chloroquine containing 1x TBE buffer. After electrophoresis, the gel was rinsed in water 3 times for 20 min with stirring and stained in ethidium bromide solution for 30 min. The stained gels were rinsed in water overnight and visualized using a CCD camera.

RESULTS

To investigate potential non-B structures within the essential *ori* elements under torsional stress, we used supercoiled pMCD plasmid, which contains a 5.8-kb fragment from DHFR *ori*-β. This fragment contains functional elements essential for the initiation activity of *ori*-β: an initiation region (IR); 60-kDa replication initiation protein-binding sites (RIP60); sequence-induced bend DNA (BEND); AT-rich sequences (AT); and a GA+CA dinucleotide repeat element (DNR) [15]. Structural analysis of the plasmid was performed by treatment with single-strand-specific mung bean endonucleases [16]. The enzyme identifies only one single-stranded region per supercoiled molecule as its first nick relaxes

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the DNA, rendering it resistant to further single-strand-specific nuclease attack. Mung bean-sensitive sites were identified by digestion with a selected combination of restriction enzymes, encompassing the fragment of interest and subsequent radioactive labeling.

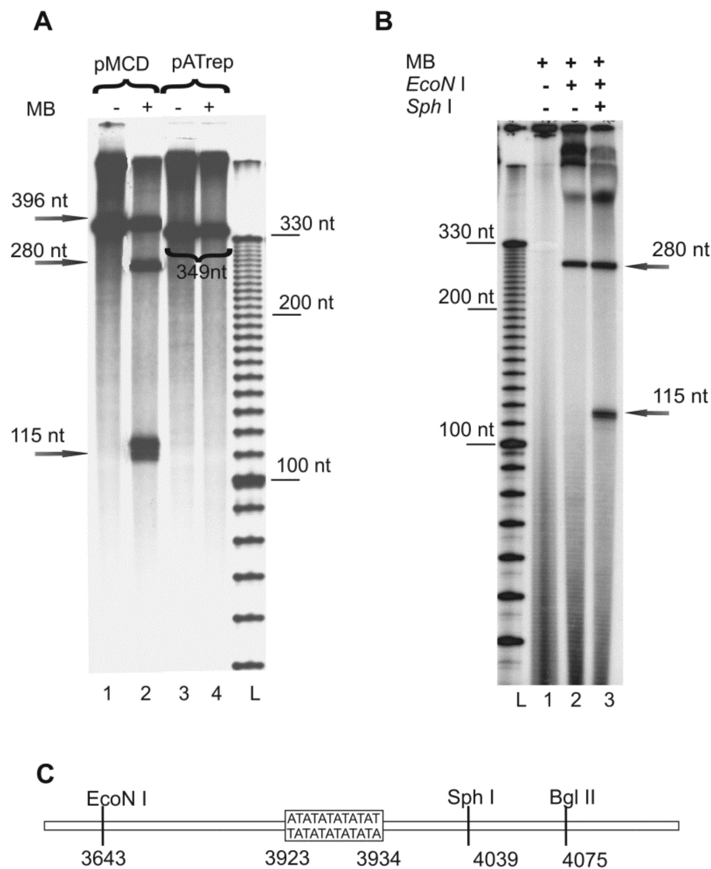

Fig. 1. Structural analysis of the AT region of the plasmids pMCD and pATrep. A – The AT regions in the plasmids pMCD (lines 1 and 2) and pATrep (line 3 and 4) were treated with mung bean nuclease and subsequently with the restriction enzymes EcoNI and SphI (2 and 4) or with only the restriction enzymes (1 and 3). B – The AT region in pMCD was treated with mung bean nuclease alone (line 1) or with mung bean nuclease and one (2) or both (3) restriction enzymes. C – The region susceptible to mung bean nuclease treatment. The position of restriction is located near the AT region of pMCD.

The plasmids (pMCD and pATrep) were first treated with mung bean nuclease and then with restriction enzymes (EcoNI and SphI). The digested fragments were radioactively end-labeled and separated in denaturing gel (Fig. 1A). The EcoNI-SphI fragment and several additional bands clustered in two groups, around 115 nt and 280 nt in length, were detected (Fig. 1A, line 2). Additional

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bands are due to the action of mung bean nuclease: they are absent in the sample not treated with that enzyme (Fig. 1A, line 1). Mung bean nuclease-treated pATrep plasmid containing the mutated AT-rich element does not show additional bands, demonstrating that they originate from the AT-rich *ori* sequence (Fig. 1A, line 4). These results together indicate the presence of an unorthodox DNA structure within the AT-rich region.

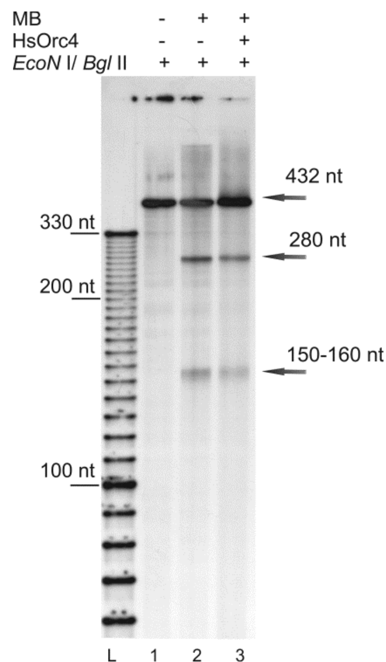

Fig. 2. Structure of the pMCD AT-rich *ori* region during interaction with HsOrc4. pMCD (line 1) treated with mung bean nuclease (line 2) in presence of HsOrc4 (line 3).

Since T4 kinase labels DNA fragments at the 5' end, labeling mung bean nuclease-treated samples before enzyme digestion enabled us to identify sites in complementary strands of the AT-rich region exposed to mung bean nuclease. pMCD was treated, radioactively end-labeled and then treated with one or both restriction enzymes. The resulting fragments were analyzed in denaturing gel (Fig. 1B). Cleavage with EcoNI produced a 280 nt band, thus locating a mung bean nuclease-sensitive site in the lower strand of the EcoNI-SphI fragment (Fig. 1B, line 2). Subsequent treatment with SphI generated a 115 nt band, indicating a mung bean nuclease-sensitive site in the upper strand of the AT-rich element (Fig. 1B, line 3). According to our results, there are several adjacent mung bean cutting sites in both complementary DNA strands spanning the region presented in Fig. 1C, at position 3923–3934 within pMCD. These results indicate that when *ori*-β is in its supercoiled state, the AT-rich region adopts a non-canonical DNA structure partly composed of single-stranded DNA.

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Structural analysis of the BEND/RIP60 and DNR element was performed, but mung bean nuclease-sensitive sites were not detected within these elements (data not shown).

Results from an earlier study revealed that recombinant HsOrc4 preferentially interacts with alternative DNA structures [10]. Therefore, we wanted to check whether HsOrc4 recognizes the unusual DNA structure detected within the AT-rich element of *ori*-β.

The binding of HsOrc4 to the AT-rich element was analyzed using the mung bean assay. Following initial plasmid incubation with the protein, the reaction mixtures were treated with mung bean nuclease, digested with EcoNI-BglII, radiolabeled, and analyzed on sequencing gels (Fig. 2). Within the 432-bp long EcoNI-BglII fragment encompassing the AT-rich element (Fig. 2, line 1), mung bean nuclease digestion produces two additional bands at positions 280 nt and 150–160 nt (Fig. 2, line 2). Former restriction mapping confirmed that mung bean nuclease cutting sites in the EcoNI-BglII fragment correspond to the same sites in EcoNI-SphI fragment (data not shown). The binding of HsOrc4 to pMCD results in modest protection of mung bean nuclease-sensitive sites in the AT-rich region of *ori*-β (Fig. 2, line 3). Possible interpretations of this outcome are that HsOrc4 directly binds and protects mung bean nuclease cutting sites; that its interaction with pMCD changes the DNA structure within the AT-rich region, abolishing the single-stranded regions; or that both occur.

To address the question of whether HsOrc4 could induce local changes in DNA structure using the free energy of negative supercoiling, we carried out topoisomerase relaxation assays in the presence of HsOrc4. In this assay, pMCD was incubated with HsOrc4 to allow complex formation, and then treated with topoisomerase to relax unconstrained regions of the plasmid. Following deproteinization, plasmid DNA was analyzed via 2D-gel electrophoresis in the presence of chloroquine in the second dimension. As presented in Fig. 3, incubation of pMCD with HsOrc4 followed by treatment with topoisomerase I resulted in a shift of the topoisomer distribution toward lower mobility relative to topoisomers generated without HsOrc4 (first dimension, Fig. 3B and A, respectively). Topoisomer separation in the second dimension revealed that the shift to lower mobility was due to the introduction of positive supercoiling in pMCD (Fig. 3A and B).

The next step was to check whether conformational changes in supercoiled pMCD induced by HsOrc4 affect the *ori*-β AT-rich region. For that purpose, topoisomers generated in the topoisomerase assay in the presence of HsOrc4 were deproteinized and subjected to mung bean nuclease treatment. Following digestion with EcoNI-SphI, samples were radiolabeled and analyzed on denaturating PAGE. The protein presence during the topoisomerase reaction generated topoisomers without mung bean nuclease sensitivity sites within the AT-rich region (Fig. 4, line 4), showing that conformational changes in supercoiled plasmid pMCD mediated by the HsOrc4 protein target at least one region critical for the initiation activity of *ori*-β.

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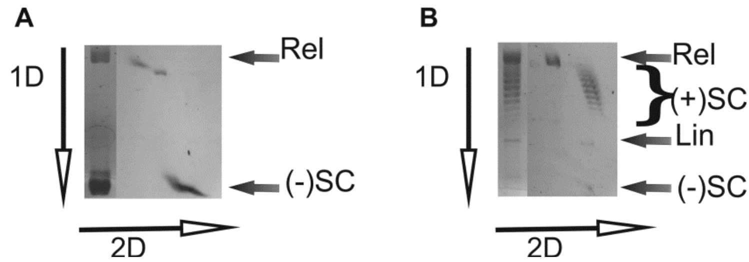

Fig. 3. Topoisomerase assay of pMCD via 2D electrophoresis without (A) and with (B) HsOrc4. 1D/2 D – first/second dimension, Rel – relaxed form of plasmid, (+/-)SC – positive/negative supercoiled forms of plasmid, Lin – linearised plasmid.

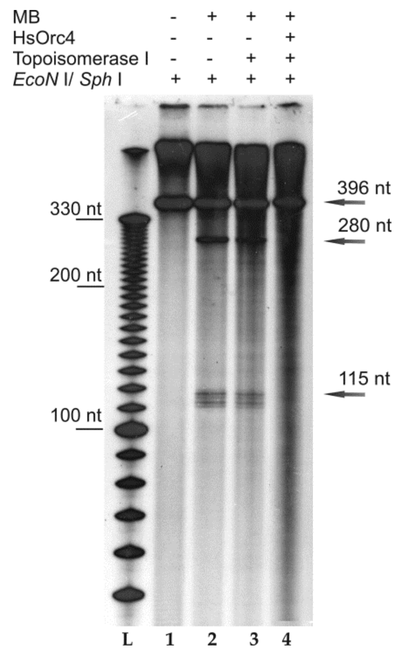

Fig. 4. Structural analysis of the pMCD AT-rich *ori* region in topoisomers generated in the presence of HsOrc4. Line 1 – EcoNI/SphI digestion of pMCD (line 1). Remaining lines – pMCD (line 2) was incubated with topoisomerase I (line 3) in presence of HsOrc4 (line 4), deproteinized, and treated with mung bean nuclease.

DISCUSSION

The formation and activation of the pre-replication complex is crucial step in the initiation of replication. One of the protein complexes involved in this process is the ORC hexamer, but the exact function of each ORC subunit is not completely understood [8, 17, 18]. Studies of functional characterization have focused on some of the ORC subunits. These studies showed that Orc2 is responsible for keeping the ORC complex bound to DNA [19, 20]. Orc6 dynamically associates

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with the core complex, but is also involved in interaction with other proteins [21–24]. The aim of our study was to reveal function of Orc4 subunit of human ORC complex (HsOrc4).

In our previous studies, we analyzed the interaction of HsOrc4 with linear DNA fragments and showed that this subunit of the human ORC complex binds to AT-rich regions and mediates the formation of non-canonical structures [10, 11, 13]. To study interaction between HsOrc4 and supercoiled origin DNA, we introduced circular DNA into our experiments. Local structural modifications were investigated using the modified mung bean nuclease assay.

To analyze potential structural changes induced by HsOrc4, we chose the DHFR *ori*-β sequence. The *ori*-β sequence of DHFR is one of the best-characterized *ori* sequences. It has several elements which were shown to be crucial for its replication activity [4]. Our structural analysis of this region showed the presence of a non-canonical structure in the AT-rich region spanning around position 3923 in pMCD. The *ori*-β AT-rich element is one of several sequence elements necessary for ectopic *ori* activity. It can be functionally substituted by an AT-rich region from the human lamin B2 initiation region that differs in length and sequence [4]. Mung bean nuclease cuts both complementary strands, indicating a possible bubble-like structure (Fig. 5A), but more complex structures could not be excluded. One chain of DNA could form triple helix strand with distant region in the plasmid [25], thus leaving one DNA strand susceptible to mung bean nuclease activity (Fig. 5B).

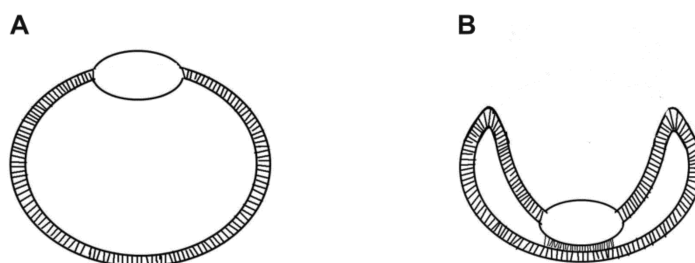

Fig. 5. Potential structures of the AT region in pMCD. A – The bubble-like structure where both DNA strands are susceptible to mung bean nuclease activity. B – The triple helix strand with a distant region, with only one DNA strand susceptible to mung bean nuclease activity.

A similar model was suggested in previous studies, based on experiments on linear fragments or on plasmid studies [26, 27]. Since the AT region melts easily and is capable of forming non-canonical structures, the triple helix is not so unexpected. Our experiments have shown that HsOrc4 protects the AT region in topologically closed origin DNA (Fig. 2). This is in accordance with previous results showing that HsOrc4 preferentially binds non-canonical DNA structures [10].

To further investigate the interaction of HsOrc4 with *ori*, we used the topoisomerase I assay to "freeze" potential topological changes introduced by the protein (Fig. 3). The different pattern of topoisomers generated in the

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presence of HsOrc4 indicates the introduction of topological changes. Using mung bean nuclease in the analysis of newly formed topoisomers (Fig. 4), we concluded that, in the presence of topoisomerase I, HsOrc4 abolishes the alternative structure of the AT-rich region. According to our results, at least one functionally important domain of DNA replication origin gets remodeled upon direct interaction with the initiation protein HsOrc4. These findings could have an impact on our understanding of the function of each ORC subunit and of the whole ORC complex in the process of initiation of replication.

Proper marking of *ori* locations and formation of pre-RC is fundamental to establishing genome integrity. Together with subunits Orc2, Orc3 and Orc5, Orc4 is a part of a core complex that is dynamically connected with the subunits Orc1 and Orc6. ORC disassembly through S phase is regulated by phosphorylation of Orc2, a crucial step in the dissociation of the core complex from DNA [17, 19]. During the cell cycle, the concentration of Orc4 is constant, but dynamic interaction with DNA is proposed [28].

The protein HsOrc4 is essential for the organism, with very few viable ORC mutations detected. Mutations in the Orc4 subunit are detected in human disorders [29–31], but the exact function of this subunit is as-yet unknown. We suggest that Orc4 plays a crucial role in ORC–DNA interaction. Remodeling of DNA by ORC subunits has previously been proposed. Archaeal Orc1 was shown to locally remodel duplex DNA using ATP [1]. In *Schizosaccharomyces pombe*, Orc4 alters DNA structure by binding to the origin of replication and is essential for DNA binding of the ORC complex [32, 33]. In human ORC, the Orc4 subunit can play a similar role. Since HsOrc4 can recognize and form non-canonical DNA structures [10, 11], this could be the first step of altering the *ori* sequence structure and function of ORC. Our results on plasmid DNA further confirm this model of interaction.

CONCLUSION

We show that the functional AT-rich domain of *ori*-β DHFR replicator has a non-B structure and is altered in its interaction with HsOrc4. These findings indicate that HsOrc4 could have a fundamental function in the process of initiation of replication by remodeling the structure of origin of replication using the energy of supercoiled DNA.

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REFERENCES

1. Dueber, E.L.C., Corn, J.E., Bell, S.D. and Berger, J.M. Replication origin recognition and deformation by a heterodimeric archaeal Orc1 complex. **Science** 317 (2007) 1210–1213.
2. Aladjem, M.I. and Fanning, E. The replicon revisited: an old model learns new tricks in metazoan chromosomes. **EMBO Rep.** 5 (2004) 686–691.
3. Aladjem, M.I. Replication in context: dynamic regulation of DNA replication patterns in metazoans. **Nat. Rev. Genet.** 8 (2007) 588–600.
4. Altman, A.L. and Fanning, E. Defined sequence modules and an architectural element cooperate to promote initiation at an ectopic mammalian chromosomal replication origin. **Mol. Cell. Biol.** 24 (2004) 4138–4150.
5. Delgado, S., Gomez, M., Bird, A. and Antequera, F. Initiation of DNA replication at CpG islands in mammalian chromosomes. **EMBO J.** 17 (1998) 2426–2435.
6. Shimizu, N., Miura, Y., Sakamoto, Y. and Tsutsui, K. Plasmids with a mammalian replication origin and a matrix attachment region initiate the event similar to gene amplification. **Cancer Res.** 61 (2001) 6987–6990.
7. Kobayashi, T., Rein, T. and DePamphilis, M.L. Identification of primary initiation sites for DNA replication in the hamster dihydrofolate reductase gene initiation zone. **Mol. Cell Biol.** 18 (1998) 3266–3277.
8. Vashee, S., Simancek, P., Challberg, M.D. and Kelly, T.J. Assembly of the human origin recognition complex. **J. Biol. Chem.** 276 (2001) 26666–26673.
9. DePamphilis, M.L. Cell cycle dependent regulation of the origin recognition complex. **Cell Cycle** 4 (2005) 70–79.
10. Kusic, J., Tomic, B., Divac, A. and Kojic, S. Human initiation protein Orc4 prefers triple stranded DNA. **Mol. Biol. Rep.** 37 (2010) 2317–2322.
11. Stefanovic, D., Kusic, J., Divac, A. and Tomic, B. Formation of noncanonical DNA structures mediated by human ORC4, a protein component of the origin recognition complex. **Biochemistry** 47 (2008) 8760–8767.
12. Bates, D.A. and Maxwell, A. **DNA Topology**, United States: Oxford University Press, 2005.
13. Stefanovic, D., Stanojcic, S., Vindigni, A., Ochem, A. and Falaschi, A. In vitro protein-DNA interactions at the human lamin B2 replication origin. **J. Biol. Chem.** 278 (2003) 42737–42743.
14. Altman, A.L. and Fanning, E. The Chinese hamster dihydrofolate reductase replication origin beta is active at multiple ectopic chromosomal locations and requires specific DNA sequence elements for activity. **Mol. Cell. Biol.** 21 (2001) 1098–1110.
15. Gray, S.J., Liu, G., Altman, A.L., Small, L.E. and Fanning, E. Discrete functional elements required for initiation activity of the Chinese hamster

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dihydrofolate reductase origin beta at ectopic chromosomal sites. **Exp. Cell Res.** 313 (2007) 109–120.
16. Baumann, U. and Chang, S. Asymmetric structure of five and six membered DNA hairpin loops. **Mol. Biol. Rep.** 22 (1996) 25–31.
17. Siddiqui, K. and Stillman, B. ATP-dependent assembly of the human origin recognition complex. **J. Biol. Chem.** 282 (2007) 32370–32383.
18. Méndez, J. and Stillman, B. Perpetuating the double helix: molecular machines at eukaryotic DNA replication origins. **Bioessays** 25 (2003) 1158–1167.
19. Lee, K.Y., Bae, J.S., Yoon, S. and Hwang, D.S. Dephosphorylation of Orc2 by protein phosphatase 1 promotes the binding of the origin recognition complex to chromatin. **Biochem. Biophys. Res. Commun.** 448 (2014) 385–389.
20. Lee, K.Y., Bang, S.W., Yoon, S.W., Lee, S.H., Yoon, J.B. and Hwang, D.S. Phosphorylation of ORC2 Protein dissociates origin recognition complex from chromatin and replication origins. **J. Biol. Chem.** 287 (2012) 11891–11898.
21. Dhar, S.K. and Dutta, A. Identification and characterization of the human ORC6 homolog. **J. Biol. Chem.** 275 (2000) 34983–34988.
22. Liu, S.X., Balasov, M., Wang, H.F., Wu, L.J., Chesnokov, I.N. and Liu, Y.F. Structural analysis of human Orc6 protein reveals a homology with transcription factor TFIIB. **PNAS** 108 (2011) 7373–7378.
23. Prasanth, S.G., Prasanth, K.V. and Stillman, B. Orc6 involved in DNA replication, chromosome segregation and cytokinesis. **Science** 297 (2002) 1026–1031.
24. Thomae, A.W., Baltin, J., Pich, D., Deutsch, M.J., Ravasz, M., Zeller, K., Gossen, M., Hammerschmidt, W. and Schepers, A. Different roles of the human Orc6 protein in the replication initiation process. **Cell. Mol. Life Sci.** 68 (2011) 3741–3756.
25. Shimizu, M., Hanvey, J.C. and Wells, R.D. Intramolecular DNA triplexes in supercoiled plasmids. I. Effect of loop size on formation and stability. **J. Biol. Chem.** 264 (1989) 5944–5949.
26. Kusic, J., Kojic, S., Divac, A. and Stefanovic, D. Noncanonical DNA elements in the lamin B2 origin of DNA replication. **J. Biol. Chem.** 280 (2005) 9848–9854.
27. Bianchi, A., Wells, R.D., Heintz, N.H. and Caddle, M.S. Sequences near the origin of replication of the DHFR locus of Chinese hamster ovary cells adopt left-handed Z-DNA and triplex structures. **J. Biol. Chem.** 265 (1990) 21789–21796.
28. McNairn, A.J., Okuno, Y., Misteli, T. and Gilbert, D.M. Chinese hamster ORC subunits dynamically associate with chromatin throughout the cell-cycle. **Exp. Cell Res.** 308 (2005) 345–356.
29. Guernsey, D.L., Matsuoka, M., Jiang, H.Y., Evans, S., Macgillivray, C., Nightingale, M., Perry, S., Ferguson, M., LeBlanc, M., Paquette, J., Patry, L., Rideout, A.L., Thomas, A., Orr, A., McMaster, C.R., Michaud, J.L., Deal, C., Langlois, S., Superneau, D.W., Parkash, S., Ludman, M., Skidmore, D.L.

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and Samuels, M.E. Mutations in origin recognition complex gene ORC4 cause Meier-Gorlin syndrome. **Nat. Genet.** 43 (2011) 360–U157.

30. Radojkovic, M., Ristic, S., Divac, A., Tomic, B., Nestorovic, A. and Radojkovic, D. Novel ORC4L gene mutation in B-cell lymphoproliferative disorders. **Am. J. Med. Sci.** 338 (2009) 527–529.
31. Shen, Z. The origin recognition complex in human diseases. **Biosci. Rep.** 33 (2013) e00044.
32. Kong, D.C. and DePamphilis, M.L. Site-specific DNA binding of the Schizosaccharomyces pombe origin recognition complex is determined by the Orc4 subunit. **Mol. Cell. Biol.** 21 (2001) 8095–8103.
33. Gaczynska, M., Osmulski, P.A., Jiang, Y., Lee, J.K., Bermudez, V. and Hurwitz, J. Atomic force microscopic analysis of the binding of the Schizosaccharomyces pombe origin recognition complex and the spOrc4 protein with origin DNA. **PNAS** 101 (2004) 17952–17957.

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