Annu. Rev. Biochemistry 1996. 65:15–42

RELATIONSHIPS BETWEEN DNA REPAIR AND TRANSCRIPTION

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KEY WORDS: nucleotide excision repair, Cockayne syndrome, xeroderma pigmentosum, RNA polymerase II, repairosome

ABSTRACT

Multiple relationships have been noted between DNA repair and transcription in both prokaryotic and eukaryotic cells. First, in both prokaryotes and eukaryotes nucleotide excision repair of the template strand of transcriptionally active regions of the genome is faster than in the coding strand. In prokaryotes the biochemical basis for this kinetic difference appears to be related to the specific coupling of repair to arrested transcription by RNA polymerase. The biochemical basis for strand-specific repair in eukaryotes is unknown. Second, in eukaryotes some or all of the subunits of transcription factor IIH (TFIIH) are required for nucleotide excision repair. The biological significance of this dual function of TFIIH proteins is not obvious. Finally, there are indications that the genes *CSA* and *CSB,* which are implicated in the human hereditary disease Cockayne syndrome, may have a role in transcription.

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0066-4154/96/0701-0015$08.00

INTRODUCTION

Free-living forms have evolved manifold mechanisms for repairing or tolerating diverse types of DNA damage. Recent reviews of many of these repair and DNA damage–tolerance mechanisms provide a thorough discussion of DNA repair (1–17). Excision repair is one of the general strategies used by both prokaryotic and eukaryotic cells for repairing multiple types of base damage. During this process, chemically altered, mispaired, or inappropriate (such as uracil in DNA) bases are physically excised from the genome and replaced by bases with normal chemistry and sequence. Excision repair is classified into two mechanistically distinct modes called base excision repair (BER) and nucleotide excision repair (NER), on the basis of differences in the excised products. During BER, damaged bases are excised as free bases, whereas NER is characterized by the excision of offending bases as nucleotides, typically oligonucleotide fragments. NER operates most efficiently on chemically modified bases which promote helix distortion, such as cyclobutane pyrimidine dimers (CPD) and bases bearing adducts derived from interactions with chemicals. However, it is increasingly apparent that the excision of nucleotides occurs in the course of several enzymatically distinct pathways. For example, strand-directed long patch mismatch correction results in the excision of mispaired bases as nucleotides, yet the biochemical mechanism of this repair mode is substantially different from that of NER. Furthermore, the excision of CPD can also be effected by at least two other known pathways which differ from one another and from conventional NER. This diversity of mechanisms for the excision of photoproducts from DNA presumably reflects selection that operated during evolution as a defense against the effects of UV radiation from the sun, a prevalent natural genotoxic agent. New insights concerning the multiple mechanisms of excision repair call for a refinement of the classification of this repair mode to clarify the relationships between transcription and individual DNA repair pathways, the primary topic of this review. BER is always initiated by a DNA glycosylase and results in the excision of free bases. NER is always initiated by endonucleolytic incisions near sites of base damage. During general NER these incisions flank sites of base damage. Multiple specific forms of NER also exist. One such repair mode

involves the action of a specific endonuclease which cleaves DNA exclusively 5′ to certain photoproducts. A second specific mode of NER is initiated by a DNA glycosylase which only recognizes CPD, and so-called long patch mismatch repair represents a third form of specific NER.

GENERAL FEATURES OF BASE AND NUCLEOTIDE EXCISION REPAIR

Base Excision Repair

BER is initiated by a specific class of enzymes called DNA glycosylases. Each such enzyme recognizes a single or a relatively small number of damaged (e.g. following simple alkylation), inappropriate (e.g. uracil in DNA), or mispaired bases [reviewed in (17)]. DNA glycosylases catalyze the hydrolysis of the N-glycosyl bonds linking bases to the deoxyribose-phosphate backbone of DNA, thereby releasing free bases and generating sites of base loss (abasic sites). The hydrolysis of phosphodiester bonds 5′ to abasic sites by apurinic/apyrimidinic (AP) endonucleases creates suitable template-primers for DNA polymerases. Hence, concerted repair synthesis, nick translation, and excision reactions can result in repair patches that may be as small as a single nucleotide.

Nucleotide Excision Repair Pathways

GENERAL NUCLEOTIDE EXCISION REPAIR NER repair pathways operate on many types of base damage caused by the interaction of DNA with physical and chemical agents. Such forms of base damage range from the simple covalent addition of methyl groups to complex chemical structures, such as covalently joined adjacent pyrimidines [CPD and so-called (6-4) photoproducts] and the covalent cross-linking of bases on opposite DNA strands through some sort of chemical bridge. Lesions that perturb the helical structure of the DNA duplex show indications of being processed more efficiently by NER than lesions that do not. So although nonhelix distortive lesions such as simple alkylated bases may be more efficiently repaired by the BER pathway, these lesions may on occasion be processed by NER.

General NER is characterized by two universal features. First, incision (nicking) of the damaged strand is effected by the action of two endonucleases, each of which operates exclusively on one side of the damaged base. Second, these incisions are located at rather precise distances from the damaged base. In *Escherichia coli* (*E. coli*) the 3′ incision is located three or four nucleotides from the site of CPDs while the 5′ incision is seven nucleotides removed. The distance relationship between the damaged base and the 3′ nick is conserved in human (and presumably in other eukaryotic) cells. However, in such cells

the 5′ nick and the damaged base are separated by about 21 nucleotides (9). Similar results have been obtained with a reconstituted yeast NER system (17a).

These precise spatial relationships are consistent with the notion that during general NER a region of the DNA in the immediate vicinity of a damaged base undergoes specific conformational changes effected by interactions with the NER machinery. In the case of *E. coli* these interactions are understood in considerable detail (3, 18–20). A complex consisting of two molecules of UvrA protein and one molecule of UvrB protein ($UvrA_2B_1$ complex) binds to sites of base damage. The formation of the $UvrA_2B_1$-DNA complex is accompanied by conformational changes in the DNA and in UvrB protein. These changes are thought to promote the dissociation of UvrA protein and the tight binding of UvrB protein to DNA. The conformation of the $UvrB_1$-DNA complex facilitates the binding of UvrC protein, an event that results in further conformational changes and ultimately leads to incision of the DNA by UvrB protein 3′ to the damaged base. This incision may further alter the conformation of the damaged DNA, resulting in a second nick catalyzed by UvrC protein 5′ to the site of base damage (3, 18).

In eukaryotic cells the molecular events that are involved in the interaction of the NER machinery with damaged DNA and culminate in dual incisions flanking sites of base damage are less well understood. Nonetheless, recent studies in the yeast *Saccharomyces cerevisiae* and in human cells have led to the identification of candidate enzymes for such dual incisions and to the elucidation of their substrate specificities (21–23a).

SPECIFIC NUCLEOTIDE EXCISION REPAIR Besides strand-directed long patch mismatch correction two other pathways have been identified for the excision of damaged bases as nucleotide structures. Both of these pathways are apparently specific for base damage produced by UV radiation. In *E. coli* infected with bacteriophage T4, and in the highly UV radiation-resistant organism *Micrococcus luteus,* excision repair of CPD can be initiated by hydrolysis of the 5′ glycosyl bond in the dimerized pyrimidine pair by a pyrimidine dimer-specific DNA glycosylase [reviewed in (17)]. Since the 3′ glycosyl bond remains intact, the dimerized pyrimidines are ultimately released as part of an oligonucleotide. Recent studies in the fission yeast *Schizosaccharomyces pombe* (24) and in the filamentous fungus *Neurospora crassa* (24a) have identified another enzyme that recognizes pyrimidine dimers. The substrate specificity of this endonuclease includes the other quantitatively major photoproduct in DNA, the (6-4) lesion (17). The endonuclease specifically catalyzes cleavage of a single phosphodiester bond 5′ to these photoproducts in an ATP-independent fashion (24, 24a). How the damaged bases are ultimately excised is not clear.

GENERAL FEATURES OF RNA POLYMERASE II TRANSCRIPTION

Transcription by the eukaryotic RNA polymerase II has also been extensively reviewed in recent years (25–38). Hence, this discussion touches only on key features of this process to complete the stage for a more detailed analysis of the relationships between transcription and DNA repair.

Initiation of Basal Transcription

Purified RNA polymerase II (RNAP II) is inactive in initiating accurate transcription in vitro. The pioneering studies of Roeder and his colleagues [e.g. see (26)] demonstrated a requirement for multiple factors, designated basal or general transcription factors (TFs), for the initiation of transcription by this enzyme. Many of these transcription factors have been purified and characterized from a variety of lower and higher eukaryotic sources and include TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, TFIIJ (25–38), and more recently TFIIK (39–41). The prevailing view is that these transcription factors (most of which are comprised of more than one polypeptide in mammalian cells) assemble with RNAP II at promoter sites in an ordered, stepwise fashion. Support for this view comes from the demonstration that various transcription factors can be assembled onto promoter DNA in vitro. This ordered assembly is believed to be initiated by a complex of TFIIB and TATA binding protein (TBP) (a component of TFIID) to the promoter, followed by the binding of RNAP II in association with TFIIF. TFIIE and TFIIH (with bound TFIIK) are thought to be added to the initiation complex last. In vivo studies in yeast also indicate that a large RNA polymerase II holoenzyme comprising RNA polymerase II, TFIIF, TFIIB, TFIIH, and additional polypeptides involved in transcription initiation may preassemble in a DNA-independent fashion (42–45).

TRANSCRIPTION FACTOR IIH The transcription factor TFIIH is particularly relevant to this review because it is required not only for RNAP II general transcription, but for NER as well (see section entitled "Some RNAP II Basal Transcription Proteins Are Required for Nucleotide Excision Repair in Eukaryotes"). In human cells TFIIH is thought to comprise nine subunits (46–51a; J-M Egly, personal communication). Five of these, with molecular masses of 89, 80, 62, 52, 44, and 34 kDa, are encoded by cloned genes designated *XPB* (*ERCC3*), *XPD* (*ERCC2*), *p62*, *p52*, *p44*, and *p34*, respectively (Table 1). Subunits of ~38 and ~37 kDa have also been identified (Table 1). Analysis of the ~38 kDa subunit showed that it is identical to a protein kinase called MO15 (49, 51a, 57a) and, more recently, Cdk7 (52). This kinase was originally identified as the catalytic subunit of cyclin-dependent kinase-activating kinase

Table 1 Composition of transcription factor IIH (TFIIH)[a]

Human		Yeast	
Subunit (kDa)	Gene	Subunit (kDa)	Gene
89	*XPB*	105	*SSL2*
80	*XPD*	85	*RAD3*
62	*p62*	73	*TFB1*
52	*p52*	55	*TFB2*
44	*p44*	50	*SSL1*
38	*MO15 (CDK7)*	33	*KIN28*
37	*CYCH*	47	*CCL1*
—	—	45	*CCL1*
—	*MAT1*	38	*TFB3*
34	*p34*	—	—

[a]Polypeptides encoded by homologous human and yeast genes are entered on the same line.

(CAK), an enzyme that phosphorylates the cyclin-dependent kinases $p34^{cdc2}$ and $p33^{cdk2}$ (53–57). Cyclin H, an ~37 kDa polypeptide that is associated with MO15 and is essential for kinase activity, has also been identified in TFIIH (49, 51a, 57a).

TFIIH from the yeast *S. cerevisiae* (designated as factor b in earlier literature) was originally purified using an in vitro transcription assay in which TFIID and TFIIH were inactivated by heat. Replenishment of inactivated TFIID with purified recombinant TATA-binding protein (TBP) facilitated the fractionation of TFIIH as a complex of three polypeptides of 85, 73, and 55 kDa (58). Cloning the *TFB1* gene encoding the 73 kDa subunit allowed the construction of yeast strains that encode histidine-tagged Tfb1 protein, and hence the use of high-resolution chromatography based on the affinity of histidine residues for a nickel-agarose matrix. These procedures revealed the presence of two other polypeptides of ~50 and ~38 kDa in purified TFIIH (59). This complex of five polypeptides is stable to extensive fractionation and has been designated as core TFIIH (41). When this form of TFIIH was tested in an in vitro transcription system reconstituted from purified transcription factors it was found to be inert. This observation led to the search for and discovery of a more complex form of TFIIH called holoTFIIH, which contains an additional four subunits of ~105, 47, 45, and 33 kDa (40). HoloTFIIH is the form of TFIIH that is believed to participate in RNAP II transcription initiation in yeast (41).

The genes encoding all of the nine subunits that have been identified in yeast holoTFIIH have been cloned and sequenced. The 105, 85, 73, and 50

kDa subunits are encoded by the *SSL2* (*RAD25*) (60, 61), *RAD3* (62), *TFB1* (63), and *SSL1* (64) genes, respectively, which are the yeast homologs of the human XPB, XPD, p62, and p44 subunits (Table 1). The 33 kDa subunit is encoded by the *KIN28* gene and is the yeast homolog of the MO15/Cdk7 kinase (39). In yeast, Kin28 protein interacts with a cyclin called Ccl1 (65). Kin28, together with the 47 and 45 kDa subunits of holoTFIIH, is designated TFIIK (39–41). Peptide and western analyses of the 45 and 47 kDa subunits of TFIIK indicate that they are both encoded by the *CCL1* gene (JQ Svejstrup, WJ Feaver & RD Kornberg, personal communication). The smaller subunit could be a degradation product of the larger polypeptide, or one of the proteins may be post-translationally modified. The genes that encode the remaining two subunits (55 and 38 kDa) of holoTFIIH are called *TFB2* and *TFB3* (Table 1) (WJ Feaver & RD Kornberg, personal communication). Recent studies suggest that the yeast *TFB2* and *TFB3* genes are represented in human cells as genes designated *p52* and *MAT1* respectively (Table 1) (J-M Egly, personal communication).

A recent study demonstrated that Kin28 protein is required for RNAP II transcription and that it phosphorylates the C-terminal domain (CTD) of RNAP II in vitro (65a). Consistent with these results, RNAP II transcription was dramatically reduced and phosphorylation of the largest subunit of RNAP II in a *kin28-ts* mutant at the restrictive temperature decreased (65b). CAK activity was not detected in the in vitro study just mentioned (65a). Similarly, CAK activity tested on appropriate yeast substrates has not been identified in purified preparations of holoTFIIH or TFIIK (WJ Feaver & RD Kornberg, personal communication). Hence, unlike its human counterpart, yeast TFIIH appears to be unendowed with CAK activity.

Promoter Clearance and Transcriptional Elongation

The biochemical events that ensue once a complete initiation complex is assembled at a eukaryotic promoter are less clear. In an attempt to determine the roles of various transcription factors in transcription initiation, promoter clearance, transcript elongation, and transcription termination in eukaryotic cells, Goodrich and Tjian (66) used an in vitro reconstituted human RNAP II transcription system. They defined transcription initiation as a state characterized by the assembly of the initiation complex at a promoter and the concomitant melting of the DNA surrounding the transcription start site. Promoter clearance was defined as a state in which the melted region (transcription bubble) begins to move away from the promoter and the first few ribonucleotides of the nascent mRNA are synthesized. Goodrich and Tjian (66) suggested that during this stage the composition of the initiation complex and its interaction with DNA are altered. Transcript elongation involves the

passage of the transcription machinery along the template DNA strand and the progressive synthesis of a complete mRNA, and transcription termination involves the release of the completed transcript and RNAP II from the template. Using an abortive initiation assay that operationally defined the initiation stage, Goodrich & Tjian (66) observed a requirement for only TBP, TFIIB, TFIIF, and RNAP II. TFIIE and TFIIH were required in addition for promoter clearance, but not for transcription initiation or transcript elongation.

Similar results were obtained in an independent study using a defined reconstituted human transcription system and several experimental strategies to define the composition of the transcription complex at various stages of the transcription cycle (66a). This study showed that TFIID, TFIIB, TFIIF, TFIIE, and TFIIH are assembled in a promoter-bound initiation complex prior to the addition of nucleoside triphosphates. TFIID remained stably bound to the promoter, whereas TFIIB was released immediately upon addition of triphosphates. TFIIF was released from the initiation complex after the first 10 nucleotides were incorporated into the nascent RNA chain. TFIIF could reassociate with a stalled transcription elongation complex, but was released once the stalled complex re-entered productive elongation (66a). This finding is consistent with the results of other studies on transcription elongation (see below).

Both TFIIE and subsequently TFIIH were released from the initiation complex. TFIIE was released before the formation of the first 10 nucleotides in the RNA, whereas TFIIH was released after the first 30 nucleotides were synthesized but before the transcript was ~70 nucleotides in length (66a). Neither the p62, XPB, nor the XPD subunits of TFIIH were detected in isolated stalled elongation complexes (66a). As noted later in this review, the apparent absence of TFIIH from the transcription machinery during the elongation phase has important implications for understanding the apparent coupling of transcription by RNAP II to DNA repair.

Much remains to be learned about the composition and functional characteristics of the complex that promotes the elongation of a properly initiated transcript. Several proteins that interact directly with RNAP II and affect the rate of transcription have been identified from various eukaryotic sources. TFIIF has been reported to stimulate the rate of elongation of RNA chains and to promote read-through of RNAP II at pause sites (67). A 38 kDa polypeptide variously referred to as TFIIS, SII, RAP38, or p37 [quoted in (68)] and a factor called TFIIX (69, 70) have also been implicated in transcriptional elongation. Additionally, a factor called YES has been purified from *S. cerevisiae* and shown to stimulate the rate of RNA elongation by RNAP II on oligo (dC)-tailed DNA templates (71). More recently, an elongation factor called SIII has been isolated from rat liver extracts (72). SIII is comprised of three subunits of 110, 18, and 15 kDa (72) and is distinct from other known transcription factors.

Purified SIII stimulates the synthesis of accurately initiated RNAP II transcripts in vitro.

3′ TRANSCRIPTION SHORTENING A process that is intimately associated with transcript elongation by RNAP II is the shortening or retraction of the nascent transcript by hydrolysis of the 3′ end (73). This hydrolytic activity is believed to be an intrinsic function of RNAP II (74), but is dependent on the presence of functional TFIIS (SII) (75). Shortening of the transcript by hydrolysis of its 3′ end appears to be essential for TFIIS-mediated read-through past natural transcriptional pause sites. By direct analogy with the proof-reading function of the 3′->5′ exonuclease function of many DNA polymerases, it has been suggested that hydrolysis of the 3′ end of the emerging RNAP II transcript may promote the fidelity of transcription (75).

A final caveat of this brief overview of RNAP II transcription is the reminder that in living cells transcription operates on chromatin rather than on naked promoter DNA, and in addition to basal and transactivating factors, multiple factors that operate in relieving chromatin-mediated repression of transcription are important elements of transcriptional regulation. Experimental systems that apparently monitor both the antirepression of genes in the so-called inactive ground state and their concurrent activation by basal and transactivating factors are just beginning to emerge (29, 76).

TRANSCRIPTIONAL ARREST IN TEMPLATES CONTAINING DAMAGED BASES

The interpretation of early studies of base damage effects on in vitro transcription was complicated by the presence of multiple base adducts produced by some types of damage, particularly by their random distribution in both template and coding strands. The use of substrates containing chemically defined single base alterations in known locations led to the demonstration that various types of base damage, and even single strand breaks in the template strand (but not the coding strand) of DNA, can act as blocks to transcription catalyzed by various prokaryotic RNA polymerases in vitro (77–86). In all of these studies the efficiency of transcriptional arrest varied with the type of base damage, and transcriptional bypass, when observed, was frequently accompanied by misinsertion of bases, suggesting that bypass of unrepaired DNA damage may be a source of mutant proteins, at least in prokaryotic cells. An observation of particular interest with respect to T7 RNA polymerase is that single strand breaks or even large gaps with 3′OH and 5′P termini do not block transcription. However, breaks and gaps with 5′P and 3′P termini do block transcription, suggesting that charge repulsion between the two phosphoryl termini may perturb the catalytic function of the polymerase (87).

Transcriptional arrest of RNAP II on damaged templates has been less extensively studied. In recent experiments the effect of CPD on RNAP II-mediated transcription elongation was investigated using a reconstituted in vitro system (88). In these studies CPD were placed in known locations downstream of the adenovirus late major promoter. These lesions proved to be potent inhibitors of transcription when present on the template DNA strand. CPD at sites of arrested transcription were resistant to repair by DNA photolyase, suggesting that the arrested transcription complex was stably bound at sites of base damage and that it shielded the damage from this repair enzyme. TFIIS promoted transcript cleavage in this system. However, unlike the situation with natural pausing, this reaction was not accompanied by bypass of CPD sites.

RELATIONSHIPS BETWEEN TRANSCRIPTION AND DNA REPAIR

Preferential Excision Repair of Transcriptionally Active Genes

In 1985 Hanawalt and his colleagues (89) devised an elegant experimental technique in which they coupled the sensitivity of Southern hybridization for examining defined regions of the genome with the substrate specificity of certain DNA repair enzymes (e.g. the specificity of the pyrimidine dimer-DNA glycosylase for CPD in DNA), thereby devising a technical strategy for monitoring the kinetics of the loss of lesions such as CPD from defined genes. The essential elements of this technique are the following. The initial presence and persistence of lesions such as CPD in the DNA of a gene of interest results in sensitivity of the DNA to degradation by the damage-specific enzyme probe and hence a loss of the relative intensity of the hybridization signal during Southern analysis of that gene. In contrast, the progressive removal of CPD by NER as a function of the time of postirradiation incubation of cells protects the DNA against such degradation and restores the hybridization signal. This general technology has been refined to facilitate measurement of the repair of other types of base damage, including interstrand cross-links and base damage that yields alkali-labile sites that can be converted to strand breaks. Using this general technique Hanawalt and his colleagues observed that the rate of loss of CPD from the transcriptionally-active *DHFR* gene of Chinese hamster ovary (CHO) cells was about fivefold faster than in the genome overall. This phenomenon is referred to as the preferential repair of transcriptionally active genes, and it has been extensively documented in multiple genes for several types of base damage in both prokaryotic and eukaryotic cells (90–103).

A facile explanation for the preferential repair of transcriptionally active genes is that it reflects a more "open" configuration of the chromatin structure of such genes compared to genes that are transcriptionally silent, thereby

providing preferential access of the DNA repair machinery to sites of base damage. In some experiments of this type this explanation may be correct. However, more refined analysis of the phenomenon using hybridization probes for each of the two DNA strands revealed that in many transcriptionally active eukaryotic genes the template strand is repaired more rapidly than the coding strand (103). Even this strand selectivity has been challenged with the caveat that arrest of the transcription elongation complex at lesions in the template strand might prolong the "open" conformation of that strand relative to the coding strand, thereby providing a kinetic advantage for its repair (104). But strand-specific repair has also been observed in the prokaryote *E. coli* (105) in which genomic organization is not believed to result in significant steric hindrance to the access of enzymes required for various metabolic transactions of DNA. Furthermore, the presence of stalled RNAP complexes at sites of base damage in the transcribed strand actually inhibited the repair of CPD by the *E. coli* UvrABC endonuclease in vitro (106). This result is consistent with the notion that an arrested transcription complex might actually protect lesions from repair (see "Coupling of Transcription and Nucleotide Excision Repair in *E. Coli*").

These observations, coupled with the observation that in mammalian cells both preferential repair and strand-specific repair of transcriptionally active genes are confined to genes which are transcribed by RNAP II, have led to the view that preferential repair and strand-specific repair are mechanistically related and reflect the operation of an NER mode that is somehow coupled to the process of transcription elongation by RNAP II at sites of base damage. Hence, the terms preferential repair, strand-specific repair, and transcriptionally-coupled repair are often used interchangeably in the literature.

The imprecision of this terminology is unfortunate because it has the potential to create considerable confusion. As already indicated, the preferential repair of transcriptionally active genes is not necessarily always predicated on a kinetic bias for the repair of the transcribed (template) strand relative to that of the nontranscribed (coding) strand. Furthermore, although the evidence that the *E. coli* NER machinery can be specifically coupled to arrested transcription in vitro is substantial (see "Coupling of Transcription and Nucleotide Excision Repair in *E. Coli*"), such coupling has not been definitively established as the biochemical correlate of all strand-specific repair in this prokaryote. In addition, the biochemical coupling of transcription and excision repair (of any type) has not been demonstrated in any eukaryotic system in vitro, and the molecular mechanism of strand-specific repair of transcriptionally active genes in higher organisms remains unknown.

Further confusion about the relationships between transcription and DNA repair stems from the evidence that many of the subunits of the RNAP II basal transcription factor TFIIH are also required for NER (see discussion

below). The obligatory loading of TFIIH onto promoter sites during transcription initiation suggests an obvious mechanism for directly coupling RNAP II transcription to NER at sites of base damage in the template strand. However, as mentioned above, several studies (66, 66a) indicate that TFIIH is not associated with the transcription elongation complex when the nascent transcript becomes longer than about 30 nucleotides. Hence, the observation that some NER proteins are also essential for RNAP II transcription may have no direct bearing on the mechanism of strand-specific repair of transcriptionally active genes.

Strand-Specific Repair of Transcriptionally Active Genes

The strand-specific repair of transcriptionally active genes has been documented in many organisms, ranging from *E. coli* to mammalian cells (90–103). The phenomenon has also been observed with many but not all types of base damage. However, the extent of the kinetic differences observed between the repair of the template and coding strands varies considerably as a function of both the cell type and the type of base damage. For example, in CHO and human cells ~80% of the CPD were removed from the transcribed strand of the *DHFR* gene within 4 hr with little loss of these lesions from the nontranscribed strand of CHO cells during the same time period (107). This kinetic difference for the repair of CPD in the transcribed and nontranscribed strands is equally striking in the *lacZ* gene of *E. coli* (105). However, in human cells significant repair of CPD from the nontranscribed strand of the *DHFR* gene was observed in addition to the rapid repair of the transcribed strand (107).

With respect to the type of DNA damage, strand selectivity for the repair of CPD is more pronounced than for (6-4) photoproducts (97). The general observation is that lesions that are typically substrates for BER (such as base damage resulting from alkylation of DNA) are not repaired in a strand-specific manner (97). However, experimental evidence conflicts on this issue. No differences in the removal of alkylation damage (measured by the loss of alkali-labile sites in the gene of interest) were detected between the transcriptionally active *DHFR* and *HPRT* genes and the transcriptionally silent Duchenne muscular dystrophy gene of cultured T-lymphocytes following exposure to methylmethane sulfonate (108). In contrast, when cells were exposed to *N*-methyl-*N*-nitrosourea, the *DHFR* gene of CHO cells was cleared of ~60% of alkali-labile lesions during a 24-hr period, whereas the transcriptionally silent *c-fos* gene was not repaired at all (109).

In experiments in which the bias for repair of alkylation damage in the transcribed strand is statistically insignificant, such a bias is nonetheless highly reproducible (110, 111). This may reflect the strand-specific repair of some but not all lesions produced by alkylation treatment. More recent studies of

the repair of both 3-methyladenine and 7-methylguanine from the amplified *DHFR* gene of CHO cells following exposure to several different alkylating agents showed no strand bias for the clearance of either lesion (112).

Strand-specific repair of transcribed genes is apparently confined to RNAP II. The repair of rDNA genes does not show a strand bias and is in fact reduced relative to genes transcribed by RNAP II (113–114). Strand-specific repair of genes transcribed by RNAP I has not been extensively investigated.

A strand bias for excision repair does in fact anticipate a bias for DNA damage–induced mutations. After cells were treated with UV radiation or the bulky chemical carcinogen benzo[a]pyrene, more mutations were observed in the slowly repaired nontranscribed strand of the human *HPRT* gene than in the more rapidly transcribed strand (115–118). Similar biases have been observed in the hamster *DHFR* gene (119).

Cells from individuals with Cockayne syndrome (CS) (discussed below) are defective in both the preferential repair of UV radiation damage and in the strand-specific repair of transcriptionally active genes (120, 121). Additionally, CS cells have been reported to be defective in strand-specific repair after exposure of cells to ionizing radiation (122). In contrast, cells from the human hereditary NER-defective disease xeroderma pigmentosum (XP) genetic complementation group C retain the capacity for the preferential repair of transcriptionally active genes [reviewed in (17)].

The evidence shows that strand-specific repair of transcriptionally active genes reflects a kinetic preference for the repair of the template compared to the coding strand of transcriptionally active genes. However, with the limited exceptions noted above, this kinetic preference is modest (two- to fivefold) and is most consistently associated with NER.

Unlike human CS cells, yeast mutants that are defective in genes called *RAD26* (the yeast homolog of the human Cockayne syndrome group B (*CSB*) gene) (123) and *RAD28* (the yeast homolog of the human Cockayne syndrome group A (*CSA*) gene) (P Bhatia & EC Friedberg, unpublished observations) are not abnormally sensitive to killing by UV radiation. These findings, and the general observation of the limited strand bias in human cells, have prompted questions as to what special advantages (if any) cells enjoy by repairing the template strand of transcriptionally active genes more rapidly than the coding strand. The most obvious advantage is the enhanced potential for remedying transcriptional arrest and hence completing the transcription of essential genes. This potential may be unlikely to provide a selective advantage on a population basis unless every cell in the population sustained "hits" in a particular gene. In any event the ability to bypass damage during semiconservative DNA synthesis may eventually yield lesion-free templates for transcription (100). However, if most cells in a population were at risk for hits in any essential gene at evolutionarily significant doses of DNA damage, those cells

which could best cope with the potential for transcriptional failure attendant on such damage might indeed enjoy an immediate growth advantage.

Coupling of Transcription and Nucleotide Excision Repair in Escherichia coli

As early as 1987 Hanawalt (92) suggested that a plausible explanation for strand-specific repair is that "a repair complex is physically coupled to the transcription machinery." This notion was systematically explored in *E. coli*, an organism in which the biochemistry of NER is well characterized (see above). Using an in vitro system in which NER of the transcribed strand was inhibited relative to that of the coding strand, Selby & Sancar (124) screened extracts of *E. coli* for a fraction that could relieve such inhibition and also reverse the kinetic bias in favor of the transcribed strand. These studies led to the purification of a protein that, in the presence of UvrABC endonuclease, resulted in a fivefold faster rate of transcription of the template strand of a *tac* transcriptional unit carrying base damage produced by either UV radiation, cisplatin, or psoralen plus UV light (124). This protein is designated transcription repair coupling factor (TRCF). Subsequent studies showed that TRCF is encoded by a gene called *mfd* (for mutation frequency decline), a gene previously characterized by the isolation of a mutant allele (125).

Mfd (TRCF) protein is present in *E. coli* cells at a level of ~500 molecules/cell (126, 127). The predicted amino acid sequence of the cloned *mfd* gene (128) revealed the presence of consensus helicase motifs. However, the purified protein (~130 kDa) has no detectable DNA·DNA or DNA·RNA helicase activity in vitro and a weak DNA-independent ATPase activity (126, 127). This region of the polypeptide is required for binding of the TRCF protein to DNA, and ATP hydrolysis is required for the dissociation of DNA-TRCF complexes (129). The translated sequence of the cloned *mfd* gene also revealed a 140–amino acid domain near the N-terminus with significant homology to a region of *E. coli* UvrB protein. This region of the polypeptide has been implicated in the binding of Mfd protein to UvrA protein, since both Mfd and UvrB proteins have been shown to bind to UvrA in vitro. This implication has been directly confirmed experimentally (129).

Purified TRCF protein specifically interacts with *E. coli* RNAP that is stalled at a site of base damage, and it dissociates the ternary mRNA/DNA/RNAP complex. Amino acid residues 379–571 of TRCF are required for this binding (129). TRCF-mediated release of the RNAP and the truncated transcript also occurs at transcriptional blocks produced by sites of base loss and at sites where proteins are bound to DNA. However, the protein does not affect rho-dependent or rho-independent transcription termination (130).

Recent studies have provided insights into the possible mechanism by which Mfd (TRCF) protein stimulates the rate of repair of the transcribed strand. Purified UvrA protein binds to a Mfd affinity column (127). Additionally, when the $UvrA_2UvrB_1$ complex was applied to such a column only UvrA protein bound. These observations suggest that, after binding to stalled RNAP, Mfd protein releases the polymerase and the aborted transcript from the template DNA and recruits the $UvrA_2UvrB_1$ complex to sites of base damage by binding to the UvrA moiety of the complex. Mfd then dissociates this complex, allowing UvrB protein to bind tightly to the damaged DNA and to initiate strand-specific incision (127). Under conditions that permit transcription in vitro, stimulation of repair in the template strand affects only those lesions located 15 or more nucleotides downstream of the transcriptional start site, suggesting that TRCF only couples NER to transcription when the *E. coli* RNAP is in an elongation mode (127).

How these in vitro observations relate to strand-specific NER in vivo in *E. coli* remains to be established. Space constraints preclude a detailed discussion of mutation frequency decline, but the phenomenon is a decrease in UV radiation–induced mutation frequency observed under specific growth conditions that are believed to reflect enhanced NER of premutational damage of suppressor mutations in tRNA genes. However, the *mfd* mutant strain is about five times more mutable by UV radiation for both suppressor and nonsuppressor mutations.

Coupling of Transcription and Nucleotide Excision Repair in Eukaryotes

Two genetic complementation groups have been defined for CS: CS-A and CS-B (131). A human gene originally called *ERCC6* (132) was cloned by phenotypic correction of the UV radiation sensitivity of a rodent cell line from genetic complementation group 6 of a series of UV radiation–sensitive rodent lines. The *ERCC6* gene was shown to also correct the cellular phenotypes of CS-B cells (133). Additionally, mutations in the human *ERCC6* gene have been detected in CS-B individuals (133; D Mallery & AR Lehmann, unpublished data). Hence the *ERCC6* gene has been renamed *CSB* (134). Examination of the amino acid sequence of the translated *CSB* gene revealed some similarity to the *E. coli mfd* gene; in particular the presence of consensus ATPase/helicase motifs (128). This observation, coupled with the observation of defective strand-specific repair in CS cells, has led to the suggestion that CSB protein may function as a TRCF in human cells (11, 100, 103, 126, 127). However, no direct biochemical evidence supports this theory at present. Fibroblasts from the Li-Fraumeni syndrome that are homozygous for mutations in the *p53* gene have recently been reported to be defective in NER of bulk

DNA and in the coding strand of transcriptionally active genes, although they retain normal levels of repair of the transcribed strand of such genes (134a). This observation suggests that transcriptionally coupled NER is biochemically distinct from the mode that processes base damage in transcriptionally silent DNA.

In an attempt to demonstrate transcriptionally dependent NER in eukaryotic cells, transcription-competent extracts of human cells were incubated in the presence of randomly damaged plasmid DNA carrying the human CMV promoter. A small (~twofold) increase in repair synthesis was observed in a 650 bp fragment located immediately downstream of the promoter compared to the same fragment in a plasmid deleted for the promoter (G Dianov & EC Friedberg, unpublished observations). This increase was not observed in extracts of CS cells (G Dianov & EC Friedberg, unpublished observations). However, whether this observation specifically reflects repair of the template strand, or whether the increased repair is directly coupled to transcription, is not known.

Some RNAP II Basal Transcription Proteins Are Required For Nucleotide Excision Repair In Eukaryotes

In recent years studies in both human cells and *S. cerevisiae* have established another relationship between transcription and NER. Specifically, most if not all of the subunits of TFIIH are also indispensable for NER (27, 33, 51, 135–137). It is intuitively compelling to conceptually link this biochemical observation to the biological observation of strand-specific repair in transcriptionally active genes. However, as indicated above, there is no direct evidence that they should be linked, and what biological significance, if any, may be assigned to the fact that subunits of the transcription initiation factor TFIIH are also indispensable for NER is not clear.

The essential features of TFIIH were summarized above. The first documented indication that components of this multiprotein complex are required for NER came from the demonstration that the 89 kDa subunit of human TFIIH is the product of a previously cloned gene called *ERCC3* (46). Like the *ERCC6* gene mentioned above, *ERCC3* was isolated by functional complementation of the UV radiation sensitivity of a mutant rodent cell line known to be defective in NER, this time from genetic complementation group 3 (138). The *ERCC3* gene was shown to specifically and uniquely correct the NER-defective phenotype of cells from the XP-B genetic complementation group of the human hereditary disease xeroderma pigmentosum (XP) (139), a disease characterized by defective NER [reviewed in (17)]. The *ERCC3* gene is now called *XPB* (134). Direct evidence that XPB protein is required for NER came from the demonstration that microinjection of purified TFIIH into XP-B cells cor-

rected defective NER in these cells in culture (140). Purified TFIIH also corrected defective NER in cell-free extracts of XP-B cells (140).

An independent study showed that the 85 kDa subunit of yeast TFIIH is the product of another NER gene called *RAD3* (59), the yeast homolog of the human *XPD* gene (141), which is also involved in XP [reviewed in (17)]. Direct evidence that Rad3 protein is required in NER came from the demonstration that purified yeast core TFIIH corrected defective NER in extracts of various *rad3* mutants (142). The additional demonstration that purified Rad3 protein alone did not correct defective NER in vitro indicates that this protein is stably bound in TFIIH (142). In an in vitro system for monitoring NER (143), the yeast Ssl2 (142) and Ssl1 and Tfb1 proteins (144) [all known subunits of TFIIH (see discussion above)], were shown to be necessary for NER. The Tfb2 and Tfb3 proteins of yeast core TFIIH have not been directly implicated in NER, but like the other four components of the core complex they are expected to be indispensable to this process. Related experiments in human cells have shown that the XPD (ERCC2), p44, and p34 protein subunits of TFIIH (see discussion above) are also required for NER (47, 48, 50).

Other RNAP II basal transcription factors show no indication of involvement in NER. However, to determine definitively whether these factors are involved in NER mutants each of the multiple genes that encode such proteins must be systematically evaluated for a NER-defective phenotype. TFIIE has been reported to negatively regulate the helicase activity of TFIIH in extracts of human cells through a direct interaction with the XPB (ERCC3) subunit (50). However, a conditional-lethal mutant of the large subunit of yeast TFIIE is not abnormally sensitive to UV radiation and is not defective in NER in vitro (Z Wang, M Sayre, X Wu & EC Friedberg, unpublished observations). This result is supported by studies showing that extracts of human cells depleted of TFIIE carry out NER at normal rates (145).

Microinjection of antibodies against the human MO15 kinase protein into normal human fibroblasts in culture caused a decrease in NER in these cells (49). However, this experiment does not provide direct evidence for a requirement of MO15 protein in NER because the antibody may have resulted in depletion of other proteins associated with MO15. Temperature-sensitive mutations in the homologous yeast *KIN28* gene yielded a very modest increase in UV sensitivity (Z Wang, G-J Valay, G Faye, X Wu & EC Friedberg, unpublished observations), which does not approach the sensitivity observed in strains carrying mutations in genes which encode proteins that are known to be required for NER. Furthermore, direct measurement of NER in vitro in several *kin28-ts* mutants at the restrictive temperature failed to reveal a significant defect (Z Wang, G-J Valay, G Faye, X Wu & EC Friedberg, unpublished observations). The *MO15* (*KIN28*) gene is unlikely to play an indispensable role in NER. However, the possibility that mutations in this and/or

other components of the TFIIH complex that participate in transcription initiation may indirectly perturb the function of TFIIH in NER, thereby leading to modest NER-defective phenotypes, cannot be ruled out.

HOW DOES TFIIH OPERATE IN NUCLEOTIDE EXCISION REPAIR?

TFIIH Is Required for Nucleotide Excision Repair in Transcriptionally Silent DNA

The cell-free system used to characterize the NER function of TFIIH components of *S. cerevisiae* measures the repair of plasmid DNA containing defined types of DNA damage (143). This system operates in the apparent absence of active transcription as evidenced by the following criteria (Z Wang & EC Friedberg, unpublished observations). First, yeast extracts are extensively dialyzed and hence devoid of ribonucleoside triphosphates. Second, the plasmid DNA used as a substrate for repair does not contain yeast RNAP II promoters. Third, normal levels of NER were observed in the presence of RNAse A. Fourth, no incorporation of radiolabel into RNA was observed, and no transcripts were detected by gel electrophoresis and autoradiography when incubations were carried out in the presence of [^{32}P]UTP under conditions optimal for NER. Hence, this in vitro NER system is apparently not coupled to or dependent on RNAP II transcription, leading to the conclusion that TFIIH is required for NER in DNA that is transcriptionally silent. Whether an additional transcriptionally-dependent NER mode exists in yeast and higher eukaryotes remains to be determined.

Studies in yeast demonstrated a specific interaction between purified core TFIIH and in vitro–translated Rad2 and Rad4 proteins (147), both of which are indispensable for NER. Neither *RAD2* nor *RAD4* are essential yeast genes [(reviewed in (17)]. Hence, they are presumably not absolutely required for RNAP II transcription. Also, Rad2 protein has been shown to interact with both Tfb1 protein (the 73 kDa subunit of core TFIIH) and, through a different interacting domain, Ssl2 protein (the 105 kDa subunit of core TFIIH). Purified core TFIIH is typically depleted of Ssl2 protein. However, Ssl2 protein is required for NER in vitro (142) and in vivo (148) and is also essential for RNAP II transcription in a fully reconstituted system (40, 41). Furthermore, the human homolog of Ssl2 protein [XPB (ERCC3)] is an integral component of human TFIIH (46). Hence, Ssl2 protein likely dissociates from core TFIIH during purification, and in fact a form of core TFIIH that includes Ssl2 protein has been isolated (142).

Purified Rad1 and Rad10 proteins do not interact with purified core TFIIH in vitro. However, extensive purification of yeast extracts has yielded fractions

that contain all the components of core TFIIH (including Ssl2 protein) as well as Rad1, Rad10, Rad2, Rad4, and Rad14 proteins (41). These observations suggest the presence of a preassembled repairosome complex in yeast comprising at least 11 polypeptides (41). Studies also suggest the existence of a repairosome complex that includes TFIIH in human cells (9, 49, 50, 149, 150), though it has not been established that such a complex is preassembled in the absence of active NER. Additionally, partially purified TFIIH from human cells has been shown to complement defective NER in extracts of XP-C cells (50). Specific interactions between human XPG protein (the human homolog of yeast Rad2 protein) and multiple components of TFIIH (N Iyer, MS Reagan, KJ Wu, B Canagarajah & EC Friedberg, unpublished observation), as well as between XPA protein (the human homolog of yeast Rad14 protein) and components of TFIIH (145; R Legerski, personal communication), have been demonstrated. Additionally, XPA protein has been shown to interact with ERCC1 protein (the human homolog of yeast Rad10) (150a).

The Role of TFIIH in Nucleotide Excision Repair in Transcriptionally Active Genes

The specific role(s) of the putative preassembled repairosome in NER in yeast is not known. No direct evidence shows that transcriptionally-independent NER detected in the cell-free system mentioned above is specifically mediated by this preassembled repairosome. An additional or alternative role for the repairosome is its participation in NER in the template strands of transcriptionally active units, a possibility equally lacking in experimental support, especially given the current absence of an in vitro system which specifically monitors such repair. Nonetheless, the possibility that a preassembled NER complex might be specifically recruited to sites of arrested transcription raises an interesting conundrum. If the dual function of TFIIH proteins in NER and transcription is indeed the biochemical correlate of strand-specific NER, one might anticipate that this pathway of excision repair exploits the obligatory loading of TFIIH onto the template strand during transcription initiation, i.e. TFIIH remains associated with RNAP II during transcriptional elongation. Such a scenario would place these NER proteins in the immediate proximity of sites of base damage when transcription was arrested, making them a potential nucleation site for the assembly of a complete functional NER complex.

This model would provide a good explanation for the more rapid repair of the template compared to the coding strand of transcriptionally active genes. However, as indicated above, several studies show that the components of TFIIH are not involved in transcript elongation (66, 66a). Hence, any model of transcriptionally coupled NER requires that TFIIH must somehow be re-re-

cruited to the template strand following arrested transcription. The requirements for the resumption of transcription after stalling of RNAP II at sites of base damage may recapitulate some of the requirements for transcription initiation at natural promoters. Hence, TFIIH may have a special affinity for arrested RNAP II complexes, providing for the rapid delivery of NER proteins to such sites. TFIIH may be recruited to sites of arrested transcription as the core complex around which a repairosome is then assembled. However, the observation of a preassembled repairosome in extracts of yeast cells suggests the alternative possibility that following transcriptional arrest TFIIH returns to the template strand as part of a larger repairosome (135).

Several interesting ramifications of the latter model of strand-specific repair merit consideration. First, the model does not require (though it certainly does not exclude) additional components for the coupling of transcription to NER (as is the case in *E. coli*.), in which case human CSB (ERCC6) protein may not be the functional homolog of the *E. coli* Mfd protein. On the other hand, CSA protein has been shown to interact with the p44 subunit of TFIIH in vitro (150b), and CSB protein has been shown to interact with XPG protein in vitro (N Iyer, MS Reagan, KJ Wu, B Canagarajah & EC Friedberg, unpublished observations). Furthermore, CSA and CSB proteins have been shown to interact with each other both in vitro and in vivo (150b). These interactions have interesting potential implications for the regulation of transcription (see "The Transcription Hypothesis of Human Hereditary Diseases"). However, in the present context these interactions have equally interesting potential implications for the coupling of NER to sites of arrested transcription. Second, the observation that TFIIH is apparently shared by two different complexes, one of which (holoTFIIH) is dedicated to transcription initiation and the other (the repairosome) to NER, suggests a means for limiting transcription initiation in the presence of DNA damage (41, 135).

Rad3 (XPD) and Ssl2 (XPB) Proteins Are DNA Helicases

Of the six components of yeast core TFIIH, catalytic functions have been identified for Rad3 and Ssl2 proteins, both of which have been shown to be DNA-dependent ATPases with DNA helicase activity (151–153). The Rad3 (human XPD) helicase has a 5′->3 directionality with respect to the strand on which it translocates (151, 152), whereas the Ssl2 (human XPB) helicase has the opposite directionality (153). Mutational inactivation of the Rad3 helicase activity is not lethal to yeast cells, but it is correlated with inactivation of NER. Hence, the helicase is presumably required for the repair function of this protein. Mutational inactivation of the helicase function of Ssl2 protein is lethal (154). This helicase is therefore almost certainly required for transcription, but may also participate in NER. Both helicases may participate in repair by

facilitating unwinding of the duplex at sites of base damage. Ssl2, but not Rad3 protein, may play a similar role during transcription initiation and promoter clearance. The observation that TFIIE [which negatively regulates the helicase activity of TFIIH (50)] leaves the promoter prior to transcription elongation (66, 66a) is consistent with such a role.

The helicase function of Rad3 protein is inhibited by many types of base damage in the strand on which it translocates in vitro, but not by base damage on the opposite strand (155–157). This observation has led to the suggestion that Rad3 protein participates in the process of base damage recognition (155–157). This function may be restricted to the repair of the nontemplate strand of transcriptionally active genes and to transcriptionally silent DNA.

The biological implications of the dual function of the TFIIH proteins remain unclear. The most obvious explanation for this evolutionary development is that it directly couples NER to arrested transcription at sites of base damage, although this theory has not been verified experimentally. Given the uncertain biological significance of strand-specific NER in general, it is imperative to entertain the less interesting notion that eukaryotic cells simply utilize the same proteins in functionally distinct multi-protein complexes. Hence, these proteins may have evolved distinct functions that are appropriate to the particular complexes in which they are assembled.

THE TRANSCRIPTION HYPOTHESIS OF HUMAN HEREDITARY DISEASES

Regardless of whether the bifunctional role of proteins in RNAP II transcription and general NER has special utility for cells following exposure to DNA damage, this bifunctionality has led to interesting new insights about the possible pathogenesis of several hereditary human diseases in which NER genes are directly implicated. Noteworthy examples are the closely related syndromes referred to by the acronyms BIDS, IBIDS, and PIBIDS [158; reviewed in (17)]. BIDS is an acronym for a clinical state characterized by brittle hair, impaired intelligence, decreased fertility and short stature [158; reviewed in (17)]. IBIDS refers to the additional presence of ichthyosis (fish-like scaliness of the skin), PIBIDS the added feature of photosensitivity. In a clinical subtype of PIBIDS, often referred to as trichothiodystrophy (TTD), the features of brittle hair and photosensitivity are especially prominent.

Some (but not all) individuals with TTD are defective in NER, and this deficiency has been correlated at the molecular level with mutations in the NER/transcription genes *XPB* and *XPD* (159–160). Mutational inactivation of the NER function of XPD protein provides an explanation for the NER-defective phenotype. However, unlike patients with XP {the other known hereditary NER-defective disease [reviewed in (17)]}, TTD patients are not extremely

prone to sunlight-induced skin cancer. Brittle hair, sterility, impaired intelligence, and growth retardation are difficult to rationalize on the basis of defective NER. This conundrum has led to the hypothesis that these and other clinical features of TTD (and perhaps of related syndromes) result from subtle defects in the transcription function of the XPB and XPD proteins and possibly other proteins endowed with bifunctional roles in RNAP II transcription and NER [161–163, reviewed in (163a)].

The *XPB* and *XPD* genes have also been implicated in a disease state in which the clinical features of XP are combined with those of Cockayne syndrome (CS). Like TTD, CS is characterized by diverse clinical features which are not obviously related to defective NER. Postnatal growth retardation leading to an appearance of so-called cachectic dwarfism is particularly prominent (164). The notion that defective transcriptional activity of the *XPB* and *XPD* genes may lead to the diverse clinical features of CS associated with XP and to features of TTD is attractive. However, the nature of such defects and their specific consequences for the biochemistry of transcription remain to be determined. Since the XPB and XPD proteins are clearly implicated in the initiation of RNAP II transcription, certain mutations in these proteins might alter the rate and/or extent of expression of a particular subset of genes that are critical for specific stages of development. Recent studies have shown that extracts of XP-B/CS cells are unable to support normal levels of RNAP II transcription from various promoters in vitro (G Dianov, N Iyer & EC Friedberg, unpublished observations).

In addition to the form of CS that is complicated by the clinical features of XP, certain individuals suffer from clinically "pure" CS [164; reviewed in (17)]. As indicated above, these individuals fall into two genetic complementation groups designated CS-A and CS-B. The *CSB* gene encodes a protein that resembles *E. coli* TRCF at the amino acid sequence level. CSB protein also bears a more pronounced resemblance to a family of proteins of which the yeast Snf2 protein and its human homolog Hsnf are notable examples (133). Yeast Snf2 is a subunit of the multi-protein SWI/SNF complex known to be involved in transcriptional activation (165–168). This scenario provides a possible explanation for how defective transcription could result in CS.

Consistent with this notion the *CSA* gene encodes a protein belonging to the WD-repeat family (150b), which comprises proteins required for diverse aspects of metabolism in eukaryotic cells, including transcription [reviewed in (170)]. The CSA and CSB proteins interact both in vitro and in the yeast two-hybrid system (150b). Additionally, CSA protein interacts with the p44 subunit of human TFIIH in vitro (150b) and CSB protein interacts with XPG protein (N Iyer, MS Reagan, KJ Wu, B Canagarajah & EC Friedberg, unpublished observations). As with extracts of XP-B/CS cells, those derived from

CS-A and CS-B cells are compromised with respect to RNAP II transcription in vitro (G Dianov, N Iyer & EC Friedberg, unpublished observations).

An observation that is more difficult to reconcile with the "transcription hypothesis" is that some patients with the combined clinical features of CS and XP belong to XP complementation group G. XPG protein encodes an endonuclease which is required for incision of DNA during general NER (22, 171). No experimental evidence supports a role for this protein in RNAP II transcription, however. Since yeast (and possibly human) TFIIH is common to both a repairosome complex and a complex required for RNAP II basal transcription (41), these complexes may exist in a regulated equilibrium, in which case mutations in any of the subunits of either complex might alter the function of the other. Thus, mutations in proteins that are functionally required for NER might result in secondary transcriptional defects and vice versa. By this argument one might anticipate that clinical features unrelated to defective NER might complicate most if not all cases of XP involving genes that encode components of the repairosome. Most cases of XP do in fact manifest with neurological complications that are not obviously related to defective NER [reviewed in (17)]. Further studies are required to establish whether this model accounts for the fact that mutations in the *XPG* gene can result in the clinical features of CS, whereas mutations in the *XPA*, *XPE*, and *XPF* genes result only (apparently) in neurological problems. Not all XP-D and XP-G patients exhibit the typical clinical features of CS. But examining such individuals, as well as patients from the XP-A, XP-E, and XP-F complementation groups, for subtle clinical features of CS that may have been ignored might be informative.

I have emphasized possible transcription defects as the underlying basis for the pathology of CS and TTD. However, CS cells are also abnormally sensitive to UV light and manifest a defect in strand-specific repair (11, 120, 121, 172, 173). Defective strand-specific repair has also been reported in CS cells exposed to ionizing radiation (122). Additionally, CS cells have been reported to be deficient in the repair of ribosomal RNA genes (174). These biochemical phenotypes are difficult to reconcile with the complex clinical features of CS, especially because the features of CS, unlike XP, are not linked to exposure to a known form of DNA damage. These features may result from defective strand-specific repair of spontaneous DNA damage such as that resulting from oxidative metabolism (11). The transcription and repair defects of CS cannot be simply reconciled at present. The CSA and CSB proteins, like some of the XP proteins, may have dual roles in transcription and DNA repair. Alternatively, transcriptional defects may result in secondary defects in repair when CS cells are exposed to agents such as UV radiation.

In addition to CS, other provocative relationships between transcription and repair are emerging in the literature. Recent studies (175, 176) have shown that p53 protein can bind to the XPB and XPD subunits of TFIIH. The protein

has also been shown to bind to sites of insertion/deletion mismatches in DNA (177), and its binding to a p53 response element in supercoiled DNA is stimulated in the presence of short single-stranded regions (178). Cells from an individual with the cancer-prone hereditary Li-Fraumeni syndrome, carrying a heterozygous mutation in the *p53* gene, were found to have a significantly reduced rate of repair of the transcriptionally active *DHFR* gene relative to normal cells following exposure of the cells to UV radiation (175). NER is also reduced in vitro in p53 mutant cell lines (179). As indicated above, Li-Fraumeni cells carrying mutations in both copies of the *p53* gene are defective in global NER and in the repair of the coding strand of transcriptionally active genes, but they retain normal NER of the transcribed strand of such genes (134a).

SUMMARY AND PERSPECTIVES

Multiple distinct relationships are evident between excision repair and transcription by RNAP II. Regions of the genome in which the configuration of chromatin is in an "open" state because of active transcription are apparently more accessible to the repair machinery than regions that are in a more "closed" state. Specific mechanisms also appear to provide for more rapid repair of the template than does the coding strand of transcriptionally active genes. Further research is required to decipher the biochemical mechanism by which this strand-specific preference is effected. Arrested transcription at sites of base damage likely couples the repair machinery to the transcription elongation complex. Several mechanisms for such coupling have been postulated. A repair complex may be assembled in steps around a core unit that is integral to the elongation machinery. Alternatively, a preassembled repairosome may have special affinity for stalled RNAP II molecules, effectively recapitulating the process of transcription initiation. The coupling of a repair apparatus to sites of stalled transcription may alternatively or additionally involve one or more dedicated coupling factors for which there is good evidence in *E. coli*. The definition of a NER mode in eukaryotes that is specifically coupled to RNAP II transcription is an important biochemical challenge for the future. Finally, the discovery that components of TFIIH have an obligatory role in NER provides an important new dimension for investigating the molecular basis of hereditary diseases such as CS and TTD, for which the complex clinical phenotypes cannot be obviously reconciled with defective DNA repair. These diseases and possibly others require detailed exploration at the level of RNAP II transcription. The experimental validation of the transcription hypothesis may provide a new dimension for defining the molecular pathology of human hereditary diseases.

ACKNOWLEDGMENTS

I thank my laboratory colleagues as well as WJ Feaver for numerous helpful discussions and insightful review of the manuscript. Special thanks are due to RD Kornberg, WJ Feaver, and JQ Svejstrup for extensive experimental and intellectual collaboration and for permission to cite unpublished observations, and to Z Wang for his unique contributions to many of the studies reviewed here. I also wish to acknowledge the collaborative efforts of AE Tomkinson, RD Wood, CS West, G Faye, J-G Valay, M Sayre, and CC Harris. I apologize to those authors whose pertinent work may not have been cited due to space constraints. Studies from the author's laboratory were supported by research grants CA12428 and CA44247 from the United States Public Health Service.

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