Biological Transformation

- transformation is taking in and expressing free DNA
  - some organisms can do this naturally
    - some, not all, bacteria can do this
    - bacteria have surface proteins that can bind to free DNA in the environmt and transport it inside themselves
    - once inside, enzymes read the new DNA & compare it to their own DNA
    - if it is similar enough, the new DNA can be substituted
      - this is recombination
    - why would an organism do this? may help survival
      - may help organism evade the immune system of a host
      - think about the rough & smooth strains of pneumococcus
  - artificial transformation
    - tinkering with cells to allow them to take-up DNA in environmt is called competent
    - to make cells competent, must change the permeability of the cell membrane

competent
- can change permeability by heating cells in presence of Ca+ (positive ions)
- cells do not want to take-up DNA that is not similar (not same species)
  - we get around this problem by using plasmids
- plasmids are small, circular pieces of DNA that contain an origin of replication
  - b/c there are origins of replication (sequence of bases that signal start), the host cell will copy the DNA
- plasmids occur naturally in bacteria and yeast

in order to transform bacteria, we must overcome 2 problems
- 1-there has to be an advantage to the host cell to take-up the DNA
  - copying the plasmid DNA takes E, when cells are grown in a mixed culture, some containing plasmids, some not, those without plasmids grow faster
- 2-we have to be able to tell who took up the DNA, we need a marker

in a typical transformation exp, only 1 in 1000 bacteria take up the plasmid DNA
when we grow bacteria on normal agar----->should see lots of colonies
a lawn of bacteria
when we grow bact on agar w/ampicillin (antibiotic that kills bacteria)
should see no growth
if we give bacteria a plasmid with a gene for ampicillin resistance, and the bacteria took up the plasmid, we should see growth on plate
some plasmids contain a second gene that has a marker
we used beta-galatosidase, a gene that makes a protein thats splits a disaccharide into 2 monosaccharides
also splits a substance called X-gal, when it does this it makes a blue color
if we grow bact that make B-galactosidase on X-gal, colonies will be blue
if bact do not make B-galact, colonies will be white

the color marker was used to see if new plasmid DNA was incorporated into bacteria, plasmid grown on X-gal shows bact that are blue