Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Progress in Biophysics and Molecular Biology 158 (2020) 1–3

Contents lists available at ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

Editorial: 2020 – The year of viruses

The award of this year's Nobel Prize in Physiology or Medicine for the discovery of Hepatitis C virus coincides with the year in which the world is suffering a deadly and economically expensive viral pandemic. Over 1 million have died, over 36 million infected, and the economies of many countries have been crippled. As this editorial is being written (October 11th, 2020), a population the size of Poland's has been infected worldwide with Coronavirus and the worldwide fatalities from COVID-19 are approaching the size of the population of Poland's capital, Warsaw.

In both cases, Hepatitis C and Coronavirus, the viruses concerned are RNA viruses. The Coronavirus (COVID-19) fatality count is already approaching that of viral hepatitis, which is estimated to be 1.4 million. At the present rate, that number is going to be well exceeded by COVID-19 before 2020 is finished. The projection for COVID-19 deaths by January 1, 2021, including the first full year of the disease, is 2.3 million and the projections for 2021 are likely to be even greater. This is a global disaster and we are still waiting for the solution, whether it be universal vaccination, virus evolution, or crowd immunity, or a combination of these.

Understanding viruses and how they replicate has clearly become urgent.

In addition therefore to congratulations to the 2020 Nobel laureates, this editorial will also introduce the first of a series of retrospective assessments of Classic Historic articles from the archive of articles published in this journal 50 or more years ago, up to and including volume 21, published in 1971.

The Classic Historic articles we have chosen for this first editorial in that series are precisely on viral replication mechanisms and therefore highly relevant to the basic research that was necessary to enable the discoveries of the new Nobel laureates to be made, as well as for the intensive work now proceeding worldwide on Coronavirus.

The 2020 laureates and their achievements.

The 2020 prize has been shared between three scientists. Harvey Alter's achievement was to show that, even after checking for Hepatitis A and Hepatitis B, an unknown infection remained in many of those suffering from blood-borne hepatitis. Michael Houghton showed that the culprit was a previously unknown RNA virus from the *Flavivirus* family, and so it naturally became known as Hepatitis C. Charles Rice was responsible for showing that Hepatitis C can replicate and cause the disease. At the least it is now possible to screen for Hepatitis C before blood transfusions and so to avoid infection.

To celebrate their discoveries we have summarized their achievement graphically as the cover image for this issue of the journal.

Classic Historic PBMB articles on virus replication mechanisms.

I had the privilege of working at Oxford University for several years with a previous Nobel Laureate in the field of viruses, Baruch Blumberg, who discovered Hepatitis B virus and won the 1976 prize for his discovery. Unlike Hepatitis C, Hepatitis B is a DNA virus. I learnt a lot about viruses while Blumberg was the Master of Balliol College in Oxford, and specifically to distinguish very clearly between the replication mechanisms of DNA and RNA viruses. But in addition to Blumberg, I was greatly helped by two PBMB review articles commissioned for the first (volume 18) and third (volume 20) issues of the journal that I edited with the founding Editor, J A V Butler. The first deals with the replication of RNA viruses, the second with DNA viruses. I still possess the bound copies of those issues. In those early days the "journal" was issued as an annual book.

The Lodish 1968 **Review article**.

Harvey F Lodish is the author of the review article concerned with the replication of RNA viruses, published in 1968 (Lodish, 1968). At that time he had recently graduated with a PhD at Rockefeller University and was working at the MRC Laboratory of Molecular Biology in Cambridge with Sydney Brenner and Francis Crick. He is now, at 78, a Professor at MIT's Whitehead Institute for Biomedical Research and the lead author of the highly successful textbook *Molecular Cell Biology*, now in its 8th edition. He is a Member of the National Academy of Sciences and his laboratory has been so successful that two of his former postdoctoral students have proceeded to win Nobel Prizes.

The 1968 article illustrates his magisterial command of his subject so early in his career. The first RNA bacteriophage had only been discovered 6 years previously but the replication process had already been worked out in considerable detail. In fact, "the work in many laboratories has provided a remarkably coherent and detailed picture of its intracellular growth." He goes on to note that "a virus RNA serves both as a *messenger* RNA to direct synthesis of virus-specific proteins, and as a *template for nucleic acid synthesis*, to replicate the viral RNA." (emphases in the original text). The replication itself is produced by "a new group of enzymes which replicate the viral RNA." He predicted that "The first organism to be completely understood in molecular terms will probably be a bacterial virus with an RNA genome." This was already almost true since "the synthesis of the viral RNA and virus proteins can be investigated in cell-free systems." The experiment is described in Spiegelman et al. (1967).

I had to stop at that point. We usually think of viruses as "dead" outside a cell. How then could this one be replicated in a cell-free system, including both RNA and the necessary proteins to enable replication? I have myself recently explained the reasons why that generally cannot be done (Noble, 2018, 2020). The main explanation lies in the frequency of random mutations during the

https://doi.org/10.1016/j.pbiomolbio.2020.10.004

replication process. The natural error rate for DNA replication is one sequence error in 10^4 bases before the cell error-correcting machinery kicks in to make even a 3 billion base pair human genome be copied accurately. Otherwise, there would be nearly 1 million errors in replicating a human genome. Only a living cell can do that highly accurate error-correcting.

The natural error rate in replicating RNA is even higher, which is why viral pathogenicity and virulence can evolve so rapidly (Novella et al., 2014). Without the error-correcting machinery for DNA, the RNA virus effectively hyper-mutates continuously. This is one of the dangers we face with coronavirus. As an RNA virus it will evolve rapidly. We may be facing the prospect of different coronaviruses for many years to come.

To return to the 1968 article, how large is the F2 phage RNA? Lodish writes: "F2 is also the smallest virus to grow independently in any host. The RNA genome, containing about 3000 nucleotides could code for only 1000 amino acids. The number of proteins is likely to be small (three to six)." That is also true of the other very similar RNA virus to be isolated in 1961, MS2. That one was also the first to be completely sequenced (Fiers et al., 1976). It contains 3569 nucleotides. Both F2 and MS2 are therefore at the very limit of size. Any smaller sequence would not be sufficient to code for the necessary replication proteins. Any larger genome would be subject to too many errors. There must be a pay-off in the evolution of RNA viruses between survival and replication.

There may also be clues here to the origin of life. An RNA based replication process that requires so few proteins could plausibly have been important in the evolution of the first cells.

The steps in the replication process are clearly described in Lodish's article. "we call the RNA which is found in virus particles the "plus", or viral strand. In infected cells there is made an RNA with the complementary base sequence, which we call the "minus" strand …. forming a double stranded RNA with a structure similar to double-stranded DNA." Later he writes "It will be recognised that the mechanism of viral RNA synthesis is similar to that of DNA-mediated nucleic aid syntheses; the unique feature is that a complementary RNA, rather than DNA, is the template molecule." I strongly approve of the use of the word "template" here. In evolutionary biology, regarding nucleotide sequences as templates avoids some of the problems with the role of genes in causation (Noble, 2008, 2016).

In summary, Lodish's 1968 article is a classic paradigm. It still reads well, and offers deep insights, more than half a century later.

The Stone 1970 **Review article**.

Alan B Stone is the author of the article in volume 20 on The Replication of DNA-containing Viruses (Stone, 1970). DNA replication is much more complex than RNA replication, so it is not surprising that the article is twice the length of the 1968 article. Alan Stone was working at the Lister Institute in London and had previously worked at the Chester Beatty institute. I suspect therefore that he was a former colleague of J A V Butler. Butler worked at the Chester Beatty on histones (Butler et al., 1968) and must have been keeping a keen watch on the nucleotide replication field.

The article begins by noting that the smallest DNA viruses are larger than the smallest RNA viruses. They can contain between around 10 and 500 genes, and so are capable of forming the templates for a much wider range of proteins. Some are linear, but many are circular. Their impact on the host cells is profound: "Immediately after infection, the synthesis of host DNA, RNA and protein totally ceases." This is a fundamental difference from RNA viruses. RNAs can be read immediately to form the template for protein formation. DNAs require transcription into RNA to do that. DNA viruses therefore must take over the DNA replication machinery of the cell.

At the time of the review it was already possible to map the virus structure to the DNA sequence (Figure 7 of Stone's article). This fact must have greatly contributed to the early ideas of 1:1 correspondence between genotype and phenotype. We now know that this is far from true in general. The GWAS work has favoured what is now called the omnigenic hypothesis, that nearly all genes contribute in one way or another to most functions (Boyle et al., 2017).

Stone's article is an exhaustive review of the many types of DNA virus already known at that time. He concludes: "It is evident that the DNA-containing viruses exploit the synthetic capacities of their hosts by a wide variety of means. Certain special structural features of viral DNA molecules … … … contribute to the virus's success in escaping the cell's degradative potential and in achieving metabolic control of the cell during the replication process … ….Some viruses create their own synthetic machinery to replace some of the normal pathways and initiate novel ones, while others rely mainly on enzymes manufactured by the host." The differences of course lie in how large the viral genome is and how many proteins it can code for. A virus of 500 genes can clearly replace much more molecular machinery than one containing just 10, or in the case of RNA viruses even fewer, genes. In all cases though, the functionality is much greater than in the RNA viruses reviewed by Lodish.

To what do viruses relate in evolution?

I have already hinted at the possible significance of very small RNA viruses in the early stages of the evolution of life. As Lodish's article makes clear, those viruses are at the very edge of what it means to be alive. The F2 bacteriophage can even be replicated without the involvement of a living cell. In the process of evolution from inanimate chemistry to the first living cells there has to have been some half-way houses. In concluding this editorial I want therefore to draw attention to two other important clues.

The first is evidence that a substantial part of the existing genomes of living organisms today derived from incorporation of viral nucleotide sequences (Moelling, 2013; Villareal, 2005). In the human genome the sequences of viral origin amounts to around 8%. The second is that lateral exchange of nucleotides is ubiquitous in living cells. In the microbe world there is a veritable promiscuity of nucleotide exchange.

All cells can also extrude tiny extracellular vesicles (EVs or exosomes) whose RNA, DNA and other contents can be taken up by other cells. These have also been shown to play a role in health and disease (Edelstein et al., 2019). They can also cross the Weismann Barrier (Spadafora, 2012; Noble, 2019). The Weismann Barrier, which was introduced by August Weismann at the end of the 19th century as the theory that the germ cells can be completely isolated from influence by the soma, is therefore no longer valid.

In terms of size, and in many other respects, viruses and EVs have a lot in common (Nolte-'t Hoen et al., 2016). The key difference is that EVs do not replicate. They are therefore similar to noninfectious defective viruses that have lost their ability to replicate. To quote the paper, "They share with viruses an important function that played a critical role in evolution, namely delivering bioactive material from one cell to another Specific combinations of lipids and proteins, in particular, tetraspanins, in the EV membrane can mediate specific targeting of vesicles to recipient cells and may determine the ability of vesicles to fuse with cellular membranes. These molecules, as well as genetic material and proteins enclosed in EVs (e.g., transcription factors and cytokines), constitute molecular signals that can affect the function of recipient cells. It is exactly this trait of being multicomponent transport units that EVs share with enveloped viruses."

We once thought that the cells in multicellular organisms do not take part in the nucleotide exchange that is rampant in the microbial world. That idea is no longer tenable. The implications for the origins of life and for the processes of health and disease are

profound. As an example, one of the Special Issues of this journal planned for 2021 will be devoted to the relations between evolution and cancer, including the mechanisms of intercellular communication.

This editorial forms part of the celebration of 70 years of the publication of *Progress in Biophysics and Molecular Biology*. We will be featuring further editorials on reviews of Classic Historic articles during 2021.

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Available online 21 October 2020