The effect of target site transcription on gene targeting in human cells in vitro

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ABSTRACT

We evaluated the effect of target site transcription on gene targeting in cultured human fibrosarcoma cells. A number of cell lines that harbored a plasmid recombination substrate within their chromosomal DNA were created. Gene targeting frequency was then measured at these different loci in the presence and absence of an agent that stimulated target site transcription. We observed that gene targeting was significantly enhanced by RNA transcription. The magnitude of transcription-stimulated gene targeting varied from 3-fold to >20-fold. No increase in gene targeting was observed, however, when transcription proceeded away from, rather than through, the recombination site. Transcription-stimulated gene targeting was also observed when single-stranded plasmid vectors complementary to either the coding or template strand were used as recombination substrates. Our results indicate that gene targeting, like other forms of DNA recombination, can be stimulated by target site transcription. The implications of our observations on current models of transcription-stimulated recombination are discussed.

INTRODUCTION

Mounting evidence indicates that in lower eukaryotes transcription stimulates both meiotic and mitotic homologous recombination. Transcription by RNA polymerase II has been shown to stimulate mitotic recombination in yeast (1). In addition, the cis-acting recombination-stimulating sequence HOT1 is identical to the promoter for RNA polymerase I (2). Meiotic recombination initiating sites have been identified at the promoter regions of several genes (3). Recently it has been shown that transcription factors are required for the meiotic recombination hotspot at the HIS4 locus in Saccharomyces cerevisiae (4). It has also been established that target site transcription determines the directionality of gene conversion at the S. cerevisiae mating type locus (5). Normally the transcriptionally silent a and α sites only serve to donate genetic information to the active mating type locus. However, mutations that derepress transcription at these two loci allow them to become the recipients of genetic information.

Similarly, DNA recombination is stimulated by transcription in higher eukaryotes. Transcriptional activation is known to precede immunoglobulin gene rearrangement (6). Two recent studies of mammalian cells in culture have demonstrated that both extra-chromosomal and intrachromosomal homologous recombination are stimulated by increased transcription (7,8). However, there is no evidence linking plasmid-chromosome recombination (referred to as gene targeting) frequency with enhanced levels of target site transcription. Thus it has been proposed that gene targeting frequency is independent of target site transcription (9).

We wanted to directly test whether a relationship exists between gene targeting and transcription in mammalian cells. Information of this nature would provide insight into the molecular mechanism of the targeting reaction and may result in the development of more efficient gene targeting protocols. This represents an important goal, in the light of the role that gene targeting plays in the generation of murine genetic models of disease (10,11). Such information may also shed light on the physiological role of homologous recombination enzymes in somatic cells. We also wanted to use the gene targeting system as a model with which to test various hypotheses, concerning why transcription and homologous recombination are linked.

To address these issues, we created a number of human fibrosarcoma-derived cell lines that harbor chromosomal plasmid recombination substrates (referred to as chromosomal plasmid targets). These target sequences, which are integrated into the cellular genome in random loci, are located downstream of the mouse mammary tumor virus (MMTV) LTR, whose transcription can be induced in the presence of the steroid hormone dexamethasone (12). We have determined that when the MMTV LTR drives transcription through the recombination substrate, there is a 3- to 20-fold increase in gene targeting frequency upon addition of dexamethasone. Furthermore, when transcription proceeds away from the target, there is no dexamethasone-associated increase in targeting frequency. Single-stranded targeting vectors behaved identically to double-stranded vectors and no difference in gene targeting efficiency was observed when either coding or template strand vectors were used.

MATERIALS AND METHODS

Plasmid construction

Plasmids were constructed by standard procedures as described by Sambrook et al. (13). The plasmid pMSGneoDL (Fig. 1A) contained a Klenow-treated 1.1 kb HindIII-Smal fragment from...
The human fibrosarcoma cell line HT1080 (15) was the parent incubated on ice for 10 min. The cells were then electroporated. Cells were harvested, washed twice in serum-free medium and bovine serum (FBS). To generate DTX cell lines, ~10^7 Modified Eagle's Medium (DMEM) supplemented with 9% fetal cell line used in all our studies. Cells were cultured in Dulbecco’s et al. described by Sambrook cloned into pKS and pSK (Stratagene) respectively. These HindHl-Xbal fragment of pSV2neoDR, HindUl-Smal fragment and BamHl fragment containing the Escherichia coli XGPRT gene used to select for integration of chromosomal targets; diagonally striped box, constitutive SV40 enhancer. Thin lines correspond to plasmid sequences, thick lines in (A-C) represent flanking human genomic DNA.

pSV2neoDL (14) introduced to the unique Smal site of pMSG (Pharmacia). pSV2neoDR (Fig. 1D) (14) was a kind gift of Dr Raju Kucherlapati (Albert Einstein College of Medicine). pneoDLMS was constructed in the following way. The 2.25 kb BamHI fragment containing the Escherichia coli XGPRT (xanthine-guanine phosphoribosyltransferase) gene was deleted from pMSG, creating pMS. The 1.1 kb HindIII–Smal fragment of pSV2neoDL was treated with Klenow and introduced into the Klenow-treated HindIII site of pMS, located upstream of the MMTV LTR, to create pneoDLMS. The plasmid pneoDLMG (Fig. 1B) was created by introducing a 1.9 kb HindIII–BamHI Klenow-treated fragment containing the GPT gene into pneoDLMS that had been digested with Xhol and BamHI and treated with Klenow. Plasmids pSKHX (Fig. 1E) and pSKHX contained the 1.1 kb HindIII–Xbal fragment of pSV2neoDR cloned into pKS and pSK (Stratagene) respectively. These constructs were used to generate single-stranded DNA as described by Sambrook et al. (13).

Cell lines

The human fibrosarcoma cell line HT1080 (15) was the parent cell line used in all our studies. Cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 9% fetal bovine serum (FBS). To generate DTX cell lines, ~10^7 HT1080 cells were harvested, washed twice in serum-free medium and incubated on ice for 10 min. The cells were then electroporated at a field strength of 400 V/cm with a custom made unit in the presence of 1 µg pMSGneoDL digested to completion with EcoRI. After electroporation the cells were again incubated on ice for 10 min and replated in DMEM with 9% FBS. Twenty four hours after electroporation, mycophenolic acid, aminopterin, xanthine, hypoxanthine and thymidine (MAXHT; 16) were added to the medium to select for stable integration of pMSGneoDL (Fig. 1A). The cells were fed fresh medium every 3 days. Following 14 days of selection, colonies were picked and expanded to form independent cell lines, which were used for further experiments. RTX cell lines were generated as above except that pneoDLMG (Fig. 1B) was used to electroporate HT1080 cells and dexamethasone at a concentration of 5 µM was added to the selection medium.

Gene targeting assays

Cells (2 x 10^7) were electroporated with 20 µg of the appropriate DNA as described above. After electroporation the cells were transferred to plates containing DME supplemented with 9% FBS and hypoxanthine, xanthine and thymidine (HXT) or HXT plus dexamethasone. After 48 h, selection for homologous recombinants was started using DME supplemented with 9% FBS and 200 µg/ml G-418 (active concentration) with 5 µM dexamethasone. Media was changed every 3 days and well-isolated, growing colonies were counted after 16–17 days. For experiments done in the presence of dexamethasone cells were incubated in dexamethasone 24 h prior to electroporation and continuously thereafter.

RNA analysis

Total cytoplasmic RNA and poly(A^+) RNA were prepared from confluent 10 cm plates as described by Sambrook et al. (13). Aliquots of 5 µg cytoplasmic or 1 µg poly(A^+) RNA were run on a 1% agarose–formaldehyde gel. The RNA was transferred onto Magnagraph nylon membranes (MSI). Hybridization to [α-^32P]CTP-labeled antisense RNA probes and washing were carried out according to the manufacturer’s recommendation. Autoradiographic images were obtained using Fuji X-ray film.

RESULTS

Experimental strategy

To determine whether transcription influences targeting frequency, we incorporated synthetic recombination target sites (referred to as chromosomal plasmids) into the genome of a human fibrosarcoma cell line. In our system the recombination targets contained an inducible promoter element, the MMTV LTR, which drives the expression of a non-functional allele of the neomycin phosphotransferase (neo) gene (see Fig. 1A). In the presence of the inducer molecule dexamethasone, transcription from the MMTV LTR is enhanced. The neo deletion allele is incapable of providing resistance to the aminoglycoside G-418 and is incapable of spontaneously reverting to wild-type function. Homologous recombination between this locus and a second plasmid molecule (pSV2neoDR, Fig. 1D) containing a different neo gene allele (harboring a non-overlapping deletion) will generate a functional neo gene, rendering the cell resistant to G-418. Gene targeting frequency is determined by counting the number of G-418 resistant colonies obtained following transfection of the plasmid pSV2neoDR into target-bearing cell lines.

![Figure 1. Homologous recombination substrates. (A–C) Chromosomal plasmid target sequences. (A) DTX series target (pMSGneoDL), (B) RTX series target (pneoDLMG), (C) constitutive promoter control substrate (pSV2neoDSLV2GPT), (D and E) Targeting plasmids. (D) Double-stranded pSV2neoDR, (E) single-stranded phagemid pKSXH (the phagemid encoding the opposite strand, pSKHX, is not shown). Symbols: open box, neo gene; black vertical bars, deletions within the neo gene coding sequence; shaded arrowhead, MMTV LTR; vertically striped box, bacterial XGPRT gene used to select for integration of chromosomal targets; diagonally striped box, constitutive SV40 enhancer. Thin lines correspond to plasmid sequences, thick lines in (A–C) represent flanking human genomic DNA.](image-url)
Due to the nature of the heteroalleles utilized in this study, homologous recombination is the only mechanism by which G-418 resistance can be acquired. To confirm this, genomic DNA from 10 G-418-resistant colonies was isolated and subjected to PCR analysis using primers which would only amplify the full-length neo gene. All 10 colonies analyzed showed an amplification product of the expected size, whereas genomic DNA isolated from target-containing cell lines did not show a specific band (data not shown). As others have previously noted (14), a number of different recombination pathways, including, but not limited to, single and double reciprocal crossovers and gene conversion can all generate a functional neomycin gene. In this report we have not attempted to determine the molecular events by which drug resistance develops, since we can be certain that homologous recombination was involved.

Dexamethasone induces expression of recombination target genes

The plasmid pMSGNeoDL (Fig. 1A) was introduced into HT 1080 cells via electroporation and MAXHT-resistant clones isolated as described in Materials and Methods. A number of these clones were analyzed for expression of the neoDL transgene in both the presence and absence of dexamethasone. In screening these clones to identify suitable candidates for our analysis, we were interested in two criteria. First, we needed cells that showed little or no transcription in the absence of dexamethasone and a robust transcriptional induction upon dexamethasone addition. Second, the gene targeting frequency at the locus had to be sufficiently high to allow us to generate a sizable number of gene targeting events. Four of 13 clones tested in this way fulfilled the first criteria. Upon further analysis, one of these lines did not support gene targeting (perhaps due to a rearrangement within the target locus) and was therefore eliminated from the analysis.

In order to measure transcriptional induction at the recombination target site, the three cell lines were cultured in the presence and absence of 5 μM dexamethasone and mRNA isolated. We measured steady-state levels of messenger RNA, rather than actually determining transcription rates. Since the messages should be identical in all three cell lines, it is unlikely that their half-lives will differ. Therefore, for practical purposes, we assume that the levels of mRNA seen in these cells reflects the amount of relative transcriptional activity occurring at the target site. Figure 2 shows the results of a Northern blot analysis on the three cell lines. In all six lanes an equivalent amount of mRNA was loaded, based upon optical density measurements. Control Northern blots performed using an internal standard (β-actin) revealed that: (i) optical density is an accurate method with which to quantify mRNA, since the β-actin hybridization signal was identical amongst the three lines; (ii) dexamethasone reduced the expression of β-actin in all three cell lines to an equivalent extent (data not shown). Dexamethasone has previously been shown to down-regulate β-actin expression in other cell types (17). As Figure 2 indicates, no target-specific hybridization was seen in the three cell lines when they were grown in media lacking dexamethasone (lanes 1, 3 and 5). However, a strong hybridization signal was seen from all three cell lines following incubation with dexamethasone. Upon longer exposure a faint hybridization signal was seen in the 5A (-) DEX lane (lane 5), while no signal was seen in lanes 1 and 3. From these results it appears that transcription of the neo gene is induced to nearly the same extent in all three cell lines. However, inability to detect the message in the absence of dexamethasone in cell lines DTX 1A and DTX 1C makes it impossible to determine the actual extent of transcriptional induction.

Gene targeting frequency is stimulated by target site transcription

We conducted a number of electroporation experiments on the three cell lines, using pSV2neoDR (Fig. 1D) as the gene targeting vector. Cells that had been cultured either in the presence or absence of dexamethasone were electroporated with identical aliquots of targeting plasmid on the same day. Following a 48 h recovery period, the electroporated cells were plated in G-418 and cells that had undergone gene targeting were identified by colony formation within 16 days. All dishes (including those which had been previously cultured in the absence of dexamethasone) were continuously cultured in dexamethasone beginning 48 h post-electroporation, to ensure recovery of recombiant neo genes driven by the MMTV LTR. Since some types of homologous recombination would generate a functional neo gene driven by the MMTV LTR, we felt it was essential to demonstrate that these clones would survive under the selection protocol employed. We therefore conducted control experiments in which G-418 selection was applied 24 h prior to and 0, 24 and 48 h after transfection with a functional neo gene construct driven by the SV40 promoter. We did not observe any significant difference in the number of G-418-resistant colonies obtained in these experiments (not shown). This result indicates that even if selection is applied 24 h prior to expression of a functional neo gene, the target cell will survive. This is consistent with the observation that sensitive cells can survive incubation with G-418 for several days (not shown). We are therefore confident that gene targeted recombinants whose neo gene expression is driven by the MMTV LTR will be recovered in our experiments.

Table 1 presents the results of gene targeting experiments performed on the three cell lines harboring a transcriptionally inducible recombination target. In all three cases gene targeting frequency is significantly higher in the presence of dexamethasone than in its absence. Interestingly, despite nearly equivalent levels of dexamethasone-induced transcription (Fig. 2), hormone-induced gene targeting is much greater in cell line DTX 5A than in DTX 1C (see Discussion). Since no colonies were obtained in cell line 1A in the absence of hormone, it is impossible to quantitate the extent to which gene targeting is stimulated by that agent. We wanted to determine whether the dexamethasone inducibility of gene targeting was reversible. DTX 5A cells that
had been previously cultured in dexamethasone were subsequently incubated in hormone-free media for 4 days and then gene targeting assays performed. Gene targeting levels were identical to those seen in cells that had never been exposed to dexamethasone (data not shown).

Table 1. Effect of target site transcription on gene targeting

<table>
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<tr>
<th>Cell line</th>
<th>Dex</th>
<th>No. of colonies</th>
<th>No. of experiments</th>
<th>Colonies per expt</th>
<th>Fold induction</th>
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<td>27</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(+)</td>
<td>4</td>
<td>27</td>
<td>0.15</td>
<td>&gt;4^b</td>
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<tr>
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<td></td>
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<td>16</td>
<td>11</td>
<td>1.45</td>
<td>0.53^b</td>
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</tbody>
</table>

(-) and (+) refer to the absence and presence, respectively, of 5 μM dexamethasone. ^aP < 0.01, ^bP < 0.025.

As an additional control, we evaluated the level of gene targeting stimulation by hormone at a locus that is not transcriptionally induced by dexamethasone. The cell line used (1-1B-B) in these experiments differs from the DTX cell lines in that the promoter element within the chromosomal plasmid is the constitutive SV40 enhancer (see Fig. 1C). As shown in Table 1, gene targeting was not stimulated by the presence of dexamethasone in 1-1B-B cells. Dexamethasone actually significantly diminished the level of gene targeting in this line. While we have not detected a significant decrease in the steady-state level of neo mRNA in 1-1B-B cells treated with dexamethasone, it has been previously noted that dexamethasone decreases, by 2–3-fold, transcription at loci driven by the SV40 enhancer (8). The targeting results observed in 1-1B-B cells indicate that there is no general increase in homologous recombination in response to dexamethasone. We also observed that non-homologous recombination is not affected by dexamethasone in HT1080 cells. HT1080 cells were incubated in the absence or presence of dexamethasone and transfected with pSV2neoWT. Selection was started 48 h post-transfection and G418-resistant colonies were counted 14–16 days after selection was started. No significant differences were observed in the number of colonies obtained in either case (data not shown).

Time course of transcription-induced recombination

To determine the kinetics of dexamethasone-stimulated gene targeting, a series of electroporations were carried out on DTX 5A cells that had been exposed to dexamethasone for times varying from 24 h prior to electroporation to 48 h following electroporation. As Figure 3 demonstrates, gene targeting is not appreciably stimulated when dexamethasone is added 24 h after electroporation. These results indicate that gene targeting occurs relatively soon after electroporation. These data further indicate that we have not appreciably underestimated the extent of hormone stimulation by applying dexamethasone to the (−) dexamethasone plates 48 h post-electroporation. Since most recombination had occurred by 48 h post-electroporation, addition of dexamethasone at this time would not have increased the frequency of recombination in the (−) DEX cells.

Transcription must proceed through the recombination site in order to stimulate gene targeting

It has been suggested that transcription stimulates gene targeting by creating a more favorable target for recombination enzymes, through the creation of supercoils within the target locus. We reasoned that were this true, adjacent transcription would introduce topological changes within the recombination target. These alterations in superhelicity might thereby stimulate gene targeting. To test this, we attempted to stimulate recombination by inducing transcription at a locus immediately adjacent to the recombination target. We therefore created a new chromosomal target plasmid that contained the MMTV LTR arranged in a reverse orientation relative to that in the DTX construct (see Fig. 1B). Cell lines that harbored this target were isolated. Three lines (RTX 2-1, 3-2 and 5-1) that showed robust dexamethasone stimulation of transcription of the GPT gene (not shown) were tested for gene targeting frequency in the presence and absence of dexamethasone. As the data in Table 2 indicate, gene targeting frequency was not stimulated by transcription.
There is no strand bias associated with transcription-stimulated gene targeting

It has been established that repair of UV photoproducts occurs more efficiently within transcribed genes than within the genome at large (18). It has further been shown that the transcribed strand of DNA is preferentially repaired relative to the untranscribed strand (19,20). Nag and Petes have shown preferential strand transfer during meiotic recombination in S.cerevisiae (21). In addition, Sherman and colleagues have shown that transformation in yeast occurs more efficiently with sense oligonucleotides than with antisense oligonucleotides (22). We therefore suspected that it might be possible to observe a similar bias in our system. To test this hypothesis, we created two additional targeting vectors, pKSHX and pSKHX (Fig. 1E), which, when used to create single-strand phagemids, encoded the template and coding strands respectively of the chromosomal plasmid target. These two single-stranded plasmids were used in a series of targeting experiments in both the presence and absence of dexamethasone and G-418-resistant colonies counted. As the results in Figure 4 indicate, we observed no apparent strand bias, since both the template strand was used, while the shaded bars represent colonies seen when the coding strand was used.

DISCUSSION

In this report we demonstrate that gene targeting in human cells is stimulated by target site transcription. Chromosomal plasmid sequences under the transcriptional control of the MMTV LTR, which is induced by the drug dexamethasone, were utilized as recombination substrates. We showed that transcription-stimulated gene targeting is relatively independent (see below) of the location of the target within the genome, since enhanced recombination was seen with three independent cell lines containing the target sequence integrated into different loci. In addition, experiments performed on a cell line harboring a constitutively transcribed target indicated that dexamethasone addition does not result in a general increase in gene targeting frequency. Results from other laboratories have indicated that a variety of different recombination events, including extrachromosomal and intrachromosomal homologous recombination in mammalian cells, are stimulated by target site transcription (1–3,5–8). Previous results indicating that non-transcribed target loci can serve as efficient substrates for gene targeting led to the suggestion that the transcriptional state of a locus may not influence gene targeting (9). Our results, however, provide evidence that transcription can stimulate gene targeting in human cells. It remains to be seen whether other inducible promoters will have a similar effect on gene targeting.

We evaluated the effect of transcription on gene targeting frequency in three different cell lines. Southern blot analysis of genomic DNA from the three cell lines showed that each line harbored a single integrated copy of the plasmid shown in Figure 1A, presumably located at a unique site within the host genome (data not shown). As Figure 2 indicates, while all three of these cell lines showed approximately equivalent amounts of transcriptional activation in the presence of dexamethasone, DTX 1C showed only a modest 3-fold induction of gene targeting, while DTX 5A showed a >20-fold hormone stimulation. Since both these cell lines appear to be equally induced transcriptionally by dexamethasone (Fig. 2), we do not believe that the relative increase in transcription correlates very well with the extent of gene targeting induced by dexamethasone. The differences in gene targeting induction could be due to other factors, such as the presence of hotspots of recombination, which may lead to an unusually high level of homologous recombination in the absence of transcription. It may be possible to directly test the hypothesis that relative increases in transcription cause a corresponding
increase in gene targeting frequency through the use of constructs containing inducible promoters of different strengths. We are currently exploring the feasibility of this approach. Until such additional information becomes available, we can only state that transcription enhances gene targeting in a locus-independent fashion. However, the relative extent of this enhancement is locus-dependent.

We have considered a number of models that explain the link between transcriptional activity and homologous recombination. First, transcription may result in topological changes in the recombination target that render it more recombinogenic. For example, unwinding of the DNA duplex and formation of single-stranded DNA during transcription may facilitate the formation of heteroduplex DNA between the chromosomal target and the targeting plasmid. To address this possibility, we employed single-stranded targeting vectors, reasoning that we might observe a strand bias in the transcription-stimulated recombinants. We believed that if single strands of target site DNA were made available, as a consequence of transcription, they could efficiently base pair with a single-stranded recombination vector. Experimentally this would be measured as an increased ability of one or the other single-stranded vector to participate in the recombination reaction. As the data in Figure 4 indicate, no such bias exists. This suggests either that both strands are made equally accessible to the targeting plasmid or that the effect of transcription on gene targeting is not mediated in this way. We favor the second interpretation, since we feel that it would be likely that either the coding or the template strand (but not both) would become preferentially available as a recombination substrate. Second, we considered that negative supercoils created by passage of the transcription machinery could create an attractive target for recombination proteins. It has been proposed that this may be responsible for the dual function of the RAD1–RAD10 complex in recombination and nucleotide excision repair, which is known to be stimulated by transcription (25). However, the absence of transcription-stimulated recombination in the KTX series cell lines, in which the MMTV promoter is located downstream of the recombination site, suggests that this hypothesis is not correct. It is conceivable, however, that negative supercoils produced by transcription do not extend upstream through the entire MMTV element into the recombination target.

It has also been proposed that transcription increases accessibility of the target sequence to recombination proteins. While this hypothesis is attractive, we believe that certain observations made by others, as well as by ourselves, may not be entirely consistent with it. We showed that transcription must proceed through the recombination site in order to stimulate gene targeting. This result is qualitatively similar to the observation in S. cerevisiae that the HOT1 recombination hotspot sequence is rendered non-functional if a transcriptional termination signal is inserted between it and the recombination target (2). In both of these cases extensive chromatin re-ordering is likely to occur immediately adjacent to the recombination target, yet no increase in gene targeting occurs. While chromatin-mediated accessibility may occur over extremely small domains, we are inclined to believe that such an effect could be expected to extend a mere kilobase beyond the boundaries of transcription. These two results may therefore suggest that rather than simply enhancing accessibility of the target locus to recombination proteins, a more intimate link between the processes of recombination and transcription may exist. Two hypotheses designed to explain such a link are presented below.

It has been suggested that transcribed DNA is more easily recognized by recombination enzymes due to transcriptional pausing (26). This mechanism has been proposed to explain the high efficiency of nucleotide excision repair (NER) at transcribed loci (27). Selby and Sancar (28) have developed an in vitro repair and transcription system in which the protein encoded by the E. coli mfd gene (mutation frequency decline) functions as a transcription–repair coupling factor (TRCF). Their data reveals that this factor displaces RNA polymerase from DNA lesions at which it has stalled. It appears that TRCF actively recruits the bacterial repair mechanism to the site of the lesion. A similar activity may exist in cultured mammalian cells and be responsible for coupling transcription and DNA repair. Transcriptional stalling (26) or other mechanisms may function to link transcription and homologous recombination in mammalian cells, as it apparently does in the mfd system.

A final model is based on analogy to NER. It has recently been shown that essential NER genes RAD25 (ERCC3, 29 and 30) and RAD3 (ERCC2 and 31) are components of the basic transcription factor BTF2 and that RAD2 (ERCC5) and RAD4 interact with it (32). Homologous recombination proteins may also be components of the transcription machinery or be capable of binding to it. Just as bi-functional enzymes may be involved in both transcription and DNA NER (24), recombination proteins may similarly be bi-functional, playing an additional role in transcription. The Rad1 and Rad10 proteins, which form an endonuclease complex (33, 34), are essential for nucleotide excision repair and are known to function in homologous recombination (35). Furthermore, a recent report has shown an association of homologous recombination and DNA replication (36). These observations provide precedent for the idea that the homologous recombination machinery may share common factors with complexes that catalyze other cellular processes, including transcription. It is therefore possible that a linkage between transcription and recombination exists, because common enzymes are shared by the two sets of machinery.

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