MBG - Revision questions

Questions that span several topics covered in the bacterial genetics course are presented here for in-class revision. Note, it is not possible have a comprehensive list of questions that covers all the topics in the course that can be discussed in two lecture time periods, therefore to do well in the final exam, it is not sufficient to just study these questions.

1. We have encountered the protein RecA frequently in this course as it is involved in many bacterial processes.
(a) What does Rec stand for and what are the processes that RecA is involved in?
Rec = recombination
1)know one of the functions from the name it helps with homologous recombination, if you have 2 non-identical genes but may have regions of the same DNA sequence, gene A can replace gene B between the homologous sites, generalized transduction gene that the phage carries is inserted into the chromosome of the bacteria, requires RecA protein which is one function
-Griffith's exp. on transformation, smooth and rough (disease and benign), smooth has extra gene encoding bacteria capsid, heat treated and killed the cells but the chromosome of some of the cells is intact and the living rough cells could take up the dead smooth cells chromosome, only way to ensure genes taken up are propagated in further generations can occur through homologous recombination, capsid genes recombine into chromosome of the rough strain,
2) lambda prophage induction when treated with UV light (ssDNA complex) - can proteolytically cleave C1
3) SOS response (ssDNA complex) – can proteolytically cleave LecA and UmnD
4) Trans lesion sometimes called recombination mediated, also involves thymine dimers, in order to transcribe region of dimer, DNA repair, RecA brings a newly synthesized strand to the template strand to read past it, the strand was replicated from the opposite template so it has the same sequence

(b) What is the common theme in the function of RecA in these processes?

2. Name all the different DNA repair pathways.
6 different DNA pathways.

1) Photo-reactivation – UV thymine dimer repair, protein enzyme photolyase reverse thymine dimer formation
2) Uracil base excision repair, once the base is removed you have a gap, polymerase removes a piece of DNA or base, polymerizes to fill in the gap that is being produced and ligase ligates the knick together, occurs in several steps, exonucleases activity removed the chunk of DNA with the base, take base from sugar, **1/2 not used in thymine dimer repair, need an exonucleases to chew up 3' end to the mutation, exonucleases cannot cut up sugars with thymine dimers on them
3) Nucleotide excision – excise nucleotides, sugar with an incorrect base, remove both base and sugar, Uvr gene encoded proteins, involved in removing thymine dimers hence the Uvr gene, cut around the dimers, fill in the gap, A binds to the damaged DNA, B and C do the cutting, doesn't use exonucleases therefore can be used for thymine dimer repairs
   ___/--TT--/_____
   _B_--A--__C__
4) Methyl directed methylated repair occurs after replication, un-methylated strand is the newly synthesized DNA, un-methylated one is the original template DNA, Polymerase proofreads to

ensure no mismatched bases, most of the time the error is in the newly synthesized strand and not the parent strand, correct and remove bases to the newly made strand, methylation is a way to know it is a new strand created, if no methylation occurs then the repair may not be able to tell the difference, genes involved start with mut – mutants
-two genes mutS and mutL – Surveillance (scanning new DNA, find where there is a base mismatch, A-G, T-C), mut H makes a knick and exonucleases removes the incorrect base with a few bases downstream and then DNA polymerase fills it in
-2/2 not used in thymine dimer repair, need an exonucleases to chew up 3' end to the mutation, exonucleases cannot cut up sugars with thymine dimers on them

5) Translesion DNA repair -
6) SOS Repair – last resort, too much damage has occurred, uses DNA pol IV which is error prone, good polymerase in some sense as it can read through the damaged DNA but that also makes it error prone

Which of these are involved in repair of thymine dimers and which are not?

3. (a) Compare and contrast IS elements, composite and non-composite transposons, using IS1, Tn5 and Tn3 as specific examples.

-Similarities = all transposons have inverted repeats – flanking regions, in order to be transposons they need to have a transposase gene,
-Differences = structural differences, presence/absence of antibiotic resistance genes, type of antibiotic resistance genes, IS elements are the simplest form – do not have antibiotic resistant genes like transposons, antibiotic resistant gene depends on transposon type
-composite transposon has IS elements on each end
-tnpR = encodes resolvase, seen only in non-composite transposase, -res = site of resolution, resolvase works on res site,
-co-integrate and replicate, then breaks into 2 component sites
-difference among the 3 kinds of transposons
*direct repeats are not transferred with the transposon, consequence of the transposition event not from transposase, direct repeats are part of the target site and not transferred therefore are not included in this question

(b) Describe the phenotypes of mutations in relevant genes/sequences in each of the above. Which could be complemented and which could not? Explain.

-Mutation in transposon gene – no transposition
-Mutation in resolvase – transposon can get into target site and forma co-integrate but it cannot resolve and return back to the original state
-mutations in both resolvase and integrase can be complemented in trans like antibiotic resistant mutations, only think that cannot be complemented is the res site as it is a cis acting element

(c) How does insertion of a transposon into *lacZ* gene in *E. coli* lead to polarity?

- Polarity = when it impacts transcription downstream and expression of the genes, stop codon for normal lack Z, sometimes the IS elements can introduce reading frame shift, may create extra stop codons downstream due to change in reading frame, pre-requisite of polar effects Is that you need to have a mutation create a stop codon, ribosome has a role in polar effects, ribosome introduces hair pin structure, if mRNA is being transcribing with IS element introduced into lacZ so a stop codon arises, several reasons for polar effects
-when you have an operon the ribosome binds so shine dolgarno sequence to translate the gene, then moves to the next shine dolgarno sequence for transcription of another gene

-mRNA with a stop codon prior to hair pin formation then it can translate next gene in operon
-if stop codon shifted further downstream then a stem-loop/hairpin structure occurs and then the ribosome can't bind and recognize the shine dolgarno sequence, one way of polar effects in translation of genes downstream - affected because of hairpin formation
-in prokaryote – transcription and translation can occur simultaneously, may be hidden rut sites (rho-depended transcription termination), rut sites are never exposed ot rho because the ribosome blocks the rut site as it translates the genome, if you have a stop codon upstream of rut site then ribosome leaves and then the rut site is no longer hidden by ribosome, can bind to rut and premature transcription termination of the operon occurs.

-If a transposon inserts in LacY – gene which it has polar effects on is lacA downstream not LacZ which is upstream from LacY, polar effects is always downstream of the mutated gene
-LEARN POLAR EFFECTS AND HOW IT WORKS**

4. (a) Using specific examples, explain why the protein CAP is a global regulator?
-catabolite activator protein, holds polymerase so it can initiated transcription at the promoter site, global expression, controls expression of multiple genes, beside the lac operon it controls the arabinose pathway, sugar metabolism genes in general, CAP binds to cyclic AMP before it can bind to the DNA to activate the transcription of the lac or ara operon, when glucose is present cAMP is low as the enzyme that makes cAMP (adenylase cyclase) responds to glucose concentration, phosphorylated 2a is needed for activation, if a mutation in CAP occurs in the lac operon – the Lac operon will not be transcribed to its maximum level, same with Ara even when either sugar is present

(b) describe what happens if a transposon is inserted into the gene encoding CAP?

5. Describe ALL the possible mechanisms of how an *E. coli bio*$^-$ strain can become *bio*$^+$.
-Bio stands for biotin, which is a vitamin required by the cell, if you have E.coli strain that can't make biotin synthesis enzymes it can acquire the genes through: transmissible plasmid – conjugation, a bio gene on an F plasmid which gets transferred into recipient E.coli, donor is F' as it has genes inserted into it, recipient must be F-

-another way conjugation can bring conjugation into F- strain, Hfr = F plasmid inserted into genome, other genes present as well, when the F plasmid can transfer itself together with the chromosome, the bio genes could go into F plasmid recipient where a cross-over event occurs, then get F= bio+, doesn't need to excise before transferring, reliant on the pilus being able to hold itself to the recipient until the bio genes have been transferred, transferred in a linear fashion, Lac genes then Bio genes etc.

-Transformation – plasmid with bio+, E.coli takes up the genes and becomes bio+, E.coli is not naturally competent so it needs to be treated by chemicals or force DNA into cell through electroporation, another method of how E.coli can receive new genes

-Transduction – introduce new genes through viral infection, Generalized transduction = P1 phage, biotin transferred by P1, P1 infects E.coli that is bio+, makes phage particles, P1 is a lytic phage, has to go through a lytic cycle and at the end you have a lot of progeny P1, some of the P1 progeny has errors instead of phage DNA packaged it has E.coli DNA, may contain bio+, general transduction, if the new phage progeny bio+ phage infects E.coli bio-, the bio+ will be inserted in to the E.coli cell, needs to undergo homologous recombination to insert into host cell genome, most of the phages have phage

DNA some package incorrectly and package bacterial DNA instead, phage now a conduit vector for taking DNA from donor to recipient cell,

-Specialized transduction = involves lambda phage, when lambda undergoes lysogeny it inserts in attB site of bacterial cell, attB is by galactose and biotin genes, it is not a random insertion, goes through lysogeny, if conditions change and it wants to go through a lytic cycle then it needs to excise itself from E.coli genome, most of the time it excises correctly and only takes out its own DNA, sometimes it can take up some of E.coli genome with it such as galactose and biotin genes surrounding the attachment site, gets packaging into the lambda phage, when it later infects an E.coli bio- the gene is transferred over, if lysogenized again then bio+ genes with phage DNA end up being inserted, get a bio+ E.coli cell,

6. (a) What is similar and different about replication of E. coli chromosome, plasmid replication, phage lambda DNA, replication of F plasmid during conjugation.

-all circular, some when replicating are very similar, theta replication where part of the circle unwinds and you have leading and lagging strand synthesis, or rolling circle = some plasmid go through wither one, E.coli chromosome is like theta, F plasmid during conjugation is like rolling circle
-the one that can go through both lytic and lambda phage is phage lambda, initial state when making a lot f its DNA it goes through theta, when ready for packaging DNA it goes through rolling circle

(b) Where do we encounter these cis elements: oriC, oriT and R6Kori and what are their functions?

7. What roles does DNA methylation play in a bacterial cell, such as in E. coli?
-Two methods of methylation we encounter in E.coli
-DAM – adenine base is methylated, G A-(m) T C
-ACN – cytosine base is methylated CC-(m) G T C

Where we see methylation:

1) Chromosome replication (timing): dictated by methylation state, during DNA rep. the newly synthesized DNA is not methylated right away, in the hemi-methylated DNA (new strand not methylated, parent strand is), in this state the second round of replication does not occur because of SecA preventing RecA from binding, need both strands methylated before new round of replication
2) Restriction and modification – protect bacterial DNA against restriction endonucleases produced by the cell itself, to recognize its own DNA vs. foreign DNA it makes RE that recognize specific sequences, the RE cannot cut methylated DNA, therefore foreign DNA is un-methylated such as phage DNA and can be digested
3) DNA Repair – methyl directed DNA repair, polymerase when it replicates DNA makes mistake along the way, parent strand is M new strand is not M, way for E.coli cell to know which strand is new, new strand would be the erroneous strand so it can tell the new strand needs to be corrected
4) Methylated cytosine makes thymine, base excision repair would not work, thymine occurs naturally in DNA, not necessarily remove that part

T4 is a linear pahge, never circularizes, leading-lagging strand, lambda circularizes when integrating into bacterial cell therefore undergo theta and rolling circle, theta and rolling circle only occur in circular DNA