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A Research Proposal to Study the Life Cycle of SARS-COV 2

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ABSTRACT

SARS-CoV2 continues to affect the lives of the majority of the world, and although vaccines are beginning to become available, much of the world will still be unable to obtain them. Furthermore, some studies have suggested that there may have to be annual vaccines and as strains of the virus continue to increase, it is essential for us to move to the next stage of research and attempt to better understand the virus. By utilizing a chemical genetics approach where numerous ligands of distinct chemical libraries are screened through high-throughput screening, we may be able to form an ordered viral cycle of metabolic events that could help identify drug targets more efficiently and coordinate drug use to improve efficacy. A modified version of the virus (to decrease its ability of infection) along with the URA3 protein is then inserted into yeast cells (Saccharomyces cerevisiae) and screened. A simple assay involving the addition of 5'- fluoroorotic acid helps to determine ligand interference and after identifying the compounds, we can order their action

Introduction

As COV-2 continues to pervade our lives, causing widespread morbidity and mortality,[1] research must begin to attempt to understand the underlying cause of the disease, SARS-CoV-2 (CoV-2) virus, to better overcome it in the long-term. Therefore, it is crucial to start researching about the life cycle of the virus: the order of its metabolic events. This is important because in many areas to this day, an end to the pandemic is still unclear. In addition, predictions that this will be a recurring virus also stresses the need for an in-depth understanding that will be more useful in the long-term.

A quintessential element research must therefore strive to understand is the unascertained life cycle of the SARS-CoV-2 virus. This knowledge is crucial to help us to identify interventions such as drugs that interfere with disease progression.[2] Although a few drugs (such as Remdesevir) have already been identified to be somewhat effective and research to identify more drugs is on-going, success rates of these drugs are limited and finding them requires great amounts of time.[3,4] However, by understanding the viral life cycle, the potential targets for drugs could be vastly increased;[4] hence, the life cycle of a virus is very helpful for the development of a vaccine or cure. This is still crucial even with the increasing number of vaccines available for there has been increasing support for the inability of antibodies to remain in the immune system for long periods of time.[5] Therefore, vaccines are required to trigger stronger immune responses which may be actually detrimental to more elderly patients. In addition, other issues with the vaccines[6] such as their specific requirement of storage conditions as well as their high demand and prices make them unattainable for the vast majority of the developing and under-developed world.

Sars-COV-2 is a positive strand RNA virus, with a genome size of approximately 30,000 bases in length (Coronaviruses possess the largest genomes of all RNA viruses).[8] It encodes four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins.[8] In addition to these structural proteins, the viral genome encodes many non-structural proteins (Nsp's) that perform numerous roles in the replication and virus assembly

processes (such as proof-reading to reduce RNA synthesis errors).[8] The main known Nsp complexes include Nsp7, Nsp8, and Nsp12, which form the 'core' polymerase (an enzyme that is key to the replication and assembly of the viral genome and cell).[8] Many other proteins also work together with the central polymerase subunit Nsp12 to increase its processivity, forming other complexes (for instance, nsp12, nsp10 and nsp14) that can add other activities to the complex.[8] The replicase-transcriptase proteins, together with other proteins, form Replication-Transcription Complexes (RTCs), which copy or produce the viral genome or sub genomic-length RNA; these complexes are then assembled together with the structural gene protein products in a novel subcellular organelle composed of double membrane vesicles, in order to form the viral particles. [9]

Compounds vary in their function depending on the step they intervene at. For instance, compounds that bind to the viral attachment molecule or to the cellular receptor can disrupt the viral particle interactions with host cells and thus prevent the first steps of the viral life cycle by thereby preventing infection. However, other compounds may affect later stages of the viral life cycle, thus interfering with a different, later function.[10]

By studying the life cycle of the virus, we may observe when different protein complexes function and what each are responsible for. Interactions between the proteins (Protein-Protein Interactions (PPIs)) are crucial to the making of essential complexes and to the functioning of necessary viral life processes.[11] Therefore, as PPIs are fundamental to the viral life cycle, they are a very good target for drugs to inhibit or interfere with the viral life cycle.[12]

Although there is a knowledge of a basic sequence of events, some of the PPIs and certain protein complexes, there is still plenty of uncertainty regarding specific events and the life cycle as a whole.[13] Another aspect that remains uncertain is which events follow others —the order or sequence of the events in the viral life cycle. One way used previously to determine the order of events in a biological pathway is by using certain genetic tests. In 1973, Jonathon Jarvik and David Botstein published a paper where they used temperature-sensitive (ts) and cold-sensitive (cs) mutants to determine the order of gene action by observing the effects — such as inhibition or continuation — on the different stages of viral infection by the bacteriophage P22.[14] By shifting the temperature differently, either from 20 to 40 degrees Celsius or vice versa, Jarvik and Botstein were able to observe which mutant was being affected by checking if the intermediate product was produced. For example, if both mutants affect the same step, then the temperature shift in either order would not permit the process to continue. If one mutant blocks a step prior to the other, then the order of temperature would reveal one of the two possible orders of inactivation would permit the process to continue, thus ordering the activity of the two genes in a pathway.

Another way to determine the order of gene action, may be by the use of chemical genetics[15], which has become more widespread relatively recently. Although similar to previous method, instead of using temperature-sensitive mutations as tools to order metabolic events and study the activity of the various proteins, chemicals are used. Much in the way that temperature can be controlled temporally by shifting the temperature (as shown by Jarvik and Botstein), chemical compounds can be easily added or removed. Often the compound, when removed, will be completely eliminated so that the block in the progression of biochemical events is reversed —permitting the progression to continue again. This way, chemical compounds might act in a similar way as conditional mutations.

The process of identifying compounds to use in chemical genetics is relatively well developed, including a variety of available compound libraries and advanced screening tools utilizing robotics that can be used to carry out high throughput chemical library screens.[15] Thus the first step will be the assembly of a set of mutation equivalents, which are ligands that are known to alter the function of proteins. After this set of ligands is ready, a high throughput screen for ligands that affect a biological process of interest—for instance, cell division — is carried out. High throughput screens are used to increase the efficiency of the screening process. There are multiple chemical libraries that can be screened, each consisting of tens of thousands of compounds. The libraries have distinct compounds, such as large heterocyclic compounds, that are often effective in interfering with PPIs; other libraries consist of approved drug compounds or are in an approval process, which make them more amenable to fast repurposing for COVID-19 treatment.[16, 17] Considering the options for libraries, and the use of robotics to conduct high-throughput, it is reasonable to consider compound screen of approximately 100,000 compounds that can be accomplished within a two-week timeframe. Finally, following the detection of which ligands are effective, the protein targets that the ligands affect

are identified and able to be altered as desired in the future. Subsequent to repeating this process for all the different essential biological events of the virus' life cycle and finding ligands that bind and alter protein functions, we can use this similar to how mutations were used (by Jarvik and Botstein) to determine the precise order of the virus' life cycle.

It is expected that we will be able to use chemical genetics in place of forward genetics to order the events in the CoV-2 life cycle. If this can be done, it also identifies a multitude of categories of potential drugs that may act at different steps and thus might act synergistically when affecting the viral infection.

A Review of Methodologies

To understand the order of events of CoV-2, it is possible to seek and identify compounds that interfere with the CoV-2 life cycle at any point and then attempt to order their action into specific steps in the lifecycle. A chemical genetics approach is better than a temperature-sensitive mutation approach in this case as it allows us to find compounds that affect the virus which could then be potentially more useful for drug or vaccine or development. They are also more suitable for organisms with more complex genomes for ligands can be added or removed at any time—enabling a kinetic analysis of the consequences of the changes of the protein activity in vivo. As a result, this allows for the analysis of the cellular events that immediately follow the altering of the activity of a specific protein. On the other hand, one of the disadvantages that exist with using the chemical approach is that it would take substantially longer and greater difficulty to ascertain which specific protein a chemical is affecting.

One method is to screen ligands holistically based on the latest possible endpoint of the viral life cycle. This will be able to spot ligands that have any effect, which will inform us if they have bound with and affected any proteins that are responsible for a biological event in its life cycle. Therefore, it would be valuable to employ as a first assay for the identification of compounds during library screening a method that reports a late step in the viral lifecycle so that we may recover compounds that interfere with any preceding and necessary step for normal reporter activity. Once an initial high-throughput screen has been conducted with a specific assay, follow-up retests and additional assays will verify the compounds' identity, activity and optimal concentration.

After a general collection of ligands is assembled, the objective shifts to then determine where in the viral life cycle that each of these compounds acts. One potential method is to use biological assays (such as using the URA3 assay described later) to assess what is happening in the viral life cycle. In this regard, a synchronous start to the viral life cycle should be used, and analysis at different time points will determine where the viral life cycle has gone awry or stopped.

To study the virus CoV-2, one approach with a lot of potential involves the use of the bakers' yeast (*Saccharomyces cerevisiae*) as its host for a multitude of reasons. First and foremost, yeast opens up the opportunity for studying the biochemistry and genetics of CoV-2 for it is easier to grow in large and homogeneous numbers in order to obtain an amount of temporally synchronous biological material for biochemical analysis.[18] Using yeast cells will allow us to control and initiate a synchronous start to the infection cycle more easily than animal cells. This is due to the methods of gene regulation, which are used to initiate the viral life cycle, and can be more precisely controlled to allow for a more precise control than is possible in an animal cell culture. Animal cells in culture tend to exhibit variation which in turn interferes with obtaining precise readouts from assay reporters and biochemically homogenous cell populations.[19] Overall, it is much more straightforward in yeast cells than animal cells. However, there are also some concerns. Although there is precedent (Price and Alquist managed to study the complete replication process of the Flock House Virus (FHV; an animal virus) in 1996)[20] the fact that CoV-2 has such a large genome (around 30000 kb–significantly larger than that of FHV), poses the risk that yeast may not be a competent host. Another issue is that because yeast cells are rather far evolutionarily from mammalian cells, chemicals that are generally non-toxic to yeast may pose problems in the intended host for COVID-19 treatment (for instance, humans).[21]

Nonetheless, yeast cells have significant advantages as alluded to above: one being its stability of its gene expression and its use as a substrate for biochemical studies, and another being its possible use for high-throughput

chemicals screens (e.g., employing tens of thousands of compounds); that is, typical chemical screens involving a yeast host are able to screen ten times as many compounds as can be done in an animal cell culture system.[22]
To ensure the safety of the researchers and the general community, suggestions call for the S-protein (the protein that creates the "corona (crown)" that plays an essential part host infection) to be removed. This is because the modification will likely decrease its infectiousness[23], which is crucial for not only could it affect researchers but also be spread easily as it is a widespread consumer product that is ingested. Therefore, not only should modifications (that are insignificant to this investigation) be made to the virus to ensure safety, but tighter regulations should also be made to guarantee that it will not escape into the community.

To be able to do a screen with yeast for these compounds, assays could be developed. In order to perform a high throughput screen, there must be a simple assay that can be done where you can have large compounds tested simultaneously.[24] To achieve a high efficiency (so that we could test a larger number of ligands in a shorter amount of time), it should also be able to be done by robots (to increase speed and reduce human error). For instance, it would be unsuitable to use a western blot or northern blot to determine certain characteristics, for the assay must be simple––such as determining if the yeast is growing or not growing. One assay that may be suitable involves using a growth assay that depends on expression of the protein URA3—a biosynthetic, metabolic protein that is part of the process of making uracil. Therefore, yeast cells that have a mutation in the URA3 gene cannot make uracil and requires uracil to be added to the growth medium. In addition, the growth of yeast cells that do have a functional URA3 gene can be inhibited by the compound 5'- fluoroorotic acid (5' FOA). The URA3 catalyzes a poison to form from this compound, inhibiting further yeast growth.[25]

The first of two possibilities that exist involves replacing the code for the S protein (that is responsible for the making of the Spike Protein) with the coding sequence for the URA3 protein. In addition, the viral genome will be expressed from a DNA construct in which the viral RNA will be expressed by RNA polymerase-mediated transcription. This transcription will be initiated at the promoter sequence Gal1. This promoter is normally repressed during yeast growth on the sugar glucose; however, when glucose is replaced with galactose, the Gal1 promoter becomes immediately active and the result will be a CoV-2 genomic RNA.[26] When the viral life cycle progresses to the point where the virus creates the S protein, this recombinant genome would synthesize the URA3 gene product instead. To determine if the life cycle reaches this stage, 5' FOA could be added, as it would kill the yeast if transcription has reached and successfully created the URA3 protein. However, since the S protein is only created after Orf1a and Orf1b and the non-structural proteins[27], it is an assay that informs about a relatively late stage. For example, if it's a drug that blocks transcription of the genetic code that would cause URA3 expression (a late transcript such as a 3 prime mRNA transcript), the virus would lack the creation of the protein URA3. Such yeast would grow in the presence of the compound 5'FOA. Thus, to perform the screen, yeast harboring the SARS-CoV-2 reporter would be grown on agar in small patches onto which various chemicals from libraries have been added robotically. The agar would also contain the growth inhibitor 5'FOA. Library chemicals that permitted yeast to grow in the presence of 5'FOA would be considered primary candidates and scheduled for secondary re-testing, as mentioned above.

However, the problem with this approach is that inhibition of any of the steps before the formation of the 3 prime nested mRNA would give the same result—resulting in the obscurity regarding which step the ligand interacted with and inhibited.

Another problem is that, as the aim is to do this holistically, the assay should ideally be able to determine whether virus particles are being formed.

Similar to the P22 paper that uses Cs and Ts mutants, an assay that could determine whether the assembling of the viral particle is very much essential. This would permit a more in-depth understanding about the life cycle as a whole and the connections between the events. Therefore, it would probably be essential to engineer the genome so that URA3 is inserted into the different areas of the genome so that we can screen any compound that blocks any of the steps up to and including of the formation of this 3 prime nested mRNA. This way, we would know more than just if the 3 prime nested mRNA has been formed successfully or not.

Another concern is the chance of having false positives. Compounds that bind and inhibit URA3 protein directly instead of the viral life cycle would also allow yeast to grow in the presence of 5'FOA, so it is crucial to analyze carefully the ligands that passes through the screening. One way to determine this is by doing a control to observe whether 'normal' yeast cells grow in the presence of the compound without an addition of uracil to the culture media. If the compound inhibits URA3, then yeast cannot grow in its presence without adding uracil to their growth media, so the growth of yeast can ensure that these false positives are identified.

A set of assays can be performed to determine where (the 'step') in the viral life cycle and in what process (such as RNA replication, protein complex formation, etc.) that each compound acts. Suggested assays that seem crucial include the following:

1) To determine whether a compound interferes with the expression of viral proteins from the RNA genome, Western blots will be used with antibodies that recognize each of the expected Nsp proteins that would be synthesized from Orf1a, Orf1b, as well as the structural proteins (aside from the deleted S protein) encoded by the 3' nested transcripts. This survey of viral protein expression would determine whether a compound interfered with gene expression and with particular stages of gene expression. Specifically, nsp proteins are synthesized from the (+) strand mRNA expressed from the viral cDNA construct, whereas structural protein expression requires synthesis of the viral (-) strand RNA by the RTC protein complex, and 3' nested (+) strand RNA synthesis by additional RTC activities. For example, if a compound interferes with the RTC complex, we would expect to observe nsp protein expression but fail to observe structural protein expression.
2) To determine whether a compound interferes in a specific aspect of viral RNA synthesis, Northern blots will be used with hybridization probes for specific viral RNA strands ("+ strand" and "- strand" probes), as well as region-specific hybridization probes for specific RNA products such as the 3' nested (+) strand RNA transcripts that encode structural proteins. As with Western blot analysis of protein expression above, these assays will determine whether a compound interferes with specific aspects of RTC function, such as (-) strand RNA synthesis or 3' nested (+) strand RNA synthesis.
3) As one of the principal targets for compound identification are those that interfere with the PPIs of the RTC protein complex, it will be especially useful to determine whether compounds that interfere with steps of viral gene expression, as revealed by the above assays, do so by interfering with the formation of viral protein complexes. To this end, yeast with synchronized induction of viral RNA expression (using galactose addition to induce viral RNA synthesis from the Gal1 promoter) will be lysed at various timepoints after galactose addition and under conditions to maintain native protein complex structure. Protein extracts will be subjected to sucrose density gradient centrifugation to sediment protein complexes according to molecular weight. The resulting centrifuged sucrose gradients will be distributed to fractions. The fractions will then be analyzed by Western blot analysis (as described above) in order to produce a time series of protein complex identity during the viral life cycle. For example, it is expected that complexes that sediment at particular molecular weights will be identified and these complexes will contain some combination of viral proteins. It is possible that multiple complexes of distinct protein composition will share the same sedimentation properties; these will not be resolved by this approach. Furthermore, it is possible that some complexes will contain host (yeast) proteins – these proteins will not be identified by the Western analysis. Additional approaches, such as mass spectroscopy, could be employed to identify host proteins in these complexes. We expect for example to identify RTC complexes that contain nsp proteins, especially Nsp12 and others that are already known to be components of the RTC. However, it is possible that additional complexes, including those that would be classified as being variants of the RTC, will be identified. After having established this framework for the analysis by identifying viral protein complexes, we will proceed to determine whether compounds identified in the chemical screen interfere with the formation of particular protein complexes.

The foregoing results will inform our understanding of the likely protein targets for a particular compound that interferes with the viral life cycle. It would also be of particular value to definitively identify the protein target of a particularly useful compound. There are two possible approaches to this end that may be taken. One is to induce mutations in the viral genome that result in resistance to the compound. The yeast DNA construct harboring the viral genome will be subjected to local mutagenesis and returned to a yeast host, and yeast that express the URA3 reporter in the presence of the compound will be identified. DNA sequencing will be used to identify mutations in viral genes. This approach might identify mutations that change protein sequences in a way as to reduce compound binding to a viral protein, hence identifying the protein target.

Another approach will be to identify proteins and/or protein complexes that bind to the compound. Proteins and protein complexes identified by sucrose gradient sedimentation, as described in (3) will be incubated with the compound. Proteins bound to a chemical compound will then identified by mass spectroscopy.

After an initial understanding and assessment of the effect of compounds on the viral life cycle, the use of compounds in combinations could potentially order the biological events. For example, combinations of two compounds A and B can be used in sequence (AB and then BA) to attempt to order the action of compounds and their corresponding biological events. Inspired by Jarvik and Botstein, this process is very similar to how they used different combinations of temperatures (up-down or down-up) to order the action of genes. Although there will be many repeats and attempts to link up the events, the final result will be a detailed order of the biological events of the viral life cycle.

Possible Findings and Interpretation

One general finding that may observed is that the RTC is in fact larger and more complex than we know currently. Some findings may also include specific proteins and PPIs so that they are easier to target—although the main aim is to spot and target weaknesses in the viral life cycle that may be targeted with more success.

One possible result that could be obtained is a successful characterization of the steps of the viral life cycle. We may be able to observe the RTC progress through a series of different complexes of different subunit composition and structures—each associated with a different step in the RNA biochemistry—such as (-) strand RNA synthesis, (+) strand RNA synthesis and capped nested 3' structural gene transcript formation. Furthermore, we may be able to precisely define the timing of the steps of the RNA 'biology' – such as the time of (-) strand synthesis, complete (+) strand and nested 3' structural gene transcripts.

By analysing both, we should seek to question if there is a correlation between the 'steps' of the RNA pathway and the RTC complexes that are present. This would be particularly beneficial as it would link the two observations beforehand and offer a much deeper understanding of what is happening and what is responsible. If such correlation is to be found, then we could even purify the RNA-RTC complexes to show which particular RTC complex is bound to the structural gene RNA which would be useful for drug formation.

This is because RTC and RNA products assemble in double-membrane vesicles where the assembly of viral particles occurs; therefore, identifying the protein complexes and protein RNA complexes present in these vesicles would provide additional therapeutic targets.

Presumably, the PPI interfering compounds would target specific RTC complexes, which could be found by determining which complexes identified in the analysis are not properly formed in the presence of the particular compound. These compounds then become drug candidates that act via interfering in PPIs.

Another possible result that could be obtained is the order of the viral pathway steps. This would be particularly helpful if more than one drug was needed and the sequencing was significant. For example, the ordering of the usage of drugs (A before B or B before A) could be aligned to the step at which drug A or drug B alone blocks the progression of RNA stages and the sequence of RTC complexes that are formed as the life cycle proceeds. This would help narrow down the large numbers of drug candidates and improve their effectivity whilst using less time.

Overall Summary and Conclusion

Although there may be a slight possibility that CoV-2 will fail to function in yeast as it would in an animal cell, this investigation may yield exceptionally interesting and significant results if the virus could perform many of its functions properly. This could be assessed by comparing observations made in the yeast system with similar experiments done in animal cells (verify complexes observed in yeast are made in animal cells).

The yeast system permits easy alteration of the sequence of the viral construct (using homologous recombination methods), so that mutations in the virus in the human population can be introduced into the yeast construct to see their effects on particular drugs. Therefore, not only will it be a quintessential to the discovery of new treatment options, but it will also prove useful in checking the effectivity of treatment options in the long-term.

The life cycle will not only permit us to know just the order of cellular events as part of the viral life cycle, but also the replication process, the different proteins and protein complexes better. This understanding is very helpful for pursuing treatment options such as vaccines or cures but is even more useful if the virus does indeed become a regular virus or a long-term issue. Both ways, this proposed investigation will be fundamental to the continued study of the virus CoV-2. Moreover, this research is essential for promoting research in the scientific community to study the long-term understanding of Sars-CoV 2.

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References

1. World Health Organisation. (2020) WHO Coronavirus Disease (COVID-19) Dashboard. *World Health Organization*. World Health Organization.

2. Poduri, R., Joshi, G., and Jagadeesh, G. (2020, October) Drugs targeting various stages of the SARS-CoV-2 life cycle: Exploring promising drugs for the treatment of Covid-19.

3. Young, B., Tan, T. T., and Leo, Y. S. (2020, November 26) The place for remdesivir in COVID-19 treatment. *The Lancet Infectious Diseases*. Elsevier.

4. Poduri, R., Joshi, G., and Jagadeesh, G. (2020, October) Drugs targeting various stages of the SARS-CoV-2 life cycle: Exploring promising drugs for the treatment of Covid-19. *Cellular signalling*. Elsevier Science Ltd.

5. Ward, H., Atchison, C. J., Whitaker, M., Ainslie, K. E. C., Elliott, J., Okell, L. C., Redd, R., Ashby, D., Donnelly, C. A., Barclay, W., Darzi, A., Cooke, G., Riley, S., and Elliott, P. (2020, January 1) Antibody prevalence for SARS-CoV-2 in England following first peak of the pandemic: REACT2 study in 100,000 adults. *medRxiv*. Cold Spring Harbor Laboratory Press.

6. Ledford, H., Cyranoski, D., and Noorden, R. V. (2020, December 3) The UK has approved a COVID vaccine - here's what scientists now want to know. *Nature News*. Nature Publishing Group.

7. (2020, November 24) The COVID vaccine challenges that lie ahead. *Nature News*. Nature Publishing Group.

8. Mittal, A., Manjunath, K., Ranjan, R. K., Kaushik, S., Kumar, S., and Verma, V. (2020, August 21) COVID-19 pandemic: Insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. *PLOS Pathogens*. Public Library of Science.

9. Astuti, I., and Ysrafil. (2020) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes & metabolic syndrome*. Diabetes India. Published by Elsevier Ltd.

10. Lee, N., Shum, D., König, A., Kim, H., Heo, J., Min, S., Lee, J., Ko, Y., Choi, I., Lee, H., Radu, C., Hoenen, T., Min, J.-Y., and Windisch, M. P. (2018, August 24) High-throughput drug screening using the Ebola virus transcription- and replication-competent virus-like particle system. *Antiviral Research*. Elsevier.

11. Kuzmanov, U., and Emili, A. (2013, April 30) Protein-protein interaction networks: probing disease mechanisms using model systems. *Genome medicine*. BioMed Central.

12. Bojadzic, D., and Buchwald, P. (2018) Toward Small-Molecule Inhibition of Protein-Protein Interactions: General Aspects and Recent Progress in Targeting Costimulatory and Coinhibitory (Immune Checkpoint) Interactions. *Current topics in medicinal chemistry*. U.S. National Library of Medicine.

13. Astuti, I., and Ysrafil. (2020) Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes & metabolic syndrome*. Diabetes India. Published by Elsevier Ltd.

14. Jarvik, J., and Botstein, D. (1973, July) A genetic method for determining the order of events in a biological pathway.

15. 15 Kao RY;Tsui WH;Lee TS;Tanner JA;Watt RM;Huang JD;Hu L;Chen G;Chen Z;Zhang L;He T;Chan KH;Tse H;To AP;Ng LW;Wong BC;Tsoi HW;Yang D;Ho DD;Yuen KY; (2004, September 11) Identification of novel small-molecule inhibitors of severe acute respiratory syndrome-associated coronavirus by chemical genetics. *Chemistry & biology*. U.S. National Library of Medicine.

16. 16.. Stockwell, B. R. (2000, November) Chemical genetics: ligand-based discovery of gene function. *Nature reviews. Genetics*. U.S. National Library of Medicine.

17. Xiao, G., Peng, K., Zhang, L., Wan, W., Zhu, S., Li, S., Shang, W., Zhang, R., Li, H., and Liu, W. (2020, November 12) High-Throughput Screening of an FDA-Approved Drug Library Identifies Inhibitors against Arenaviruses and SARS-CoV-2. *ACS Infectious Diseases*.

18. Zhao, R. Y. (2017, September 18) Yeast for virus research. *Microbial cell (Graz, Austria)*. Shared Science Publishers OG.

19. Denny, P. (2018, October 16) Yeast: bridging the gap between phenotypic and biochemical assays for high-throughput screening. *Taylor & Francis Online*. Taylor & Francis.

20. Price, B. D., Rueckert, R. R., and Ahlquist, P. (1996, September 3) Complete replication of an animal virus and maintenance of expression vectors derived from it in Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences of the United States of America*. U.S. National Library of Medicine.

21. Välimaa, A.-L., Kivistö, A., Virta, M., and Karp, M. (2008, October 16) Real-time Monitoring of Non-specific Toxicity Using a Saccharomyces cerevisiae Reporter System. *Sensors (Basel, Switzerland)*. Molecular Diversity Preservation International (MDPI).

22. Denny, P. W., and Steel, P. G. (2014, August 13) Yeast as a Potential Vehicle for Neglected Tropical Disease Drug Discovery 2015.

23. Huang, Y., Yang, C., Xu, X.-feng, Xu, W., and Liu, S.-wen. (2020, August 3) Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Nature News*. Nature Publishing Group.

24. Attene-Ramos, M. S., Austin, C. P., and Xia, M. (2014, April 14) High Throughput Screening. *Encyclopedia of Toxicology (Third Edition)*. Academic Press.
25. Huang, J., and Schreiber, S. L. (1997, December 9) A yeast genetic system for selecting small molecule inhibitors of protein-protein interactions in nanodroplets. *Proceedings of the National Academy of Sciences of the United States of America*. The National Academy of Sciences of the USA.

26. Flick, J. S., and Johnston, M. (1990, September) Two systems of glucose repression of the GAL1 promoter in Saccharomyces cerevisiae. *Molecular and cellular biology*. U.S. National Library of Medicine.

27. Romano, M., Ruggiero, A., Squeglia, F., Maga, G., and Berisio, R. (2020, May 20) A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. *Cells*. MDPI.