Nucleotide excision repair : complexes and complexities : a study of global genome repair in human cells

Volker, Marcel

Chapter 4

More interplay between NER and transcription: inhibition of transcription following DNA damage

4 More interplay between NER and transcription: inhibition of transcription following DNA damage

Although NER and transcription are quite distinct processes several intimate links exist between them, two of which – TCR and the dual role of TFIIH in NER and transcription – have been described in chapters 2 and 3. A third link between NER and transcription is the temporary inhibition of transcription that occurs after the introduction of several types of DNA damage.

4.1 Transcription inhibition *in cis* and *in trans*

Two fundamentally different mechanisms can underlie an inhibition of transcription. First, transcribing RNA pol II may stall on a lesion (the same phenomenon that is thought to initiate TCR). This direct inhibition is referred to as *cis*-inhibition and is known to occur for various types of lesions, e.g. UV-induced photolesions (Giorno and Sauerbier, 1976). It is tempting to envisage TCR coming about as a direct consequence of this stalling, i.e. *cis*-inhibition of transcription. However, the severity of transcription-interference by a lesion does not correlate with its rate of removal by TCR (McGregor et al., 1995) indicating that other factors – subsequent steps in the TCR pathway, such as the ease of removal of RNA pol II from the lesion, or damage verification – are also important in determining its repair kinetics.

In addition to inhibition of transcription *in cis*, several observations indicate the existence of another mechanism, resulting in transcription inhibition *in trans*. In normal cells transcription returns to its normal levels within several hours following the induction of DNA damage (a phenomenon usually referred to as recovery of RNA synthesis or RRS). Remarkably even XP-D cells lacking TCR of UV lesions (van Hoffen et al., 1999) display complete RRS 24 hours after UV, albeit a low dose (van Hoffen et al., 1999). In contrast, in cells from CS and XP/CS patients transcription remains permanently inhibited following UV irradiation (Mayne and Lehmann, 1982; Moriwaki et al., 1996; Rockx et al., 2000; van Hoffen et al., 1999) or NA-AAF treatment – despite the complete repair of the NA-AAF-induced dG-C8-AF adducts by GGR (van Oosterwijk et al., 1996a; van Oosterwijk et al., 1996b). It is thought that this phenomenon contributes considerably to the high cell killing by UV irradiation in these cells (Andrews et al., 1978; van Hoffen et al., 1999), and underlies the sensitivity of CS cells to NA-AAF (van Oosterwijk et al., 1996b).

Other experiments show that the *trans*-effect actually occurs through inhibition of transcription at the initiation level. Using cell extracts competent for transcription initiation on plasmids Rockx and co-workers showed that in cell extracts from UV-irradiated cells, transcription did not initiate from plasmids that were themselves not damaged (Rockx et al., 2000). *In sum*, there is a number of indications arguing for additional inhibition of transcription acting *in trans*; more specifically, acting at the initiation level.

Finally, this phenomenon appears to be exclusively linked to lesions that are (at least potentially) subject to TCR, as UV-induced photolesions (Mellon et al., 1987) as well as dG-C8-AF adducts (van Oosterwijk et al., 1996a) are a target for TCR, although in the case of dG-C8-AF, repair by TCR is masked in normal cells due to the higher kinetics of repair via GGR (van Oosterwijk

et al., 1996b) whilst these lesions at the same time induce an overall reduction in transcription (Mayne and Lehmann, 1982; van Oosterwijk et al., 1996a; van Oosterwijk et al., 1996b). On the other hand, publications reporting TCR of oxidative lesions such as thymine glycols and 8-oxoguanine have been retracted or are under strong suspicion, and the introduction of such lesions does not lead to a reduction in overall transcription initiation (D.A.P. Rockx, unpublished observations).

4.2 A role for TFIIH in transcription inhibition *in trans*?

The dual involvement of TFIIH in transcription and NER lies at the basis of the so-called 'TFIIH-shuttling' model (van Oosterwijk et al., 1996b). In this model, in the presence of DNA damage TFIIH is preferentially tethered to repair sites, depleting it from sites of transcription initiation and so resulting in reduced transcription. *In vitro* research provided conflicting evidence on this matter. On the one hand, in a HeLa cell-free assay optimised to support NER and transcription simultaneously, Satoh and Hanawalt found that NER and transcription did not interfere with each other (Satoh and Hanawalt, 1996). In contrast, in yeast cell extracts capable of both NER and transcription You and co-workers found transcription to be inhibited when the system was simultaneously presented with NER substrates (You et al., 1998). Addition of TFIIH relieved this inhibition, leaving the authors to conclude that the preferential mobilisation of TFIIH to sites of repair was the primary cause of the transcriptional inhibition (You et al., 1998).

Subsequent experiments however have provided more evidence against the TFIIH-shuttling hypothesis. First, Rockx and co-workers tested the hypothesis in the abovementioned *in vitro* transcription assay (Rockx et al., 2000). The prior inclusion of additional TFIIH was found to have a non-specific positive effect on transcription initiation in extracts from both unirradiated and irradiated cells (Rockx et al., 2000). Additionally, using GFP-tagged XPB, Hoogstraten et al. found that the maximum fraction of TFIIH that could be tethered to repair was ~40% (Hoogstraten et al., 2002). Since in non-damaged cells only 20-40% of TFIIH is involved in transcription at any one time (Hoogstraten et al., 2002) these results together reinforce the idea that recruitment of TFIIH to repair sites does not cause an inhibition of transcription. Finally, we tested whether TFIIH, or any other freely diffusible factor, is involved in propagating the signal for transcription inhibition throughout the nucleus. One early experiment suggested that transcription inhibition not only takes place *in trans*, but also spreads throughout the nucleus. As early as 1967, Takeda and co-workers used a UV microbeam to induce UV damage in a small volume of HeLa cell nuclei and measured the effect of this irradiation on transcription with 3H-uridine pulse-labelling. Transcription decreased in the entire nucleus, suggesting that transcription was inhibited *in trans* and the signal for inhibition was distributed by a factor that can freely diffuse through the nucleus (Takeda et al., 1967). However, using a different UV irradiation method and run-off transcription labelling with BrUTP, followed by immunofluorescent labelling, we were unable to reproduce a nucleus-wide transcription inhibition. Instead, we found the inhibition to be confined to the area of damaged DNA, and we concluded that the *trans*-inhibition in fact has a limited range (chapter 7).

4.3 What causes the transcription inhibition *in trans*?

As yet the exact cause for the *trans*-effect of transcription inhibition is not known, but some indications exist. Most notably, following UV irradiation, the hypophosphorylated initiating form of RNA pol II (RNA pol IIa) is reduced and the amount of hyperphosphorylated elongating form (RNA pol IIo) increases (Rockx et al., 2000), coinciding with the reduction in transcription. Conversely, as transcription returns to its pre-UV levels, RNA pol IIa reappears (Rockx et al., 2000). In sharp contrast, in CS-A and -B as well as XP-A cells both transcription and the levels of RNA pol IIa remain low after UV (Rockx et al., 2000). Thus, the *trans*-effect of transcription inhibition reveals itself as a shift in RNA pol II from predominantly the initiating to the non-initiating/elongating form. Whether the stalling of RNA pol II on a lesion is the leading cause for this shift in phosphorylation state or whether the shift is brought about by other means, e.g. a UV-sensing signalling pathway, is unknown. It is however curious that only those DNA lesions that are a target for TCR appear to give rise to *trans*-inhibition of transcription (see above); it is therefore tempting to speculate that it is the stalling of RNA pol II that, directly or indirectly, triggers the transcription inhibition. The conceptual problem that arises from models in which another damage sensor (such as a signalling pathway utilising receptors in the cell membrane) evokes the *trans*-effect is that this sensor seemingly is able to predict if a type of DNA damage is a target for TCR.