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# Cockayne syndrome: defective repair of transcription?

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**In the past years, it has become increasingly evident that basal metabolic processes within the cell are intimately linked and influenced by one another. One such link that recently has attracted much attention is the close interplay between nucleotide excision DNA repair and transcription. This is illustrated both by the preferential repair of the transcribed strand of active genes (a phenomenon known as transcription-coupled repair, TCR) as well as by the distinct dual involvement of proteins in both processes. The mechanism of TCR in eukaryotes is still largely unknown. It was first discovered in mammals by the pioneering studies of Hanawalt and colleagues, and subsequently identified in yeast and *Escherichia coli*. In the latter case, one protein, the transcription repair-coupling factor, was found to accomplish this function *in vitro*, and a plausible model for its activity was proposed. While the *E.coli* model still functions as a paradigm for TCR in eukaryotes, recent observations prompt us to believe that the situation in eukaryotes is much more complex, involving dual functionality of multiple proteins.**

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## Transcription-coupled repair

Nucleotide excision repair (NER) is a cut and paste mechanism that utilizes the non-damaged strand as template for an error-free resynthesis of the excised lesion-containing part of the damaged strand (for recent reviews on NER, see Hoeijmakers, 1994; Wood, 1996; and, for repair in general, Friedberg *et al.*, 1995). The importance of this repair pathway is illustrated by its remarkable versatility in eliminating a very broad class of structurally diverse lesions, including UV-induced cyclobutane pyrimidine dimers and 6/4 photoproducts.

One of the most urgent problems originating from damage to the DNA template is that the vital process of transcription becomes hampered. For over a decade it has been known that mammalian cells take special precautions and prioritize elimination of many types of DNA injury from active genes (Bohr *et al.*, 1985; Madhani *et al.*, 1986). The preferential repair of the active genome compartment is accounted for largely by the faster repair of the transcribed strand (Mellon *et al.*, 1987). This specialized strand-directed form of NER, designated transcription-coupled repair (TCR), occurs for a number of lesions for which the default NER mechanism, the global genome subpathway, is too slow, such as for the above-mentioned UV-induced cyclobutane pyrimidine dimers. 6/4 Photoproducts, on the other hand, exemplify a class of damage for which removal by the global genome system is already very fast; a contribution by TCR only becomes visible when the global genome NER pathway is impaired (van Hoffen *et al.*, 1995).

The universal nature of TCR is suggested by its conservation from man to *Saccharomyces cerevisiae* (Smerdon and Thoma, 1990; Leadon and Lawrence, 1992; Sweder and Hanawalt, 1992) and *Escherichia coli* (Mellon and Hanawalt, 1989). Enhanced repair of transcribed strands seems to be restricted to genes transcribed by RNA polymerase II, since no strand bias could be demonstrated for RNA polymerase I- and III-transcribed sequences (Vos and Wauthier, 1991; Christians and Hanawalt, 1993; Damman and Pfeifer, 1997). However, again, inactivation of the global repair system revealed a contribution of TCR to RNA polymerase I-transcribed genes in yeast (Verhage *et al.*, 1996a) and in human cells (van Hoffen *et al.*, 1995). Finally, the special system for repair of active genes is not only found for *in vitro* cultured cells but is also observed in UV-exposed skin *in situ* (Ruven *et al.*, 1993), underlining its biological relevance.

## Dual involvement of proteins in transcription and repair

An additional but distinct link between transcription and repair is represented by proteins with a direct role in both processes. The prototype example of dual involvement is the basal transcription factor TFIIH, consisting of nine subunits. The TFIIH complex is also indispensable for DNA excision repair (reviewed by Hoeijmakers *et al.*, 1996). Mutations in the XPB and XPD subunits of human TFIIH were found previously to cause some forms of the DNA repair disorder xeroderma pigmentosum (XP, see below) (Weber *et al.*, 1990; Weeda *et al.*, 1990; Flejter *et al.*, 1992). Moreover, mutagenesis of several subunits of yeast TFIIH rendered UV-sensitive alleles, while mammalian and yeast mutants have reduced DNA repair activities *in vivo* and *in vitro*, clearly indicating an involvement of TFIIH in nucleotide excision repair ( Feaver *et al.*, 1993; Drapkin and Reinberg, 1994; van Vuuren *et al.*, 1994; Sweder *et al.*, 1996a).

**Table I.** Proteins involved in each step of the core of nucleotide excision repair in *E.coli*, yeast and man

| Function | *E.coli* | Yeast | Human |
|---|---|---|---|
| Lesion recognition | $UvrA_2B$ | Rad14 | XPA |
| Unwinding damaged region | UvrB | TFIIH | TFIIH |
| 3′ Incision of damaged strand | UvrB(?)/UvrC | Rad2 | XPG |
| 5′ Incision of damaged strand | UvrC | Rad1/Rad10 | ERCC1/XPF |
| Excision and repair synthesis | UvrD,DNA pol I | DNA pol II/III | DNA pol δ/ε |
| Ligation | DNA ligase | DNA ligase | DNA ligase I |
| Specific for global genome repair | – | Rad7, Rad16 | XPC/HHR23B |
| Specific for TCR | TRCF | Rad26, ? | CSA, CSB |

It is tempting to assume that the dual involvement of TFIIH in transcription and repair provides the basis for enhanced repair of the transcribed strand of an active gene. However, it should be stressed that *in vivo* and *in vitro* repair analysis of yeast and human TFIIH mutants indicated a requirement for TFIIH in both transcription-coupled and transcription-independent, global genome DNA repair (van Vuuren *et al.*, 1994; Vermeulen *et al.*, 1994; Wang *et al.*, 1995; Sweder *et al.*, 1996a). Clearly other factors are required for the coupling between transcription and repair, and these will be discussed below.

## Transcription-coupled repair in *E.coli*

NER in *E.coli* is performed by the UvrABC endonuclease (Table I) that is able to recognize and repair a surprisingly wide variety of lesions (reviewed by van Houten, 1990). The occurrence of TCR in *E.coli* was shown by strand-specific repair analysis of the lactose operon under induced conditions *in vivo* (Mellon and Hanawalt, 1989). Using an *in vitro* system, a 130 kDa transcription–repair coupling factor (TRCF) was purified, containing RecG-like helicase motifs (Selby and Sancar, 1991). TRCF possesses an ATPase activity and was shown to recognize, bind and displace an RNA polymerase stalled on a lesion (Selby and Sancar, 1993). Subsequently, the protein stimulates recruitment of the repair machinery, presumably via an interaction with the damage recognition component UvrA (Selby and Sancar, 1993).

Intriguingly, in addition to TRCF, mismatch repair proteins are reported to be involved *in vivo* in TCR in *E.coli* and also in man, thus providing a link between mismatch repair and TCR (Mellon and Champe, 1996; Mellon *et al.*, 1996). However, *in vitro*, purified mismatch repair proteins MutS and MutL could not mediate TCR, nor were *mutS* and *mutL* cell-free extracts found to be deficient in TCR (Selby and Sancar, 1995). Moreover, various yeast mismatch repair mutants failed to exhibit any defect in TCR (Sweder *et al.*, 1996b). It is therefore unclear at present whether the observed TCR defects associated with mismatch repair deficiencies have a direct or an indirect origin.

## Transcription-coupled repair in yeast

The first indications of TCR in *S.cerevisiae* were provided by repair studies of the two mating-type loci, *MAT*α and *HML*α, which are identical in sequence, but the *HML*α locus is transcriptionally silenced by the SIR proteins (Terleth *et al.*, 1989). It was found that *MAT*α was repaired more efficiently than *HML*α. However, in this case, the difference in local chromatin structure turned out to be the predominant determinant (Brouwer *et al.*, 1992), indicating that chromatin conformation also influences NER. Bona fide TCR subsequently was demonstrated for *URA3* (Smerdon and Thoma, 1990), *RPB2* (Sweder and Hanawalt, 1992) and induced *GAL7* (Leadon and Lawrence, 1992). The dependency of fast strand-specific repair on active RNA polymerase II transcription was revealed by the absence of TCR in temperature-sensitive RNA polymerase II mutants under non-permissive conditions (Leadon and Lawrence, 1992; Sweder and Hanawalt, 1992).

At present, only one factor, Rad26, is known to be involved specifically in TCR in yeast (Table I) (van Gool *et al.*, 1994). The *RAD26* gene was isolated based on homology with human *CSB* whose product, together with CSA, is selectively required for TCR in man (see below). By *rad26* disruption, the preferential repair of the transcribed strand of the active *RPB2* gene (van Gool *et al.*, 1994) and of the *PHO5 PHO3* locus (our unpublished results) is severely diminished but not completely lost. In contrast to its human equivalent (Troelstra *et al.*, 1992), but in analogy to the *E.coli trcf* mutant (Selby and Sancar, 1993), *RAD26* disruption did not lead to enhanced sensitivity to UV irradiation. This can be explained by the higher efficiency of global genome repair in yeast compared with man, which compensates for the loss of TCR. Indeed, evidence was obtained for an overlap between the two pathways in yeast (Verhage *et al.*, 1996b). The *rad26* mutant displayed a slower recovery of growth after UV irradiation, which might indicate a functional role for TCR in the biology of yeast under non-laboratory conditions (van Gool *et al.*, 1994).

Surprisingly, a residual level of TCR in the absence of Rad26 was still found when the global genome repair system was additionally inactivated by disruption of the *RAD7* or *RAD16* gene (Verhage *et al.*, 1996b). The latter gene products are implicated selectively in repair of inactive DNA, including the non-transcribed strand of active genes (Verhage *et al.*, 1994). The double mutants displayed a synergistic increase in UV sensitivity, but were not as sensitive as a total repair-deficient mutant such as *rad1* (Verhage *et al.*, 1996b). By analysing repair in triple yeast mutants, it could be excluded that the second TCR activity is encoded by Rad28 (the yeast homologue of human CSA) (Bhatia *et al.*, 1996), by the elongation factor SII (Verhage *et al.*, 1997) or by yeast mismatch repair proteins (Sweder *et al.*, 1996b). Thus, the nature of this second CS-independent TCR activity remains unknown, stressing the complexity of the transcription–repair interface.

**Table II.** Features of NER-deficient XP, CS and TTD complementation groups

| Complementation group | Clinical | | | Repair | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Associated syndrome | Skin cancer | Neurological abnormalites[a] | UV sensitivity | Residual UDS (%)[b] | NER defect GGR[c] | NER defect TCR[d] | Yeast homologue | Remarks |
| XP-A | XP | + | $++^{I}$ | +++ | <5 | + | + | Rad14 | binds damaged DNA |
| XP-B | XP/CS, TTD | +/– | $++/+^{II}$ | ++ | 10–40 | + | + | Rad25/Ssl2 | 3′→5′ helicase; subunit of TFIIH |
| XP-C | XP | + | – | + | 15–30 | + | – | Rad4[e] | ssDNA binding; complex with HHR23B |
| XP-D | XP, XP/CS, TTD | +/– | $++/\pm^{II}$ | ++ | 15–30 | + | + | Rad3 | 5′→3′ helicase; subunit of TFIIH |
| XP-E | XP | +/– | – | ± | ⩾50 | + | + | ? | binds UV-damaged DNA |
| XP-F | XP | +/– | $-/\pm^{I}$ | + | 15–30 | + | + | Rad1 | 5′ incision; in complex with ERCC1 (Rad10) |
| XP-G | XP, XP/CS | +/– | $++^{II}$ | ++ | 2–25 | + | + | Rad2 | 3′ incision |
| CS-A | CS | – | $+^{II}$ | + | 100 | – | + | Rad28 | WD repeat protein |
| CS-B | CS | – | $+^{II}$ | + | 100 | – | + | Rad26 | Swi2/Snf2-like ATPase |
| TTD-A | TTD | – | $+^{II}$ | + | 10 | + | + | ? | unidentified subunit of TFIIH |

[a]Classified as I: neurological degeneration or II: neuro-dysmyelination.
[b]Unscheduled DNA synthesis (% of wild-type cells).
[c]Global genome repair.
[d]Transcription-coupled repair.
[e]XP-C and Rad4 show clear sequence homology, however phenotypically XP-C is more reminiscent of yeast *Rad7* and *Rad16* mutations.

## Transcription-coupled repair in man

At present, three distinct genetically heterogeneous human disorders have been associated with a defect in NER: XP, Cockayne syndrome (CS) and PIBIDS, the photosensitive form of trichothiodystrophy (TTD) (Lehmann, 1987; Nance and Berry, 1992; Stefanini *et al.*, 1993; for a recent review, see Bootsma *et al.*, 1997). Cell complementation studies revealed seven gene products to be involved in XP (XPA–XPG), and two in CS (CSA and CSB) (Table II). In addition, XP groups B, D and G include some patients with CS symptoms in addition to XP features. Furthermore, NER-deficient TTD patients are assigned to three complementation groups, two of which correspond to XP and combined XP/CS groups (XPB, XPD and TTDA), again revealing overlap of syndromes. Repair analysis of cell lines derived from patients showed that most XP and TTD gene products are required for both NER subpathways, with the notable exception of XPC (Hoeijmakers, 1993). XP-C cells are deficient in global genome repair in man, while possessing normal TCR activity (Venema *et al.*, 1990b). On the other hand, CS-A as well as CS-B cells are deficient in enhanced repair of the transcribed strand of the active genes *ADA* and *DHFR*, while having normal global genome repair (Venema *et al.*, 1990a; van Hoffen *et al.*, 1993). Moreover, microinjection of antibodies raised against the CSA and CSB proteins inhibited the residual repair in XP-C fibroblasts, indicating their requirement for TCR (A.J.van Gool *et al.*, submitted). These findings reveal the existence of NER subpathway-specific human factors.

The consequences of TCR deficiency in man are severe, in contrast to the same mutations in *E.coli* and yeast (Selby and Sancar, 1993; van Gool *et al.*, 1994). CS patients typically suffer from developmental and neurological abnormalities, including neuro-dysmyelination, immature sexual development, mental retardation and impaired physical development manifested by cachectic dwarfism, microcephaly, skeletal and retinal abnormalities and a characteristic ‘bird-like’ face. Death results from progressive neurological degeneration, in most cases before the age of 20 (Lehmann, 1987; Nance and Berry, 1992). In accordance with their defect in DNA repair, most CS patients display an increased photosensitivity of the skin. However, in contrast to XP, they have not been reported to develop skin tumours (Lehmann, 1987; Nance and Berry, 1992). When compared with totally NER-deficient XP patients, it is remarkable that CS individuals exhibit many additional symptoms whereas their NER defect is only restricted to the mechanism for faster repair of the transcribed strand. Most XP individuals have a normal development but display predominantly cutaneous features, including photosensitivity, pigmentation abnormalities and predisposition to skin cancer. The non-XP characteristics in CS point to an additional function of the CS proteins.

## The Cockayne syndrome A and Cockayne syndrome B proteins

The *CSA* gene recently was isolated and predicted to encode a protein of 44 kDa containing five WD repeats (Henning *et al.*, 1995). Such domains are found in a large family of proteins implicated in a diverse range of cellular activities, and may function by stimulating formation of multi-protein complexes (Neer *et al.*, 1994). The *CSB* gene, previously isolated as *ERCC6* (Troelstra *et al.*, 1990), encodes a protein of 168 kDa with a strongly conserved middle part (Troelstra *et al.*, 1992; van Gool *et al.*, 1994). This region contains motifs shared with a large family of helicases and, more specifically, with the entire ATPase domain of the still expanding Swi2/Snf2 subfamily (Troelstra *et al.*, 1992; Gorbalenya and Koonin, 1993). Interestingly, this family includes participants in all major multi-enzyme DNA repair systems (including both NER subpathways), transcription activation and repression as well as preservation of chromosome stability

(reviewed in Carlson and Laurent, 1994; Eisen *et al.*, 1995). No overt DNA unwinding function has been shown for any of these proteins but, recently, compelling evidence was obtained that the common domain may disrupt protein–DNA interactions in an ATP-dependent fashion (Hirschhorn *et al.*, 1992; Peterson and Tamkun, 1995). The yeast Swi2/Snf2 protein is part of a large complex of at least 10 proteins (Cairns *et al.*, 1994) which is able to disrupt a nucleosome *in vitro*, thus allowing binding of the GAL4 transcription activator (Côté *et al.*, 1994; Kwon *et al.*, 1994) or the TATA-binding protein (Imbalzano *et al.*, 1994). Nucleosome disruption might be induced by changes in helical twist that are generated by Swi/Snf binding to promoter DNA (Quinn *et al.*, 1996). A similar, yet distinct activity has been reported for the human nucleosome remodelling factor as well as for the yeast RSC complex, containing the Swi2/Snf2-like ATPases ISWI and STH1 respectively (Tsukiyama *et al.*, 1995; Cairns *et al.*, 1996). Finally, the yeast Mot1 protein is able to disrupt binding of the TATA-binding protein to a promoter sequence, and thus inhibits transcription initiation (Auble *et al.*, 1994). In all cases, disruption required the ATPase activity of the Swi2/Snf2 family members, which is stimulated by naked DNA (Swi2/Snf2), or a fully assembled nucleosome (ISWI), indicating a target specificity. Recently, it was shown that purified recombinant Rad26 (Guzder *et al.*, 1996) as well as purified CSB protein (Selby and Sancar, 1997; our unpublished results) possesses a strong ATPase activity which is stimulated by double- (and to a lesser extent single-) stranded DNA. However, in none of the above cases has a standard helicase activity been found. The recent report on the crystal structure of a DEXX helicase (Subramanya *et al.*, 1996) reveals a structural similarity of the helicase domain with the ATP-binding core of the strand exchange protein RecA. All together, this opens up the possibility that the CSB protein also performs some type of local DNA strand separation, influencing DNA topology and thus remodelling its target protein–DNA complex.

Using immunoprecipitations of *in vitro* translated proteins or GST pull-down assays, interactions between CSA and p44 (a subunit of TFIIH), CSA and CSB (Henning *et al.*, 1995), CSB and XPG (Iyer *et al.*, 1996), CSB and XPA, CSB and the p34 subunit of TFIIE, and CSB and XPB (Selby and Sancar, 1997) were claimed. On the other hand, no physical interactions of significant quantities of these proteins with CSA and CSB could be demonstrated in cell-free extracts that are competent in performing NER and transcription *in vitro* (A.J.van Gool *et al.*, submitted). Thus, associations between the CS proteins and some of the other transcription and repair proteins may have a transient character, which occur, for example, when the cell is challenged with genotoxic agents.

A tentative model for TCR in humans, which is analogous to the proposed mechanism of TCR in *E.coli*, has been suggested previously (Hanawalt *et al.*, 1994). The initial step is formed by an elongating RNA polymerase II complex blocked by a lesion in the transcribed strand. The stalled RNA polymerase has to retract or dissociate to permit access of repair proteins to the injury (Selby and Sancar, 1990; Donahue *et al.*, 1994). *In vitro*, the elongation factor SII is able to stimulate retraction, preceding transcriptional read-through, by promoting the $3'\rightarrow5'$ exonuclease activity of the RNA polymerase II molecule (Reines *et al.*, 1989; Izban and Luse, 1992). However, by itself, this appeared insufficient for repair proteins to reach the lesion (Donahue *et al.*, 1994). In analogy with other Swi2/Snf2-like proteins, CSB may mediate the partial disruption of RNA polymerase binding to DNA, thereby facilitating the backtracking. The recent report that recombinant CSB, on its own or with CSA, was unable to remove a stalled RNA polymerase *in vitro* (Selby and Sancar, 1997) suggests that a combination of proteins is required to mediate efficient upstream translocation. The WD repeats in CSA could serve to stabilize transient interactions between CSB and the stalled transcription complex. In addition, these domains, perhaps together with unidentified regions in CSB, may be involved in the transient interaction with the repair machinery, and thus stimulate repair of the lesion.

## Dual functionality of the CSA and CSB proteins?

The question remains whether the requirement for CSA and CSB in TCR reflects their function as factors that couple repair to blocked transcription, as described above, or whether they are (also) required for the transcription process itself. In the latter scenario, mutations in *CSA* and *CSB* would abolish transcription, and consequently also TCR. However, the fact that both CS genes can be disrupted completely in yeast, mice and man (Troelstra *et al.*, 1992; van Gool *et al.*, 1994; Henning *et al.*, 1995; Bhatia *et al.*, 1996; van der Horst *et al.*, 1997, and unpublished results) directly indicates that neither CSA nor CSB is essential for transcription. Moreover, microinjection of CSA and CSB antibodies had no effect on transcription *in vivo*, whereas they did inhibit recovery of RNA synthesis after UV exposure. Furthermore, complete depletion of CSB from active cell-free extracts did not significantly affect transcription *in vitro* (A.J.van Gool *et al.*, submitted), indicating that CSA and CSB do not largely contribute to basal transcription. However, several observations support the possibility that these proteins may have a non-essential, auxiliary function in transcription.

(i) The phenotype of CS patients cannot be rationalized easily on the sole basis of a repair defect (Bootsma and Hoeijmakers, 1993). As outlined above, the characteristic clinical features of CS merely reflect neurological and developmental abnormalities, which are not apparent in XP-A patients that are totally deficient in NER (Bootsma *et al.*, 1997). Comparable features have also been found in XP-B, XP-D, TTD-A and XP-G patients, of which the first three have been shown to carry mutations in subunits of the transcription/repair factor TFIIH (Schaeffer *et al.*, 1993; van Vuuren *et al.*, 1994; Vermeulen *et al.*, 1994). Moreover, there are patients that display many of the CS hallmarks except for sun sensitivity and underlying TCR-related defect in RNA synthesis recovery after UV exposure (Nance and Berry, 1992; our unpublished results). This is consistent with the idea that the CS proteins have an additional function beyond their involvement in NER. The striking phenotypic parallels with the CS symptoms resulting from mutations in the repair/transcription factor TFIIH suggests that the non-XP CS features are derived from influencing basal transcription.

(ii) *CSB*–/– knock-out mice have been generated to study the function of CSB in a multicellular organism (van der Horst *et al.*, 1997). Fibroblasts derived from these mice display a repair deficiency that is very similar to yeast *rad26* and human CS-B cells, primarily reflected in a severe defect in TCR of UV-induced CPD lesions and concomitantly in recovery of RNA synthesis. Phenotypically, the *CSB*–/– mice do not display the characteristic hallmarks of CS as dramatically as the human patients, but minor growth disturbance and neurological deficits have been noted (van der Horst *et al.*, 1997). Unexpectedly, when the *CSB*–/– mice were crossed with knock-out mice of either the *XPC* or the *XPA* gene, which do not show any obvious developmental abnormalities (De Vries *et al.*, 1995; Nakane *et al*., 1995; Sands *et al.*, 1995), a strong synergistic effect was observed. The *CSB*–/–*XPC*–/– as well as the *CSB*–/–*XPA*–/– mice display a very severe growth impairment, suffer from neurological problems and die before weaning (our unpublished observations). Since crossings between *XPA*–/–*XPC*–/– mice yielded normally developing offspring, the CSB defect in combination with a total NER deficiency apparently leads to dramatically pronounced CS features. This suggests that CSB has an additional cellular function besides its involvement in NER. As mentioned above, XP complementation group G also includes patients with very severe CS features, which are clearly more pronounced than those associated with classical CS-A and CS-B (Vermeulen *et al.*, 1993; our unpublished observations). This suggests that XPG, one of the endonucleases of NER (Table II), also has a CS-like additional function. Since the XPG NER defect involves TCR as well as a global genome repair, the same synergistic effect may underlie the severe clinical outcome of human XPG mutations as that found in the CSB–XPA and CSB–XPC double knock-out mice. A prediction of these considerations is that XPG-deficient mice show essentially the same symptoms as the CSB–XPA or –XPC double mutant mice.

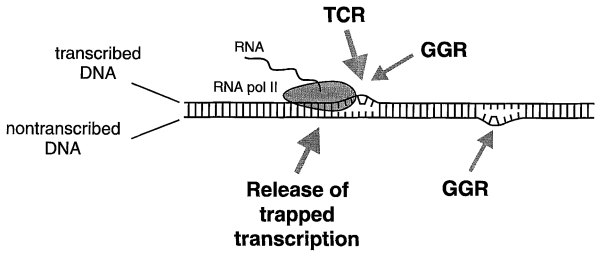
(iii) Ionizing radiation induces a variety of lesions that for a large part are repaired by means other than NER, since totally NER-deficient XP-A cells are proficient in repair of such lesions (Leadon and Cooper, 1993). Among these are oxidative damages, such as thymine glycols, that have been shown to block ongoing transcription (Htun and Johnston, 1992) and that are repaired in a transcription-coupled way (Leadon and Lawrence, 1992). Surprisingly, CS cells display a slightly increased sensitivity to ionizing radiation, and were shown to be impaired in strand-specific removal of ionizing radiation-induced lesions (Leadon and Cooper, 1993). Since oxidative damage can also arise from intracellular metabolic processes, it was suggested that failure of transcriptional bypass of these lesions leads to the observed clinical features in CS patients (Leadon and Cooper, 1993). Recently, it was shown that cells from those XP-G patients that also display severe CS features are defective in TCR of thymine glycols as well (Cooper *et al.*, 1997). Thus, in analogy with CS, this form of XP-G may also be caused by impaired transcriptional bypass of damaged DNA.

(iv) The genotoxic agent *N*-acetoxy-2-acetylaminofluorene (NA-AAF) is converted mainly into a dG-$C^8$-AF adduct in human cells (van Oosterwijk *et al.*, 1996). It is known that TCR does not contribute significantly to repair of dG-$C^8$-AF lesions in normal cells, and consequently repair rates of active genes in CS cells are very similar to those in normal cells. However, CS-A and CS-B cells show clearly hypersensitivity and an inability to recover their RNA synthesis after NA-AAF treatment (van Oosterwijk *et al.*, 1996). This suggests that the NA-AAF sensitivity of CS cells is not caused by impaired TCR *per se*, but instead may reflect trapping of essential transcription factors preventing re-initiation of transcription after NA-AAF treatment.

(v) A recent study revealed a role for CSA and CSB in UV-induced modification of RNA polymerase II. After exposure of cells to UV irradiation or cisplatin, the large subunit of RNA polymerase II becomes ubiquitinated (Bregman *et al.*, 1996). Ubiquitin conjugation of proteins has pleiotropic consequences for many cellular pathways, including DNA repair, sporulation, cell cycle progression and transcription (reviewed in Jentsch, 1992). Besides proteolytic degradation, ubiquitination can also result in modification and alternative processing of protein complexes (Chen *et al.*, 1996; Hicke and Riezman, 1996; Hochstrasser, 1996; Wang *et al.*, 1996). Surprisingly, the UV-induced ubiquitination of RNA polymerase II was specifically reduced in CS-A and CS-B cells, but could be restored by transfection of the corresponding cDNAs, strongly implying a direct or indirect role for CSA and CSB (Bregman *et al.*, 1996). The RNA polymerase II ubiquitination defect seems to be correlated with absence of CSA or CSB rather than with defective TCR, since wild-type ubiquitination is observed in totally repair-deficient XP-A, XP-B and XP-D fibroblasts (D.B. Bregman, personal communication). Interestingly, a low level of ubiquitination of RNA polymerase II is also observed without genotoxic treatment. Assuming that this type of modification has a functional role, mutations in CSA or CSB might affect the regulation of the enzyme and thus influence transcription. In agreement with these observations, we recently found evidence that in whole cell extracts a significant portion of RNA polymerase II is associated specifically with CSB (A.J.van Gool *et al.*, submitted).

The above considerations strongly suggest that the CS and XPG proteins have additional functions besides their role in DNA repair. The fact that the non-XP CS symptoms become more severe when a CSB defect is placed in a total NER-deficient background indicates that unremoved damage synergizes with the additional CSB function. One non-NER function therefore might be to detrap blocked transcription machinery either by dissociation of a stalled RNA polymerase complex or by promoting bypass via translesion RNA synthesis. This step could involve the CSA/CSB-dependent ubiquitination of RNA polymerase. The main objective of this procedure might be the release of ‘trapped’ transcription to make it again available for RNA synthesis. The second (NER) function would be to recruit the NER machinery to accomplish TCR.

The non-NER CS features may arise primarily from transcription insufficiency that develops in the course of time in specific tissues as the consequence of the accumulation of persisting lesions in the genome. Without TCR, such lesions trap essential transcription factors, e.g. elongating RNA polymerase complex, thereby interfering with transcription of the same and possibly other genes.




| disorder | transcription-coupled repair | global genome repair | release of trapped transcription |
|---|---|---|---|
| WT | + | + | + |
| CS-A, CS-B | - | + | - |
| XP-A | - | - | + |
| XP-C | + | - | + |
| XP-G | - | - | - |
| CSB-/-XPA-/-* | - | - | - |
| CSB-/-XPC-/-* | - | - | - |

* mouse mutants

**Fig. 1.** Speculative model for the functions and clinical impact of factors involved in transcription-coupled repair, global genome repair and release of the transcription machinery blocked by a DNA lesion in the transcribed strand. See text for further explanation.

Endogenous cellular metabolism is known to be a cause of DNA injury (e.g. oxidative damage) that may comprise, in part, substrates for the NER system and, in part, for other repair processes such as base excision repair. When global genome repair is still intact (like in the case of classical CSA and CSB mutations), this NER subpathway can function as a backup that takes care of removal of accessible lesions from transcribed strands, albeit more slowly. The rate of removal then depends on the activity of this NER subpathway in the specific cell type for the specific lesion. However, not all types of DNA damage are recognized by global genome repair or other repair mechanisms. Consequently, in CS, inevitably such types of lesions will still accumulate, particularly in post-mitotic cells with a long lifespan and no cell renewal system, such as neurones. Tissues like skin with continual proliferation, will 'dilute' their damage because of DNA replication and thus not suffer from this problem.

In conclusion, we hypothesize that CS patients suffer from two defective processes (Figure 1): impaired TCR and an inability to release trapped transcription. In an XP-A patient, lacking both TCR and global genome repair, the latter 'backup' system is not available but the transcription trapping problem does not occur because the CSA/CSB-dependent turnover of transcription components is still active. In XP-C, the active TCR mechanism compensates for the detrapping as well as for efficient removal of the lesions from the transcribed strand of active genes. Finally, as argued above, the severe clinical CS features resulting from XPG mutations might be explainable by the synergistic combination of a defect in both NER subpathways as well as in the release of trapped transcription. Future research should reveal whether this scenario is a reality.

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