Advantages Of DNA Damage: AID, SHM, CSR

The human body is infected by a massive variety of different pathogens new and old every day and so a huge collection of different specific antibodies are needed to fight against them. Once a B cell is activated it is converted into an antibody producing factory, the plasma cell. They contain more cytocolic component (as the cytoplasm is the ribosomal workbench for producing proteins) and less area for the nuclear regions. The consequence of genetic alterations can lead to cancer or disease and so needs to be carfully coordinated using the Activation Induced Deamination (AID) protein which can turn cytosine to uracil in the antibody loci affecting CSR but mainly SHM. AID is a hydrolase that deaminates the cytosine base and replaces it with a uracil by hydrolytic removal of the amino group on the 4th position of the pyrimidine ring creating a U:G mismatch. If this mismatch is not repaired before it is replicated then one strand with a completely incorrect segment will have been made picture here (animated biology with Arpan) In the germline centre B cells where the mutation is being repaired, instead of recruiting polymerases with minimal error, highly error-prone polymerases are recruited leading to more variety. It could be processed as a point mutation in SHM or the DNA lesion caused by this mismatch could lead to DSBs in CSR. The switch regions have a high density of the WRCY motif which is where AID likes to mutate. The 14-3-3 transcription associated protein is recruited to this area and pulls in AID with it. Either MMR or BER mechanisms will then cause DSBs on each switch region allowing a segment to be excised and ends to recombine using error prone polymerase in NHEJ leading to more chance of mutation.

Somatic Hypermutation (SHM)

To create a great variety of antibodies able to interact with the maximum number of pathogens, the cell can tweak the variable region (Fab region) of the antibody picture here by changing the proteins that make up the variable region, more varieties of antigens can be recognises. These are not post translational modification, it is mediated during the transcription process. Site specific recombination plays an important role in producing a diversity of immunoglobulin genes in the bodies B cell. An immunoglobulin heavy chain gene consists of several families of coding regions called; Variable, Diversity, Joining (VDJ, the variable portion) and Constant (the constant region). Mutation is very active in the genes of the variable region, also present but less in the constant region. The B cell starts to modify the antibody by editing the VDJ (Variable Diversity Joining) region by SHM; deletion and recombination of remaining ends. In humans there are 65 functional V sections, 25 D sections and 6 J sections of genes and therefore this means there are 10,530 different possible combinations of these to give a broad spectrum of variable regions. The light chain does not have the D segment. The rearrangement is done by the random selection and joining of one gene from each of the V, D and J sections. First the D and J regions join and the intervening DNA is discarded, next the V and DJ regions join and again the intervening DNA is discarded. This rearrangement process is done by somatic recombination; gene segments that can be recombined have specific sequence motifs adjacent to them the RSS motif (recombination signal sequence motif). A protein complex containing the product of the recombination activator genes are RAG-1 and RAG-2 bind specifically to the RSS motifs, however it is random to which gene segments RRS motif they bind to. The RAG proteins bring together the gene segments to be combined and cleave the DNA at the junction of the gene segment and its RSS motif. The cleavage forms a hairpin of DNA at the end of the gene

segemts and double stranded breaks at the ends of there RSS motifs. DNA dependant protein kinase (DNA-PK), Ku, Artemis and a DNA ligase/XRCC4 dimer form a large protein complex with the RAG proteins. These RSS ends are joined to form the signal joint and a closed ring of DNA that no longer plays a role in the recombination process. The DNA hairpins at the end of the segments are cleaved and TdT (Terminal deoxynucleotidyltransferase) is recruited and adds additional nucleotides to the ends of the DNA strands. The other enzymes of the complex ligate together the two ends of the gene segments and completing the recombination process.

Class Switch Recombination

The constant region is responsible for determining what type of antibody will be formed. In the human body there are 5 main isotypes of immunoglobulin IgA, IgD, IgE, IgG and IgM. The antibody is named after the region that is present directly after the variable region eg IgG (Immunoglobulin G), IgM is the first type of antibody made in response to an infection by a virgin B cell. When a known pathogen enters the body the complementary antibody recognises it and binds. T cell confirms it is a complementary pathogen/antibody and activates the B cell using interleukin 4 (IL-4) to start producing more of that type of antibody. ENGULFING SENDS SIGNAL, MHC COMPLEX, SHOMUS BIOLOGY ANTIBODY CLASS SWITCHING… If the pathogen is not recognised by IgM then the aintobody class can be changed to anotother immunoglobulin isotype by class switch recombination (CSR). The antibody class is defined by the heavy chain isotype and the CSR is driven by an enzyme complex recombinase wgich always makes two cuts and recombine the DNA. The first cut and recombination will always happen just before the mu chain . The second cut is dependant on the cytokines that are present in the germinal centre which are produced by T follicular helper cells (Tfh). The cut will happen at just 1 of 3 recombination sites within the constant region; right before the gamma chain, epsilon chain or alpha chain. If IFNgamma is in high concentration the recombinase enzyme will cut firstly before the mu chain and then a second cut before the gamma chain therefore the excised piece of DNA will have the genes coding for IgM and IgD. The cut ends will recombine then gene will express firstly the variable region and then the next in line would be the gamma chain therefore giving the IgG isotype. IL-4 secreted from Tfh the recombinase will make its second cut before the epsilon chain so IgE will be expressed. If TGFbeta is secreted recombinase will make its second cut before the alpha chain so IgA will be expressed. The Tfh cell will secrete the cytokine depending where the pathogen was found; If the pathogen was located in the spleen or non mucosal lymph node INFgamma would be secreted so as to increase the population of IgG as it is a broad spectrum antibody able to get into peripheral tissue and can exert a lot of different effector functions. If the pathogen was detected in mucosal lymph nodes more TGFbeta is produced to make more IgA which can be secreted into mucosal lymph nodes and clear infection more efficiently. IgE isotypes are predominantly in mucosal tissues bound to the mast-cell surface on the Fc?RI receptor and production increases using IL-4 and IL-13 in an allergic response. Also very good in defense against parasites because it coats eosinophils which degranulate their content onto the parasite which kills it. (picture here from katherina brandl)

As the immunoglobulin genes rearrange, the B cell matures and the immunoglobulin genes are expressed. The heavy chain gene still contains DNA between the VDJ region and the constant region, rewordthis is left in the DNA but is spliced out of the RNA after transcription has taken place. The Mrna strands for the light and heavy chains are both translated and arranged to form an immunoglobulin unique to the B cell and which will fight against a specific antigen. Therefore

the B cell will try a massive array of antibodies with a different constant region and VDJ genus combination so that it can find the specific antibody that is most effective for neutralising the antigen. Once the most effective antibody is found its population is increased to fight the infection.

Causes of DNA damage, BER, NER and SOS

There are 4 main types of DNA damage; the presence of unwanted bases and single strand breaks by damage induced from X-rays or oxygen radicals. UV or Polycyclic aromatic hydrocarbon damage causing bulky DNA adducts of predominantly thymine dimers or 6,4-photoproducts. Interstrand links or double strand breaks by X-ray or antitumor agent damage. Fourthly mismatch damage where incorrect nucleotides are paired together (T-C and G-A) is simply due to replication errors by the cell itself.

With the help of SOS response the cell can stop the cell cycle progression, check for the DNA damage, repair with error prone repair by reassembling the replication fork and restarting the process of DNA replication or using error free repair. A universal DNA repair response by both prokaryotic and eukaryotic cells. When the cell is under stress and the DNA gets DAMAGE OR A DAMAGE RESPONSE causes by UV radiation or oxidative stress from ROS for instance. The moment the cell is under stress the first step the cell needs to undertake is to halt the cell cycle progression. This is very important as if the cell was to progess to the next stage with said damage it will amplify in the next generation. The SOS response uses two main proteins; lexA and recA. LexA is an inhibitiory protein made up of one activator domain and one repressor domain which while bound can inhibit the expression of the SOS genes. While the cell is under no stress and the DNA is undamaged, the lexA dimer is able to bind to the promotor region (Olex) of the SOS box (a collection of genes coding for repair processes). Some of the proteins encoded by these SOS genes are uvrD, umuD, recA, uvrA, polB and lexA itself. So when there is no need for the DNA repair processes the genes are inhibited however when there is damage the recA proteases function is activated. RecA destroys the lexA protein therefore stopping the inhibition of the SOS genes. Uvr portion codes for error free repair like NER while umu codes for error prone repair. 2 umuD proteins combine with umuC to form a checkpoint effector complex. It checks the DNA for damage at DNA damage checkpoints. If damage is found it will stall the replication fork and stops the cell cycle progression (cell cycle checkpoint arrest). 2 umuD' proteins, when combined with umuC will activate the translesion synthesis of the DNA and rearrange the replication fork so it can continue the process of replication again

Base excision Repair (BER) is a method used to removed unwanted bases caused by X- rays and oxygen radicals. The glycosylase enzyme is used to remove unwanted bases without cutting the sugar phosphate backbone of the DNA. If an unwanted uracil is left in the DNA it can lead to transition mutations, uracil glycosylase cuts out uracil. Hypoxanthine can lead to mutations and halting of protein production, hypoxanthine glycosylase is used to remove this unwanted base. 3 methyl adenosine (by deamination, check sallys 1st vid @ 30 min) is an even more damaging base which will stop the cells replication process, 3 methyl adenosine glycosylate cuts out 3 methyl adenosine. Now the DNA is lacking a single base, this is called an AP site (apurinic/apyrimidinic site). AP endonuclease is used to make a small nick on the 3' side of the AP site in the sugar phosphate backbone, this allows DNA polymerase I to be recruited. It uses its 3' to 5' exonuclease activity to cleave a small stretch of the DNA around 2-5 nucleotides. Simultaneously the DNA Pol I fills in the gap behind it with the correct

nucleotide sequence. Only a small nick remains which a ligase enzyme will repair. Hand drawn diagram

Nucleotide Excision Repair (NER) is a high energy consuming repair process of lesions formed by UV radiation. The radiation causes the bases to form covalent bonds with an adjacent base. Commonly 'T' dimers are formed, these lesions are detected by specific enymes uvrB and uvrC. These two enzymes use energy from energy from ATP hydrolysis ? ADP + Pi to from a dimer; the uvrB/C complex. This complex binds to the DNA lesion using another hydrolysis of a second ATP molecule. uvrB will load uvrC to the specific part of the lesion with energy from a third ATP molecule. uvrC acts like an endonuclease and makes two cuts around 15-20 nucleotides apart either side of the lesion to the sugar phosphate backbone. A helicase like activity protein, uvrD, is then recruited and uses energy from a final ATP molecule to separate the lesion region from the opposite strand of the DNA by breaking Hydrogen bonds between bases. DNA Polymerase synthesises the strand to fill the gap while still leaving a nick in the backbone. Finally DNA ligase enzyme is used to form the phosphodiester bond to repair the nick. Hand drawn diagram

Double strand break repair

X rays and antitumor agents are the cause of double strand breaks and they can be repaired ny recombinational processes HR and EJ 3 different functionalities required for repair of DSBs are detection of damage. Ability to control cell cycle and transcriptional progams in response to damage. Thirdly a mechanmism for catalysing the repai of lesions. The MRN complex(Mre11, Rad50 and Nbs1) recognisesthe break and recruits ATM (ATR usually for a single stranded break). This will bind to the Nbs1 region of MRN to creae the ATM homodimer. ATX is a new member of the ATM/ATR kinase family which can be actibated by UV light and in response to DSB. Ataxia Talengiactasia Mutated (ATM) is a Ser/Thr kinase which once damaged autophosphorylated itself which initiatesd a signalliog cascade by phosphorylating Ser/Thr residues ion other proteins. ATM kinase phosphorylatyes Seribne 139 on histone H2A.Z to form gamma symbol2A.X. gamma symbolh2a.x recruits a huge number of different proteins, for example Checkpopint kinase 2(ChK2) which is now an active Ser/Thr kinase. ChK2 along with ATM activates/phosphorylates a protein, P53 (encoded by tumor supressior protein 53 in the cytogenic location; 17p13.1) by adding a phosphate group to Ser 395 MDM2 protein therefore unbinds from P53. P53 increases expression of genes for DNA rep[air, stopping the ncell cycle and inducing apoptosis. MDM2 is a P53 inhibitor therefore P53 is only active when DNA damage occurs. MDM2 is bound to the transactivation domain which stops function, it also adds a uniquitin group to P53 meaingiong it can be destroyed by a proteasome.

The role of chromatin

DNA is compacted into chromatin around histones, its configuration undergoes dramatic remodelling to allow access of DNA repair factors anmd removal of lesions; "Acess,Repair,Restore". Chromatin relexation and recondensation is highly regulated and has highATP consumption. This requires action of 4 chromatin remodelling complexes; Imitation switch, Chromatin DNA helicase binding, Switch/sucrose non-fermentable and INO80 subfamily.

Chromatin remodelling

MRN complex binds and recruitsATM kinase, C-Abl phosphorylates TIP60 which acetylates ATM leading to the full activation of kinase. TIP60 binds to and acetylates methylated histones leading to the formation of the open relaxed structure. ATM kinase phosphorylates histone H2A.X to form gamma symbol H2A.X which spreadfs away from the DNA break into megabase sized domains. gamma symbol H2A.X is recognised by MDC1 adaptor proteins. Phospho-MDC1 recruyits RNF8 ubiquitin ligase. RNF8 ubiquitinates histones H2A on K13/K15 which facilitates recruitment of key adaptor protein 53BP1 which serves a critical regulationof DSB repair pathway choice and promotes NHEJ repair by inhabitating DNA resection.

Non Homologous End Joining

A quick, errorprone repair involving deletion of a sequence and chromosomal translocation. NHEJ is mutagenic as it removes several base pairs out ofg the break site. This is mediated y sensor Ku protwein heterodimer Ku80/Ku70. Ku recruits the DNA-PKcs (protein kinase catalytic subuinit) DNA-PKca is actuivated and phosphorylates proteins required for NHEJ e.g Artemis enzyme; this makes a clean breakl by removing single stranded ends. Protein complex of 3 proteins (Ligase 4 enzyme, XRCC4, XCR)bind to l,ink the blunt ends together. In the G1 phase therefore no BRCA1 is madde so doesn’t dephosphoryklate 53BP1 therefore allowing NHEJ

Homologous Recombination: DNA information copied accurately from sister chromatids. During S/G2 phase; 53BP1 phosphorylates and binds angain but now BRCA1 present therefore dephosphorylates and pushes 52BP1 off the DNA which allows CtIP to bind. MRN recruitys CtIP after 53BP1 is pushed off, CtIP recruits EXO1 which removes ends from each strand. Single strands get coated in RPA which is then switched to Rad51 by BRCA2 (BRCA2 facilitates exchange of RPA off and addition of Rad51 on to makle the Rad51-ssDNA nucleofilament) Rad51 pairs the 3’ end with the sister chromatid. DNA polymerase elolongatwes the strands, DNA bridges the gap and is ligates, this is the Holliday Junction. This is finally cuit and resealed to separate the chromatids.