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Mini-review

Damage control: DNA repair, transcription, and the ubiquitin–proteasome system

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ABSTRACT

The presence of DNA damage within an actively transcribed gene poses an immediate threat to cellular viability. Bulky DNA adducts, such as those induced by ultraviolet light, can profoundly influence patterns of gene expression by causing the irreversible arrest of RNA polymerase II at sites of DNA damage. It is critical that processes exist to either specifically repair transcribed genes or clear stalled RNA polymerase, so that general repair can occur and transcription resume. A growing body of evidence indicates that clearance of stalled polymerase is achieved, in part, by ubiquitin-mediated destruction of the largest subunit of RNA polymerase II. In this review, we shall discuss how an intimate connection between RNA polymerase II and the ubiquitylation machinery acts to restore normal transcription after DNA damage, and other forms of transcriptional arrest, has occurred.

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1. Introduction

The integrity of the genetic information is constantly under threat from DNA damage. Not only can DNA damage result in the passage of heritable genetic mutations, but it can also result in immediate changes to the gene expression profile of a cell. Bulky DNA adducts that occur within transcribed regions of the genome can lead to rapid and irreversible arrest of RNA polymerase II (pol II), attenuating the expression of damaged genes, and promoting a cell death response (reviewed in [1]). The maintenance of normal cellular homeostasis in response to DNA damage, therefore, requires that cells either preferentially repair active genes, or possess the ability to quickly clear arrested pol II complexes so that DNA can be repaired and transcription resumed.

In the last 20 years, considerable progress has been made in understanding the mechanisms through which eukaryotic cells respond to DNA damage within active genes. Preferential repair of DNA in transcribing genes was first described in the mid-1980s [2,3], and it is now clear that this process occurs via the transcription-coupled DNA repair (TCR) pathway (reviewed in [4]). TCR is a type of nucleotide excision repair (NER) that, in essence, employs transcribing pol II as a sensor of DNA damage and directs the NER machinery to aberrant sites in the genome. This process allows rapid removal of DNA adducts from active genes, and permits transcription to continue. But what happens if TCR fails? Under these conditions, components of the TCR machinery appear to engage a failsafe mechanism that specifically removes stalled pol II complexes from damaged DNA, allowing the more general global genome repair (GGR) pathway to restore DNA to as-new condition. One of the most effective mechanisms through which stalled pol II complexes can be stripped from DNA involves pol II destruction by the ubiquitin (Ub)–proteasome system (UPS [5]).

Ubiquitin is a highly conserved, 76 amino acid, protein that can be conjugated to lysine residues within a substrate protein. This process—which is referred to as ‘ubiquitylation’—requires the sequential action of three sets of proteins: an E1 (Ub-activating

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enzyme), that provides the energy for conjugation by forming a thioester bond with Ub; an E2 (Ub-conjugating enzyme), that accepts Ub from the E1 and is responsible for carrying out the chemistry of substrate ubiquitylation; and an E3 (Ub-protein ligase), which specifically recognizes the substrate and brings it together with the E2 for ubiquitylation to occur [6]. Typically, the Ub that is linked to a substrate protein will itself become ubiquitylated, giving rise to a poly-Ub-chain that, if of the correct type, will lead to rapid destruction of the substrate by a large protease complex called the 26S proteasome [7]. The utility of the UPS stems from the high degree of substrate selectivity that can be achieved via specific E3s, which allows substrates to be targeted for destruction under very specific circumstances—such as in response to a stalled RNA pol II complex.

In this review, we shall discuss how the UPS interacts with pol II and with components of the TCR machinery to specifically clear stalled transcriptional complexes from sites of DNA damage. We describe current understanding of the molecular processes at work, and present a model for how the unique features of the UPS could act to switch between TCR- and GGR-mediated DNA repair.

2. RNA polymerase II destruction as a failsafe for transcription-coupled repair

The importance of TCR in protecting cells against cytotoxic, transcription-blocking, DNA lesions is obvious. What has been somewhat less obvious, however, is how ubiquitylation and destruction of pol II features in this process. We argue here in support of a model, originally proposed by Svejstrup [8], in which pol II turnover is not part of TCR *per se*, but rather functions to facilitate DNA repair if TCR cannot occur.

The initiating event for TCR is the encounter between a transcribing pol II and a helix-distorting DNA lesion (reviewed in [4,9]). In mammalian cells, this arrest of pol II leads to the recruitment of two critical proteins—CSA and CSB [10]. CSB, which is the mammalian homolog of *Saccharomyces cerevisiae* Rad26 (discussed later [11]), is related to the SWI/SNF family of ATP-dependent chromatin remodelers [12]. Its recruitment to DNA is primarily responsible for recruitment of CSA, a WD-40 domain-containing E3 ligase [13,14]. Together, CSA and CSB recruit components of the NER machinery to the sites of DNA damage, where they excise the affected zone and repair the DNA using the undamaged strand as a template. Once the DNA is repaired, the CS proteins recruit chromatin remodeling enzymes (e.g., the p300 histone acetyltransferase [15]) and elongation factors (e.g., TFIIS [16]) to the stalled polymerase [10], CSB is targeted for destruction via the E3 function of CSA [17], and transcription resumes.

In the mid-1980s researchers first noted that treatment of eukaryotic cells with DNA-damaging agents such as cisplatin, UV light, and methyl methanesulfane, resulted in the ubiquitylation and the destruction of the largest subunit of RNA polymerase II, Rpb1 [18–20]. At first, it was reasonably concluded that Rpb1 destruction is part of the TCR process, and is required to remove pol II from the DNA before NER can occur [18,19]. This notion, however, was challenged by the demonstration that NER can repair a DNA lesion *in vitro* without having to displace pol II from the template [21,22]. This result strongly suggested that Rpb1 ubiquitylation and destruction is not an integral part of the TCR response.

If Rpb1 turnover is not required for TCR, then why does it occur? The answer to this question has come following identification—and genetic analysis—of the cellular machinery that regulates pol II ubiquitylation in response to DNA damage. In *S. cerevisiae*, a number of E3s have been implicated in this process, including the Ub-ligase Rsp5 [20]—which works together with the targeting factor Def1 [23]—and a complex composed of Elongin A (Ela1), Cullin 3 (Cul3), Elongin C (Elc1) and Roc1, which resembles the mammalian VHL Ub-ligase (an E3 for Rpb1 [24,25]). Following identification of these factors, genetic analyses were able to separate TCR from the pol II ubiquitylation machinery. For example, neither Def1 [23] nor Elc1 [26] are required for repair of DNA damage at active genes, and—conversely—Rpb1 destruction occurs normally in yeast defective for either TCR or the entire NER pathway [23,27]. Despite the separation, however, Rbp1 turnover is clearly important for the DNA damage response, as mutations within the Rpb1 ubiquitylation pathway are strongly synthetic with mutations that disrupt TCR. For example, simultaneous deletion of *RAD26*, together with deletion of either *ELA1*, *CUL3*, or *ELC1*, produces a much more pronounced UV sensitivity than deletion of either gene alone [24]. Genetic interactions such as these support the idea that TCR and Rpb1 ubiquitylation/destruction are both important for the DNA damage response, but function via separate pathways. The most logical interpretation of these results is that Rpb1 ubiquitylation/degradation functions as a 'failsafe' mechanism to insure rapid repair of damaged transcription units in the event that TCR does not proceed in a timely manner. As depicted in Fig. 1, destruction of Rpb1 at sites of failed TCR would clear the damaged template DNA of stalled transcription complexes, allowing DNA repair via GGR [8]. By abandoning non-productive TCR through this mechanism, the cell creates for itself a final opportunity to restore transcription before cellular damage can occur.

3. Rpb1 ubiquitylation as a general remedy for RNA polymerase arrest

Implicit in the above discussion is the notion that the Rpb1 ubiquitylation/destruction machinery has the capacity to zero on a population of transcribing polymerase complexes that have encountered an impassable segment of DNA. Although it is conceivable that a dedicated factor could recognize the DNA lesion and liaise with the ubiquitylation machinery, it appears as though the signal for Rpb1 ubiquitylation may be generated, in part, simply by the stalling (arrest) of RNA polymerase II at a transcribed gene. Support for this concept comes from the finding that agents that inhibit transcription (e.g., α-amanitin) or compromise pol II elongation (e.g., 6-azauracil) lead to Rpb1 ubiquitylation and destruction [5,28,29]. Importantly, the same cellular machinery appears to mediate Rpb1 ubiquitylation in response to both DNA damage and elongation arrest [23,30], arguing that stalling of pol II is sufficient to engage the process of Rpb1 ubiquitylation and destruction.

If transcriptional arrest is sufficient to engage the machinery of pol II ubiquitylation, then one might expect that the same ubiquitylation pathway might also participate generally in the transcription process, to ameliorate endogenous blocks to transcription—such as a natural pause in the polymerase at a particular sequence of DNA, or when the path of the enzyme is blocked by factors such as a recalcitrant nucleosome. In this regard, it is interesting to note that the Elc1 protein was originally characterized based on its ability to promote transcriptional elongation [31]. It is also informative that disruption of the *DST1* gene—which encodes for the elongation factor TFIIS—induces pol II ubiquitylation [5], and is synthetic with disruption of genes required for pol II ubiquitylation after DNA damage. For example, the 6-azauracil sensitivity of a *Δdst1* strain is enhanced by simultaneous deletion of *DEF1* [23], whereas deletion of *DST1*, together with disruption of one of the RSP5-dependent ubiquitylation sites on Rpb1, is synthetically lethal [30]. These genetic interactions support the concept that UPS-mediated destruction of Rpb1 occurs not just after DNA damage, but also during transcription in unperturbed cells. Svejstrup [8] have proposed

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