

## Order of Assembly of Human DNA Repair Excision Nuclease\*

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Mitsuo Wakasugi and Aziz Sancar‡

From the Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599-7260

**Human excision nuclease removes DNA damage by concerted dual incisions bracketing the lesion. The dual incisions are accomplished by sequential and partly overlapping actions of six repair factors, RPA, XPA, XPC, TFIIH, XPG, and XPF-ERCC1. Of these, RPA, XPA, and XPC have specific binding affinity for damaged DNA. To learn about the role of these three proteins in damage recognition and the order of assembly of the excision nuclease, we measured the binding affinities of XPA, RPA, and XPC to a DNA fragment containing a single (6-4) photoproduct and determined the rate of damage excision under a variety of reaction conditions. We found that XPC has the highest affinity to DNA and that RPA has the highest selectivity for damaged DNA. Under experimental conditions conducive to binding of either XPA + RPA or XPC to damaged DNA, the rate of damage removal was about 5-fold faster for reactions in which XPA + RPA was the first damage recognition factor presented to DNA compared with reactions in which XPC was the first protein that had the opportunity to bind to DNA. We conclude that RPA and XPA are the initial damage sensing factors of human excision nuclease.**

In human nucleotide excision repair, 14 polypeptides in six repair factors act in concert to excise DNA damage in the form of 24–32-nucleotide long oligomers (1, 2). The six repair factors are XPA, RPA, XPC, TFIIH, XPG, and XPF-ERCC1 (3, 4). The excision reaction has been characterized extensively: the XPG endonuclease makes the 3' incision (4–6) followed by 5' incision by the XPF-ERCC1 endonuclease (4, 7). However, the critical step of damage recognition remains poorly understood. Three proteins have been implicated in damage recognition: XPA (8, 9), RPA (10–12), and XPC (13). All three proteins have been reported to have moderate preference for damaged DNA compared with undamaged DNA as tested by electrophoretic mobility shift assay, filter binding assay, or damaged DNA affinity chromatography. Furthermore, using a pull-down assay it was found that the combination of RPA + XPA conferred increased selectivity for damaged DNA (14, 15). These findings led to a model whereby the initial damage sensing was performed by XPA + RPA, which subsequently recruited the other repair factors to the site of damage (1, 2). Indeed, a comprehensive study of the excision nuclease assembly revealed that the first high-specificity complex is formed in the presence of

XPA + RPA + XPC + TFIIH (16) under conditions known to cause specific unwinding of substrate at the site of the lesion (17, 18).

A recent study (19), however, raised some questions about the validity of published models for the assembly of human excision nuclease. In this study (19), it was found that an XPC footprint was readily obtained on a (6-4) photoproduct containing DNA fragment in contrast to reported failures to obtain DNase I footprint of XPA and RPA (18). Furthermore, it was reported that preincubation of damaged DNA with XPC protein followed by addition of other repair factors in the form of a partially purified cell extract resulted in a higher rate of repair relative to reaction conditions in which damaged DNA was incubated first with XPA or XPA + RPA prior to addition of extract containing the additional repair factors (19). Independently, it was reported that the yeast homologue of XPC, the Rad4-Rad23 complex, also bound to damaged DNA with high specificity (20, 21). These findings led to an alternative human excision nuclease assembly model (19) which suggests that XPC is the first repair protein to bind to DNA damage and, thus XPC is the initiator of excision repair which recruits the other repair factors following binding to the damage site.

Previous studies on damage recognition were carried out by several groups using a variety of both DNA damage and methods to detect DNA-protein interactions (8–21). In order to address the issue of specificities and order of assembly of the three proteins implicated in damage recognition we decided to investigate the binding of XPA, RPA, and XPC to a DNA duplex containing a (6-4) photoproduct by using a band mobility shift assay and DNase I footprinting. In addition, we used repair factors purified to homogeneity to conduct repair kinetic experiments under a variety of “order of addition” conditions. Of the three damage recognition proteins tested, we find the best discrimination between damaged and undamaged DNA with RPA protein followed by comparable levels of discrimination for the XPA and XPC proteins. Significantly, we find that preincubation of damaged DNA with XPA, RPA, or XPA + RPA results in about 5-fold faster rate of repair relative to DNA preincubated with XPC prior to addition of other repair factors. Our results are consistent with initial damage recognition by RPA or the RPA-XPA complex followed by formation of a higher stability DNA-protein complex by XPC recruited independently or in association with TFIIH to form a four repair factor preincision complex of high specificity.

### MATERIALS AND METHODS

**Substrate**—The substrate for DNase I footprinting and repair assay was a 136-bp<sup>1</sup> duplex containing a (6-4) photoproduct in the center. The sequence and preparation of this substrate have been described elsewhere (22). For the excision repair assay the duplex contained <sup>32</sup>P label at the 4th phosphodiester bond 5' to the photoproduct and the duplex for the footprinting assay contained the radiolabel at the 5' terminus of

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‡ To whom correspondence should be addressed: Dept. of Biochemistry and Biophysics, Mary Ellen Jones Bldg., CB 7260, University of North Carolina, Chapel Hill, NC 27599-7260. Tel.: 919-962-0115; Fax: 919-843-8627.

<sup>1</sup> The abbreviations used are: bp, base pair(s); MBP, maltose-binding protein; TBP, TATA-binding protein.

the complementary strand. The substrate for electrophoretic mobility shift assay was a 49-mer duplex which was obtained by digesting the internally labeled 136-mer with *PvuII* and *HindIII* restriction endonucleases followed by purification of 49-mer after electrophoresis through a 5% nondenaturing polyacrylamide gel. The concentrations of all substrates were determined by Cerenkov counting.

**Repair Factors**—MBP-XPA, (His)<sub>6</sub>-XPA, RPA, XPC-HR23B, XPC, XPG, XPF-ERCC1, and TFIIH were purified as described previously (4, 7, 13, 23). For purification of MBP-XPC-HR23B protein, a baculovirus construct was prepared by inserting the *maleE* gene 5' to the *XPC* gene in a construct previously used for making XPC-HR23B heterodimer (13). The fusion protein was purified by chromatography on amylose resin (New England Biolabs), followed by single-stranded DNA cellulose (Sigma) and DEAE-agarose (Bio-Rad) resins. The fusion protein was as active as native XPC-HR23B in complementation assay or reconstitution assays. For clarity in the presentation we will refer to the XPC-HR23B heterodimer as "XPC" in this paper. When necessary, the three forms of XPC used in this study will be identified explicitly as XPC monomer, XPC-HR23B, and MBP-XPC-HR23B, respectively.

**Antibodies**—Monoclonal antibodies against XPA were from Neomarkers, polyclonal antibodies against the p34 subunit of RPA were from Santa Cruz Biotechnology, and the monoclonal antibody against MBP was purchased from Sigma.

**Electrophoretic Mobility Shift Assay**—The 49-bp duplex containing the (6-4) photoproduct or control DNA without the photoproduct were incubated at a concentration of 0.12 nM with the indicated concentrations of repair proteins in 25  $\mu$ l of reaction buffer which contained 32 mM Hepes-KOH, pH 7.9, 64 mM KCl, 6.44 mM MgCl<sub>2</sub>, 0.16 mM dithiothreitol, 0.16 mM EDTA, 2 mM ATP, and 4% (v/v) glycerol. After a 30-min incubation at 30 °C, the samples were loaded directly onto 3.5 or 5% nondenaturing polyacrylamide gels in 1  $\times$  TBE (50 mM Tris borate, pH 7.9, 1.2 mM EDTA). Electrophoresis was carried out at room temperature at 20 mA for 1.5 h. Mobility shift assays to identify various forms of preincision complexes 1, 2, and 3 were performed as described previously (16). The DNA-protein complexes were visualized by autoradiography and quantitative analyses were performed using an AM-BIS scanner system. In supershift assays (mobility shift with antibody) DNA and protein were preincubated, antibody was added to the reaction mixture, and following an additional 10-min incubation, the samples were loaded onto the polyacrylamide gel.

**DNase I Footprinting**—3 fmol of DNA was incubated with the repair factors at the indicated concentrations in 25  $\mu$ l of reaction buffer at 30 °C for 30 min. CaCl<sub>2</sub> was added to 3.8 mM followed by 0.01 unit of DNase I (Life Technologies Inc.) and incubated at 22 °C for 3 min. The DNA was extracted with phenol:chloroform, precipitated with ethanol, dissolved in formamide:dye loading reaction mixture and separated on 10% denaturing polyacrylamide gels along with Maxam-Gilbert G + A chemical sequence ladder.

**Excision Repair Assay**—All the repair factors used were purified to near homogeneity (4, 7, 13, 23) and, with the exception of TFIIH, we used recombinant proteins purified from *Escherichia coli* or baculovirus-infected insect cells for all of our assays. A standard reaction contained 3 fmol of substrate and 50 ng of MBP-XPA (or His-XPA), 300 ng of RPA, 10 ng of XPC-HR23B, 150 ng of TFIIH, 10 ng of XPG, and 20 ng of XPF-ERCC1 in 25  $\mu$ l of reaction buffer. The reaction mixture was incubated at 30 °C for the indicated times and then DNA was extracted with phenol:chloroform and separated on 8% denaturing polyacrylamide gels. Since the role of XPC in damage recognition is a critical question addressed in this study the amount of XPC necessary for optimal excision reaction was titrated carefully. Under our experimental conditions, 10–20 ng of XPC in a reaction mixture constituted saturating amounts, increasing XPC to 60 ng/reaction severely inhibited excision (4). Quantitative analyses were done by PhosphorImager (Molecular Dynamics) or by scanning the autoradiograms with a Molecular Dynamics Computing Densitometer Series 300 instrument. In addition to the standard excision assay, we conducted "omission type" and "sequential addition type" assays. In omission type assays, the substrate was incubated with all but one of the repair factors for 10 min, then the omitted factor was added and further incubation continued at 30 °C. In sequential addition assays, the substrate was first incubated with one (or two) repair factor for 10 min, and then the rest of the repair factors were added and incubation was continued at 30 °C. The reactions were stopped by quick-freezing on dry ice, and the excision products were analyzed by autoradiography following resolution in 8% sequencing gels.

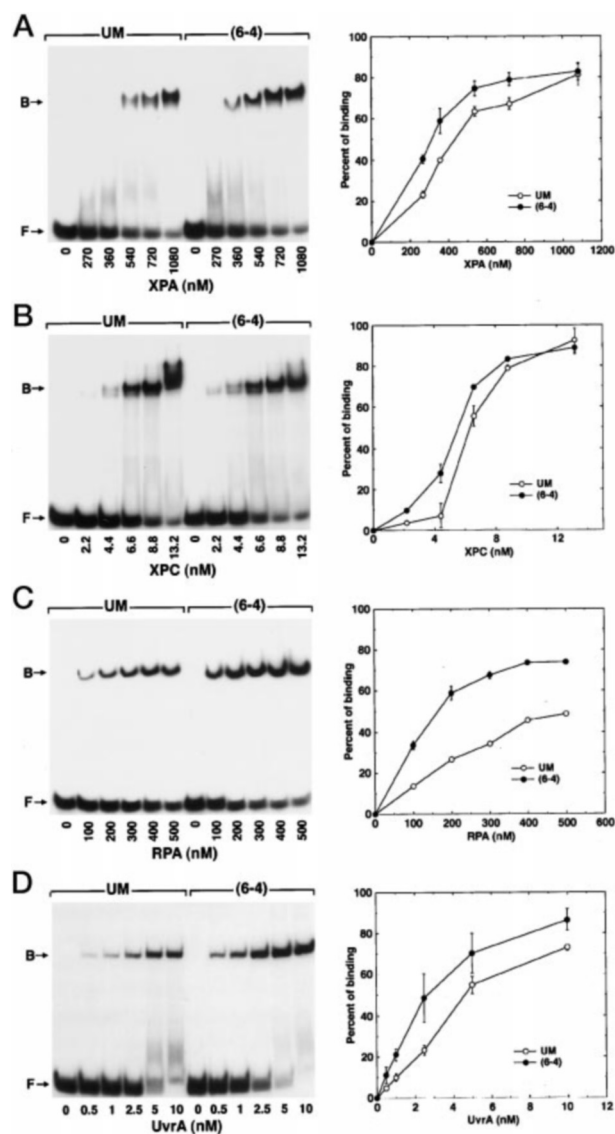


FIG. 1. Binding of XPA, XPC-HR23B, RPA, and UvrA to undamaged and damaged DNA. A 49-bp duplex (0.12 nM) either with no damage (open symbols) or with a centrally located T-T (6-4) photoproduct were incubated at 30 °C for 30 min with the polypeptides at the indicated concentrations and the free (F) and bound (B) fractions were separated on 5% nondenaturing polyacrylamide gel. The quantitative analyses of the data are shown in the right panels. Bars indicate the standard error of two independent sets of experiments including the ones shown in this figure.

## RESULTS

### Damage Recognition by Excision Repair Proteins

Although XPA (8, 16), RPA (10–12), and XPC (13, 19) have been shown previously to bind to damaged DNA preferentially, there have been no quantitative comparisons of the binding affinities of these three repair proteins to a defined substrate under identical reaction conditions. To learn about the contributions of these repair proteins to damage recognition by human excision nuclease, we performed binding assays with unmodified and (6-4) photoproduct containing 49-bp duplex DNAs using the electrophoretic mobility shift assay for quantitative analysis. As a positive control, we used the *E. coli* UvrA protein which is known to be the damage recognition subunit of *E. coli* excinuclease (24). Fig. 1 shows the results of these experiments. The approximate specific and nonspecific binding constants calculated from the data in Fig. 1 are summarized in

FIG. 2. DNase I footprinting of XPC-HR23B and the effects of XPA and RPA on the footprint. A, terminally labeled 136-bp control duplex (lanes 2–6) or DNA containing a T-T (6-4) photoproduct and radiolabel in the complementary strand were incubated with the indicated concentrations of XPC-HR23B, digested with DNase I, and separated on 10% denaturing polyacrylamide gels. B, the (6-4) photoproduct containing duplex was incubated with 2.2 nM XPC-HR23B or with 180 nM XPA, 100 nM RPA at the combinations shown on the top and following 30 min at 30 °C the DNA was digested with DNase I and separated on 10% denaturing polyacrylamide gel. Open arrow, site of (6-4) photoproduct; closed arrow, XPC-induced DNase I-hypersensitive site. G + A indicates Maxam-Gilbert sequencing reaction for purines.

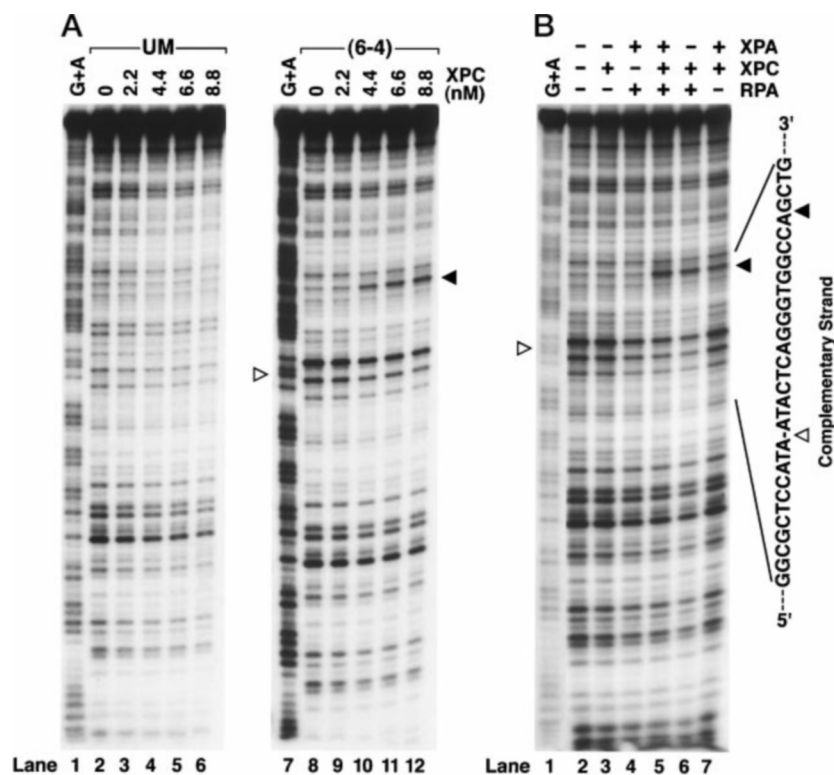


TABLE I

Specific and nonspecific binding constants of excision repair proteins

The equilibrium dissociation constants for nonspecific DNA ( $K_{NS}$ ) were estimated from the protein concentrations which bound 50% of the substrate. To calculate the  $K_S$  the measured binding constants with damaged DNA were adjusted for the frequency of the photoproduct (1:49) in a nonspecific DNA matrix (25). The  $K_{NS}$  values agree with the previously published values for XPA (8), RPA (56), and XPC (13) within a factor of two.

	$K_{NS}$	$K_S$	Selectivity
XPA	$4.2 \times 10^{-7}$	$6.0 \times 10^{-9}$	70
RPA	$4.0 \times 10^{-7}$	$3.0 \times 10^{-9}$	133
XPC	$5.0 \times 10^{-9}$	$1.2 \times 10^{-10}$	42

Table I. XPC is the protein with the highest affinity for both damaged and undamaged DNA and RPA has the best discriminatory power. The selectivity factor, which is defined as the ratio of equilibrium dissociation constant for the (6-4) photoproduct to the equilibrium constant to a non-damaged base pair (25), is about 133 for RPA, 70 for XPA, and 42 for XPC. Clearly, based on these values, none of the three human damaged DNA-binding proteins have enough specificity to function as the “damage recognition subunit” of the human excision nuclease. We reasoned that the three subunits may act in a cooperative fashion to achieve the high specificity observed *in vivo*.

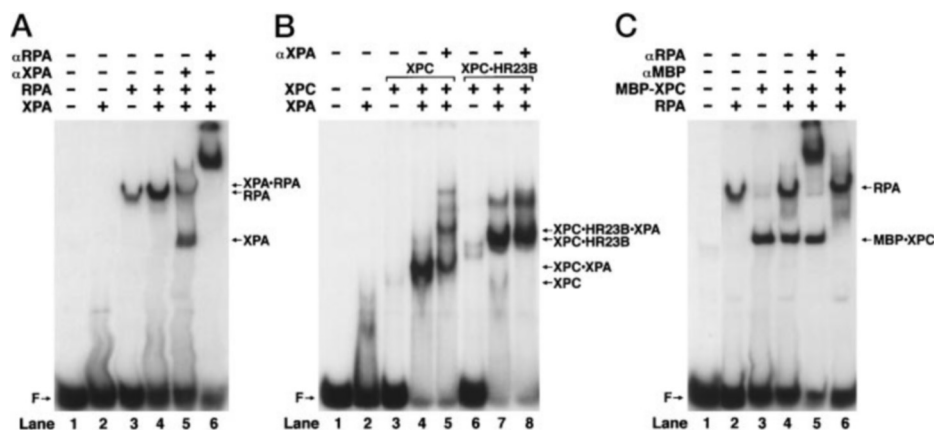
#### Footprinting of XPA, RPA, and XPC

We wished to confirm the band mobility shift data by footprinting experiments and, in addition, to use footprinting to detect any potential cooperative interactions between these three subunits. We were unable to obtain a damage-specific footprint with XPA, RPA, or XPC under a variety of conditions where the concentrations of DNA, the binding proteins, or of DNase I were the variables. Under all reaction conditions tested, the three factors inhibited the DNase I digestions of both undamaged or damaged DNA to the same extent. Since an XPC footprint on a (6-4) photoproduct substrate was reported previously (19) we show the results of our footprinting experi-

ment with a similar substrate in Fig. 2A. We were unable to observe either a specific footprint or a hypersensitive site when the damaged strand was labeled. However, with label in the complementary strand, a specific XPC effect was seen. Visual inspection of this figure as well as quantitative analysis of densitometric scans show no difference between the degree of protection of undamaged and damaged DNA by XPC protein. Using up to 10-fold higher concentrations of XPC than the one used in this experiment caused progressively stronger inhibition of DNase I uniformly and with no preference for damage DNA (data not shown). Interestingly, however, in agreement with the previous report (19), we do find that the 16th phosphodiester bond 3' to the AA across from the (6-4) photoproduct becomes hypersensitive to DNase I digestion at XPC concentrations that cause overall protection of damaged and undamaged DNA (compare Fig. 2A, lanes 4–6 and lanes 10–12). Since the comparison of lanes with vastly unequal overall digestions could give rise to artificial “footprints” we took special precautions to compare only lanes with comparable overall DNase I digestion as evidenced by the amount of undigested full-length fragment. To our surprise, neither under these conditions nor under conditions of unequal DNase I digestion, were we able to obtain an XPC DNase I footprint as defined by specific protection of the area around the photoproduct.

Equally surprising was the observation that even though RPA exhibited the best discrimination between undamaged and damaged DNA by the band shift assay it failed to exhibit a specific footprint either in the form of protection from DNase I around the photoproduct or in the form of induction of a DNase I-hypersensitive site only in the damaged DNA. XPA, which exhibits less selectivity by the gel retardation assay, also failed to elicit a specific DNase I footprint (data not shown). Hence we decided to test combinations of two and three damage recognition factors to find out if such combinations improve the selectivity and give rise to damage-specific footprints.

Fig. 2B shows DNase I footprints for various combinations of XPA, RPA, and XPC. The XPA + RPA combination (lane 4)



**FIG. 3. Detection of specific XPA-RPA-DNA and XPA-XPC-DNA complexes by electrophoretic mobility shift assay.** These band mobility shift assays were done with the 136-bp duplex (3 fmol) containing a T-T (6-4) photoproduct and the complexes were analyzed on 3.5% nondenaturing polyacrylamide gels. *A*, DNA was incubated with XPA (180 ng) and RPA (300 ng) as indicated and where appropriate anti-RPA ( $\alpha$ RPA) or anti-XPA ( $\alpha$ XPA) antibodies were added to the binding mixtures before loading onto the gel. Note that XPA-RPA-DNA complex (*lane 4*) migrates slightly slower than RPA-DNA complex (*lane 3*). The XPA antibody partly supershifts and partly disrupts the ternary complex giving rise to three bands in the following decreasing order of mobilities: supershifted ternary complex, XPA-RPA-DNA complex not bound by antibody superimposed onto RPA-DNA complex and XPA-DNA complex. *B*, DNA was incubated with XPC monomer (20 ng), XPC-HR23B (10 ng), and XPA (360 ng) as indicated. In *lanes 5* and *8*, anti-XPA antibody was added to the binding mixtures before loading onto the gel. The position of various complexes inferred from the mobility differences are indicated. *C*, DNA was incubated with RPA (300 ng) and MBP-XPC (180 ng) as indicated. Note that the combination of two proteins gives rise to two discrete bands (*lane 4*) of mobilities identical to those obtained with individual proteins (*lanes 2* and *3*). The RPA antibody supershifts the RPA-DNA band only (*lane 5*); and the MBP antibody partly supershifts and partly disrupts the MBP-XPC-DNA band only (*lane 6*).

causes uniform inhibition of DNase I as is seen with individual factors. The combinations of XPC + RPA (*lane 6*) or XPC + XPA (*lane 7*), however, reveal an interesting phenomenon: although at the XPC concentration used in this particular experiment the XPC-induced hypersensitive site was only marginally evident (*lane 3*), the intensity of the hypersensitive band in reactions with XPC + RPA (*lane 6*) or XPC + XPA (*lane 7*) was increased and the combination of all three proteins gave rise to the most intense hypersensitivity (*lane 5*) consistent with additive stimulatory effects of XPA and RPA on the XPC-induced hypersensitive site. Under no conditions, however, were we able to obtain a specific footprint around the damage site. As is apparent in *lanes 4-6*, when DNase I protection was seen, it extended over the entire fragment and manipulations of the concentrations of the three proteins did not confer further specificity (data not shown). The footprinting experiments, nevertheless, revealed a specific effect of XPA and RPA on XPC-DNA interactions never seen before in studies aimed at understanding the assembly of human excision nuclease. The data in Fig. 2*B* raised the possibility that XPC, XPA, and RPA may form a specific complex on damaged DNA. We wished to search for such a complex by conducting gel mobility shift assays.

#### Ternary Complexes of XPA-RPA-DNA and XPA-XPC-DNA

The results of gel mobility shift assays with the pairwise combinations of XPA, RPA, and XPC are shown in Fig. 3. In agreement with earlier reports (14, 15) the XPA + RPA combination produces a DNA-protein complex with higher stability than the complex formed with either protein alone (Fig. 3*A*, *lanes 2-4*). Although the retarded DNA band seen when both proteins are present is only marginally slower than the RPA-DNA band (*lanes 3* and *4*) it appears to contain both XPA and RPA: the XPA antibodies partially supershift this band but mostly disrupt it giving rise to a faster migrating DNA band which corresponds to XPA-DNA and to a slower migrating band which is the undisrupted band XPA-RPA-DNA complex superimposed onto the RPA-DNA band (*lane 5*). The anti-RPA antibodies supershift the entire XPA-RPA-DNA band (*lane 6*) consistent with the interpretation that this complex contains RPA. Fig. 3*B* shows the result of XPA + XPC combinations. To probe

for the presence of XPC in DNA-protein complexes: we employed two forms of XPC; "XPC monomer" which is as active in the excision assay as the heterodimer (13) and XPC-HR23B heterodimer which is the natural form of XPC (26, 27). To better discern the cooperative interaction of the two repair factors, reactions were performed under conditions where band shift by the individual factors was minimal (*lanes 2, 3, and 6*). The combination of XPC monomer + XPA yields a retarded band (*lane 4*) which can be supershifted by anti-XPA antibodies (*lane 5*) and thus must contain XPA. Furthermore, the retarded band generated by XPA + XPC monomer (*lane 4*) migrates faster than the band generated by XPA + XPC-HR23B (*lane 7*) and hence the retarded bands in *lanes 4* and *7*, in addition to XPA, must also contain the XPC protein either as a monomer or as a heterodimer.

Fig. 3*C* shows the result of a band shift assay performed in an attempt to detect an RPA-XPC-DNA ternary complex. We used MBP-XPC in these experiments so that we could conduct supershift experiments with commercially available anti-MBP antibodies. Under the assay conditions used in this experiment both RPA (*lane 2*) and MBP-XPC (*lane 3*) give rise to discrete retarded bands. The combination of the two proteins yields the same two bands (*lane 4*) indicating the lack of a ternary complex containing both repair factors. In agreement with this interpretation, anti-RPA antibodies supershifted the band assigned to RPA only (*lane 5*) and anti-MBP antibodies partly supershifted but mostly disrupted the band assigned to the MBP-XPC-DNA complex (*lane 6*). The MBP antibodies disrupt other DNA-protein complexes as well in gel retardation assays in a nonspecific manner and hence were not particularly useful for further characterization of this and other DNA-protein complexes which contain the MBP tag. We attempted to form an RPA-XPC-DNA ternary complex under a variety of conditions including using XPC without the MBP tag but failed to find any evidence for such a complex by the band shift assay (data not shown). Apparently, the enhancement of XPC-induced DNase I-hypersensitive site by RPA seen in Fig. 2*B* (*lane 6*) occurs by a relatively transient interaction between these two proteins or by an unknown mechanism. Similarly, the band shift assays

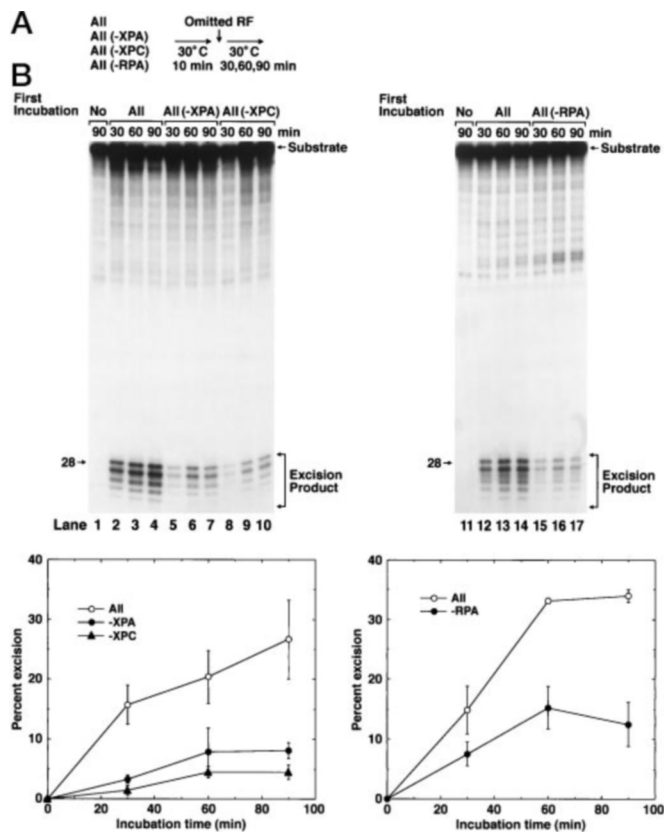


FIG. 4. Omission type repair kinetic experiments. The 136-mer (T-T (6-4) photoproduct) was incubated either with the six pre-mixed repair factors, or with five factors first followed by the addition of the omitted repair factor and samples were taken at the indicated time points. *A*, reaction scheme. *B*, excision reaction performed under three different conditions. The top panels show the excision products detected by autoradiography following electrophoresis on 8% denaturing polyacrylamide gels. The bottom panels show quantitative analyses of the data from the experiments in the top panel plus a second set of experiments performed under identical conditions. Bars indicate standard error.

with the three repair factors, XPA, RPA, and XPC only revealed evidence for XPA·RPA and XPA·XPC complexes; there are no convincing data for formation of XPA·RPA·XPC complexes on DNA.

#### Order of Addition Experiments

We performed two types of order of addition experiments analogous with the types of experiments that were performed with partially purified cell extracts and which led to the “XPC first” model (19) for the assembly of human excision nuclease: omission type and sequential addition type assays.

**Omission Type Excision Assays**—Fig. 4 shows the results of omission type excision assays. The substrate was incubated with 5 repair factors in separate reactions missing XPA, XPC, or RPA; then the missing factor was added and incubation was continued. In all three instances the reaction rates were comparable. Each rate was significantly slower than the rate achieved when substrate was added to the mixture of all six repair factors. These results differ from those obtained with partially purified extracts where it was found that an XPA omission type of experiment yielded a repair rate at least 5-fold faster than the XPC omission type of experiment (19). Clearly our results do not support the XPC first model. Hence we proceeded to test this model more directly by “addition type” assays.

**Sequential Addition Experiments**—DNA was incubated first

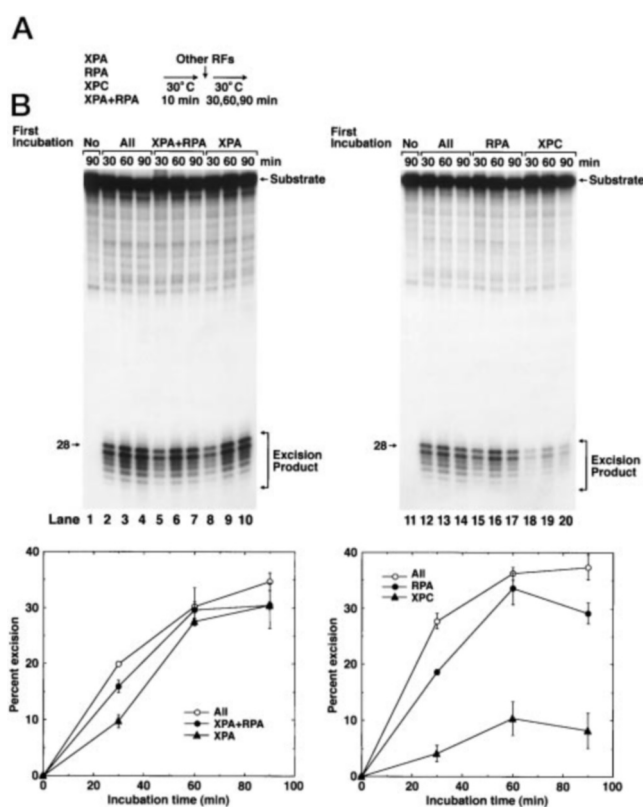


FIG. 5. Sequential addition type repair kinetic experiments. *A*, scheme of experimental protocol. DNA was incubated with XPA, RPA, XPC, or XPA + RPA for 10 min, the remaining 5 (or 4) repair factors (RFs) were added and incubation continued. Samples were taken for analysis at the indicated times. *B*, the top panels show the excision products detected by autoradiography following electrophoresis on 8% denaturing polyacrylamide gels. Quantitative analyses of data from top panels and a second set of experiments conducted under identical conditions. Bars indicate standard error.

with XPA, RPA, XPA + RPA, or XPC; then the remaining repair factors were added and the excision kinetics were measured. As seen in Fig. 5 DNAs preincubated with XPA, RPA, or XPA + RPA are repaired at essentially the same rate as DNA incubated with pre-mixed six repair factors. In contrast, DNA preincubated with XPC was repaired about 5-fold slower. These findings are not consistent with an XPC first model and actually suggest that, although XPC is capable of damage recognition, its binding to the lesion interferes with the proper assembly of the excision nuclease.

#### Effect of XPC Concentration on Reaction Rates

Of all the human excision nuclease subunits, XPC has the highest affinity for undamaged DNA (13) (Table I). Therefore, we were concerned that in the sequential addition experiment shown in Fig. 5 the slow kinetics of repair of DNA preincubated with XPC might have been caused by nonspecific binding of XPC to the substrate which could have interfered with proper assembly of the excinuclease at the site of specifically bound XPC protein upon later addition of the other repair factors. To address this point we titrated XPC in a standard excision assay. Fig. 6A shows that excision increases linearly over the range of 10 to 20 nM XPC and levels off at the higher concentrations. No inhibition was observed at the highest concentration (66 nM) used in this experiment (compare lanes 3–5). Since the sequential addition type experiments shown in Fig. 5 were performed with saturating XPC concentration (40 nM) we wished to find out if the addition type assays would yield

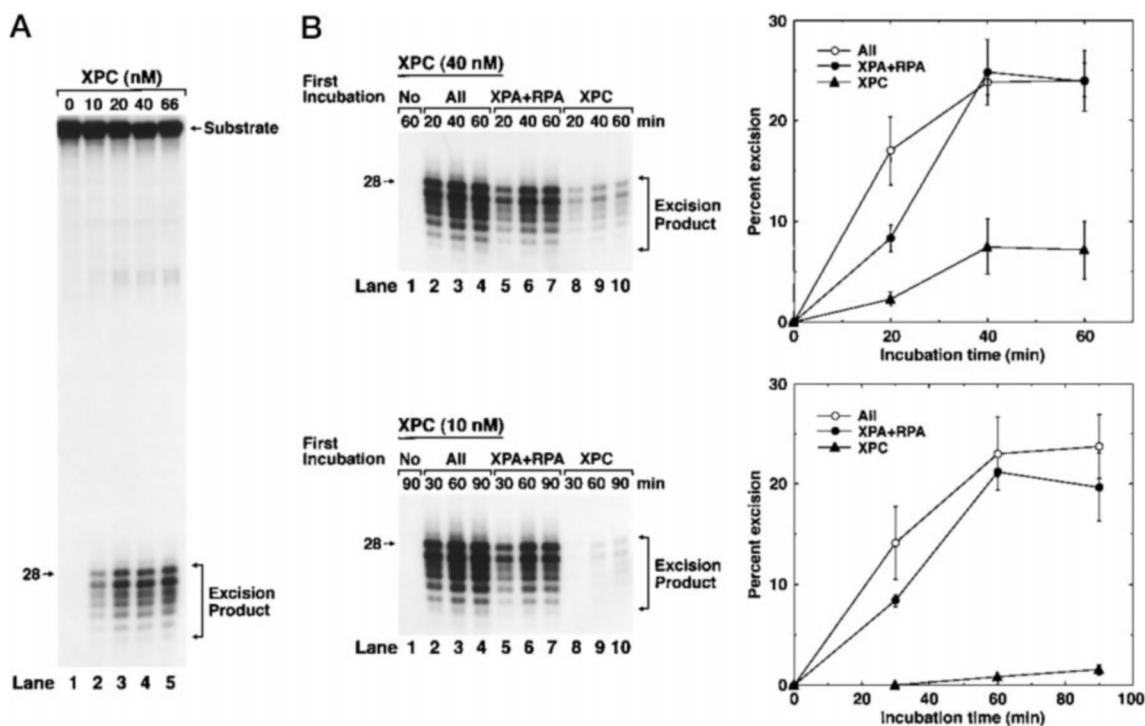


FIG. 6. **Excision kinetics under conditions of saturating and limiting XPC concentrations.** *A*, determination of limiting XPC concentrations. Excision reactions were done with the standard concentrations of all repair factors except XPC which was included in the reaction mixtures at the indicated concentrations. The reactions were carried out at 30 °C for 2 h and the excision products were separated on an 8% denaturing polyacrylamide gel. The levels of excision were (as percent of input substrate): 17.4 (lane 2), 42.6 (lane 3), 44.8 (lane 4), and 46.5 (lane 5). *B*, sequential addition type excision assay with saturating and limiting concentrations of XPC. The left panels show only the parts of the autorads containing the excision products; the top panel shows the results of different orders of addition performed with 40 nM XPC and the bottom panel shows the same experiment performed with 10 nM XPC. The right panels are the quantitative analyses of data in the left panel and of a second set of experiments conducted under identical conditions.

different results when conducted under reaction conditions where XPC was limiting. Fig. 6B shows that, in fact, under conditions of limiting XPC the difference between the rates of “XPA + RPA first” versus XPC first becomes much more drastic. We conclude, then, that under no conditions tested is DNA preincubated with XPC repaired faster than DNA preincubated with XPA or XPA + RPA repair factors.

**Two-factor Preincubations**—Data presented so far fail to provide any evidence for the XPC first model of assembly. Since the band shift assays with two factors reveal ternary complex formation with certain combinations (XPA + RPA and XPA + XPC) but not with others (RPA + XPC) we wished to carry out excision assays following such two-factor incubations in order to find out if there was correlation between the results obtained in gel shift experiments and excision kinetic assays, and hence if the ternary complexes detected by the band shift assays were on the pathway for assembly of the excision nuclease. Fig. 5 showed that the XPA + RPA preincubated DNA was repaired at a rate comparable to six-factor preincubation, consistent with the notion that the XPA·RPA·DNA complex is on the pathway for the assembly. The results of kinetic assays with the other two combinations (Fig. 7) shows that XPA + XPC first reaction yields an excision rate comparable to the XPA first reaction (*cf.* Fig. 5, left panel). In contrast, the XPC + RPA combination slows the rate of excision relative to the preincubation with RPA alone (Fig. 5, right panel, and Fig. 7). Thus, the two-factor preincubation results are consistent with those of the band shift assays in that two-factor combinations (XPA + XPC) which appear to form specific complexes with damaged DNA lead to a faster rate of repair relative to the two-factor combination (RPA + XPC) which failed to produce a specific ternary complex.

**Four-factor Assembly**—We previously reported that the first high specificity complex detectable with electrophoretic mobility shift assays was the one formed with XPA + RPA + XPC + TFIIH (16). Based on the DNA binding and excision kinetics results presented in this study it appears that this complex, which we have named “preincision complex 1,” represents the step subsequent to formation of the XPA·RPA·DNA ternary complex. Formation of PIC1 requires XPC but, due to technical problems, we were unable to address the issue of whether XPC is actually a component of PIC1. By using improved experimental conditions we are now able to answer this question. Fig. 8A shows that PIC1 formed with a maltose-binding protein (MBP) tag on either XPC (lane 3) or XPA (lane 4) migrates slower than PIC1 formed without MBP-tag on either protein (lane 2). This indicates that PIC1 contains both XPA and XPC. In contrast, PIC2 and PIC3 formed with five- and six-repair factors, respectively, exhibited the slower mobility when the MBP tag is on XPA but not when it is on XPC, relative to complexes formed without tagged repair factors (Fig. 8, B and C, lanes 3 and 4 versus lane 2). These data lead to the conclusion that in the human excision nuclease assembly pathway, the XPA·RPA·DNA complex is followed by the XPA·RPA·XPC·TFIIH·DNA complex (PIC1), followed by XPA·RPA·XPG·TFIIH·DNA complex (PIC2), and finally the dual incision complex containing XPA·RPA·XPG·TFIIH·XPF·ERCC1·DNA (PIC3). Thus, data in this paper as well as other evidence from previous studies (16, 19) indicate that XPC is a key player in the early steps of excision nuclease assembly at the site of damage and as such makes critical contributions to damage recognition but it is a molecular matchmaker which is not present in the ultimate dual incision complex.

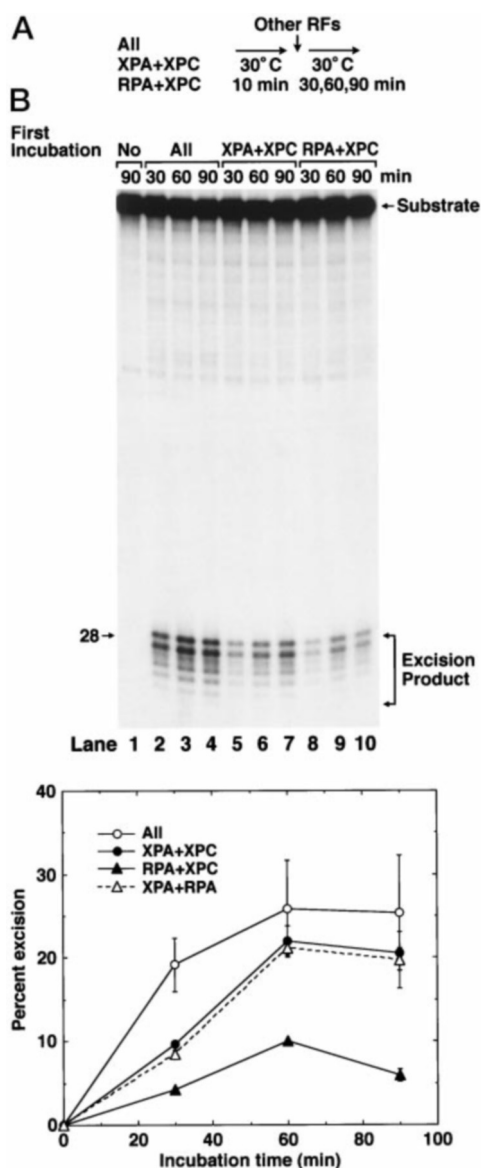


FIG. 7. **Sequential addition type excision assays after two-factor preincubation.** A, scheme of experimental procedure. B, excision assay performed with premixed six repair factors (lanes 2–4) or with DNA preincubated with XPA + XPC (lanes 5–7) or RPA + XPC (lanes 8–10). The bottom panel shows quantitative analysis of data from the top panel and a duplicate experiment. The XPA + RPA data points from Fig. 6 are included in this figure as well for direct comparison of all two-factor incubation experiments.

#### DISCUSSION

Human excision nuclease removes DNA damage in three basic steps: the lesion is recognized, the DNA around the lesion is unwound (17, 18), and dual incisions bracketing the lesion are made by two separate endonucleases (1, 2). We wish to discuss the problem of damage recognition and the current models for high specificity complex formation, and propose a model which incorporates data from previous experiments and findings presented in this paper.

#### Human Excision Nuclease and Damaged DNA-binding Proteins

The fact that the damage recognition step remains poorly understood is not for lack of damaged DNA-binding proteins, but rather because there are too many mammalian “damaged

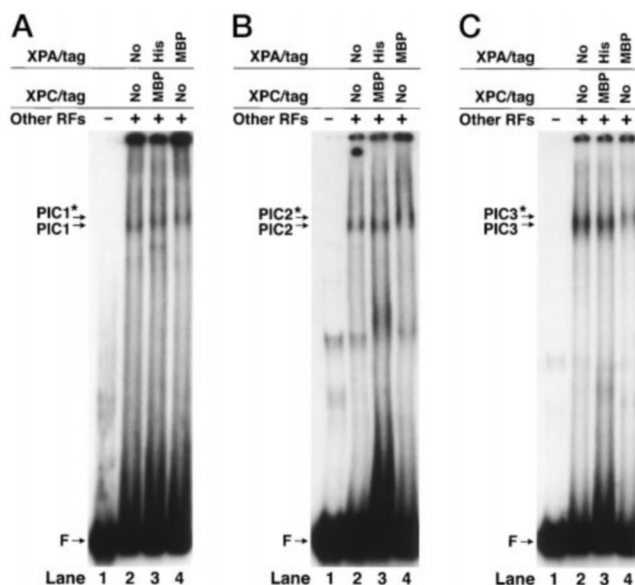


FIG. 8. **XPC is present in preincision complex 1 (PIC1) but not in preincision complexes 2 and 3.** A, PIC1, which is formed by XPA, RPA, XPC, and TFIIF, contains XPC. Note that the complexes formed with MBP-XPA (lane 4) and MBP-XPC (lane 3) have slower mobility than the complex formed with (His)<sub>6</sub>-XPA and non-tagged XPC (lane 2). B and C, PIC2, which is formed by XPA + RPA + XPC + TFIIF + XPG, and PIC3, which forms with all 6 repair factors including XPF-ERCC1, lack XPC. When these complexes form with MBP-XPA they migrate slower than complexes formed with (His)<sub>6</sub>-XPA (compare lanes 2 and 4) whereas complexes formed with MBP-XPC or non-tagged XPC show identical mobilities (lanes 2 and 3). These are autoradiograms of 3.5% nondenaturing band shift assay gels. PIC 1\*–3\* indicates supershifted PIC 1–3.

DNA-binding proteins.” The following proteins have been found to bind to damaged DNA preferentially and have been implicated in damaged DNA repair at one time or another: XPA (8, 9), RPA (10–12), XPC (13, 19), MutS $\alpha$  (28, 29), HMG1 (30, 31), UV-DDB (32–35), and TBP/TATA-binding protein (36). There is no genetic or biochemical evidence that HMG1 or TBP play any role in nucleotide excision repair. In addition, *in vitro* studies have shown that MutS $\alpha$  does not affect excision repair (22), that HMG1 inhibits the repair of cisplatin intrastrand cross-links (37), and that UV-DDB, depending on the type of assay used, either inhibits (38) or does not affect damage removal (39) by the human excision nuclease. Thus we consider the binding of damaged DNA by MutS $\alpha$ , HMG1, and TBP to be incidental to their mode of binding to their DNA targets or substrates and hence to be irrelevant to their physiological function. The role of UV-DDB in repair remains an enigma. It has been suggested that DDB fails to stimulate repair *in vitro* either because the density of lesions on the DNA substrate is too high to require the discriminating power of UV-DDB or because the UV-DDB plays a role in recognizing lesions in the context of chromatin (33, 39). These models await experimental verification. In contrast, both genetic and biochemical data implicate XPA, RPA, and XPC proteins in excision repair. Even though all three proteins bind to damaged DNA preferentially, the selectivity achieved by any one of the three is insufficient to explain the high specificity of the excision nuclease for damaged DNA (40, 41). Three models can be advanced to explain the high specificity of the excision nuclease within the context of poor selectivity of these three damaged DNA-binding proteins: the repairsome model, the concerted binding model, and sequential recognition model. These models are summarized below.

### Three Models for High Specificity Damage Recognition

**Repaosome**—It has been suggested and some evidence has been reported for the existence of a complex of all six-repair factors of excision nuclease (42, 43) and perhaps of a complex of all excision repair and mismatch repair factors capable of carrying out the entire excision reaction or, indeed, both the excision repair and mismatch repair reactions (44). While such a model is esthetically pleasing we consider it unlikely for the following reasons. First, most of the six-excision repair factors are readily separated from one another under relatively mild purification conditions (1–4) which makes the notion of a repaosome a semantic rather than a biochemical issue. Second, and more importantly, this model can be rejected in light of findings presented here and elsewhere (16) that it is not possible to form a preincision-incision complex which contains XPC and XPG simultaneously. While a complex of moderate stability of a subset of excision nuclease subunits is still a possibility we believe that the preponderance of available data are against the existence of a supramolecular repaosome complex.

**Concerted Recognition Model**—In this model the XPA, RPA, and XPC proteins make a complex which by additively combining the moderate selectivities of all three proteins achieves higher selectivity. Indeed, data exist which show that XPA and RPA make a complex with higher selectivity than either factor alone (14, 15). However, even though we have obtained XPA-RPA-DNA and XPA-XPC-DNA complexes we have been unable to detect an XPA-RPA-XPC-DNA complex under a variety of conditions tested in the band shift assay. In the footprinting assay the XPC-induced DNase I-hypersensitive site was enhanced by both XPA and RPA and more so by the XPA + RPA combination. However, the latter observation cannot be taken as evidence for the presence of a DNA-protein complex encompassing all three repair factors. Thus, while the concerted damage recognition model has not been rigorously eliminated, neither is there direct experimental support for such a model.

**Sequential Recognition Model**—In its most explicit form this model means that there is a rigid order for assembly of the repair factors at the damage site. High specificity binding is achieved by a cascade whereby the selectivities of XPA, RPA, and XPC are multiplied instead of being added up as in the concerted model. Since there are three proteins with documented specificity for damaged DNA, theoretically, there are 6 possible orders of assembly to make a DNA-protein complex containing all three proteins should such a complex exist. However, because two studies (14, 15) have demonstrated that XPA and RPA make a relatively tight complex with a higher discriminatory power than either subunit alone, the possibilities can be reduced to two: either XPA-RPA bind first or XPC binds first. Based on repair kinetics under a variety of order of addition experiments, a previous study concluded that when damaged DNA was incubated with XPC first the reaction proceeded faster and therefore it was concluded that XPC is the first protein to bind to damaged DNA (19). In the present study we find the opposite: when the substrate is incubated with XPA, RPA, or XPA + RPA first, the reaction proceeds at a faster rate than the reaction in which the DNA was first incubated with XPC. We do not have a satisfactory explanation for these contradictory results. However, it might be useful to point out the differences in the ways the two studies were performed.

The previous study used RPA-depleted cell extracts from XPA- or XPC-mutant cell lines as the source of excision nuclease subunits; it employed heavily damaged plasmid DNA (1 acetylaminofluorene-guanine adduct per 100 bp) as substrate;

repair was assessed by a coupled-enzyme assay whereby the excision nuclease generated gaps were filled in by T4 DNA polymerase; only about 0.1% of the DNA was repaired in 30 min; and, finally, the background “repair synthesis” (incorporation of radiolabel into undamaged DNA) could be as high as 50% of the repair signal in some of the experiments. In contrast, in this study we did the following: (i) we used repair factors purified to near homogeneity; (ii) the substrate was a 136-bp duplex with a single (6-4) photoproduct in the center; (iii) repair was quantitated by measuring the primary reaction, the excision of the damage; (iv) in standard reactions 10–30% of the damage was excised in 30 min; (v) there is virtually no background in the excision assay (40, 41) employed in our study. Whether these experimental differences explain the contradictory results remains to be determined. However, under no circumstances, including using cell-free extract for the repair assay, have we observed faster repair rates by incubating the substrate first with XPC and then supplementing with the other repair factors in the form of cell-free extract from an XPC mutant cell line (data not shown). Thus, we conclude that if the assembly of human excision nuclease is by a sequential mechanism XPC cannot be the first factor to bind to the damage sites.

### Model for Assembly of Human Excision Nuclease

In the following we present a model for human excision nuclease assembly with a brief summary of supporting evidence for each step (Fig. 9).

**Closed Complex**—RPA alone or the RPA-XPA complex binds to the damage site. In either case, eventually an RPA-XPA-DNA complex forms at the site of DNA lesion. The DNA is not unwound at this stage beyond the intrinsic unwinding caused by certain lesions (45, 46), hence the designation of “closed complex.” Experimental evidence for this step include the following. First, RPA is an abundant cellular protein (47) and, of the three excision repair proteins with preference for damaged DNA, it is the one with the highest selectivity factor. Second, RPA and XPA are required for recognition and removal of all DNA lesions regardless of the type of lesion or the DNA structure around or in the vicinity of the lesion (4, 18, 48) including transcription bubble (49). Third, in the defined excision nuclease system preincubation of DNA with RPA or RPA + XPA leads to faster rates of repair relative to DNA preincubated with XPC.

**Preincision Complex 1**—The RPA-XPA-DNA ternary complex recruits the TFIIH-XPC complex (50), to the site of damage through specific interactions of XPA with TFIIH (51, 52) and possibly XPC. The resulting complex contains the four repair factors, and the DNA is actively unwound hence the name preincision complex 1 (16, 18). The following findings are consistent with PIC1 being the second step along the pathway of excision nuclease assembly. First, although XPC has the highest affinity for damaged DNA among all repair factors it has poor selectivity for damage and, as the kinetic experiments in this paper reveal, it must enter the complex after XPA and RPA for an optimum excision reaction. Second, damage recognition and excision occur in the absence of XPC under a variety of conditions: certain synthetic lesions (4), thymine dimers adjacent to or within 10–20 bp of mismatch bubbles (18, 48), and thymine dimers blocking progression of RNA polymerase II (49) are recognized and removed without XPC. Third, we have been unable to detect an XPA-RPA-XPC-DNA complex by a variety of methods but we do detect a specific XPA-RPA-TFIIH-XPC-DNA complex suggesting that TFIIH and XPC are recruited together. Indeed, XPC and TFIIH make a moderately stable complex (50) and the C terminus of XPA specifically



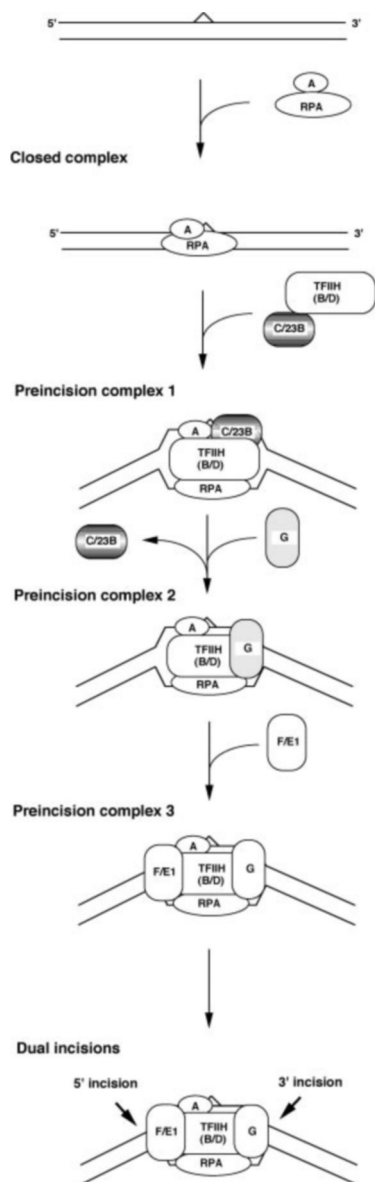


FIG. 9. **Model for assembly of human excision nuclease.** XPA and RPA make a low specificity complex at the damage site which becomes more discriminatory upon recruiting XPC and TFIH (PIC1). Specificity is sequentially enhanced by the addition of XPG (PIC2) and the entry of XPF-ERCC1 (PIC3). PIC3 is a short-lived complex as the repair factors begin to dissociate as soon as the dual incisions are made.

interacts with TFIH (51, 52).

**Preincision Complex 2**—The XPG protein is recruited to PIC1 to form the more stable PIC2. Entry of XPG to PIC1 is accompanied by departure of XPC from the complex (16). However, it is doubtful that XPC protein plays a direct role in recruiting XPG because PIC2 (XPA-RPA-TFIH-XPG-DNA) also forms under conditions (e.g. a thymine dimer adjacent to a bubble) where "PIC1" does not actually contain XPC but otherwise is similar to regular PIC1 in the sense of having XPA, RPA, TFIH, and unwound DNA. XPG is known to interact with TFIH specifically (3) and it is most likely that it is recruited to PIC1 by TFIH. It is possible that the XPC-binding site on TFIH overlaps with the XPG-binding site such that entry of one of these repair factors into the complex forces the other one out. The failure to detect XPC in PIC2 by supershift assays provides direct experimental evidence of this step.

**Preincision Complex 3**—The XPF-ERCC1 complex is re-

cruited to PIC2 by specific interaction of ERCC1 with XPA (53, 54) and of XPF with RPA (7, 23). Although free XPA can bind XPF-ERCC1, this heterodimer cannot bind PIC1 (XPA-RPA-XPC-TFIH-DNA) to make a stable complex but binds to PIC2 (XPA-RPA-TFIH-XPG-DNA) readily (16) to form PIC3. Whether or not XPG plays an active role in recruiting XPF-ERCC1 is not known at present but RPA appears to play a secondary role (7).

**Excision**—Dual incisions are made by XPG and XPF-ERCC1. Although under certain experimental conditions 3' incision (which occurs first) can be uncoupled from 5' incision (4) and in PIC3 formed with an active site mutant XPG 5' incision can occur in the absence of 3' incision (55), under optimal reaction conditions the dual incisions occur in a concerted manner. The dual incisions result in excision and release of the damage containing oligonucleotide (24–32 nucleotides) without the aid of additional repair factors (4). The status of the postexcision complex prior to gap filling by repair synthesis awaits further studies.

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#### REFERENCES

- Sancar, A. (1996) *Annu. Rev. Biochem.* **65**, 43–81
- Wood, R. D. (1996) *Annu. Rev. Biochem.* **65**, 135–167
- Mu, D., Park, C.-H., Matsunaga, T., Hsu, D. S., Reardon, J. T., and Sancar, A. (1995) *J. Biol. Chem.* **270**, 2415–2418
- Mu, D., Hsu, D. S., and Sancar, A. (1996) *J. Biol. Chem.* **271**, 8285–8294
- O'Donovan, A., Davies, A. A., Moggs, J. G., West, S. C., and Wood, R. D. (1994) *Nature* **371**, 432–445
- Matsunaga, T., Mu, D., Park, C. H., Reardon, J. T., and Sancar, A. (1996) *J. Biol. Chem.* **270**, 20862–20869
- Matsunaga, T., Park, C. H., Bessho, T., Mu, D., and Sancar, A. (1996) *J. Biol. Chem.* **271**, 11047–11050
- Jones, C. J., and Wood, R. D. (1993) *Biochemistry* **32**, 12096–12104
- Asashina, H., Kuraoka, I., Shirakawa, M., Morita, E. H., Miura, N., Miyamoto, I., Ohtsuka, E., Okada, Y., and Tanaka, K. (1994) *Mutat. Res.* **315**, 229–237
- Clugston, C. K., McLaughlin, K., Kenny, M. K., and Brown, R. (1992) *Cancer Res.* **52**, 6375–6379
- Burns, J. L., Guzder, S. N., Sung, P., Prakash, S., and Prakash, L. (1996) *J. Biol. Chem.* **271**, 11607–11610
- Patrick, S. M., and Turchi, J. J. (1998) *Biochemistry* **37**, 8808–8815
- Reardon, J. T., Mu, D., and Sancar, A. (1996) *J. Biol. Chem.* **271**, 19451–19456
- He, Z., Henrickson, L. A., Wold, M. S., and Ingles, C. J. (1995) *Nature* **374**, 566–569
- Li, L., Lu, X., Peterson, C. A., and Legerski, R. J. (1995) *Mol. Cell. Biol.* **15**, 5396–5402
- Wakasugi, M., and Sancar, A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 6669–6674
- Evans, E., Fellows, J., Coffey, A., and Wood, R. D. (1997) *EMBO J.* **16**, 625–638
- Mu, D., Wakasugi, M., Hsu, D., and Sancar, A. (1997) *J. Biol. Chem.* **272**, 28971–28979
- Sugasawa, K., Ng, J. M. Y., Masutani, C., Iwai, S., van der Spek, P. J., Eker, A. P. M., Hanaoka, F., Bootsma, D., and Hoeijmakers, J. H. J. (1998) *Mol. Cell* **2**, 223–232
- Guzder, S. N., Sung, P., Prakash, L., and Prakash, S. (1998) *J. Biol. Chem.* **273**, 31541–31546
- Jansen, L. E. T., Verhage, R. A., and Brouwer, J. (1998) *J. Biol. Chem.* **273**, 33111–33114
- Mu, D., Tursun, M., Duckett, D. R., Drummond, J. T., Modrich, P., and Sancar, A. (1997) *Mol. Cell. Biol.* **17**, 760–769
- Bessho, T., Sancar, A., Thompson, L. H., and Thelen, M. P. (1997) *J. Biol. Chem.* **272**, 3833–3837
- Van Houten, B., Gamper, H., Sancar, A., and Hearst, J. E. (1987) *J. Biol. Chem.* **262**, 13180–13187
- Von Hippel, P. H., and Berg, O. G. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 6443–6447
- Masutani, C., Sugawara, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., Takio, K., Tanaka, K., van der Spek, P. J., Bootsma, D., Hoeijmakers, J. H. J., and Hanaoka, F. (1994) *EMBO J.* **13**, 1831–1843
- Masutani, C., Araki, M., Sugawara, K., van der Spek, P. J., Yamada, A., Uchida, A., Maekawa, J., Bootsma, D., Hoeijmakers, J. H. J., and Hanaoka, F. (1996) *Mol. Cell Biol.* **17**, 6915–6923
- Duckett, D. R., Drummond, J. T., Murchie, A. I. H., Reardon, J. T., Sancar, A., Lilley, D. M. J., and Modrich, P. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 6443–6447
- Yamada, M., O'Regan, E., Brown, R., and Karran, P. (1997) *Nucleic Acids Res.* **25**, 491–496
- Pil, P. M., and Lippard, S. J. (1992) *Science* **256**, 234–237
- Hughes, E. N., Engelsberg, B. N., and Billings, P. C. (1992) *J. Biol. Chem.* **267**, 13520–13527

32. Feldberg, R. S., and Grossman, L. (1976) *Biochemistry* **15**, 2402–2408
33. Hwang, B. J., and Chu, G. (1993) *Biochemistry* **32**, 1657–1666
34. Reardon, J. T., Nichols, A. F., Smith, C. A., Taylor, J. S., Linn, S., and Sancar, A. (1993) *J. Biol. Chem.* **268**, 21301–21308
35. Nichols, A. F., Ong, P., and Linn, S. (1996) *J. Biol. Chem.* **271**, 24317–24320
36. Coin, F., Frit, P., Violet, B., Salles, B., and Egly J. M. (1998) *Mol. Cell. Biol.* **18**, 3907–3914
37. Huang, J. C., Zamble, D. B., Reardon, J. T., Lippard, S. J., and Sancar, A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 10394–10398
38. Kazantsev, A., Mu, D., Nichols, A. F., Zhao, X., Linn, S., and Sancar, A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 5014–5018
39. Paric Otrin, V., Kuraoka, I., Nardo, T., McLenigan, M., Eker, A. P. M., Stefanini, M., Levine, A. S., and Wood, R. D. (1998) *Mol. Cell. Biol.* **18**, 3182–3190
40. Huang, J. C., Svoboda, D. L., Reardon, J. T., and Sancar, A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 3664–3668
41. Huang, J. C., Hsu, D. S., Kazantsev, A., and Sancar, A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 12213–12217
42. He, Z., and Ingles, C. J. (1997) *Nucleic Acids Res.* **25**, 1136–1141
43. Rodriguez, K., Talamantez, J., Huang, W., Reed, S. H., Wang, Z., Chen, L., Feaver, W. J., Friedberg, E. C., and Tomkinson, A. E. (1998) *J. Biol. Chem.* **273**, 34180–34189
44. Bertrand, P., Tishhoff, D. X., Fioshi, N., Dasgupta, R., and Kolodner, R. D. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 14278–14283
45. Guntz, D., Hess, M. T., and Naegeli, H. (1996) *J. Biol. Chem.* **271**, 25089–25098
46. Hess, M. T., Schwiffer, V., Petretta, M., Giese, B., and Naegeli, H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 6664–6669
47. Wold, M. S. (1997) *Annu. Rev. Biochem.* **66**, 61–92
48. Mu, D., and Sancar, A. (1997) *J. Biol. Chem.* **272**, 7570–7573
49. Venema, J., van Hoffen, A., Karcagi, V., Natrajan, A. T., van Zeeland, A. A., and Mullenders, L. H. F. (1991) *Mol. Cell. Biol.* **11**, 4128–4134
50. Drapkin, R., Reardon, J. T., Ansari, A., Huang, J. C., Zawel, L., Ahn, K., Sancar, A., and Reinberg, D. (1994) *Nature* **368**, 769–772
51. Park, C. H., Mu, D., Reardon, J. T., and Sancar, A. (1995) *J. Biol. Chem.* **270**, 4896–4902
52. Nocentini, S., Coin, F., Saijo, M., Tanaka, K., and Egly, J. M. (1997) *J. Biol. Chem.* **272**, 22991–22994
53. Li, L., Elledge, S. J., Peterson, C. A., Bales, E. S., and Legerski, R. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 5012–5016
54. Park, C. H., and Sancar, A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 5017–5021
55. Wakasugi, M., Reardon, J. T., and Sancar, A. (1997) *J. Biol. Chem.* **272**, 16030–16034
56. Kim, G., and Wold, M. S. (1995) *Biochemistry* **34**, 2058–2064