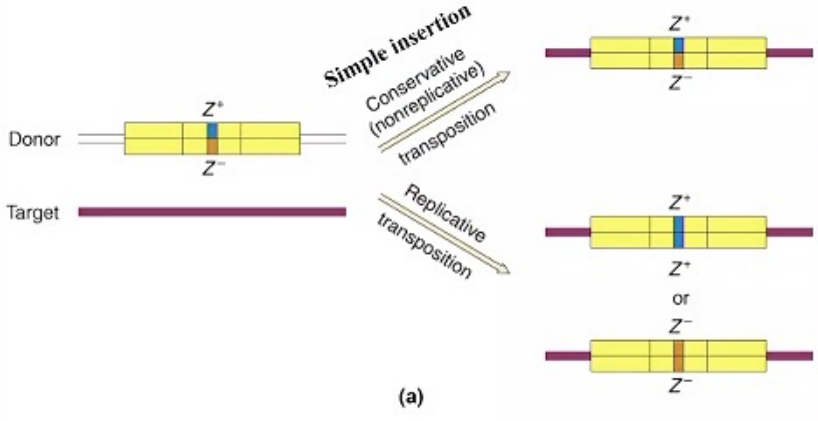
lecture 11: site specific recombination II

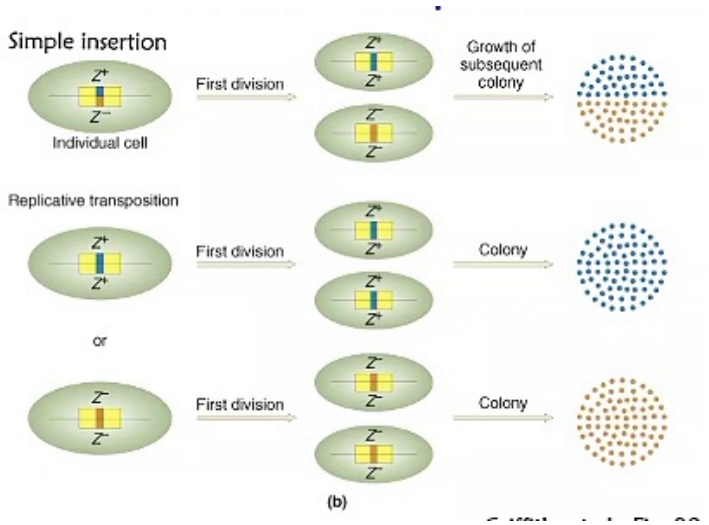
review
- simple insertion
  - transposon is completely excised from original site and inserted into new site
- replicative insertion
  - transposon is replicated as it inserts
  - a co-integrate is formed
  - co-integrate is resolved - resolvase
  - process
    - staggered cut at target site, and transposon cut
    - strands transferred - DDE transposase catalyses this
    - hot fixes up mess
    - replicates transposon with this process = cointegrate
    - replicons could be chromosome, plasmid etc.
    - if you fuse them they often aren't happy - due to having two forks going in opposite direction coming together
    - so want to separate these replicates = co-integrate is resolved
    - E.coli
      - chromosome has method for resolving co-integrates that happen during replication
      - when get replication, occasionally get dimer formed, way that is resolved - site specific system which recognises sites on replicons which are fused, and brings them together and separates into two molveulse
      - resolution reaction
      - resolvase protein - recombines those sites and separates molecules

Tn10
- compound transposon
- large inverted repeats - is10 on left and right
- some mutations in is10 left (stabilises the structure) the entire transposon is able to mobilise due to this mutation
- encodes tetracycline resistance

is it replicative or simple?
- process 1 - bender and Kleckner experiment
  - took donor molecule with tn10 in it and put in lac z gene
  - lac z - beta galactosidase - used for cloning - blue/white selection on x-gal
    - when clone into it you disrupt lac z gene and get white colonies
    - intact lac z = blue colonies
  - made a heteroduplex - two strands of DNA with differences between the two strands
  - took tn10 derivative which they put lacZ into, and made a mutation in one copy of the Lacz gene
    - would have had a plasmid with the tn10 gene with two effective copies of lacZ, and a plasmid with two copies of mutated lacZ genes
    - so they heated to denature, mixed together and re-anneal, in a certain proportion would have a heteroduplex - one strand effective lacZ, one strand ineffective lacZ
  - introduced all 3 phenotypes of lacZ into e.coli - mutated, wild type and heteroduplex

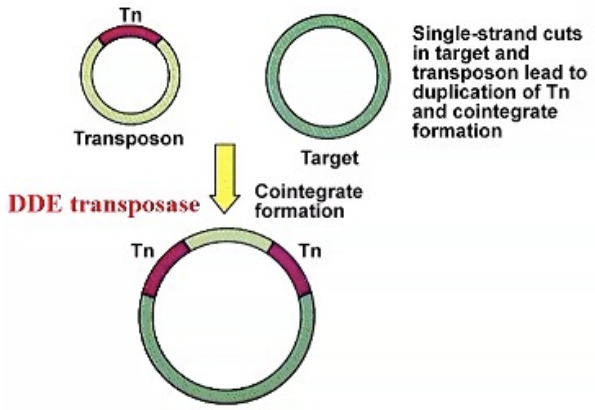


- predicted
  - simple insertion you would get the whole transposon cut out from donor and inserted into target without replication occurring, so you could get heteroduplex into chromosome
  - replicative transposition - replication occurs as split transposon in half, so if it is a heteroduplex you'd only get one strand - so you'd only get either mutation or wild type
  - X gal example:
    - Replicative transposition: you'd get only blue or white colonies on X-gal
    - simple insertion: when bacterial cell with heteroduplex splits during replication, one chromosome goes into one offspring, and the other will go into the other offspring therefore you will get half blue colonies, half white colonies along the plane where the first replication event occurred
  - simple insertion occurred!!



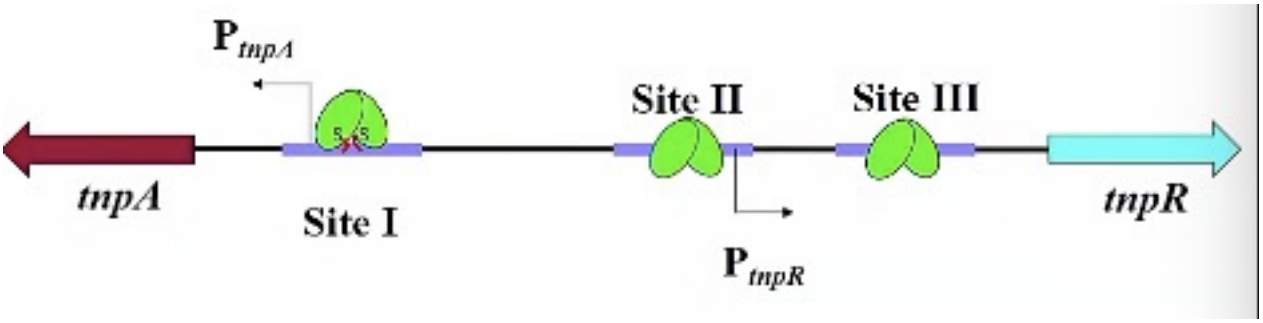
tn10 simple insertion
- cleavage of single strand at either end of the transposon
- every enzyme family of transposases deals with the other strand in a different way
- Tn10 uses water so not covalently attached to DNA when cleavage occurs, it uses hydroxyl group on the cleaved stand to nick the other strand which forms a stem-loop at the ends of the molecule
- trasnposase must cleave again to get it into the cell

Tn3
- replicative transposon
- ampicillin resistance
- end up with duplication of transposon in the co-integrate - DDE transposase does this
- resolvase reaction
  - res sites located internal to the transposon
  - able to synapse - brought together by resolvase protein
  - get donor molecule again and transposon inserted into target molecule also
- involves TWO different site-specific recombinase families
  - DDE transposase - responsible for cleavage of transposon ends and new target site
  - resolvase responsible for recombination between res sites on duplicated transposons (co-integrated resolution)
- resolvase belongs to serine recombinase family



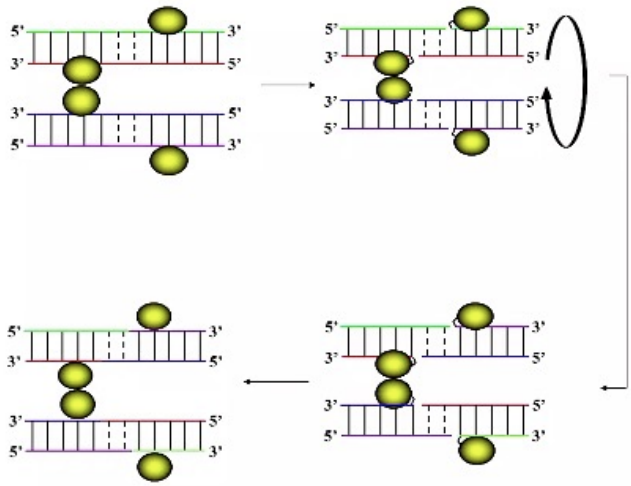
serine recombinase family
- sequence conservation within enzyme catalytic domain - end terminus of proteins
- different subtypes named from function
  - resolvase proteins - resolve or excise only
  - invertase proteins - invert only
  - phage integrases - excise and integrate (insert)
- mechanism
  - covalently attach to DNA via Ser-OH
    - form 5' phosphoserine covalent linkage to cleaved DNA strand
  - cleavage of each DNA molecule staggered by 2bp
  - recombination reaction is a single step process
  - all four strands cleaved at the same time, rotated 180 and re-sealed
  - NO holliday junction formation

- res site for Tn3
  - site of cointegrate resolution, two res sites (duplicated Tn)
  - each res site contains three resolvase binding sites
  - two bp staggered cut by resolvase at site 1
- each site made up of inverted repeats that the enzyme binds to
  - site 1 - where cleavage occurs
  - site 2 and 3 - synapse, resovlase doesnt cleave DNA at these sites
- need two res sites per transposon - diagram is only one



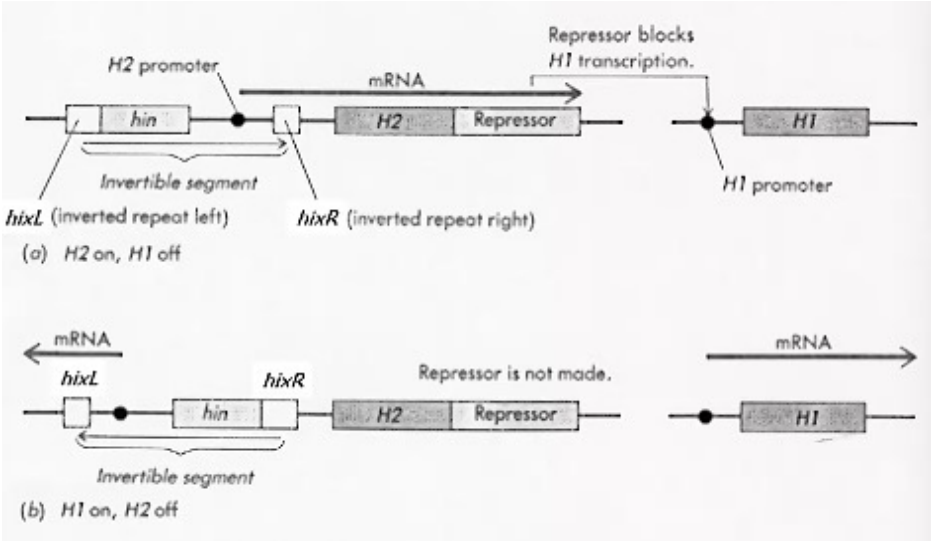
- synapse allows DNA to stay in place - it doesnt go away before recombination can occur
- processes dont require active energy as such - only transfer of energy
- however to get rotation you need kinetic energy - negative supercoils in synapse
- PROMOTOR is located at site 2 for TnpR, and at site 1 at TnpA
  - when resolcase protein is bound, it shuts down promotor activity, so when have enough protein you don't get more production of transposase or resolvase
- 12 resolvase involved in recombination, however only 4 are involved in cleavage

serine recombinases
- resolvase/invertase family
- like tyrosine recombinases they also can carry out conservative site specific recombination
  - no energy is required for reactions, and DNA is same before and after reaction - sequence is identical unlike tyrosine family
- two bp staggered cut at nick site
- recombination involves nucleophilic attack by serine residue - forms 5' phosphoserine linkage
  - no use of water like tyrosine
- N-terminal domains of these proteins are highly conserved
- serine recombinase-mediated site-specific recombination
  - differs from integrase mediated recombination
  - nucleophilic attack of phosphodiester bond occurs via serine residue
  - forms 5'-phosphoserine linkage
  - all 4 strands are cleaved before strand exchange occurs
- reaction process
  - 'site 1' - have inverted repeats which protein is binding to, and have two base pairs
  - cleavage occurs and you get attachment at 5' end
  - once all 4 strands are cleaved you get rotation
  - have recombinant formation of DNA still attached
  - attack of covalent link between DNA and protein by hydroxyl group on cleaved DNA strand
  - phosphate backbone is resealed



DNA inversion

- involves inversion of a defined segment of DNA
- site-specific recombination process
- invertase mediated
- inverses are members of serine recombinase family or site specific recombinases
- e.g. bacteriophage Mu
  - when inverted it cant infect E.coli K12, however infects other E.coli
  - involves S and U proteins
  - some phage particles produced
  - inverted repeats are DNA recognition sequences for gin protein = GIX sites
  - need to be inverted orientation = inversion
  - if they are directly repeated = incision
- summary of inversion process
  - mediated by Gin invertase (serine recom.)
    - located outside region that is inverted
  - involves 34bp repeated sequences at ends of G region (gix sites Gin binding site)
  - also involves FIS protein
    - factor for inversion stimulation
    - binds to enhancer site located within G region (synapse)
- e.g. inversion of salmonella flagella antigens
  - salmonella produces two different flagella antigens
    - H1 and H2
    - antigenically distinct
    - only one expressed at a time
  - phase variation between H1 and H2 involves inversion of 970bp fragment
  - mediated by Him invertase binding to *hix* sites
  - process
    - him gene within the region that is inverted
    - hixL and hixR and h2 promotor
    - when promotor goes in one direction you get production of H2 protein, and repressor for H1 is activated
    - when inverted then H2 is not made, and the repressor is not activated therefore H1 is produced
    - hin gene - expresses hin protein which catalyses recombination between the two sites and switches it between one or the other



- synapse models
  - for the invertases you have hixL and hixR and have enhancer site and FIS binds to that
  - binding of Him protein to HixL and HixR they are bound together and enhancer is rectuitted to make sure that DNA doesnt float away after cleavage of 4 strands at the same time
  - role of Hin and FIS proteins
  - formation of nucleoprotein complex
  - two alternative models
  - FIS does not appear to be required for *hix* sites to come together
  - one synapse is formed, Hin catalyses cleavage and strand exchange
  - change in confirmation occurs between binding and cleavage, and this cant happened until activation of these enzymes after a synapse is formed - a control mechanism to ensure the right thing occurs