

# Interactions Involving the Human RNA Polymerase II Transcription/Nucleotide Excision Repair Complex TFIIH, the Nucleotide Excision Repair Protein XPG, and Cockayne Syndrome Group B (CSB) Protein<sup>†</sup>

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Received October 9, 1995; Revised Manuscript Received December 19, 1995<sup>⊗</sup>

**ABSTRACT:** The human basal transcription factor TFIIH plays a central role in two distinct processes. TFIIH is an obligatory component of the RNA polymerase II (RNAP II) transcription initiation complex. Additionally, it is believed to be the core structure around which some if not all the components of the nucleotide excision repair (NER) machinery assemble to constitute a nucleotide excision repairosome. At least two of the subunits of TFIIH (XPB and XPD proteins) are implicated in the disease xeroderma pigmentosum (XP). We have exploited the availability of the cloned *XPB*, *XPD*, *p62*, *p44*, and *p34* genes (all of which encode polypeptide subunits of TFIIH) to examine interactions between *in vitro*-translated polypeptides by co-immunoprecipitation. Additionally we have examined interactions between TFIIH components, the human NER protein XPG, and the CSB protein which is implicated in Cockayne syndrome (CS). Our analyses demonstrate that the XPB, XPD, p44, and p62 proteins interact with each other. XPG protein interacts with multiple subunits of TFIIH and with CSB protein.

Transcription mediated by RNA polymerase II (RNAP II)<sup>1</sup> in human cells requires the participation of multiple initiation factors (Chalut et al., 1994). Among these is a complex of polypeptides called transcription factor IIH (TFIIH)<sup>1</sup> comprising nine polypeptides encoded by the *XPB* (*ERCC3*), *XPD* (*ERCC2*), *p62*, *p52*, *p44*, *p34*, *CYCH*, *MO15*, and *MAT1* genes [reviewed in Drapkin and Reinberg (1994), Roy et al. (1994), Sheikhattar et al. (1995), and J.-M. Egly, personal communication]. The *XPB* (*ERCC3*) and *XPD* (*ERCC2*) genes encode polypeptides of 89 and 80 kDa respectively (reviewed in Drapkin & Reinberg, 1994). Both genes are required for the process of nucleotide excision repair (NER)<sup>1</sup> in addition to their role in RNA II transcription. Consistent with this requirement individuals from the XP-B and XP-D genetic complementation groups of the hereditary disease xeroderma pigmentosum (XP)<sup>1</sup> are defective in NER and are exceptionally prone to skin cancer associated with sunlight exposure [reviewed in Friedberg et al. (1995)].

Individuals from the XP-B, XP-D, and XP-G complementation groups sometimes manifest clinical features typical of a second hereditary disorder called Cockayne syndrome (CS)<sup>1</sup> (Broughton, et al., 1995; Nance & Berry, 1992; Vermeulen et al., 1993, 1994; Weeda et al., 1990). The combined XP/CS state results in severe developmental and neurological problems in addition to the dermatological features of XP (Nance & Berry, 1992). CS can also present

without the clinical features of XP (Nance & Berry, 1992). Such patients belong to one of two genetic complementation groups designated CS-A and CS-B [reviewed in Friedberg et al. (1995)]. The *CSA* and *CSB* genes have recently been cloned and can encode proteins of ~168 and ~46 kDa, respectively (Henning et al., 1995; Troelstra et al., 1992). The function of these proteins is presently unknown. Cells from CS-A and CS-B individuals are abnormally sensitive to UV radiation and are defective in the preferential repair of the template strand of transcriptionally active genes (Venema et al., 1990).

A further genetic complexity stems from the observation that certain individuals with a third hereditary disorder called trichothiodystrophy (TTD)<sup>1</sup> are also defective in NER [reviewed in Friedberg et al. (1995)]. Some of these TTD patients have been shown to carry mutations in the *XPD* gene (Broughton et al., 1994; Stefanini et al., 1986, 1993a,b). Additionally, the NER-defective phenotype of cells from a TTD individual has been shown to be corrected by micro-injection of purified XPB protein, thereby implicating the *XPB* gene in TTD (Vermeulen et al., 1994b).

The multiple subunits of human TFIIH are genetically and functionally conserved in the yeast *Saccharomyces cerevisiae*. The human XPB and XPD proteins, are represented as the Ssl2 (Rad25) and Rad3 proteins respectively (Gulyas & Donohue, 1992; Park et al., 1992), and the yeast homologs of the p62 and p44 proteins are called Tfb1 (Gileadi et al., 1992) and Ssl1, respectively (Humbert et al., 1994). As is the case with human TFIIH components, the yeast Rad3, Ssl2, Tfb1, and Ssl1 proteins are required for both NER and RNAP II transcription (Feaver et al., 1993; Wang et al., 1994, 1995). The architecture of yeast TFIIH (designated factor b in earlier literature) has been partially defined by analyzing interactions between some of its subunits (Bardwell, L., 1992; Bardwell, L., et al., 1994; Bardwell, A. J., et al., 1994). Ssl2 protein interacts specifically with Rad3 protein, which in turn

<sup>†</sup> Supported by Research Grant CA44247 from the USPHS to E.C.F.

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<sup>⊗</sup> Abstract published in *Advance ACS Abstracts*, February 1, 1996.

<sup>1</sup> Abbreviations: RNAP II, RNA polymerase II; TFIIH, transcription factor IIH; NER, nucleotide excision repair; XP, xeroderma pigmentosum; CS, Cockayne syndrome; TTD, trichothiodystrophy; RPA, replication protein A.

interacts with Ssl1 protein. The latter also interacts with Tfb1 protein.

Both human and yeast TFIIH stably associate with other proteins required for NER but which have no known function in RNAP II transcription. For example, purification of human TFIIH often yields a complex which is stably associated with XPG (Mu et al., 1995) or XPC protein (Drapkin et al., 1994). Additionally, human XPA protein interacts with XPB protein and with purified TFIIH *in vitro* (Park et al., 1995). XPA protein also interacts with the NER proteins ERCC1 and XPF and with replication protein A (RPA)<sup>1</sup> (Matsuda et al., 1995), which is also required for NER (Aboussekhra et al., 1995; Mu et al., 1995). A stable multiprotein complex designated the nucleotide excision repairosome has been identified in extracts of yeast cells (Svejstrup et al., 1995). This complex includes the six known subunits of yeast TFIIH (Ssl2, Rad3, Ssl1, Tfb1, Tfb2, Tfb3) as well as the NER proteins Rad1, Rad2, Rad4, Rad10, and Rad14 (Svejstrup et al., 1994, 1995). Yeast Rad2 protein has been shown to specifically interact with both the Ssl2 and Tfb1 subunits (Bardwell, A. J., et al., 1994b).

A definition of protein–protein interactions in human TFIIH is expected to facilitate an understanding of the assembly of the putative human nucleotide excision repairosome. Additionally, a definition of such interactions may provide insights as to how mutations in the *XPB*, *XPD*, and *XPG* genes can result in the simultaneous clinical features of XP and CS or the clinical features of TTD. In the present study we have utilized the techniques of *in vitro* transcription and translation of the cloned human *XPB*, *XPD*, *p62*, *p44*, and *p34* genes to examine interactions between the proteins they encode and to map domains required for these interactions. Our results indicate interactions involving all five of these subunits of TFIIH. However, we have identified some differences between specific protein–protein interactions in human and yeast TFIIH. Additionally we report here that the human NER protein XPG interacts with multiple components of TFIIH and, surprisingly, with the protein encoded by the *CSB* gene.

## EXPERIMENTAL PROCEDURES

Plasmid pGEM4Z and RNasin RNase inhibitor were purchased from Promega Biotechnology. *In vitro* synthesis of mRNA was carried out using either SP6 or T7 mMessage mMachine mRNA synthesis kit from Ambion. Rabbit reticulocyte lysates for *in vitro* translation of mRNA were obtained from Novagen. *In vitro* translation grade [<sup>35</sup>S]-methionine, Amplify fluorographic reagent, and prestained molecular weight markers were from Amersham. Ready protein scintillation cocktail was from Beckman. Buffer-saturated phenol was from Amresco. Caffeine, isopropyl thiogalactoside (IPTG), and 5-bromo-4-chloro-3-indoyl phosphate (X-gal) were from Sigma. Monoclonal antibodies to c-Fos (Ab-2) and to c-Myc, recognizing the 9E10 epitope (Ab1), and protein A-agarose were from Oncogene Science. Restriction enzymes were from Bethesda Research Laboratories, New England BioLabs, or Promega. Chemicals and reagents for SDS–polyacrylamide gel electrophoresis (SDS–PAGE) were from Bio-Rad.

**Transcription Plasmid Constructions.** All 9E10 epitope-tagged constructs were generated by cloning the gene encoding a particular TFIIH component into the pGEM4Z–

9E10 vector described previously (Bardwell, L., 1992; Bardwell, L., et al., 1992; Bardwell, A. J., et al., 1994). This vector contains a sequence encoding 10 amino acids of the 9E10- epitope (Kolodziej & Young, 1991) followed by the polylinker in the SP6 orientation. Cloning in-frame in these sites followed by *in vitro* transcription from the SP6 promoter generated mRNA that was translated to yield 9E10 epitope-tagged fusion proteins. These constructs are designated with the prefix 9E. Cloning in the same polylinker but in the opposite orientation resulted in constructs that produced nontagged mRNA following transcription from the T7 promoter. For some genes mRNA was synthesized by cloning in the vector pT3/T7 (Bardwell, 1992) in the T7 orientation. These were translated into native proteins. All constructs were verified by restriction mapping.

**XPB Gene.** Plasmids p9E-XPB and pXPB were constructed by cloning two PCR- amplified products representing the *XPB* gene. The 5′-end of the gene was amplified using primers 5′-ATAGGGATCCATGGGCAAAGAGACCGA-3′ (A) and 5′-GTAGCCATTATTCTGCAGCTCCATC-CAG-3′ (F). Primers (A) and (F) carried *Bam*HI and *Pst*II restriction sites (underlined), respectively. The 3′-region of the gene was amplified by PCR using primers 5′-CTG-GATGGAGCTGCAGAATAATGGCTAC-3′ (E) and 5′-TATCGGATCCTGCCTAAGCATCATTTTCCT-3′ (B). These have restriction sites for *Pst*II and *Bam*HI, respectively (B). The stop codon introduced by primer B at the end of the *XPB* coding region is highlighted. These two PCR products represent the entire coding region of the *XPB* gene and were used to reconstitute full-length *XPB* cDNA. Digestion of the 9E-XPB and XPB clones with the restriction enzymes *Bgl*II, *Eco*RV, and *Afl*III generated run-off transcripts that were translated *in vitro* to yield the epitope-tagged or nontagged proteins XPB<sub>1–387</sub>, XPB<sub>1–305</sub>, and XPB<sub>1–198</sub>, respectively.

**XPD Gene.** The plasmid construct that encodes the cDNA for the *XPD* gene has been previously described (Frederick et al., 1994). This construct, designated pXPD, was used to synthesize mRNA from the T7 promoter to yield native XPD protein following *in vitro* translation. Plasmid p9E-XPD was constructed by three-way ligation of the 5′-end of the gene on a *Bam*HI-*Sph*I fragment and the 3′-region on an *Sph*I-*Hind*III fragment. *In vitro* translation of mRNA templates derived from pXPD digested with *Pvu*II, *Eag*I, *Hinc*II, and *Nco*I generated the polypeptides XPD<sub>1–434</sub>, XPD<sub>1–324</sub>, XPD<sub>1–248</sub>, and XPD<sub>1–162</sub>, respectively. Similarly the 9E-XPD construct yielded the epitope-tagged polypeptides 9E-XPD<sub>1–434</sub>, 9E-XPD<sub>1–324</sub>, and 9E-XPD<sub>1–248</sub>.

**p62 Gene.** The *p62* gene was amplified by PCR using primers 5′-GGGGGATCCACCATGGCAACCTCATCT-GAA-3′ and 5′-GGGGATCCGTAAGCATCATGGCCAC-CTCA-3′. Both primers have a *Bam*HI site (underlined) that facilitated cloning of this gene in-frame into the *Bam*HI site of pGEM4Z9E10, to generate p9Ep62. Epitope-tagged p62 protein was synthesized from this construct by translation of mRNA directed from the SP6 promoter. Plasmid constructs carrying the *p62* gene in the opposite orientation yielded native p62 mRNA. Nontagged p62 mRNA did not translate full-length polypeptide of 62 kDa, unlike epitope-tagged p62 mRNA.

**p44 Gene.** The *p44* gene was amplified using the primers 5′-TGAAAGGATCCATGAAGAACCTGAAAGAACT-3′ and 5′-GACCGGATCCTCAAACACCTGAAGGAGCT-

GGA-3'. The *Bam*HI sites (underlined) allowed cloning of the gene in either orientation in the pGEM9E10 vector. The plasmid construct p9Ep44 was used to produce 9E10 epitope-tagged mRNA from the SP6 promoter. A clone carrying the *p44* insert in the opposite orientation (pNTp44) generated native *p44* mRNA from the T7 promoter. Run-off transcription from either construct linearized at the *Nhe*I and *Sca*I restriction sites yielded the corresponding truncated polypeptides, *p44*<sub>1–240</sub> and *p44*<sub>1–166</sub> or 9E-*p44*<sub>1–240</sub> and 9E-*p44*<sub>1–166</sub>.

*p34 Gene.* The *p34* gene was amplified by PCR using primers 5'-TATGAATTCATGGTTTCAGACGAAGATGAATTGAAT-3' and 5'-TGGTCTAGATCAGTTTCTTTCTTGCTTTCAGCAC-3'. These primers introduced *Eco*RI and *Xba*I sites (underlined). This allowed directional cloning of the *p34* gene in the pGEM9E10 vector (p9E-*p34*) to produce epitope-tagged mRNA from the SP6 promoter and in the pT3/T7 vector to produce *p34* mRNA from the T7 promoter. Run-off transcription from either constructs linearized at *Hpa*II and *Bam*HI restriction sites generated the truncated polypeptides *p34*<sub>1–245</sub> and *p34*<sub>1–134</sub>, respectively, or 9E-*p34*<sub>1–245</sub> and 9E-*p34*<sub>1–134</sub>.

*XPG Gene.* Plasmids p9E-XPG and pXPG were constructed using two truncated clones isolated from a HeLa cell library. Restriction mapping, sequencing, and subcloning yielded a truncated clone missing a 1.2 kb region from the N-terminus. This region was recovered by RT-PCR of human B-cell RNA using a 3' gene specific primer XPG.GSP2R, 5'-GGCGGATCCGTCTGCACATCATCC-CAG-3' and further amplification of the first-strand cDNA using a 5' primer, XPG.GSP22F, 5'-CCCCCGGGATC-CGCTCTTAGGACGCAG-3'. The *Bam*HI restriction sites used in cloning the RT-PCR product is underlined. This product was built into the truncated construct to go in-frame and generate a full-length XPG cDNA. This cDNA was subcloned in two steps, first as a *Bam*HI-*Xba*I (3'-end) fragment followed by a *Bam*HI fragment covering the remaining 5'-region, into the *Bam*HI site of either pGEM 4Z vector or pGEM 4Z-9E10 vector carrying the epitope-tag. Therefore, the synthesis of both the epitope-tagged and nontagged XPG mRNA was directed from the SP6 promoter. Digestion of the 9E-XPG and XPG gene with the restriction enzyme *Acc*I generated run-off transcripts that were translated *in vitro* to yield 9EXPG<sub>1–377</sub> or XPG<sub>1–377</sub>, respectively.

Plasmid constructs expressing the epitope-tagged internal domains of the *XPG* gene were generated by specifically amplifying an appropriate region and cloning it in-frame. The C-domain was amplified using the primer CDM.FOR 5'-CGGGATCCAACAGCAGAATTCAGTAAAGC-3' and primer CDM.REV 5'-CGGGATCCAGTTTTCAGTGAAT-TCTGCTGTGC-3'. The acidic region preceding the C-domain was amplified using the primer AD.FOR 5'-CGGGATCCAGCATGAGAATTTTCTGGAAAC 3' and primer AD.REV 5'-CGGGATCCAGTTTTCAGTGAAT-TCTGCTGTGC-3'. The *Bam*HI restriction site involved in cloning these fragments is underlined. All constructs were linearized at the *Sal*I site before transcription from the SP6 promoter to generate specific epitope-tagged mRNA.

*CSB Gene.* Plasmid p9E-CSB used in this study was constructed by cloning two PCR products representing the CSB gene that were amplified from a human cDNA library. The 5' portion of the gene was amplified using primers (CSB.FOR) 5'-CAGGGATCCATGCCAAATGAGGGAAT-CCCCACTCA-3' and (6IRR) 5'-TTGTAAGCAGTTTTGA-

CCTGTACTGGGGAAGCATTGGAATATC-3'. The 3' half of the CSB gene was amplified with primer (6ILF) 5'-CTGTGTTTATGGAGCAGTTCTCCGTCCCCATCAC-3' and (CSB.Rev) 5'-AAGGGATCCTGTTTAGCAGTAT-TCTGGCTTGAGTTT-3'. These two PCR products represent the entire coding region of the CSB gene. The two fragments were cloned in-frame by a three-way ligation using an internal unique *Nco*I site in the overlapping region. The flanking *Bam*HI restriction sites built into the CSB.FOR and CSB.Rev primers that were used for cloning the full-length CSB gene into the pGEM 4Z-9E10 vector is underlined.

*In Vitro Transcription.* All templates for *in vitro* transcription using either SP6 or T7 polymerases were generated by linearizing with an appropriate restriction enzyme at the 3'-end of the coding region, or internally for C-terminal deletions. Templates were purified by phenol-chloroform extraction followed by passage through spin columns. mRNA was synthesized *in vitro* by using an SP6 or T7 mMessage mMachine kit per the manufacturer's recommendations (Ambion). The mRNA was purified by phenol-chloroform extraction and passed through a G-50 spin column.

*In Vitro Translation.* Purified mRNA was used to program specific translation reactions with rabbit reticulocyte lysates (Novagen) in the presence of [<sup>35</sup>S]methionine at 30 °C. Translation of epitope-tagged mRNA and control translations that lacked mRNA were performed under identical conditions except that these reactions did not contain labeled methionine. All translation products were partially purified by ammonium sulfate precipitation as described previously (Bardwell et al., 1992).

*Immunoprecipitation.* Immunoprecipitation reactions were carried out in immunoprecipitation buffer (buffer A), consisting of 20 mM Tris (pH 7.5), 75 mM KCl, 5 mM sodium bisulfite, 4 mM MgCl<sub>2</sub>, 0.5 mM EDTA, 1 mM DTT, 0.1% Tween 20, 12.5% glycerol, and the protease inhibitors pepstatin (1 µg/mL), leupeptin (1 µg/mL), and PMSF (1 mM). Immunoprecipitation was carried out essentially as previously described (Bardwell, L., et al., 1992, 1994; Bardwell, A. J., et al., 1994). Briefly, partially purified *in vitro*-translated proteins were resuspended in buffer A at three times the translation volume. Aliquots (60 µL) each of the two interacting proteins under analysis were mixed with 80 µL of buffer A and incubated at 30 °C for 30 min. These reactions were incubated for 1 h at room temperature in the presence of 9E10 antibody-protein A-agarose complexes on a rocking platform. Protein complexes were pelleted and washed at least three times with buffer A. Following resuspension, aliquots were taken for measuring radioactivity by liquid scintillation counting. Samples were further analyzed by SDS-PAGE by running equal volumes of test and control reactions on 10% or 15% gels followed by fluorography. Protein-protein interactions were evaluated by comparing the amount of radioactivity immunoprecipitated by the antibody in the presence of epitope-tagged protein to that precipitated nonspecifically in the absence of epitope-tagged protein (background). Levels of radioactivity at least 3-fold above background were considered positive after multiple independent experiments.

*Yeast Two-Hybrid System.* All of the genes were cloned into either the pAS1 (Durfée et al., 1993) vector, which when introduced into yeast cells results in production of the Gal4 DNA-binding domain fused to the human protein, or into

Table 1: Summary of Protein–Protein Interactions

proteins tested	interaction observed by co-immunoprecipitation
XPB/XPB	+
XPB/p62	+
XPB/p44	+
XPB/p34	+
XPB/p62	+
XPB/p44	+
XPB/p34	+
p34/p44	+
p34/p62	–
p44/p62	– (positive in two-hybrid system)
XPD/XPB	+
XPD/p62	+
XPD/CSB	+
XPD/XPB	+
XPD/p44	+
XPD/p34	–

the pACT (Durfee et al., 1993) vector, resulting in the production of the Gal4 transcriptional activation domain fused to the human protein. The plasmids were introduced into the strain Y190 [a *cyh<sup>r</sup>* derivative of Y153 (Durfee et al., 1993)], and  $\beta$ -galactosidase activities were determined as described (Bardwell, A. J., et al., 1993). For each combination of plasmids eight to ten individual transformants were grown and assayed. The plasmids were constructed as follows:

**pACTXPB:** The XPB gene was amplified by PCR from pGEMXPB using primers 5'-GGGGATCCGAATGGG-CAAAAGAGACCG-3' and 5'-GGGGATCCTCATTTCTAAAGCGCTT-3'. Both primers have a *Bam*HI site (underlined) that facilitated cloning of the gene in-frame into the *Bam*HI site of pACT.

**pAS1p62:** The p62 gene was amplified by PCR from pGEMp62 using primers 5'-GGGGATCCGAATGGCAACCTCATCTGA-3', containing a *Bam*HI site (underlined) and 5'-GGGGATCCGTCGACTCACGTTTCTTCATCAG-3', containing a *Sal*I site (underlined). The PCR product was cloned into the pAS1 vector at the *Bam*HI and *Sal*I sites.

**pACTp44:** The p44 gene was PCR amplified using primers 5'-GGGGATCCGAATGGATGAAGAACCTG-3', containing a *Bam*HI site (underlined) and 5'-GGGGATCCGTCGACTCAAACACCTGAAGGAG-3', containing a *Sal*I site (underlined). The PCR product was cloned into the pACT vector using the *Bam*HI and *Xho*I sites.

## RESULTS

**Protein–Protein Interactions in the TFIIH Complex.** We examined co-immunoprecipitation of TFIIH subunits incubated in multiple pairwise combinations following their translation *in vitro* as epitope-tagged or native proteins. Native (nontagged) proteins were radiolabeled with [<sup>35</sup>S]-methionine and monitored for co-precipitation with epitope-tagged proteins by monoclonal antibodies to the human-specific c-Myc epitope 9E10 (see Materials and Methods). Protein–protein interactions were considered positive only when they reproducibly yielded levels of radioactivity in the precipitates more than 3-fold over background (see Materials and Methods). This general technique for monitoring protein–protein interactions is well established, and specific refinements of the technique (Bardwell, L., 1992; Bardwell, L., et al., 1992) have been successfully used in previous studies on interactions between yeast NER proteins which

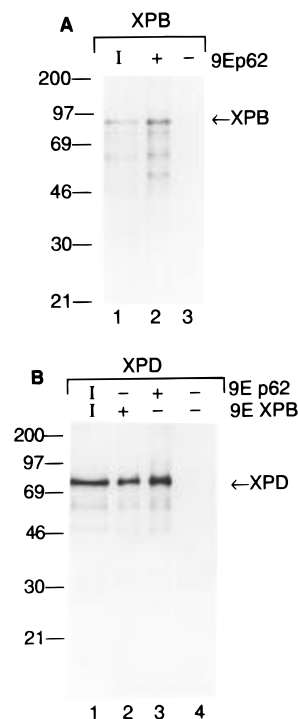
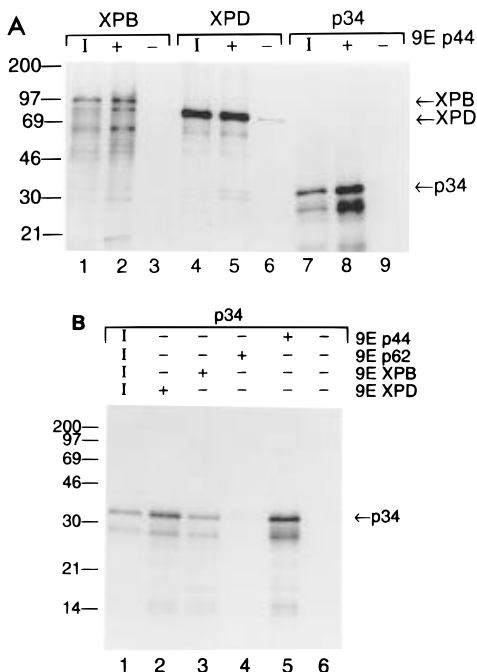


FIGURE 1: Interactions between XPB, XPD, and p62 proteins. (A) XPB protein was translated *in vitro* in the presence of [<sup>35</sup>S]-methionine and precipitated with ammonium sulfate (lane 1; I = input) or mixed with *in vitro*-translated epitope-tagged p62 protein (9Ep62) and precipitated in the presence of monoclonal antibodies to the 9E10 epitope (lane 2). Lane 3 is a control precipitation without *in vitro*-translated epitope-tagged p62 protein. Equal volumes (15  $\mu$ L) of the immunoprecipitates with 9E10 monoclonal antibody were analyzed. (B) The experiment described in A was repeated using *in vitro*-translated radiolabeled XPD protein and epitope-tagged p62 (9Ep62) or XPB (9EXPB) proteins. Lane 1, radiolabeled XPD protein; lane 2, radiolabeled XPD protein co-precipitated with 9EXPB protein; lane 3, radiolabeled XPD protein co-precipitated with 9Ep62 protein, lane 4, control without epitope-tagged proteins. Samples were analyzed by 10% SDS–PAGE and by autoradiography. The positions of molecular mass markers (kDa) are indicated on the left.

were independently confirmed *in vivo* (Bardwell, L., et al., 1992, 1994; Bardwell, A. J., 1993, 1994). In our experience false positive reactions are not encountered. However, the technique may fail to detect relatively weak protein–protein interactions with a  $K_d < \sim 10^{-7}$ . The results of these experiments revealed specific interactions among a number of the TFIIH subunits. These are detailed in the ensuing paragraphs. Specific interactions are shown in representative figures, and the collective results are summarized in Table 1. In selected cases protein–protein interactions were also examined *in vivo* using the yeast two-hybrid genetic screen.

**Interactions between XPB, p62, XPD, and p44 Proteins.** *In vitro*-translated radiolabeled XPB (ERCC3) protein co-precipitated with 9E10-tagged p62 protein (9Ep62) in the presence of monoclonal antibody to the 9E10 epitope (Table 1, Figure 1A, lane 2). Precipitation of XPB protein was not observed in the absence of 9Ep62 protein (Figure 1A, lane 3). An interaction between these two proteins was also observed *in vivo*. Specifically, when a yeast reporter strain was transformed with two plasmids carrying the *XPB* gene fused to the transcription activation domain of the yeast *GAL4* gene and the *p62* gene fused to the DNA-binding domain of *GAL4*, respectively, we observed and quantitated (see Materials and Methods) increased expression of  $\beta$ -ga-

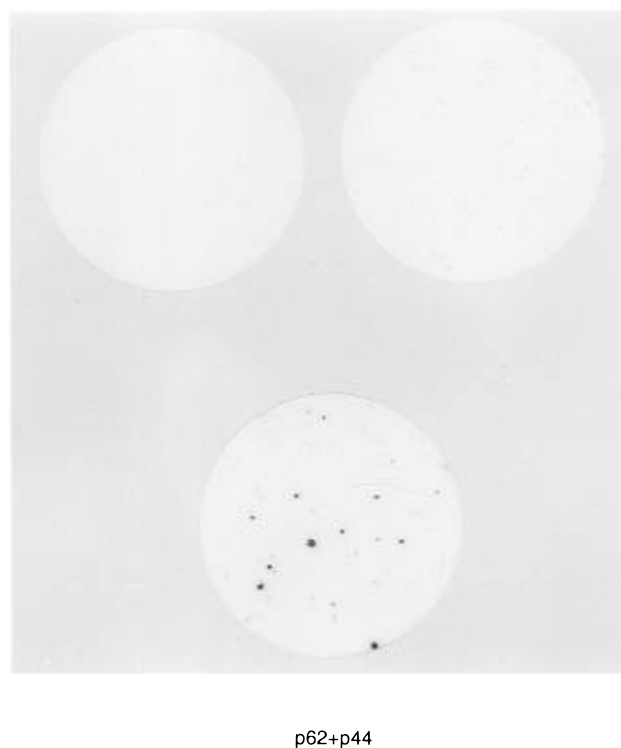


**FIGURE 2:** Interaction between p44 and p34 and their interaction with XPB, XPD and p62 proteins. (A) Radiolabeled *in vitro*-translated XPB (lane 1), XPD (lane 4), and p34 (lane 7) proteins (*I* = input) were examined for co-immunoprecipitation with epitope-tagged p44 protein (9Ep44) as described in the legend to Figure 1. Equal volumes (15  $\mu$ L) of immunoprecipitates (using 9E10 antibody) from reactions with XPB, XPD, or p34 proteins in the presence (+) (lanes 2, 5, and 8, respectively) or absence (–) (lanes 3, 6, and 9, respectively) of 9Ep44 were analyzed. (B) Radiolabeled *in vitro*-translated p34 protein (lane 1) was examined for co-immunoprecipitation with epitope-tagged XPD protein (9EXPD) (lane 2), XPB protein (9EXPB) (lane 3), p62 protein (9Ep62) (lane 4), or p44 protein (9Ep44) (lane 5). Lane 6 is a control without epitope-tagged protein. Samples were analyzed by 10% SDS–PAGE and by autoradiography. The positions of molecular mass markers (kDa) are indicated on the left.

lactosidase relative to that observed with either plasmid alone (data not shown). Several prematurely terminated XPB polypeptides also co-precipitated with epitope-tagged p62 protein (Figure 1A, lane 2). Some of these truncated polypeptides are about half the size of full-length XPB protein, suggesting that the C-terminal half of XPB protein is not required for its interaction with p62. This result was directly confirmed by mapping domains of XPB protein required for its interaction with p62 (see below).

Epitope-tagged XPB protein co-immunoprecipitated with radiolabeled XPD protein (Table 1, Figure 1B, lane 2), whereas XPD protein alone was not precipitated by the antibody (Figure 1B, lane 4). In light of the observation that XPB interacts with p62 protein (see above), we explored an interaction between the XPD and p62 proteins. Radiolabeled XPD protein was indeed co-precipitated with 9Ep62 protein (Table 1, Figure 1B, lane 3). When epitope-tagged p44 (9Ep44) was incubated with radiolabeled XPB or XPD protein, we observed interactions in each case (Table 1 and Figure 2A). We did not detect an interaction between p44 and 9Ep62 proteins by this technique (data not shown). However, a strong interaction between these proteins was observed in the two-hybrid system using plasmids expressing p44 protein fused to the Gal4 transcriptional activation domain and p62 protein fused to the Gal4 DNA binding domain (Figure 3).

p44 (transcription activation domain) p62 (DNA-binding domain)



**FIGURE 3:** Interactions between p62 and p44 proteins in the yeast two-hybrid system. Transformants containing pAS1p62, pACTp44, or both plasmids together were transferred to nitrocellulose filters, which were immersed in liquid nitrogen and incubated in the presence of 5-bromo-4-chloro-3-indolyl  $\beta$ -D-galactoside indicator. A positive interaction is indicated by colonies turning blue (p62+p44).

In summary, our results suggest that the four human TFIIH components p62, XPB, XPD, and p44, whose known yeast homologs Tfb1, Ssl2, Rad3, and Ssl1 respectively, are required for NER and RNA polymerase II transcription, are organized in a complex in which all four proteins interact with each other.

**p34 Interacts with p44, XPD, and XPB Proteins.** p34 is the smallest subunit of the TFIIH complex and has limited amino acid sequence homology with p44 protein in the region of a putative zinc-finger motif (Humbert et al., 1994). A yeast homolog of p34 has not yet been identified. When radiolabeled p34 was tested for its interaction with epitope-tagged p44, p62, XPB, or XPD proteins, co-precipitation was observed in all cases except with p62 (Table 1 and Figure 2A,B). A particularly strong interaction was observed between p34 and p44 proteins on the basis of quantitative measurements of radioactivity in immunoprecipitates (see Materials and Methods).

**XPG Protein Interacts with Multiple Components of TFIIH and with CSB Protein.** Purified preparations of TFIIH have been reported to contain XPG protein (Mu et al., 1995). Additionally, the yeast XPG homolog Rad2 has been shown to interact with the Tfb1 (human p62) and Ssl2 (human XPB) subunits and with purified yeast TFIIH (Bardwell et al., 1994b). Radiolabeled *in vitro*-translated XPB protein co-precipitated with epitope-tagged XPG protein (9E-XPG) in the presence of monoclonal antibody to the epitope (Table 1, Figure 4A, lane 2). No precipitation of XPB protein was observed in the absence of 9E-XPG protein (Figure 4A, lane

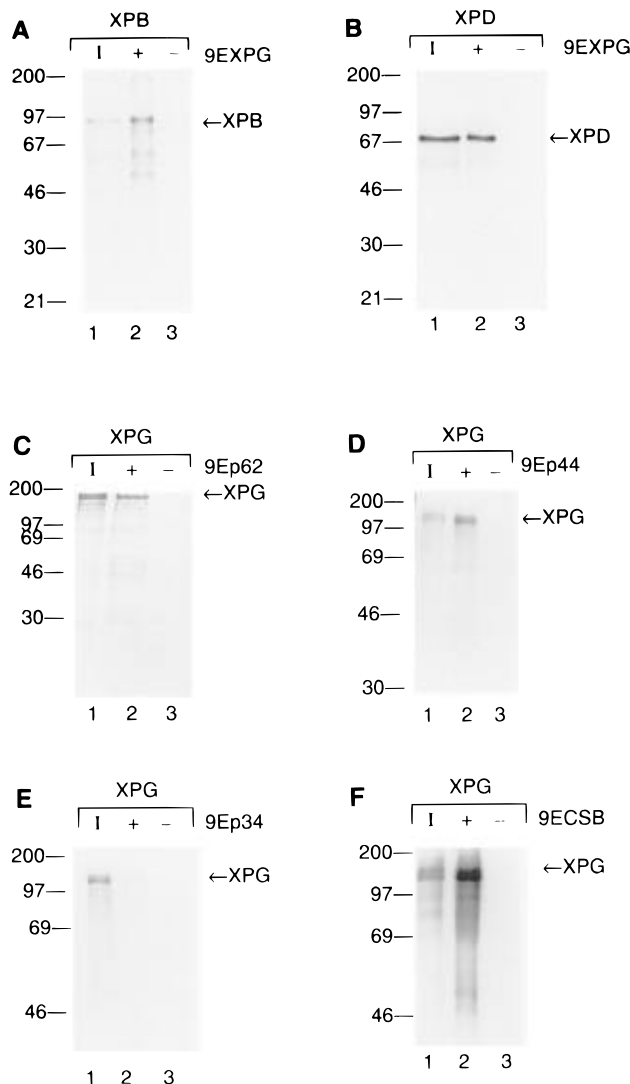


FIGURE 4: XPG interacts with multiple proteins *in vitro*. (A) *In vitro*-translated radiolabeled XPB protein co-precipitated with epitope-tagged XPG (9EXPG) protein (lane 2). XPB protein was not detected in the absence of 9EXPG. (B) *In vitro*-translated radiolabeled XPD protein co-precipitated with epitope-tagged XPG (9EXPG) protein (lane 2). XPD protein was not detected in the absence of 9EXPG. (C) Similar results are shown for the interaction between *in vitro*-translated radiolabeled XPG protein and epitope-tagged p62 (9Ep62) protein. (D) Similar results are shown for the interaction between *in vitro*-translated radiolabeled XPG protein and epitope-tagged p44 (9Ep44) protein. (E) *In vitro*-translated radiolabeled XPG protein did not interact with epitope-tagged p34 (9Ep34). (F) *In vitro*-translated radiolabeled XPG protein co-precipitated with epitope-tagged CSB (9ECSB) protein (lane 2).

3). Further experiments established that XPG protein also interacts with epitope-tagged p62 protein (Table 1, Figure 4C). Hence, as in yeast, human XPG protein (yeast Rad2) interacts with the TFIIH subunits XPB (yeast Ssl2) and p62 (yeast Tfb1). We also observed interactions between XPG protein and XPD and p44 proteins (Figure 4B,D). However, we did not observe an interaction between XPG and p34 protein (Figure 4E).

Previous studies from this laboratory have demonstrated an interaction between the proteins encoded by the human CSA and CSB genes (Henning et al., 1995). CSA protein also interacts with the p44 subunit of TFIIH, but not with the p62, XPD, or XPB subunits (Henning et al., 1995). These and other observations (see Discussion) suggest possible roles of CSA and CSB proteins in RNAP II

transcription. In the present studies we observed that radiolabeled XPG protein was precipitated by the 9E10 antibody in the presence of epitope-tagged CSB protein (Table 1, Figure 4F, lane 2) but not in its absence (Figure 4F, lane 3).

**Specificity of Protein-Protein Interactions.** The examination of protein interactions by co-immunoprecipitation of *in vitro*-translated polypeptides is a well-established technique. However, this technology can be subject to both false positive and false negative results that must be kept in mind when interpreting results. We have attempted to minimize the potential for spurious results in several ways. First, the interactions reported above were all observed in the presence of a several thousand-fold excess of nonspecific proteins contributed by the reticulocyte lysates. Such a vast excess of irrelevant protein is expected to effectively block "sticky" sites which may contribute to nonspecific interactions. Second, we have used a modified experimental system in which translation products were partially purified by ammonium sulfate precipitation prior to mixing for immunoprecipitation. This procedure improves the sensitivity of the detection of interacting proteins and reduces the potential for nonspecific interactions (Bardwell, L., et al., 1992). Third, in all cases the pairs of proteins tested represent equal volumes of a single translation reaction. While the efficiency of translation of individual mRNAs was not evaluated quantitatively, it is unlikely that the relative amount of each pair of proteins tested differed vastly. Fourth, the reticulocyte lysates used were treated with micrococcal nuclease by the manufacturer. Hence, the potential for false positive results resulting from the binding of proteins to DNA rather than to each other is significantly reduced. Fifth, the interactions observed obeyed appropriate specificity. Thus, the functionally irrelevant proteins c-Fos and c-Jun from human cells, Swi10 protein from *Schizosaccharomyces pombe* or Phr1 protein from *S. cerevisiae* did not co-precipitate with several epitope-tagged subunits of TFIIH (Figure 5A) or with XPG protein (data not shown), whereas c-Fos protein was readily precipitated by specific anti-Fos antibodies (Figure 5A). Similarly, consistent with the observation that *in vitro*-translated or purified Rad1 and Rad10 proteins do not directly associate with purified yeast TFIIH (Bardwell, L., et al., 1994), we failed to observe interactions between *in vitro*-translated radiolabeled ERCC1 protein (the human homolog of Rad10 protein) and either XPB or p62 protein (Figure 5B). Sixth, as noted above, our analysis of components of the human TFIIH complex revealed interactions between some but not all of them. Finally, it is conceivable that some protein pairs may yield a false positive interaction because they are bridged by interacting protein(s) in the rabbit reticulocyte lysate. We know of no way to definitively rule out such a possibility. This issue may be of serious concern in cases where the test proteins in question are of unknown relationship. However, the proteins tested in these experiments are all established members of the human TFIIH complex, and hence the interactions observed are not unexpected.

**Mapping Protein Interaction Domains of TFIIH Subunits.** In order to identify domains in individual polypeptides required for interactions between various TFIIH subunits and between XPG protein and TFIIH proteins we linearized DNA templates at appropriate restriction sites, expressed run-off transcripts, and translated them *in vitro*. In many instances these studies independently validated the results obtained

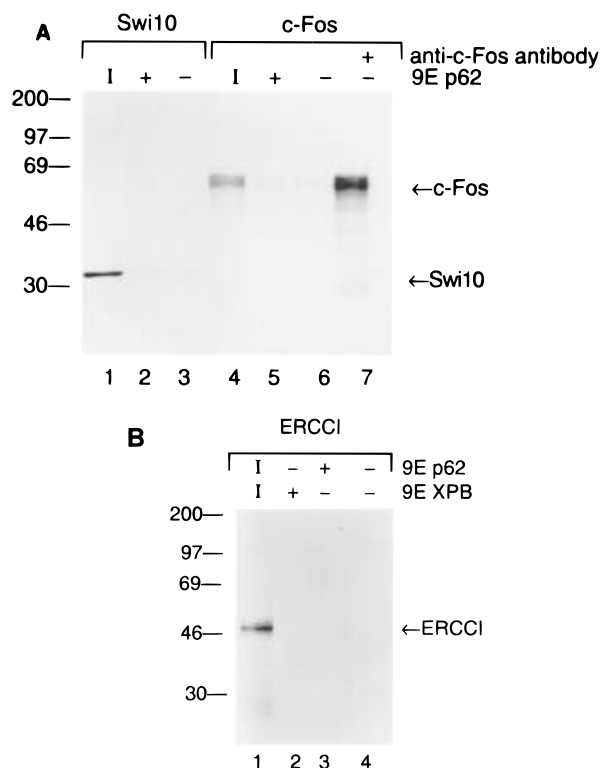


FIGURE 5: (A) Epitope-tagged p62 protein does not co-immunoprecipitate with either radiolabeled *in vitro*-translated Swi10 protein of *S. pombe* (lane 2) or c-Fos protein (lane 5). However, c-Fos protein is precipitated in the presence of antibodies to the protein (lane 7). (B) *In vitro*-translated human ERCC1 protein does not co-precipitate with either epitope-tagged XPB (lane 2) or p62 (lane 3) proteins.

with full-length proteins. All truncated proteins were verified for their reduced size. The results of these studies are detailed below. A comprehensive summary of the mapping data including a reiteration of interactions between full-length proteins is presented in Figure 6, and the interaction domains identified are represented schematically in Figure 7.

**The p62<sub>1-65</sub> Polypeptide Is Sufficient for Interaction with XPB and XPD Proteins.** The smallest truncation of p62 tested (representing the N-terminal 65 amino acids) precipitated with both radiolabeled XPB and XPD proteins (Figure 6A). Quantitative analysis of the precipitates indicated that these interactions were stronger than those observed using full-length p62 protein. The observation that truncation of a polypeptide can result in improved binding affinity with cognate proteins has been previously reported (Bardwell, L., et al., 1992). Our data suggest that the N-terminal region of p62 protein between amino acids 1 and 65 is required for interaction with XPD and XPB proteins (Figure 7).

**XPB and XPD Proteins Have Multiple Interaction Domains.** The truncated polypeptide XPB<sub>1-387</sub> interacted with both p62 and p44 proteins, but the smaller polypeptides XPB<sub>1-305</sub> and XPB<sub>1-198</sub> did not (Figure 6B). We conclude that amino acid residues 305–387 of XPB protein are required for interactions with both p62 and p44 proteins (Figure 7). All three truncated XPB polypeptides interacted with XPD and p34 proteins (Figure 6B). Hence, the domain required for the interaction of XPB with both XPD and p34 proteins apparently resides within the first 198 amino acid residues of the XPB polypeptide (Figure 7).

The truncated polypeptides XPD<sub>1-434</sub>, XPD<sub>1-324</sub>, and XPD<sub>1-162</sub> did not co-precipitate with p62 protein (Figure 6C).

Apparently the region of the XPD protein between amino acids 434 and the C-terminus is required for its interaction with p62 protein (Figure 7). All three truncated XPD polypeptides interacted with XPB protein (Figure 6C), indicating that the XPB-interacting domain of XPD protein is within the N-terminal 162 amino acids (Figure 7). Since XPB protein also interacts with XPD protein through an N-terminal domain (see above), we inquired whether just these domains are sufficient for their interaction. We indeed observed that XPD<sub>1-162</sub> and XPB<sub>1-198</sub> interacted strongly (Figure 6C). The three truncated XPD polypeptides also co-precipitated with p44 protein. Polypeptides XPD<sub>1-434</sub> and XPD<sub>1-324</sub> additionally interacted with p34 protein (Figure 6C). For technical reasons the smallest polypeptide XPD<sub>1-162</sub> could not be tested. However, polypeptide XPD<sub>1-248</sub> co-precipitated with p34 (Figure 6C), placing the p34-interaction domain of XPD protein in the N-terminal 248 amino acids (Figure 7). Thus, the N-terminal region of the XPD polypeptide is involved in multiple interactions (Figure 7).

**Mapping Interactions of p44 and p34 with Other Proteins.** The truncated polypeptides p44<sub>1-240</sub> and p44<sub>1-166</sub> co-precipitated with XPD, XPB, and p34 proteins (Figure 6D), defining an interaction domain in the N-terminal 166 amino acids of p44. The C-terminal region of p44 containing a putative zinc-finger motif (Humbert et al., 1994) is apparently not required for these interactions. However, our results with full-length p44 and p34 proteins indicated a particularly strong interaction. Hence, the region of p44 distal to amino acid 240 may also be involved in its interaction with p34.

Regions involved in the interaction of p34 with other TFIIF proteins were mapped using the truncations p34<sub>1-245</sub> and p34<sub>1-134</sub>. The larger polypeptide co-precipitated with both XPB and XPD proteins, but the smaller polypeptide did not (Figure 6E), suggesting that the region of p34 between amino acids 134 and 245 is required for its interaction with these proteins (Figure 7). Both p34 truncations co-precipitated with p44 protein (Figure 6E), indicating the presence of a p44-interacting domain in the N-terminal 134 amino acids of p34 protein (Figure 7).

**Mapping Domains for Interaction of XPG Protein with p62, XPB, and XPD Proteins.** To define domains in XPG protein required for its interactions with p62, XPB and XPD (see above) we generated the polypeptides XPG<sub>1-377</sub> (containing an evolutionarily conserved N-terminal motif) (Scherly et al., 1993), XPG<sub>747-928</sub> (containing a conserved C-terminal motif) (Scherly et al., 1993), and XPG<sub>611-753</sub> (containing a conserved motif rich in acidic amino acids) (Scherly et al., 1993). XPG<sub>1-377</sub> and XPG<sub>747-928</sub> co-precipitated with full-length XPB and XPD proteins (Figure 6F). Hence, both the N-terminal 377 amino acids of XPG protein and the C-terminal region comprising amino acids 747–928 are required for the XPG–XPB and XPG–XPD interactions (Figure 7). Polypeptides XPG<sub>1-377</sub> and XPG<sub>611-753</sub> did not interact with p62 or p44 proteins, but XPG<sub>747-928</sub> did (Figure 6F). Thus the conserved C-terminal region of XPG protein is required for interactions with the XPB, XPD, p62, and p44 subunits of TFIIF (Figure 7). Polypeptide XPG<sub>747-928</sub> did not precipitate with the irrelevant proteins ERCC1, Swi10, C-Fos, or C-Jun (data not shown).

Further confirmation of these XPG interactions was obtained by examining pairwise interactions between full-length XPG protein and various truncated TFIIF subunits. Polypeptide p62<sub>1-65</sub> co-precipitated with XPG protein (Figure

**MAPPING PROTEIN INTERACTION DOMAINS**

<b>A p62 Protein</b>		<b>Interactions</b>				
		XPD	XPB	p44	p34	XPG
Full length (549 aa)		+	+	-	-	+
p62 <sub>1-65</sub>		+	+			+

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<b>B XPB Protein</b>		<b>Interactions</b>				
		p62	p44	XPD	p34	XPG
Full length (782 aa)		+	+	+	+	+
XPB <sub>1-387</sub>		+	+	+	+	+
XPB <sub>1-305</sub>		-	-	+	+	
XPB <sub>1-198</sub>		-	-	+	+	-

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<b>C XPD Protein</b>		<b>Interactions</b>						
		p62	XPB	XPB(1-198)	p44	p34	XPG <sub>747-928</sub>	XPG <sub>1-377</sub>
Full length (760 aa)		+	+			+	+	+
XPD <sub>1-434</sub>		-	+			+	+	+
XPD <sub>1-324</sub>		-	+			+	+	-
XPD <sub>1-248</sub>							+	
XPD <sub>1-162</sub>		-	+	+		+	+	-

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<b>D p44 Protein</b>		<b>Interactions</b>				
		XPD	XPB	p34	p62	XPG
Full length (395 aa)		+	+	+	-	+
p44 <sub>1-240</sub>		+	+	+		
p44 <sub>1-166</sub>		+	+	+		

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<b>E p34 Protein</b>		<b>Interactions</b>				
		XPD	XPB	p44	p62	XPG
Full length (303 aa)		+	+	+	-	-
p34 <sub>1-245</sub>		+	+	+		
p34 <sub>1-134</sub>		-	-	+		

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<b>F XPG Protein</b>		<b>Interactions</b>						
		XPB	XPD	p62	p44	p34	XPB <sub>1-305</sub>	XPB <sub>1-387</sub>
Full length (1186 aa)		+	+	+	+	-		
XPG <sub>1-377</sub>		+	+	-	-			+
XPG <sub>747-928</sub>		+	+	+	+		+	
XPG <sub>611-753</sub>				-	-			

FIGURE 6: Qualitative summary of interaction domains in the XPB, XPD, p34, p62, and p44 subunits of TFIIH and in XPG protein, based on pairwise interactions observed *in vitro*. In each panel the full-length protein is represented above.

6A). As already indicated this region of p62 is also required for its interaction with XPB and XPD proteins (Figure 7). XPB<sub>1-387</sub> but not the smaller polypeptide XPB<sub>1-198</sub> interacted with XPG protein (Figure 6B), defining a region of XPB protein between amino acids 198 and 387 which is required for its interaction with XPG protein (Figure 7). The truncated polypeptide XPG<sub>747-928</sub> but not XPG<sub>1-377</sub> precipitated with XPB<sub>1-305</sub> (Figure 6F), suggesting that a region of XPB protein between amino acids 198 and 305 is required

for interaction with the C-terminal region of XPG protein. Additionally, XPG<sub>1-377</sub> interacted with polypeptide XPB<sub>1-387</sub> (Figure 6F), suggesting that a region of XPB protein between amino acids 305 and 387 is required for interaction with the N-terminal region of XPG protein.

The truncated polypeptide XPD<sub>1-162</sub> co-precipitated with XPG<sub>747-928</sub> containing the conserved C-terminal motif (Figure 6C) but not with XPG<sub>611-753</sub> containing just the acidic motif (data not shown). Hence, the N-terminal 162 amino



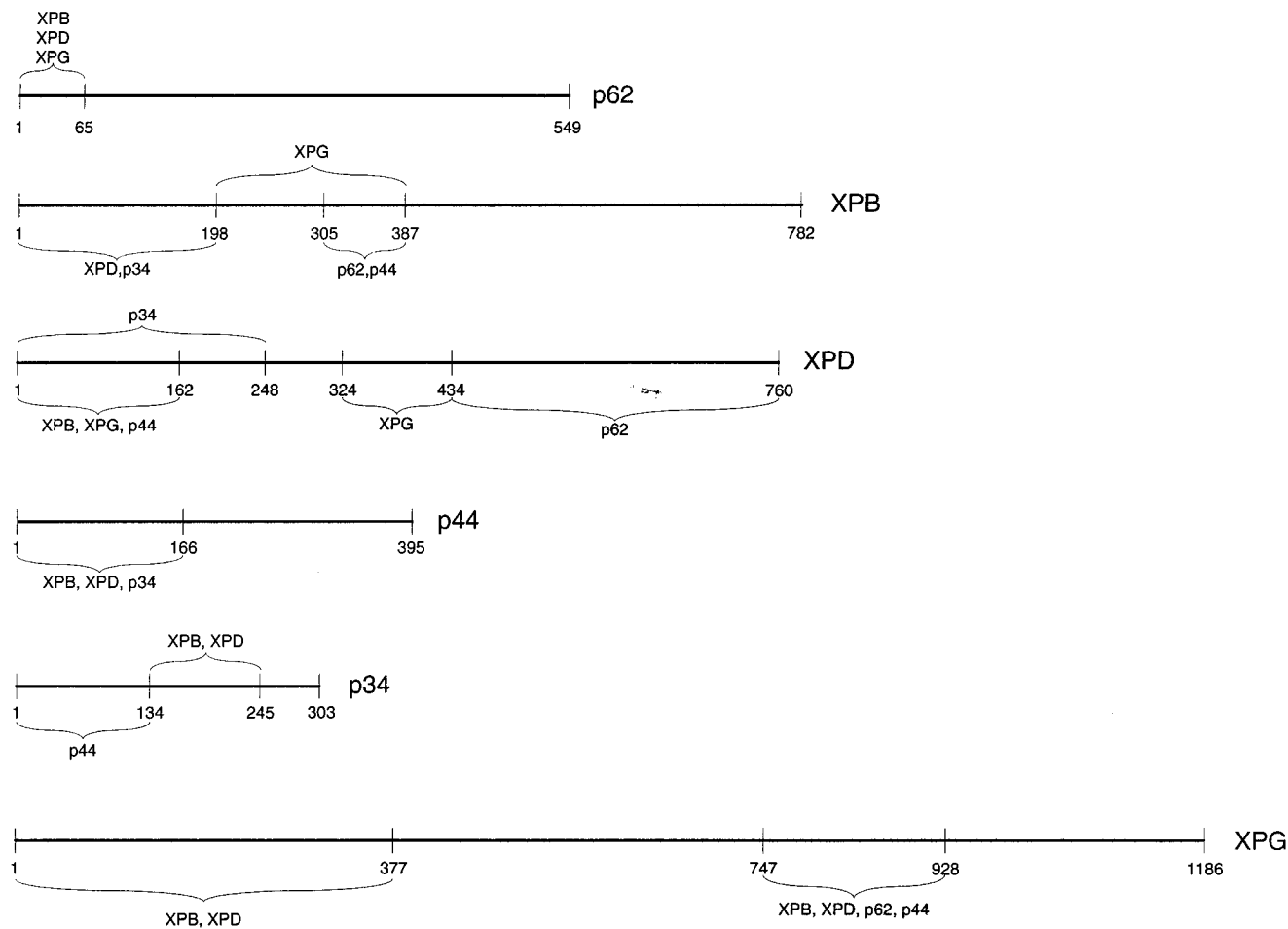


FIGURE 7: Diagrammatic representation of regions of the p62, XPB, XPD, p44, p34, and XPG linear polypeptides which are required for interactions with each other. These regions are necessary for the interactions shown, but it has not been determined that they are sufficient.

acids of XPD protein are required for interaction with a C-terminal region of XPG protein. Since XPD protein also interacted with an N-terminal domain in XPG protein (see above), we examined the interaction between various truncated XPD polypeptides and the N-terminal fragment  $\text{XPG}_{1-377}$ . Polypeptide  $\text{XPD}_{1-434}$  co-precipitated with  $\text{XPG}_{1-377}$ , but the smaller polypeptides  $\text{XPD}_{1-324}$  and  $\text{XPD}_{1-162}$  did not (Figure 6C). The region between XPD amino acid residues 324 and 434 is thus apparently required for its interaction with the N-terminal region of XPG (Figure 7).

## DISCUSSION

Systematic examination of the immunoprecipitation of proteins derived by *in vitro* translation of multiple cloned genes indicate interactions between five of the subunits of the human RNAP II transcription/NER complex TFIIH, between four of these subunits and the NER protein XPG, and between the XPG and CSB proteins. In addition, polypeptide domains required for these interactions have been approximately mapped. An extension of these studies, including definitive demonstration of their biological relevance, will require further experiments that are beyond the province of the present study, and these are planned for the future. It is additionally important to bear in mind in living cells the pairwise interactions defined in this study may be substantially modified by the presence of additional proteins which are members of the TFIIH complex and/or proteins involved in NER.

The human XPB, XPD, p62, and p44 proteins were found to interact with each other in each pairwise combination tested. An interaction between XPB and XPD has been previously implicated in the literature (Drapkin et al., 1994; Shaeffer et al. 1994). In those experiments (Reardon et al., 1993) mixing extracts of XP-B and XP-D cells resulted in only marginal complementation of defective NER, although each corrected defective repair in other XP cell extracts, thus suggesting a stable complex between the two proteins. However, these studies involved the use of crude extracts and could not exclude the possibility that the XPB–XPD interaction was mediated through another protein(s). The present experiments suggest that XPB and XPD interact directly, though we cannot definitively rule out the possibility of a third “bridging” protein. Furthermore, the use of truncated polypeptides indicates that just the N-terminal regions of both proteins are required for their interaction. An interaction between p62 and XPB protein also suggested in earlier studies (van Vuuren et al., 1994) suffers from the same interpretive limitation. Additionally, those studies failed to detect an interaction between p62 and XPD proteins. Our analysis indicates that p62 protein interacts directly with both XPB and XPD and that the N-terminal 65 amino acid region of p62 is sufficient for these interactions. The regions of the XPB and XPD proteins required for their interactions with p62 protein differ from those required for interaction between them. The 34 and 44 kDa subunits of TFIIH apparently interact strongly, possibly through distinct do-

mains located near the N- and C-terminus of each protein. Additionally, p44 protein interacts with XPB, XPD, and with p62 (in the two hybrid system), and p34 interacts with XPB and XPD proteins. We did not detect interactions between p62 protein and p34 protein (Table 1). However, recent indirect studies suggest that these proteins may indeed interact *in vivo* (Tong et al., 1995).

The structure of yeast TFIIH has also been investigated (Aboussekhra et al., 1995; Bardwell, A. J., et al., 1993; Bardwell, L., et al., 1994). A comparison with the results of the present study indicates some differences. Unlike XPB protein which interacts with the XPD, p62, and p34 proteins, the homologous yeast Ssl2 protein was shown to interact only with Rad3 (human XPD) protein, and this interaction required the use of relatively high concentrations of purified Rad3 protein (Bardwell, L., et al., 1994). This result is consistent with the independent observation that yeast Ssl2 protein is apparently loosely bound to the core TFIIH complex, since highly purified yeast TFIIH is depleted of Ssl2 protein (Svejstrup et al., 1995; Wang et al., 1994). The interaction between human p44 and XPD proteins is mirrored in the yeast Ssl1/Rad3 interaction previously documented (Bardwell, L., et al., 1994). However, in contrast to the interaction between the human p62 and XPD proteins observed in the present study, no interaction was observed between the homologous Tfb1 and Rad3 proteins (Bardwell, L., et al., 1994), and the interaction between the yeast TFB1 and Ssl1 proteins (Bardwell, L., et al., 1994) was not mirrored by a detectable interaction between the corresponding human p62 and p44 proteins. Hence, the precise architectural organization of the human and yeast TFIIH complexes may differ.

A notable similarity with respect to human and yeast TFIIH is its apparent association with human XPG (yeast Rad2) protein. *In vitro*-translated Rad2 protein has been shown to interact with purified TFIIH (Bardwell, A. J., et al., 1994). More refined studies demonstrated that Rad2 interacts specifically with both Tfb1 and Ssl2 proteins (Bardwell, A. J., et al., 1994). In the present study we have observed that XPG protein interacts with the human homologs of Ssl2 (XPB) and Tfb1 (p62) proteins. Additionally, we observed interactions between XPG and the XPD and p44 subunits of TFIIH. Thus XPG protein apparently associates with multiple subunits of human TFIIH. XPG is a junction-specific endonuclease required for incisions 3' to sites of base damage during NER in human cells (O'Donovan et al., 1994). Additionally, as indicated in the introduction, the *XPG* gene as well as the *XPB* and *XPD* genes are implicated in the combined clinical syndrome of XP/CS, and mutations in the *XPB* and *XPD* genes can also result in the NER-defective form of the disease TTD.

The complex clinical phenotypes of CS and TTD cannot be obviously reconciled with defects in the NER function of the XPB and XPD proteins. This has led to the hypothesis that these diseases may reflect defects in the transcription function of these proteins (Vermeulen et al., 1994). Such defects are presumably subtle, otherwise they would not be expected to be compatible with embryogenesis. The hypothesis that the molecular pathology of XP-B/CS and XP-D/CS derives from defects in proteins with known functions in transcription suggests a similar role for the XPG protein. This protein has no experimentally proven function in transcription. However, its intimate association with multiple

subunits of TFIIH suggests that certain mutations in XPG protein may affect the function of TFIIH. In this regard it may be informative to map mutations in the *XPG* gene in individuals from the XP-G complementation group who are and are not simultaneously afflicted with CS. It has also been suggested that XPG protein may be a critical link in the postulated exchange of TFIIH between a complex required for transcription (holoTFIIH) and one required for NER (the nucleotide excision repairosome) (Svejstrup et al., 1995). Mutations in XPG protein may interfere with such exchange and thereby compromise the availability of TFIIH for RNA polymerase II transcription.

The clinical features of CS in individuals who also manifest XP are indistinguishable from those observed in individuals who harbor the syndrome without XP (Nance & Berry, 1992). This suggests that the products of the *CSA* and *CSB* genes may also be involved in RNAP II transcription. *CSA* protein has indeed been shown to interact with the p44 component of TFIIH and with *CSB* protein (Henning et al., 1995). The demonstration in the present studies that, in addition to its interaction with multiple components of TFIIH, XPG protein also interacts with *CSB* protein lends support to a possible role(s) of XPG protein in transcription.

## ACKNOWLEDGMENT

We are grateful to Drs. Jane and Lee Bardwell for their help during the early phases of this study. We also thank our laboratory colleagues for stimulating discussions and for critical evaluation of the manuscript.

## REFERENCES

- Aboussekhra, A., Biggerstaff, M., Shivji, M. K. K., Vilpo, J. A., Moncollin, V., Podust, V. N., Protic, M., Hübscher, U., Egly, J.-M., & Wood, R. D. (1995) *Cell* 80, 859–868.
- Bardwell, A. J., Bardwell, L., Johnson, D. K., & Friedberg, E. C. (1993) *Mol. Microbiol.* 8, 1177–1188.
- Bardwell, A. J., Bardwell, L., Iyer, N., Svejstrup, J. Q., Feaver, W. J., Kornberg, R., & Friedberg, E. C. (1994) *Mol. Cell. Biol.* 14, 3569–3576.
- Bardwell, L. 1992. Ph.D. Thesis, Stanford University, Stanford, CA.
- Bardwell, L., Cooper, A. J., & Friedberg, E. C. (1992) *Mol. Cell. Biol.* 12, 3041–3049.
- Bardwell, L., Bardwell, A. J., Feaver, W. J., Svejstrup, J. Q., Kornberg, R. D., & Friedberg, E. C. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 3926–3930.
- Broughton, B. C., Steingrimsdottir, H., & Lehmann, A. R. (1994) *Nat. Genet.* 7, 189–194.
- Broughton B. C., Thompson, A. F., Harcourt, S. A., Vermeulen, W., Hoeijmakers, H. J., Botta, E., Stefanini, M., Kings, M., Weber, C., Cole, J., & Lehmann, A. R. (1995) *Am. J. Human Genet.* 56, 167–174.
- Chalut, C., Moncollin, V., & Egly, J.-M. (1994) *BioEssays* 16, 651–655.
- Drapkin, R., & Reinberg, D. (1994) *Trends Biochem. Sci.* 19, 504–508.
- Drapkin, R., Reardon, J. T., Ansari, A., Huang, J.-C., Zawal, L., Ahn, K., Sancar, A., & Reinberg, D. (1994) *Nature (London)* 368, 769–772.
- Durfee, T., Becherer, K., Chen, P.-L., Yeh, S.-H., Yang, Y., Kilburn, A. E., Lee, W.-H., & Elledge, S. J. (1993) *Genes Develop.* 7, 555–569.
- Feaver, W. J., Svejstrup, J. Q., Bardwell, L., Bardwell, A. J., Buratowski, S., Gulyas, K. D., Donahue, T. F., Friedberg, E. C., & Kornberg, R. D. (1993) *Cell* 75, 1379–1387.
- Fields, S., & Song, O. (1989) *Nature* 340, 245–246.
- Frederick, G. D., Amirkhan, R. H., Schultz, R. A., & Friedberg, E. C. (1994) *Human Mol. Genet.* 3, 1783–1788.

- Friedberg, E. C., Walker, G. C., & Siede, W. (1995) *DNA repair and mutagenesis*, ASM Press, Washington, D.C.
- Gileadi, O., Feaver, W. J., & Kornberg, R. D. (1992) *Science* 257, 1389–1392.
- Gulyas, K. D., & Donahue, T. F. (1992) *Cell* 69, 1031–1042.
- Henning, K. A., Li, L., Iyer, N., McDaniel, L. D., Reagan, M. S., Legerski, R., Schultz, R. A., Stefanini, M., Lehmann, A. R., Mayne, L. V., & Friedberg, E. C. (1995) *Cell* 84, 555–564.
- Humbert, S., van Vuuren, H., Lutz, Y., Hoeijmakers, J. H. J., Egly, J.-M., & Moncollin, V. (1994) *EMBO J.* 13, 2393–2398.
- Kolodziej, P. A., & Young, R. A. (1991) *Methods Enzymol.* 194, 508–519.
- Lehmann, A. R. (1982) *Mutat. Res.* 106, 347–356.
- Matsuda, T., Saijo, M., Kurasaka, I., Kobayashi, T., Nakatsu, Y., Nagai, A., Injoji, T., Masutani, C., Sugawara, K., Hanaoka, F., Yasui, A., & Tanaka, K. (1995) *J. Biol. Chem.* 270, 4152–4157.
- Mu, D., Park, C.-H., Matsunaga, T., Hsu, D. S., Reardon, J. T., & Sancar, A. (1995) *J. Biol. Chem.* 270, 2415–2418.
- Nance, M. A., & Berry, S. A. (1992) *Am. J. Med. Genet.* 42, 68–84.
- O'Donovan, A., Davies, A. A., Moggs, J. G., West, S. C. & Wood, R. D. (1994) *Nature* 371, 432–435.
- Park, C.-H., Mu, D., Reardon, J. T., & Sancar, A. (1995) *J. Biol. Chem.* 270, 4896–4902.
- Park, E., Guzder, S. N., Koken, M. H., Jaspers-Dekker, I., Weeda, G., Hoeijmakers, J. H. J., Prakash, S., & Prakash, L. (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89, 11416–11420.
- Reardon, J. T., Thompson, L. H., & Sancar, A. (1993) *Cold Spring Harbor Symp. Quant. Biol.* 58, 605–617.
- Roy, R., Adamczewski, J. P., Seroz, T., Vermeulen, W., Tassan, J.-P., Schaeffer, L., Nigg, E. A., Hoeijmakers, J. H. J., & Egly, J.-M. (1994) *Cell* 79, 1093–1101.
- Scherly, D., Nospikel, T., Corlet, J., Ucla, C., Bairoch, A., & Clarkson, S. G. (1993) *Nature* 363, 182–185.
- Shiekhattar, R., Mermelstein, F., Fisher, R. P., Drapkin, R., Dynlacht, B., Wessling, H. C., Morgan, D. O., & Reinberg, D. (1995) *Nature* 374, 283–287.
- Stefanini, M., Lagomarsini, P., Arlett, C. F., Marioni, S., Barrone, C., Crovato, F., Trevisan, G., Cordone, G., & Nuzzo, F. (1986) *Hum. Genet.* 74, 107–112.
- Stefanini, M., Lagomarsini, P., Giliani, S., Nardo, T., Botta, E., Peserico, A., Kleijer, W. J., Lehmann, A. R., & Sarasin, A. (1993a) *Carcinogenesis* 14, 1101–1105.
- Stefanini, M., Vermeulen, W., Weeda, G., Giliani, S., Nardo, T., Mezzina, M., Sarasin, A., Harper, J. I., Arlett, C. F., Hoeijmakers, J. H. J., & Lehmann, A. R. (1993b) *Am. J. Hum. Genet.* 53, 817–821.
- Svejstrup, J. Q., Feaver, W. J., LaPointe, J., & Kornberg, R. D. (1994) *J. Biol. Chem.* 269, 28044–28048.
- Svejstrup, J. Q., Wang, Z., Feaver, W. J., Wu, X., Donahue, T. F., Friedberg, E. C., & Kornberg, R. D. (1995) *Cell* 80, 21–28.
- Tong, X., Drabkin, R., Reinberg, D., & Kieff, E. (1995) *Proc. Natl. Acad. Sci. U.S.A.* 92, 3259–3263.
- Troelstra, C., Van Gool, A., de Wit, J., Vermeulen, W., Bootsma, D., & Hoeijmakers, J. H. J. (1992) *Cell* 71, 939–953.
- van Vuuren, A. J., Vermeulen, W., Ma, L., Weeda, G., Appeldoorn, E., Jaspers, N. G., van der Eb, A. J., Bootsma, D., Hoeijmakers, J. H. J., Humbert, S., Schaeffer, L., & Egly, J. M. (1994) *EMBO J.* 13, 1645–1653.
- Venema, J., Mullenders, L. H., Natarafan, A. T., van Zeeland, A. A., & Mayne, L. V. (1990) *Proc. Natl. Acad. Sci. U.S.A.* 87, 707–711.
- Vermeulen, W., Jaeken, J., Jaspers, N. G., Bootsma, D., & Hoeijmakers, J. H. J. (1993) *Am. J. Hum. Genet.* 53, 185–192.
- Vermeulen, W., Scott, R. J., Rodgers, S., Muller, H. J., Cole, J., Arlett, C. F., Kleijer, W. J., Bootsma, D., Hoeijmakers, J. H. J., & Weeda, G. (1994a) *Am. J. Human Genet.* 54, 191–200.
- Vermeulen, W., van Vuuren, A. J., Chipoulet, M., Schaeffer, L., Appeldoorn, E., Weeda, G., Jaspers, N. G. J., Priestly, A., Arlett, C. F., Lehmann, A. R., Stefanini, M., Mezzina, M., Sarasin, A., Bootsma, D., Egly, J.-M., & Hoeijmakers, J. H. J. (1994b) *Cold Spring Harbor Symp. Quant. Biol.* 59, 317–329.
- Wang, Z., Svejstrup, J. Q., Feaver, W. J., Wu, X., Kornberg, R. D., & Friedberg, E. C. (1994) *Nature (London)* 368, 74–76.
- Wang, Z., Buratowski, S., Svejstrup, J. Q., Feaver, W. J., Wu, X., Kornberg, R. D., Donahue, T. F., & Friedberg, E. C. (1995) *Mol. Cell. Biol.* 15, 2288–2293.
- Weeda, G., van Ham, R. C. A., Vermeulen, W., Bootsma, D., van der Eb, A. J. A. J., & Hoeijmakers, J. H. J. (1990) *Cell* 62, 777–791.

BI9524124