What have we learned from Current Topics in Molecular Biology Class

- Group 3

1. Anusara Jitsatja 5837554 MBMG/M
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2. Supanee Nimsanor 5837549 MBMG/M
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3. Voranat Wongnat 5837552 MBMG/M
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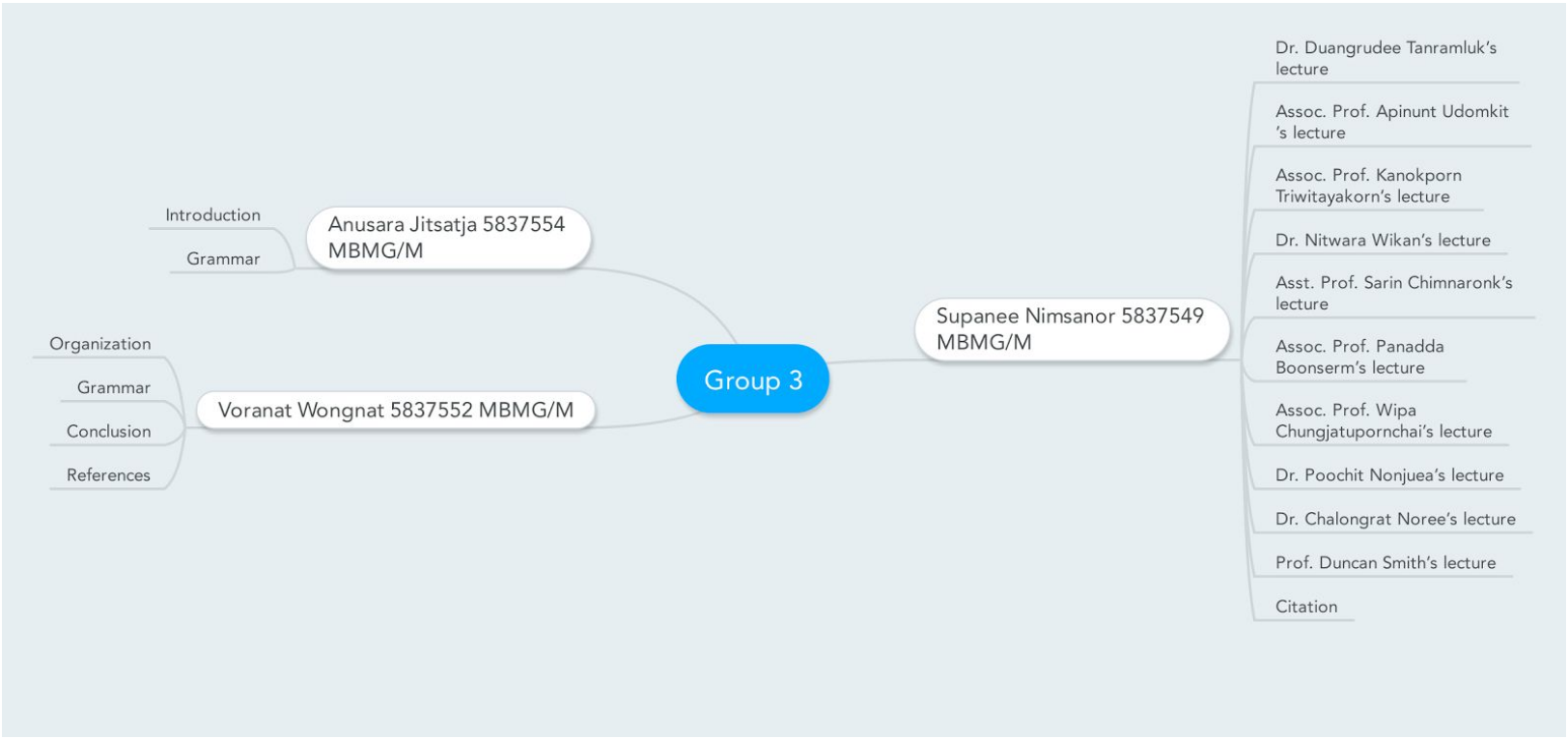
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Introduction

Nowaday, we can search knowledge from many sources such as text books, news or videos. Publication is one of the knowledge source to study interested subject. Reading journal articles is necessary for Master's degree. Therefore, Master's students should have the skills to read publications because there are many online publications that are including suitable and unsuitable article. This course "Current Topic in Molecular Biology" is the subject that involves techniques for analysis of scientific publications based on molecular biology principles. In this class we read several publications from different fields in order to practise interpreting data from each figure and graph. We studied some paper which manipulated the figures and duplicated the results from previous study as an example of retract publication. In addition, we practise writing and presentation. Finally, we will learn techniques to interpret and discuss publications and we can distinguish between suitable and unsuitable publication. We can also apply knowledge that we gain from this course to write own publication in the future.

Content

October 5, 2016

Dr. Duangrudee Tanramluk's lecture: Course orientation

The first class of this course, Dr. Duangrudee explained the details of this course which are the schedule and activities in the class. Before the class

ended, we were divided into 3 groups with 3 persons for each group. We received a writing assignment about what we will learn from this class in 2,500-3,000 words by using Google Doc applications. Before that, some group members have no idea about online Google Doc applications until it was introduced by Dr. Duangrudee. This online application is very useful for collaborating with other people every where.

Assoc. Prof. Apinunt Udomkit 's lecture: Research ethics

In the class, Assoc. Prof. Apinunt showed some scientific misconduct articles for our discussion. We discussed about frequent mistakes in research publications such as common definition, importance and scope of ethics which are necessary for scientists before writing scientific articles. For example, we should refer to any previous work that we used their information and avoid any other plagiarism such as making up the image, delete or edit some band on gel. We should not submit the same paper to different journals at the same time and should not duplicate the previous publications (1). The knowledge obtained from this class are very useful for writing scientific papers.

<u>October 12, 2016</u>

Assoc. Prof. Kanokporn Triwitayakorn's lecture: Evaluation of genetic diversity in chinese wild Apple species along with apple cultivars using SSR markers

In this class, we discussed on the topic of "Evaluation of Genetic Diversity in Chinese Wild Apple Species Along with Apple Cultivars Using SSR marker". First of all, Dr. Kanokporn explained about background of this research article and the students were divided into 4 groups in order to

explain and discuss each part of the article including introduction, methods, results and discussions. After discussion, every groups had to present the main idea of each part. From that article, we learned about the simple sequence repeat (SSR) marker. This technique are used for classifying organisms based on amplification of repetitive sequences in plant's genome and comparing them to related organism (2).

Later in the class, Dr. Kanokporn taught us about the easier technique for reading scientific publication by focusing on the results and discussions in order to assess the main point of the articles.

October 19, 2016

Dr. Nitwara Wikan's lecture: An infectious cDNA clone of Zika virus to study viral virulence mosquito transmission, and antiviral inhibitors

In this class, we learned about "An infectious cDNA clone of Zika virus to study viral virulence, mosquito transmission, and antiviral inhibitors". It is a new topic and popular in scope of virology. Dr. Nitwara presented the slides to us, which is easy to understand. She demonstrated by showing the background of the paper then focusing on the objective, result, discussion and conclusion, respectively. In the each part, she gave a break by asking some questions. She gave some point or keywords to us which allowed us to follow and she helped us to understand more.

From the class, we concluded that Zika virus (ZIKV) is a member of the Flavivirus genus. ZIKV is transmitted by *Aedes* spp. ZIKV infection led to Guillain-Barré syndrome and congenital microcephaly. In this study, they aimed to study on construction and characterization of a full-length cDNA clone. The cDNA clone are derived from Asian linkage ZIKV strain. It causes neurological disease in A129 mice (lacking interferon α/β receptors) and

AG129 mice (lacking interferon α/β and γ receptors. The mutational cDNA clone has an effect on the A129/AG129 mouse and on microcephaly development because it lacks herd immunity and led to greater susceptibility for ZIKV infection and efficient mosquito transmission. The interaction between viral and host factors determines the efficiency of infection, pathogenicity, transmission and epidemic potential. Therefore, variation of critical host factors among infected individuals may contribute to different disease severity. The differences in replication and virulence between the parental and recombinant viruses might come from the genetic heterogeneity diversity of the parental virus when compared to the homogeneity genetic. For the mosquito experiments, the replication of the recombinant virus was reduced in mosquito C3/C6 cells. Its yield of infection rate in *A. aegypti* mosquitos is similar to the parental virus, indicating that the cell culture system does not necessarily recapitulate *in vivo* outcomes. So, we can conclude that the recombinant ZIKV is highly infectious for *A. aegypti* mosquitoes. In addition, the infectious cDNA clone could be used to generate a luciferase reporter ZIKV that exhibited sensitivity to a pan flavivirus inhibitor, so it is necessary for antiviral drug discovery (3).

October 26, 2016

Asst. Prof. Sarin Chimnaronk's lecture: Genetic dissection of *Flaviviridae* host factors through genome-scale CRISPR screens

Asst. Prof. Sarin gave us a research paper published on the title of " Genetic dissection of *Flaviviridae* host factors through genome-scale CRISPR screens" and discussed with us in the class. The paper that he gave to us was very difficult. After Asst. Prof. Sarin's lecture and discussed in class, we could summarize the oligosaccharyltransferase (OST) complex,

which contain STT3 subunit that can divided into two paralogues including STT3A and STT3B, is important for viral replication because it has partially redundant function in N-linked glycosylation. DENV-2 requires both STT3A and STT3B but other flavivirus needs only STT3A. West Nile virus (WNV) does not require the translocon associated protein (TRAP) complex, whereas Yellow Fever Virus (YFV) and Zika virus (ZIKV) do not need the ER-associated protein degradation (ERAD) complex. An intact structures of the OST complexes are necessary for interactions with DENV NS3 in viral replication. In addition, Asst. Prof. Sarin gave us an answer to the topic of "Differences and advantages/disadvantages of CRISPR/Cas9 beyond siRNA screens" such as how CRISPR-Cas9 works together. It induces double strand RNA break which led to non-homologous end joining (NHEJ) and causes the gene to be knocked out. On the other hand, the siRNA interferes the expression of specific genes and causes gene silencing by knocking down mRNA transcription (4).

November 2, 2016

Assoc. Prof. Panadda Boonserm's lecture: The opportunistic marine pathogen *Vibrio parahaemolyticus* becomes virulent by acquiring a plasmid that expresses a deadly toxin

Assoc. Prof. Panadda gave assignment to us one week before the class started. Students were divided into 3 groups to discuss the paper and present in class on the topic "The opportunistic marine pathogen *Vibrio parahaemolyticus* become virulent by acquiring a plasmid that expresses and deadly toxin". After we presented, she summarized the paper again to make us understand the topic more. It can be concluded that the *V. parahaemolyticus* can produce photorhabdus insect-related (Pir) toxin

including PirA and PirB which causes acute hepatopancreatic necrosis disease (AHPND) in shrimp. This study aims to identify and confirm the AHPND virulence factor and the important role of *V. parahaemolyticus* PirAB toxin in AHPND pathogen. The AHPND-causing strains were identified and confirmed by purified plasmid and Southern blot hybridization. They can detect plasmid about 70 kbp (pVA1) in AHPND-causing strain when compared with the non-AHPND strain which indicated that pVA1 encoded Pir toxin. pVA1 leads to shrimp death via AHPND induce sloughing of hepatopancreas (HP) epithelial cell into the HP tubule lumens that can be detected by histochemistry. For the PirAB toxin, they found that PirA and PirB interacted to form a complex such as a binary complex. In order to form complex, the two proteins needed to be coexpressed. They crystallized PirA and PirB for structure determination by using x-ray crystallography. The structural alignment showed that PirA and PirB are very similar to the Cry insecticidal toxin of *Bacillus*, which is toxin that can induce cell death. The structure of N-terminal domain of PirB similar to Cry domain I, which has pore forming activity, the C-terminal domain similar to Cry domain II, which is receptor binding, and the PirA, similar to Cry domain III, that relate to receptor recognition and membrane insertion. These data suggested that PirAB might induce cell death by forming a pore in the cell membrane like the mechanism of Cry insecticidal toxin (5).

November 9, 2016

Assoc. Prof. Wipa Chungjatupornchai's Lecture: Altered lipid composition and enhanced lipid production in green microalga by introduction of brassica diacylglycerol acyltransferase 2

For this week, we learned about the lipid production by microalgae on the title "Altered lipid composition and enhanced lipid production in green microalgae by introduction of brassica diacylglycerol acyltransferase 2". In the class, emphasized the result and discussion by assigning us explain the result, 1 person per 1 figure, by focusing on "Why did they do?" and "How did they do?" the result and also the conclusion for each picture. Over all, we can conclude that the research published aim to study the function of enzyme diacylglycerol acyltransferase (BnDGAT2) encoded by aph7 gene of rapeseed of *Brassica napus*. The *Chlamydomonas reinhardtii* was transformed with vector containing BnDGAT2–eGFP to produce higher lipid. The result shows higher neutral lipid in the *C. reinhardtii* containing BnDGAT2 when compared with the wild-type cells that do not containing the BnDGAT2 as a control. However, the transgene integration and expression of BnDGAT2 was confirmed by PCR and Southern blots and the expression was confirmed under fluorescence microscope analysis of lipid droplets by Nile red staining. They quantified the total neutral lipid by FAME analysis which can show that the level of saturated fatty acid of transformed alga was decreased whereas that of unsaturated fatty acid was increased when compared with the wild type. In addition, they investigated of long-term stability that showed the cryopreservation could produce higher lipid than the subculture continuously on the solid medium. It can be concluded that the BnDGAT2 was introduced into *C. reinhardtii* to increase the production of the lipid and it should be maintained in liquid or cryopreserved for long-term stability (6).

November 16, 2016

Dr. Poochit Nonjuea's lecture: TarO-specific inhibitors of wall teichoic acid biosynthesis restore β-lactam efficacy against methicillin-resistant staphylococci

Dr. Poochit gave so little time to us to read the published research and he gave 1 page summary assignment. In this class, he explained the background of the topic "TarO-specific inhibitors of wall teichoic acid biosynthesis restore β-lactam efficacy against methicillin-resistant staphylococci". Then we discussed the result and Dr. Poochit helped us to understand extended results and focus on the main point. The slide presentation and the techniques that he taught is modern, so we can understand easily. From the class, we can summarize this topic that Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium resistance to β-lactam antibiotics, which include penicillins such as methicillin, cephalosporins, and carbapenems. The cell wall of MRSA compose of teichoic acid, which are synthesized by two pathways including the non-essential wall teichoic acid early-stage genes (*tar*O, *tar*A, and *mna*A) and the conditionally essential wall teichoic acid late-stage genes (*tar*B, *tar*D, *tar*F, *tar*I, *tar*J, *tar*L, *tar*G, and *tar*H). This study, they aim to identify early-stage wall teichoic acid inhibitors in MRSA strain COL. Wall teichoic acid early-stage subunits, TarO and TarA, were inhibited by chemical compounds leads to cell survival whereas TarG, which is wall teichoic acid late-stage subunit, was inhibited with tarG inhibitor leads to cell death because it is susceptible to β-lactam antibiotics. It depends on the position of mutation, which are TarO specific inhibitors of wall teichoic acid. Finally, to study synergistic effect of TarO inhibitor and other antibiotic, they observed synergistic effect to imipenem and dicloxacillin to both *in vitro* and *in vivo* treatment. In this study, they tried to develop new target to increase β-lactam in MRSA that is tarO gene associate with wall teichoic acid biosynthesis. Therefore, when the wall of bacteria are weaker by TarO inhibition, it led to susceptibility to β-lactam and showed synergistic effect in other antibiotic.

This study is useful for a better understanding of antibacterial drug resistance (7).

In addition, before the end of class, Dr. Poochit gave resources to the commentaries in learning and share his experiences with us, which can be applied in everyday life.

Dr. Chalongrat Noree's lecture: Supramolecular assembly of metabolic enzymes

Dr. Chalongrat gave the research publication and also the supplementary documents about "Spatial colocalization and functional link of purinosomes with mitochondria". Moreover, he gave the assignment by asking us to write a one page summary. This paper was difficult and the words were very complicate, but he had the modern techniques in teaching to make it very fun. Dr. Chalongrat teached us through game by giving one whiteboard and one dice per one person and showed the question on the slide. Before we started the every question, we had to throw dice to get the point, which were the scores for question. If the answer was correct, we would get the plus score. If the answer was wrong, we would get the minus score according to the point from the dice. After we throw the dice,he would showed the question and we had one minute to write the answer. For each question, we have to answer the main point to get the score. This technique made us feel alert and excited, and it is also thought-provoking all the time.

By learning, we know that the purinosomes is biosynthetic enzyme complex, they carry the enzyme for synthesizing purine in *de novo* pathway. Purinosomes responses to changes in purine levels as purine depletion can cause an increase in *de novo* purine biosynthesis. The presence of ATP during *de novo* purine biosynthesis is important to mitochondria. They proposed that there is a synergistic relationship between purinosomes and mitochondria. The hypothesis of this study is that there are a functional link and a

relationship between purinosomes and mitochondria. They use 3D STROM super resolution fluorescence microscope to investigate the colocalization of purinosome and mitochondria. The result indicated that purinosomes distributed at spatial mitochondria and cytoplasm. They confirmed an interaction between purinosomes and mitochondria by disturbing mTOR as inducer of purinosome formation by using rapamycin. The amount of purinosome were decreased, the colocalization of purinosome and mitochondria were decreased and the purinosome formation were stimulated by mitochondria dysregulation. It can be applied to medical purposes, e.g. developing the drug for cancer cell by inhibiting purinosome which causes a reduction in ATP synthesis and also purine as nucleotide. The nucleotide and ATP are essential to DNA synthesis. Therefore, if there is less nucleotide and ATP, the cancer cells cannot grow (8).

November 30, 2016

Prof. Duncan Smith's lecture: Novel piperazine core compound induces death in human liver cancer cells: possible pharmacological properties

Prof. Duncan gave the research published on the title of "Novel piperazine core compound induces death in human liver cancer cells: possible pharmacological properties" (9). In this class, he taught us to analyse the paper. This paper was published on 13 April 2016 and then it was retracted on 22 June 2016 due to all data in this paper was completely fabricated. When look closely at figures, there was various fabrication such as inversion, duplication, rotation, impossible merges, photoshopping to edit or delete band, questionable error bar, multiple panel reuse and some of the data were stolen from other paper. The misconduct in science does not include the error of judgment, recording, analysis of data which arises from different in opinions involving the interpretation of data. There were some type

misconduct that are unrelated to the research process, so the paper were still published. Published papers does not mean it was correct.

Conclusion

In current topics in molecular biology course, we learned several techniques about scientific articles including which articles that we should select, writing techniques, references, reading principles, interpretation and presentation techniques, including honesty and responsibility in every step of scientific works. In addition, we obtained knowledges and novel techniques that involve in molecular biology both of familiar field and other field from the assigned reading articles.

Everything that we have learned from this course make us can analyse scientific articles in molecular biology field and efficiently get logical and scientific ideas of these articles. If we can apply the knowledges and techniques that we get from this course to our current or future experiment as well as scientific publication writing, we will be a honest and ideal scientist.

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