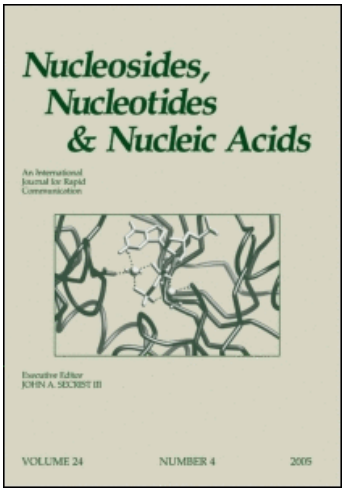

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TRIPLEX FORMATION-BASED ARTIFICIAL TRANSCRIPTION FACTOR TO REGULATE TARGET GENE EXPRESSION

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□ *A triplex formation-based artificial transcription factor to recognize any upstream sequence of target genes was developed to regulate the target gene expression. The artificial transcription factor contains a single-stranded RNA to bind with duplex DNA of the upstream sequence of the target gene to form triplex, and an effecter domain, such as activation or repression domain, of transcription factor. Reporter β-galactosidase activity in yeast was increased 1.5–2 times by introduction of the artificial transcription factor. The novel artificial transcription factor may be a useful tool to regulate the target gene expression and reveal unknown function of the target genes.*

Keywords triplex; transcription factor; gene expression

INTRODUCTION

Genome projects identified many open reading frames (ORFs) in the genome of various organisms. Analyses of phenotypes upon activating or repressing the expression of the target ORFs are useful to reveal their unknown function. Natural transcription factors bind to the upstream sequence of target genes with high specificity, but they cannot recognize any upstream sequence of target genes. In the present study, artificial transcription factor to recognize any upstream sequence of target genes was developed to regulate the target gene expression.

To recognize any upstream sequence of target genes, triplex formation was used, where a single-stranded RNA added from outside (triplex forming RNA: TFO) binds to duplex DNA of the upstream sequence through the sequence-specific interaction to form triplex involving A-A:T and G-G:C base triplets (Figure 1).[1–3] When an RNA binding protein (RBP) is translationally fused to an effecter domain (ED), such as activation or repression domain, of transcription factor, and the target RNA sequence of RBP (RS)

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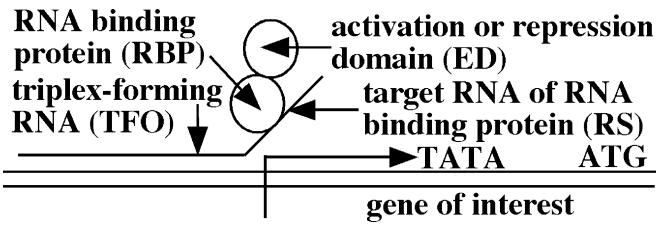

FIGURE 1 Overview of artificial transcription factor.

is transcriptionally fused to TFO in vivo, the specific association of RBP with its target RS assembles ED-RBP fusion protein and TFO-RS (or RS-TFO) fusion RNA to form the artificial transcription factor (Figure 1). The TFO binds specifically to the upstream sequence of the target gene, and recruits ED to regulate the target gene expression. We constructed expression plasmids for the component of the transcription factor, ED-RBP and TFO-RS (or RS-TFO), and reporter plasmid to estimate the effect of the transcription factor. We measured the reporter activity in yeast with or without the transcription factor.


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                Target of TFO1
CAACTTCTTTTCTTTTTTTTTCTTTTCTCTCTCCCCCGTTGTTGTCTCACCATATCCGCAAT
GTTGAAGAAAAGAAAAAAAAAGAAAAGAGAGAGGGGGCAACAACAGAGTGGTATAGGCGTTA
                          Target of TFO2
GACAAAAAAAAATGATGGAAGACACTAAAGGAAAAAATTAACGACAAAGACAGCACCAACAGA
CTGTTTTTTTTACTACCTTCTGTGATTTCCTTTTTTAATTGCTGTTTCTGTCGTGGTTGTCT
                                                  Target of TFO3
TGTCGTTGTTCCAGAGCTGATGAGGGGTATCTTCGAACACACGAAACTTTTTCCTTCCTTCA
ACAGCAACAAGGTCTCGACTACTCCCCATAGAAGCTTGTGTGCTTTGAAAAAGGAAGGAAGT
                                        Target of TFO4
TTCACGCACACTACTCTCTAATGAGCAACGGTATACGGCCTTCCTTCCAGTTACTTGAATTT
AAGTGCGTGTGATGAGAGATTACTCGTTGCCATATGCCGGAAGGAAGGTCAATGAACTTAAA

GAAATAAAAAAAGTTTGCCGCTTTGCTATCAAGTATAAATAGACCTGCAATTATTAATCTTT
CTTTATTTTTTTCAAACGGCGAAACGATAGTTCATATTTATCTGGACGTTAATAATTAGAAA
                             Target of TFO5
TGTTTCCTCGTCATTGTTCTCGTTCCCTTTCTTCCTTGTTTCTTTTTCTGCACAATATTTCA
ACAAAGGAGCAGTAACAAGAGCAAGGGAAAGAAGGAACAAAGAAAAAGACGTGTTATAAAGT

AGCTATACCAAGCATACAATCAACTCCAAGCTTTGCAAAGATGGATAAAGCGGAATTAATTC
TCGATATGGTTCGTATGTTAGTTGAGGTTCGAAACGTTTCTACCTATTTCGCCTTAATTAAG
                                        Initiation codon
CCGAGCCTCCAAAAAAGAAGAGAAAGGTCGAATTGGGTACCGCCATG
GGCTCGGAGGTTTTTTCTTCTCTTTCCAGCTTAACCCATGGCGGTAC
```

FIGURE 2 Upstream base sequence of yeast alcohol dehydrogenase gene. Five potential sites to form triplex with TFO (TFO1-TFO5) are shaded. Initiation codon is underlined.

RESULTS AND DISCUSSION

We constructed the following three kinds of plasmids: 1) a reporter plasmid (pADH-LacZ) encoding the upstream sequence of yeast alcohol dehydrogenase gene (*ADH*) (Figure 2) and a reporter β-galactosidase gene (*LacZ*); 2) an expression plasmid for ED-RBP fusion protein (pVP16-RBP) consisting of VP16 activation domain and MS2 phage RNA binding protein;[4] and 3) expression plasmids for TFO-RS fusion RNA (pTFO-RNA) and RS-TFO fusion RNA (pRNA-TFO) consisting of TFO RNA to form triplex with the upstream sequence of *ADH* (Figure 2) and the target RNA of MS2 phage RNA binding protein.[4] For control experiments, a vector plasmid without the genes coding for ED-RBP fusion protein (pTRP) and a vector plasmid without the genes coding for TFO-RS (or RS-TFO) fusion RNA (pURA) were constructed.

The reporter β-galactosidase activity was examined in yeast transformants with various combinations of the plasmids (Table 1).[5] The upstream sequence of yeast *ADH* contains five potential sites to form triplex with TFO (Figure 2). When pTFO-RNA or pRNA-TFO involving TFO1 was introduced with pVP16-RBP (Exp. No. 3 and 13), the values of β-galactosidase activity

TABLE 1 β-galactosidase activity in yeast transformants with various combinations of the plasmids

	TFO-RS or RS-TFO			ED-RBP		Repoter	Activity
Exp. No.	pURA	pTFO-RNA	pRNA-TFO	pTRP	pVP16-RBP	pADH-LacZ	β-gal units
1	+	-	-	+	-	+	23.6±1.7
2	+	-	-	-	+	+	18.3±1.3
3	-	+(TFO1)	-	-	+	+	34.3±4.4[a]
4	-	+(TFO2)	-	-	+	+	16.1±1.5
5	-	+(TFO3)	-	-	+	+	17.6±3.3
6	-	+(TFO4)	-	-	+	+	20.7±1.1
7	-	+(TFO5)	-	-	+	+	20.8±1.5
8	-	+(TFO1)	-	+	-	+	18.9±4.6
9	-	+(TFO2)	-	+	-	+	22.2±4.0
10	-	+(TFO3)	-	+	-	+	22.1±1.4
11	-	+(TFO4)	-	+	-	+	22.4±2.6
12	-	+(TFO5)	-	+	-	+	24.3±2.4
13	-	-	+(TFO1)	-	+	+	41.1±4.5[a]
14	-	-	+(TFO2)	-	+	+	23.9±5.5
15	-	-	+(TFO3)	-	+	+	22.1±4.6
16	-	-	+(TFO4)	-	+	+	19.2±3.0
17	-	-	+(TFO5)	-	+	+	23.7±3.6
18	-	-	+(TFO1)	+	-	+	24.9±2.9
19	-	-	+(TFO2)	+	-	+	23.6±2.4
20	-	-	+(TFO3)	+	-	+	27.7±2.5
21	-	-	+(TFO4)	+	-	+	27.1±3.9
22	-	-	+(TFO5)	+	-	+	21.5±3.0

[a]Significantly large values as compared with those of control experiments (Exp. No. 1, 2, 8, and 18) by the Mann-Whitney *U*-test.

were significantly increased by 1.5–2 times in comparison with those observed for the control experiments (Exp. No. 1, 2, 8, and 18). The VP16 activation domain in the artificial transcription factor successfully enhanced the target gene expression by the association between the artificial transcription factor and the upstream sequence of the target gene. On the other hand, the introduction of pTFO-RNA or pRNA-TFO involving the other TFOs (TFO2, TFO3, TFO4, and TFO5) in combination with pVP16-RBP (Exp. No. 4–7 and 14–17) did not significantly change the values of β-galactosidase activity, compared with those observed for the control experiments (Exp. No. 1, 2, 9-12, and 19-22). The length of the target sites and the distance between the target sites and the initiation codon may influence the effect of the artificial transcription factor to regulate the target gene expression. The novel artificial transcription factor may be a useful tool to regulate the target gene expression and reveal unknown function of the target genes.

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