

A Triplet-plus-One DNA Base recognition Scheme for TFIIIA Zinc fingers 1, 2, 3 and 5.

Much effort has been put into establishing a triplet code for zinc finger protein-DNA recognition. Such a code would in theory match 64 (4^3) short DNA sequences (three base pairs) with 8,000 (20^3) alpha helix variants. It was originally hoped that this triplet code [1] could be the basis for the design of artificial zinc finger proteins for academic, medical and commercial applications [2]. However, the triplet code ignores other known contacts frequently observed between zinc fingers and DNA base pairs [3].

The interactions of TFIIIA zinc fingers 1, 2, 3 and 5 with DNA triplets are shown in the tables below. The canonical alpha helix contacts are compared to those found in the co-crystal structure of TFIIIA and DNA [4]. Amino acids colored in red do not conform to the triplet code and bind instead to a nucleotide base on the complementary DNA template strand. Critical contacts (not shown here) are often made to phosphates on the DNA coding strand by the amino acids in alpha helix positions $\alpha 7$ and $\alpha 10$. The 5'-3' DNA coding strand has the same nucleotide sequence as 5S rRNA.

α -helix positions	ZnF 1	ZnF 2	ZnF 3	Znf 5
-3	tyr	phe		phe
-2				
-1	lys	ser		leu
$\alpha 1$		leu	lys	pro
$\alpha 2$	trp	his	ala	ser
$\alpha 3$	lys	his	asn	arg
$\alpha 4$				
$\alpha 5$			lys	
$\alpha 6$		arg	lys	arg
$\alpha 7$	his	his	his	his

$\alpha 8$				
$\alpha 9$				
$\alpha 10$	lys	thr	arg	val
$\alpha 11$			phe	
$\alpha 12$				

Amino acids in the recognition alpha helices of TFIIIA that bind to DNA.

DNA	dA	dG	dA	dG
alpha helix	$\alpha 6$ ala	$\alpha 3$ lys	-1 lys	$\alpha 2$ trp
ZnF 1	$\alpha 2$ trp	$\alpha 2$ trp	$\alpha 2$ trp	-1 lys

DNA	dG	dG	dG	dT
alpha helix	$\alpha 6$ arg	$\alpha 3$ his	-1 ser	$\alpha 2$ his
ZnF 2	$\alpha 6$ arg	$\alpha 3$ his	$\alpha 2$ his	-1 ser

DNA	dG	dA	dT	dC
alpha helix	$\alpha 6$ lys	$\alpha 3$ asn	-1 thr	$\alpha 2$ ala
ZnF 3	$\alpha 6$ lys	$\alpha 3$ asn		$\alpha 2$ ala

DNA	dG	dG	dT	dA
alpha helix	$\alpha 6$ arg	$\alpha 3$ arg	-1 leu	$\alpha 2$ ser
ZnF 5	$\alpha 6$ arg	$\alpha 3$ arg		$\alpha 2$ ser

Contacts between the 5'-3' DNA coding strand and TFIIIA alpha helices are shown colored in black in the table above. The predicted alpha helix interactions are compared with those observed in TFIIIA zinc fingers (ZnF1, 2, 3 and 5). A fourth nucleotide that is contacted on the DNA template strand is shown colored in red.

Lessons learned

An exact relationship between positions in the recognition alpha helix and positions in a DNA sequence is not apparent in TFIIIA. This "flexibility" can be explained by the different sizes, shapes, charges and reach of amino acid side chains. Therefore, any code should be based on overall tetramer-tetramer interactions. Four amino acids in the alpha helices (at positions -1, $\alpha 2$, $\alpha 3$ and $\alpha 6$) should be considered to act as a single unit in binding to a four-base pair DNA sequence. However, the ability to form an alpha helical structure may require other amino acids

to be present. Four DNA quartets and their respective TFIIIA amino acid tetramers are shown in the table below.

Coding strand quartets	dAdGdAdC	dGdGdGdA	dGdAdTdG	dGdGdTdT
-1, α 2, α 3 and α 6	KWKA	SHHR	TANK	LSRR
Zinc finger alpha helix	KNWKLQAH	SLHHLTRH	TKANMKKH	LPSRLKRH

“Four of the residue positions on the helix N-terminus and exposed face are predicted to provide base specific ligands”. *Toby J. Gibson (1988)* [5].

Bibliography

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