MIIM30014 MST01 Mock Paper Solutions

MULTIPLE CHOICE

SHORT ANSWER QUESTIONS

Apart from Retroviruses, no other +ssRNA viruses carry a polymerase in their capsid. Why don't (+)ssRNA viruses need to carry a polymerase in their capsid?

(+)ssRNA viruses can be directly translated by the host cell ribosome because they are like mRNA. In order to replicate their genomes, their genome encodes a RNA-dependent-RNA-polymerase, which is translated immediately upon infection.

What is the ribosome/RdRp clash problem? Give examples of how certain viruses have evolved to avoid this problem.

The ribosome/RdRp clash problem applies to (+)ssRNA viruses. The ribosome will translate the (+) strand in a 5'-->3' direction, but the RdRp will replicate the strand in a 3'-->5' direction. At some point, the RdRp and the ribosome will collide into each other.

Strategies around this:
1) Temporal separation of translation and transcription by bringing the 3' and 5' ends together. This inhibits ribosome binding, and facilitates RdRp access.
a) By protein-protein interaction: Polioviridae
b) By RNA-RNA- interaction: Flaviviridae

2) Separating replicative and structural protein synthesis between (+) and (-) strands.
First ORF translated only on the (+) strand. The replicative proteins are made from this strand. Subgenomic (-)RNA has a "hidden promoter." Structural genes lie downstream of this promoter: Togaviridae

3) Producing many subgenomic RNAs. RdRp skips to each different reading frame after transcription of a common leader: Coronaviridae

Explain the role of the IRES. What is the advantage of having this? Give examples of viruses that use this.

The IRES is a secondary RNA structure that replaces the need for a 5' CAP. The ribosome recognises this structure, and will begin translation. Having an IRES negates the need to have methyltransferases. Poliovirus and Hepacivirus use this.

Poliovirus also has a viral protease that cleaves host elongation factor elF4G. What does this do?

This inhibits protein synthesis from capped mRNAs. It preferentially recruits the ribosome to the IRES. Because host cell proteins aren't being made, the cell begins to die.

Does Hepacivirus have the same viral protease?
No. This is reflected in the chronic status of infection. The host cells don't die.

Norovirus uses the VPg, which is linked to the 5' end. It is like IRES in that it substitutes the role of the CAP. However, VPg is PROTEIN, not RNA.

Describe how the nucleoprotein enables non-segmented (-) ssRNA viruses to replicate their genome.

Early on: RdRp transcribes mRNAs from the (-)ssRNA template. The genes that are closest to the 3' end of the (-) strand encode for replicative proteins and get translated more frequently than those that are further away, which encode for structural proteins. In between each gene, there is a long sequence of UUUUU, which causes the RdRp to stop transcribing.

Later on: NP is the most abundantly made protein because its gene is located the closest to the 3' end. NP's role is to bind to the intragenic sequences, which prolongs the processivity of the RdRp. As it begins to accumulate, longer transcripts will be made more frequently. When all the intragenic sequences are bound by NP, RdRp can make a full length transcript, which can be replicated into a negative sense progeny of the genome.

Reoviridae is a family of dsRNA viruses. Name a member of this family. Describe the key events that happen to the virus from entry to replication. State how it gains entry into the cell, and where it replicates and how it does so.

Rotavirus:
Outer capsid proteins bind to the cell receptor to initiate receptor mediated endocytosis.
The virus capsid undergoes proteolytic processing in the late endosome. It is now referred to as an infectious subviral particle, and will exit the endosome by bursting it. It makes mRNA, which will secreted out out the pore of the capsid. This mRNA will be translated to make capsid proteins. These capsid proteins encapsidate the mRNA. Within the capsid, the genome is replicated using the (+) sense RNA as the template. The dsRNA genome is never exposed to the cytoplasm, and thus will not trigger the interferon response.

What is the genome and family of SV40? Describe the role of the LT protein in Simian virus 40. When is it made (early or late)? Where and how does it perform its function?
Simian virus 40 is a papovavirus. It has a double stranded DNA genome.
The LT protein is encoded by an early gene. After being transcribed by host machinery, the mRNA encoding LT exits the nucleus to become translated. It then returns to the nucleus to facilitate genome replication, which subsequently results in the transcription of late genes. It does this by binding near the Ori, so as to open it up, creating a single bidirectional replication fork. This allows the genome to replicate much like cellular DNA, using host machinery. Late genes were previously repressed by cellular inhibitory binding proteins, but as the ratio of viral genome to inhibitory binding proteins of late gene promoters increases, the late genes will begin to be synthesised.

Briefly explain the 'end-of-replication problem' and its overall consequence on the virus. Name a ssDNA virus and describe how it solves the end-of-replication problem.
The end of replication problem refers to the loss of nucleotides from the ends of linear double stranded DNA. Without telomerases, the viruses must find alternative methods to fill the gap in nucleotides when the primers are inevitably degraded after replication is complete. Otherwise, the genome of the virus will become progressively shorter after each round of replication.
Parvovirus uses 'fold-back initiation' to overcome this problem. The ITRs at the ends of the genome pair back on themselves, so as to function as primers.

For Poliovirus-infected cells, there is a distinct timeline that can be visualised by Western Blotting which shows protein synthesis (i) dropping, (ii) picking up, and then (iii) rapidly dropping later.

(a) Explain this phenomenon.

Solution Pathway

The initial drop is due to the blocking of host cell protein synthesis by viral factors. PV stops CAP-dependent translation by cleaving the transcription factor (TF) eIF4G (that is a part of the host's translational complex) with its 2A protease. Consequently, this allows preferential binding to PV's Type I IRES (Internal Ribosome Entry Site) which is recognised by the host's ribosome to initiate translation. Thus, PV has achieved dominant viral protein production by blocking host cell protein synthesis. This is reflected in the 2nd stage of the distinct timeline where protein production is now rapidly active. Note that PV's action of blocking host cell protein synthesis marks that host cell for death as host cell's cannot survive without translating proteins, and thus PV has a limited timeframe to generate proteins. The third stage of the timeline (i.e. drop in protein synthesis) is due to host cell death. Note that PV (*Picornaviridae* family) is a non-enveloped virus and hence lyses the host cell to release infectious progeny.

(b) PV is characterised as an acute infection. In contrast, Hepatitis C Virus is characterised as a chronic infection. Why is this the case? Be sure to compare the similarities and differences of HCV and PV.

Solution Pathway

HCV follows a similar protein synthesis strategy to PV in that it utilises an IRES (in addition to 5' and 3' UTRs) to facilitate efficient replication. However, where IRES in PV is used to initiate translation, a VPG-like protein (in addition to the IRES element) is used to initiate translation for HCV. HCV does not use a protease to cleave the TF eIF4G (c.f. PV) and thus host protein synthesis can still continue. As a result, HCV is a rather chronic infection compared to the acute infection of PV that prevents host protein production and lyses the cell upon release.

Emerging Infectious Disease (EID) are defined as an infectious disease whose incidence has increased in the past 20 years and threatens to increase in the near future. What features of a virus make it ideal to infect multiple species/populations to ultimately result in an EID in humans?

Solution Pathway

First, RNA viruses have higher mutation rates than DNA viruses due to lack of proofreading ability of RdRp and thus a mutation may result in the ability for the virus to cross species or infect vaccinated populations (by evolving against the vaccine-induced immune response). In fact, most EID are RNA viruses. Second, viruses should ideally have a broad host range (spanning several mammalian orders) which will allow the virus to have a greater potential to transfer to new hosts and infect different populations. Third, if a virus possesses a secondary receptor shared between closely related species, this may be an effective mechanism to cross from one species to another and initiate infection of a new host. (Note that this information has been obtained from the papers allocated to us in the flipclass).

The *Reoviridae* family is characterised by being an RNA virus (specifically segmented dsRNA) with an icosahedral and non-enveloped structure. Viruses that belong to the *Reoviridae* family include Rotavirus and Orthoreovirus.

(a) Explain the replication process of *Reoviridae* viruses including the entry process. A part of their replication involves replication of its genome within a inner core. Be sure to explain the purpose of this replication in the inner core (c.f. cytoplasm) in your answer.

Solution Pathway

Reoviridae viruses enter via endocytosis and then undergoes multiple changes in structure. The structures in order are referred to as the Virion, ISVP, and Core. dsRNA is a PAMP which will elicit an immune response, and hence replication occurs concealed in the inner core to prevent an immune response (especially the antiviral IFNgamma response). mRNA is transcribed within the capsid and then (+)ssRNA leaves via the 5-fold axis in the capsid structure. The (+)ssRNA

can either be used to translate virus-specific proteins or be packaged in newly formed virion particles. Once the (+)ssRNA is packaged, (-)ssRNA strand synthesis occurs inside the virion and thus the dsRNA is now formed.

(b) Rotavirus primarily replicates in the intestinal tract and is acquired by ingestion, and hence is classed as an Enteric Virus. How does Rotavirus promote its infectivity by passing through the gut?

Solution Pathway

The passage through the gut allows for processing of its capsid proteins to promote infectivity. In particular, this is the gut protease trypsin that allows for fusion activity (performed by VP4).

SV40 is a DNA virus and thus utilises a DdDp which requires primers to initiate replication. In addition, the virus' genome is circular and uses cellular replication proteins but viral *ori*-binding proteins. The replication of SV40 has been described below. Dissect each key stage of the replication process by answering the following questions.

SV40 attaches and enters a host cell to release its genome. The virus initially undergoes early RNA transcription utilising host RNAPII. Note that alternative RNA splicing of transcripts can occur to produce variations of a protein. Early proteins are translated and this includes LT. The LT protein moves to the nucleus and binds the *ori* sequence to initiate DNA replication and late RNA transcription. In regards to DNA replication, LT binds to the *ori* to create a replication bubble to allow for cellular machinery to localise and perform replication. Note that late RNA transcription is achieved via viral LT and host RNAPII. The late RNA transcripts (structural components) now move to the cytoplasm for translation into structural proteins (VP1-3). VP1-3 move back to the nucleus and together with the viral replicated DNA, the virion assembles for the final stage of progeny release.

(a) Describe the viral genome structure of SV40 and how it maximises its coding potential. (Structure)

Solution Pathway

The circular genome of SV40 can replicate in both directions where early genes are transcribed counterclockwise while late genes are transcribed clockwise. The overlapping of genes and RNA splicing (to generate variations of a protein) help to maximise coding potential despite a relatively small genome. (Note that different ORFs will result in different AAs sequences in comparison to genes that overlap and are in the same ORF to possess same AA sequences in the case of VP2 & VP3.)

(b) Explain how SV40 is able to regulate mRNA transcription to allow timing of transcript production. (Transcription)

Solution Pathway

SV40 initially uses the host cell machinery (i.e. RNAPII) for early viral mRNA but the proteins produced from the early viral transcripts later modulate the response for production of late transcripts. This initial use of host cell machinery before switching to viral factors is observed for Papovaviruses. The products of the early mRNA transcripts include the protein LT (Large T) which binds to the *ori* sequence of the DNA to drive DNA replication. Initially, the late promoter is repressed from late gene RNA transcription due to Cellular Inhibitory Binding Proteins (Ibp) bind to the DNA. However, upon increasing amounts of LT, this generates more DNA copies, and thus with increasing no. of DNA copies, the concentration of Ibp is decreased where it eventually becomes insignificant. Thus, the SV40 late promoter is now released from repression of Ibp and late gene RNA transcription can occur to the full extent.

Influenza Virus causes the common flu but can cause death in immunocompromised individuals (e.g. the very young and the very old). It is a helical segmented (-)ssRNA virus which is considered as an re-emerging virus. In particular, the strains H1N1, H5N1, H10N8, H7N9, H3N2v have shown signs of re-emergence or emergence.

(a) In order to identify the amount of virus in a patient's sample, researchers can perform a Haemagglutination Assay. The ability to measure the amount of viral particles is based on the virus' ability agglutinate RBCs. Why is the HA Assay considered as not sensitive? Furthermore, there are other assays that can be used to give a readout of the amount of viral influenza particles. This includes Electron Microscope, Quantal Assay (in Eggs), and

Plaque Assay (in MDCK Cells). It is important to understand the technique/assay to interpret the results correctly. Why is this the case?

Solution Pathway

The HA Assay is not sensitive as it requires a large amount of viral particles (10^4) for agglutination of RBCs and hence detect any visible changes for diagnostics. Different techniques/assays produce different viral counts. For example, Electron Microscopy measures all viral particles including those that are dead. In comparison, the Quantal only measures alive cells. In a similar fashion, the Plaque Assay only measures viral particles that are both alive and capable of infecting the MDCK cells. Understanding this difference in how the technique measures viral particles, helps the researcher understand the sensitivity and accuracy of the results.

(b) The Influenza Virus attaches to sialic acid on cell glycoproteins using the envelope protein HA. Once this interaction is established, the next phase is entry into cells and this virus achieves it via fusion. The Class I Fusion Protein is required to achieve fusion of host and viral membranes. Briefly describe the process by which this fusion protein operates. In addition, identify the key difference between Class I and Class II Fusion Proteins.

Solution Pathway

Class I Fusion Proteins are composed of three identical protein subunits and this trimer is only functional upon cleavage due to the acidification of the endosome. The functional trimers then attach to the host membrane and multiple trimers then also localise to the same area. The trimers pull the membrane together for hemi-fusion, which ultimately leads to fusion of the two membranes. Class II Fusion Proteins differ in that the function of the protein is activated upon conformational change while Class I Fusion Proteins are dependent on a cleavage event.

(c) Following Membrane Attachment, replication is vital to produce viral progeny and spread the infection for virus survival. Although Influenza is an RNA virus (of which most replicate in the cytoplasm), it replicates in the nucleus. The transcripts of influenza virus do not include a CAP sequence yet it is able to translate on the host's ribosome. Name

this phenomenon that allows influenza to compensate for the lack of CAP and explain the process.

Solution Pathway

Influenza Virus uses ‘cap-snatching’ to steal the CAP on host transcripts and add it on its own viral mRNAs. Thus, the virus is dependent on transcription of host new mRNAs. The process can be broken down in three steps. First, the 5’ (-)ssRNA genome binds PB1 to activate PB2 and is in loop conformation to aid in replication (i.e. 3’ end bottom so that 5’ → 3’ viral mRNA synthesis can occur). Second, PB2 binds m7ppG-CAP structure from cellular mRNA. Third, PA cleaves CAP to serve as a primer for viral mRNA. (See diagram for a clearer explanation.)