

Binding sites of miRNAs with transcription factors' genes of *Camelus ferus* and *Homo sapiens*

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The nucleotide sequences of mRNAs of ZNF family 157 TF genes of *Camelus ferus* were taken from GenBank (<http://www.ncbi.nlm.nih.gov>). The nucleotide sequences of 2563 hsa-miRNAs were taken from the miRBase database (<http://mirbase.org>). We next evaluated human miRNAs since camel miRNAs have not yet been identified. The search for miRNA binding sites in target gene mRNAs was performed using the MirTarget program. This program defines: the beginning of binding sites of miRNAs with mRNAs; the location of sites in the 5'-untranslated region (5'UTR), a protein-coding portion (CDS) and the 3'-untranslated region (3'UTR) of mRNA; the free energy of hybridization (ΔG , kJ/mole) and patterns of interaction miRNAs nucleotides with mRNAs. We expected value of $\Delta G/\Delta G_m$ (%), where ΔG_m is the free energy of miRNA binding with fully complementary nucleotide sequence. Binding sites of miRNAs with mRNAs selected with the value of $\Delta G/\Delta G_m$ equal or more than 90%. The position of the binding sites was shown from the first nucleotide (n.) of mRNA.

We searched for the binding sites of 2,563 *Homo sapiens* genome miRNAs and the mRNA of 157 *Camelus ferus* zinc finger family transcription factor (ZNF family TF) genes. The table 1 shows the characteristics of some of the miRNAs that bind to human orthologous transcription factors of the ZNF family of *C. ferus* and *H. sapiens*. There are 16 binding sites in the 5' UTRs, 210 binding sites in the CDSs, and 34 binding sites in the 3' UTRs of 96 transcription factor gene mRNAs. Eight miRNAs bind to the mRNA of the *Gli2* gene, and there are seven miRNA binding sites in the *ZNF236* and *GLIS1* mRNAs. In the 3' UTR of the *ZFP91* mRNA, 13 binding sites for miR-574-5p were identified, located through two nucleotides. The *VEZF1* gene mRNA has eight binding sites for miR-1322, located through three nucleotides. miRNAs that are 25 and 26 nucleotides in length have the greatest binding energy. The $\Delta G/\Delta G_m$ values, which characterize the degree of affinity of miRNAs to mRNA,

vary from 90% to 96% in these miRNAs. miR-1322 has one binding site in the mRNA of *Gli1*, *HINFP*, *HIVEP1*, *MTF1*, *SALL4*, *SP1*, *ZNF335* and *ZNF451* target genes, two sites in the *ZNF142* mRNA, and three sites in the *EGR1* mRNA. The ΔG values ranged from -114.6 kJ/mole to -138.0 kJ/mole. Some miRNAs that are 23 and 24 nucleotides in length also have high ΔG values, which range from -112.5 kJ/mole to -129.5 kJ/mole. The results demonstrate a strong dependence of transcription factor gene expression on their associated miRNAs.

Table 1. The characteristics of miRNA binding sites in mRNAs of transcription factor genes of ZNF family

Gene	miRNA	Position in mRNA, n.	
		<i>C.ferus/H.sapiens</i>	<i>C.ferus/H.sapiens</i>
<i>E4F1</i>	miR-6861-3p	1037/1205	92/92
<i>EGR1</i>	miR-4318	454/689	93/93
<i>EGR4</i>	miR-6867-3p	182/1253	92/92
<i>GF11</i>	miR-4481	43/293	93/93
<i>GLI2</i>	miR-665	454/466	93/94
<i>GLI2</i>	miR-6763-5p	3650/4313	92/92
<i>HINFP</i>	miR-877-3p	146/384	93/95
<i>KLF4</i>	miR-4478	394/969	91/93
<i>KLF9</i>	miR-4492	289/1646	94/94
<i>MECOM</i>	miR-466	5158/5100	93/95
<i>PLAGL2</i>	miR-4279	203/306	93/93
<i>PRDM10</i>	miR-4516	2861/724	91/94
<i>TRERF1</i>	miR-3960	1669/2253	93/93
<i>WIZ</i>	miR-1260a	182/396	94/94
<i>WIZ</i>	miR-7155-3p	497/711	92/92
<i>ZFPM1</i>	miR-1306-3p	942/1020	94/91
<i>ZKSCAN4</i>	miR-6828-3p	899/1143	94/94
<i>ZNF143</i>	miR-4313	25/25	93/93
<i>ZNF335</i>	miR-548av-5p	1366/1468	95/95
<i>ZNF423</i>	miR-3155b	2918/3236	92/92
<i>ZNF652</i>	miR-6892-5p	1946/2150	96/91

The data show great similarity to the affinity of orthologous mRNAs of camel and human transcription factor genes. The vast majority of binding sites are located in the protein coding portions of the mRNAs, indicating that regulation of the expression of transcription factor genes by miRNAs is evolutionarily conserved.

Orthologous miRNAs of *Equus caballus* and *Bos taurus* interacted with mRNAs orthologous to the MYB family of transcription factors, which also indicates that regulation of transcription factor gene expression by miRNAs is evolutionarily conserved across the species studied.