Zinc fingers Nucleases (ZFNs)

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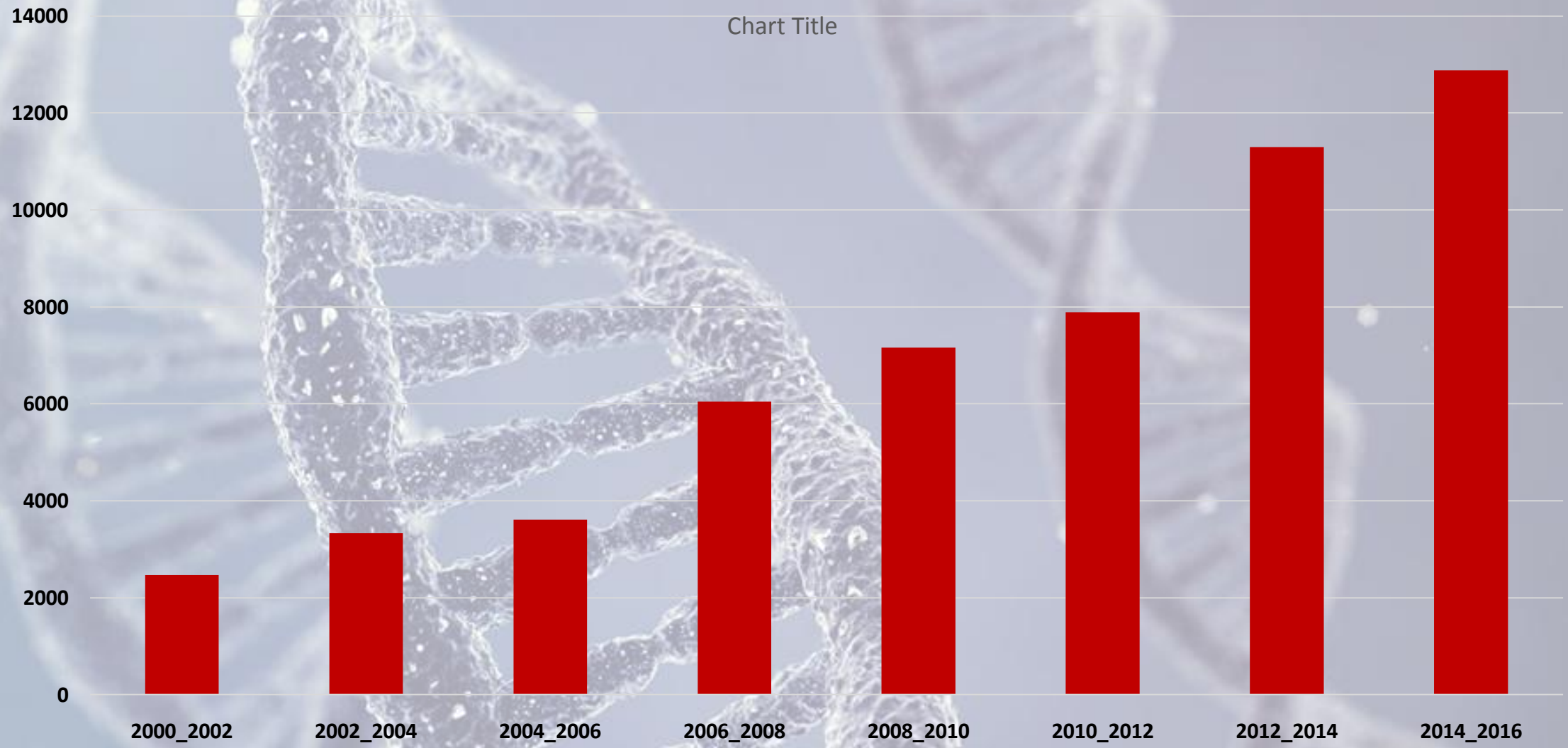
contents

- Introduction

*History

*Features

- Components
- Zf
- Application
- ZFN delivery
- Hydrodynamic delivery method
- Novel uses
- ZFNs &sicke cell anemia
- Cure
- conclusion



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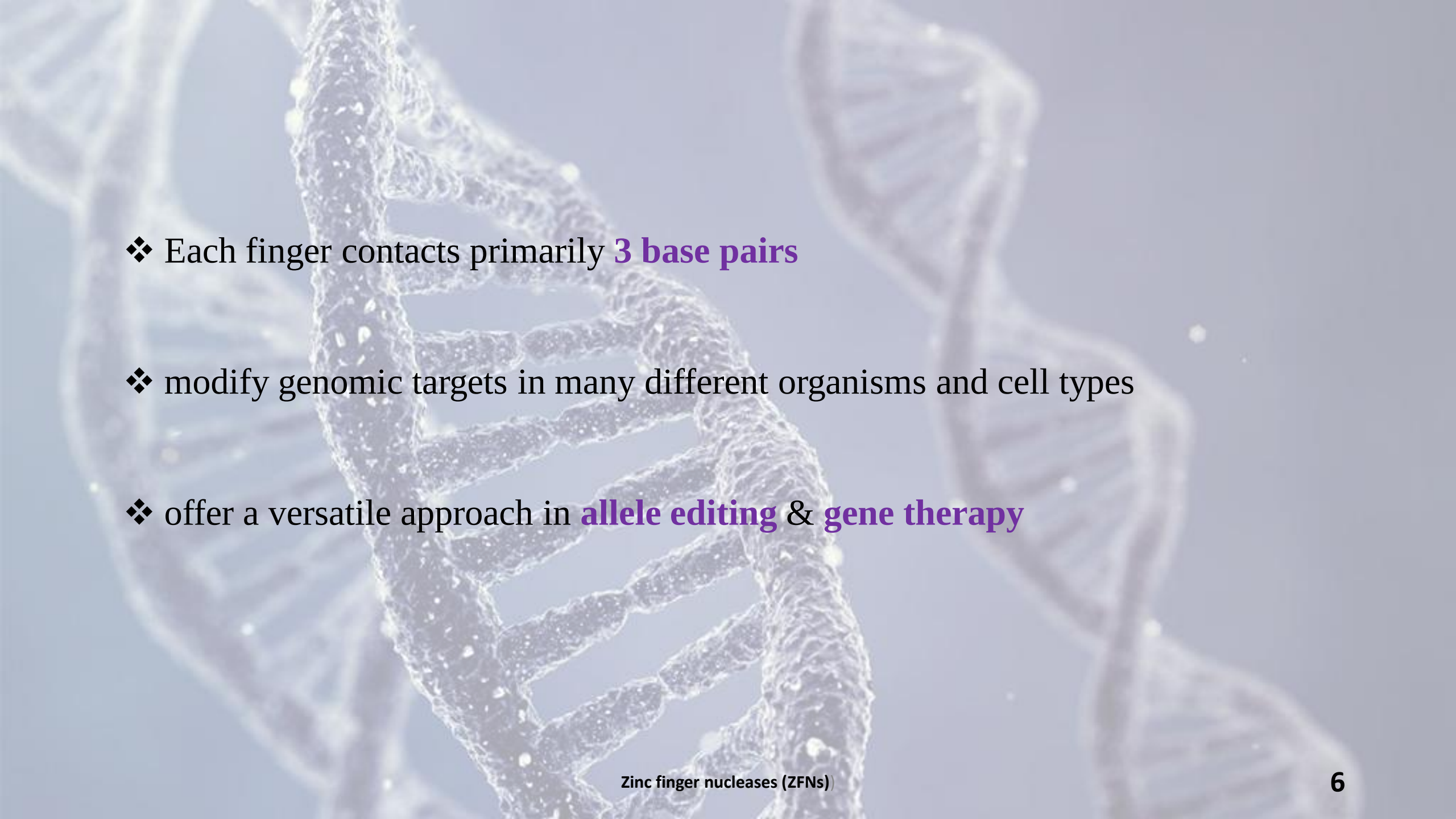
Zinc finger nucleases (ZFNs)

application

- **Diabete**
- **Cystic fiberosis**
- **Lych nyhan syndrome**
- **ALS(Amyotrophic Latera Sclerosis)**
- **Sickle cell anemia**
- **Glioblastoma**
- **Resistance to apoptosis**

Introduction (1) cont…….

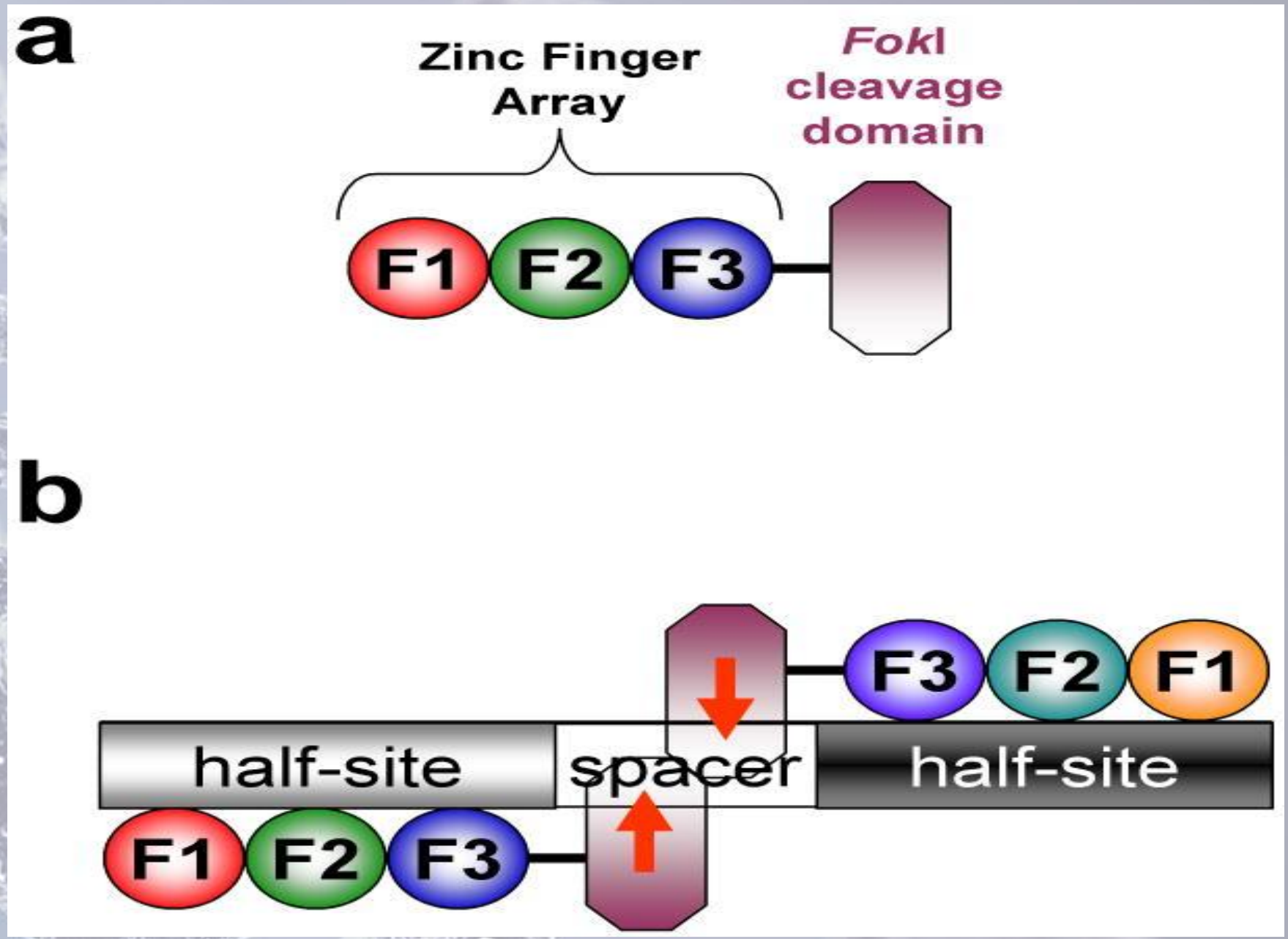
- ZFNS are engineered restriction enzymes designed to target specific DNA sequences within the genome
- They are **hybrid proteins**
- The cleavage domain must **dimerize** to be active



- Each finger contacts primarily **3 base pairs**
- modify genomic targets in many different organisms and cell types
- offer a versatile approach in **allele editing** & **gene therapy**

Structure of ZFNs

(https://www.google.com/)



Zinc finger nucleases (ZFNs)

Introduction(fok1) (1)

- Recognizes a 5bp sequences
- Cuts 9&13 bases away with no sequence specificity
- Cleavage redirected by nucleases

Components (3)

- 1.non.sequence.specific cleavage domain
- 2.DNA binding zinc-finger domain
- 3.Peptide linker

ZF(1)

- Discovered in transcription factor III A
- Specific DNA binding in Eukaryotic cells
- The binding domain alpha-helix into major Groove

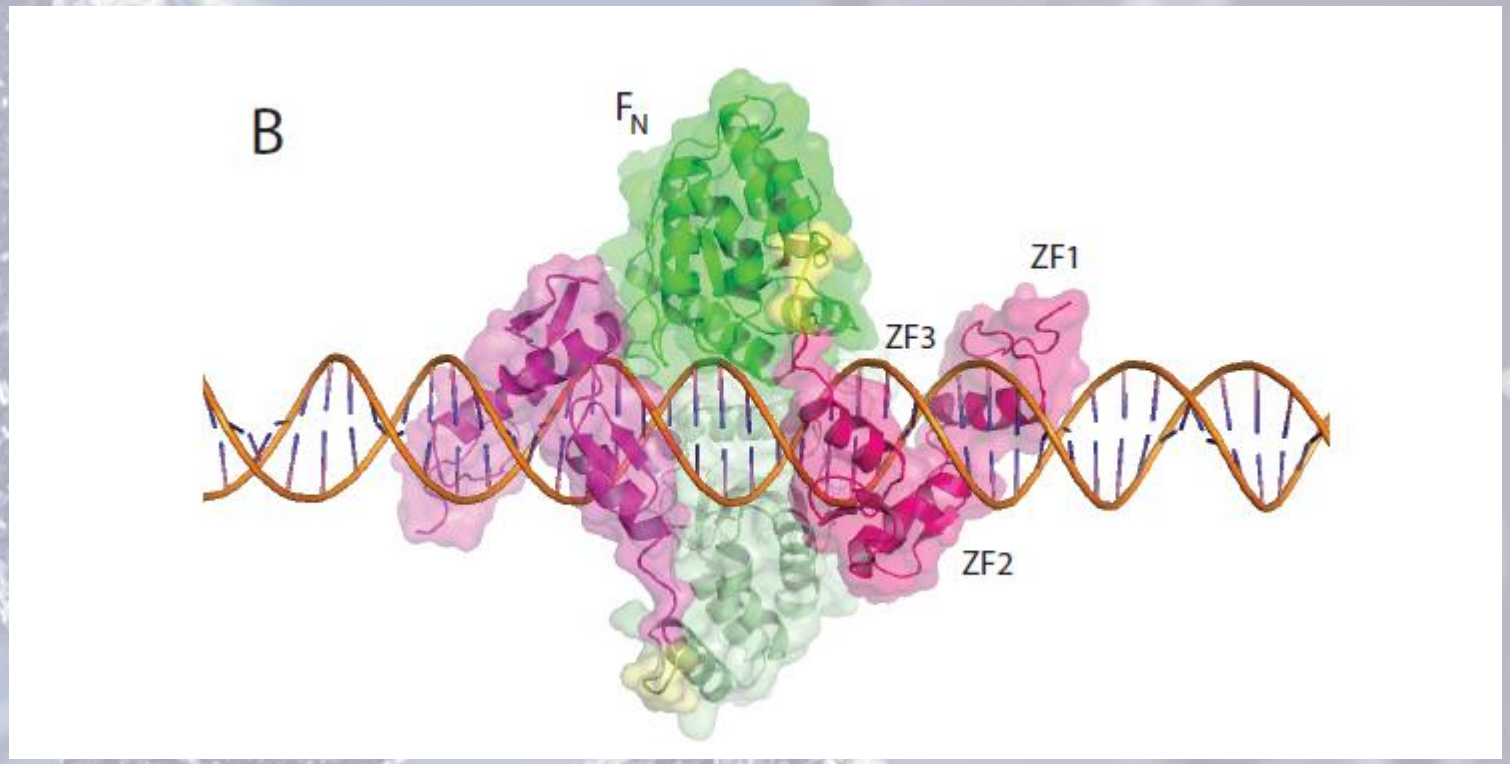
Zf (1)

- The C2h2 is the most common DNA binding domain

Higher number of zinc fingers increase specificity

GUO & colleagues :

subunit affinity more important than the number of fingers



3-dimensional model of a pair of ZFNs on DNA(1)

Functional domains attached to zinc finger proteins. This figure presents a summary of the functional domains that have been demonstrated
to be targeted to specific DNA regions by zinc finger proteins.(4)

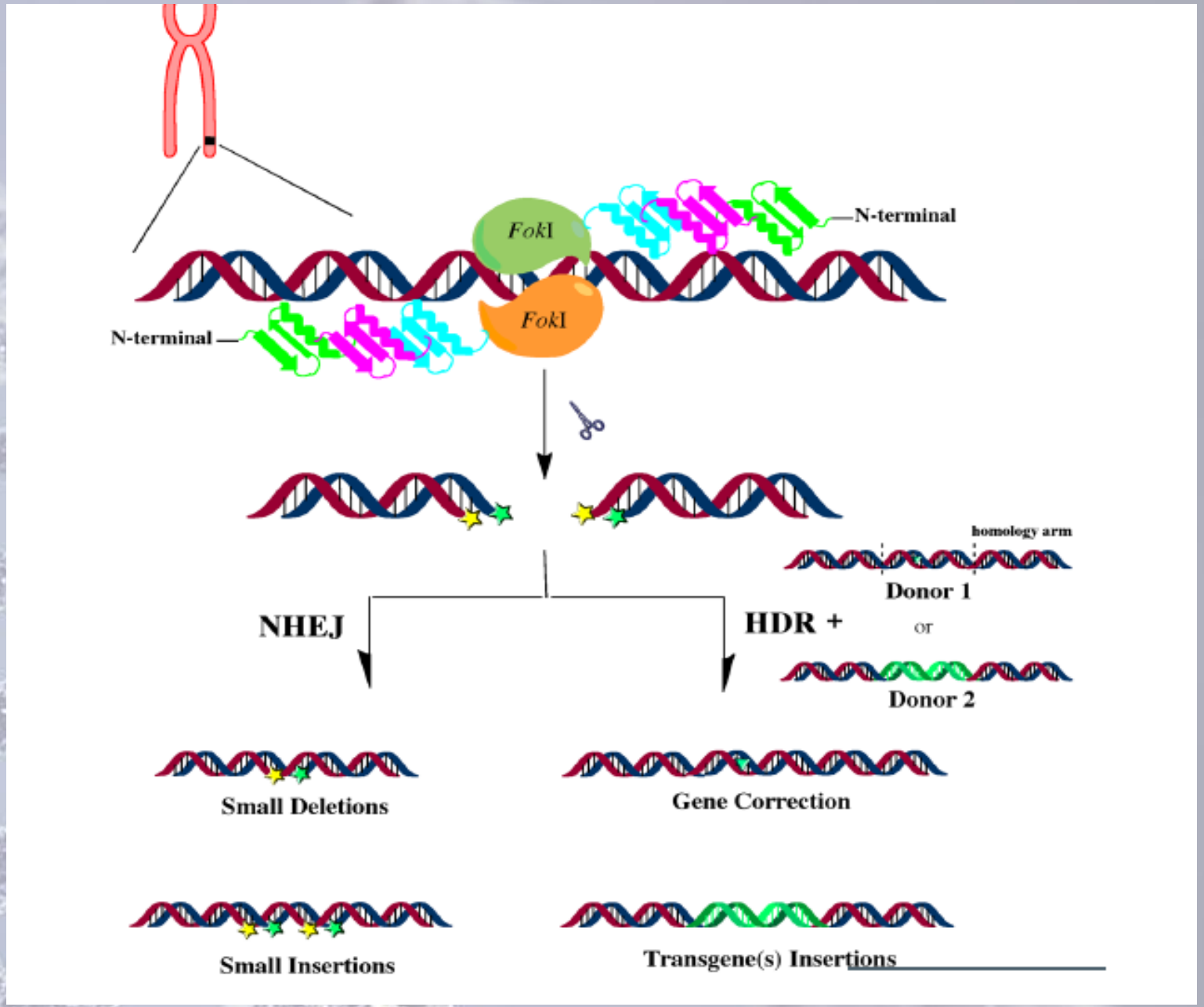
Fusion	Function
VP16[a]	Transcriptional activator
KRAB-A[b]	Transcriptional repressor
Progesterone Receptor and p65	Ligand dependent transcriptional activator
Protein methyl transferase (Suv39H1)[c]	Transcriptional repressor
DNA methyl transferase (M.SssI)[d]	Transcriptional repressor
DNA endonucleases (Fok1)[e]	DNA cleavage

Zinc finger nucleases (ZFNs)

Emerging of ZFNs (2)

- Efficient method for creating targeted genetic modifications have long been used
- using homologus recombination,the efficiency is extremely low!!
- ZFNS make **double strand** breaks at specific sequences

The components and mechanisms of Zinc Finger Nuclease (ZFN) (3)



History (1)

- is not deep
- The production & use of ZFNS represent the merging of several different research threads.
- Manipulation & application of ZFNS depend on advances in **molecular technology**

Application (2)

❖Correct the gene mutation in **oncogenes** and **tumor supressor** genes

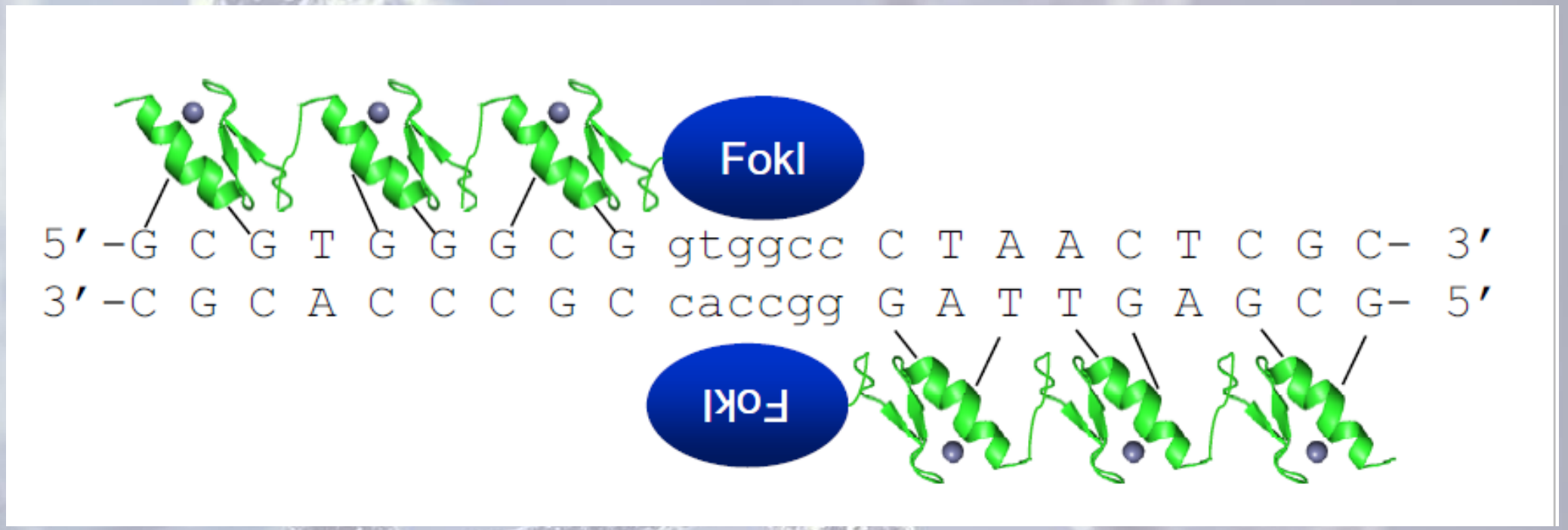
❖ Knockout of the gene for the HIV-1 coreseptor,ccr5

❖Genetic disease like **lesch-Nyhan** syndrome & **cystic fibrosis**

CF

Features (2)

- ZFNS have a number of beneficial characteristics
- Dimerization is require for cleavage
- A tremendous benefit for gene targeting as a monomer is not an active nuclease



heterodimerization of two independently designed ZFNs

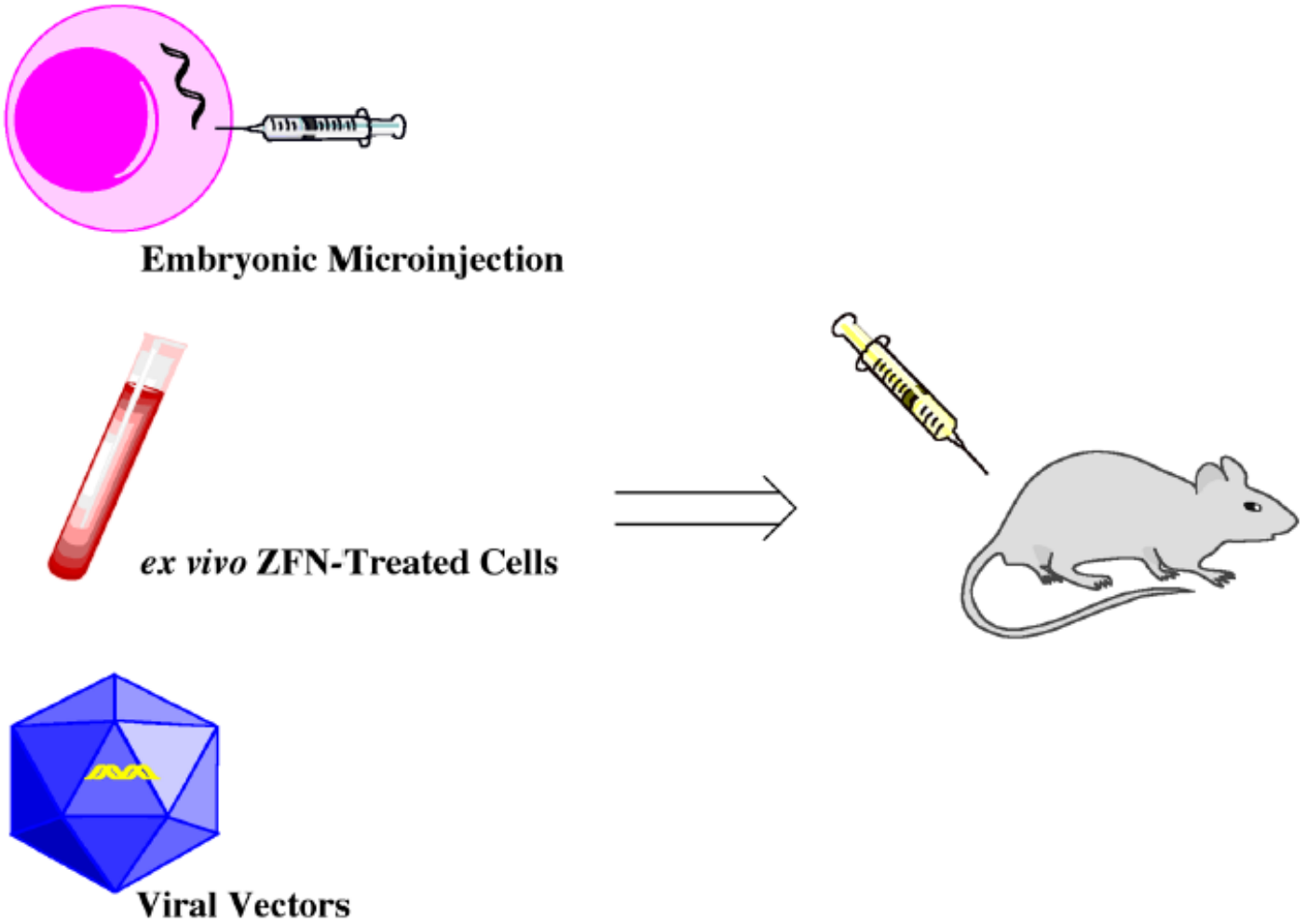
(4)

ZFN delivery: (3)

- An efficient transient transfection agent is required

- **Electroporation** has been widely used

- Several less frequently : adeno viruses,adeno associated viruses,lentiviruses & lipofectamine



(3)

Therapeutic applications of ZFN

ZFN-mediated Gene modification *In vitro*

Cell line	Target gene	Selection	Mechanism	Transfection
CHO cells	*DHFR*	2 + 2	NHEJ	Electroporation
	BAK/BAX	2 + 2	NHEJ	Electroporation
	DHFR/Gs/FUT8	2 + 2	NHEJ	Electroporation
	FUT8	2 + 2	NHEJ	Electroporation
	GS/BAK	2 + 2	HDR/NHEJ	Electroporation
HEK293	*IL2R- γ*	2 + 2	HDR	Lipofectamine/Electroporation
	βglobin/IL2R- γ/CD8	Modular assembly	HDR	Electroporation
	VEGF/HoxB13/CFTR	OPEN	NHEJ	Electroporation
	CCR5	2 + 2	NHEJ	Electroporation
	CCR5	Modular assembly	NHEJ	Lipofectamine
	erbB2/BCR-ABL/*HIV*§	Context	HDR	Calcium phosphate precipitation
K562	*IL2R- γ*	2 + 2	HDR	Lipofectamine/Electroporation
	VEGF/IL2R- γ	OPEN	NHEJ	Electroporation
	IL2R- γ	2 + 2	HDR	IDLV

ZFN-mediated Gene modification In Vitro(3) cont….

Human T cells	*IL2R- γ*	2 + 2	HDR	Electroporation
	CCR5	2 + 2	NHEJ	Electroporation
	CXCR4	2 + 2	NHEJ	Ad5/F35
Human lymphoblastoid cells	*IL2R- γ*	2 + 2	HDR	IDLV
Mouse ESC	*H3f3b*	2 + 2	HDR	Electroporation
Human ESCs	*IL2R- γCCR5*	2 + 2	HDR	IDLV
	OCT4/AAVS1	2 + 2	HDR	Electroporation
	PIG-A	OPEN	HDR	Electroporation
	CCR5	2 + 2	NHEJ	Electroporation
Human iPSCs	*PITX3*	2 + 2	HDR	Electroporation
	PIG-A	OPEN	HDR	Electroporation
	AAVS1	2 + 2	HDR	Electroporation
	βglobin	OPEN	HDR	Electroporation

(3)

In vivo				
Organism	**Target gene**	**Selection**	**Mechanism**	**ZFN Delivery (Treatment)**
Drosophila	*yellow*	Modular assembly	NHEJ	Embryonic microinjection
	yellow	Modular assembly	HDR	Embryonic microinjection
	rosy/brown	Modular assembly	NHEJ/HDR	Embryonic microinjection
	coil/pask	Modular assembly	NHEJ/HDR	Embryonic microinjection
Zebrafish	*kdr*	Context	NHEJ	Embryonic microinjection
	gol/ntl	2 + 2	NHEJ	Embryonic microinjection
	tfr2/dat/telom erase/hif1aa/g ridlock	OPEN	NHEJ	Embryonic microinjection
	actn1¶	CoDA	NHEJ	Embryonic microinjection
Rats	*IgM/Rab38*	2 + 2	NHEJ	Embryonic microinjection
	IL2R- y	2 + 2	NHEJ	Embryonic microinjection
	Mdr1a/PXR	2 + 2	HDR	Embryonic microinjection

(3)

ZFNs delivery: (1)

- Hydrodynamic delivery method Efficient for delivery to **liver**
- Non-viral methods may not currently be successful for **invivo** application
- RNAi therapies
- Dual targeting

Novel uses (4)

- Phase 2 trials for diabetic &ALS(Amyotrophic lateral sclerosis)
- Targeted HIV co-receptor CCR5
- AS a therapy for Glioblastoma

Novel uses

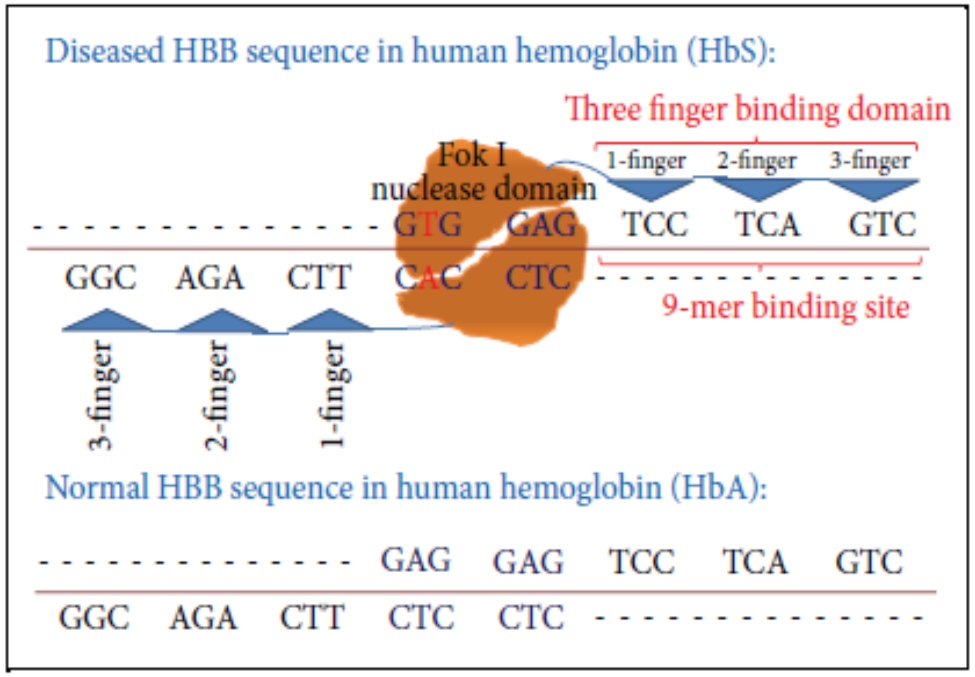
- Modify the oct4 locus in stem-cells
- Delete Bax and Bak from CHO cells
- Making resistance to **apoptosis**

Zfns & sickle cell anemia (4)

- *off-target* binding with unacceptable side effects was a problem
- Limited cytotoxic effects in engineered zinc fingers
- Limited understanding cause slow progress

cure

- Ips repaired by a healthy HBB(hemoglobin) gene
- Cutting gene at the specific location
- Introducing a healthy donor gene



Mutated HBB diseased gene. Normal HbA targetsequence versus single point mutation of diseased HbS gene& target sequence of a three-finger binding domain (1)

Curing with zfns

- zfns bind to the specific DNA sequences
- Two nuclease domains at the same location on opposite strands
- Successfully in mouse models

Increase specificity with sp1

- Ubiquitous transcription factors
- Exchange of amino acids in alpha helical region of the 2nd finger of sp1
- The EMSA-assay show significant changes in the bindimg

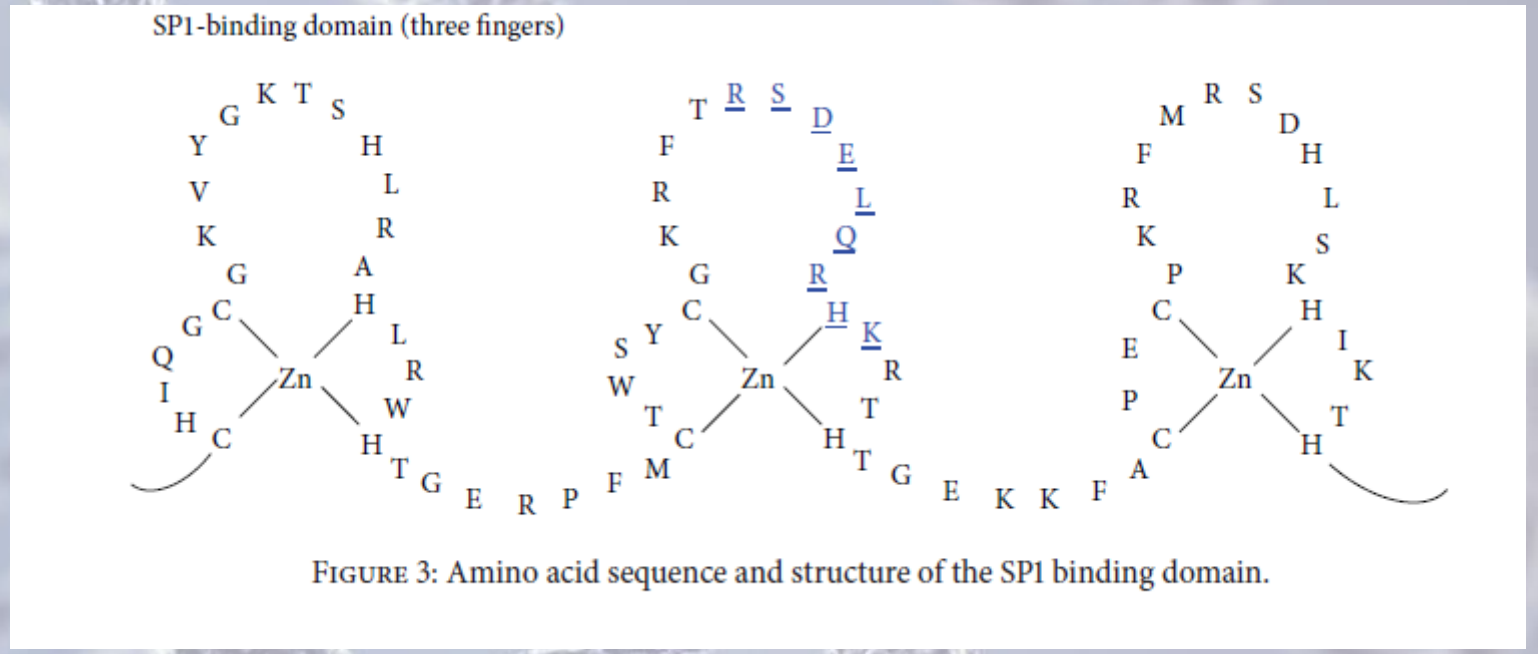


FIGURE 3: Amino acid sequence and structure of the SP1 binding domain.

(1)

Table 1: List of exchanged amino acids in 2nd finger of SP1.

2nd finger	Amino acids in alpha helical region
SP1 (wild type)	R S D E L K R H K
	Exchanged Amino Acids
CB1	H S S R L I R H E
MR14	R S S T L I Q H K
MQ91	Q S S Y L I K H K
MQ135	Q S S H L I Q H K
MQ151	Q S S Y L T Q H K

(1)

Conclusion(3)

❖Low frequency of homologus recomibination in cells

❖The proficiency of precise gene modification,bolestered using of them

❖In contrast to ***RNAi*** methods cant be readily used by many lab

Conclusion(3)

- Safe and robust viral and non-viral vectors desirable for ***in vivo*** use
- Enable their use on less accessible target cells
- Development of improved screening for ***off target*** effect and ***potential toxicity***

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