

## GTG as a Potential Translation Initiation Codon in Mitochondrial F<sub>1</sub> ATPase $\alpha$ -Subunit Gene (*atpA*) of Korean Ginseng

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**Abstract**—The complete open reading frame (ORF) of  $\alpha$ -subunit of the F<sub>1</sub> ATP synthase (*atpA*) in Korean ginseng mitochondria was identified by the sequence similarity with *atpA* genes in other plant mitochondria. The sequence alignment showed that the common translation initiation codon, ATG, in plant genes was replaced with GTG valin codon in Korean ginseng. The *atpA* gene from GTG to TGA termination codon was 1524 nucleotides long, and the sequence homology of nucleotides and deduced amino acids revealed high values of 92~97%. A deletion event of 6 nucleotides was observed at the 1468th nucleotide from the GTG in Korean ginseng, in contrast to that at the 1450th in other plants such as pea, common bean, soybean, sugar beet, and radish. An unidentified open reading frame (*orf27*) was observed upstream of *atpA* ORF. No other ATG as an initiation codon was detected in the region between *orf27* and *atpA* ORF in Korean ginseng, although a pyrimidine cluster "TTTTCTTTT" was located in this region as in *Oenothera* and maize genes. It could be supposed that GTG codon in *atpA* gene of Korean ginseng mitochondria would act as an initiation codon as in microbial genes.

**Key words**—ATPase  $\alpha$ -subunit, mitochondrial DNA, *Panax ginseng*.

### Introduction

The mitochondrial ATP synthase complex is a multisubunit enzyme complex composed of two distinct sectors, the F<sub>o</sub> and F<sub>1</sub> moieties.<sup>1,2)</sup> The hydrophobic F<sub>o</sub> sector is embedded within the inner mitochondrial membrane to form proton channel and three subunits (subunit 6, 8 and 9) in F<sub>o</sub> moiety are encoded in plant mitochondrial genome.<sup>3)</sup> The hydrophilic F<sub>1</sub> sector is required for nucleotide binding and the interconversion of ATP and ADP. In seed plants, mitochondrial F<sub>1</sub>-ATPase complex consists of five ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ )<sup>4,5)</sup> or six ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\delta'$ )<sup>6,7)</sup> subunits, and only  $\alpha$ -subunit gene is localized in mitochondrial genome.<sup>3)</sup> The mitochondrial gene for the  $\alpha$ -subunit has been identified and analysed in many kinds of plant species.<sup>8-20)</sup> Among them, *Oenothera*,<sup>8)</sup> maize,<sup>9)</sup> pea,<sup>10,11)</sup> and soybean,<sup>12)</sup>

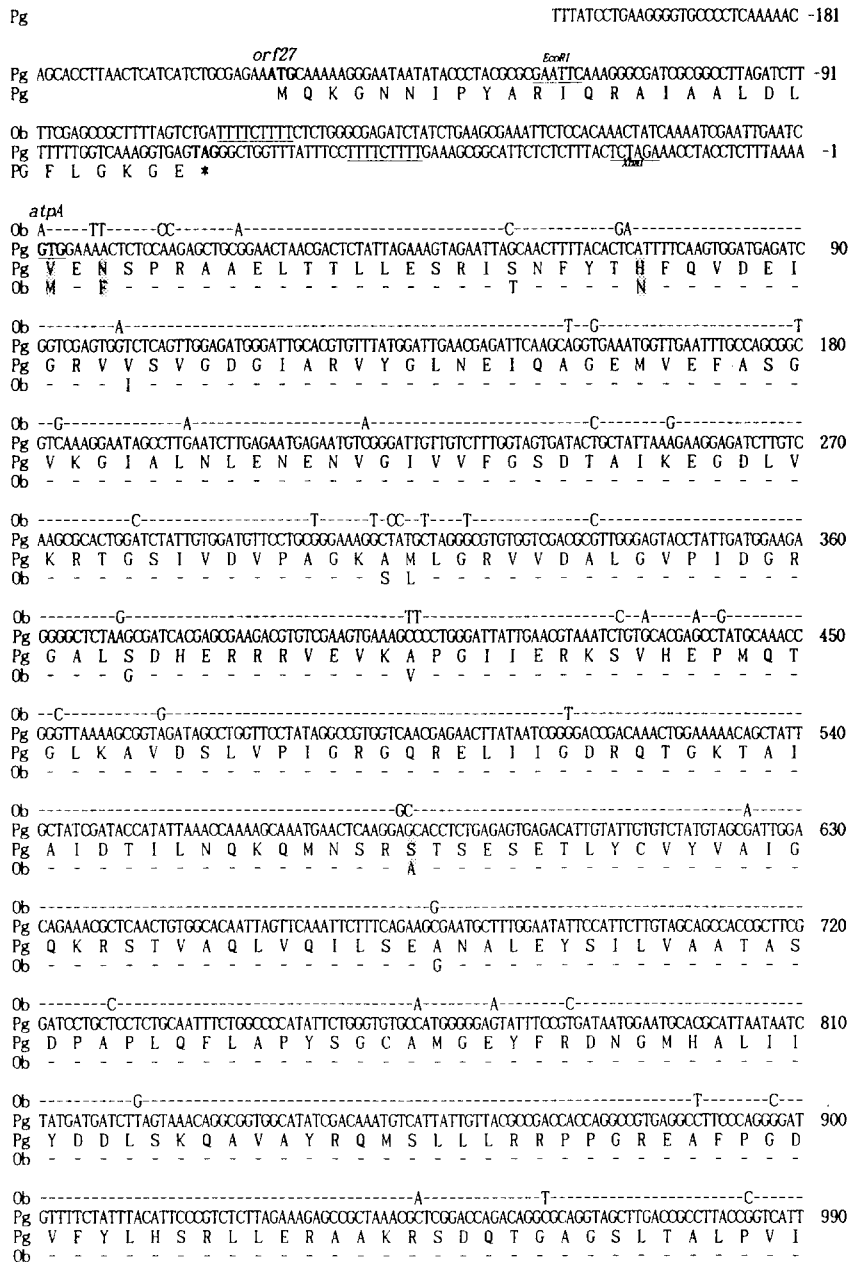
contain the multiple copies of this gene and pseudogenes in their mitochondrial genome.

We reported the presence of this gene as a single copy in Korean ginseng mitochondria in the previous paper.<sup>24)</sup> In this paper, the nucleotide and deduced amino acid sequences of the mitochondrial F<sub>1</sub> ATPase  $\alpha$ -subunit gene of Korean ginseng are presented and the possibility of GTG codon as a translation initiation codon is suggested.

### Materials and Methods

Ginseng mtDNA restriction fragments homologous to *Oenothera atpA* gene were subcloned into pBS II vector from previously documented 13 kb *atpA* clone, pGinP13. The subclones were sequenced by the dideoxy chain-termination method with Sequenase™ (Pharmacia) in the presence of [<sup>35</sup>S]dCTP or [<sup>35</sup>S]dATP as described by Sambrook *et al.*<sup>21)</sup> Sequencing of a subclone with 0.9 kb fragment

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**Fig. 1.** Nucleotide and deduced amino acid sequences of the ATPase  $\alpha$ -subunit gene and *orf27* from Korean ginseng mitochondria. Dash indicates identical sequence in the Korean ginseng (Pg) and the *Oenothera*<sup>+</sup> (Ob) genes. A putative GTG initiation codon and TGA termination codon in bold letters was underlined and numbering of nucleotides is from the predicted translation start of *atpA*. The open reading frames were translated using the universal code. The TTTTCTTTT feature common to the upstream region of initiation codon in both species were also underlined. Small asterisks indicate nucleotides deleted in sequence alignment. The shaded codons indicate amino acids different from those highly conserved in other 12 species and putative pre-editing sites in nucleotides.

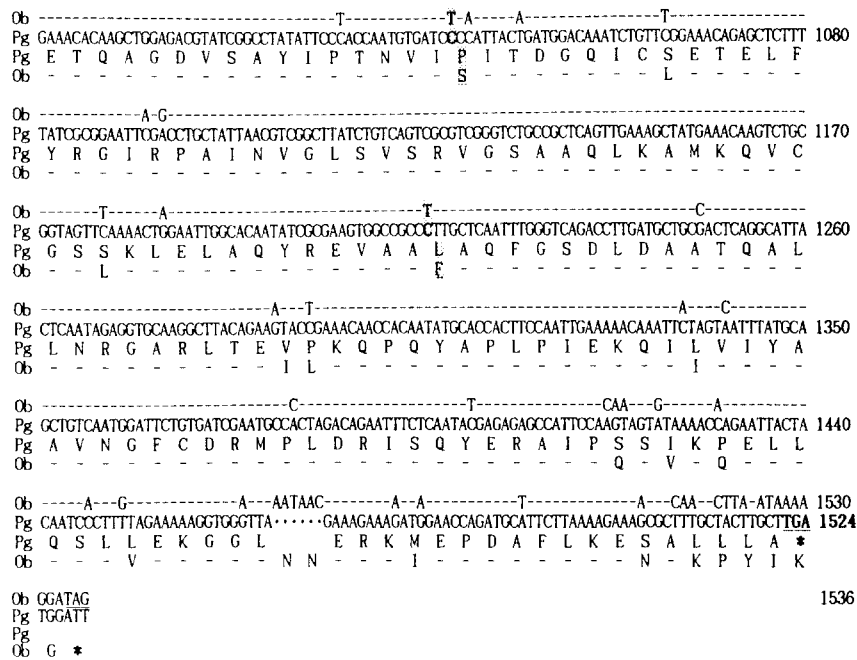


Fig. 1. Continued.

containing 3' terminus of *atpA* coding sequence was performed by a series of deletion method. Similarities of these sequences and other plant genes were identified by searching the GenBank and EMBL Database.

## Result and Discussion

### 1. Nucleotide sequence analysis of ATPase $\alpha$ -subunit gene

From the pGinP13 clone (13 kb *Pst*I fragment) of the mitochondrial DNA, *atpA* open reading frame and upstream region were sequenced (Fig. 1). Unexpectedly, we found that a GTG codon was localized at the position of the common translation initiation codon, ATG, in other plant mitochondria as clearly shown in Fig. 1 and 4A. This observation suggests that at least the translation efficiency of this gene in Korean ginseng mitochondria is not so high as in other plants containing ATG codon. The entire coding sequence from GTG to TGA termination codon was 1524 bp long, 12 bp shorter than that of *Oenothera atpA* gene and 3 bp longer than radish (Fig. 3).

In nucleotide sequence alignment of the Korean ginseng mitochondrial *atpA* gene with those identified in the 12 kinds of plant species (data not shown), only Korean ginseng contained GTG codon (valine) instead of ATG and the 15 bp sequence from GTG in *atpA* ORF revealed low homology (70~75%), although this region and the entire sequence were highly conserved in other plants as shown in the sequence similarity (*Oenothera* : 93%, sunflower : 97%, *Nicotiana* : 96%, maize : 92%, rice : 93%, wheat : 93%, pea : 94%, *Phaseolus vulgaris* : 93%, soybean : 93%, sugar beet : 94%, rapeseed : 94%).<sup>8, 19)</sup> There were 6 amino acids (shaded codon in Fig. 1) in Korean ginseng, which were different from those highly conserved in all other plants. The alterations of nucleotides and amino acids in the plant mitochondrial gene can occur by RNA editing phenomenon such as post-transcriptional nucleotide switches, in most instances from C to U, but occasionally from U to C,<sup>22)</sup> which plays a role in the conservation of protein sequences during evolution.<sup>23, 24)</sup> Only two events from 6 amino acid conversions might be explained as the preedited site, in which T nucleotide in other plants is replaced with C in

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Panax ginseng : VENSPPAAEL TTLLESRISN FYTHFQVDEI GRVVSVDGI ARVYGLNEIQ 50
Pisum sativum : M-F-V-----T-----N-----
Zea mays      : M-F-----MI-----NLK-----

AGEMVEFASG VKGIALNLEN ENVGIVVFGS DTAIKEGDLV KRTGSIVDVP AGKAMLRV 110
--L-----S-----

DALGVPI DGR GALS DHERRR VEVKAPGII E RKS VHEPMQT GLKAVDSLVP IGRGQRELII 170
-----K-----

GDRQTGKTAI AIDTILNQKE MNSRSTSESE TLYCVYVAIG QKRSTVAQLV QILSEANALE 230
-----G-N-----

YSILVAATAS DPAPLQFLAP YSGCAMGEYF RDNGMHALII YDDLKQAVA YRQMSLLRRR 290
--M-----

PPGREAFPGD VFYLSRLL E RAAKRS DQTG AGSLTALPVI ETQAGDVSAY IPTNVIPITD 350
-----S-----S-----

GQICSETELF YRGIRPAINV GLSVSRV GSA AQLKAMKQVC GSSKLELAQY REVAALAQFG 410
--L-----L-----F-----F-----
--L-----L-----F-----

SDLDAATQAL LNRGARLTEV PKQPQYAPLP IEKQILVIYA AVNGFCDRMP LDRISQYERA 470
-----L-----E-----V-----K-A-----D-----K-----

IPSSIKPELL QSLLEKGG L# ERKMEPDAF LKESALLA* 507
-L-T-Q---##---T G---I-----K-S-I*
-L-T-N---K-F-----T N-----N-*

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Fig. 2. Comparison of amino acid sequences of mitochondrial *atpA* gene of Korean ginseng with those of pea, maize. Dash and # indicate the identical and deleted sequences in the Korean ginseng and pea, respectively. Termination codon is shown as asterisk. Numbering of amino acids is from the predicted translation start codon GTG for valine of *atpA* gene. The open reading frames were translated by using the universal code. The shaded codes indicate the conserved amino acids involved in ATP binding.<sup>13)</sup>

Korean ginseng (Fig. 1).

## 2. Comparison of amino acid sequence

The protein sequences of  $\alpha$ -subunit of ATPase in plant mitochondria as deduced from the nucleotide sequences are highly conserved among many species with the exception of the C-terminal region (Fig. 2 and 3). Among 24 and 25 different amino acids between Korean ginseng and pea or maize genes, 13 and 11 are found, respectively, in the C-terminal region beyond the 460th amino acid. The homology between Korean ginseng and other plants was above 94% (*Oenothera* : 94%, pea and maize : 96%).

There was no tryptophan residue (W) in this protein like in other plants, that means the absence of TGG codons. The essential sequence regions for ATP binding function were also highly conserved

in Korean ginseng (Fig. 2, shaded residues).

## 3. Sequence rearrangement in ATPase $\alpha$ -subunit gene

In contrast to the highly conserved sequence in the *atpA* gene, the upstream region of GTG codon (data not shown) and the C-terminal ca. 150 bp from TGA showed an extensive divergence in nucleotide sequences among plant species (Fig. 3), which indicated a possible sequence rearrangement.

As shown in Fig. 3, there were three types of sequence structure in *atpA* gene of plant mitochondria. In the first type such as *Oenothera*, sunflower, *Nicotiana*, maize, rice, and wheat, nucleotide deletion in 3'-terminal region was not detected. The second group of plants such as pea, common bean, soybean, sugar beet, and radish showed a 6 bp deletion at 1450th nucleotide from the ATG initiation

A: Nucleotide sequences

	1402		1450	1455	1467
	#		#	#	
Pg	GAGAGAGCCATTCCAAGTAGTATAAAACCAGAATTACTACAATCCCTITTTAGAAAAAGGTGGGTTA				
Ob	-----CAA--G-----A-----A--G-----A---				
Ha	-----TT--AG-----A-----A-----A-----C---				
Np	-----A--G-C-----T-----C---				
Zm	--A-A-AA---T---C---T---T---A---T-C-----C---				
Os	--A-A---T---C---T---T---A---T-CAAC-----G--A---				
Ta	--A-A---T---C---T---T---A-A---T-C-----C---				
Ps	--A--A---T---C-C---A-----A-A#####-AC-				
Pv	--A--A---T-CG-C---T---T---A---A#####-AC-				
Gm	--A--A---T-CG-C---T---T---A---A#####-AC-				
Bv	--C-A---A--G-----T-----#####-G-G-				
Bn	--A-----A--G-C---T-----G-----#####-				
Rs	--A-----A--G-C---T-----G-----#####-A---				
	1468	1473		1524	
	#	#		#	
Pg	#####GAAAGAAAGATGGAACCAGATGCATTCTTAAAAGAAAGCGCTTTGCTACITGCTTGA				
Ob	AATAAC-----A-A-----T-----A---CAA--CTTA-ATAAA-GGATAC				
Ha	ACTAAC-----A-----A-----G--T-----CTTACA-AAT-TAA				
Np	ACTAAC-----A-----A-----T-----AGCIT--AT--A-				
Zm	ACTAAC-----T---T---T-CT---C---CT--GAA-GAGA--AA--AG				
Os	ACTAAC-----T---T---T-CT---C---CT--GAA-GAGA--AA--AG				
Ta	ACTAAC-----T---T---T-CT---A-----CTTACCTG--				
Ps	ACTAGC-----A-A---T---AAA-----AA--AAAAATTACA-A-T-TGA				
Pv	ACTAGC-----A-A---T---AAA-----AA--AAAAATTACA-A-T-TGA				
Gm	ACTAGC-----A-A---T---AAA-----AA-G-GGAACCTACTA-AT-TGA				
Bv	ACTAAC-----A-----T---T-C-----T-----AATTACTAA				
Bn	ACTAAC-----A-----T-----G-----A-GTT-AAT--AG				
Rs	ACTAAC-----A-----T-----A-----AGCIT-AAT--AG				

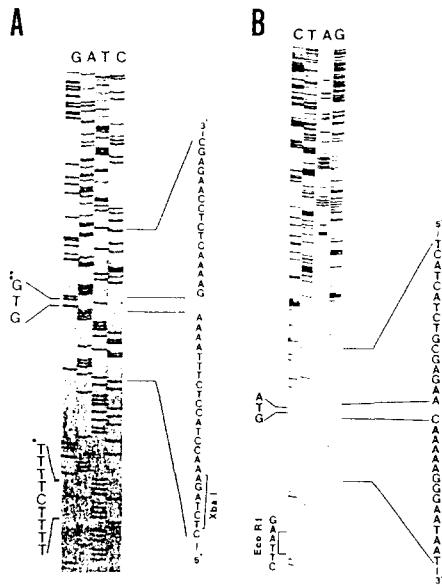
B: Amino-acid sequence

	467	484	490	507
	#	#	#	
Pg	ERAIPSSIKPELLQSLLEKGGI##ERKMEDPAFLKESALLA*			
Ob	ERAIPQSVKQELLQSLVEKGGI##ERKMEDPAFLKENAKPYIKG*			
Ha	ERAILKSIKTELLQSLLEKGGITNERKMEDPDTFLKECALPYTI*			
Np	ERAIPNSVKPELLQSFLEKGGITNERKMEDPDTFLKESALAFI*			
Zm	EKNILSTINPELLKSFLEKGGITNERKMEDPDSLKESALNL*			
Os	EKAILSTINPELLKSFNEKGGITNERKMEDPDSLKQTAKEIN*			
Ta	EKAILSTINPELLKSFLEKGGITNERKMEDPDSLKKESTLPYL*			
Ps	ERDILSTIKQELLQSL##KGGLTGERKIEPDAFLKEKALSII*			
Pv	ERDILTTIKPELLQSL##KGGLTSERKIELEKFLKEKAKNYTL*			
Gm	ERDILTTIKPELLQSL##KGGLTSERKIELEKFLKEKGGTYVI*			
Bv	ERTIPNSVKPELLQSL##KGGLTNEKMEKELDSFLKECALNY*			
Bn	EKAIPNSVKPELLQAL##KGGLTNERKMEDPAFLKERALRLI*			
Rs	EKAIPNSVKPELLQAL##KGGLTNERKMEDPAFLKERALALI*			

**Fig. 3.** Comparison of nucleotide and amino acid sequences in C-terminal region of mitochondrial *atpA* gene of Korean ginseng with those of 12 other plants. Dash and # indicate identical and deleted sequences in the Korean ginseng (Pg) and the other plants, respectively. Termination codon is underlined and shown as asterisk. Homologous amino acids were shaded and numbering of nucleotides and amino acids is from the predicted translation start codon of *atpA*. Pg: Korean ginseng, Ob: *Oenothera*,<sup>8)</sup> Ha: sunflower,<sup>14)</sup> Np: *Nicotiana*,<sup>12)</sup> Zm: maize,<sup>9)</sup> Os: rice,<sup>15)</sup> Ta: wheat,<sup>16)</sup> Ps: pea,<sup>10, 11)</sup> Pv: *Phaseolus*,<sup>17)</sup> Gm: Soybean,<sup>13)</sup> Bv: sugar beet,<sup>18)</sup> Bn: rapeseed,<sup>19)</sup> Rs: radish.<sup>20)</sup>

codon. But in Korean ginseng as a third group, a 6 bp fragment was deleted at 1468th nucleotide from GTG, not at 1450th like in the second group. Particularly, the amino acid sequences in both sides

of deletion events were highly conserved in all plants. The sequence in this region of Korean ginseng was more similar to those of sunflower and *Nicotiana* than those of other plants.



**Fig. 4.** Sequence analysis of the upstream region containing a possible translation initiation codon, GTG, and a cluster of 5'-TTTTCTTT-3' pyrimidines (A), and ATG codon of *orf27* in upstream region (B) of *atpA* gene in Korean ginseng.

#### 4. GTG codon in *atpA* gene of Korean ginseng as a translation initiation codon?

Is it possible that GTG codon acts as a translation initiation codon in plant mitochondrial genes?

Up to date, it has been known that translation from GTG codon is observed mostly in prokaryotes. Recently, Brennicke group found that the first codon of *rpl16* gene of *Oenothera* mitochondria was GTG (valine codon) as the only potential translational start codon. This codon is also conserved at the same position in maize, *Petunia* and *Marchantia* mitochondria, while sequences diverge upstream. These observations suggest that GTG at least at this position may act as translation initiation codon in plant mitochondria (personal communication).

We tried to find other ATG codon in upstream region. As shown in Fig. 1 and 4A, there is no ATG codon within 150 bp from GTG codon. The first ATG codon was located at 153th nucleotide upstream (Fig. 1 & 4B). But the translation initiation from this must be stopped by TAG termination codon at -71th nucleotide (Fig. 1, *orf27*). Although

there was no ATG codon in the region between *orf27* and GTG, a pyrimidine cluster 5'-TTTTCTTT-3' existed at -45th nucleotide from GTG in Korean ginseng as in *Oenothera* and Maize. But there was no pyrimidine cluster in this sequence region in most other plants such as rice, *Nicotiana*, soybean, sugar beet, etc. and the function of pyrimidine cluster in translation is not yet known. In consideration of the active function of Korean ginseng mitochondria for the growth, the presence of *atpA* gene as a single copy, and the absence of other possible ATG codon in upstream region, it could be suggested that GTG codon might function as a translation initiation codon as in prokaryotes.

#### 요약

고려인삼 미토콘드리아 DNA 함유 clone인 pGinP 13에서 ATPase  $\alpha$ -subunit 유전자(*atpA*) 부위의 염기 서열을 분석하고 타 식물과 비교하여 *atpA*임을 동정하였다. 염기서열을 비교하였던 바, 타 식물 11개 종류에서 해석시작 codon이 모두 ATG이었으나 인삼에서는 valine codon인 GTG이었다. 이 유전자는 1524 염기로 이루어졌으며 해석종료 codon은 TGA이었다. 타 식물의 핵산 및 아미노산서열과의 상동성은 92~97%로 아주 높았다. 달맞이꽃, 담배, 해바라기, 옥수수, 벼, 밀과 비교해서 C-terminal 부위에 6개 염기가 인삼 및 완두, 콩, 사탕무, 무, 등에서 공히 결실되어 있었지만, 그 결실 위치가 인삼에서는 GTG로부터 1468번째 염기로서, ATG로부터 1450번째인 완두, 콩, 사탕무, 무와는 달라서, DNA 재배치 과정에서 인삼은 차이가 있음을 알 수 있었다.

이 유전자 앞부분에는 아미노산 27개의 단백질인 미확인 유전자 *orf27*이 존재하였고 이 *orf27*과 GTG 사이에는 ATG codon이 없어 인삼의 *atpA* 유전자에서는 GTG codon이 translation initiation codon으로 작용할 것으로 생각되었다.

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