Lecture_11: Identifying Host Factors

October 22, 2019

- Why screen for Host Factors
  - Basic knowledge to understand how viruses hijack cells
  - Applied Aspect
    - Most antiviral drugs target viral proteins, and are usually virus-specific and vulnerable to resistance.
    - Drugs that target a host protein important/essential for viral infection might have broader spectrum and less susceptible to resistance as human cells evolve more slowly than viruses. The Anti-HIV drug that target cellular protein CCR5, the cellular coreceptor for virus entry evolves less slowly than the virus. This is a host protein that can be trageted to counter attack virus infection
    - Genome-scale screening technologies allow for comprehensive analysis of host factors involved in virus infection and thus help select potential targets for antiviral drug therapy
  - Common Screens
    - Y2H screens can be used to detect virus-host interactions. Here, a virus is interrogated against each of the host factor from library of genes and then checked for interaction.
    - Interactions maybe indirect or not required.
    - Proteomics is able to detect indirect interactions via assays like pull down assays and consecutive mass spectrometry. Viral proteins can be tagged and then the proteins that come down can be detected. This gives information on both direct and indirect actors. Proteins are then determined by mass spectrometry.
    - RNAi can detect all important essential proteins. It is commonly used to knock down, not knock out protein. RNAi assays identify:
      - Host factors in protein complexes
      - HFs involved in signalling pathways and cellular processes relavant for infection
      - HFs binding to viral non-protein components
    - The first RNAi screen in mammals was performed for HCV cells in 2007. Later on its was used for HIV, WNV and Influenza.
  - RNAi Screens: Sources of siRNAs
    - Long dsRNAs are potent and routinely used in insect cells screens. Dicer cleaves the sequence and is loaded into RISC. Used in insect cells as it contains a potent RNAi system.
    - Due to induction of type I interferon response, long dsRNAs cannot be used in verterbate systems as this would lead to induction of viral anti-viral system which would prevent infection. Infection is necessary to identify host factors.
    - siRNAs and shRNAs (short hairpin RNAs) which knockdown genes in sequence specific manner are used in mammalian systems. siRNAs are potent, but require transfection and are transient neccessitating a short assay window. shRNAs are derived either in plasmid form or viruses (lentiviral or retrobviral) and can provide long term knock down. Viral vectors are not preferrable as they will trigger the immune system.
    - Overtime siRNAs will be eventually degraded and the level of specific host genes will go up which would allow virus to replicate.
    - Cells must be then challenged with a virus and assay-read out chosen, typically using micro-titer plates or image based GFP. These are the tools used for high throughput measuring of viral replication. Viral ag production can also be used, but it is harder and more laborious process as it requires the use of Abs
    - Luciferase and GFP offer instant readout of the virus infection. The more luciferase is active, the better is viral replication.

        - RNAi suffers from weak silencing and/or off target effects. Sometimes the protein is not knocked down properly (about 70 -80% of time). The goal should be to knock down at least 50% at minimum. The lower the better. The off target issues, like CRISPR is problematic here. There is a possibility that another mRNA that encodes a similar sequence can be targeted leading to compound effect. The high specificity allows for targeting but does not preclude the possibility of off target effects.
    - Screen Strategies
        - Techniques for interrogating the importance of protein in infection.
        - In microtiter plates, each of the 96 well contains siRNA for a specific gene from the host. The siRNAs enter the cell and then there is a wait for 48-72 hours. This is required for siRNA to achieve their role, that is knock down the gene. Thereafter the virus is introduced, and then there is 18-48 hour incubation. The virus contains some sort of reporter gene to measure replication. The signals from each are then measured. Array cell-based sequencing → GFP read and Pooled based Cell-Screening → Luciferase. The intent is to look for genes that cause decrease in virus replication which suggests that specific protein required by virus has been knocked down from the system.
        - Off-target can be tested using siRNA that targets protein but from a different region which indicates that specific protein of interest was knocked down. Different cell lines can lead to different and conflicting results, so an independent cell line is used.
        - Pool Cell based Screening → Transfection of shRNA library of plasmids. There can be libraries for entire genomes, i.e. for each specific gene. Transfection is performed such that each cell gets only one plasmid or none. This achieved by having a higher number of cells than plasmids. Next, cells that have been transfected are selected using some antibiotic resistance genes to isolate cells containing plasmid. Thus, each of the remaining cell produces a specific group of siRNAs from the plasmid. Thereafter, the cells are infected with cytolytic viruses that kill the cells. The cells in which an important host factors is knocked down will allow for survival for cells. If the cell dies, then the specific gene product is not critical for virus replication. Thereafter, plasmids are isolated and sequenced to determine what shRNAs are formed. This must be typically followed by further systemic testing.
        - There is the complication that cells might die if the expression of essential genes is knocked out.
- Reproducibility
    - Reproducibility of independent large scale screens is very low → even if the same technology is used.
    - General → large-scale methods suffer from low sensitivity and specificity. A lot of times they miss the genes that are important. Sometimes things cannot be knocked down to get the results. There is also a problem of false positive.
    - Specific → differences in the experimental setup, cell culture systems, virus isolates (mild vs virulent strains) and siRNA pools (3'UTR vs coding region).
    - Other factors that influence the outcome of RNAi screens are criteria used for filtering false positives and identifying the final set of interactions.
- Reproducibility of HIV RNAi screens
    - From the three studies, only 3 proteins were found to be in common. There is a difference in level of commonality between two groups. These results put a lot of doubt about reproducibility.
    - Differences likely due to reasons mentioned above.
    - Largest overlap between the studies was for studies that used the same cell lines and focused on the entire life cycle.
- Positive and Negative Factors can be identified
    - Negative factors are Restriction factors that inhibit viral growth in the host. Positive factors aka host factors allow the virus to replicate in the cell.

- RNAi screens do not only identify HFs, but also antiviral HFs that inhibit virus replication. Knockdown of these antiviral HFs lead to increase in infection rates.
- In normalized RNAi screening results, the number of inhibitory HFs that represent proteins involved in intrinsic host defense is about as high the number of stimulatory HFs.
- Statistics are very important in designating any gene as HF or IHF status. Ideally, the a robust Z score with $p < 0.05$ and 2-fold cutoff is suitable for determining the nature of gene under question.

- Organizing and Understanding Results
  - The protein needs to be placed in the cellular context to understand the mechnaisms involved the help in replication: like microtubules, translation etc.
  - If multiple proteins are identified are part of complex, then it provides support for the hypothesis that protein is involved in interaction with the virus.
  - Multiple proteins identified for a specific process can increase confidence.
  - However, the more important the protein for cell, the less likely it is that the protein is a good target for drugs due to likely side effects.

Lecture_12: Identifying Host Factors
October 29, 2019

- A whole genome screen for HIV Restriction Factors
  - Host factors → help virus in replication. While restriction factors function against virus. When these proteins are knocked down, the virus replication increases.
  - The goal of screening was to identify HIV restriction factors.
  - Method involved using RNAi screen of 19,000 human genes
  - Results: 52 genes validated to reduce HIV infection and studied Paf1 complex proteins in detail
  - It was found that PAF1 complex establishes an anti-viral state to inhibit HIV.
    - Hela-cells with CD4 receptors were pooled with 4 siRNA for each gene in a single well
    - From the initial screen, 192 primary hits were obtained
    - Thereafter, 183 were selected for further screening
    - The 183 genes were then pooled with 4 siRNA in triplicate, out of these 114 hit primary hits
    - Out of 114, 52 primary hits were chosen with 4 deconvoluated siRNA/gene
- Readout of Assay
  - Fluorescent green means that virus has replicated, but green intensity does not change and it was already too high. Thus, they modified the HIV.
  - Modified HIV with limited viral replication allowed for detection of changes in intensity of fluorescence. This involved viral attenuation. Thus, 89.6R is used to observe changes in fluorescene.
  - The virus is limited to single round of infection in the modified form.
  - Proteins in the same pathway will display similar results.
- Examples of Results
  - AP2M1 helps with trafficking and knocking it down results in increase of viral replication; knocking PAF1 down results in an increase in virus replication.
- Primary Screen Hits
  - The more GFP foci, the more the cells that are infected it and z-score tells us the percentage of population that is green.
- Validation of selected 52
  - Deconvulating siRNA involves using single siRNA to test for RNAi against host factors. So if there are 4 siRNAs each siRNA is individually tested against the gene. So if all four siRNA for a single gene consistently knock down IHF, that provides concrete support for the hypothesis that mRNA of a host restriction factor is being knocked out.
  - With siRNAs, sometimes there is a possibility of off target effect where siRNA.
  - All four deconvoluted siRNA should show similar results → an increase in GFP foci.
  - The more the results are seen, the better it is.
  - It is unlikely that all four can have inhibitory effects via suppression of restriction factors.
  - Ideally, the proteins with 4/4 deconvoluted are best for results.
  - CTR9 + PAF1 + RTF1 + CDC73 + LEO1 have consistent effect as they are part of the same protein
  - Ideally, we also need to perform RT-PCR to confirm mRNA levels are actually decreased in case of treatment with deconvoluted mRNA.
  - Lower mRNA does not always corrleate to protein degradation. Hence, the protein should be interrogated using western blot. In this case, Paf1 levels did go down.
  - They can be used as potential antiviral drugs by upregulating their expression.

Yeast Hybrid Systems

- Advantages of using Yeast as Model Host
    - Small eukaryotic genome with only 6000 genes
    - 75% of 6000 genes have been characterized and their functions are known. Yeast cellular processes have 50% genes homolog in plants and animals
    - Deletion libraries → yeast knock out libraries (YKO) of non-essential genes covers 80% of yeast gene
    - Down-regulate library: yeast Tet-promoters Hughes Collection of essential cover 15% of gene. The tet-promoter lowers transcription level in presence of tetracycline. The more the tetracycline is added, the lower the transcription level.
    - Thus, yeast is a powerful system for genome-wide screens for identifying the host factors involved in replication

- Disadvantages of using Yeast as a Model Host
    - Yeast may only support replication of a limited number of plant and animal viruses which restricts its widespread use.
    - Yeast does not have any antiviral RNAi or innate response pathways. Thus, results may not reflect the natural cellular environment.
    - Tissue tropism and tissue specific host factors cannot be identified host.
    - Some host proteins might functionally different in yeast vs host cells → not a real concern most of the times
    - Genetic manipulation of yeast cells by introducing plast or mammalian gene into yeast might address some of the above issues and expand the use of yeast in viral studies.

- Virus genome replication can be studied in these systems. Downregulators are restriction factors and up-regulators are host factors. There is not a great deal of similarity between genes that are down-regulated and up-regulated during infection by RNA virus. In the figure above, only the proteins required for replication are mentioned.
- Categorizing TBSV Host Factors
    - Host factors are from diverse set of functional roles.
- TBSV
- P33 is encoded as the first protein and stops at UAG. To make p92, there must be readthrough through the stop codon. The latter makes the components for RdRp. Both p33 and p92 are essential for genome replication.
- A movement protein is important for infection of plants. The virus is unable to go through plasmodesmata, so it uses movemenet protein (p22)
- P22 and p19 are made from same mRNA using leaky scanning. The full genome is not replicated.
- To replicate the genome in the vitro, four proteins are needed along with p33 and p92.
- TBSV Yeast System
    - His is used for protein isolation using chromatography
    - In the second plasmid, stop codon is removed and replaced with UAC (Tyr)
    - The minigenome makes repRNA. The 5' AND 3' sequenes should be same to allow for functional promoter.
    - Rz sat is a ribozyme from satellite RNA is used. It is encoded in the genome, but is only functional in its mRNA form.

    - The ribozyme will fold into a complex structure and cleave itself. This results in formation of proper 5' and 3' end. This results in formation of replicon that can be replicated by p33 and p92.
    - P33 has a membrane anchor at its N terminus and can bind to the inner membrane. The protein p92 also has NTD, basically its p33.
- YKO Library Screen for TBSV
    - Parental → wild type yeast
    - When the host factors are knocked down, they contribute to decrease in replication of virus.
    - Usually, a probe complementary to repRNA is used in Northern blot to quantify the changes in expression.
    - For northern blot, repRNA probe is used → can be quantified if desired to compare changes in tc'n levels.
    - Normalization is used in comparison with repRNA. And then the factor of change is used for comparison.

- Validation of Identified Host Factors
    - Need to confirm host protein's importance for natural viral replication
    - Validation in Yeast
        - Express orthologous host factors from plants or animals in yeasts → should complement the function of KO'ed yeast protein.
        - Validation in Natural Host
            - Treating cells with a specific inhibitor of a host factor and see if replication goes down. The problem with using inhibitors is that they are not specific enough.
            - Knocking down endogenous host protein level by RNAi and again test for decrease in viral replication levels.
            - Overexpressing a dominant-negative mutant of host protein → basically the protein from the mutant binds to the wt and inhibits its function in dominant manner.
            - Think of Protein X-RdRp binding → a protein required for initiating transcription via binding to hairprin loop

Lecture_13 : Virus Hunting: Metagenomics

- Virus Prevalance
  - DON'T HAVE TO KNOW THE NUMBERS OF VIRUSES
  - Most viruses are thought to reside in ocean subsurfaces on both ocean and terristerial instances.
  - Biosphere → biologically based entities on earth. Viruses are definitely biologically based.
  - Holobiont → used to describe an organism and its associated microbes. Holo generally means everything.
  - There are 10-fold more bacteria than human cells and there are 100-fold more viruses in human cells than human cells.
  - Human viruses form a human virome.

- Metagenomics in Virus Discovery
  - Traditionally virus discovery requires propagation of virus in cell culture. Propagation of viruses requires amplification in cell cultures. The Nobel prize was given to researchers who discovered ways to grow poliovirus in culture.
  - Many viruses are not easily propagated in cell culture.
  - Viral culturing is critical for basic studies and medical aspect as well
  - HCV identified in 1989: Initially, cultivation was variable and very low required highly sensitive detection techniques for viral proteins. An efficient system is required for detection. Detection of viral proteins by Western Blot or immunofluorescence analysis or demonstration of infectious virus production was nearly impossible.
  - Usage of powerful tool like RT-PCR indicates that virus is not replicating properly. Also need to check for virion formation.
  - Took until 2005 to get HCV replication in cell culture (both virus and cell are important). The virus subtype was important and so were the cells important.

- Metagenomics in Virus Discovery
  - Effective virus exploration requires culture-independent methods as many viruses are not easily cultured.
  - For cells, 16S rRNA is used for classification as all cells have them. It divides life in three forms. Viruses do not share a common gene that could be used for unified phylogenetic classification.
  - Within viruses there are some common genes. For example, retroviruses can be classified using RT and RNA viruses can be classified using RdRp.
  - In last decade, culture independent and sequence independent metagenomic approaches have permitted the discovery of new viruses.
  - 60-99% of the sequences generated in different viral metagenomic studies are not homologous to known viruses
  - Agricultural and medical aspect have been the main focus of virology.
  - Many plant viruses do not cause disease → ideal for virsues as it allows virus to replicate more often without killing the host.

- Why Viral Metagenomics?
  - Biosphere virome → all the present viruses in the world
  - This is important for understanding viral ecology, viral diversity and community structure → viruses can interact with other group of viruses
  - Viruses do not hangout by themselves → hangout with host

- Understanding the viral ancestry can help in understanding the complexity of the virus replication → discovery of novel viral species helps in elucidating evolutionary gaps
- Design virus surveillance strategies for emerging pathogens → bats are used as examining new pathogens, also seeking out viruses that might infect plants.
- Discover new viral enzymes with biochemical and commerical value. Examples of important discoveries from basic studies: RT, use of viral vector and CRISPR and also being used for production of vaccines

- Selected Viral Metagenomic Discoveries
  - An explosion in metagenomic research that can aid in discovery of new viruses has taken place since 2003. 12, 000 references for metagenomics in the present times.
  - First metagenomic analyses was done for ocean using filter and concentration techniques and mega sequencing → viruses from the sample were isolated and sequenced → lead to discoveries of giant algaviruses
  - Gut microbiome is beneficial to the host and is also an important for development of autoimmune disease in some cases and several other physiological functions. Equally important are the phages that can influence the bacteria composition and population.
  - Plant viruses can be recovered from feces suggesting that humans could be vectors for plant virues.
  - Honeybee colony collapse disorder is important as fruit supply that is depdendent on pollination can be devastated by virus infection. Many farmers hire beekeepers for pollination.
  - Novel viruses have been found in hotsprings at really high temperature (74-93˚C). The viruses in these conditions are different from the normally infectious viruses. These viruses need to be significantly more stable.
  - Wild living chimpanzees are close relatives of humans and viruses infecting them can infect us.
  - High-density farm lead to massive loss of pigs due to Niphan virus infection. Nipah virus infect bats, pigs and humans. High density farms provide an avenue for rapid transfer for virues. African swine fever viruses has been transmitted around due to high density farms.

- Molecular methods for virus discovery
  - Sequence-dependent can use known primers to amplify viral genome using PCR. With this approach we can only find existing strains and species of virus.
  - Sequence-independent does not rely on prior knowledge of virus genome.
    - Random primer → amplification → sequencing → stitching of the sequences to generate a viral genome.
    - Requires Next generation/high throughput sequencing that are based on different chemistry than the standard dideoxy sequencing. This reaction can sequence tremendous amount of DNA in short amount of time and relatively cheaply compared to old mechanisms.
    - NGS has also made ancestry studies possible.

- Next Generation Sequencing
  - Illumin and 454 are earliest and proven system for NGS in viral metagenomics. Ion torrent and PacBio are relatively new method for sequencing.
  - There are different sequencing priniciples for each technique and the systems have different speed with different error rate, read length and cost.
  - 454
    - Long reads and a mature system → homopolymer misreads and very expensive. Homopolymers are when we have multiple consecutive identical nucleotides.

- For all three techniques other than PacBio there is amplification of sequence using PCR. PacBio directly sequences the DNA, but it is really expensive and has a higher error rate.

- Metagenomic Analysis
  - Sample is prepared from liquid fluid or tissues and viscous liquid samples. Thereafter, the sample is homogenized, filtered and subjected to choloroform and Dnase treatment. The DNA/RNA are extracted from the sample and are then amplified (DNA)/cDNA synthesized (cDNA synthesis) and are later sequenced using high throughput sequencing.
  - The analysis involves three main steps → sample preparating, high-throughput sequencing and bioinformatic analysis.
  - Filters can have very small pore sizes that are around 20 nm in size which allows for differential filtering.
  - Homogenization, filtration, and ultracentriguation are often necessary to concentrate viral particles
  - In ultracentrifugation, heavy particles settle first and then the organelles (medium – spin) and then the viruses in supernatant (high-spin). 100, 000 rpm is used for isolating the virus.
  - Microscopy is used to monitor the presence of VLPs → only for big viruses does light microscopy work.
  - Chloroform treatment followed by DNase digestion removes contaminating DNA and then followed by high throughoput sequencing
- Most Metagenomic Discoveries are Unknowns
  - Sequences are assembled using overlapping reads as sequencing performed using NGS generates short sequences.
  - Sequences generated by HTS are queried against homology search tools to known sequences in databases like GenBank.
  - However homology searches against known sequences cannot characterize unknown viruses as 60-99% of sample is composed of unknown samples. The unknown values are from earlier stages of metagenomics where new viruses were unkown, as more searches are performed, unknown viruses are found.
  - Depending on the similarity criteria of unknown sequence against a known sequence, the definition of known and unknown will vary. There are standards for this criteria nowadays.
- Virus Discovery and Diseases
  - Historically, diseases caused by viruses have been known before the discovery of the causative virus. Ex: AIDS, poliomyelitis and cervical cancer
  - Virus discovery were biased due to use of convenient samples from patients. Nowadays, the virus can be identified and be matched to a disease.
  - The metagenomic approach generates information from unkown viruses, some of which could be associated with diseases. With this new approach, the discovery of virus will precede the characterization of the diseases they cause, well before the pathogenicity of these agents is defined.
- Not all viruses are pathogenic
  - Most plant viruses are asymptommatic leading to minimal discovery of these viruses
  - A fungal virus that is beneficial
    - Mutualistic association between a fungus and tropical grass allow grass to grow at high temperature → unable to grow at high temperatures by itself
    - A virus in this fungus is required for high temperature tolerance → NOT THE FUNGUS
    - Fungal isolates cured of the virus are unable to confer the tolerance to the grass, but heat tolerance is restored after virus introduction
    - Infection with the virus can cause changes in fungal gene expression of proteins like anti-viral proteins etc. This effect is trickling down to plant.

- Hepatitis virus G commonly infects humans. The infection is asymptommatic and no disease is associated with the virus. HGV has been associated with a more favorable prognosis for patient with HIV infection by slowing the progression of AIDS (neutral and beneficial) → HGV is doing something to the immune system that is beneficial in minimizing the damage caused by HIV.
- Similarly, Dengue virus, a known pathogen has been shown to limit HIV-1 replication and to reduce viral load (pathogenic and beneficial) and also lowers DNV infection when co-infected with HIV. DNV caused fever and hemorrhaging. The HIV is pushing dengue down and dengue is pushing HIV down.

Lecture_14: The Human Virome and Disease

- Human Virome
  - The body can be thought of as an ecosystem with viruses and bacteria as hitchhikers.
  - A variety of creatures can be present in humans like fungi, protists and metazoans. There are virueses that in turn can infect these group of organisms.
  - Studies of virome component are in their infancy; priority has been given to bacterial studies due to their medical importance.
  - Human viriome consists of
    - Viruses that infect human cells → transient and chronic. It is estimated that an individual healthy harbors >10 permanent chronic systemic viral infections. Ex: Herpes virus, VZ virus, polyamvirus, GB Virus, HPV
    - Viruses that infect the broad array of other types of organisms that inhabit humans
    - Virues derived elements in our chromosomes (endogenous retro/viral elements → almost 8% of the genome is virally derived.) Virus derived elements in chromosomes can change host-gene expression, expression proteins, or even generate infectious virues.
    - Virus can insert in new site and use their promoters to control expression of specific genes. They allow for faster genome evolution.
    - The virome is dynamic as there are going to be several different transient virus infection and infection by different microbes resulting in evolving virome.
- Why study the human virome?
  - Virus discovery and emerging pathogen → identification of potential threats using NGS
  - Viruses associated with diseases of unknown etiology → some viruses cause infection and lead to diseases. Use it to identify virus that cause similar disease in different individuals.
  - Components of the microbiome interact with and affect other microbes →see how viruses effect other members of the microbiome.
- Virus discovery and emerging pathogens
  - In 2012, the first CV infection in Saudi Arabia spread to other parts. This CV was different because it infected multiple hosts like primates, pigs and bats. This allowed for jumping and evolution. The infection was eventually contained. This multiplicity suggested that it infects hosts using the same protein.
  - Corona virus originated from camels. These were identified using lots of sequencing. Although bats are thought to be permanent reservoirs, bats might have affected camels and transmitted it further.
  - SARS has 10% death rate because it transmitted it more rapidly than MERS and Corona virus had 40 %. Sars infected cilliated cells and MERS affected non-cilliated cells that affected their ability to aerosolize and cause infection.
- Viruses associated with disease of unknown etiology
  - If a virus that is common to several people displaying similar symptoms, it can possibly be the cause of the disease. Correlation is only part of the picture, it doesn't imply cause and effect. The virus might not cause the disease but it can predispose individuals to disease by precipitating on already existing defective state.
  - Certain disease correlate with certain viruses.
  - Many of these diseases are immune related disease suggesting that individuals might have certain genetic predisposistion that together with an overactive immune system can contribute to the symptomology.
  - XMRV has been linked to chronic fatigue syndrome. However, it was eventually proved to be a contaminant in the experiment.
- Koch's Postulate
  - These four conditions need to be met for cause and effect confirmation

    - The pathogen must be present in all cases of the disease
    - The pathogen can be isolated from the disease host and grown in pure culture. Virus are not easilly cultured in pure form as they need to rely on host cells. NGS might remedy this limitation.
    - The pathogen from pure culture must cause the disease when inoculated into a healthy, susceptible laboratory animal.
    - The pathogen must be reisolated from the new host and shown to be the same as the original pathogen.
    - If these postulates are met, there is strong evidence in support for viral agents as the causative agent of the disease.
    - Viral cultures cannot be neccesarily pure.
    - However, viruses can be positively identified as causative agent without satisfying all the criteria.
- Metagenomic Koch's Postulate
    - The concept is similar to genetic ancestry studies.
    - The letters in the figures correspond to different viruses. After the initial sequencing, there are differences in the levels of viruses between the healthy and disease mouse.
    - The arrow is indicating that certain viruses are more abundant in diseased mice than normal. Some viruses can also operate via dose type effect.
    - The diseased mice serum can then be taken and innoculated in normal mice and observed for phenotype in normal mice.
    - Thereafter, the purification of trait is carried out from the diseased individual. And there is no culturing to amplify.
    - Next, the inoculation of purified trait must induce disease in another healthy control subject.
- Components of the microbiome interact and affect other microbiomes
    - The idea that viruses act as obligatory pathogens is beginning to give way to the concept that viruses are part of the normal flora of the human body
    - Viruses can also be in harmonious equilibrium with their host.
    - Viruses that affect bacteria
        - The abundance of phage in lung may reflect their beneficial role in controlling bacterial population. There are several pahges in lung that can help in controlling bacterial populations to prevent overgowth of cells. Thus, phages are part of the environment that keep bacteria under control.
        - Phage are one the major mobile genetic elements that spread antibiotic resistance.
        - Thus, phages can be beneficial or harmful indirectly through their association with bacteria. A three way communal interactions.
- Bacteria can affect viruses
        - Depleting commensal bacteria in airways of mice by antibiotic treatment dampens the immune response to influenza virus , likely due to the role of commensal bacteria in developing and regulating immune system. The influenza viruses can affect the host more effectively as the bacteria that conferred protection against the virus are lost.
        - Poliovirus has enhanced ability to infect mice in presence of gut microbes. Administeration of antibiotics that killed bacteria made it difficult for the poliovirus to infect the host.
- Virobiota Image
    - Phages can defend the mucosal surface.
    - There are phages and mammalian viruses in the blood.
- Mouth Virome
    - Composed largely of phages that infect bacteria in the biofilms.
    - There are differences in virus composition between healthy and peridontal diseased individual.
    - There is an expansion of myoviruses in subgingival biofilm, suggesting that peridontal disease favors lytic phage.

    - These plaque viruses were predicted to be significantly more likely to kill their bacteria than those found in healthy mouths.
- Skin Virome
    - Skin is a major organ with important functions in protection and immunity.
    - Some keratinocytes release peptides that are antimicrobial. Skin is little bit hard to study.
    - Difficult to distinguish between contaminant and commensal.
    - The skin is colonized by polyomaviruses and pappilomavirus that are not believed to be associated with a disease. It has been hypothesized that the ubiquitious skin colonizing beta human papillomavirus persists on human skin due to its potential promote skin healing.
    - Also found phages using indirect evidence → viral genome in CRISPR array of bacteria.
- Lung Virome
    - Respiratory tract of healthy children has been found in both picornavirouses and coronaviruses in the absence of symptoms.
    - Studied primarily in setting of CF with a mutation in cftr Cl-channels that results in inefficient removal of mucus allowing for bacterial infection. Thus, most viruses characterized in the CF airways have been phage. CF are very different from healthy controls.
    - Phages carry genes for survival in anaerobic conditions as the amount of O2 exchange is very limited. A limited, not strict, anaerobic environment.
- Gut Virome
    - Phage have recently shown to have a unique role in human immunity as defeneder of mucous barried from bacteria .
    - Dietary habits → influence gut virome → food could be a common reservoir from which each individuals is colonized with viruses
    - Gut virome from healthy host are highly specific to individuals and fairly stable over time.
    - The gut virome evaluate over 2.5 years showed that 80% of the phages were the same. The proportions might have shifted.
    - Over the 2.5 years, a lytic phage from family of Microviridae evolved so rapidly that it could be classified as a new species by the end of the study
    - Thus, the body can serve as vessel for evolutionary event to occur.
    - The epithelial cells of mucosal barriers continously produce mucin glycoproteins that are rich in nutrients for microbiota.
    - Mucosa are highly populated by phage which bind to mucus via their capsid protein. Phages bound decrease bacterial attachement to the mucus resulting in significantly decreased damaged to the tissue.
    - The phages protect the mucus from the bacteria by infecting them as well.
- Blood Virome
    - Eukaryotic viruses including TTV and SEN virus have been found in the healthy human bloodstream, neither of which has been associated with pathogenesis.
- VirScan
    - Take a blood drop and look for specific antigens in the blood
    - Exploration detects only known viruses, not new viruses
    - Oligos are made against different coding regions of viral protein and then the viral peptides are cloned into phage genome that is associated with a capsid protein. Thus, the phage capsid protein expresses the protein on the phage. This is an instance of phage display.
    - The antibodies bind to the phage and the Fc parts of Ab are isolated using IP and the viral sequences from the phage are sequenced to identify the source of the virus based on the peptide sequence.
    - The more the peptides, the stronger the evidence by that specific virus.
    - HHV4 is the most common virus.

- Provides historical footprint of who have been infected by specific viruses.
- Limitations: cannot detect epitopes that require post-translocation mods and it cannot detect discontinuous epitopes.

Lecture_15: Beneficial Use in Agriculture

- Viruses in Agriculture
  - Natural benefits
  - Biological pest control
  - Vaccine and Ab production → viruses can be used as vector to produce tons of a protein.

- Virus Benefits in Nature
  - Polydnavirus → not really a virus, a psuedovirus (virus like particle) that is hijacked by the host (wasp) resulting in a symbiogenetic relationship. The virus is required for survival of wasp eggs in the insect larvae.
  - Pararetrovirs are found in plants. Para means beside, or like. These are not quite retroviruses, but have RT stage and are DNA virus. Provides protection against pathogenic viruses by forming a symbiogenic relationship with the plant.
  - Plant viruses form a conditional mutualism with plants in which the plant benefits under certain conditions like drought and cold.
  - Symbiogenic is when a new species is formed by the fusion of symbiotic organisms. This also explains the origin of eukaryotic cells from prokaryotes. Mitochondria and chloroplast were intially independent organism that became incorporated in the cell. In this case, viruses become incorporated within the host.
  - Mutualistic → a symbiosis that is beneficial to all of the partners.
- Viruses of Endoparasitoid Wasps
  - The progeny of infected wasps are parasitic. These polydnaviruses are described as VLPs as they can get into the host cell, but do not reproduce within the host.
  - The relationship between wasp and virus is interlinked suggesting that it started a long time ago.
  - The wasp genome has genes for replication and packaging that are no longer present in the virus. Wasp genes are also found in VLPs. There are certain wasp genes that are really important for this process.
  - Within wasps, we can get virus like particles replicating and being packaged. Once these VLPs infect some other cells other than the wasp, that is a dead end infection.
  - The wasp releases its eggs and some of the VLPs. The wasp genes in VLPs express protein that suppress the immune system of the larave. Noramlly, an immune reaction would encapsulate the eggs and prevent the eggs from developing. However, the wasp genes suppress the response which allows the egg to develop.
  - The wasp larvae hatch and consume the insect the laravae.
  - VLPs express the wasp genes that suppress the innate immune response. The infection of insect larvae does not lead to replication of a new viruses.
  - Polydnaviruses are sufficiently integrated to be considered part of the wasp, rather than independent from wasp. Thus, the relationship is symbiogenic. The mode of transmission and reproduction for the wasp have changed in this relationship.
  - Wasp need VLPs for infecting the insect larvae, but the virus can still survive without the wasp (?)
- Endogenous Pararetroviruses of Plants
  - These viruses package DNA rather than RNA. The genome is circular dsDNA. The intermediate in replication is ssRNA that is converted to cdsDNA by RT.
  - Defective endogenous pararetroviruses are integrated into plant genomes. A lot of recombination when DNA is broken occurs via NHEJ where the repair system is just looking for DNA to patch up. This is how the virus genome can get integrated into the host genome.
  - The virus *does not normally* get integrated into the host genome. This also occurs with defective proviruses of retroviruses that get into cellular genome. The virus genome that is integrated is not functional, but is rather a part of the viral genome that is missing some of the sequences.

- DEP sequences integrated into the host genome can be transcribed and the transcribed viral sequences can confer resistance against the same virus or related group of viruses by serving as siRNA in antiviral RNAi.
- DEPs can get transcribed and form hairpin via complementary interactions that can be cleaved to make viral siRNAs. Similarly, opposing promoter can make RNA sequences that are complementary that can then be cleaved to obtain vsiRNAs. Thus, vsiRNAs can be obtained from two different RNA sequences or a single RNA sequences.
- The vsiRNAs will be loaded into RISC and used to target other viruses.
- This approach is used to make transgenic plants that are resistant to viral infection.
- The plant naturally bioenginereed a strategy for generating resistance against viruses.
- There is some equivalence to the CRISPR system here.

- Plant Viruses can display conditional mututalism
  - When the virus is present, there is some sort of benefit conferred on the host. TMV infected tobacco plants survive longer after water is withdrawn than uninfected plants.
  - Mechanism is unclear but the levels of several plant osmoprotectants were higher in virus-infected plants than in uninfected plants (eg: amino acids (carobxyl and NH2 groups) and sugars (OH groups) that bind to water and retain it more efficiently.)
  - This is an instance of conditional mutualism as normally the interaction is only one way where the virus benefits, but during drought the plant can benefit from the virus. This is advantageous for virus as it allows it replicate and spread more often.
  - Cucumber mosaic virus infected beets survived cold treatments that killed uninfected plants.
  - A peristent virus, white clover cryptic virus, encodes a genes that the host uses under certain condition. This protein is actually the capsid protein.
  - Clover plant is able fix nitrogen → convert nitrogen gas to ammonia. It uses its roots and attracts rhizobium bacteria from soil that can form nodules in the roots and serve as the nitrogen fixation machinery. These bacteria require energy that is supplied by the plant. It sends sugar to the roots during nodulation and nitrogen fixation by the bacteria. This process is metabolically expensive and therefore would be suitable for the plant if this activity could be suppressed when sufficient nitrogen is present.
  - The virus somehow tells the virus to stop nitrogen fixation when there is enough nitrogen present.
  - The plant has adopted the virus due to this benefit and therefore persists. The virus is transmitted by seed (contain viruses) and pollen which maintains it in the population.
- Biological Pest Control
  - There is a limited use of biological control agents due to:
    - High specificity, limiting the range of insects that can be controlled and often requires identification of the pest insect before use. In contrast, the insecticide have a borader spectrum and work on several different pests.
    - Relatively slow effects, compared to chemical agents allowing crop damage from an infestation to continue for some days after treatment
    - Relatively high cost for initial treatment, although control maybe long lasting as the virus can persist in the population for quite a long time even after the insect is dead.
    - Lack of support for their use from large pesticide companies
    - More effort is going to be put into biological methods in future due to growing concerns about chemical use.
- Biological Control Agents
  - Using biological organisms to control damaging pests is quite limited and most of its is chemical based.
  - Biological pesticides: non-microbiological, bacteria, fungi and viruses. Bacteria Bacillus thuringiensis is quite commonly used as it produces a crystalline toxin that interferes with the gut activity of pest and

causes it to die. Bacterial use is followed by fungi and then virus. Viruses are the least used and proven efficient against multiple species of insects.
    - The non-microbiological techniques include use of predator that prey on other pests, parasites or other competing species that are not as damaging.
- Virus based Pest Control
    - Viruses that are used against pests include various baculoviruses and use of myxoma poxvirus against rabbits.
- Baculoviruses
    - Rod shaped nucelocapsid with circular dsDNA.
    - They have matrix around NC called occlusion bodies that make them extremely stable. Therefore they can last for months to years.
    - Nucleopolyhedrovirus with single or multiple genome copies are surrounded by OB. Granuloavirus have a single genome copy surrounded by OB (not giant OB). Non-occluded form are responsible for spread of infection.
    - The virus produces two types of progeny: one with OB for long time survival and the other one without OB for infection of insects and subsequent spread.
- Baculovirus Life cycle and Application to Biocontrol
    - Occluded virus are applied to plants or are released by insects. Insects eats the virus with occlusion bodies. OB in gut is dissolved and virus is released which kills the insect. Insect dies from resulting infection then disintegrates, releasing occluded virus onto plant where it can infect new hosts. Caterpillar can also shed baculoviruses by defecating and vomiting.
- Viruses to Control Rabbits
    - European rabbits introduced in Australia as a source of food in 1859. Dingles prey on rabbit but did not cull effectively resulting in population burst.
    - The myxoma pox virus gave mild disease in American rabbit, but a fatality rate of 90-100% in European.
    - Virus population released in 1950 was highly effective and reduced the rabbit population by an estimated 500 million in 2 years.
    - By 1957, only 25% in Australia were killed by myxoma due to development of resistance.
    - In constrast, resistance to viral insectidies in insect is very rare probaly due to more limited immune system in insects.
- Plant Dervied Vaccines and Antibodies
    - Infectious disease account for approximately 25% of all deaths worldwide and 45% of death occur in low income countries as they cannot afford expensive drugs or health care system.
    - New technology is needed to create additional vaccines with decreased cost of delievery, needle free delivery and heat stable vaccines.
    - Plants can be easily fed to individuals for protection against infection and they can be stable.
    - Plants carry out post-translational modifications similar to mammalian version, and minimize the risk of contamination from human pathogens (antigens and antibodies from human can be contaminated with other pathogen). Vaccines can also be delivered via plant tissues with no additional purification without any additional procesisng. This can be achieved by freeze-dried plant leaves or edible vaccines (an idea pionnered by Charles Hutchinson). Lastly, plants also afford convenient storage and elimination of health profression for delivery.
- Diagnostics
    - For TBSV, P region of protein stands for protruding domain. S is shell domain and R is RNA binding domain. T = 3 and 180 subunits of protein.

    - Peptide derived from V3 loop of HIV-1 gp120 was attached as a CTD of TBSV CP. In doing so, each of the protruding domain would be decorated by V3 loop, with 180 in total.
    - TBSV is a ssRNA virus that is cloned in DNA form and DNA sequence is added to form a modified protein.
    - Viral capsids were a little bit less in yield. The purpose was not to create vaccines, but to validate the idea that it can be used to create an assay for HIV.
    - Indirect ELISA
        - Virus particles with modified CP are attached to microtiter plate and serum from HIV patient is applied. If the serum contains anti-HIV antibody, then secondary Ab bind to Fc region and undergo chemical reaction when a substrate is added. The reaction is carried out by enzyme present in secondary Ab
        - From HIV+ patients, they would have antibodies against HIV. The more anti-HIV antibody, the greater the reaction in indirect ELISA.
- Antigens
    - The idea is to make the entire antigen. Here, an entire sequence of TBSV CP was replaced with HIV p24.
    - TBSV makes two sgRNA (the arrows indicate initiation site). P24 is translated from the larger subgenomic RNA 1.
    - Plants that have been infected with modified TBSV produce p24. The virus did not replicate as well as wt, but it did with production of both subgenomic RNA 1 and 2.
    - Biggest band is RNA, second is subgenomic 1 and then subgenomic 2.
    - Western blot on plant showed p24 was made in plants. 5% of the soluble leaf protein.
    - Similar approaches are being used for different viruses to make antigens for viruses.
- Antibodies
    - Zmapp is a form of passive immunotherapy.
    - A genetically engineered Gemini virus is injected into a tobacco plant (Nicotina bethamiana).
    - As the plant starts turning yellow, harvest the leaf material before the plant dies from viral infection.
    - Cloned humanized ab are separated from the plant, purified and turned into doses.
    - Zmapp was first tested in NHP (macaques) infected with Ebola. Six monkeys that received Zmapp survived, 3 that didn't receive Zmapp died.
    - In humans, trials led to inconclusive results as it was not efficient enough.

Lecture 16: Nanotechnology
November 12, 2019

- Nanoreactors
  - Below 100 nm → things at the atomic and molecular levels. Obviously, viruses fall into this category
  - Need organization at this level, things cannot be random.
  - Nanocontainers and nanoreactors → reaction could be on the surface of virus; some viruses are useful for imaging blood vessels and drugs can loaded in viruses and be targeted to specific tissues
  - Novel shaped nanostructure → shapes at nano level obtained from viruses
  - Batteries → viruses can be used to improve battery design
  - Virus Imprinting → taking cast of virus that serves as an artificial antibody
  - Triggered disassembly goes in hand with being able to assemble the virus
  - Surface modifications was achieved by molecular techniques (genetic tecnhiques) ??
  - Templates Synthesis → same thing as nanoreactor
  - Some RNA plant viruses can be reversibly disassembled/assembled by varying the pH and salt concentration of the medium
- Nanoncontainers and Nanoreactors
  - A lot of these technologies focus on the capsid, while the RNA in the inside does not matter that much. The focus is changing shape, attaching something to it, or loading something into it.
  - Virus provides us with scaffold for nanotechnological application. Viruses also come in different sizes 12-500 nm in terms of icosahedral capsids.
  - There are three important interfaces that can be exploited
    - Inside: Need mechanisms for cargo/substrate inclusion and a minimum size of this species to stop diffusion out of the cage through the shell pores. Viruses particles are small, but they have pretty leaky parts which can allow something to leak out of the capsid.
    - Outside: Something that projects away from the surface
    - Surface: something could be attached to the interface of capsid
  - Some plant viruses have reversible assembly and disassembly. Using changes in pH and ionic concentration, capsids can be disassembled into subunits. Subunits can be purified and then reassembled. The inside of the capsid will be positively charged which will help in driving the assembly of the particle and will stabilize the particle. Assembly can also be achieved without NA: some assemble easily while other can do it easily. This cannot be done on TBSV (capsid protein just does not work)
  - Instead of NA, some other negatively sensed particle can also be included in the particle.
  - Swelling and contraction can also be used for loading of substances using pH changes. The swelling of virus cause pore to open through which substances can diffuse and then the pores can be caused to contract.
  - Limitations of this approach include thermal stability (viruses are stable only to certain extent at different temperatures) and in organic solvent.
- Nanoreactor
  - Cowpea chlorotic mottle virus, T = 3
  - Diameters: outer of 28 nm and inner diameter of 18 nm (side to side)
  - CCMV undergoes a reversible pH dependent swelling resulting in a 10% increase in virus dimension
  - An increase in Ph can be used to cause opening of triangular pores, and circular pores (5 – fold)
  - Pores occurs at 3-fold symmetry → 60 pores open up
  - Disovery was by serendepidity
  - Mineralization of two polyoxometalate species and encapsulation of an anionic polymer inside this virion. At lower pH, these metallic compounds aggregate inside the virion that acts like a mold.

- Step 1 involves the removal of viral RNA and purification of the empty virus particle by ultracentriguation.
- Step II involves the selective mineralization of an inorganic species within the confines of the virus particle
- The diversity in size and shapes of virus particle make this a versatile strategy for material synthesis and molecular entrapement
- Anionic polymer can be encapsulated without any reaction. This allow substrates to react with other substrate,
- One of the first example of integration between technology and natural viruses.

- Nanoreactor: Organic
  - Enzyme based nano-reactor
  - Used disassemly and assembly properties of CCMV.
  - Incorporation of a single horeradish peroxidase enzyme molecule in inner cavity of CCMV
  - It can react with non-fluorescent substrate to form fluroescent substrate.
  - Viral capsid is permeable for substrate and product. This permeability can be altered by changing pH. The availibility of substrate can be controlled using the pore size.
  - The size of the particle is proportional to the pH of the solution.
  - Y axis is the reaction time that is related to enzyme kinetic. Enzymes with smaller reaction time means the enzyme is turning over more rapidly.
  - The upper panel has higher pH and more fluorescene. The lower panel has lower pH and also has lower fluorescence.
  - Enzyme reaction rate can be controlled by substrate availability which in turn is controlled by pore size.
  - Control : use control with enzymes at different pH.
- Nanocontainer
  - Bromegrass Mosaic Virus (BMV), T = 3
  - Interaction between cargo and the positive N termius of the coat protein has also been used to encapsulate gold nanoparticles
  - Tethered carobxylated polythylene glycol to nanoparticles and the subsequent self-assembly of the CP around gold nanoparticles
  - Gold particle size influences capsid proteins → size can guide the size of cage that formed around the particle. Virus particles can be encouraged to form other forms of symmetries depending on the gold size particles.
- Novel Shaped Nanostructures
  - Different types of capsids can spontaneously form in vitro
  - ___
  - Interaction between the anionic viral RNA and cationic CP residues play an important role in this process.
  - But empty capsids can also assemble
  - CCMV coat protein can form depending on the ph and salt concentration, different icosahedral capsids with Casper- Klug triangulation numbers
  - The presence of anionic polymers strongly influences the CP self-assembly process
  - PSS (poly styerene sulfonate)
  - TMV → T = 1, 3, 4 and 7.
  - When DNA sequence is fed, it can be coaxed to form a totally different structure → long Rod shaped helical capsid.
  - The presence of anionic polymers strongly influence the self assembly process.
- Batteries

- The goal is to increase SA to allow more reactions to take place.
- The tiny electrode viruses are added to nanowires during the production stage
- Phage M13 works by increasing the SA of the wire which increases the area where electrochemical activity takes place.
- Viruses can be used to produce manganeses oxide nanowires. Viruses also spontaneously align with each other: more like polymerization. These polymers were then used as anode.
- Li ions are good for battery, LiO2 can hold 10 times more charge, but does not discharge easily → can't give a lot of power back.

- Virus Imprinting
  - TBSV and TYMV both are T = 3, the general organization with different capsid proteins and different surfaces. The fundamental architecture is the same, but the surface is different.
  - Step 1: Immobilization of template virions at the surface of SNPs that is spherical.
  - Step 2: Addition of OM to build recognition layer
  - Step 3 : Removal of immbolizied virions using ultrasonic treatment to free the viron imprint particles.
  - Purely synthetic materials that simultaneously possess high affinity and high selectivity for a virus. This VIP can be used to bind back to the virus particle. So TBSV can only bind to VIP prepared using this technique.
  - Used VIPs of TBSV and then solution containing both the viruses. The VIPs of TYMV allowed only TYMV to bind but not TBSV. The interaction was likely driven by complementary surface.
  - TBSV binding might be non-specific and 80% is free which means it is not bound. For TYMV, almost 100% of the virus was bound.

Lecture_17: Medical Applications
14th November, 2019

- Medical Applications
  - Gene Therapy → there is a defective gene that needs to replaced with a correct version to eliminate the disease. It can involve replcaing, complementing or repairing the gene.
  - Oncolytic viruses → Viruses can also be used to combat cancer as some viruses have high affinity for rapidly replicating cells. It involves inducing an immune response to clear cancer.

- Gene Therapy

  - FDA Definition: products "that mediate their effects by transcription and/or translation of transferred genetic material and/or by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microrganisms. The products maybe used to modify cell in vivo or transferred to cells ex vivo prior to adminstriation to the recipient.
  - The replaced entities does not have to be protein. It can also be mRNA. With viruses there is transient expression (adenoviruses) or constitutive expression (retroviruses).
  - In vivo approaches involve infection of individuals with viruses and modification takes place in individuals. In ex vivo, modifications are made outside, and then the cells are placed back in the individual.
  - Two main categories
    - Somatic gene therapy → genetic material is inserted in some target cells, but the change is not passed on to the next generation hence it is effective only to that respective person.
    - Germ line gene therapy → the therapeutic or modified gene will be passed on to the next generation because modification is made an early cell stage like blastula. This was the case with the Chinese girls in which delta CCR5 copy was modified.
    - There are benefits to getting the gene corrected in germ line as it reduces likelihood of passing disease, but care needs to be taken as the change is permanent.
    - Germ line therapy can also take place during early cell stage or through modification of progenitor cells that give rise to the germ cells.

- Gene therapy trials
  - Cancer diseases are at the top of list of diseases that can be targeted. N is the number of trials done in 2014. 64% of resources are dedicated to curing cancer. HST transfer was used to cure leukemia in the HIV patient.
  - The diagram shows gene therapies for all possible approaches, not just viral gene therapies. Viruses are important, but there are other ways of making genetic modifications.
  - Monogenic disease (9.1%) is a disease that is caused by mutation in single gene. This would be realtively easy to target as repairing the gene should remedy the issue. Ex: Cystic Fibrosis
  - Infectious Disease like HIV that are actually chronic (chronic infectious disease).
  - The first country to approve gene therapy for human use was China. **Gendicine** is a non-replicative adenoviral vector wherein the E1 gene is replaced with a wt human p53 for a head and neck squamous cell carcinoma. The cancer is caused by mutated p53.
  - E1 gene is absoulutely essential for replication of the virus, hence the Gendicine adenovirus is non-replicative. E1 is an early gene essential for control of later stages of infection, regulation and replication.
  - P53 is required for stimulation of apoptotic pathway in tumour cells.
  - The virus introduces p53 that detects unregulated replication in the cell and causes apoptosis in those cells.

- Early trials
  - The very first successful demonstration was in 1990 when a 4 year girl was treated for adenosine deaminase deficiency which causes sever immuno-deficiency. Accumulation of adenosine is one of the consequence of ADA deficiency.
  - Transduction of her purified T lymphoid cells with a retroviral vector carrying functional copy of the ADA enzyme temporarily restored her immune system efficiency. Positive side: temporary solution for immune system efficacy elevation; negative: not permanent (Ex vivo treatment used as a proof of concept)
  - In 1999 and 18 year old died after receiving viral gene therapy. He had partial deficiency of OTC, a liver enzyme that is required for removal of the excessive nitrogen from aa and protein. Large dose of viruses hyperactivated the immune response resulting in multi-organ failure from cytokine storm (similar to Ebola virus).
  - Ideally, should use viruses that are not good ag for the immune system. Adenovirus is a really good Ag. This would make it easy for the virus to perform their function with relative fewer obstructions.
  - In 2003, individuals with SCID-X1 mutant was treated with GT (ex vivo). Mutations results in low NK cell count of the immune system. Treatment worked on 8 of 9 individual, but 4 of 9 developed leukemia due to insertional mutagenesis of retroviral vector.
  - Two important disadvantages of GT: adenovirus is very antigenic and retroviruses insertion can lead to insertional mutagenesis as there is no way to control for the insertion site.

- Viral Vectors
  - Two top contenders for GT are adenovirus and retrovirus. Naked DNA plasmid can also be used as they can be transcribed and translated. Lipofection involves using lipid droplets with cargo in liposome and the liposome will fuse with PM and release the cargo.

- Adenovirus Vectors
  - Linear dsDNA genome (36 kB) with non-enveloped icosahedral capsid. It replicates in the nucleus. Attachment to host is done by capsid proteins.
  - The projections from the virus are the major attachment proteins that bind to the cell. They are called fibers.
  - The genome has ITR on either end that have multiple functions: replication and efficiency of transcription of vector, and facilitating the packaging of virus genome. They are *not* the packaging signal, but they contribute to the process.
  - Psi region contains the actual packaging signal.
  - E1 following ITR is deleted, and becomes replication defective. The region is filled in by an expression casette. This is like an extra little chromosome.
  - Once the vector is in the nucleus, it only transcribes RNA and will be translated, but no replication will take place. If it were wt virus, genome replication would take place.
  - The issue with using adenovirus is that 80% of healthy people have antibodies against one or more serotype by six years of age. It is one of the three viruses that causes cold. Rhinoviruses causes half of cold cases and coronaviruses and adenoviruse are the other half for causing cold.
  - The solution is to select rare serotypes that are also associated with mild infections. This ensures that the immune response to virus is delayed allowing adenovirus to deliever its target before being attacked.
  - The other problem is high immunogenicity of AdV proteins. After the first virus infection, there will be memeory against it and it will launch an immune response. The response might cause an anaphylactic shock when administerd in high dose.
  - Treatment requires repeated adminstration with typically diffed serotypes on subsequent occasions.

- Adenovirus can stay there for 2 weeks and keep transcribing.
- Turnip crinkle virus → looking for virus with different resistance against → repeated exposure resulted in anaphylactic shock
- Adenovirus useful as it is a stable virus due to DNA and low pathogenicity. It gets safer and safer you remove more and more genes.

- Adenovirus Vectors
  - Advantages
    - It can be used on both dividing and non-dividing cells unlike retroviruses. Simple retroviruses have to wait till nuclear envelope breakdown hence they only work on dividing cells. Complex retroviruses are pretty dangerous so gotta stay away from that.
    - Carry upto 8kb heterologous DNA
    - Ensure high levels of transgene expression
    - Well suited as an oncolytic vector
    - Vector particles produced at high titer. A lot of viruses replicate at extremely low level, while Adenovirus can be made in lots of amounts. This step is useful for making the vector.
  - Disadvantages
    - High immunogenicity due to adaptation which is not beneficial because the virus will be attacked
    - The vector genome does not integrate into the host cell genome
    - Transient expression of the transgene
    - High levels of pre-existing immunity

- Adenovirus Vectors
  - First generation vector removed early gene 1A, a regulatory gene essential for replicaton was deleted – abolished replication competence and at the same time make more room for the transgene.
  - Current generation vectors are devoid of most viral genes contain only terminal repeats and encaspsidation signal. The more you take out, the more you can put in.
  - Viral vector particles made only in presence of a helper virus or DNA plasmids provide the missing function in trans
  - Currently we use packaging cells that stably express most, if not all viral genes required for viral assembly.
  - Target cells are treated to consistently produce viral components. Packaging cells produce the vectors.
  - E1 is provided by the packaging cell that the viral vector does not lack. Thus, the viral vector will be able to replicate and produce viral vectors.
  - The AdV vector persists as an episome in that transduced cells. The transgene expression is transient. The viral vector is replication defective due to removal of a critical viral gene as the goal is not to infect cells.
  - The replication cell episome gets diluted. The effective expression for adenovirus vectors in human peaks at 1 weak and is limited to about 2 weeks
  - The packaging cell genome contains E1A gene and hence the virus will be replicated and packed, but will not contain any E1 which will make them replication defective. For safety reason, the viral vector is inactivated.
  - Over time the replication will be lost as it is diluted with increasing division. Late genes are important for making the structural protein.

- Example Study

- Analogous to actual human disease as the gene in non functional. The virus in lung cell was able to synthesize alpha1 trypsin. Alpha trypsin blocks elastase protease which attack elastin, a major component of lung. Alpha1 trypsin protects the elastin.
- A preliminary study.
- Beta gal was used in transgene initially to test the effect. Beta gal is a hydrolase that converts xgal into blue colored substance, colorimetric reaction. When used in lungs, this protein turned lungs blue. Thus, this proved it was possible to conduct gene therapy. Thereafter, the transgene was replaced with alpha1 anti-trypsin and lung recovered somewhat from the effect.
- With proper enginerring, AdV is able to infect a great variety of cells. Generally, Fibers are important for attachment. Hexon and pIX are generally not important for binding but can be modified to bind the cell.
- Adenovirus fiber has tail, shaft and knob (C terminus). The knobs at the end bind to receptors on the cell. Approaches to target cell binding
  - Adaptors: Adaptors come in different forms. Two adaptors that are antibody are used. One binds to tail knob, while the other bind to the cell receptor. This can also achieved using peptides that mimics a natural receptors. It is also possible to use trimerized adapter where one of each monomers bind to fiber and the other end binds the cell. This would be the most effective approach. Each one end binds to one of the tip at the knob.
  - Chimeric Fibers
    - In the adenovirus genome, the sequence of the knob can be modified. In psudeotyped fibers, the knob is modified such that it can bind to a new target. Psuedotype involves using the same type of virus, but different strain. In xenotype, a different adenovirus protein is used. In knobless the knobs can be replaced with some other form of peptides. This requires enginerring one's own virus.
    - With pseudotyped Ad vectors, the fiber knob domain or the entire fiber is genetically replaced with its structural counterpart from a different human serotype that recognizes an alternative cellular surface receptor.
    - The strategy was also expanded to include the insertion of fiber elements from nonhuman Ad serotypes. This strategy, termed xenotyping, has yielded a variety of non-CAR-targeted Ad vectors including vectors with fiber elements from avian, bovine, canine, murine, and porcine Ad vectors
    - The knob-less Ad platform provides the ability to move beyond small ligands and into the use of proteins as targeting ligands.
    - Hexon is the most abundant protein in the Ad capsid and as such is an ideal candidate for ligand incorporation. The potential 720 copies of hexon could allow for a "coating" of the Ad capsid in any incorporated ligand. Although most of the hexon sequence is highly conserved among serotypes, nine hypervariable regions are found within the hexon and have solvent-exposed loops. As such, these loops lay in an ideal location for modification. Vigne et al. (1999) genetically modified hypervariable region 5 (HVR5) and inserted an integrin-binding RGD domain. This RGD motif had no effect on hexon structure or capsid stability but increased CAR-independent transduction of vascular smooth muscle cells. Further HVRs 2, 3, and 5–7 were found to be amenable to insertion of a 6-His moti
    -
  - Peptide Interactions
    - The C terminus folds last and is most likely going to be on the surface. The hexon HVR can also be used and replaced to include a receptor binding protein.

- Adenovirus Vector: In Human Use

- Cerepro® is an adenoviral vector with gene for the Herpes simplex virus thymidine kinase (HSV-tk), converts the nucleoside analogue Gancyclovir (GCV) to GCV-monophosphate. GCV-monophosphate is further converted by cells own kinases to GCV-diphosphate and finally to its toxic metabolite GCV-triphosphate which inhibits cellular DNA polymerase (→ apoptosis)
- First phosphate added by HSV-tk and next two by the cell.
- Treatment of malignant brain tumours (approved in European Union)
- Infusion of GCV → ph by HSV-TK to form GCV-P followed by conversion to GCV-PPP → halted DNA replication due to usage of GCV-PPP leading to apoptosis.
- Tumour is first removed and the surrounding region is infused with Gancyclovir and Cereprovirus. Virus and Gancyclovir targets all cells (general). Healthy brain tissue are non-replicating, hence they are unaffected. Tumour cells are actively replicating which results in DNA replication being stalled which induces apoptosis and kills the cancerous cell.

Lecture_18: Medical Applications I

- Retroviral Vectors
  - Advantages
    - It integrates into target cell genome that is permanent and can be passed down to progeny.
    - Amount of information that can be included is around 8 kb.
    - Enginnering is fairly simple to introduce and remove genetic sequences.
    - Wide cellular tropism → both good and bad depending on the purpose → can affect multiple cell types.
    - Low immunogenicity unlike adenovirus.
    - There is often no pre-existing immunity. Retroviral vectors are derived from mouse hence the likelihood of antibodies against it is pretty low.
    - Vector particles can be produced at high titers → just like adenoviruses so that a large enough dose can be generated.
  - Disadvantages
    - Mouse retrovirus that is simple and hence must wait for nuclear envelope to breakdown before it can access the DNA.
    - Ceullar targeting is difficult to achieve due to wide tropism.
    - Unsuitable for non-replicating cells.
    - Random integration of retroviral gene can lead to insertional mutagenesis. There are preferred sites, but it is relatively random.
    - Low stability in the blood stream and as a result do not last for a long time.

- Retroviral Vectors
  - The virus vector that is used must be controlled and should not be able to infect like wt. Its replication machinery is disabled.
  - LTR are important for integration and so is the packaging site (psi)
  - Replication defective vector is made and is complemented by packaging virus.
  - Vector contains LTR, transgene, psi and EP.
  - In the vector there is a transgene with eu promoter, as well as in Packaging and env DNA plasmids.
  - Env and packaging constructs are co-transfected with vector. These do not have packaging signal and thus will only provide machinery required for building the virus.
  - The packaging construct has a deletion in Env and is instead provided as an additional construct. To limit the likelihood of functional virus formation, the packaging and env are provided as separate construct. This minimizes the chances of recombination.
  - The packaging cell genome is not providing any of the virus genome particles they are all provided by transfection.
  - Within the vector particles, only transgene viral vector is present as it is the only one with psi.
  - Viral vector paritlce also contains RT and integrase along with transgene.
- Nature Paper
  - MMLV was used to to cure epidermolysis bullosa. The diagram shows the extent to which the skin was damaged.
  - Normal cells isolated → transduced with LAMB3 an epidermal anchoring protein using viral vector → grew the cells in layers in vitro → layers were assembled and grafted → overtime the skin regenerated well → skin completely regenerates about once every month
  - Both stem cells and differentiated cells were used to regenerate the skin.
- Oncolytic Viruses

- Overtime it has been noticed by physicians that some cancers can undergo remission following infection by a virus. Leukemia remission following infection by influenza virus.
- The remission was transient by viruses, but it returned after infection was cured.
- Oncolytics viruses can divided into natural and synthetic viruses designed by genetic enginerring.
- These viruses are able to preferentially infect cancer cells and multiply within the cells. This is similar to chemotherapy but with viruses.
- The difference is that viruses can self-replicate and the viruses can spread to progeny → self perpetuation like baculoviruses.
- Oncolytic viruses (OVs) have the targeted ability, either naturally or via genetic engineering, to preferentially infect cancer cells and multiply within them
- Initially it was thought that the viruses infected cancer cells and killed them. However, it became clear with additional studies that the best response occurs when tumour suppressor genes are reactivated. Cancer cells have an abnormal function and produces protein that are not normally produced. Tumour specific antigens are produced, secreted and positioned on surface. These ags can be detected by the immune system and signals the cancerous cell for death.
- Virus can infect the cells and release tumour antigens in blood, increasing the chances of detection and launching a response to tumour cells.

- Oncolytic Virus Selectivity
  - Cancer cells can't generate IFN response (some can, but most cannot generate IFN response)
  - Normal cells launch an IFN response and kill the virus.
  - In cancer, the IFN response is down and as a result it kills the cells and can spread the further
  - The absence of an IFN response in cancer makes them susceptible to cell death via viruses.
  - Targeting of cancer cells generates an anti-tumour immune response due to release of tumour antigens.
  - Depending on the type of cancer cells, it can affect normal signaling pathways. Certain viruses will thrive under this altered pathways. In cells with dysregulated Ras protein, the virus cells can thrive. The pathway can vary depending on the virus. Viruses with different cellular attack mechanism will have to be matched with pathway specific cancer cell defects.
- Animal Cancer Models
  - Xenograft Models of Human cancer in immunodeficient mice
    - Graft from a different source → xenograft
    - In this cases, human tumour is implanted in mouse that are immunodeficient to prevent attack from the immune system.
    - This is ideal because the cancer cells from humans are studied, but the negative condition is that the mouse are immunodeficient (unnatural conditions). Also, mouse immune system is quite different from humans.
- Cancers within immunocompetent animal hosts
    - The host animal immune system can be intact and even accurately mimic some of the immune tolerance aspects found in human cancers
    - The negative thing is that the cancer cells are not humans and may exhibit very different properties with respect to test viruses
    - In lab with particular mouse models, oncolytic viruses can be exceedinly effective with a single dose leading to complete and long-lasting cures.
    - In 2015, Talimogene was approved for melanoma in US. It is a modified Herpes virus and is directly injected into melanoma where it replicates and kills the tumour cells via induction of immune response. Although it has been proved, the clinical trials show that the treatment with virus lead to decrease in lesions in 16% of the patient. Of those 16%, 30% disapperaed and 70% showed decreased in size by 50%.

    - These treatments are generally used in combination with some other therapies to get a synergistic effect.
- Results from clinical support several KEY CONCEPTS
    - Oncolytic viruses can be delievered intravenously to systemic sites of metastatic disease
    - Delievery of viruses can be systemic
    - Barriers that limit intravenous drug delievery can be overcome by dose escalation (for example blood brain barrier and placenta)
    - Tumour cells are not the only targets of many oncolytic viruses. It can also attack tumour vasculature. Vasculature of tumour can be targeted to starve cancer cells of nutrients. Pox virus is one of the exmaple used for this approach of targeting angiogenesis.
    - Immune cells can be dose-limiting barriers of oncolytic viruses. Immune system that is robust can help in elimination of tumour but it can also attack the virus which can limit the virus infection.
    - Virus initiated anti-tumour immune response to tumour ag is critical for effective treatment. This works best during early stages when the immune system is highly robust.
    - This is a form of treatment that cannot be cured by itself, it needs several different approaches simultaneously.
- Adenovirus Oncolytic Therapy
    - Oncorine is a conditionally replicative adenovirus that does not replicate too much.
    - The very first oncolytic virus to be approved in China in 2005.
    - It is used in combination with chemotherapy for treatment of late-stage refractory (not responsive to treatment; resistant) nasopharyngeal cancer
    - Vector contains deletion in E1B 55K region which normally inactivates wt p53 that is responsible for inducing apoptosis.
    - Without E1B 55K replication in normal cells is prevented by p53 resulting in apoptosis.
    - Replication only in p53 deficient cells and viral replication in cancer leads to cell death.
    - 50% of cancers have mutations in p53
- Table
    - Need to know Adenovirus, Retrovirus and Herpesvirus
    - As of last year, only Herpesvirus and Adenovirus have been approved.

Medical Applications II

- Medical Applications
    - Targeted Delievery → used for drug delivery
    - Imaging → viruses that are sticky to enodthelial cells
    - Phage Therapy → resurgence due to CRISPR discovery and antibiotics resistance (phages are cool again and can be used to modify gut microbiota)
- Targeted Delivery
    - Accurate targeting is needed for gene delivery, drug delivery and imaging. Vectors deliver genetic information, and can also be used for delivering drugs and for imaging specific regions.
    - Potential Advantages of targeted delivery:
        - Reduce harmful side effects → ex: targeting cancer cells only
        - Less drug and/or treatments needed → all drug ends up at the desired site
        - Able to use drugs with short in vivo half-lives
    - Targeting requires presence of receptor-binding domain on carriers:
        - Genetically modifying viral capsid protein
        - Chemically attaching the targeting domain via bioconjugation

- Different cancer cell targeting ligands have been attached to VLPs using chemical bioconjugation reactions: small molecules, ab, peptides, proteins and DNA aptamers
- Chemical Bioconjugation
  - Linkage chemistry involves surface-exposed lysine residues on a carmovirus
  - Folic Acid → small molecular ligand that has widely used in targeted drug delivery to cancer cells → required for proper nerve growth
  - Uptake of FA into cells is mediated by FR (Folate Receptors). Most normal cells have low levels of FR, whereas in malignant cancer cells FR expression is elvated (Ex: overaia, uterian and mesothelium).
  - Approximately 360 folic could be attached to the outside of virus like particle, corresponding to roughly two ligands per coat protein
  - Doxorubicin is loaded in the viral capsule. It targets topoisomerase that is responsible for relieving strain in DNA during replication. Thus, build up of tension halts DNA replication leading to apoptosis.
- Carmovirus
  - Carmovirus is used and folic acids are added in B1 step. In B2, the virus is disassembled, RNA is removed, CP is isolated and the virus is reassembled in presence of doxorubicin. **In addition to doxorubicin, polyacid at neutral pH is used as a bulk agent to get the drug into the virus particle**. The drug and polyacid end up in the virus. Polyacid is negatively charged.
  - In A1, no folic acid is added, but the drug is present inside the capsule.
  - Addition of free folic acid acts as a competetior and reduces drug uptake.
  - In normal cells, there is no preference for Folic Acid decorated carmovirus.

Lecture_19: Medical Applications II

- Carmovirus
  - Euorpean medicine agency approved Zebov on November 11.
  - There is a chance that when virus are conjugated folic acid might add folic acid to key regions that might affect assembly. Hence, the modification is carried out prior to conducting the disassembly so the folic acid is only added to sites that do not affect assembly severely.
  - By itself, doxorubicin enters in a limited amount. PC-Dox is not decorated with FA and gets in it about at the same rate. In fPC-Dox, there was 2.5 fold increase in drug entry following 2.5 hour.
  - Free folic acid competes with binding for FR on cancer cells thereby decreasing the entry of drugs from fPC-Dox
  - There is no preferential uptake in case of normal cells.
- Chemical Bioconjugation of DNA Aptamer
  - As DNA is relatively flexible, it is possible to generate structures that mimic wild type ligand. DNA aptamers are small molecule that fold in specific configuration and binds PTK7 receptors.
  - PTK7 receptors are present on surface of Jurkat leukemia T cells.
  - MS2 is single stranded RNA virus (a spherical phage with T = 3). The idea is to put ligand (DNA Aptamer) on the surface of the virus. MS2 coat protein dimer (top is outside, and bottom is going to be inside)
  - To attach DNA aptamers to outside of the particle, DNA apatamers was modifed and R group allows for attachment to the virus. Porphyrin is a molecule that is covalently attached to the inside of the cell. In this case, the cargo is being attached to the inside of the particle. Porphyrin is a photoactivated compound.
  - The goal of delivery is different. Porphyrin activation causes production of a highly reactive singlet oxygen (in excited state) that causes nonspecific damage to the cell membranes - resulting in necrotic or apoptotic cell death. Singlet oxygen is produced by activated porphyrin
  - Washing allows for selecting particles that entered the cell, while unattached particles are removed.
  - Annexin allows for staining dead cells and are then separated from other live cells using Flow cytometry.
  - MS2-1B does not have any DNA aptamer.
  - Blue is control cell line which should not be expressing the receptor.
  - Treatment was specific for Jurkat cells.
  - SELEX → enrichement of DNA sequences using iterative binding and PCR with increasingly stringent conditions.
- Imaging
  - Non-invasive imaging has great potential for early detection and treatment of disease versus biopsy, surgery, or other invasive techniques
  - Current common imaging technologies: MRI (Gadolinium or Iron as image contrasting agents)
  - PET → Radioactive isotopes are taken up by metabolically active cells. A good way to track down cancer cells.
  - CAT → X rays slices that are reconstructed.
  - Important goal is to use sensitive imaging sensors and the ability to specifically target cells and tissues of interest
- CPMV as an Imaging Agents
  - CPMV VNPs are covalently conjugated to fluorescent dye was used for vascular imaging. Both blood flow and vasculature can be visualized.
  - Fluorescent CPMV sensors allow the vasculature and blood flow to be visualized in living mouse and chick embryos to a depth of 500 um and for upto 72 h.

- Internalization of VNPs by vasculature endothelial cells is possible due to interaction with vimentin on the cell surface (endocytosis).
- CPMV VNPs specifically label endothelial cells by interacting with vimentin on the cell surface and uptake is enhanced in the tumour endothelium.
- CPMV-specific feature has been used to image tumour angiogenesis.
- CPMV covalently modified with imaging dyes and bombesin peptides to target gastrin-releasing peptide receptors overexpressed on proastate carcinoma cells.
- The bottom panel allows for tumour identification. After pre-injection the virus particles were targeted specifically to tumours. This shows the potential of VNP tissue specific imaging reagents.

- Phage Therapy
  - Treatment of bacterial disease with phage that will infect and kill bacteria. In most cases, lytic phages are used.
  - There has been renewed interest in this therapy due to anitibiotic resistance.
  - There are different ways of treating. Treatment is carried out by directly applying phages to the wound area. In cases with bacterimia (systemic infection in blood) systemic delivery via IV can be used.
  - Potential benefits of phage therapy
    - Phages have activity against all bacteria including MDR-pathogens. Basically, there is no limit to what can be achieved with phages especially with recent rise in genetic manipulation technique.
    - Narrow antibacterial spectrum allow preservation of the existing microbiome. Heavy antibiotic treatment can cause destruction of gut microbiome.
    - Potential low level of side effects
    - Wide distribution of synthetic adminstration → limited side effects of viruses and can be spread throughout the body.
    - There is a possibility of inflammatory response. Stronger inflmmatory response can occur. Mild can be useful in clearing the pathogen.
    - Cost effectiveness → ensure that phages are purified, impurities can results from bacterial phages already present.
    - There is an improved efficacy as compared with antibiotics.
- Potential Drawbacks
  - There is a need to rapidly identify microorganism causing infection to ensure specific phages are delivered. Like the wide spectrum antibiotic, phage cocktail can be used but this can also kill good bacteria.
  - Lytic phages induce the lysis of bacteria releasing various bacterial substance. Ex: LPS (endotoxin) from gram -ve bacteria can initiate an inflammatory cascade that can result in organ failure.
  - There is potential possiblity to transfer of DNA from one bacterium to another. This can lead to development of a new microbe or even a more resistant bacterium
- Phage Therapy Studies
  - Patients with uclers → failed to respond to convential therapies.
  - In 2002: Applied a biodegradably polymer saturated with antibiotics and lytic bacteriophages to wounds of 107 patients. 96 patients were available to follow up. 67 people recovered completely and other showed significant improvement. 5 had confounding factors such as uncontrolled diabetes who are predisposed to infection.
  - This study indicated that using phages could be highly effective.
  - Problem is that phage activity is not isolated. The effect could have been due to combination.
- Examples of Studies showing Potential Applications
  - Salads are good source of infection. The idea was to treat leafy green with phages and kill the bacteria. Thus, it allows for disinfection.

- E coli contaminated surfaces can also be killed with phages. Bacteriophages are pretty hardy
- Campylobacter jejuni caused food poisoning. By treating the chicken, bacterial load in feces is reduced. It reduces chances of bacterial transfer.
- Clostridium pferigines → mortality rate is high → chicken treated with virus have 92% reduced mortality
- Kp → burn wound → increased survival from 6 to 94%

- Acienobacter baumanii
  - Opportunistic pathogen primarily associated with hospital-acquired infections.
  - Phage therapy was used to cure a 68 year diabetic patient with pancreatic patients. Antibiotics were inefffective as the bacteria was MDR.
  - Patient deteriorated over a 4 month period
  - Two labs identified nine different phages that could infect Ab and had lytic activity.
  - Phages were adminstered IV and percutaneously into abscess cavities
  - Bacteria can become resistant to phages so they first used 4 different phages on the abscess site and then other 4 phages IV and then a single phage with one from the previous 4 IV. Multi step treatment reduces chances of resistance.
  - Patient was treated for 18 months and returned to normal health.
  - Development and Use of personlized Bacteriophage based therapeutic cocktails to treat patient with a disseminated

Lecture_20: Controversial Experiments

Experiments with: Poliovirus and Influenza virus will be the focus. Medical aspects of the virus can be used for bioterrorism.

- Poliovirus
  - Picornaviridae, Enterovirus
  - At the 5'end it has Vpg, a protein that helps with replication . In term of translation, it requires IRES on 5'UTR. This is an internal ribosome entry point that is critical for ribosome recruitment. Proteins made as polyproteins that are made into structural protein. The mRNA ends with a polyA sequence.
  - The RNA itself can initiate the infection when placed in plasmid under the control of T7 promoter. There is no need for the particle itself.
  - T=3 capsip. Through the five fold, the genome is inteserted into the cell.
- Poliomyelitis
  - Affects mainly children under 5 years → lots of infection and paralysis
  - The virus is transmitted by person-to-person - mainly by faecal-oral route or by contaminated water or food → then multiplies in the intestines
  - Symptoms range from asymptomatic (most) to headache, stiff neck, pain in the limbs, gastroenteritis, malaise and severe forms of paralysis
  - Most infected cases are asymp as immune system clears the virus
  - It can invade the nervous system and cause complete paralysis in some cases
  - 1 in 200 infection lead to irreversible paralysis → usually the lower body region
  - There is positive association between poliovirus and bacteria → poliovirus binds to polysacc on bacteria → stabilizes the virus particle and helps it attach to the intestinal epithelium
  - Iron lung contains a belows that moves in and out which can change the pressure in the can. When bellows goes in, there is exhalation as pressure increase. The pressure is dealing with the chest and diaphragm; the face is not contained.
  - People can survive for a short period outside the iron lungs.
  - There will be different degress of recovery of patients.
  - Due to vaccines (both killed and live), there has been decrease in level of virus significantly.
    - Salk → killed
    - Sabin → live, but attenuated
    - Wild poliovirus population had been removed near 1973, a few imported cases were present. There were also cases vaccine associated paralytic poliomyelits as the vaccine is live attenuated as the attenutation might not have been completely effective.
- The Gloabal Poliovirus Eradication Initiative
  - Bill Gates (Bill and Melinda Gates foundation) initiated the clearing → 0.5 bn invested
  - Only 3 countries that are polio-endemic → Pakistan, Afghanistan, and Nigeria → war in this region prevents eradication as health workers are confronted with hostile environment
  - Once wild poliovirus transmission has been stopped, effective containment will depend on biosafety level 3 (BSL-3) for storing & handling wild poliovirus stocks
  - Some poliovirus is maintained for research purposes, as it might be used as biological weapon
  - Even if all wild poliovirus stocks are eventually destroyed, it would still be possible to synthesize infectious poliovirus in molecular biology laboratory
  - Therefore, the risk of resurgence of polio in future cannot be completely eliminated.
  - Eckard Wimmer did chemical resynthesis of virus with plus and minus strand

- The poliovirus genome was composed from three sections of overlapping genome. The sequence is cloned in vector.
- Phage T7 promoter is commonly used for in vitro expression.
- Plaque assay showed that virus synthesized chemically could induce plaque formation.
- Need common restriction site for ligation.
- Most viruses can be chemically synthesized.

- Influenza Virus
  - Family: Orthomyxoviridae, Genus: Influenzavirus A
  - - segmented RNA genome; helical capsids and enveloped
  - HA → attachment and fusion; NA → cleaves sialic acid → prevent clumping on cell surface
  - H N strain determined based on HA and NA
  - PA, PB1, PB2 are required for replication → components of polymerase complex. Also, NP is required for replication. NP always coats the RNA.
  - PB1-RdRp, PB2 – binds 5'cap for Transcription

- 1918 Influenza H1N1
  - Pandemic that affected 1/3 of world population at that time → about 50 million died
  - Approximately 10-20% of infected people die. A lot of healthy people were also dying due to induction of cytokine storm → the better your immune system, the worse the outcome.
  - 2000 - 2005: determined virus sequence based on viral RNA fragments from formalin-fixed lung tissues from one patient and from tissues of another victim buried in the Alaskan permafrost
  - Reverse genetics was used for construction of the genome. Using reconstruction, it was found that virus caused death in chicken, mice and replicating bronchical epithelial cells.
  - Proteins that were necessary for replication (PA, PB1 and PB2, NP) were constructed and 8 plasmids with RNA genome for replication and transcription was provided.
  - The virus most likely originated in birds (avian). It is also believed that the virus has bounced around a lot between humans and pigs (still happening today).
  - Swine flu is descendant of 1918 H1N1

- What was learned from the 1918 H1N1 Resurrection?
  - Genes from the 1918 influenza strain, specifically HA, NA, and PB1 proteins, significantly contributed to its replication efficiency and virulence
  - 150,000 -600,000 people died from H1N1 in 2009 → initially underestimated.
  - Genes from the 1918 influenza strain, specifically HA, NA, and PB1 proteins, significantly contributed to its replication efficiency and virulence
  - Two genes from the 1918 influenza strain, HA and PB2, conferred greater transmissibility of the virus in ferrets
  - Swine flu was descendant of H1N1.
  - H1 and PB2 were important for virulence and transmissibility
  - Dual use of discvorey → both good and evil

- More Influenza virus Controversy: H5N1 (bird flu)
  - In 1997 in Hong Kong, the first human deaths directly attributable to avian H5N1 virus → 334 died from infection
  - H5N1 was highly pathogenic with 60% mortality

- Sustained human to human transmission of H5N1 has not been detected → all cases of transmission were from bird to virus
- Aerosol particles facilitate human to human transmission
- Ferrets are good example for studying human influenza virus. For virus to become transmissible, 4 substitution in PB2 and two in HA are needed.
- This basically provides a template for preparation of biological weapon.

- The Ferret Animal Model for Influenza

  - Ferrets are highly susceptible to both human and avian influenza viruses
  - Ferrets are well suited for vaccine efficacy studies
  - Numerous clinical signs of human infection with influenza viruses are present in the ferret (e.g. sneezing, fever, and nasal discharge)
  - Pathogenesis and transmission studies can both be performed in the ferret model (e.g. highly pathogenic strains cause severe diseases)
  - Ferrets show similar symptoms compared to human and are used for vaccine testing as well

- Selecting For and Testing Transmission
  - Serially passaged virus in ferrets to allow adaptation for efficient airborne transmissionin mammals
  - P1 virus is non-transmissible using aerosol
  - P1 is killed → nasal tissue isolated and used as innoculum for infection of P2 ferret
  - A passage is basically transmission from one individual to another
  - After 5 passage, P6 could transmit the virus. NW is nasal wash.
  - Selection of virus from nasal region was used as these viruses are what will be selected for.
  - First you select for virus selected in nasal tissue (virus that replicate well in nasal region) and then the one that were released in nasal tissues.

- Biosecurity
  - Examples: recreating virus, virus archeaology, making a deadly virus more deadly
  - Carefulness has gone up for pathogens that can be used for bioterrorism
  - Dec. 2011: initally, NSABB decided that the article would be published with redaction of the methodological design in order to avoid potential misuse of the mutant virus. Access to all the details of the research would be provided to certain authorized researchers.
  - Feb 2012: a meeting between WHO and NSABB members took place. The result was that the article needed to be published fully
  - June 2012: The article was published
  - This controversy led to the introduction of policy changes that now allow Government Officers to review & monitor biological research involving:
    - Pathogens present on the list of 15 biological entities of potential DUAL-USE concern
    - Certain types of Experiments involving pathogens

- U.S.A. Government Policy on Life Sciences Dual Use Research
  - Foot and mouth disease virus → highly pathogenic virus that is highly stable