The potential of genome editing to strengthen local food production.

FSN Webinar
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Genetic diversity: source for trait improvement

- Natural variations

Genome is dynamic and fluid

Modern strawberries never existed in nature

Fragaria chiloensis
Chile

Fragaria virginiana
Eastern North America

X

Fragaria ananassa
Europe, 1740's

Gene transfer, 1950's style

Ln-9 *gene for leaf rust resistance from* Aegilops *to wheat*

Need to move gene from grass to wheat

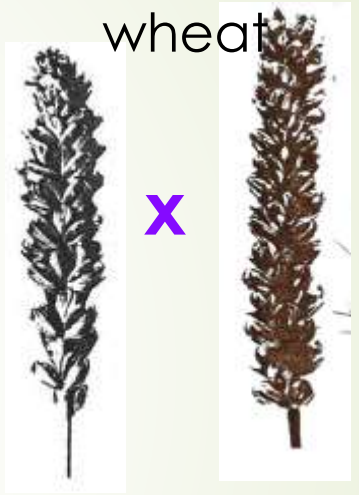
Grass with trait

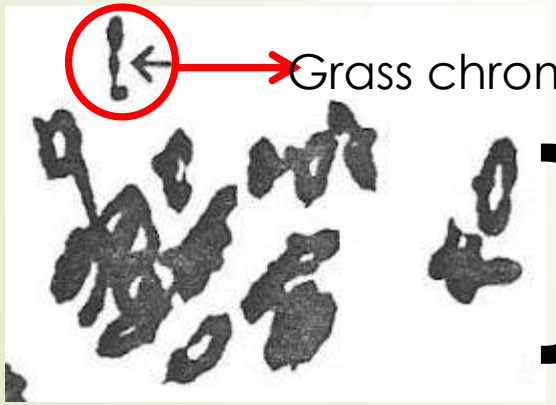
Wheat

Problem: Grass will not cross with wheat

So cross with wheat relative

Cross hybrid repeatedly to wheat





Grass chromosome with desired gene

- Break grass chromosome with X-Rays
- Let pieces integrate into wheat
- Get wanted gene + many unknown

Wheat chromosomes

Final product

Sears, E.R. 1956. The transfer of leaf-rust resistance form *Aegilops umbellulata to wheat. Genetics in Plant Breeding Brookhaven Symposium #9*.

Mutation breeding

> **3200 known varieties** developed from mutation breeding

- FAO/IAEA database (http://www-infocris.iaea.org/MVD/)

DNA changes

- 4 bp to 8 kb deletions
- Inversions of up to 1.5 kb
- Insertions ~200 bp
- Frame-shift mutations
- Premature stop codons

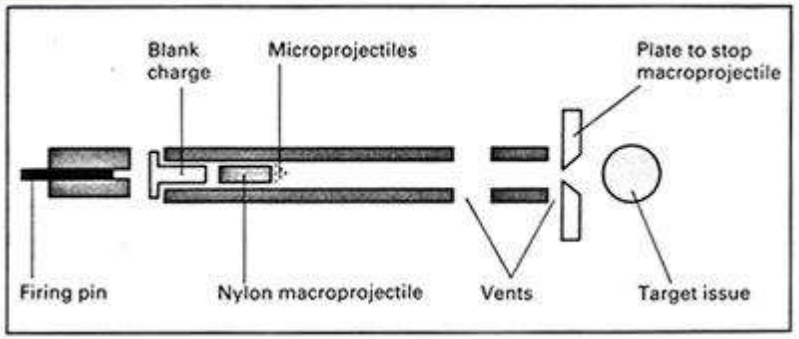
Institute of Radiation Breeding
Ibaraki-ken, JAPAN
www.irb.affrc.go.jp/

Gene transfer, 1950's style

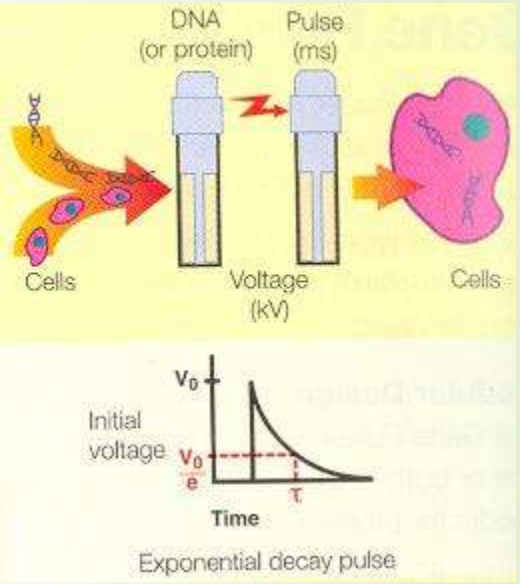
- **Things to notice**
  - Move many thousands of genes
  - No one knows what genes got moved / mutated
  - No regulations

1980s: Direct DNA transfer

Introduce DNA directly into cells through chemicals, electroporation, particle bombardment or a bacterium called Agrobacterium



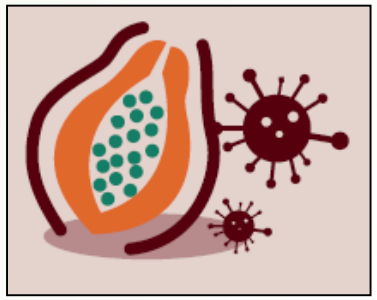
Original biolistic gun – a modified 22 caliber (J. Sanford & T. Klein, 1988).

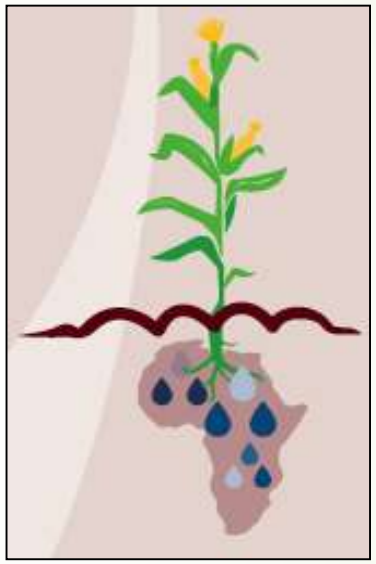


- **Things to notice**
  - Move a few, well characterized genes
  - Regulations

Research delivers biotech solutions

Virus resistant **papaya**

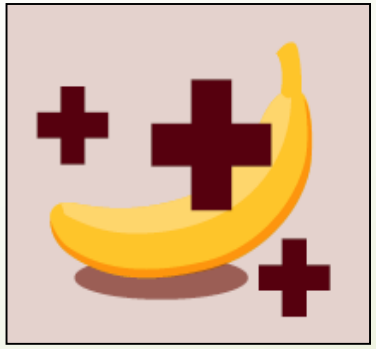




Drought-tolerant maize

Gluten-free wheat

Golden rice (enriched in β-carotene)



Bananas enriched in vitamin A

Genome editing

A genetic manipulation approach in which DNA is inserted, removed or replaced **at a precise location** within the genome.

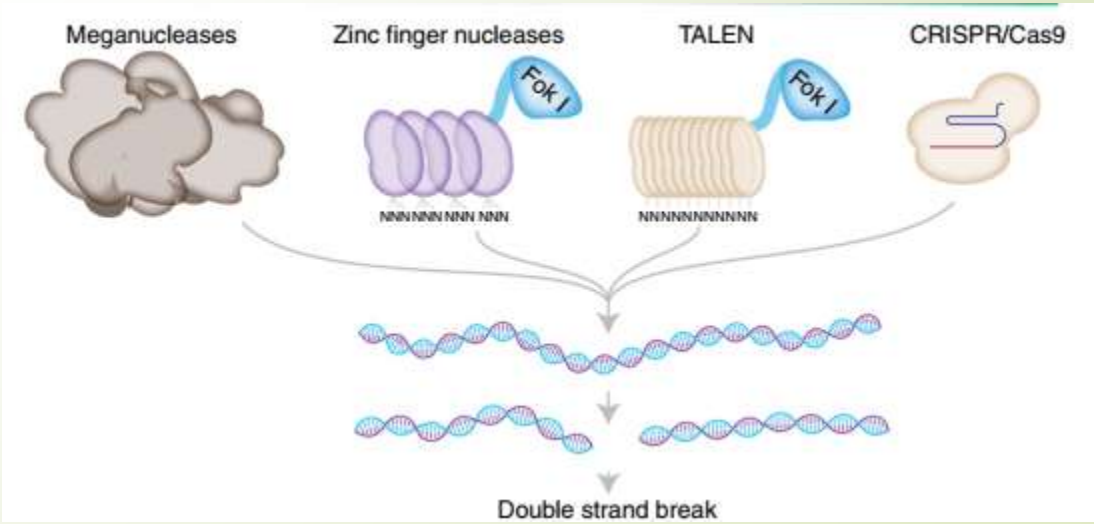
Genome editing tools

Based on protein – DNA binding specificity:

- **Meganucleases** are engineered restriction enzymes that recognize long stretches of DNA sequences.
- **Zinc Finger Nucleases (ZFNs)** – engineered DNA binding proteins made up of a chain of two-finger modules, each recognizing a unique hexamer (6 bp) sequence of DNA.
- **T**ranscription **A**ctivator-**L**ike **E**ffectors bound **N**ucleases **(TALENs) -** each TALE recognizes an individual base.

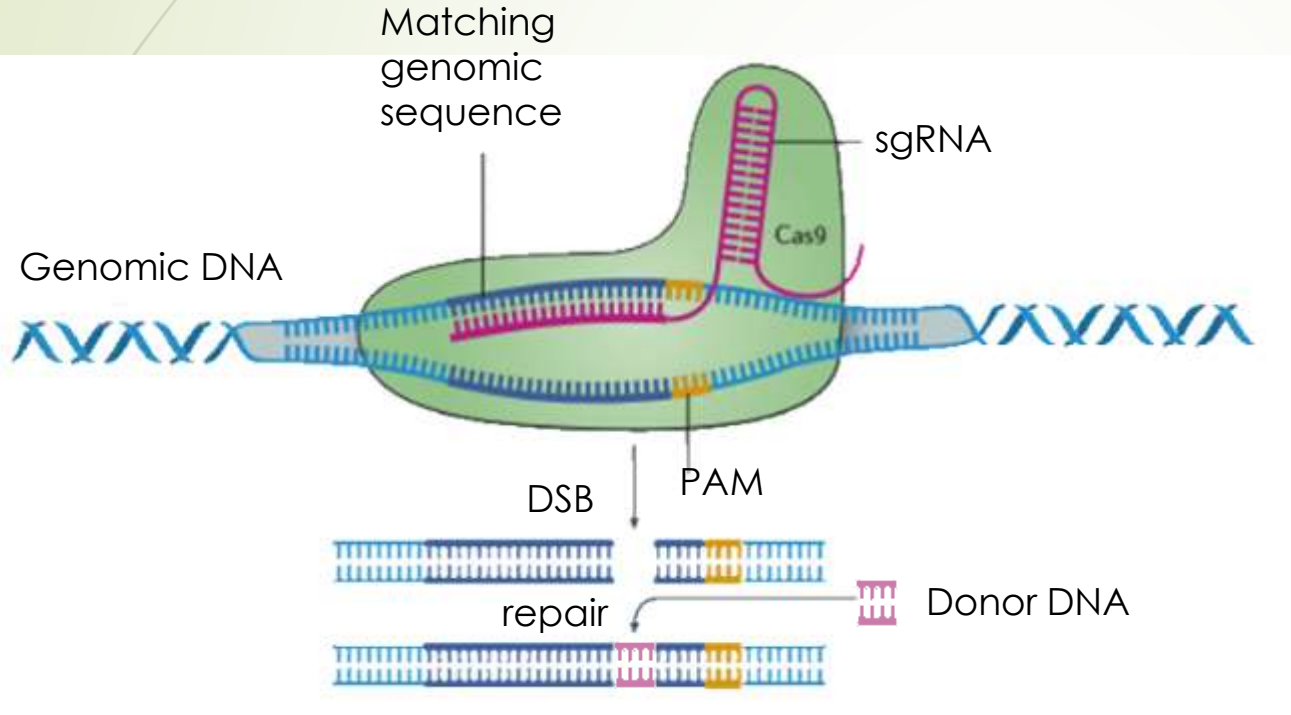
Based on RNA – DNA binding specificity:

- CRISPR-Cas9
- CRISPR-Cpf1 (now CRISPR-Cas12)
- ……

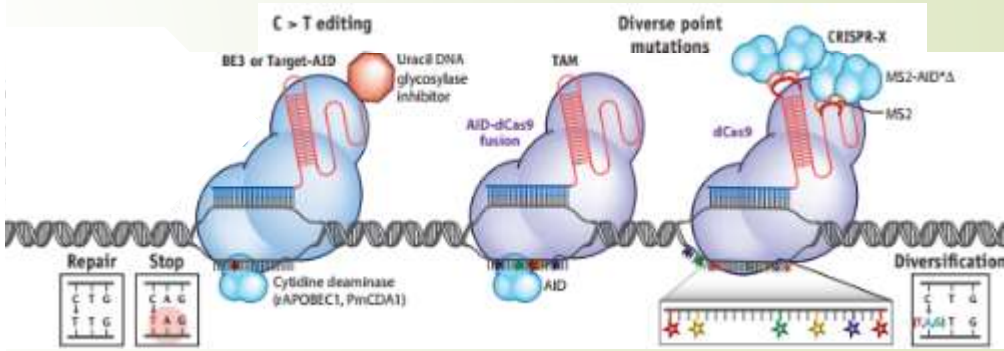


Targeted genome editing

CRISPR-Cas9

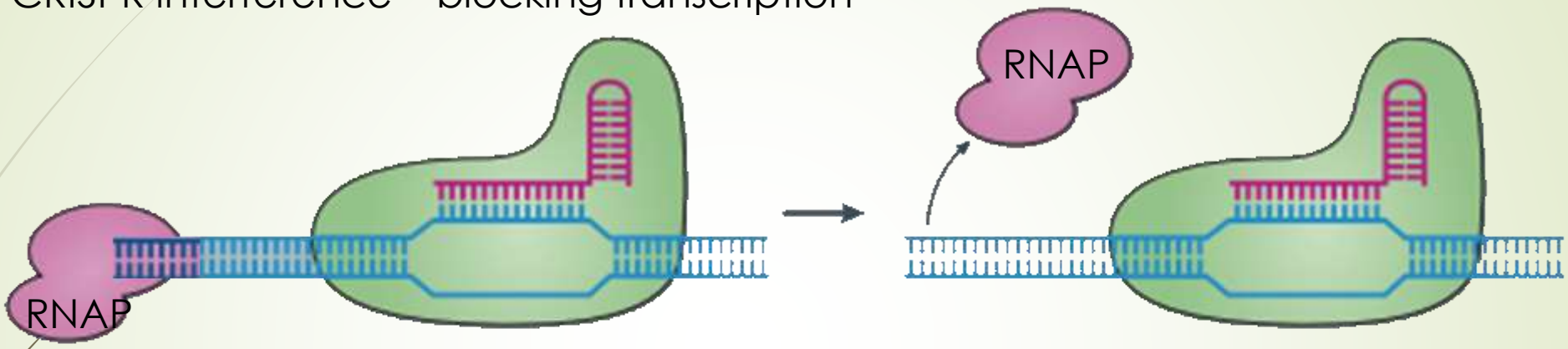


Base editing

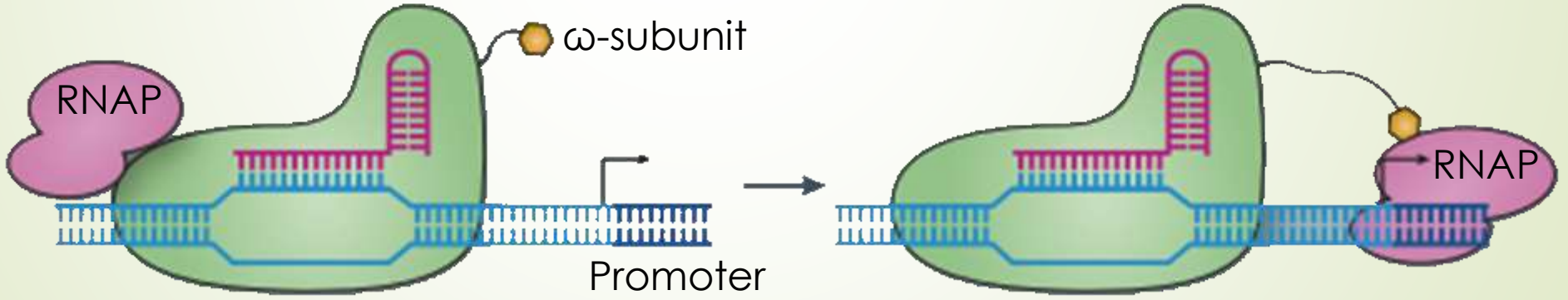


Gene regulation

CRISPR interference – blocking transcription



CRISPR-mediated gene activation – promoting transcription



The potential of genome editing to strengthen local food production – Example I

Disease resistance in local malting barley

The problem – net blotch disease

- no local resistant varieties

Potential solution

- Obtained resistant/tolerant genotypes from NORGENE and added to our core collection
- GWAS for resistance loci
- Planned gene editing to introduce identified resistant loci in BG genotypes

The potential of genome editing to strengthen local food production – Example II

Improved antioxidant contents in local tomato varieties

The problem

- Lower storage capacity of local varieties thus producing homogenous lots is problematic – sold mostly at farmers markets; difficult brand development
- Increasing demand for local varieties, especially if they can claim added health benefits

Proposed solution

- Perform metabolomics study of markedly different local varieties (yellow, red, pink, blue)
- Identify loci responsible for increased antioxidant content (carotenoids, polyphenolics, etc.)
- Pyramid various antioxidants into improved genotype(s) by genome editing

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