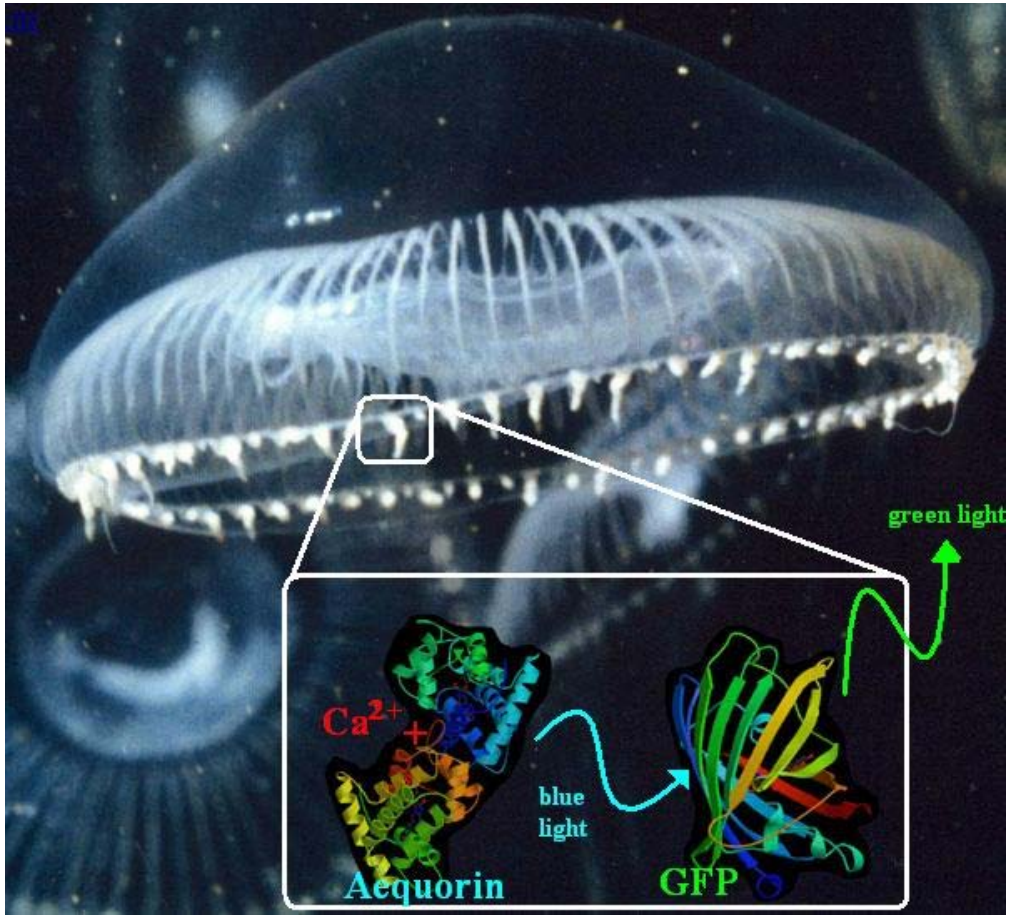
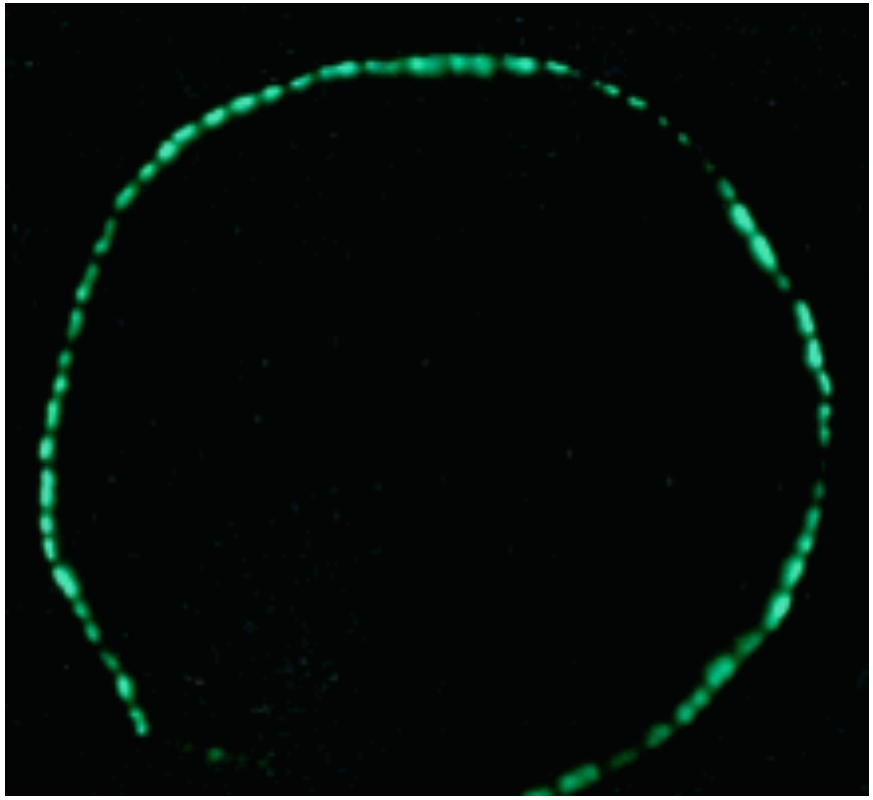
GFP Expression

Green Fluorescent Protein

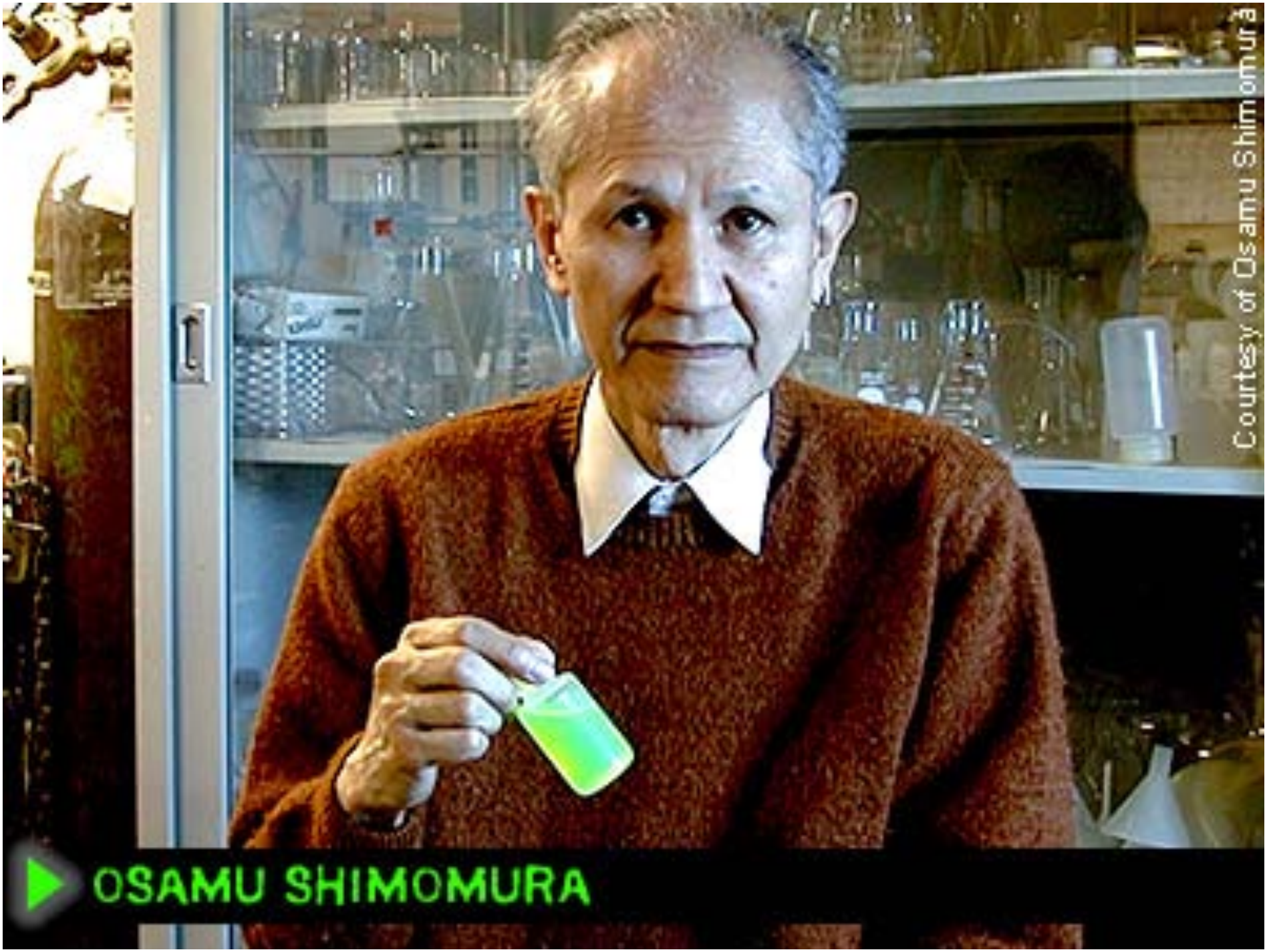


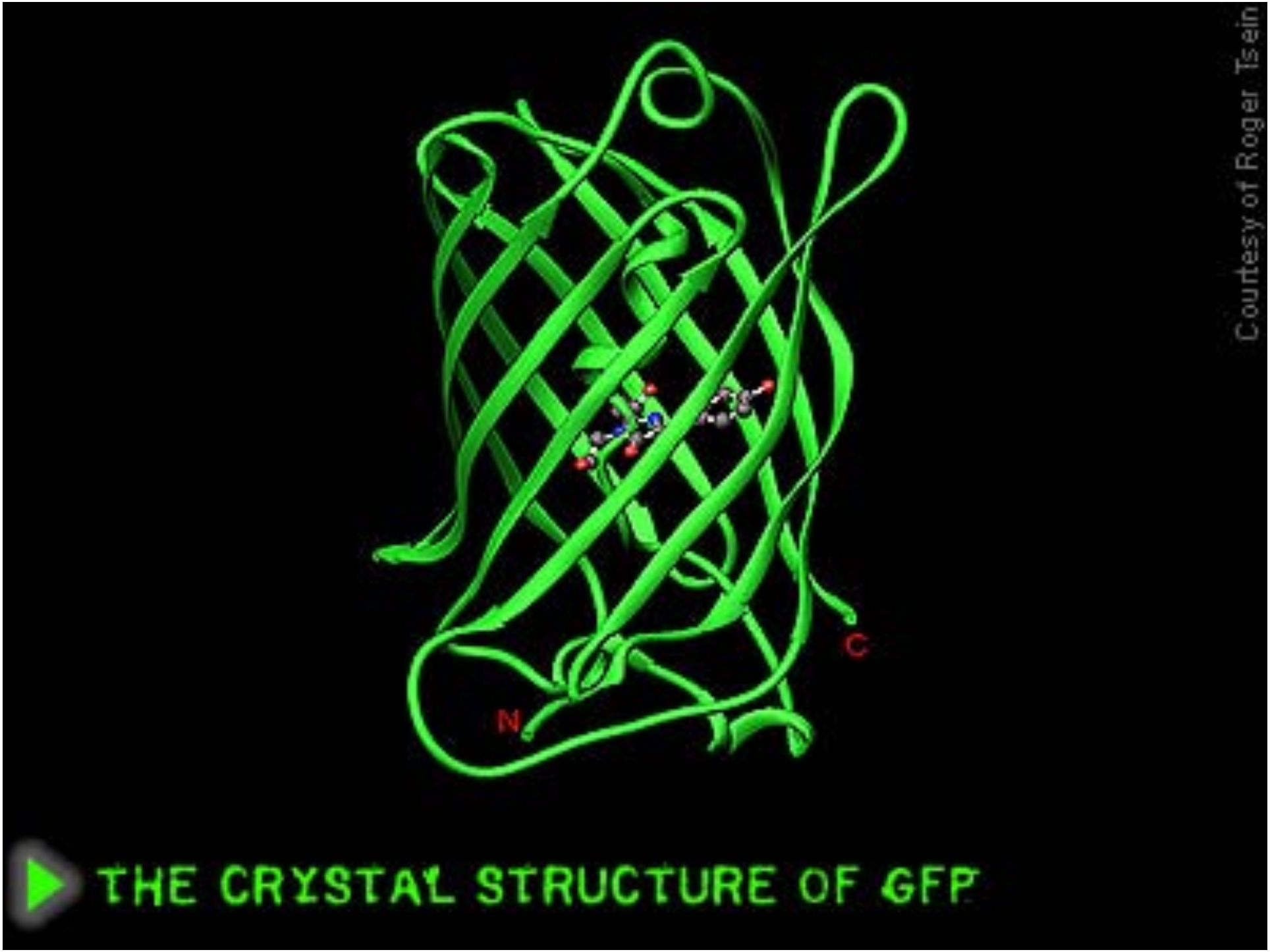
- Normally found in the rim of Jellyfish

jellyfish produces green bioluminescence from small photoorgans located on its umbrella



- When the rings of twenty to thirty jellyfish are squeezed through a rayon gauze, a faintly luminescent liquid called “squeezate” is obtained
- GFP Green Fluorescent protein can extracted from “Squeezate”


OSAMU SHIMOMURA
Courtesy of Osamu Shimomura


Courtesy of Roger Tsein
C
N
THE CRYSTAL STRUCTURE OF GFP


THE MANY COLORS OF GFP
Courtesy of Roger Tsein

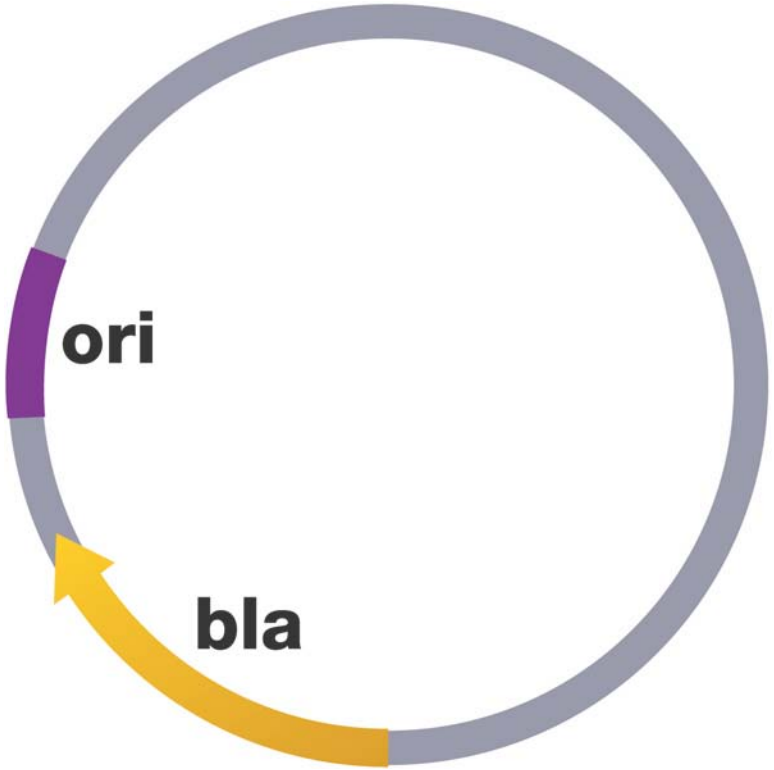
Our system

- GFP gene is on a plasmid (small DNA molecule) with the selectable marker of Amp and under an inducible Arabinose promoter.

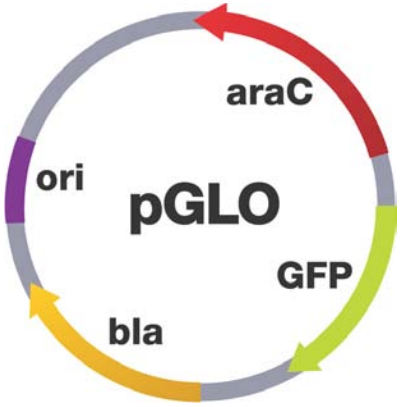
What is a plasmid?

- **A circular piece of autonomously replicating DNA**
- **Originally evolved by bacteria**
- **May express antibiotic resistance gene**

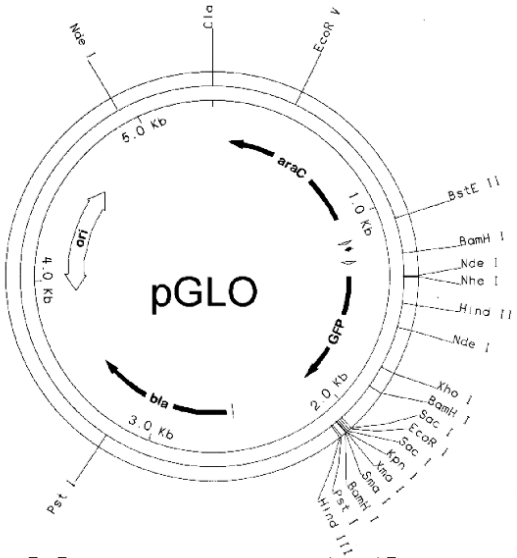
  or be modified to express proteins of interest



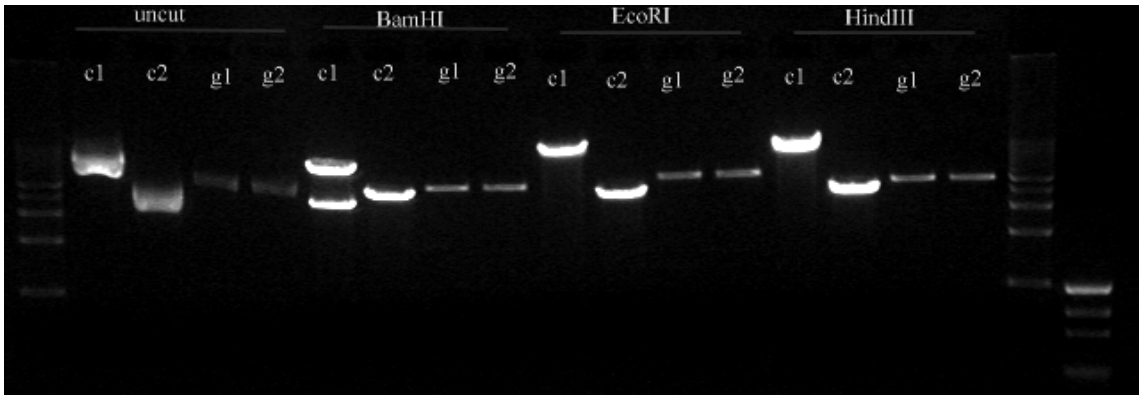
The Many Faces of Plasmids



Transmission electron micrograph



Graphic representation



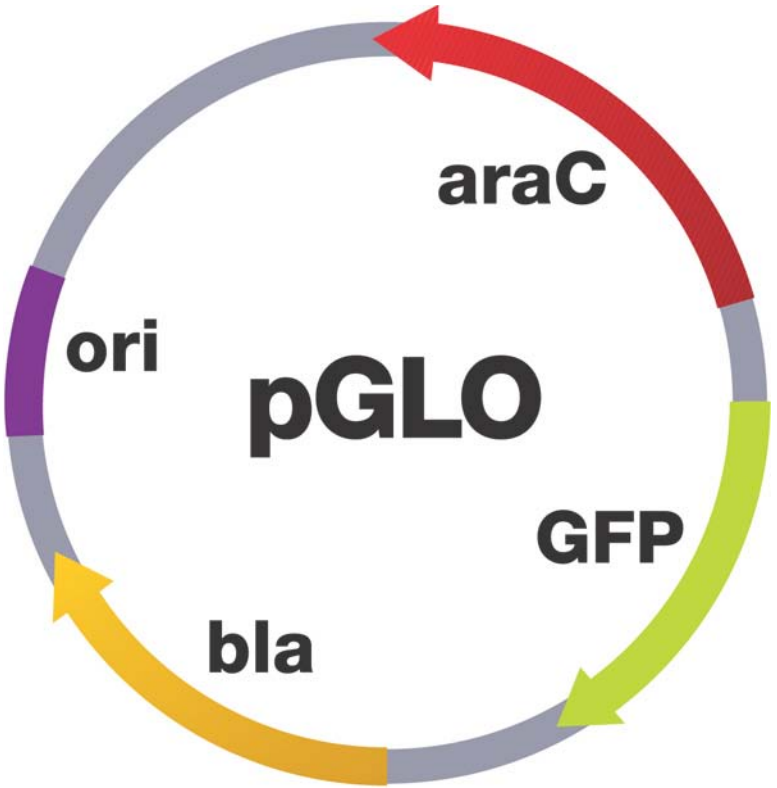
Agarose gel

BLA= beta lactamase

- BLA encodes for an enzyme Beta lactamase
- The function is to cleave lactam group off Beta lactams (penicillin , ampicillin and related molecules) to render it in active
- Confers antibiotic resistance
- Used as a means of selecting for tranformed cells

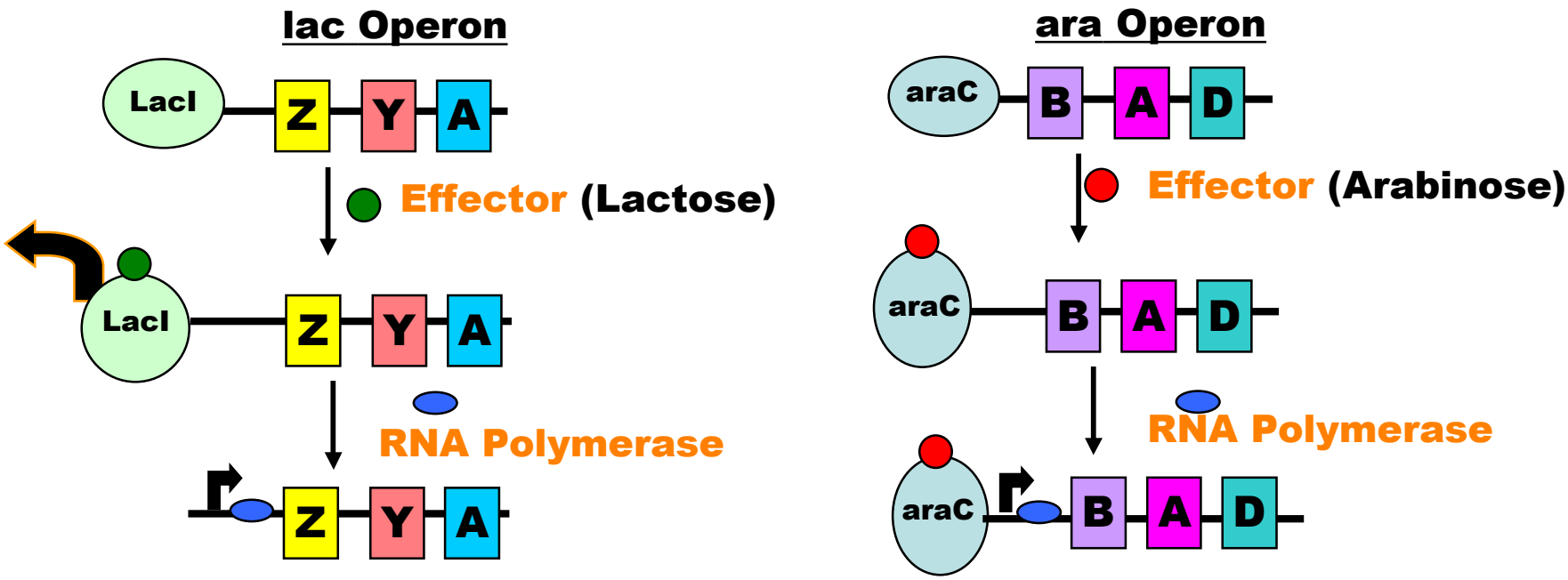
Transcriptional Regulation

- pGLO plasmid
- Arabinose operon
- BLA Ampicillinase

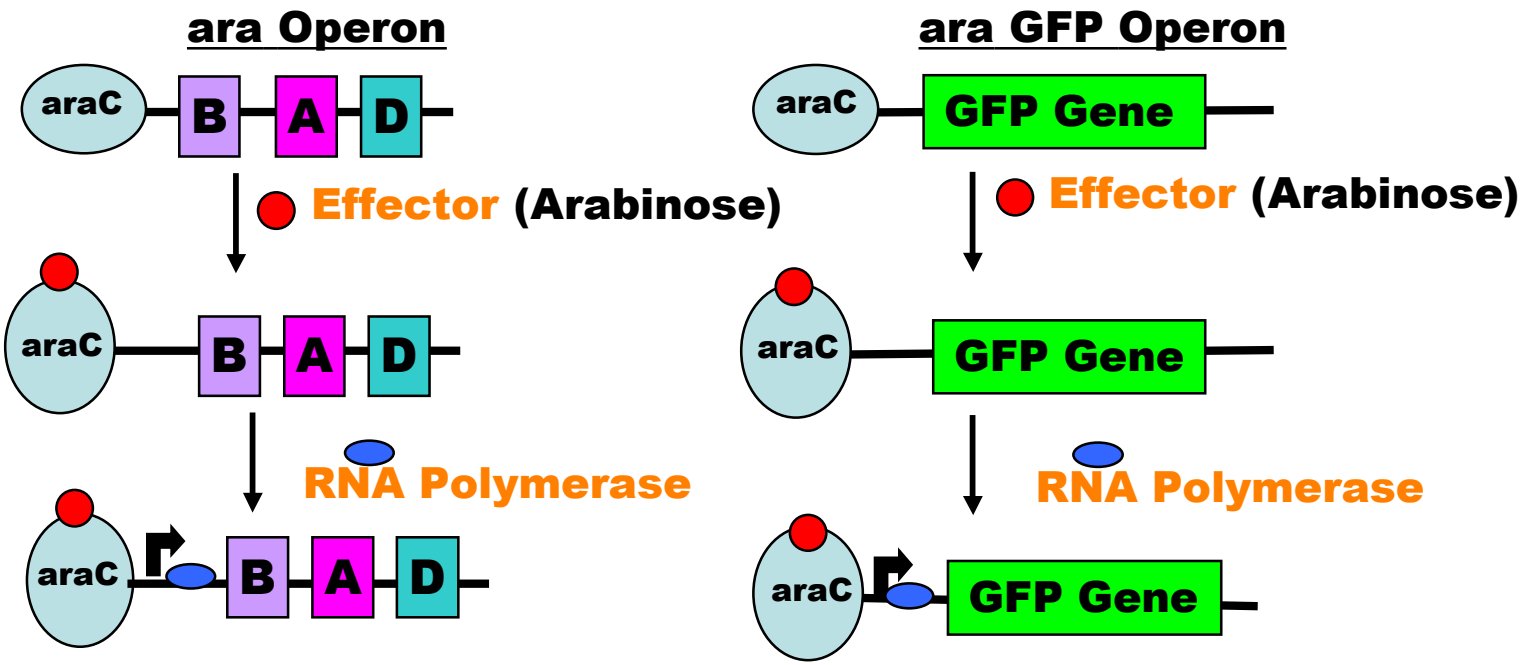


Transcriptional Regulation

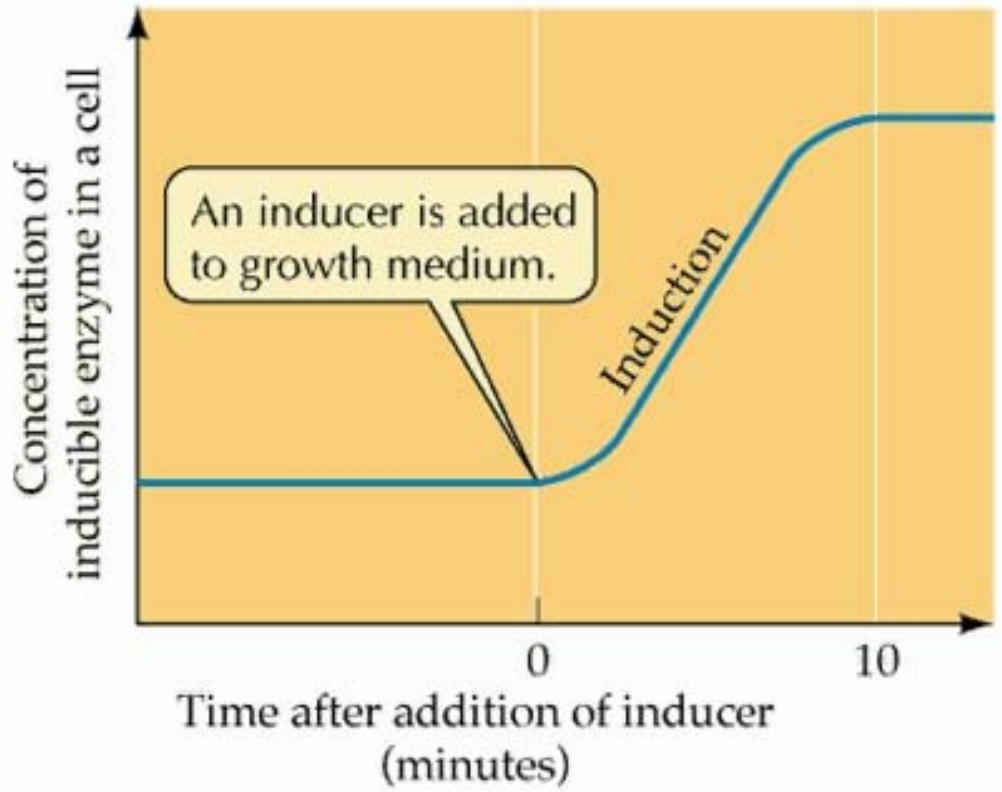
Arabinose gene control is similar to Lac operon



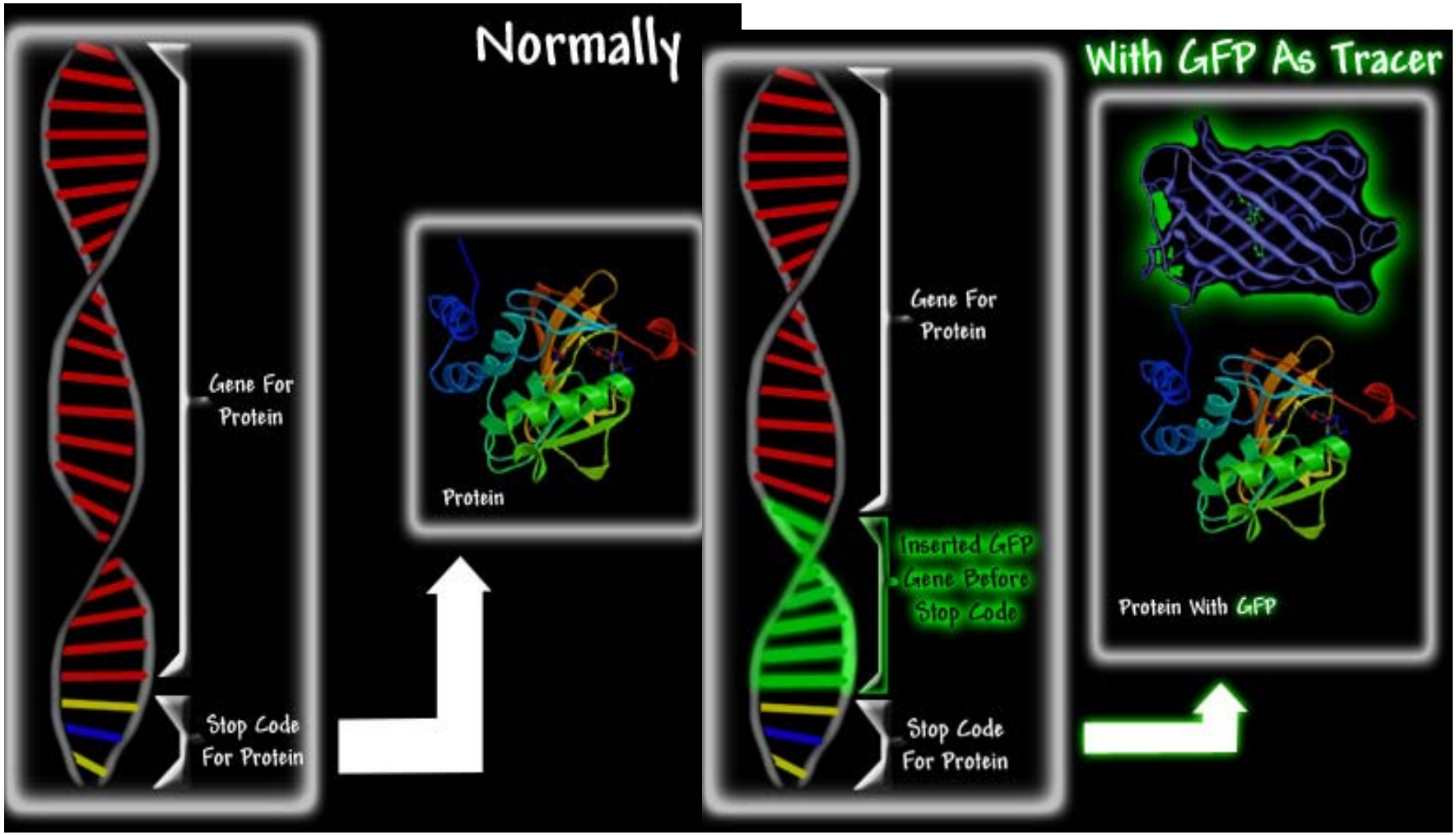
Gene Regulation



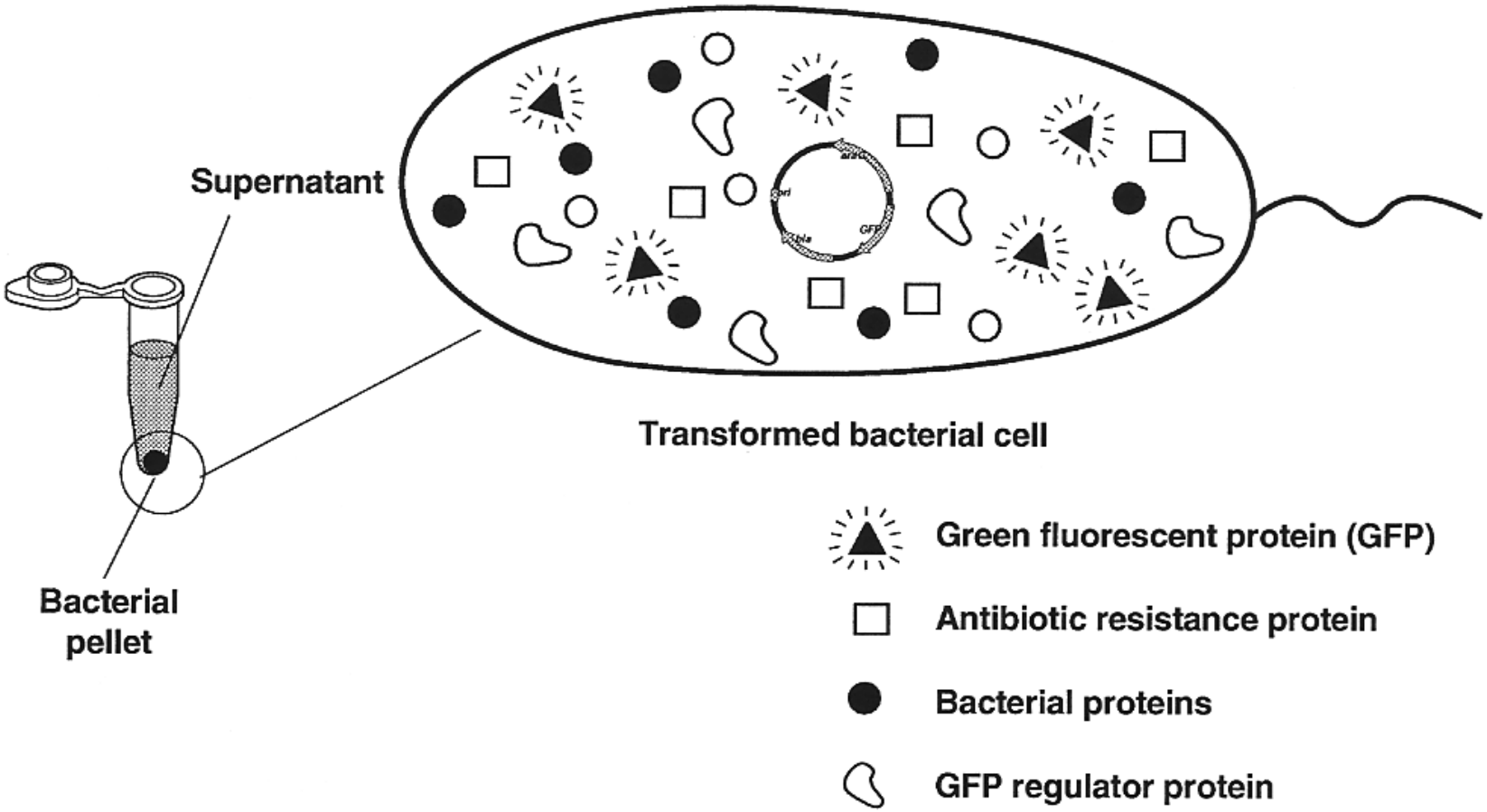
Example of an induction



Expression of GFP can be linked with another gene

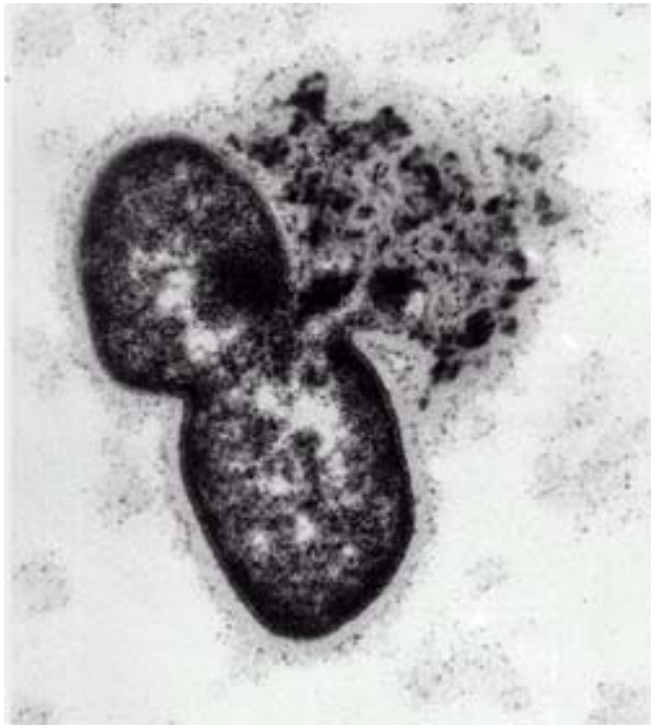


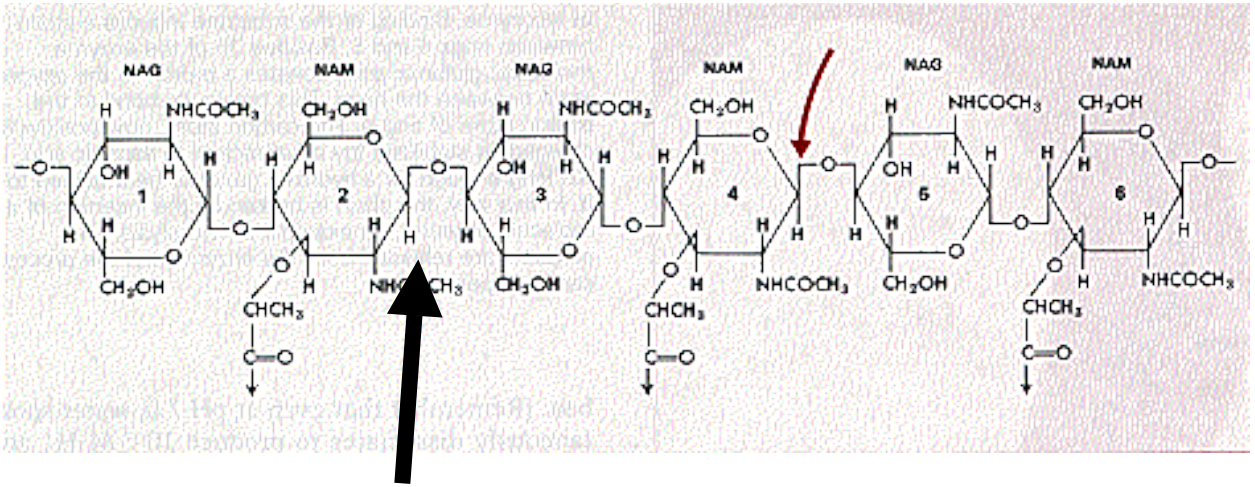
E.Coli cell is like a bag containing all sorts of proteins



Strategy for GFP purification

- Harvest cells
- Lyse cells
- Precipitate protein to reduce volume
- Apply to chromatography columns; selectively desorb





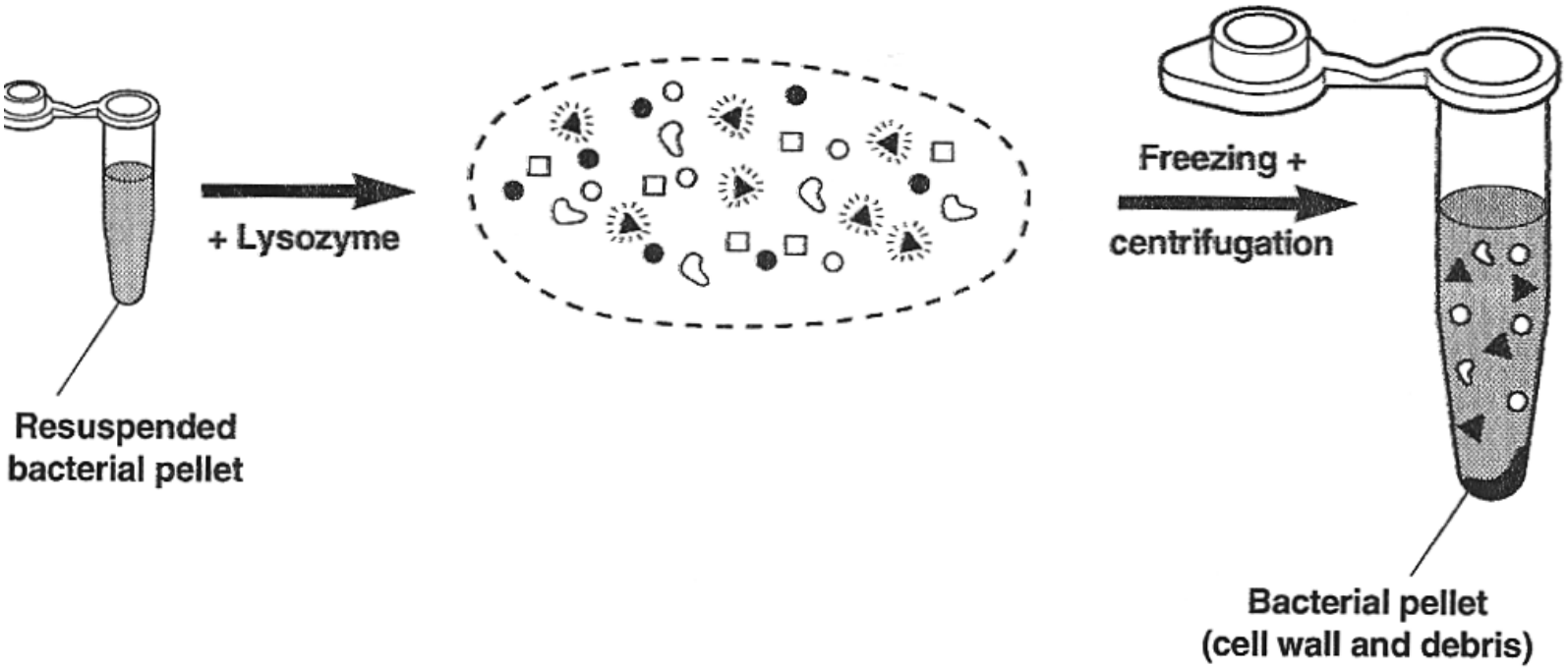
Lysozyme cuts bonds between Sugars on the cell walls

Cell Wall weakens and the cell bursts open

Lysozyme facts:

- Lysoszyme is an enzyme that fights infections (kills certain types of bacteria)
- Found in Tears, Saliva and Nasal Drippings
- Also found in Chicken egg whites
- Used to prevent Bacterial contamination in Beer making
- Cows secrete lysozyme into their stomaches as a digestive enzyme

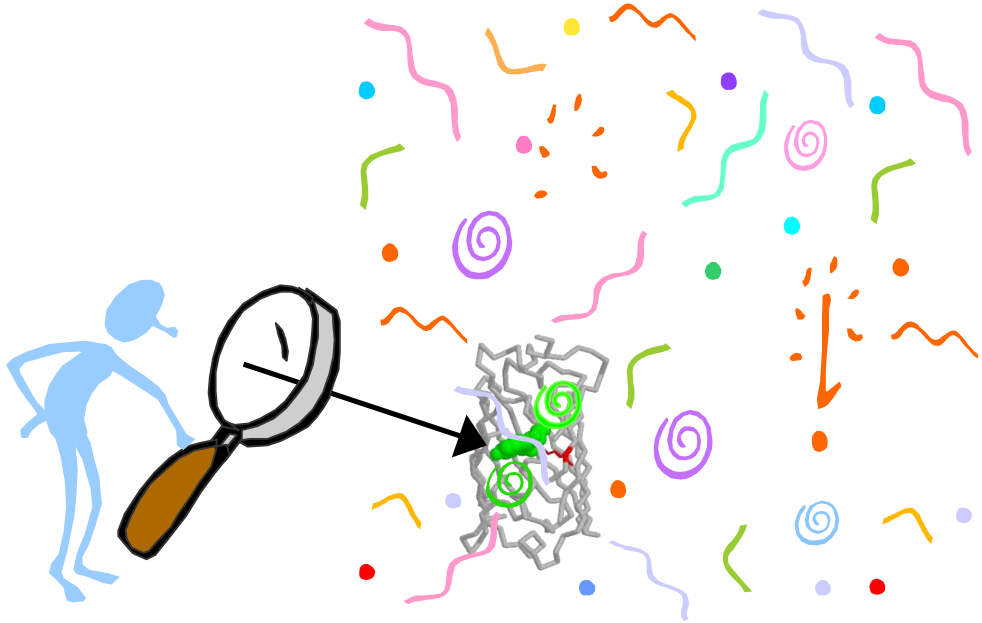
Lysozyme in action



Why Use Chromatography?

After Lysis A mixture of proteins is released

- To purify a single recombinant protein of interest from over 4,000 naturally occuring *E. coli* gene products.



Hydrophobic Interaction Chromatography:
Steps 1–3

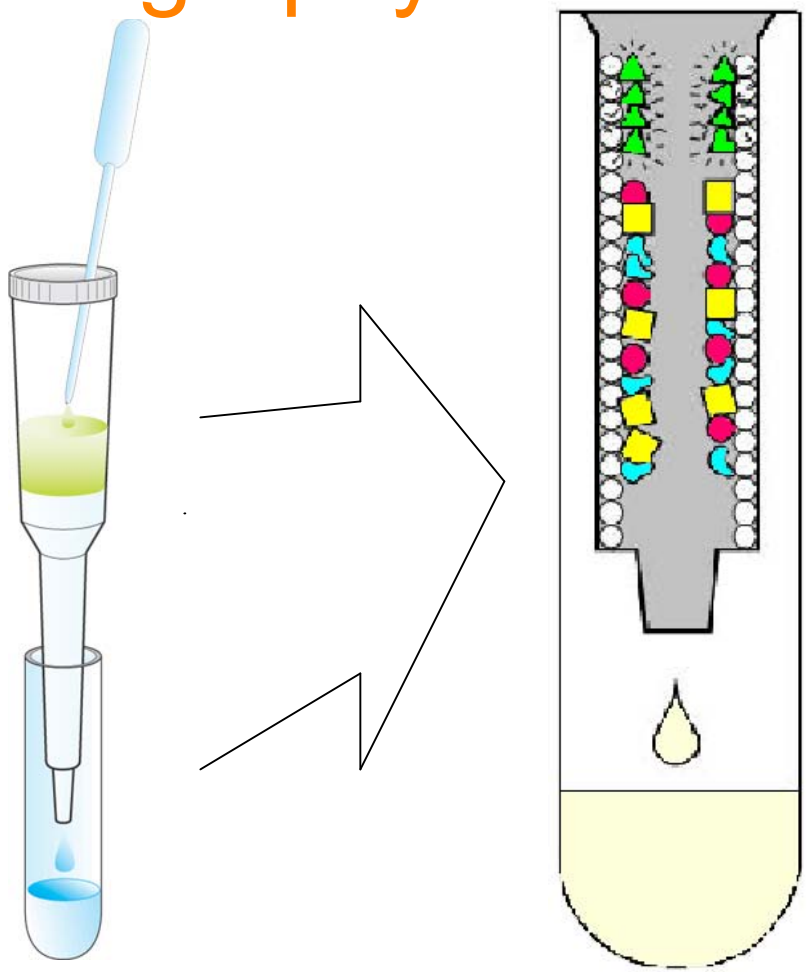
Add bacterial lysate to column matrix in **high** salt buffer

2. Wash less hydrophobic proteins from column in **low** salt buffer

3. Elute GFP from column with **no** salt buffer

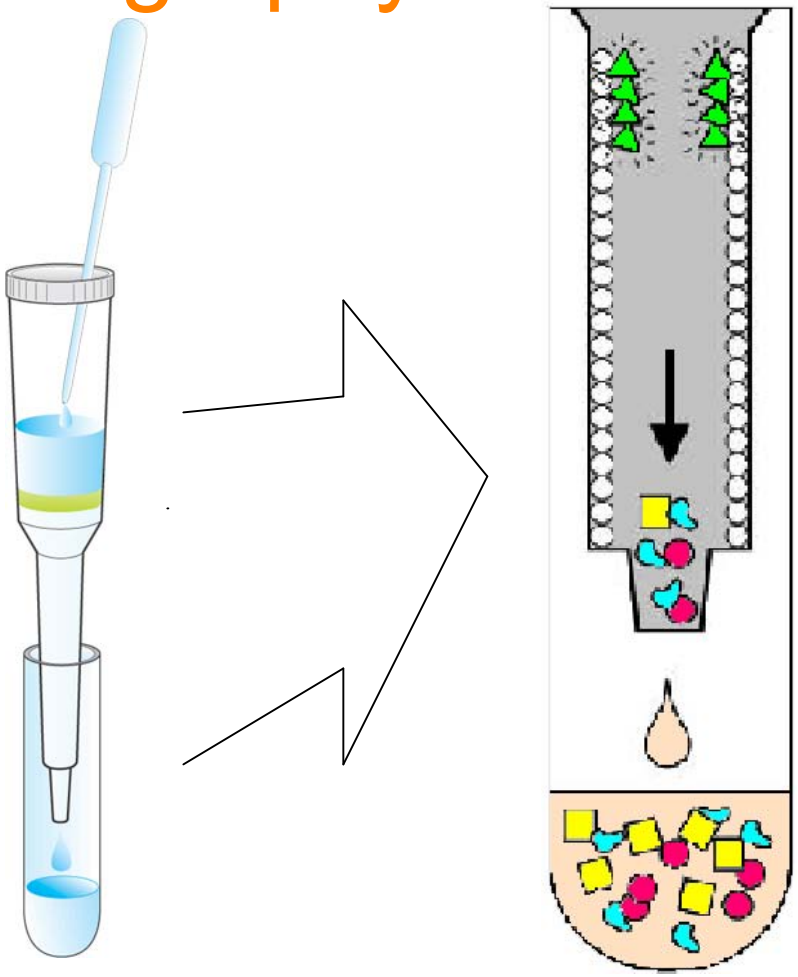
Step 1:
Hydrophobic Interaction Chromatography

- Add bacterial lysate to column matrix in high salt buffer
  - Hydrophobic proteins interact with column



Step 2:
Hydrophobic Interaction Chromatography

- Wash less hydrophobic from column with low salt buffer
  - Less hydrophobic E. coli proteins fall from column
  - GFP remains bound to the column



Step 3:
Hydrophobic Interaction Chromatography

- Elute GFP from column by adding no salt buffer

GFP

  - Released from column matrix
  - Flows through the column