S-1 [10:00-10:30]

An early transcription checkpoint ; A dual role of capping enzyme in RNA polymerase II transcription

Eun-Jung Cho

College of Pharmacy, Sungkyunkwan University

Abstract

Recently, data from several groups have raised the concept of "checkpoint" in transcription. As capping of nascent RNA transcript is tightly coupled to RNA polymerase II transcription, we seek to obtain direct evidence that transcripiton checkpoint via capping enzyme functions in this early regulatory step.

One of temperature sensitive (ts) alleles of *ceg1*, a guanylyltransferase subunit of the *Saccharomyces cerevisiae*capping enzyme, showed 6-azauracil (6AU) sensitivity at the permissive growth temperature, which is a phenotype that is correlated with a transcription elongational defect. This ts allele, *ceg1-63* also has an impaired ability to induce *PUR5* in response to a 6AU treatment. However, this cellular and molecular defect is not due to the preferential degradation of the transcript attributed from a lack of guanylyltransferase activity. On the contrary, the data suggests that the guanylyltransferase subunit of the capping enzyme plays a role in transcription elongation. First, in addition to the 6AU sensitivity, *ceg1-63*is synthetically lethal with elongation defective mutations of the largest subunit of RNA polymerase II. Secondly, it exhibited a lower *GAL1* mRNA turn-over after glucoseshut off. Third, it decreased the transcription read through a tandem array of promoter proximal pause sites in an

orientation dependent manner. Interestingly, this mutant also showed lower pass through a pause site located further downstream of the promoter. Taken together, these results suggest that the capping enzyme plays the role of an early transcription checkpoint possibly in the step of the reversion of repression by stimulating polymerase to escape from the promoter proximal arrest once RNA becomes appropriately capped.

Introduction

The eukaryotic mRNAs produced by RNA polymerase II (pol II) are capped with an inverted 7-methyl-guanosine (m^7G) linked to the first residue of the mRNA. This event occurs by a series of three enzymatic reactions; The 5' triphosphate end of the nascent RNA pol II transcript is cleaved by 5' RNA triphosphatase to produce the diphosphate ends. RNA guanylyltransferase forms a covalent enzyme-GMP complex and subsequently caps the RNA substrate by adding a guanosine residue in a 5'-5' triphosphate linkage. Capping, the first mRNA modification occurs by the time the transcript is only 25-30 nucleotides long in an early transcription phase. Such cotranscriptional capping is mediated by recruitment of capping enzyme machinery to the phosphorylated carboxy-terminal domain (CTD) of the largest subunit of pol II. The capping enzyme binds directly and specifically to the phosphorylated CTD of pol II via the Ceg1 subunit (yeast) or the guanylyltransferase domain (metazoan). The capping enzyme interacts with pol II immediately after the serine 5 of CTD is phosphorylated. As serine 5 phosphorylation decreases in an early elongation phase, the capping enzyme dissociates from the transcription complex.

In the same range of windows, pol II transcription is subject to a checkpoint

control for the coordinated transcription with mRNA capping. Within this time and spatial interval, while pol II with the hypophosphorylated CTD is paused at the promoter proximal region, many factors are intended to target the capping enzymes to increase their recruitment or to enhance their catalytic activities. In the case of HIV, DSIF/Spt5-induced transcription arrest allows HIV-encoded Tat to interact with each capping enzyme domain and stimulates their catalytic activities. Pol II CTD phosphorylation is critical for the transition to the elongation phase. At this step, the elongation factor, P-TEFb phosphorylates the pol II CTD and Spt5. HIV Tat is also known to interact with P-TEFb. The phosphorylation of pol II CTD stimulated by Tat is meant to lead to the formation of the processive transcription elongation complexes. Therefore, CTD phosphorylation via P-TEFb and cotranscriptional capping are believed to be a major transactivation mechanism of the HIV Tat. How does the capping enzyme fit into the complicated scheme to delineate it in the order of pausing, capping, and the reversion of pausing? Because elongation competitive CTD phosphorylation does not occur before the RNA is capped, it is conceivable that a capping enzyme may play a role in overcoming the arrest condition either directly or via the positive elongation factors such as P-TEFb only when the RNA has been capped.

In this study we used a well-characterized yeast system to show whether or not the capping enzyme also plays a key role in coordinating mRNA processing and transcription elongation.

Results

A mutation in the guanylyltransferase subunit gene (*CEG1*) of the capping enzyme

confers an allele specific sensitivity to 6-azauracil.

In order to test the possibility that a capping enzyme plays a role in the transcription elongation independent of its ability to form a cap structure, we examined the sensitivity of various alleles of the *CEG1* guanylyltransferase subunit gene to 6AU. Yeast strains carrying a variety of the *ceg1* temperature sensitive (ts) alleles have been reported previously. The growth of each ts allele was tested on the 6AU (50 μg/ml) plates. Among different ts alleles, only *ceg1-63* showed reduced growth on the media containing 6AU (Fig. 1A). However, it had no effect on the other alleles. This shows that the Ceg1 guanylyltransferase capping enzyme subunit might be needed for the efficient transcript elongation in addition to its known activity in cap formation.

In parallel, the induction of *PUR5* by 6AU was monitored in the different *ceg1* alleles (Fig. 1B). As a control, *SED1* mRNA was amplified from the same batch of RNA samples. As shown in Figure 1B, a large induction of *PUR5* mRNA was observed in the wild type and other ts alleles except for *ceg1-63*. The level of *PUR5* induction in *ceg1-63* was much lower compared to the others, while *SED1* mRNA was detected at levels that were relatively unchanged. This suggests that Ceg1 may play a role in the transcription elongation as observed for the typical elongation factors.

***Ceg1-63* mutant shows defects in the transcription elongation due to a reduced guanylyltransferase activity.**

Although the above results suggest that the Ceg1 capping enzyme subunit serves as an elongation factor, alternatively, they can be explained in another way such that the decreased GTP level might impair the ability of *ceg1-63* to transfer GMP to a diphosphate end of the nascent RNA. As a result, the RNA transcript without the 5' cap

might be subject to preferential degradation. Therefore, we next examined whether or not a low level of RNA is attributed from a lack of mRNA stability or from the impaired ability of transcription elongation. The wild type *CEG1*, *ceg1-63, and ceg1-250* yeasts were grown in raffinose media followed by the galactose induction for an hour. During this period wild type and both mutants reached the maximum level of *GAL1*. Glucose was then added to repress further *de novo* initiation of transcription from the *GAL1* promoter. Figure 2A shows that wild type *CEG1* and *ceg1-250* have decreased level of *GAL1* in the time course once the glucose was added. However, the *GAL1* transcript lived longer after the glucose shut off in *ceg1-63*. It required more than 40 minute to drop to 50 % of the starting level. This result shows that *ceg1-63* generated more long-lived RNA than either the wild type or *ceg1-250*. Consequently, it is unlikely that lower level of the transcript from *PUR5* (Fig.1B) is due to an increase in the degradation of mRNA from the lack of a guanosyl cap. It is rather likely that, as reported in other transcription elongation defective mutants, *ceg1-63* has a lower rate of RNA synthesis. Therefore, the guanylyltransferase subunit of the capping enzyme has a distinctive role in transcription elongation.

Allele specific synthetic lethal assay shows a genetic interaction of *ceg1-63* with the transcription elongation factors.

To determine if Ceg1 plays a role in transcription elongation and to examine whether they behave allele specifically, we constructed *rpo21(rpb1)* deletion strain in the background of *ceg1-250* or *ceg1-63* suitable for plasmid shuffling. The defects in the transcription elongation activity must display synthetic interactions with defects in the other known elongation factors. Several *RPO21* (*RPB1*) alleles, which encode for

the largest subunit of RNA polymerase II, are well characterized to display 6AU sensitivity and confer compromised elongation. The above yeast strains were shuffled to introduce the elongation defective *rpo21-7*, *rpo21-17*, *rpo21-18*, *rpo21-23*, and *rpo21-24* alleles as well as the wild type *RPO21* (*RPB1*) in the *ceg1-63* or *ceg1-250* background. When *ceg1-63* was combined with each of these alleles it resulted in synthetic lethality while *ceg1-250* did not exhibit an additive growth defect in combination except for *rpo21-7* who has stringent ts phenotype (Fig. 3). This result shows an allele specific functional link between Ceg1 and Rpb1, in further support of the notion that the capping enzyme Ceg1 subunit has a dual role in both the transcription elongation and cap formation.

***Ceg1-63* cells are unable to transcribe passing through the artificial pause sites *in vivo*.**

Since the *ceg1-63* mutant has defects in transcription elongation *in vivo*, we attempted to determine the mutational effect on the transcription of the reporter gene with a strong pause site. One of the well-characterized pause sequences, which were originally derived from the human histone gene, H3.3 (Ia site), has been studied both *in vitro* and *in vivo*. At least *in vitro*, this Ia site efficiently attenuated transcription by eukaryotic polymerase as well as by prokaryotic polymeras. The high copy plasmid carrying two repeats of the Ia pause site under the *GAL1* promoter was used for this study (Fig. 4A). Plasmids with a correct or reverse orientation of the pause sites were transformed into the wild type *CEG1*, *ceg1-250*, or *ceg1-63* separately. As previously reported, although the pause sites have a strong effect on the transcription read through *in vitro*, it did not decrease the transcription *in vivo* in the wild type *CEG1* background

(Fig. 4B). However, surprisingly, the *ceg1-63* has dramatically decreased the level of transcript only from the reporter with pause in the arresting orientation. The transcription that passed through neither the reverse orientation nor the one without was significantly affected.

Discussion

This study showed that one of the ts alleles of *CEG1* capping enzyme guanylyltransferase subunit gene displayed the transcription elongation defective mutant phenotypes. *Ceg1-63* showed 6AU sensitivity and had a lower *PUR5* induction level in response to 6AU. It displayed a genetic interaction specifically with the elongation defective pol II mutants. Interestingly, *Ceg1-63* failed to pass through the artificial pause sites inserted in the promoter proximal region in an orientation dependent manner. Overall, these results demonstrate that the capping enzyme plays a role in transcription elongation presumably by releasing the temporal arrest in the early elongation phase once RNA 5' end is successfully capped.

The capping enzyme associates with the pol II transcription complexes, apparently during the transition from initiation to elongation. At the same time and in the same place, transcription checkpoint occurs. This early transcriptional arrest is supposed to ensure a temporal window so a timely modification of the 5' end of the mRNA can be obtained. The temporal arrest must be resumed by the subsequent recruitment of positive elongation factors such as P-TEFb. P-TEFb (CTDK-I and/or Bur1/Bur2 complex in yeasts) adds phosphates onto CTD of pol II and allows it to proceed into a productive elongation. Therefore, repression and reactivation must be

precisely coordinated with the transfer of the cap onto the nascent RNA. This observation raises an interesting possibility that the capping enzyme might play a role as a checkpoint protein with Spt4/5 to ensure that pol II does not extend uncapped RNA. In this regards, the Human Immunodeficiency Virus (HIV) encoded Tat protein binds a capping enzyme and stimulates both catalytic activities. It has also been shown independently to interact with P-TEFb to override the Spt5-induced promoter proximal arrest. Therefore the Tat protein might mediate the coupling of mRNA capping to pol II transcription by targeting capping enzymes not only to stimulate their catalytic activity but also to enhance their elongation activity.

In our scheme, the capping enzyme plays a key role in differentiating the present condition of pol II (Fig. 5). Indeed, none of the proteins in this early checkpoint window are known to interact with the RNA transcript or differentiate the capped messages from those without. Accordingly, the capping enzyme might be the only candidate to determine the time when to reverse the repression. Interestingly, the capping enzyme undergoes a large conformational change during transfer of the guanylyl moiety. The conformational change might allow a microenvironmental reconfiguration of the transcription complex to signal that the situation is to be reversed.

Figures

FIG. 1. The mutation in the guanylyltransferase subunit (*CEG1*) of the capping enzyme shows an elongation defective phenotype. (A) *Ceg1-63*, one of the ts alleles of the gaunylyltransferase subunit displays an allele specific sensitivity to 6AU and caffeine. (B) *Ceg1-63* mutant allele was defective in the *PUR5* induction.

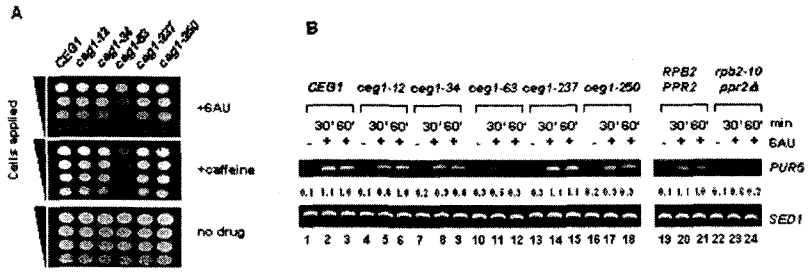

FIG. 2. *Ceg1-63* mutant shows defects in transcription elongation. (A) *Ceg1-63* produces the *GAL1* transcript with a longer half-life following the glucose shut off. (B) The guanylyltranferase activity of alleles.

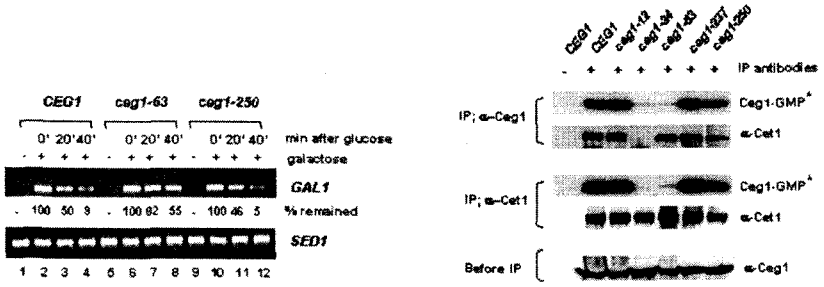

FIG. 3. The *ceg1-63* allele is synthetically lethal with *rpo21* mutations that are specifically compromised in the transcription elongation.

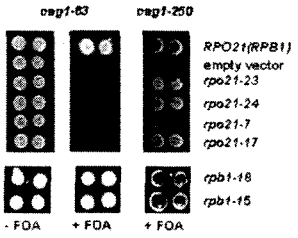

FIG. 4. The *ceg1-63* cell is unable to transcribe by passing through the promoter proximal pause sites.

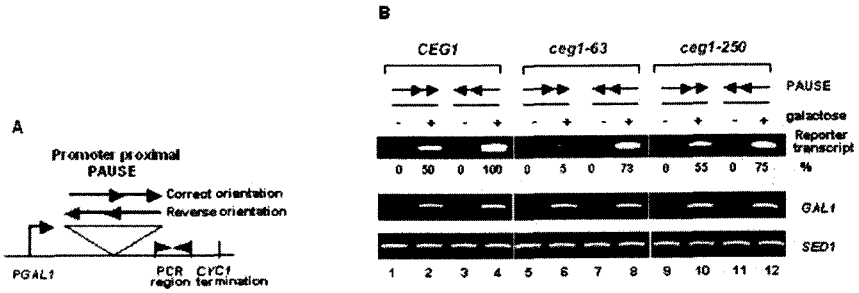

FIG. 5. Schematic model of the early transcription checkpoint by the capping enzyme