Review

S-II mediated gene regulation

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ABSTRACT: S-II, also designated as TFIIS, was the first identified transcription elongation factor and is widely found in eukaryotes. Among known elongation factors, S-II has a characteristic biochemical activity: it facilitates the elongation process by allowing RNA polymerase II (RNAPII) to read through transcriptional blocks *in vitro*. While the biochemical and molecular mechanism of stimulating elongation *in vitro* has been shown in detail, the *in vivo* role of S-II long remained unclear. Recent research has revealed that S-II has a crucial role in the activation of expression of a set of genes *in vivo*. In this review, we summarize recent studies focusing on the regulation of gene expression by S-II and discuss the biological functions of S-II.

Keywords: Transcription, Elongation, Proofreading

1. Introduction

Regulation of gene expression has to be carried out precisely in order to maintain cellular functions for development and differentiation. Because gene expression is highly regulated at the level of transcription, this step has a great impact on cellular functions. The transcription process contains multiple steps designated as pre-initiation, initiation, elongation, and finally termination. Among these, the elongation step is one of the key steps in the activation of gene expression (*1*). Research has shown that expression of certain cellular or viral genes is regulated at this transcriptional step (*2-4*).

S-II was originally identified as a stimulation factor of RNAPII from an extract of Ehrlich ascites tumor cells in 1973 (*5*) and then purified in 1979 (*6*). Its activity was later found to be due to the ability to suppress pausing and to increase the yield of long transcripts *in vitro* (*7-10*). During mRNA elongation, RNAPII can encounter DNA sequences that cause transcriptional arrest (*11-13*). In some cases, RNAPII backtracks by several nucleotides along the template DNA. As a result, the 3'-end of the transcript is extruded from the catalytic center of RNAPII, leading to transcriptional arrest. In such cases, S-II helps RNAPII pass through the arrest sites by stimulating the 3'→5' nuclease activity intrinsic to RNAPII, designated as "cleavage" activity. Then, S-II induces cleavage of the nascent RNA leading to the resumption of transcription elongation. This characteristic activity of S-II *in vitro* may contribute to maintenance of mRNA quality, thus regulating gene expression. Indeed, it has been proposed that genetic mutations in S-II cause transcriptional arrest and produce truncated transcripts as mentioned below.

Another point of view concerning transcription arrest is that it occurs not only when RNAPII encounters arrest sites on template DNA but also when incorrect ribonucleotides are incorporated into the nascent transcripts. S-II relieves this arrest by stimulating cleavage of these mis-incorporated nucleotides (*14,15*). Thus, S-II potentially proofreads the nascent transcript and maintains transcriptional fidelity (*15-17*).

Moreover, a recent study demonstrated that homozygous inactivation of the S-II gene in mice leads to embryonic lethality with impaired definitive erythropoiesis in fetal liver (*18*). In addition, S-II contributes to oxidative stress resistance (*17*). These findings provide evidence that S-II has a distinctive role in biological processes.

2. Genes with expression levels that are changed by the inactivation of S-II

What follows in Table 1 is a summary of the reported genes with transcriptional levels that are regulated by S-II.

2.1. *SSM1 (suppressor of 6-azauracil sensitivity of the S-II null mutant 1)*

Although the yeast S-II null mutant is viable, loss of S-II activity leaves the mutant sensitive to a drug that inhibits IMP dehydrogenase (IMPDH), 6-azauracil (6AU) (*19*).

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Table 1. Genes regulated by S-II

Species	Gene	Ref.
Yeast	*SSM1/SDT1*	*19*
	IMD2/PUR5	*22*
	GAL1	*26*
Drosophila	*hsp70*	*29*
Mouse (fetal livers)	*Bcl-x_L*	*18*

In the course of identifying multi-copy suppressor genes of this phenotype, the *SSM1* gene, which encodes a 280-amino-acid polypeptide, was identified (*19*). Subsequently, *SSM1* was first described as a highly specific pyrimidine 5'-nucleotidase in *S. cerevisiae* (*20*). Shimoaraiso *et al*. also demonstrated that *SSM1* has two transcriptional arrest sites within the transcription unit of this gene *in vitro* that can be relieved by S-II, suggesting that S-II regulates the expression of *SSM1* through relief of the arrested RNAPII at the intrinsic arresting site of this gene.

2.2. *IMD2/PUR5*

The *IMD2/PUR5* gene, which encodes an IMPDH, is induced by IMPDH inhibitor 6AU and mycophenolate (*21-23*). The induction of the *IMD2/PUR5* gene in the yeast S-II null mutant is defective in response to 6AU (*22*,*24*). Cleavage activity of S-II is required for the induction of the *IMD2/PUR5* gene in response to 6AU (*25*). Reporter gene analysis indicates that the open reading frame of the *IMD2/PUR5* gene is responsible for S-II-mediated transcriptional activation in response to 6AU (*22*,*24*). These results suggest that S-II is involved in the transcription elongation of the *IMD2/PUR5* gene in response to nucleotide depletion treatment, although whether or not the *IMD2/PUR5* gene has intrinsic transcriptional arrest sites is still unclear.

2.3. *GAL1*

In the course of investigating S-II function using the *S. cerevisiae GAL1* gene, S-II was found to be strongly associated with the *GAL1* upstream activating sequence *in vivo* (*26*). S-II was also reported to be necessary for the optimal recruitment of the TATA-binding protein and RNA polymerase II to the *GAL1* promoter region. These results suggest that S-II acts as an activator of the transcriptional initiation step of *GAL1* or as an enhancer of the elongation step just downstream of the initiation site.

2.4. *hsp70*

The heat shock response gene 70 (*hsp70*) of *D. melanogaster* has been shown to be primed for activation in a dormant state without stimuli. In this uninduced condition, RNAPII is engaged but paused at the position just downstream of the initiation site (*27*,*28*). This RNAPII arrest within the promoter-proximal region of *hsp70* is dependent on S-II function, suggesting that S-II relieves the arrested RNAPII from the promoter region of *hsp70* (*29*).

2.5. *Bcl-x_L*

Homozygous inactivation of the S-II gene in mice leads to embryonic lethality with impaired definitive erythropoiesis in the fetal liver (FL) (*18*). A dramatic increase in apoptotic cells has been observed in S-II$^{-/-}$ FL. Consistent with this result, *Bcl-x_L* gene expression is reduced in FL. Reporter gene analysis also provides evidence that S-II participates in the transcriptional activation of the *Bcl-x_L* gene. These data provide one possible explanation that S-II stimulates the elongation step of *Bcl-x_L* transcription, although the possibility that S-II participates in certain transcriptional processes other than elongation, such as initiation, cannot be ruled out.

3. Contribution to oxidative stress resistance

In the course of transcription, nascent transcripts accidentally incorporate abnormal nucleotides. This mis-incorporation of abnormal nucleotides stops the transcriptional elongation chain, causing transcriptional arrest. S-II facilitates the intrinsic exonuclease activity of RNAPII when in arrest sites, and this activity leads to proofreading of nascent transcripts incorporating abnormal nucleotides during transcription.

Oxidized nucleotides such as 8-oxoguanine and 2-oxoadenine are generated in the cellular nucleotide pool by oxidative stress (*30*). Some are mis-incorporated into RNA during transcription (*31*,*32*). Proofreading systems have to be carried out correctly in order to prevent cells from producing aberrant proteins caused by translation using abnormal templates containing incorrect nucleotides. S-II potentially contributes to the maintenance of transcriptional fidelity in the presence of oxidative stress. Additionally, oxidative stress induces the direct oxidation of nucleotides in genomic DNA or mature transcripts. Research has shown that 8-oxoguanine in template DNA causes transcriptional arrest *in vitro* and that S-II relieves RNAPII at the arrest site (*33*). These results strongly suggest that S-II is involved in the oxidative stress response to maintain genetic information.

4. Discussion and Perspective

What follows is a summary of recent *in vivo* studies on gene regulation by S-II as was discussed above.

Research has found that there are genes which have arrest sites and that have expression regulated by the transcription elongation factor S-II *in vivo*.

This supports the idea that S-II makes RNAPII read through the arrest sites present in the gene, promoting transcription elongation *in vivo*. In addition, S-II is also reported to be involved in the initiation complex (*34*,*35*). In this regard, some of the genes mentioned above might be regulated by S-II participating in the initiation process. Additionally, S-II is reported to synergize with p300 (histone acetylation) in productive activator-dependent transcription *in vitro* (*36*). These S-II related findings may expand the view of gene regulation.

Those genes with expression controlled by S-II tend to be regulated in response to external stimuli, which might be a feature they share in common. *SSM1* and *IMD2* are induced by drugs that inhibit nucleotide metabolism. *GAL1* and *hsp70* are induced by external nutrients and heat shock, respectively. Additionally, *Bcl-x*$_L$ participates in anti-apoptotic responses. Therefore, one could speculate that S-II is a regulator for stress response genes when cells are exposed to stress, and this may be the biological function of S-II. S-II may be needed for regulation of genes specific to stress responses to avoid cellular dysfunction.

In terms of stress response, the reduction of *Bcl-x*$_L$ gene expression levels in S-II$^{-/-}$FL may be due to oxidative stress. Reactive oxygen species (ROS) are involved in Erythropoietin-mediated erythroid differentiation (*37*). This situation increases the possibility of generating oxidized nucleotides, leading to mis-incorporation of these nucleotides into DNA or RNA. S-II is assumed to promote read-through at the arrest sites made by oxidized DNA or to eliminate RNA containing mis-incorporated nucleotides *via* its cleavage activity. This may result in the maintenance of transcriptional fidelity, RNA quality, and oxidative stress resistance. The possible involvement of S-II in the oxidative stress response requires further study.

All cellular biochemical processes rely on the accuracy of expression of genetic information. To ensure the fidelity of genetic information flow, organisms have evolved monitoring systems to assess the quality of the units of heredity. In this sense, S-II has an important role in biological processes by contributing to the proofreading of nascent transcripts. If S-II is inactivated, genes will produce frequently aberrant transcripts such as nonstop mRNAs lacking a termination codon (*38-40*). Moreover, the inactivation of S-II results in reduced expression of genes containing arrest sites. Therefore, S-II plays significant roles in regulating gene expression and maintaining cellular functions in living cells. More information about S-II mediated gene regulation *in vivo* and *in vitro* may expand our knowledge of gene expression.

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