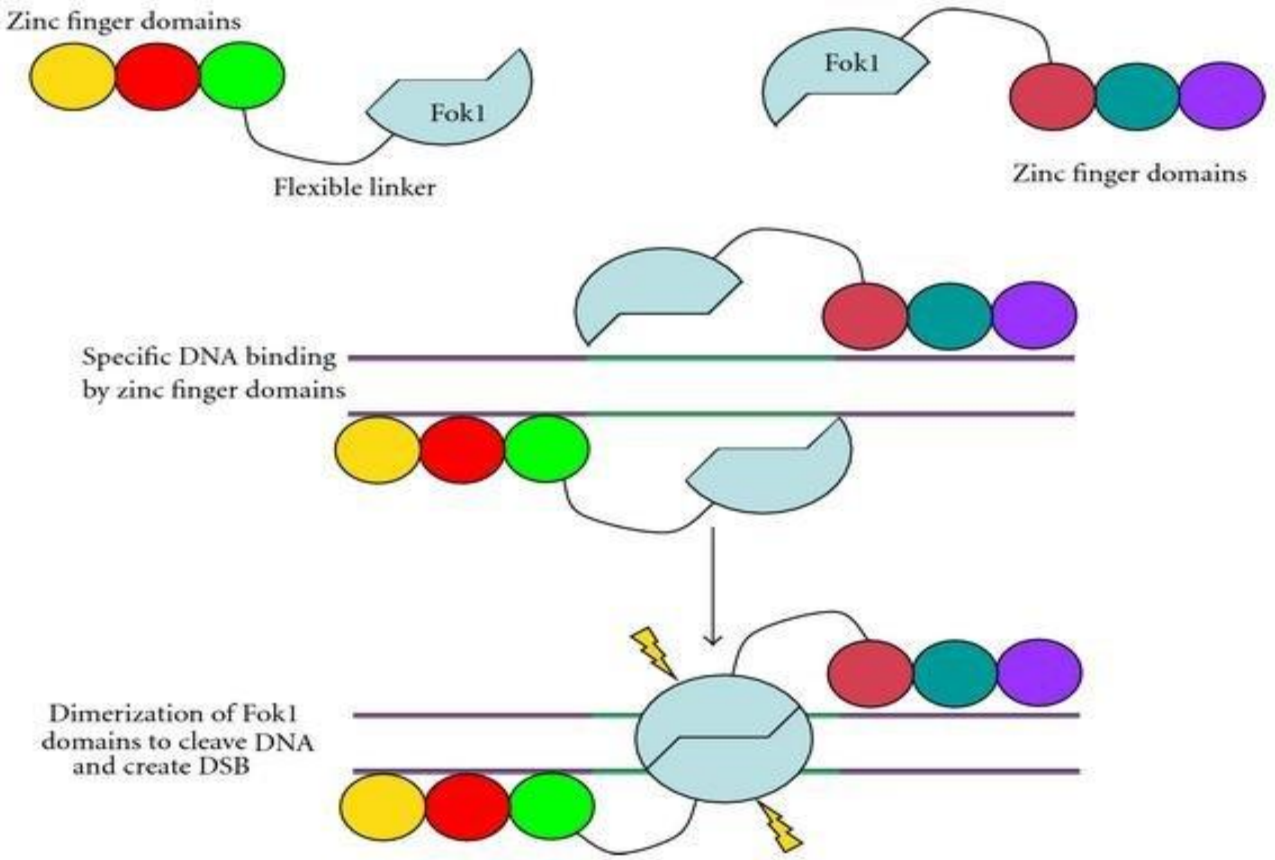
# Manipulation of DNA- 3

# Artificial Restriction Enzymes

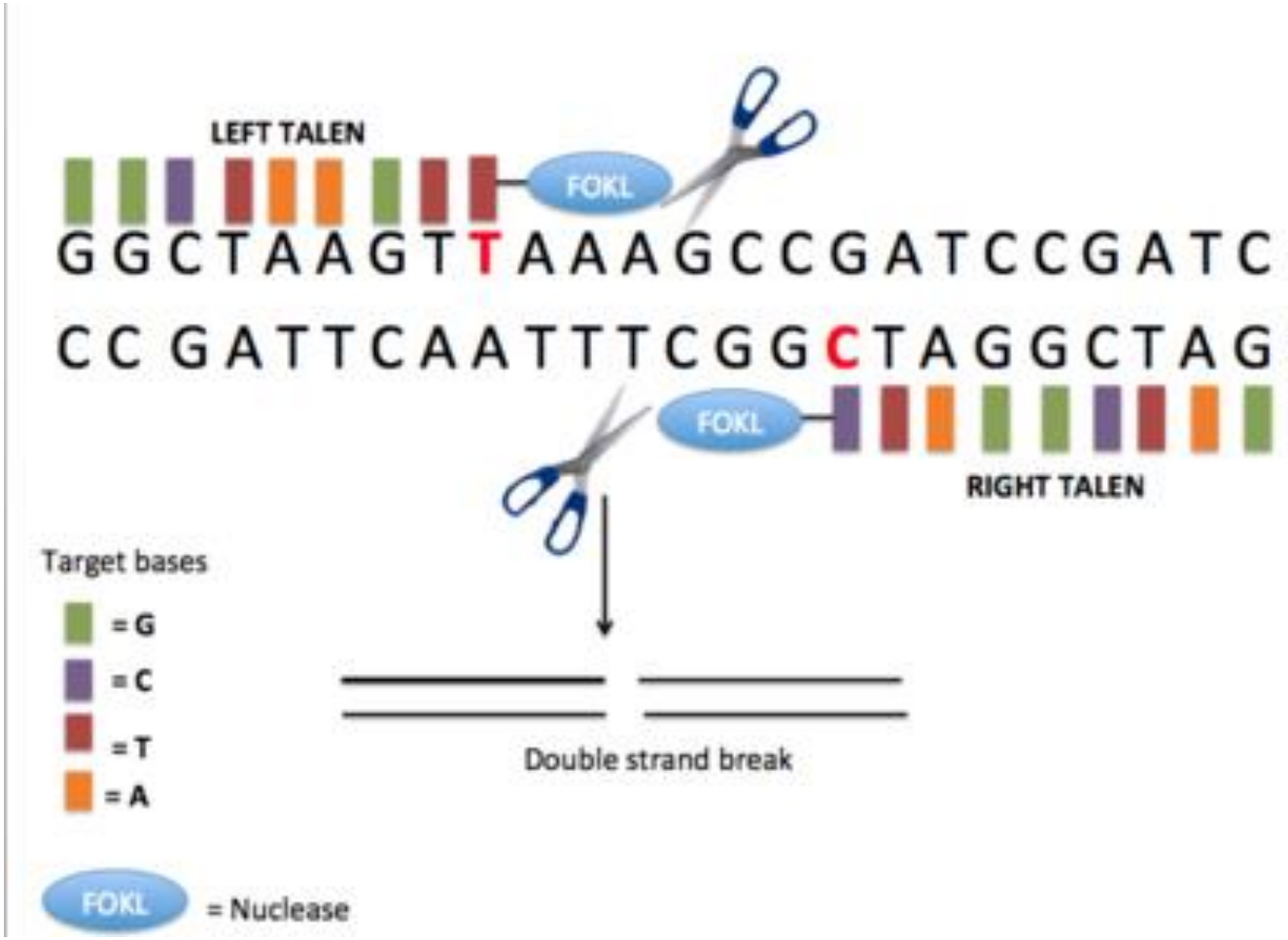
# ZINC FINGER NUCLEASES




Basic structure and design of a zinc finger nuclease (ZFN). ZFNs are created by joining a DNA-binding region to the catalytic domain of the nonspecific Fok1 endonuclease. Zinc fingers are a protein motif capable of DNA binding, whose sequence specificity can be predetermined. Each zinc finger, illustrated by an individual circle, recognizes 3-4 nucleotides, and, by assembling three or four suitable zinc finger motifs, a sequence-specific DNA-binding domain can be created. Fok1 nuclease activity requires dimerization, and so the customized ZFNs function in pairs. As shown, the zinc finger-binding domain brings two Fok1 units together in the right orientation over the target sequence; this induces Fok1 dimerization and target sequence cleavage.

# Transcription Activator-like Effector Nucleases (TALENs)

Transcription activator-like effectors (TALEs) are DNA-binding domains that can be linked together modularly and fused with nuclease domains to create TALE nucleases, or TALENs



# Pioneers of revolutionary CRISPR gene editing win chemistry Nobel

Emmanuelle Charpentier and Jennifer Doudna share the award for developing the precise genome-editing technology.

Heidi Ledford & Ewen Callaway

Jennifer Doudna and Emmanuelle Charpentier share the 2020 Nobel chemistry prize for their discovery of a game-changing gene-editing technique. Credit: Alexander Heinel/Picture Alliance/DPA

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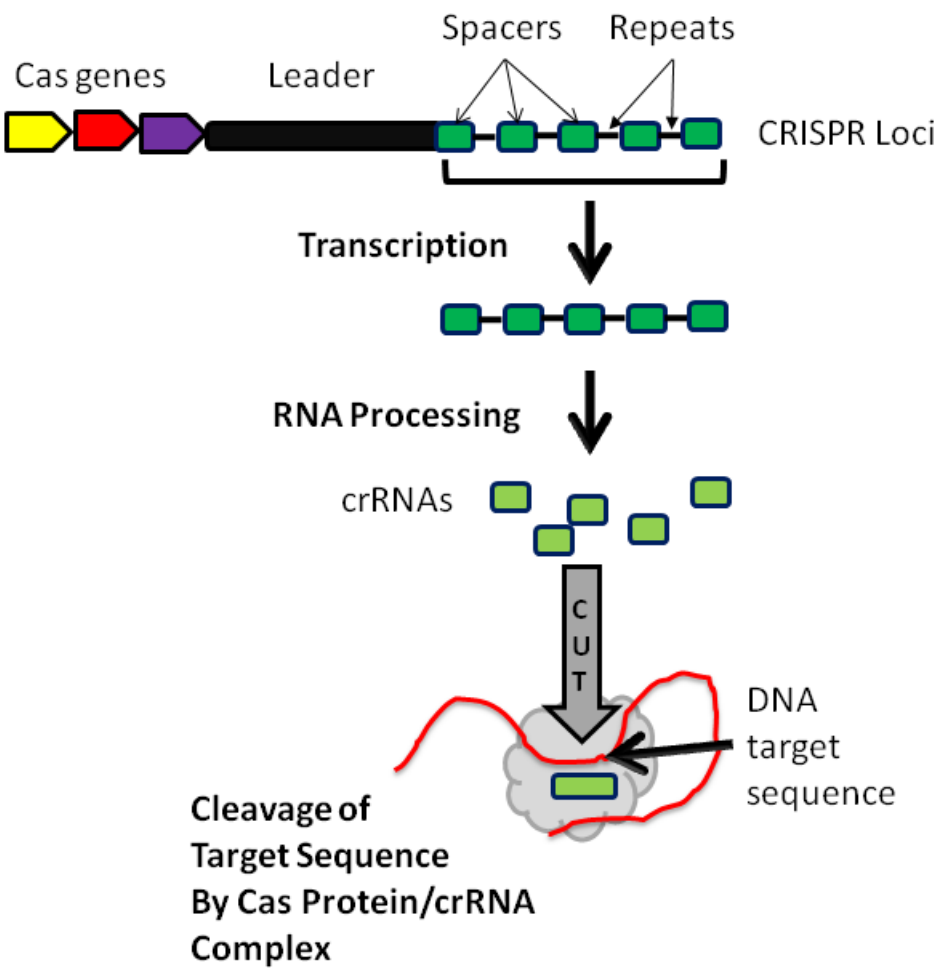
https://www.nature.com/articles/d41586-020-02765-9

**CRISPR (clustered regularly interspaced short palindromic repeats)** is a family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria and archaea. These sequences are derived from DNA fragments of bacteriophages that had previously infected the prokaryote




The CRISPR loci include Cas genes, a leader sequence, and several spacer sequences (in green) derived from engineered or foreign DNA that are separated by short repeat sequences (in black). Individual mature CRISPR RNA (crRNAs) are created by processing of the CRISPR transcript. Cas proteins and the crRNA make an effector complex that recognizes a target nucleic acid sequence via complementary base pairing to the crRNA. Cleavage of the target sequence occurs and is followed by DNA repair by the endogenous cellular repair machinery.

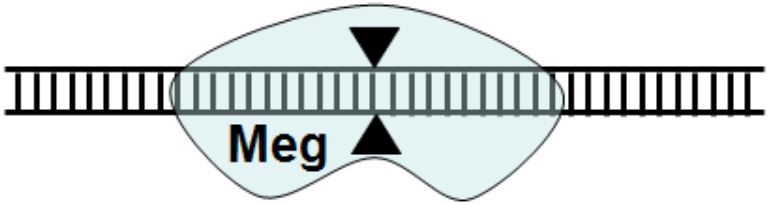
**For Zinc Finger Nuclease**
https://www.youtube.com/watch?v=iGmq1O6mIy0

**For transcriptional activator-like effector nucleases (TALENs)**
https://www.thermofisher.com/in/en/home/life-science/genome-editing/geneart-tals.html

**For CRISPR**
https://www.youtube.com/watch?v=MnYppmstxIs

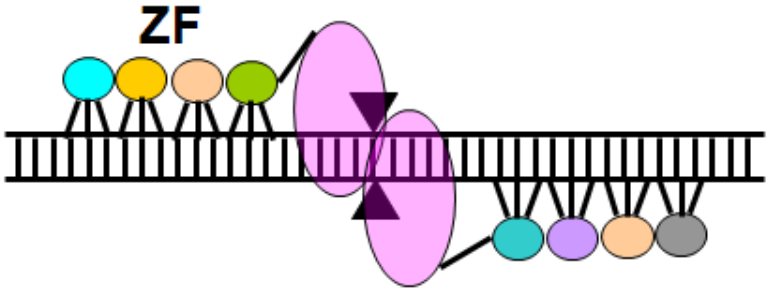
https://www.livescience.com/58790-crispr-explained.html

**For ethical concerns read this news**
https://www.sciencemag.org/news/2019/12/chinese-scientist-who-produced-genetically-altered-babies-sentenced-3-years-jail



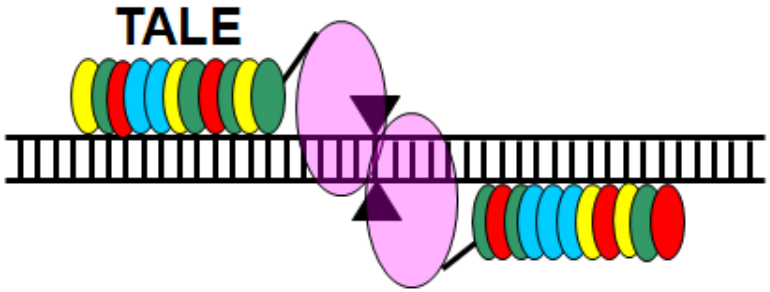

Meganuclease

20-40 bp/Enzyme



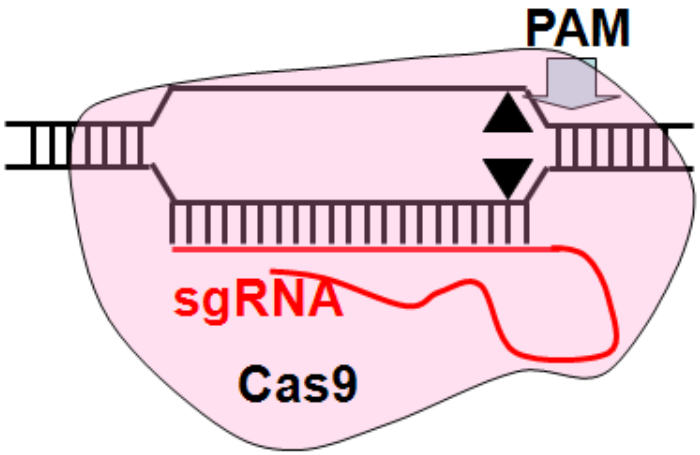

ZFN

3 bp/Finger



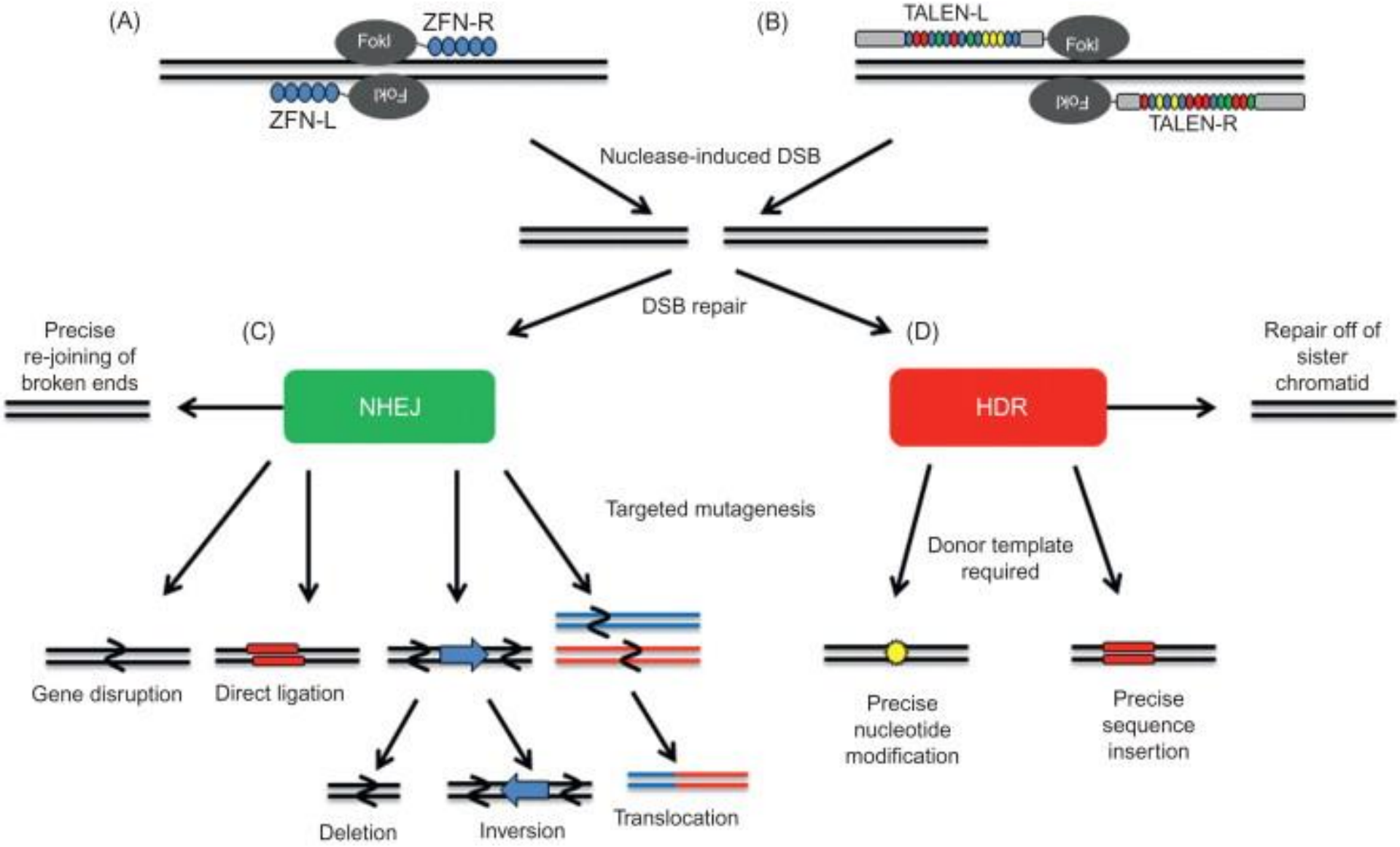

TALEN

1 bp/Module




CRISPR-Cas

1 bp/Base


(A)
ZFN-R
FokI
FokI
ZFN-L
(B)
TALEN-L
FokI
FokI
TALEN-R
Nuclease-induced DSB
DSB repair
Precise re-joining of broken ends
(C)
NHEJ
(D)
HDR
Repair off of sister chromatid
Targeted mutagenesis
Donor template required
Gene disruption
Direct ligation
Deletion
Inversion
Translocation
Precise nucleotide modification
Precise sequence insertion