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Review

RNA polymerase II pausing and transcriptional regulation of the *HSP70* expression

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ABSTRACT

Heat-shock proteins (HSPs) belong to the chaperone protein family whose expression is induced by different stresses including heat-shock. In response to the extracellular or intrinsic stimuli and stresses, HSPs play important roles in the maintenance of cellular homeostasis. HSP70, a major HSP protein (molecular weight, 70 KDa), regulates diverse cellular pathways including protein quality control, translation, immune response, and cancer survival. As a critical cellular defense system to minimize damages from cellular stresses, *HSP70* expression and transcriptional activation are rapidly regulated, mainly through the action of a transcription activator, Heat shock factor 1 (HSF1). Eukaryotic *HSP70* genes are well-characterized; they utilize a transcriptional mechanism termed as RNA polymerase II (Pol II) promoter-proximal pausing. Pol II promoter-proximal pausing enables synchronized gene expression in a number of mammalian protein-coding and non-protein coding genes upon the reception of gene activating signals. In particular, *Drosophila* and human *HSP70* genes serve as a *bona fide* model system to understand the mechanisms of Pol II pausing and pause release. In this review, we will discuss *HSP70* transcription and the newly discovered mechanisms that regulate *HSP70* gene expression.

1. Introduction

Heat shock proteins (HSPs) are molecular chaperones that assist the folding and assembly of macromolecules in the cell (Zhang et al., 2015; Vabulas et al., 2010). The chaperone function of HSPs is required for maintaining the quality of proteins, particularly under the cellular stresses (Chen et al., 2011). Protein quality control is achieved by regulating misfolded proteins- through refolding, degrading, or sequestering (Chen et al., 2011; Richter et al., 2010; Schlecht et al., 2011). The diverse sources of stresses such as osmotic pressure, hypoxia, hyperthermia, and chemical stresses can threaten proper folding of proteins and induce *HSP70* gene expression (Petronini et al., 1993; Hammerer-Lercher et al., 2001; Blake et al., 1991; Liu et al., 1994). In turn, the expressed HSP70 proteins are involved in controlling the physiological and pathological cellular pathways ranging from unfolded protein response, protein biosynthesis to autophagic cell death (Hetz, 2012; Cuesta et al., 2000; Dokladny et al., 2013).

Transcription is a critical cellular process by which RNA is generated from the DNA template (Young, 1991; Bunch et al., 2016). RNA is an intermediate macromolecule that participates and modulates biological pathways as it is (RNA form) or is translated into proteins to function (Bunch et al., 2016; Horning and Joyce, 2016; Li and Izpisua Belmonte, 2015; Hocine et al., 2010; Lee and Young, 2013). The former includes transfer RNA (tRNA), ribosomal RNA (rRNA), and non-coding RNA (ncRNA) and the latter messenger RNA (mRNA). Studies have proposed that approximately 74.7% of the mammalian genome is transcribed, although only approximately 2%–3% of the transcribed genes encode proteins (Graur et al., 2013). Transcription is the primary step to control gene expression; therefore, it is important to understand the mechanisms that regulate transcription.

RNA polymerase II (Pol II) promoter-proximal pausing has been known since it was discovered at some genes including *HSP70* a couple of decades ago (Brown et al., 1996; Li et al., 1996). However, in recent years, Pol II pausing has been observed in a large number of protein-coding and non-protein coding genes (Bunch et al., 2016; Nechaev et al., 2010; Rahl et al., 2010). The discovery was achieved through the development of genomic analyses, and the significance of Pol II pausing in transcription and gene expression has been shown in different organisms such as humans, mice, and flies (Bunch et al., 2016; Nechaev et al., 2010; Rahl et al., 2010; Gilchrist et al., 2010). In early 2010s,

Abbreviations: HSP, heat shock protein; HSF1, heat shock factor 1; Pol II, RNA polymerase II; UPR, unfolded protein response; HSE, heat shock element; P-TEFb, positive transcription elongation factor b; PARP, poly(ADP)-ribose polymerase; PAF1, polymerase associated factor 1; GTF, general transcription factors; HP1γ, heterochromatin protein 1γ; NELF, negative elongation factor; DSIF, DRB sensitivity-inducing factor; DNA-PK, DNA-dependent protein kinase; ATM, ataxia telangiectasia mutated; GAF, GAGA factor; TRIM28, tripartite motif-containing 28; STRAP, stress-responsive activator of p300; PIC, pre-initiation complex; snRNP, small nuclear ribonucleoprotein; DNA-PKcs, DNA-PK catalytic subunit; eRNA, enhancer RNA; H3Kme3, H3 trimethylated at lysine 4; m^6A, N6-methyladenosine

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some elaborate genomic studies reported an unexpected prevalence of Pol II pausing, and were followed by mechanistic studies for the understanding of this phenomenon (Bunch et al., 2016; Schweikhard et al., 2014; Bunch et al., 2014, 2015; Bunch and Calderwood, 2015; Bunch, 2016; AJ et al., 2016; Jonkers et al., 2014; Jonkers and Lis, 2015). *Hsp70* has been serving as an established model gene to study Pol II pausing. Pol II pausing before gene activation was discovered using *Drosophila Hsp70* gene (Rougvie and Lis, 1988). In addition, our recent study utilized the native human *HSP70* gene to identify the mechanisms underlying Pol II pausing regulation in mammalian cells (Bunch et al., 2014, 2015; Bunch and Calderwood, 2015; Bunch, 2016).

Transcriptional activation and gene expression of *HSP70* is largely dependent on heat shock factor 1 (HSF1), a 57 KDa protein (Hentze et al., 2016). HSF1 is present in the cytoplasm as an inactive complex with other chaperon proteins such as HSP70/HSP90 (Vabulas et al., 2010; Zou et al., 1998). Cellular stresses such as elevated temperature releases HSF1 to translocate into the nucleus. The protein is trimerized and hyper-phosphorylated to become active before the translocation (Hentze et al., 2016; Neef et al., 2011). In turn, nuclear HSF1 trimers bound to the promoter of *HSP70* gene trigger the downstream activating cascade to release Pol II from the pausing site (Bunch et al., 2014; Neef et al., 2011; Zobeck et al., 2010).

Understanding the expression system of the *HSP70* gene is critical because the protein encoded by it has diverse physiological and pathological roles in the cell. In addition, this is an exemplary gene that is regulated by Pol II pausing, and therefore the recently identified mechanisms of transcriptional elongation at *HSP70* will help getting an insight into transcription and gene expression regulation in a large number of genes in the mammalian cells. For these purposes, we focus on these recent findings of transcriptional regulation, the novel mechanisms of Pol II pausing and pause release regulation at metazoan *HSP70* genes in this review.

2. *HSP70* transcription

In humans, at least thirteen HSP70 proteins exist (Radons, 2016). The representative members of the *HSP70* family are *HSPA1A* and *HSPA1B*. Both these genes are located in the chromosome 6, possibly through gene duplication, and encode an identical protein comprising 641 amino acids. There are 3 and 5 members of *Hsp70* known in yeast and *Drosophila*, respectively (Slater and Craig, 1987; Tower, 2011). Although a substantial amount of *HSP70* is expressed in normal physiological conditions without heat-shock, a constantly elevated level of HSP70 has been reported in cancer cells (Murphy, 2013; Jagadish et al., 2016; Rohde et al., 2005). The basal level of *HSP70* gene expression suggests a leaky transcription as well as a measurable stability of its mRNA and protein. Studies presented the half-life of *HSP70* mRNAs to be approximately 50 min and that of HSP70 protein to be over 60–120 min (Theodorakis and Morimoto, 1987; Mao et al., 2013; Li and Duncan, 1995). Continuous heat shock increased the half-life to 7 h (Li and Duncan, 1995).

Transcriptional regulation of *HSP70* was previously discovered and has been vigorously studied in *Drosophila* (Rougvie and Lis, 1988; Zobeck et al., 2010; Wu et al., 2003; Lee et al., 2008). Upon heat-shock, decondensation of chromosomes (Polytene), appearing like puffs, occurs in the loci of *hsp70* genes (Zobeck et al., 2010; Schlesinger, 1986). Hyper-phosphorylated HSF1 trimer binds to the DNA sequence called Heat shock element (HSE), which is necessary for heat- induced gene expression (Neef et al., 2011). In human, the consensus sequence is nTTCnnGAAnnTTCn and the promoter of *HSP70* gene includes multiple HSEs, achieving effective and cooperative HSF1 binding (Neef et al., 2011). The mutations of HSE abolish HSF1-dependent transcriptional activation and the spacing and position of Guanine is known to be important for the function of HSE despite some variations (Sarge et al., 1991).

A significant number of Pol II is already engaged with and accumulated in the promoter-proximal site of *HSP70* genes during the uninduced state of transcription (Adelman and Lis, 2012). We will discuss Pol II pausing and pause release at *HSP70* gene in more detail below. In brief, Pol II initiates transcription and polymerizes short RNA chains with lengths ranging between 20 bp and 50 bp (*Drosophila*) and 30 bp and 100 bp (mammals) before being paused (Brown et al., 1996; Nechaev et al., 2010; Bunch et al., 2014; Adelman and Lis, 2012). The nascent RNA molecule is associated with the paused Pol II and the pausing is known to be stable, and resistant to salts and detergents such as sodium lauroyl sarcosinate (also called sarkosyl) (Li et al., 1996; Gnatt et al., 1997). Pol II pausing is established and stabilized by different transcription factors as described below.

Protein factors involved in transcriptional activation are recruited to the *HSP70* gene upon heat induction. The hyper-phosphorylated and trimerized HSF1 proteins bind to HSE; subsequently, Pol II is recruited along with Positive transcription elongation factor b (P-TEFb), Spt6, Polymerase associated factor 1 (PAF1), Poly [ADP-ribose] polymerase (PARP), and topoisomerase I in *Drosophila* (Zobeck et al., 2010; Adelman et al., 2006). The recruitment of these factors is rapid. A study reported that HSF1 is recruited within 20 s and Pol II and the other factors mentioned above within approximately 2 min (Zobeck et al., 2010; Murawska et al., 2011). This synchronized and rapid induction appears to be conserved in human *HSP70*. In our study, the recruitment of HSF1 to the promoter site was detectable within 30 s by Chromatin immune precipitation (ChIP) analysis with Human embryonic kidney 293 (HEK293) cells (Bunch et al., 2014). Transcription activation in human *HSP70* recruits various transcription factors: General transcription factors (GTFs), nucleosome remodeling factor SWI/SNF complex, P-TEFb, topoisomerases, γH2AX, Histone 3.3 (H3.3), and Heterochromatin protein 1γ (HP1γ) (Fig. 1) (Brown et al., 1996; Bunch et al., 2014, 2015; Kim et al., 2011; Yoon et al., 2011). In addition, some factors are bound to the inactive *HSP70* before transcriptional activation. These include Negative elongation factor (NELF) and DRB sensitivity inducing factor (DSIF) that induce Pol II pausing in *Drosophila* (Fig. 1) (Adelman and Lis, 2012; Gilchrist et al., 2008; Missra and Gilmour, 2010). NELF knock-down (KD) reduces Pol II occupancy in the promoter-proximal region in *HSP70* and other genes harboring Pol II pausing (Gilchrist et al., 2008). In human cells, Tripartite-motif containing 28 (TRIM28, KAP1, TIF1β) is associated with the promoter-proximal site of *HSP70* and other inducible genes to regulate Pol II pausing and pause release before the gene is activated (Fig. 1) (Bunch et al., 2016, 2014, 2015; Bunch and Calderwood, 2015; McNamara et al., 2016).

In *HSP70* gene, Pol II is paused between the early and processive elongation stage before *HSP70* is activated. Therefore, the rate-limiting step of *HSP70* transcription is to release Pol II from the pausing site. P-TEFb is a critical protein in regulating this step. Our recent study also showed the activation of DNA repair enzymes, DNA-dependent protein kinase (DNA-PK) and Ataxia-telangiectasia mutated (ATM) (Bunch et al., 2015; Bunch, 2016). Interestingly, the transcriptional elongation of *HSP70* appears to be coupled with DNA damage response signaling, nucleosome remodeling, and translation (Brown et al., 1996; Bunch et al., 2015; Bunch and Calderwood, 2015; Bunch, 2016; Zobeck et al., 2010; Kim et al., 2011; Gilchrist et al., 2008; Vera et al., 2014). DNA damage response signaling was visualized by the enrichment of γH2AX and phosphorylated TRIM28 (S824) on the transcribing units of human *HSP70* gene (Fig. 1) (Bunch et al., 2015; Bunch, 2016). The recruitment of γH2AX, PAF1, and H3.3/HP1γ, and nucleosome release suggest nucleosome remodeling in the gene body of *HSP70* (Fig. 1) (Bunch et al., 2015; Adelman et al., 2006; Kim et al., 2011). In *Drosophila*, spontaneous eviction of nucleosomes was reported in *Hsp70* upon transcriptional activation (Petesch and Lis, 2008). This study showed a marked loss of the Histone H3 and the increased access of Micrococcal nuclease (MNase) to the DNA in *Hsp70* during heat shock (Petesch and Lis, 2008). Interestingly, this study indicated that nucleosomes are evicted independently of the transcription activity (or Pol II progression) but