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석사학위논문

**Role of Nrd1 CTD-Interacting Domain
in RNA Polymerase II Termination**

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생물물리□및□화학생물학과

공 지 원

Abstract

Role of Nrd1 CTD-Interacting Domain in RNA Polymerase II Termination

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Carboxy-terminal domain (CTD) of RNA polymerase II (RNAP II) consists of multiple hepta-peptide repeats well conserved in eukaryotes. At each step of transcription, phosphorylation pattern of CTD gradually changes and orchestrates binding of various proteins for RNA processing. When transcription ends, RNAP II dissociates and becomes ready for a new transcription event. This "termination" process is aided by two distinct pathways, which have different affinities for CTD phosphorylation states. Rtt103-Rat1-Rai1 dependent pathway is responsible for long mRNA gene termination where serine 2 phosphorylation of CTD (CTD-S2P) dominates at 3'-end of the gene, whereas Nrd1-Sen1-Nab3 dependent pathway takes the role in short gene termination at which CTD serine 5 phosphorylation

(CTD-S5P) prevails. This distinction primarily comes from CTD-interacting domain (CID) protein of each complex: Rtt103 and Nrd1. The specific CID-CTD interaction might affect RNAP II in choice of termination pathway. To verify this idea, swapping CID of Nrd1 with that of Rtt103 was carried out in yeast *Saccharomyces cerevisiae*. CID swapping changes the affinity of Nrd1 for CTD phosphorylation states from CTD-S5P to CTD-S2P *in vivo*. In chromatin immunoprecipitation (ChIP) assay, *nrd1*[CID^{Rtt103}] successfully binds to 3'-end of both snoRNA and mRNA genes. Under CID swapped condition, snoRNA genes showed mild termination defects and read-through transcripts were shown on Northern blot. On the other hand, mRNA genes were classified into two different categories. ADH1 showed proper termination by *nrd1*[CID^{Rtt103}] and this result was supported by genome-wide microarray (ChIP-chip) data, which showed that about 30% of mRNA genes could be terminated by *nrd1*[CID^{Rtt103}]. On the contrary, PMA1, a typical mRNA gene, showed severe termination defect. In this case, recruitment of *nrd1*[CID^{Rtt103}] at 3'-end of gene might result in interruption of proper termination. In ChIP-chip analysis, about 70% of mRNA genes showed defective termination like PMA1. To verify the assumption that CID-CTD interaction was responsible for the termination failure on PMA1, CTD-S2P kinase null mutant and defective RNA-binding mutant of Nrd1-

dependent pathway were prepared and restoration of termination was confirmed by ChIP assay. Furthermore, other interactions of Nrd1-dependent pathway were also examined. In co-immunoprecipitation (Co-IP) assay, *nrd1*[CID^{Rtt103}] still formed complex with Sen1 and Nab3, and interacted with exosome core components Rrp4 and Rrp41, like wild type Nrd1 does. These results show that the specific CID-CTD interaction plays a major role in choice of termination pathway for RNAP II and not in other interactions involved in Nrd1-dependent pathway.

Keywords : Nrd1, Rtt103, CID, CTD, RNAP II, Termination

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Abstract in Korean

I. Introduction

CTD of RNAP II mediates a physical and functional coupling between mRNA transcription and processing (Meinhart et al. 2005). CTD is required for efficient mRNA processing, such as capping, splicing, cleavage, and polyadenylation, by means of recruiting various RNA processing factors (McCracken et al. 1997b; Hirose and Manley 1998, 2000; Proudfoot et al. 2002).

CTD phosphorylation mainly occurs at serine 2 and serine 5 residues (Corden et al. 1985; Zhang and Corden 1991) among five potential phosphorylation sites in a CTD consensus repeat (tyrosine 1, serine 2, threonine 4, serine 5, and serine 7), and the phosphorylation pattern of serine 2 and serine 5 changes throughout the transcriptional cycle. Serine 5 phosphorylation mainly takes place in promoter-proximal region (Cho et al. 1997; McCracken et al. 1997a; Ho et al. 1998; Komarnitsky et al. 2000) and decreases in promoter-distal region. On the contrary, serine 2 phosphorylation is low at promoter-proximal region and gradually becomes predominant at

promoter-distal region (Komarnitsky et al. 2000; Cho et al. 2001). The combination of different CTD phosphorylation states specifies state of RNAP II within the transcriptional cycle: initiation, elongation, and termination (Buratowski 2003). This “CTD code” can determine the binding of specific processing factors at each step of transcription and orchestrate nuclear mRNA biogenesis events (Dahmus 1995; Maniatis and Reed 2002; Palancade and Bensaude 2003; Proudfoot 2004; Sims et al. 2004; Zorio and Bentley 2004).

There are two distinct pathways for termination process RNAP II can choose to use. Long mRNA gene, which has dominating amount of CTD-S2P at the 3'-end, is known to be terminated by Rtt103-Rat1-Rai1 dependent pathway (Kim et al. 2004). On the other hand, Nrd1-Sen1-Nab3 dependent pathway is responsible for short gene termination where CTD-S5P prevails at the 3'-end (Vasiljeva and Buratowski 2006). Both pathways have CID proteins, Rtt103 and Nrd1, and these might be important determinants for the different affinities between CTD and each pathway. CID swapped Nrd1 protein which had CID of Rtt103 was constructed in yeast

Saccharomyces cerevisiae to verify this idea and consequent effects on distribution of Nrd1 and RNAP II, or interactions among Nrd1-dependent pathway caused by CID swapping were examined.

II. Material and methods

1. Yeast strains and plasmids

All yeast strains used in this study are in the BY4741 genetic background unless otherwise noted. Strains constructed in this study were prepared by standard methods (Brachmann et al., 1998; Longtine et al., 1998). Unless otherwise indicated, cells were grown at 30°C into mid-log phase (3.0×10^7 cells ml⁻¹) in standard synthetic complete medium (pH 6.5) with the appropriate amino acids and 2% glucose supplemented. *nab3-20* mutant was made by inserting restriction fragments into pBluescript SK+. And *nab3-20* shows temperature-sensitive phenotype, so cells were grown at 25°C, and then further incubated at 37°C. All other mutants were targeted on genomic DNA.

2. Western blotting

Cells were incubated until OD₅₉₅ reached at 1.0 to 1.2 and lysed in 25 mM Tris (pH 7.4), 100 mM NaCl, 5% glycerol, 1 mM EDTA, 0.5 % Triton X-100 lysis buffer. And then immunoprecipitation was done using PAP antibody (Nrd1-TAP). For Nab3, mouse anti-Nab3p antibody (Invitrogen) was used for blotting. Sen1-myc13 was blotted by mouse 9E10 (COVANCE), and mouse 16B12 (COVANCE) was used for Rrp4-HA and Rrp41-HA. CTD phosphorylation states were detected by mouse 8WG16 (MILLIPORE, unphosphorylated CTD), rat 3E8 (MILLIPORE, CTD-S5P), and rat 3E10 (MILLIPORE, CTD-S2P) antibodies.

3. Chromatin Immunoprecipitation

ChIP procedure and quantification were done as previously described (Kim et al., 2004). For temperature-shift experiments, cells were incubated until OD₅₉₅ reached at 1.1 ~ 1.2 at 25°C, and then equal amount of media pre-warmed to 55°C was added, before further incubation at 37°C for 30 minutes. Primers used in real-time PCR are listed in Table 1.

Table 1.

Primer	Sequence
ADH1 #1-up (-235)	TTCCTTCCTTCATTACGCACACT
ADH1 #1-low (-13)	GTTGATTGTATGCTTGGTATAGCTTG
ADH1 #2-up (+844)	TTCAACCAAGTCGTCAAGTCCATCTCTA
ADH1 #2-low (+1013)	ATTGACCCCTTTTCCATCTTTTCGTAA
ADH1 #3-up (+1231)	ACCGGCATGCCGAGCAAATGCCTG
ADH1 #3-low (+1400)	CCCAACTGAAGGCTAGGCTGTGG
ADH1 #4-up (+1690)	CAAGTAACTACCAGCACCATAACACCG
ADH1 #4-low (+1940)	CTCCGGCTTTTTATTGGCTTGCTC
ADH1 #5-up (+3993)	CATTTAGGTCTACTTCTGGTCGTGC
ADH1 #5-low (+4250)	CAGATGATCTTAGTTTGTATGAGTG

PMA1 #1-up (-304)	CAAATGTCCTATCATTATCGTCTAAC
PMA1 #1-low (-47)	CAATGATTTTCTTTAACTAGCTG
PMA1 #2-up (+168)	CGACGACGAAGACAGTGATAACG
PMA1 #2-low (+376)	ATTGAATTGGACCGACGAAAAACATAAC
PMA1 #3-up (+584)	AAGTCGTCCCAGGTGATATTTTGCA
PMA1 #3-low (+807)	AACGAAAGTGTGTGCACCGGTAGC
PMA1 #4-up (+1010)	GTTTGCCAGCTGTCGTTACCACCAC
PMA1 #4-low (+1250)	TTCTTCTTTCTGGAAGCAGCCAAAC
PMA1 #5-up (+2018)	CTATTATGATGCTTTGAAGACCTCCAG
PMA1 #5-low (+2290)	TGCCCAAATAATAGACATACCCCATAA
PMA1 #6-up (+2866)	GATACACTAAAAAGAATTAGGAGCCAAC
PMA1 #6-low (+3098)	CAAGAAAGAAAAAGTACCATCCAGAG
PMA1 #7-up (+3287)	GAAAATATTTGGTATCTTTGCAAGATG
PMA1 #7-low (+3500)	GTAAATTTGTATACGTTTCATGTAAGTG
PMA1 #8-up (+3448)	GCGCCCATACAGACACTCAAGATAC
PMA1 #8-low (+3652)	GGCCTGGCGATTTGTTTGCTTTCTTG
PMA1 #9-up (+3745)	ACTACCCTGGCGCTATGATG
PMA1 #9-low (+3913)	GGAAACAGCGTGATGAGTGA
PMA1 #10-up (+4129)	GTTTCATGAAGTCACCTCTCCACAAG
PMA1 #10-low (+4353)	CATACCGAAATATGGAACGCCGAAC TG
PMA1 #11-up (+5300)	CTGATATTTTGATGCCTATGAACG
PMA1 #11-low (+5562)	GTCTTCGGGTGGACAGGTTTCAG
snR13 #1-up (-60)	TTATAAATGGCATCTCAAATCGTC
snR13 #1-low (+124)	GGTCAGATAAAAGTAAAAAAAGGTAGC

snR13 #2-up (+119)	CTGACCTTTTAACTTCCCCGTAG
snR13 #2-low (+358)	CTGTCGCTTCCGTGTCTCTTGTCTG
snR13 #3-up (+345)	CACGGAAGCGACAGAAAGACAGGGAG
snR13 #3-low (+537)	CTAGAGGGAATGTATGTTGTTGAAG
snR13 #4-up (+747)	GAGCATCTGCTTTCCTTTCAC
snR13 #4-low (+922)	ATCACGGCGCCTCATCTTTG
snR13 #5-up (+1284)	AAAACCAAGAAAAGGATAAAGAG
snR13 #5-low (+1524)	TCGGTGTCTACAAAATGATACGC
snR33 #1-up (-87)	CGGAACGGTACATAAGAATAGAAGAG
snR33 #1-low (+106)	TTCAATCTCTGCTCCTCCAAAC
snR33 #2-up (+108)	GCCTAGCTTTTACACCGGTTTGAGTC
snR33 #2-low (+288)	TAAAGAAAACGATAAGAATAACC
snR33 #3-up (+343)	CAATTGCAGTAACCAGAAGCGAACAG
snR33 #3-low (+556)	CGGCAGTGATCTCGTTCCATTG
snR33 #4-up (+1123)	GCTAATAAACCCGCAAGAAAATC
snR33 #4-low (+1294)	AGAGTCCCAGGATTTACAAGGTAG
snR33 #5-up (+1447)	GTATTTCACTTGCACTATTCTTCTATC
snR33 #5-low (+1668)	TGGAAGATGIGTTAGGATTAGG
TEL (VI)-up	GCGTAACAAAGCCATAATGCCTCC
TEL (VI)-low	CTCGTTAGGATCACGTTCAATCC

4. Northern blotting

Northern blotting was performed as previously described (Kim et al. 2006). Primers used for generating probes are listed below in Table 2.

Table 2.

Primer	Sequence
snR13 #1-up (-60)	TTATAAATGGCATCTCAAATCGTC
snR13 #2-up (+119)	CTGACCTTTTAACTTCCCCGTAG
snR13 #2-low (+358)	CTGTCGCTTCCGTGTCTCTTGCCTG
snR33 #1-up (-87)	CGGAACGGTACATAAGAATAGAAGAG
snR33 #2-up (+108)	GCCTAGCTTTTACACCGGTTTGAGTC
snR33 #2-low (+288)	TAAAGAAAACGATAAGAACTAACC

III. Results

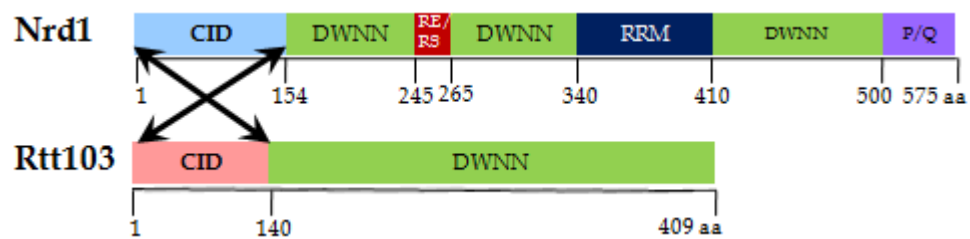
1. CID swapping changes specific CID-CTD interaction between Nrd1 and CTD

Wild type Nrd1 and Rtt103 more likely bind to 5'- and 3'-end of gene, respectively, resulting from their specific CID-CTD interactions (Kim et al. 2004; Vasiljeva et al. 2008). Despite their totally different affinities for CTD phosphorylation states, these CIDs have highly conserved sequences and superimposable structures (Lunde et al. 2010). Thereby CID swapping between Nrd1 and Rtt103 might cause no harm for other functions of proteins except the specific CID-CTD interaction. Under this assumption, *nrd1*[CID^{RTT103}], a chimeric protein which had CID of Rtt103 (aa 2-140) instead of its own CID (aa 2-154), was constructed (Figure 1A).

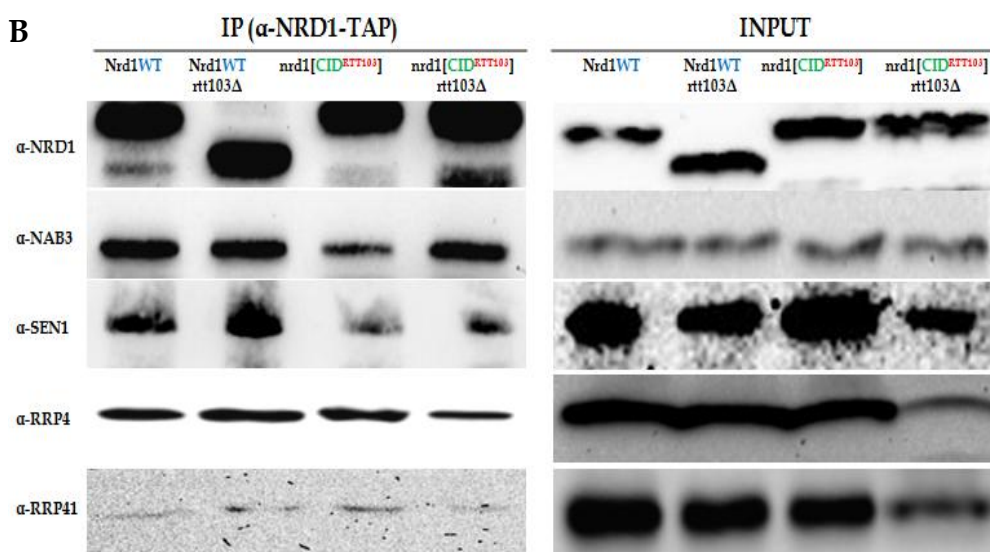
In Co-IP assay, Nrd1-Sen1-Nab3 complex remained intact when CID of Nrd1 was swapped (Figure 1B). Furthermore, interactions between Nrd1-Sen1-Nab3 complex and exosome core subunits, Rrp4

and Rrp41, were not altered by CID swapping (Figure 1B). However, *nrd1*[CID^{RTT103}] showed more affinity for CTD-S2P than wild type Nrd1 did, while its level of interaction with CTD-S5P decreased when CID was swapped (Figure 1C). Additionally, Rtt103 knockout was also performed to see the influence of Rtt103 as competitor for *nrd1*[CID^{RTT103}]. There were slight competitor effects shown, compared to their wild type Rtt103 strains (Figure 1C). These results show that CID swapping changes the preference of Nrd1 for CTD phosphorylation state from CTD-S5P to CTD-S2P, and does not affect other interactions involved in Nrd1-dependent termination pathway.

A



B



C

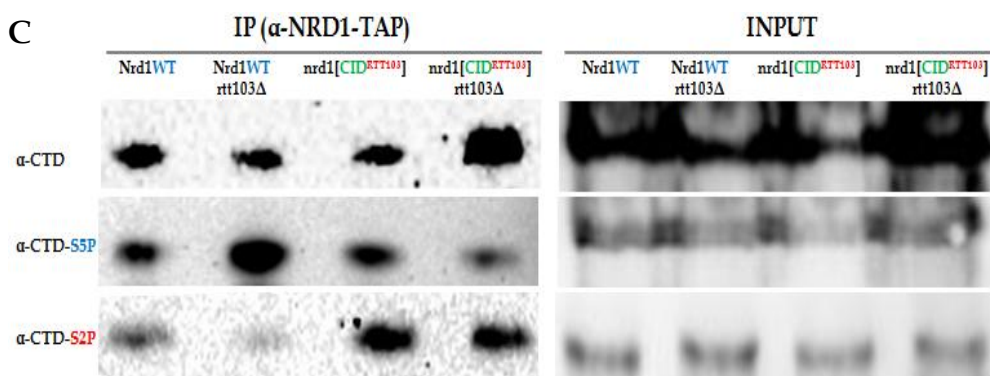


Figure 1. Affinity of Nrd1 to CTD phosphorylation state depends on CID.

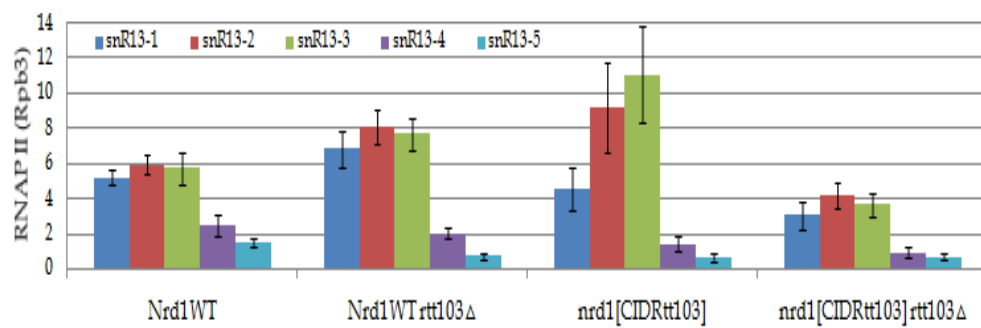
- (A) Schematic representation of the construction of *nrd1*[CID^{RTT103}]. The CTD-Interacting (CID, aa 2-154 and aa 2-140), RNA binding (RRM, aa 340-410) domains, and RE/RS region (arginine-, serine-, and glutamate-rich, aa 245-265) are marked; P/Q is a proline-, glutamine-rich region (aa 500-575). Domain with no name is indicated as DWNN.
- (B) Nrd1-TAP was immunoprecipitated by PAP antibody, and then each component was blotted using different antibodies: α -Nab3p (Nab3), α -c-myc (Sen1-myc13), and α -HA.11 (Rrp4-HA and Rrp41-HA).
- (C) Co-IP assay was done as in B. Blotting were performed by 8WG16 (CTD), 3E8 (CTD-S5P), and 3E10 (CTD-S2P) antibodies.

2. CID swapping causes mild termination defects on snoRNA genes

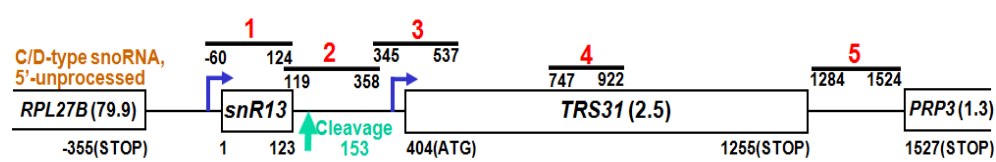
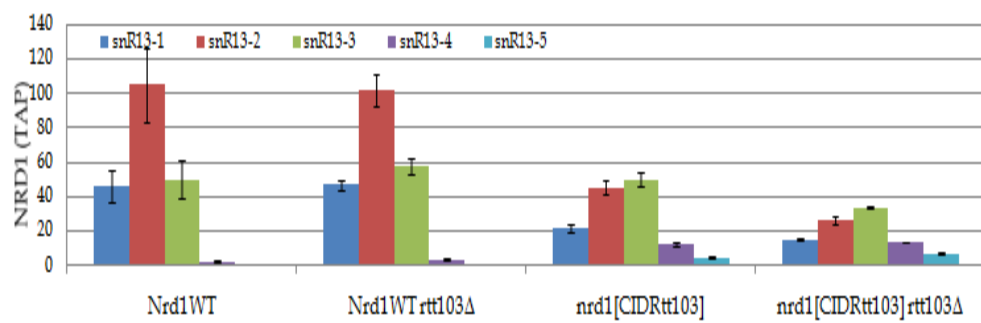
In addition to the identification of interactions *in vivo*, the consequent influence on the functionality of Nrd1-dependent pathway by CID swapping still needs be verified. To this purpose, snR13 and snR33 genes were chosen to represent non-coding short RNAs transcribed by RNAP II. In ChIP analysis, *nrd1*[CID^{Rtt103}] successfully bound to 3'-end of genes compared to wild type Nrd1 (Figure 2B and 2D). According to this pattern, RNAP II slightly shifted to the 3'-end, but still was terminated after position 3 on ChIP assay regardless of CID swapping (Figure 2A and 2C). CID swapped Nrd1-dependent pathway appears to be able to terminate RNAP II. Competitor effect of Rtt103 is hardly recognizable since the level of CTD-S2P on 3'-end of snoRNA gene is not high enough for the binding of Rtt103 (Figure 2B and 2D). Since resolution of ChIP is limited for showing read-through transcripts generated by RNAP II slightly shifted to 3'-end of gene, however, Northern blotting is needed to be performed. Exosome and TRAMP complex are known to be responsible for

degradation of read-through transcripts. Therefore, to see read-through transcripts visually by Northern blotting, Rrp6, exosome component having exonuclease activity, and Trf4, one of TRAMP complex component were knocked-out. Under these conditions, CID swapped strains showed read-through transcripts implying that RNAP II terminated slightly downstream compared to CID wild-type strains (Figure 2E).

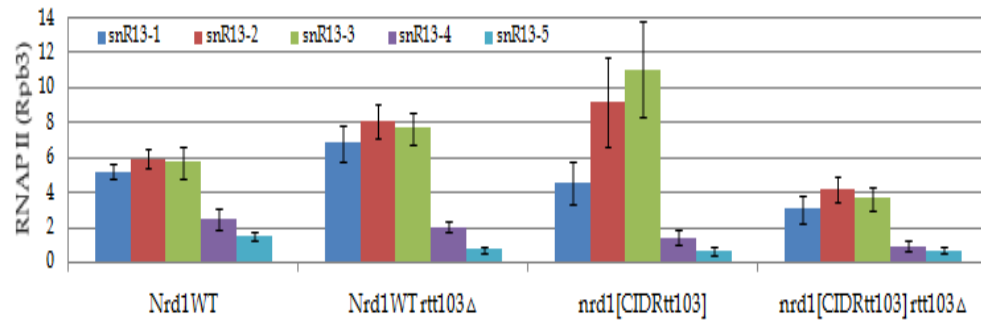
A



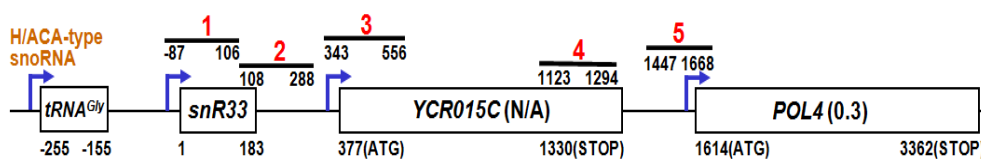
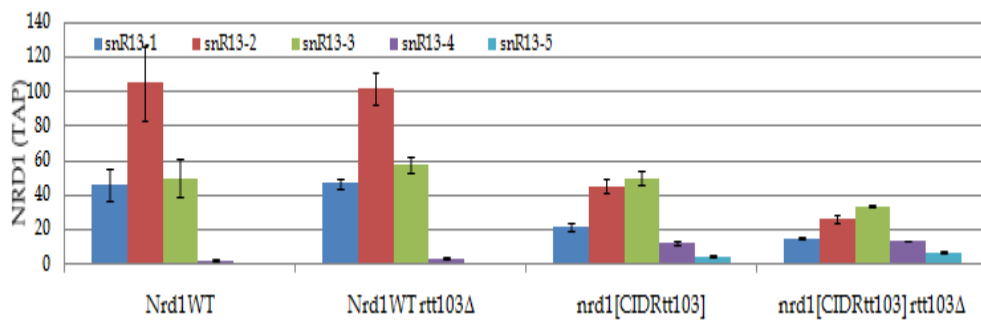
B



C



D



E

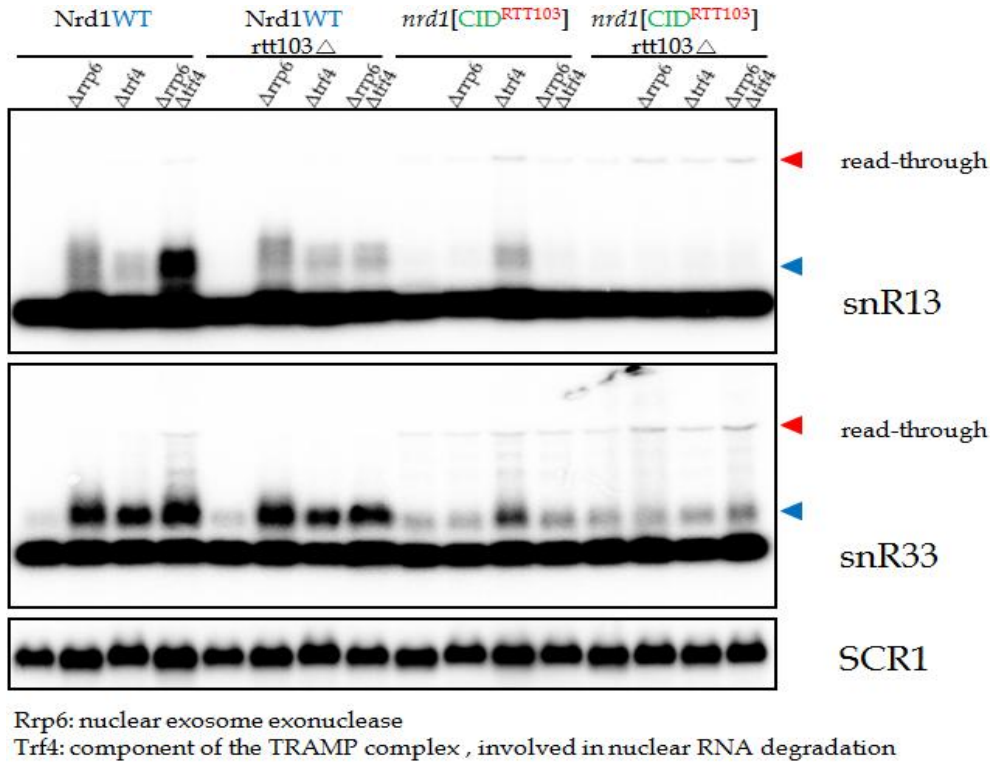


Figure 2. *nrd1*[CID^{RTT103}] terminates RNAP II slightly downstream on snoRNA genes

(A to D) ChIP assays were carried out using α -Rpb3 (RNAP II) and PAP (Nrd1-TAP) antibodies. Quantification data were obtained by real-time PCR. Schematic diagrams of the gene-flanking regions are shown below ChIP data. Numbers indicate primers used in real-time PCR. (A) RNAP II on snR13, (B) Nrd1 on snR13, (C) RNAP II on snR33, and (D) Nrd1 on snR33.

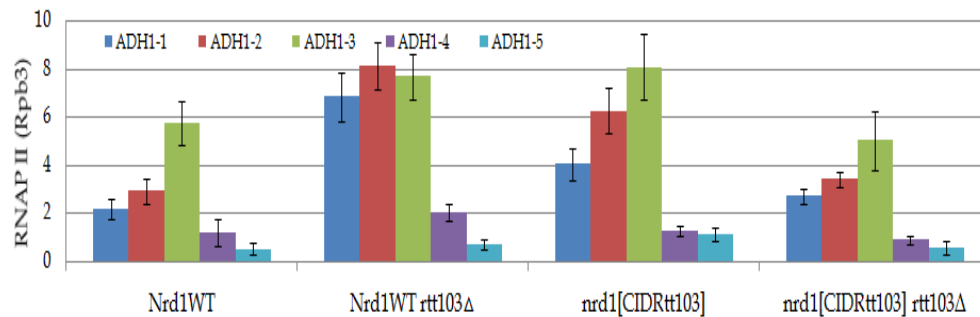
(E) Northern blotting was done using probes which covered position 1 and 2 on ChIP map. SCR gene was chosen for control.

3. Effects on mRNA termination caused by CID swapping are classified into two categories

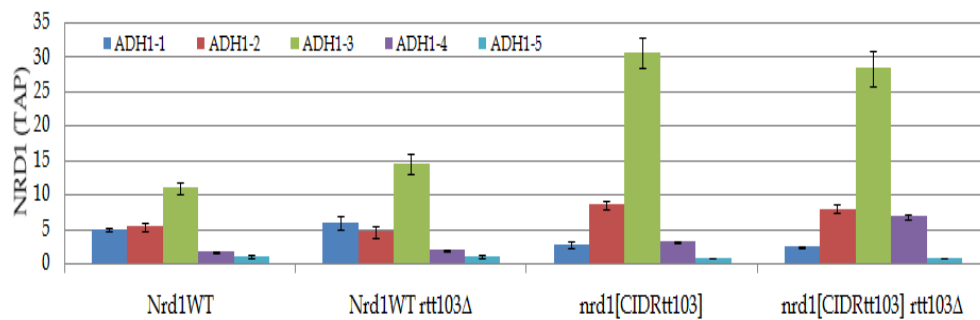
Interestingly, termination profiles of mRNA genes appeared in two different patterns. ADH1, relatively short mRNA gene, showed that RNAP II dissociated after poly (A) signal (Figure 3A, position 4 and 5 on ChIP map of ADH1) under large amount of *nrd1*[CID^{RTT103}] recruited (Figure 3B). On the contrary, PMA1, long length mRNA, resulted in defective termination meaning RNAP II remained at relatively high level after poly (A) signal (position 8, 9, 10, and 11 on ChIP map of PMA1) compared to promoter region (position 1 on ChIP map of PMA1) (Figure 3D). Analysis of ADH1 sequence revealed cluster of Nrd1 binding sequences (GTAA/G) and Nab3 binding sequences (TTCT) around poly (A) site (Figure 3C). It seems that short length mRNAs, such as ADH1, can recruit Nrd1 and Nab3 supported by these binding sequences and be terminated by Nrd1-dependent pathway in mechanism not yet discovered. This assumption is supported by genome-wide data which show that about 30% of mRNA genes could be terminated by *nrd1*[CID^{RTT103}]

(Figure 3F). In case of long length mRNA termination, on the other hand, *nrd1*[CID^{RIT103}] competes Rtt103 for binding at 3'-end of gene and disturbance of Rtt103-Rat1-Rai1 pathway might lead to termination failure. In genome-wide ChIP-chip analysis, about 70% of mRNA genes showed defective termination like PMA1 (Figure 3F).

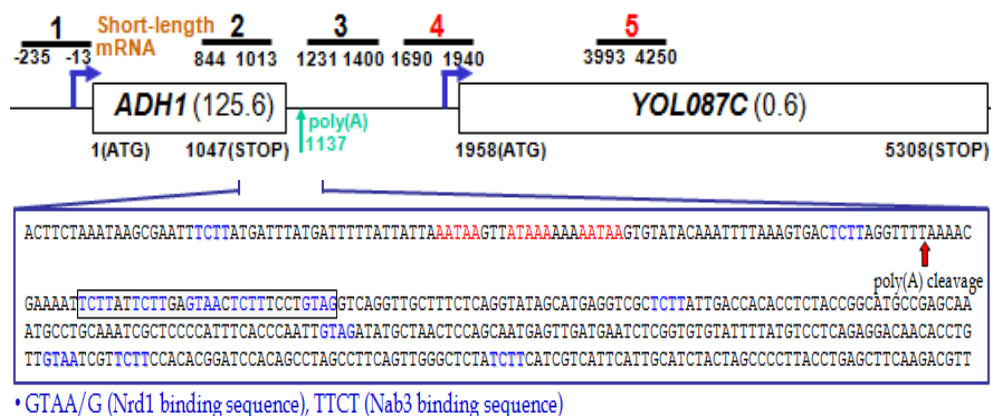
A



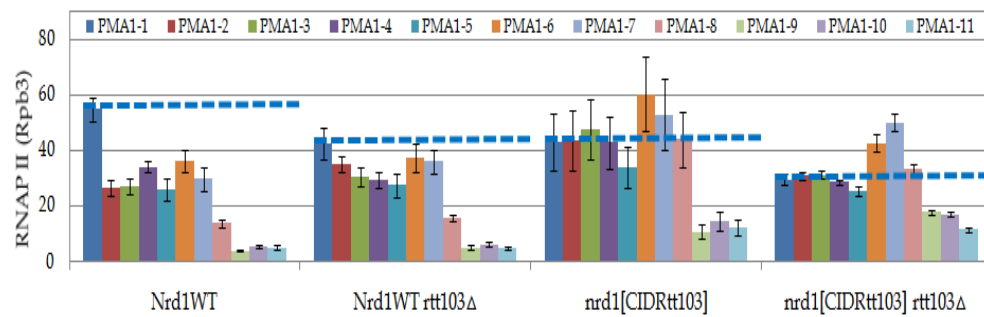
B



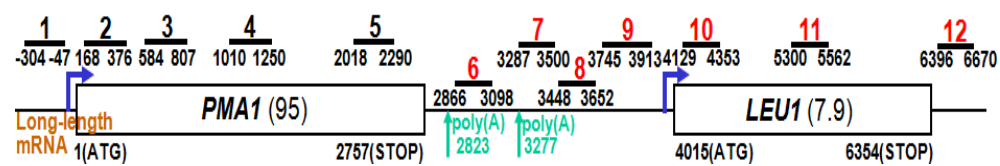
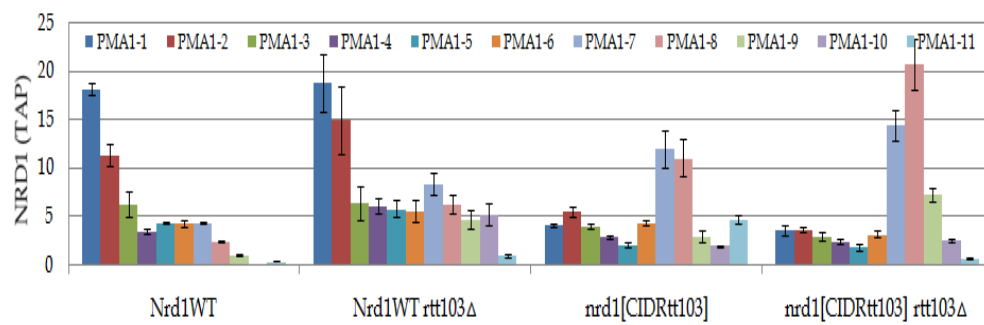
C



D



E



F

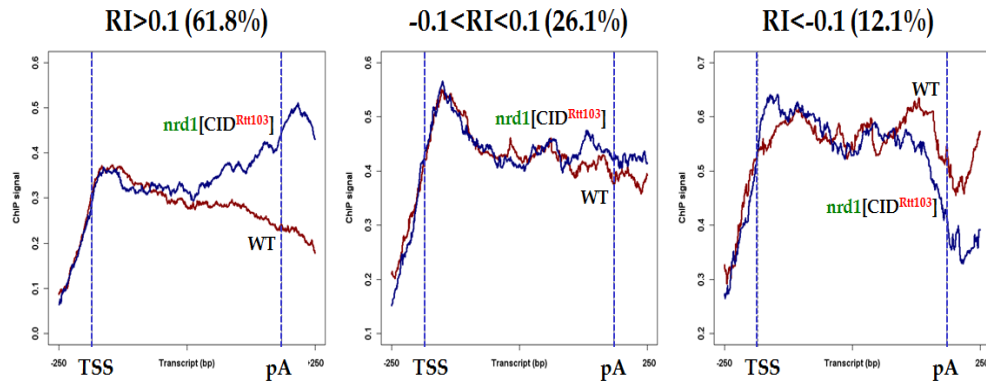


Figure 3. *nrd1*[*CID^{RTT103}*] is able to terminate RNAP II on snoRNA and short length mRNA

(A to E) ChIP assays were carried out as Figure 2. (A) RNAP II on ADH1, (B) Nrd1 on ADH1, (C) ChIP map of ADH1 and sequence analysis indicating Nrd1 and Nab3 binding sequences (between +1036 and +1449 bp from transcription start site (TSS)), (D) RNAP II on PMA1, and (E) Nrd1 on PMA1.

(F) mRNA genes were clustered into three categories in *nrd1*[*CID^{RTT103}*] strains. Genome-wide RNAP II ChIP-chip analysis was performed by Affymetrix Yeast Genome tiling arrays and stringent filtering was done to get rid of spill-over effect from overlapping genes. Median ChIP-signal over a region starting from poly (A) site (pA) to 250 nt downstream is shown. Read-through Index (RI) = (median ChIP-signal from *nrd1*[*CID^{RTT103}*] strains) - (median ChIP-signal from wild-type strains).

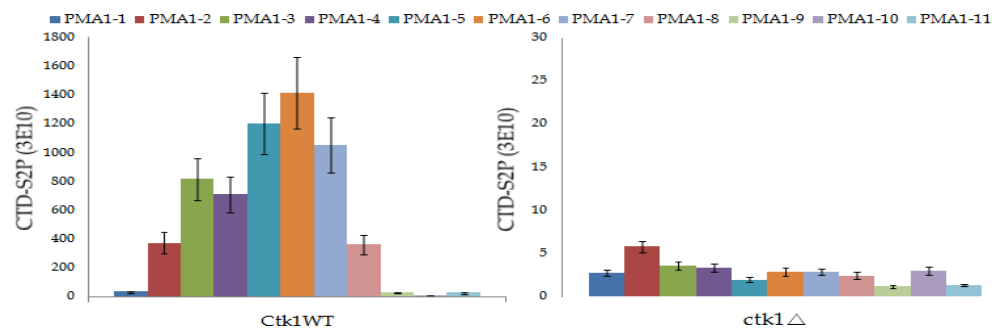
4. Defective termination due to CID swapping can be restored by reduction of interactions between CID swapped Nrd1-dependent pathway and RNAP II

If RNAP II chose termination pathway depending on the interaction between CTD and CID proteins, when the interaction decrease, termination process would be back to normal on PMA1 under the presence of *nrd1*[CID^{RTT103}]. There are two kinds of CTD-S2P kinase, Ctk1 and Bur1. Bur1 is essential for cell growth (Irie et al. 1991) and deletion of Ctk1 is known for not altering RNAP II distribution along coding sequences (Ahn et al. 2004). Therefore Ctk1 knockout was chosen for minimizing amount of CTD-S2P and recruitment of *nrd1*[CID^{RTT103}] to 3'-end of gene. Under Ctk1 deletion, the amount of CTD-S2P was dropped about 10³ fold and its relative enrichment at 3'-end of gene compared to 5'-end greatly decreased (Figure 3A). And when Rtt103 was present, recruitment of *nrd1*[CID^{RTT103}] to the 3'-end of gene successfully reduced to similar level of 5'-end (Figure 3C) and consequently termination defect was rescued (Figure 3B). When

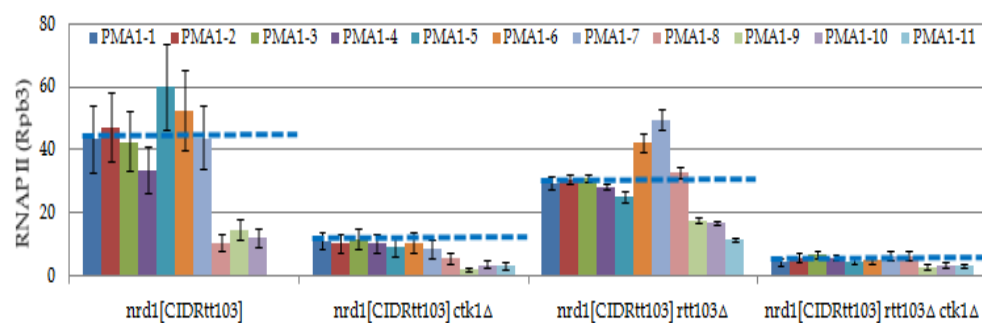
Rtt103 was deleted, however, binding of *nrd1*[CID^{RTT103}] on 3'-end of gene was restored (Figure 3C) and defective termination still occurred (Figure 3B). Therefore we can conclude that basal amount of CTD-S2P primarily recruit Rtt103, but when Rtt103 is absent, *nrd1*[CID^{RTT103}] is able to bind on minimal level of CTD-S2P.

Other interaction between Nrd1-dependent pathway and RNAP II besides CID-CTD interaction was also examined. Nab3 is a RNA binding protein in Nrd1-Sen1-Nab3 complex and has RNA recognition motif (RRM) which mediates interaction between Nrd1-dependent pathway and RNAP II. *nab3-20* mutant has three point mutations in RRM (Figure 3D). *nab3-20* mutant rescued termination in *nrd1*[CID^{RTT103}] strains regardless of the presence of Rtt103 (Figure 3E). These two kinds of disturbance to Nrd1-dependent pathway reveal CID swapped Nrd1 can be a strong competitor to Rtt103. From these results we can assume that the specific CID-CTD interaction might act as primary determinant for RNAP II in choice of termination pathway.

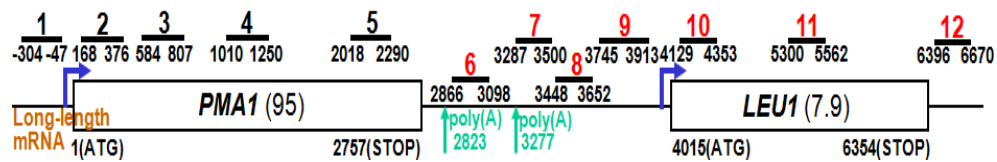
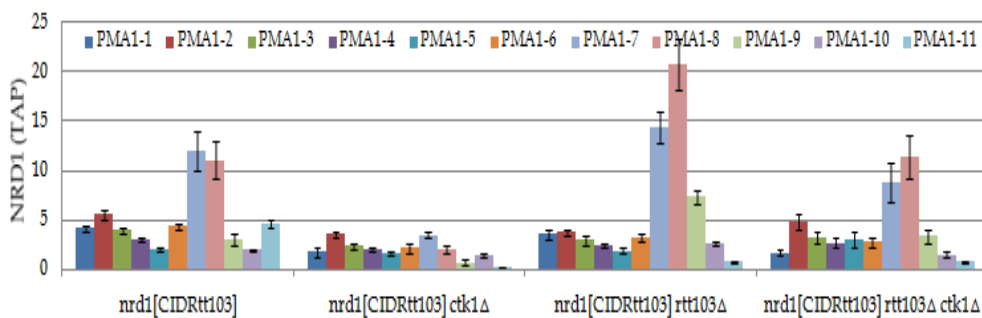
A



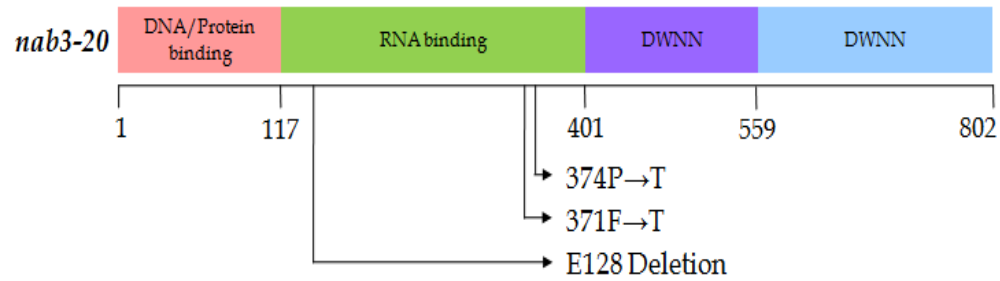
B



C



D



E

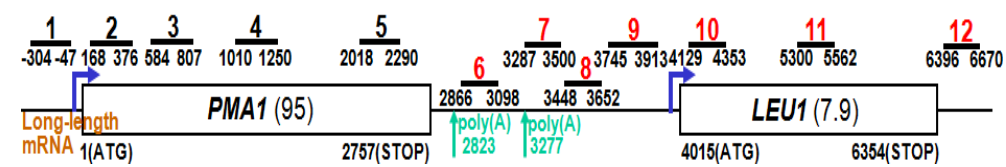
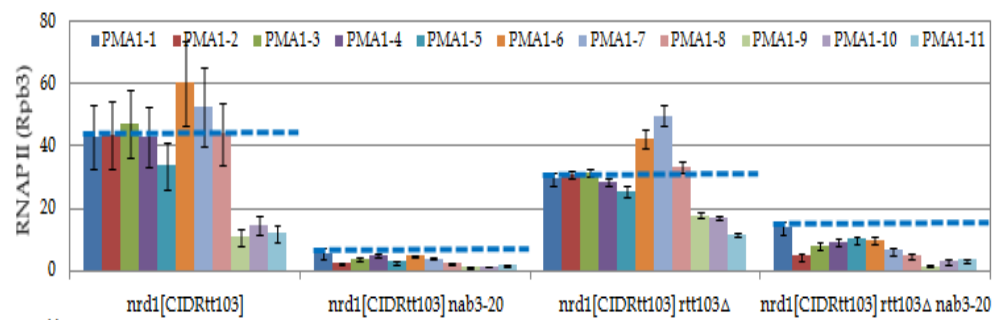


Figure 4. Interruption of Rtt103-dependent pathway caused by CID swapping can be rescued by disturbance of interaction between Nrd1-dependent pathway and RNAP II

(A) CTD-S2P kinase Ctk1 knockout greatly reduced the amount and the pattern of CTD-S2P throughout coding sequence. ChIP assay was carried out using (α-CTD-S2P).

(B and C) ChIP assays of Ctk1 knockout strains. (B) RNAP II and (C) Nrd1 on PMA1.

(D) Schematic representation of the *nab3-20* mutant construction.

(E) RNAP II distribution of *nab3-20* mutants were analyzed by ChIP.

IV. Discussion

CID swapping experiment elucidated role of CID-CTD interaction for RNAP II in choosing termination pathway. First of all, CID swapping only affected specific interactions between CTD phosphorylated states and CID. The affinity of Nrd1 for CTD apparently changed from CTD-S5P to CTD-S2P (Figure 1B). Other interactions involved in Nrd1-dependent pathway were not influenced by CID swapping (Figure 1C). Therefore *nrd1*[CID^{RTT103}] successfully bound to 3'-end of all examined genes (Figure 2B, 2D, 3B, and 3E) and showed functionality (Figure 2A, 2C, and 3A). In case of snoRNA, *nrd1*[CID^{RTT103}] shifted RNAP II slightly downstream (Figure 2A and 2C) and led to generation of read-through transcripts (Figure 2E). On the other hand, mRNA genes were classified into two distinct categories in CID swapped strains. ADH1 having Nrd1-Nab3 binding cluster terminated properly by CID swapped Nrd1-dependent pathway and about 30 % of mRNA genes showed normal termination property as ADH1 (Figure 3A, 3B, 3C, and 3F). On the contrary,

PMA1 showed severe termination defect (Figure 3C) and most of mRNA genes had RNAP II at high level after their poly (A) signals like PMA1 (Figure 3F). Rtt103-dependent pathway might be disturbed on these genes by binding of CID swapped Nrd1 at 3'-end of gene, because defective termination could be rescued when other interactions between Nrd1-dependent pathway and RNAP II were prevented (Figure 4). Therefore we can conclude that termination of genes which can be terminated by Nrd1-dependent pathway, like snoRNA genes and mRNA genes like ADH1, occurs properly under CID swapped condition, and genes which cannot be terminated by Nrd1-dependent pathway show defective termination due to disruption of Rtt103-dependent pathway caused by CID swapping. RNAP II might choose termination pathway depending on CID-CTD interaction upon 3'-end of these mRNA genes. Consequently, CID swapped Nrd1 could act as competitor of Rtt103-dependent pathway and lead to defective termination of RNAP II.

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국문 초록

Nrd1 CTD-Interacting Domain 이 RNA 중합 효소 II 의 전사 종결 과정에서 수행하는 역할에 대한 연구

RNA 중합 효소 II (RNAP II) 의 carboxy-terminal domain (CTD) 는 7 개의 아미노산으로 이루어진 중합체가 반복되는 구조로써 진핵 생물의 진화과정에서 잘 보존되어 있다. RNAP II 에 의한 전사의 각 과정에서 CTD 는 인산화 상태가 점차적으로 변하면서 RNA processing 을 담당하는 다양한 단백질들과의 결합을 매개한다. 전사가 종결될 때 RNAP II 는 전사 복합체에서 떨어져 나와 새로운 전사 과정에 참여할 수 있는 상태가 되는데, 이러한 “전사 종결” 과정을 일으키는 두 종류의 기작은 각각 서로 다른 CTD 인산화 상태에 결합하여 이루어진다. Rtt103-Rat1-Rai1 복합체에 따른 기작은 긴 mRNA 유전자의 3' -말단에서 CTD serine 2 인산화가 많이 일어난 상태 (CTD-S2P) 의 전사 종결을, Nrd1-Sen1-Nab3 복합체에 따른 기작은 길이가 짧은 유전자의 3' -말단에서 CTD serine 5 인산화가 많이 일어난 상태 (CTD-S5P) 에서의 전사 종결을 각각 담당하고 있다. 이러한 차이점은 각각의 기작을

담당하는 복합체가 공통적으로 가지고 있는 CTD-Interacting domain (CID) 에서 비롯된다. CID 를 가진 단백질들은 각각 Rtt103 그리고 Nrd1 이다. 본 연구는 특정한 CID-CTD 상호작용이 RNAP II 가 전사 종결 기작을 선택함에 있어 영향을 줄 것이라는 아이디어에서 시작되었으며 이러한 가정을 확인하기 위하여 효모의 한 종류인 *Saccharomyces cerevisiae* 의 Nrd1 CID 와 Rtt103 CID 를 서로 바꾸었을 때 전사 종결 과정에 미치는 영향을 알아보았다. CID 가 바뀐 Nrd1 은 생체 내에서 결합하는 CTD 인산화 상태가 CTD-S5P 에서 CTD-S2P 로 달라졌다. 나아가 ChIP 실험에서 *nrd1*[CID^{Rtt103}]는 유전자의 3' -말단에 성공적으로 결합할 수 있었다. 이러한 조건에서 snoRNA 는 공통적으로 전사 종결이 좀 더 3' -말단에서 일어난 read-through transcript 를 Northern blot 으로 확인할 수 있었지만, mRNA 는 서로 다른 결과를 보여주었다. 상대적으로 길이가 짧은 mRNA 인 ADH1 은 *nrd1*[CID^{Rtt103}]에 의해 정상적으로 전사 종결이 일어났으며, genome-wide ChIP-chip 분석에서 30 %의 mRNA 가 이와 같은 패턴을 나타냈다. PMA1 과 같은 상대적으로 길이가 긴 mRNA 유전자에서는 *nrd1*[CID^{Rtt103}] 의 결합이 정상적인 전사 종결 기작을 방해하는 것으로 보였고, genome-wide ChIP-chip 분석에서 70 %의 mRNA 유전자들이 PMA1 과 같은 전사 종결 실패를 보임을 확인할 수 있었다. PMA1 의 전사 종결 실패가 CID-CTD 상호작용에 의한 것인지 알아보기

위하여 CTD-S2P 인산화 효소를 제거하거나 Nrd1 에 따른 기작의 RNA 와의 결합을 약하게 만든 돌연변이들의 ChIP assay 를 수행하였고, 전사 종결 실패가 정상적으로 돌아올 수 있음을 확인하였다. 또한, CID 와 Nrd1 에 따른 전사 종결 기작에 참여하는 다른 인자들과의 상호작용들도 확인하였다. 생체 내에서 *nrd1*[CID^{RTT103}]는 정상적인 Nrd1 과 마찬가지로 Sen1, Nab3 와 복합체를 형성하였으며 exosome 중심 인자들인 Rrp4, Rrp41 과 여전히 상호작용하는 것으로 나타났다. 이러한 결과들은, CID 의 가장 중요한 역할은 CTD 와의 상호작용이며 CID-CTD 상호작용은 RNA 중합 효소 II 가 전사 종결 기작을 선택하는 데 있어 중요한 결정 요소로 작용함을 보여준다.

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주요어 : Nrd1, Rtt103, CID, CTD, RNA 중합 효소 II,

전사 종결

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