ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 4, pp. 346-353. © Pleiades Publishing, Ltd., 2012.
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Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM11-294, February 19, 2012.

Influence of Centrin 2 on the Interaction of Nucleotide Excision Repair Factors with Damaged DNA

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Received November 1, 2011
Revision received December 13, 2011

Abstract—We have examined the influence of centrin 2 (Cen2) on the interaction of nucleotide excision repair factors (XPC-HR23b, RPA, and XPA) with 48-mer DNA duplexes bearing the dUMP derivative 5-{3-[6-(carboxyamidofluoresceinyl)amidocapromoyl]allyl}-2′-deoxyuridine-5′-monophosphate. The fluorescein residue linked to the nucleotide base imitates a bulky lesion of DNA. Cen2 stimulated the binding and increased the yield of DNA adducts with XPC–HR23b, a protein recognizing bulky damages in DNA. Stimulation of the binding was most pronounced in the presence of Mg^{2+} and demonstrated a bell-shaped dependence on Cen2 concentration. The addition of Cen2 changed the stoichiometry of RPA–DNA complexes and diminished the yield of RPA–DNA covalent crosslinks. We have shown that Cen2 influences the binding of RPA and XPA with DNA, which results in formation of additional DNA–protein complexes possibly including Cen2. We have also found some evidence of direct contacts between Cen2 and DNA. These results in concert with the literature data suggest that Cen2 can be a regulatory element in the nucleotide excision repair system.

DOI: 10.1134/S0006297912040050

Key words: nucleotide excision repair, centrin 2, photoaffinity modification, binding of repair protein factors with DNA

Nucleotide excision repair (NER) removes from DNA various damages caused by environmental factors such as UV and chemical carcinogens or by chemotherapeutic agents. NER involves more than 30 polypeptides [1], i.e. it is a complicated supramolecular machine responsible for DNA repair with participation of an ensemble of enzymes and other proteins. Later, additional proteins contributing to this process were discovered, and some of these factors seem to play a regulatory role. Thus, these proteins include centrin 2 (Cen2), which is a component of a complex with XPC, a factor responsible for the primary recognition of DNA lesions during NER [2]. In the cell XPC is in a complex with two proteins, HR23b and Cen2. These small subunits do not have DNA-binding activity but stimulate the binding with DNA of the major subunit of the complex (XPC) and thereby increase the efficiency of NER *in vitro* and *in vivo* [3, 4]. In contrast to HR23b, Cen2 is not absolutely required for NER *in vitro*, but it seems to regulate XPC functions as a sensor of lesions. In addition to stimulation of XPC binding with DNA, HR23b and Cen2 cooperatively stabilize XPC structure [5]. Moreover, both proteins perform some regulatory functions including those associated with protein degradation by the 26S proteasome [6-8]. Cen2 is supposed to modulate the interaction of XPC with the TFIIH complex (which was discovered as transcription factor IIH) containing helicases responsible for DNA unwinding around the lesion and, thus, for control of the involvement of TFIIH in NER [4].

Human Cen2 is a small (19.7 kDa) acidic (p*I* 4.91) Ca^{2+}-binding protein belonging to the highly conserved calmodulin superfamily. Cen2 consists of two domains

Abbreviations: Cen2, centrin 2; Flu-dUMP, 5-{3-[6-(carboxyamidofluoresceinyl)amidocapromoyl]allyl}-2′-deoxyuridine-5′-monophosphate; 5I-dUMP, 5-iodo-2′-deoxyuridine-5′-monophosphate; NER, nucleotide excision repair; RPA, replicative protein A; XPA, *Xeroderma pigmentosum* factor of complementation group A; XPC–HR23b, *Xeroderma pigmentosum* factor of complementation group C complexed with yeast protein Rad23 homolog.

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# Deceased.

containing structural "helix–loop–helix" ("EF-hand") motifs. The Ca^{2+}-binding site is located inside the "loop" motif of the C-terminal domain of Cen2, and this domain is involved in the interaction with XPC (region 847-863 a.a.) [9]. This region of XPC belongs to the C-terminal fragment (815-940 a.a.), which is highly disordered in the native state. Specific binding with Cen2 induces a local folding of the 17-mer peptide of XPC (847-863 a.a.), namely, formation of α-helix [10] and consequent stabilization of the XPC structure. In the XPC–Cen2 complex, intermolecular interface is formed by hydrophobic amino acid residues of the XPC α-helix and the nonpolar surface of the C-terminal domain of Cen2 [11-13].

The cellular pool of Cen2 is *in vivo* distributed between the cytoplasm and cell nucleus. In the cytoplasm Cen2 is bound with the microtubule organizing centers (centrosomes in animal cells and spindle pole bodies in yeast) and is required for the normal centriole duplication. The subcellular distribution of Cen2 between the nucleus and cytoplasm is regulated by the SUMOylation. Modification of Cen2 by SUMO2/3 results in its accumulation in the cytoplasm [13]. Only 10% of the total amount of Cen2 is included in the XPC–HR23b–Cen2 complex, and in this complex Cen2 stabilizes the structure of XPC and increases its affinity for DNA [4, 14]. The Cen2 functions, other than its interaction with centrosomes and XPC, are still unclear. Moreover, up to now there is no clear comprehension of the mechanism of the stimulatory effect of Cen2 on NER.

In the present work we studied the influence of Cen2 on the interaction of the key factors of NER (XPC–HR23b, XPA, and RPA) with damaged DNA.

MATERIALS AND METHODS

Materials. The following materials and reagents were used in the work: [γ-^{32}P]ATP (3000 Ci/mmol) produced by the Laboratory of Radiochemistry of the Institute of Chemical Biology and Fundamental Medicine (Siberian Branch of the Russian Academy of Sciences), T4 phage polynucleotide kinase from Biosan (Russia), stained markers of protein molecular weights from BioRad (USA), reagents for electrophoresis and components of buffers were from Sigma (USA) or domestic products of special purity. Oligonucleotides containing a photoactive analog 5I-dUMP or a fluorescein derivative of dUMP were synthesized by V. N. Silnikov (Nanotech-C, Russia). Structures of oligonucleotides and nucleotide analogs are presented in Fig. 1.

a

DNA duplex for experiments on binding

```
5'-p*-CTAT GGCG AGGC GATT AAGT TGGG CAAC GUCA GGGT CTTC CGAA CGAC-3′
   3′-GATA CCGC TCCG CTAA TTCA ACCC GFTG CAGT CCCA GAAG GCTT GCTG-5′
```

DNA duplex for photoaffinity modification

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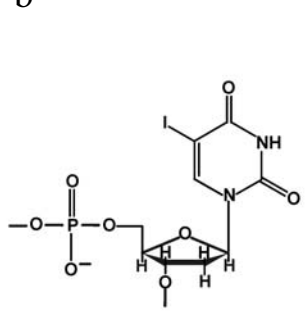
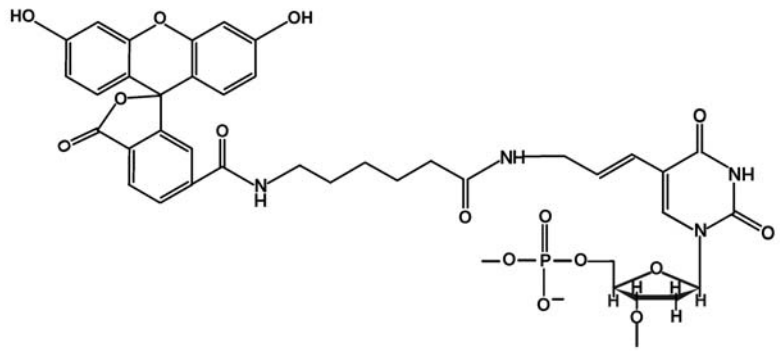
5′-p*-CTAT GGCG AGGC GATT AAGT TGGG TAAC GTCA GGGT CTTC CGAA CGAC-3′
   3′-GATA CCGC TCCG CTAA TTCA ACCC AIFG CAGT CCCA GAAG GCTT GCTG-5′
```

b



I: 5I-dUMP

5-iodo-2′-deoxyuridine-5′-monophosphate



F: Flu-dUMP

5-{3-[6-(carboxyamidofluoresceinyl)amido-capromoyl]allyl}-2′-deoxyuridine-5′-monophosphate

Fig. 1. a) Sequences of the studied DNA duplexes. b) Structures of nucleotide analogs.

Protein preparations. Recombinant human Cen2 was expressed in *E. coli* and isolated as described in [12]. To prepare a stock solution, a sample of lyophilized protein was dissolved in buffer containing 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, and 1 mM $CaCl_2$. Further dilution to the desired concentration was performed in PB1× buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 1 mM DTT). Recombinant human RPA was isolated as described in [15]. Recombinant human XPA containing a polyhistidine fragment on the N-terminus was isolated as described in [16]. Recombinant heterodimer XPC–HR23b (Flag-XPC and 6HisTag-HR23B) was prepared as described in [4] with a slight modification. Recombinant DNAs encoding subunits of the heterodimer were kindly provided by K. Sugasawa (Kobe University, Japan).

Preparation of 5′-^{32}P-labeled DNA duplexes. The 5I-dUMP-containing oligonucleotides were 5′-^{32}P-labeled using the phage T4 polynucleotide kinase according to [17]. Labeled oligonucleotides were purified by electrophoresis in denaturing polyacrylamide gel with subsequent elution from the gel [18]. To prepare DNA duplexes, 5′-^{32}P-labeled oligonucleotide was annealed with a complementary oligonucleotide at the ratio of 1 : 1 by heating a solution to 95°C for 5 min followed by slow cooling to 75°C, maintaining for 15 min at this temperature, and further slow cooling to room temperature. The hybridization degree was monitored by electrophoresis in 10% polyacrylamide gel (acrylamide/*bis*-acrylamide = 40 : 1). As electrode buffer, TBE buffer consisting of 50 mM Tris-HCl, 50 mM H_3BO_3, and 1 mM EDTA (pH 8.3) was used.

Binding of proteins with DNA was analyzed by the gel retardation technique. The reaction was performed in 10 μl of reaction mixture containing buffer PB1×, 2 mM $MgCl_2$ (if the reaction was performed in the presence of Mg^{2+}), 0.6 mg/ml BSA, 1 nM 5′-^{32}P-labeled DNA, and different concentrations of the studied proteins. The proteins were preincubated with DNA at 37°C for 20 min. Then the specimens were supplemented with the loading buffer (1/5 volume) consisting of 20% glycerol, 0.015% Bromophenol Blue, and buffer PB1× (the loading buffer temperature was also 37°C). The reaction mixtures were subjected to previously cooled gels (4°C). Electrophoresis was performed in nondenaturing 5% polyacrylamide gel (acrylamide/*bis*-acrylamide = 60 : 1) at the constant potential of 17 V/cm and 4°C. TBE was used as the electrode buffer. Positions of the radiolabeled oligonucleotide and protein–nucleic complexes were determined by radioautography using a Molecular imager FX Pro+ device (BioRad).

Anisotropy of fluorescence was measured using a POLARstar Optima multifunctional microplate reader (BMG Labtech, Germany). A fluorescein residue inside the strand was used as a fluorophore too (excitation at 495 nm, emission at 520 nm). A decrease in anisotropy indicated changes in the level of XPC–HR23b binding. Reaction mixtures (100 μl) contained buffer PB1×, 0.6 mg/ml BSA, increasing concentrations of Mg^{2+}, 20 nM XPC–HR23b, and 3 nM damaged DNA duplex. The protein binding with DNA and the anisotropy measurement were performed at 37°C.

Photoaffinity modification of proteins by DNA structures containing 5I-dUMP residues and a bulky lesion was performed in a reaction mixture (10 μl) containing buffer PB1×, 0.6 mg/ml BSA, 10 nM 5′-^{32}P-labeled photoactive DNA duplex or a mixture of proteins at studied concentrations. The mixtures were incubated for 20 min at 37°C and exposed to UV light at the wavelength of 312 nm and power of 5 mJ/(cm^2·sec) for 60 min in ice bath. UV irradiation was performed using a Bio-link BLX-312 cross-linker (Vilber Lourmat, France). The reaction was terminated by adding 1/5 volume of "stop"-buffer (5% SDS, 5% 2-mercaptoethanol, 0.3 M Tris-HCl, pH 7.8, 50% glycerol, 0.005% Bromophenol Blue). The modification products were separated by Laemmli electrophoresis [19] in 10% SDS-polyacrylamide gel (acrylamide/*bis*-acrylamide = 60 : 1) with subsequent radioautography.

RESULTS AND DISCUSSION

It is known that Mg^{2+} decreases the efficiency of XPC–HR23b binding with DNA [16]; therefore, we initially analyzed the influence of Mg^{2+} on the binding of XPC–HR23b with damaged DNA by fluorescence titration. We used the fluorescein residue introduced into DNA as a bulky lesion as a reporter group too. The concentration of Mg^{2+} in the reaction mixture was varied from 0.3 to 20 mM. The fluorescence intensity remained constant during the experiments, and changes were detected in the signal of fluorescence anisotropy. On increase in the Mg^{2+} concentration in the reaction mixture, the fluorescence anisotropy decreased (Fig. 2). This indicated a decrease in the amount of damaged DNA in the complex with XPC–HR23b. In the presence of Mg^{2+} concentrations from 0 to 1 mM, the anisotropy signal decreased insignificantly (~10-12%). However, in the presence of 2 mM Mg^{2+} the signal was decreased already by 30%. Note that Mg^{2+} concentrations from 0.3 to 2 mM correspond to the concentration of unbound Mg^{2+} in the cell nucleus [20]. Further increase in the Mg^{2+} concentration to 20 mM led to decrease in the anisotropy signal to 20% of its initial level. Inhibition of the complex formation was shown earlier in the presence of 3-10 mM Mg^{2+} for some other DNA-binding proteins [20]. However, a decrease in the affinity of the protein for DNA is accompanied by an increase in the specificity of their binding even at 2 mM Mg^{2+} [16]. Based on our findings and the literature data, in further experiments we used 2 mM Mg^{2+} as the concentration corresponding to opti-

mal conditions providing for an increase in the specificity of binding with damaged DNA, along with an insignificant decrease in the affinity of XPC–HR23b for DNA.

In the cell only a small part of Cen2 is associated with XPC–HR23b, whereas the major part of this protein is located in the cytoplasm [14] and the migration of the protein from the nucleus into the cytoplasm is strictly regulated [13]. To elucidate whether a definite ratio between concentrations of the nuclear centrin and XPC is maintained, we analyzed the influence of various Cen2 concentrations on the interaction of the XPC–HR23b complex with damaged DNA using methods of gel retardation and photoaffinity modification. The binding of XPC–HR23b with DNA was analyzed in both the absence and presence of Mg^{2+} (2 mM). Figure 3a shows that centrin stimulates the binding of XPC–HR23b with DNA independently of the presence of Mg^{2+}. The stimulatory effect is bell-shaped (Fig. 3a, bottom panel). In the absence of Mg^{2+} (Fig. 3a, *1-9*) the complexing levels without centrin (Fig. 3a, *1*) and at the maximum stimulation (Fig. 3a, *6* and *7*) are different by about 4-fold. In the presence of Mg^{2+} (Fig. 3a, *10-17*) the stimulatory effect is manifested more clearly, and the complexing without Cen2 (Fig. 3a, *10*) and at the maximum stimulation (Fig. 3a, *14* and *15*) is different by an order of magnitude. In the absence of Mg^{2+} and at 50-fold excess of Cen2 relative to XPC–HR23b, the binding of XPC–HR23b with DNA returns to the initial level (Fig. 3a, compare *9* and *1*). However, even at a high concentration of Cen2 in the presence of Mg^{2+} the complexing level of XPC–HR23b with DNA is 2-3 times higher than the initial one (Fig. 3a, *17*). Note that Mg^{2+} decreases efficiency of the binding of XPC–HR23b with DNA as show earlier [16]; however, in the presence of Cen2 the negative effect of Mg^{2+} is abolished, and the complexing levels in both the presence and absence of Mg^{2+} become equal, at least at a small excess of Cen2 relative to XPC–HR23b.

According to the literature data, at equimolar ratio of XPC and Cen2 the latter increases 20-fold the affinity of XPC for DNA containing a thymine dimer (pyrimidine-(6-4)-pyrimidone ((6-4)-photoproduct) as a lesion [4]. A higher stimulation obtained in [4] at equimolar ratio of Cen2 and XPC could be caused by either the other type of DNA lesion or the higher concentration of Mg^{2+} (5 mM) used in the study, as well as by different activities of proteins used. Moreover, in the Na^+-containing buffer used in the referred study Cen2 binds Ca^{2+} with higher affinity than in K^+-containing buffer used in our experiments [21], and this also can influence the activity of Cen2.

Stimulatory effect of centrin was also observed in experiments on modification of XPC–HR23b by damaged DNA containing a photoactive residue 5I-dUMP near the lesion (Fig. 3b). One can see that the level of XPC modification increases in the presence of Cen2. The formation of covalent XPC–DNA adducts depends on the Cen2 concentration similarly to the dependence of the binding of XPC–HR23b with DNA on the Cen2 concentration (compare curves in Figs. 3a (in absence of Mg^{2+}) and 3b). But the absolute stimulation in the case of photocrosslinks was approximately twofold lower, which could be due to specific features of the affinity modification method. The level of formation of DNA–protein adducts can depend on conformational changes in the XPC structure influencing the mutual orientation of amino acid residues of the protein and of the photoreagent in DNA.

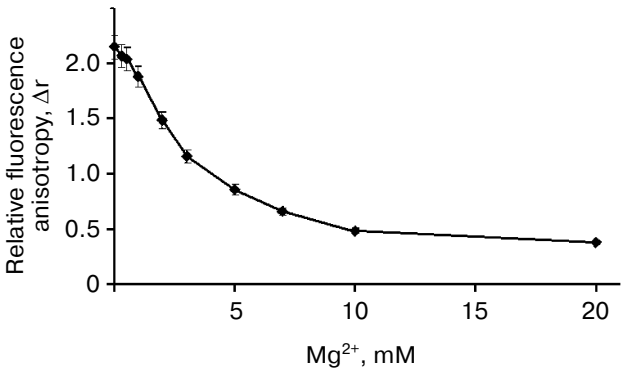


Fig. 2. Changes in fluorescence anisotropy during titration by Mg^{2+} of reaction mixture containing XPC–HR23b and 3 nM DNA duplex bearing a bulky lesion. The reaction mixtures (100 μl) contained PB1× buffer, 0.6 mg/ml BSA, increasing concentrations of Mg^{2+}, 20 nM XPC–HR23b, and 3 nM DNA duplex. The binding of the protein with DNA and measurement of fluorescence anisotropy were performed at 37°C. The plot was constructed using results of three experiments.

A decrease or complete absence of stimulatory effect with the increase of the Cen2 concentration can be caused by interaction of Cen2 excess with DNA resulting in inhibition of the binding of XPC to DNA. But using the gel retardation method, centrin–DNA complexes were not detected (data not presented). Such complexes seem to be insufficiently stable and can dissociate during electrophoresis.

However, the hypothesis about the interaction of Cen2 with DNA was confirmed by studies on the influence of the order of component addition into the reaction mixture on the binding of XPC–HR23b with DNA (Fig. 4). The Cen2 concentration was chosen to provide for the maximum stimulation of the binding. In the previous experiments the complex between XPC–HR23b and Cen2 was formed first, and then DNA was added. This order of component addition corresponds to experiments shown in Fig. 4 (*1* and *2*). In two variants of the addition, the levels of protein binding with DNA were the same. In the first case the complex of XPC–HR23b with DNA was prepared first and then Cen2 was added (Fig. 4, *3* and *4*). In the other case all components were introduced simultaneously (Fig. 4, *7* and *8*). On incubation of DNA with Cen2 and the subsequent addition of XPC–HR23b (Fig. 4, *5* and *6*) the binding level was two-three times lower than under the other conditions. This finding supported

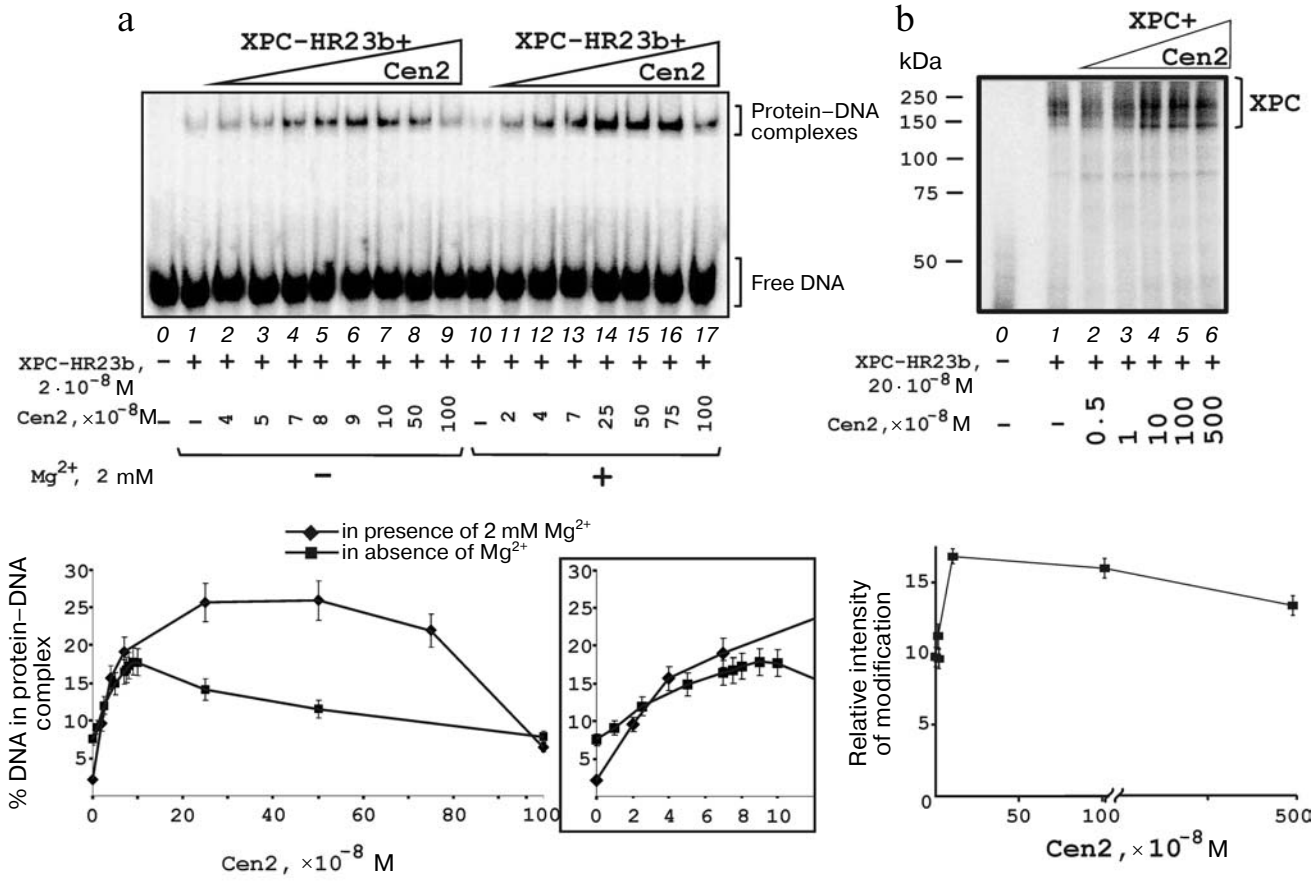


Fig. 3. Influence of Cen2 on the interaction of XPC–HR23b with DNA bearing a bulky lesion. a) Influence of Cen2 on the binding of XPC–HR23b with a damaged DNA in the absence of Mg^{2+} (lanes *0-9*) and in the presence of 2 mM Mg^{2+} (lanes *10-17*). The reaction mixtures (10 μl) contained PB1× buffer, 0.6 mg/ml BSA, 2 mM Mg^{2+} (if the reaction was performed in the presence of Mg^{2+}), 20 nM XPC–HR23b, increasing concentrations of Cen2, and 1 nM 5′-^{32}P-labeled damaged DNA duplex. Radioautograph of 5% polyacrylamide gel (acrylamide/*bis*-acrylamide = 60 : 1). b) Influence of Cen2 on the photoaffinity modification of XPC–HR23b. The reaction mixtures (10 μl) contained PB1× buffer, 0.6 mg/ml BSA, $20 \cdot 10^{-8}$ M XPC–HR23b, increasing concentrations of Cen2, and 10 nM 5′-^{32}P-labeled DNA. Radioautograph of 10% SDS-polyacrylamide gel (acrylamide/*bis*-acrylamide = 60 : 1). The plots were constructed using results of three experiments.

the existence of interactions between Cen2 and DNA, which can result in abolishment of stimulation by Cen2 of the XPC–HR23b binding with DNA at high concentrations of centrin. Despite the absence in the literature of information about protein–nucleic contacts between Cen2 and DNA, some proteins are known that similarly to centrin are members of the structural superfamily of calmodulin and can interact with specific sequences in DNA [22].

Proteins RPA and XPA are indispensable components of the NER system. These proteins are components of a complex that is formed on a damaged DNA during different stages of the repair. Although these proteins are not considered now as factors responsible for the primary recognition of damage, their involvement during this stage of the repair is very likely because there are data on their influence on the interaction of XPC–HR23b with damaged DNA [16, 23]. Therefore, it was very important to analyze the influence of Cen2 on the interaction of RPA and XPA with damaged DNA duplex. It was important to elucidate whether the stimulatory effect of Cen2 was extended onto other participants of NER or this effect was specific and limited only to the interaction with XPC. Figure 5 shows data on the binding of RPA and XPA with DNA and on the affinity modification of these proteins in the presence of different concentrations of Cen2. RPA forms three types of complexes with different electrophoretic mobility, which indicates different stoichiometry of RPA–DNA complexes [16]. On increase in the Cen2 concentration, the intensity of the band corresponding to the complex with the intermediate mobility also increases, along with decreases in the intensity of the bands corresponding to the complexes with the low and maximal mobility (Fig. 5a, *5* and *6*). Although the pattern of RPA–DNA complexes changed in the presence of Cen2, the total binding level was constant. Cen2 had virtually no influence of the binding of XPA with DNA under the experiment conditions (Fig. 5a, *7-10*).

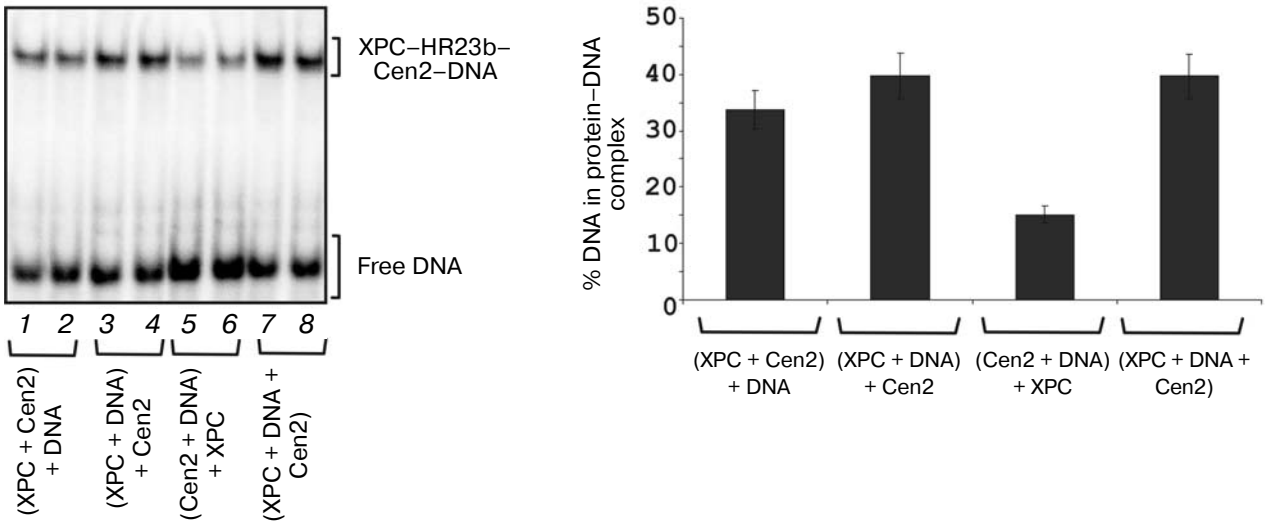


Fig. 4. Dependence of yield of the XPC–HR23b–Cen2–DNA complexes on the order of component addition into the reaction mixture. The components in parentheses were added into the reaction mixture first and incubated at 37°C for 10 min, then the third component was added and incubation was continued for 10 min. In the case of simultaneous addition (lanes *7* and *8*) the mixture was incubated for 20 min. Two identical lanes in the radioautograph correspond to each variant of the reagent addition. The histogram was constructed using results of three experiments.

There are data in the literature on the interaction of RPA and XPA with DNA during NER. The RPA–XPA complex has much higher affinity for damaged DNA than each of the proteins separately [24, 25]. The importance of interaction between RPA and XPA is evidenced by the absence of support for NER by XPA deletion mutants incapable of interacting with the large subunit of RPA [26]. During the experiment with the simultaneous presence of RPA and XPA in the reaction mixture, a band appeared that corresponded to the RPA–XPA–DNA complex (Fig. 5a, *11*). Increase in the Cen2 concentration is accompanied by appearance of additional bands corresponding to protein–DNA complex with electrophoretic mobility other than that of the RPA–DNA and XPA–DNA complexes (Fig. 5a, *13-15*). In the presence of maximal concentration of Cen2 (Fig. 5a, *15*) two bands of XPA–DNA complexes and one band of the RPA–DNA complex with the maximal mobility are recorded. However, the three bands can be assigned to the RPA–XPA–Cen2–DNA complexes.

Figure 5b presents results of electrophoretic separation of products of modification of RPA (Fig. 5b, *1* and *3-7*) and XPA (Fig. 5b, *2* and *8-12*) by a photoreactive DNA duplex in the presence of Cen2. Cen2 did not influence XPA modification, and this correlated with the results of binding (Fig. 5a, *2* and *7-10*). Titration with Cen2 of a reaction mixture containing RPA and DNA decreased the yield of RPA adducts with DNA (Fig. 5b, *1* and *3-7*). Because Cen2 does not influence the total level of binding, it was suggested that a decrease in the modification level could be associated with conformational changes in the complex of RPA with DNA in the presence of Cen2 (Fig. 5a, *5* and *6*), with resulting changes in positions of the amino acid groups of the protein relatively to the photoreagent.

Thus, results of the present work demonstrate the influence of Cen2 on interaction of the factors XPC–HR23b and RPA with damaged DNA. According to the literature data, Cen2 stimulates the binding of XPC–HR23b with DNA even at equimolar and lower ratio of the XPC and centrin concentrations [4]. Experiments performed *in vivo* on the HeLa cells have shown that overexpression of XPC induced translocation of Cen2 from the cytoplasm to the nucleus [27]. The inhibition of XPC gene expression leads to decrease in Cen2 gene expression and protein amount [28]. The influence of the Cen2 excess (relative to XPC) on XPC and a whole NER system was not studied earlier. Our data *in vitro* have indicated that a slight excess of Cen2 stimulates the binding of XPC with damaged DNA. However, with a pronounced excess of Cen2 its stimulatory effect disappears. Therefore, it is suggested that a mechanism should exist responsible for maintaining in the nucleus the Cen2 concentration sufficient to provide for the stimulatory effect of this protein on the NER system. In the presence of Mg^{2+} the stimulatory effect of Cen2 on the binding of XPC–HR23b with DNA is manifested most clearly. This effect seems to be explained by a supposed functioning of the heterotrimer XPC–HR23b–Cen2 in cells with involvement of Mg^{2+}.

The influence of Cen2 on the interaction of RPA and XPA with a damaged DNA was not studied earlier. The data presented in this work are the first to indicate the

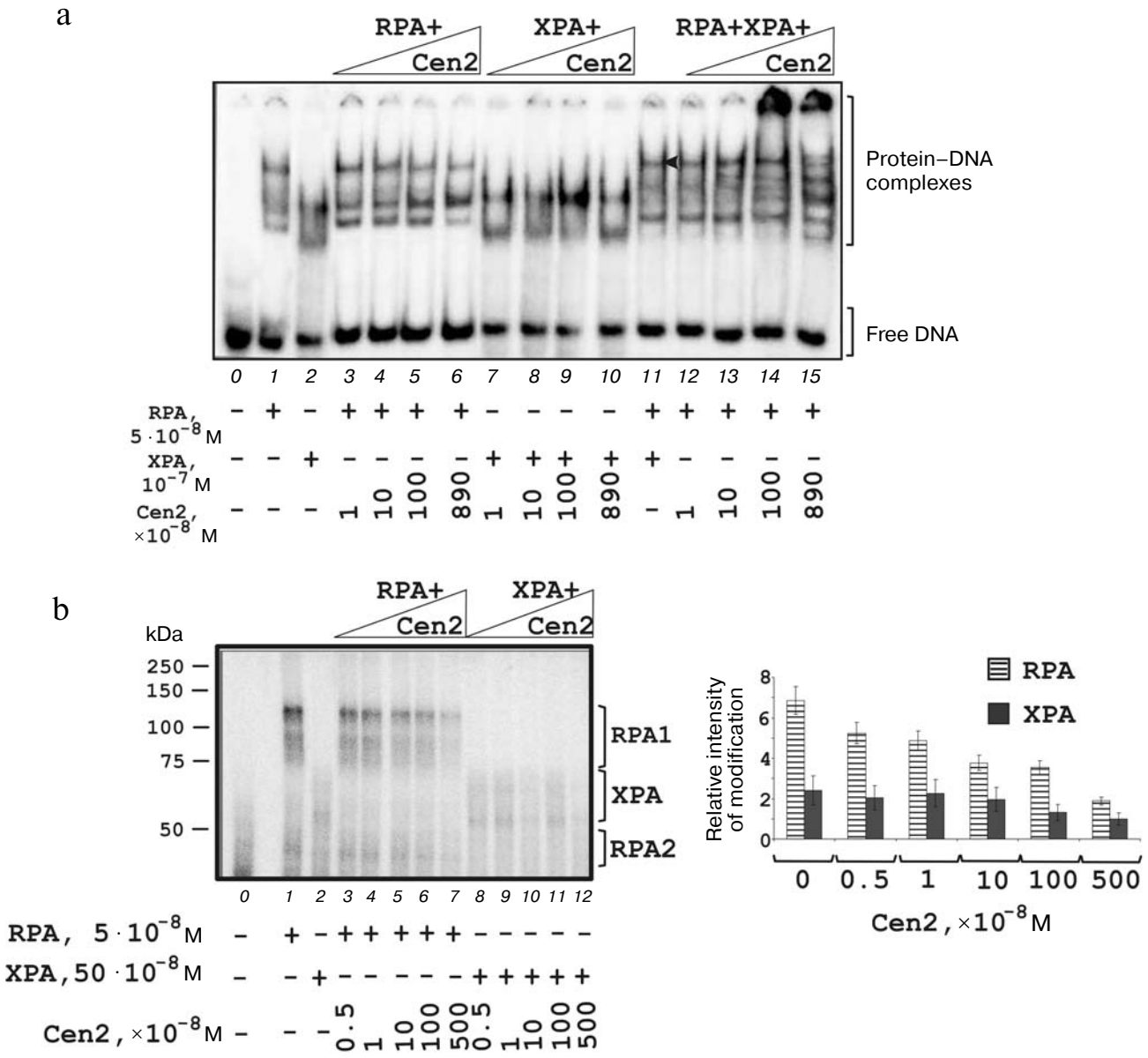


Fig. 5. Influence of Cen2 on the interaction of RPA and XPA with DNA duplex containing a bulky lesion. a) Influence of Cen2 on the binding of RPA and XPA with damaged DNA duplex. The reaction mixtures (10 μl) contained buffer PB1×, 0.6 mg/ml BSA, different concentrations of the studied proteins, and 1 nM 5′-^{32}P-labeled damaged DNA duplex. Radioautograph of 5% polyacrylamide gel (acrylamide/*bis*-acrylamide = 60 : 1). b) Influence of Cen2 on the RPA and XPA modification. The reaction mixtures (10 μl) contained buffer PB1×, 0.6 mg/ml BSA, different concentrations of the studied proteins, and 10 nM 5′-^{32}P-labeled damaged DNA duplex. Radioautograph of 10% SDS-polyacrylamide gel (acrylamide/*bis*-acrylamide = 60 : 1).

interaction between Cen2 and RPA and also the influence of Cen2 on the cooperative binding of RPA and XPA with DNA manifested in appearance of additional DNA–protein complexes, which seem to include Cen2.

The influence of Cen2 on the binding of RPA with damaged DNA found by us can be mediated by protein–protein interactions between Cen2 and RPA or be a result of contacts of Cen2 with DNA. It was shown earlier that RPA stimulated the binding of XPC–HR23b with damaged DNA [16]. Therefore, the specific influence of Cen2 on formation of a definite type of RPA–DNA complexes is supposed to indicate a specific role of protein–protein interaction of RPA with the XPC–HR23b–Cen2 during the assembly of the repair machine at an early stage of NER.

Perhaps the influence of Cen2 on the interaction of RPA with damaged DNA is important not only for NER, but also for regulation of transcription, because both proteins are involved in this process. The role of RPA and Cen2 in transcription is not clear, but RPA is known to be

involved in transcription through interaction with specific regulatory sequences of DNA [29], and Cen2 influences the transcription of genes encoding the NER factors [6].

Based on our findings in concert with the literature data, Cen2 is supposed to be a regulatory element of the NER system.

This paper is dedicated to the memory of Constantine Craescu (C. T. Craescu, Institut Curie-Recherche, Orsay, France).

The authors are grateful to James Angulo (CEA, Fontanay aux Roses, France) for his help in organization of this work and to Kaoru Sugasawa (Biosignal Research Center, Kobe University, Kobe, Japan) for presenting recombinant DNAs encoding XPC and HR23b.

The work was supported by the Russian Foundation for Basic Research (project No. 10-04-00837) and by the Russian Academy of Sciences Presidium Program on Molecular and Cell Biology.

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