Johns Hopkins Engineering

Molecular Biology

Posttranslational Processing and Genetic Engineering Tools

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Outline

- Posttranslational processing
- Protein sorting and targeting
  - The signal hypothesis
- Genetic Engineering
  - CRISPR/Cas9
  - Gene Drives

Posttranslational Processing

- After polypeptide chains are synthesized, they often must undergo **posttranslational modification** before they can perform their functions

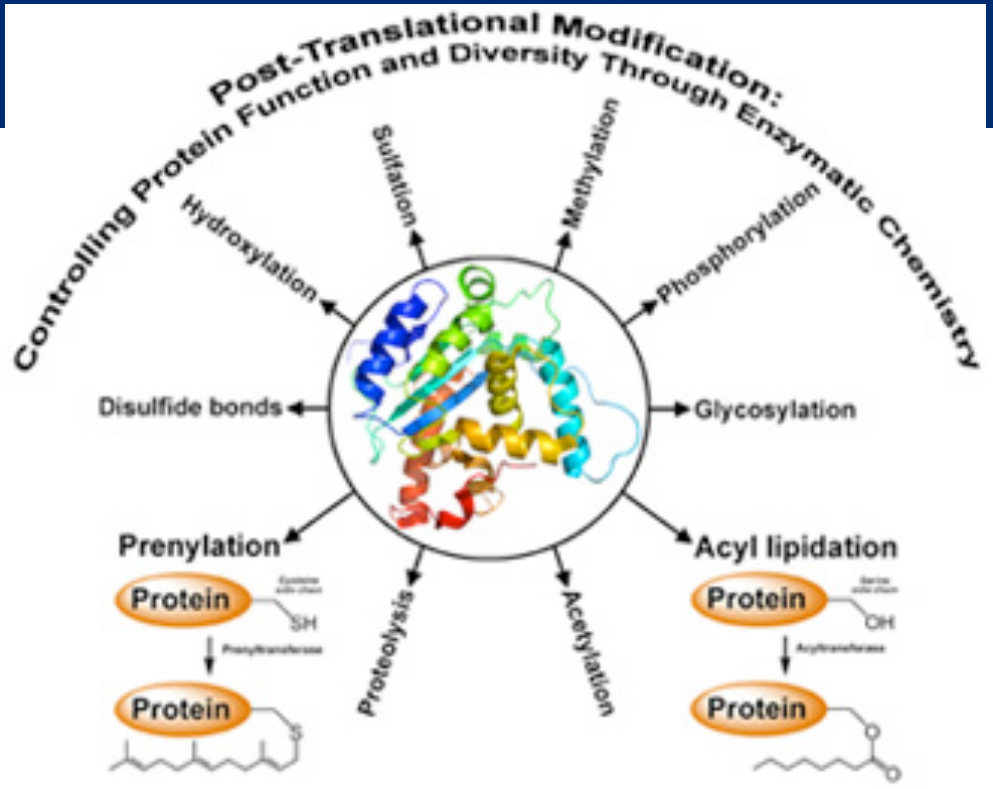
- In bacteria, the N-formyl methionine at the N-terminus is removed

- In eukaryotes, the methionine at the N-terminus is released

Posttranslational processing (continued)

- Sometimes whole blocks of amino acids are removed from the polypeptide, for instance certain enzymes synthesized as inactive precursors
- These are activated by removal of sequences from one end of the protein
- Transport of proteins across membranes may require removal of a **signal sequence** and some have internal amino acids that must be removed (e.g. insulin)

Other common processing events include chemical modification of amino acids—methylation, phosphorylation, acetylation



http://asfaculty.syr.edu/pages/chem/Hougland-James.html

Translation is mainly cytosolic

- Most polypeptide synthesis (~90%) takes place in the cytosol with transcripts leaving the nucleus through nuclear pores and associating with *free ribosomes*

- Shortly after translation begins, two pathways for routing the protein products diverge

Protein Targeting and Sorting

- As proteins are synthesized, they must be sorted and directed to their final locations
- The compartments of eukaryotic cells can be divided into three categories: 1) the endomembrane system (ER, Golgi, endosomes, & lysosomes); 2) the cytosol; and 3) the mitochondria, chloroplasts, and peroxisomes and interior of the nucleus
- Polypeptides are routed to compartments via several mechanisms

Cotranslational import

- The first pathway is utilized by ribosomes synthesizing polypeptides destined for the endomembrane system or for export from the cell

- These ribosomes become attached to ER membranes early in translation, and polypeptide chains are transferred across the ER membrane as synthesis takes place

- This is called **cotranslational import**
  - Movement of the polypeptide across the ER membrane is directly coupled to the translational process (trafficking from the ER to final destination occurs via vesicles or the Golgi)

The signal hypothesis

- The *signal hypothesis*, proposes that proteins that move into the ER during synthesis possess an intrinsic signal, directing them to the ER
- The **ER signal sequence** directs the mRNA polypeptide complex to the rough ER surface
- It is now established that only polypeptides with ER signal sequences can be inserted into or across the ER membrane as their synthesis proceeds
  - This has been demonstrated with recombinant DNA methods, where adding a false ER signal sequence results in the polypeptides being directed to the ER

Protein Folding and Quality Control Take Place Within the ER

- After polypeptides are released in the ER lumen, they fold into their final shape
- Protein folding is often accompanied by formation of disulfide bonds
- Proteins that repeatedly fail to fold properly activate various quality control mechanisms
  - **unfolded protein response** (**UPR**), in which sensor molecules in the ER lumen detect the misfolded proteins
  - **ER-associated degradation** (**ERAD**) mechanism recognizes misfolded or unassembled proteins and exports them to the cytosol where they are degraded *proteasomes*

Proteins Released into the ER Lumen Are Routed to the Golgi Complex, Secretory Vesicles, Lysosomes, or Back to the ER

- Most proteins synthesized on rough ER are *glycoproteins*
- The initial glycosylation takes place in the ER as the polypeptide is being synthesized
- In the Golgi complex, further glycosylation and processing of carbohydrate side chains occurs, and the proteins are sorted and distributed to other locations

Soluble proteins

- Soluble proteins move from the Golgi complex to secretory vesicles for secretion from the cell

- Those that are not destined for secretion have specific side chains or signal sequences that target them to destinations within the endomembrane system

  - e.g., many lysosomal enzymes have side chains with *mannose-6-phosphate*

Post-translational Import Allows Some Polypeptides to Enter Organelles After They Have Been Synthesized

- Proteins destined for the nuclear interior, mitochondrion, chloroplast, or peroxisome are imported into these organelles after completion of translation
- These are synthesized on free ribosomes and released into the cytosol
- Each protein released to the cytosol has localization signals specific to the destination
  - e.g. import into the nucleus requires *nuclear localization signals* that target proteins for transport through nuclear pores

Summary video: *The Inner Life of a Cell*, an eight-minute animation created in NewTek LightWave 3D and Adobe After Effects for Harvard biology students

https://www.youtube.com/watch?v=FzcTgrxMzZk

- Leukocyte responding to an an environmental signal
- Extracellular matrix (ECM)
- Lipid rafts
- Cholesterol molecules
- Secreted chemokines
- 7 transmembrane receptors
- Integral membrane proteins
- Cytoskeleton
- Microvilli/actin
- Actin polymerization
- Microtubules
- Motor proteins
- Mitochondria
- Golgi
- Exocytosis
- G-protein coupled receptors
- Leukocyte extravasation (diapedesis)

Genetic Engineering & Synthetic Biology

- CRISPR/Cas9
- Gene Drives

CRISPR/Cas9- What is it & how does it work?

- CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats
- Cas9 = CRISPR associated protein (nuclease)
- Timeline of discovery: https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/crispr-timeline

- These are critical to 'adaptive immunity' in select bacteria and archaea
- Most studied CRISPR mechanism:
  - Invading DNA from viruses or plasmids is cut into small fragments and incorporated into a CRISPR locus amidst a series of short repeats (around 20 bp). The loci are transcribed, and transcripts are then processed to generate small RNAs (crRNA – CRISPR RNA), which are used to guide effector endonucleases that target invading DNA based on sequence complementarity
- Resource: https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

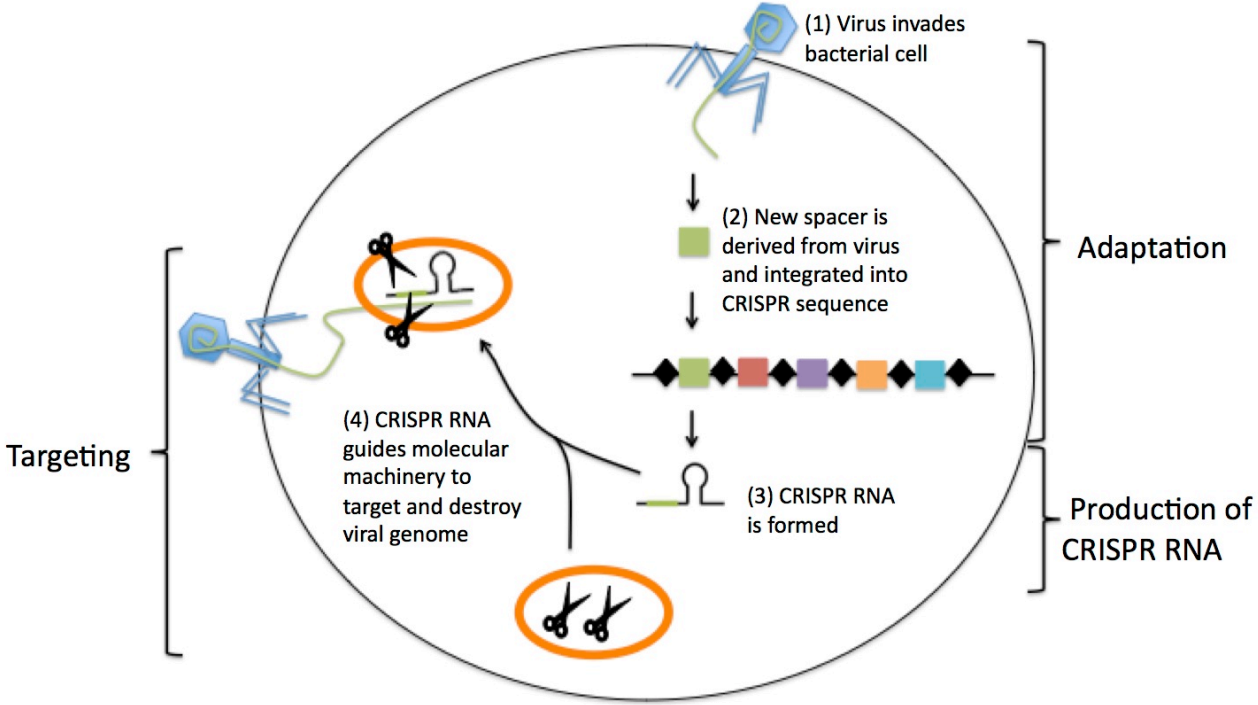
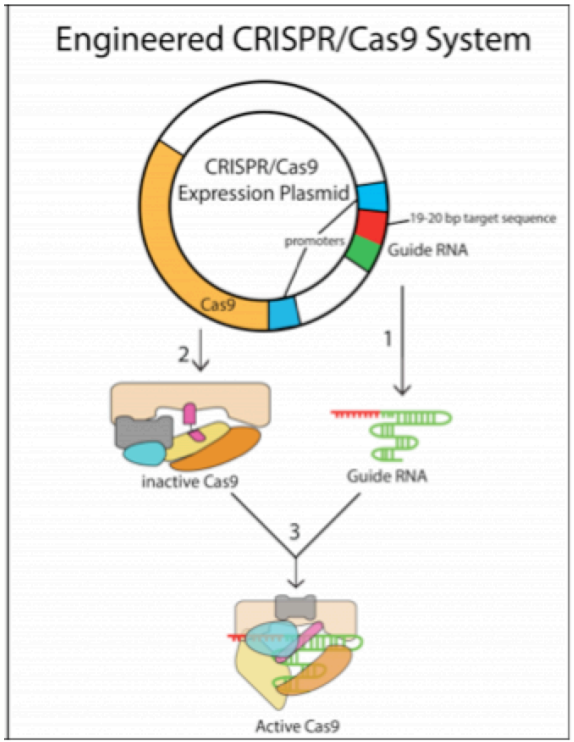
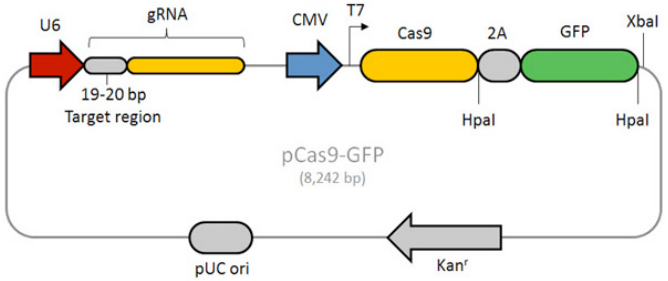


Image credit: http://www.helpbiotech.co.in/2016/02/crispr-genome-editing-technique.html

CRISPR/Cas9- How is it used as a tool?

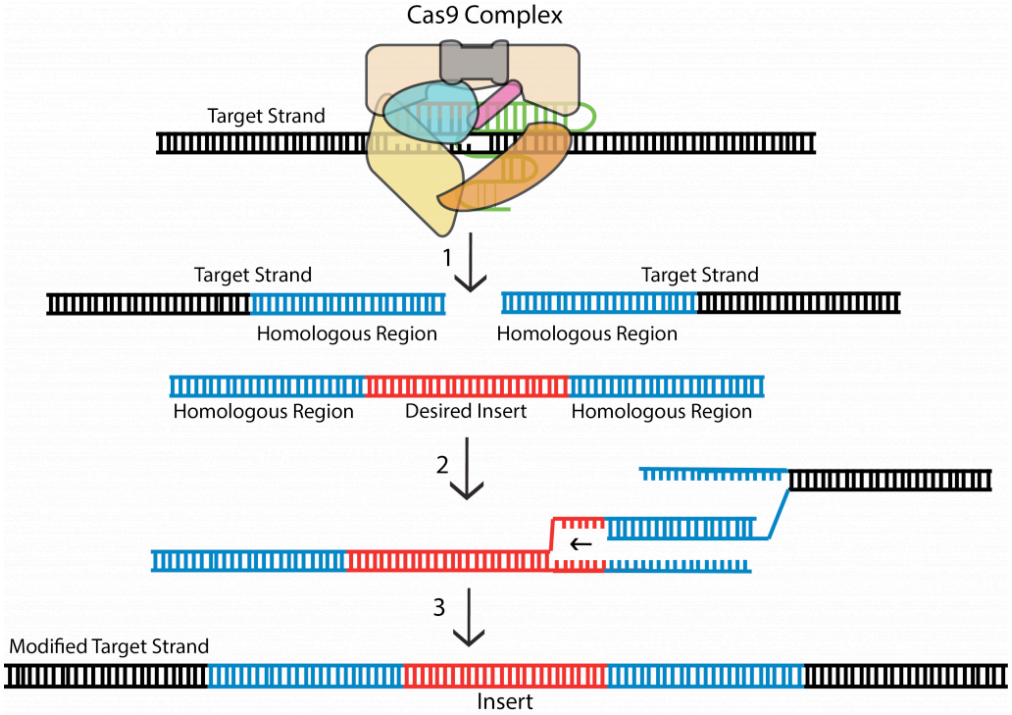


https://sites.tufts.edu/crispr/genome-editing/



Example GFP CRISPR/Cas9 plasmid from Sigma Aldrich

Homologous End Joining for Gene Insertion



https://sites.tufts.edu/crispr/genome-editing/homology-directed-repair/

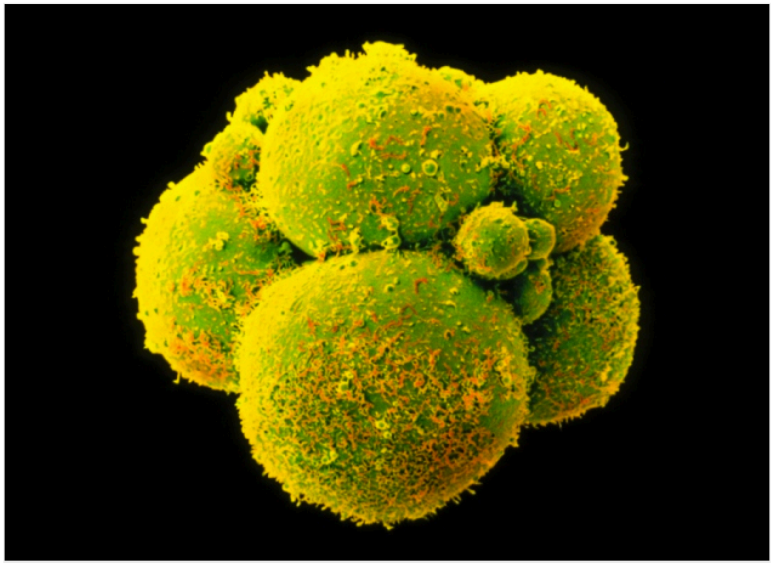
NATURE | NEWS

Chinese scientists genetically modify human embryos

Rumours of germline modification prove true — and look set to reignite an ethical debate.

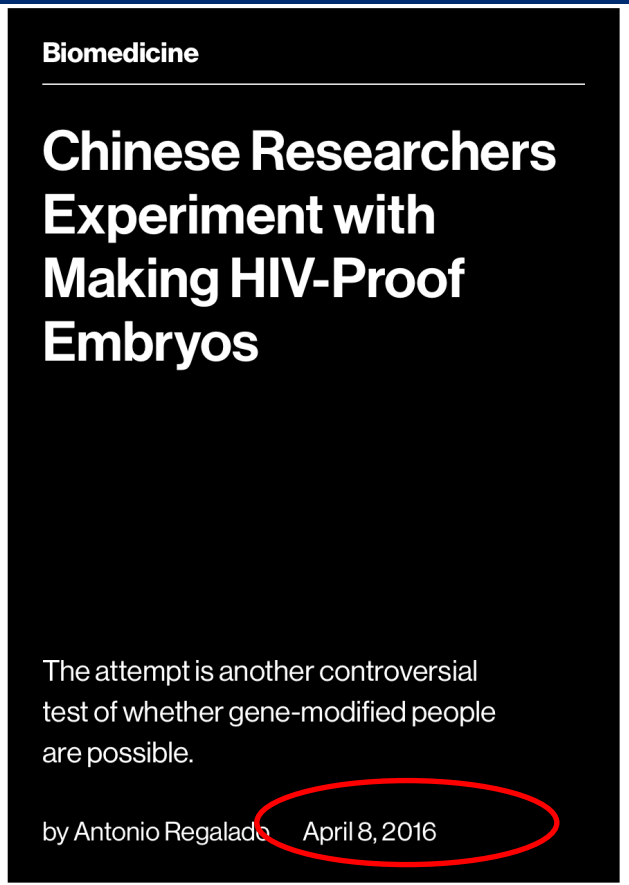
David Cyranoski & Sara Reardon

22 April 2015



Dr. Yorgos Nikas/SPL

http://www.nature.com/news/chinese-scientists-genetically-modify-human-embryos-1.17378



- The new report is the second time researchers in China revealed that they tried to make genetically modified human embryos.
- They collected more than 200 one-cell embryos and attempted to alter their DNA to install a gene that protects against HIV infection. The study, published two days ago in an obscure reproductive journal was first spotted by reporters at *Nature.*
- The Chinese scientists tried to make human embryos resistant to HIV by editing a gene called CCR5. It's known that some people possess versions of this gene which makes them immune to the virus, which causes AIDS. The reason is they no longer make a protein that HIV needs to enter and hijack immune cells.
- Using the gene-editing method called CRISPR, Fan and his team tried to change the DNA in the embryos over to the protective version of the CCR5 gene in order to show, in principle, that they could make HIV-proof people.

How Gene Drives Could Change the World


Image credit: BBC.com

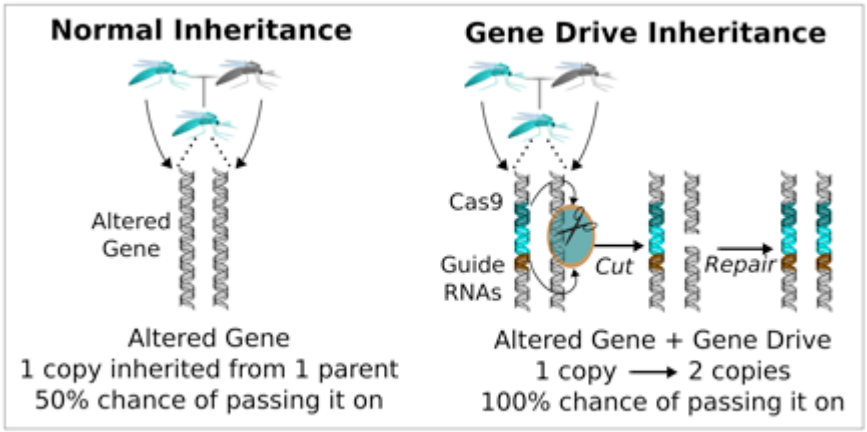


Image credit: Kevin Esvelt

- Scientists have made a breakthrough that has the potential to revolutionize the fight against malaria, a disease that still affects more than 200 million people a year and claims over 400,000 lives.
- Using the gene editing tool, known as CRISPR-Cas9, researchers genetically modified mosquitoes to make them resistant to malaria. This means that it may now be possible to eradicate the malaria parasite from the mosquito population and stop it spreading to humans.
- The discovery is a major milestone in the development of a controversial technology called a “gene drive” that works by making a modified gene spread rapidly through a population.

Summary

- Posttranslational processing
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  - Gene Drives
- Bioethics