

Answers To Replication And Protein Synthesis Webquest

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Rad4TopBP1, a multiple BRCT domain protein, is essential for initiation of replication and participates in checkpoint responses following genotoxic treatment during the S and G2 phases of the cell cycle. Rad4 interacts with several proteins that are involved in initiation of DNA replication (i.e. Sld2) or activation of Chk1 and/or Cds1 checkpoint kinases (i.e. Rad9, Crb2, and Rad3). However, it remained unclear how Rad4 and its homologues are regulated to coordinate these diverse functions. This PhD project presents a comprehensive structure/function analysis of the fission yeast Rad4TopBP1. In order to obtain separation of function mutants of Rad4, a hydroxylamine random mutagenesis genetic screen was performed. However, we were not able to separate a checkpoint activation function from replicative function. Rad4 being phosphorylated in a Cdc2-dependent manner, the role of Rad4 phosphorylation by Cdc2 was investigated. A mutant strain containing multiple mutations at Rad4 Cdc2 consensus phosphorylation sites does not exhibit significant sensitivity to DNA damage or HU. In addition, Rad4 Cdc2 phosphorylation sites do not play a role in DNA re-replication. There is no significant phenotypic effect observed after DNA damage in *S. pombe* strains expressing a Rad4 protein deleted for a putative domain (RXL motif) interaction with cyclin, or harboring mutations in putative sumoylation motifs, or C-terminus truncation. In higher eukaryotes, TopBP1 binds and activates the ATR-ATRIP complex via an ATR-activating domain (AAD) in order to activate a checkpoint function. We identified a potential AAD in C-terminal of Rad4 in *S. pombe*. I show that Rad4 physically associates with Rad3 in vitro in an AAD-dependent manner. *S. pombe* strains mutated in the AAD show a slight sensitivity to DNA damage and HU. The rad4 AAD mutants do not completely prevent Rad3-mediated G2/M checkpoint activation after DNA damage. The sensitivity in a rad4 AAD mutant increases when damage occurs in S-phase, when histone H2A phosphorylation is defective. I established an artificial checkpoint induction system in the absence of exogenous lesions by targeting checkpoint proteins onto chromatin in *S. pombe*. Interestingly, Rad4 AAD is essential for checkpoint activation in this system. Because this checkpoint activation is independent of ssDNA-RPA formation, the data suggest that the AAD plays a role in chromatin-

mediated checkpoint maintenance/amplification. Altogether, this pathway seems to play an important role in S-phase when DSBs resection is limited.

Alkylating minor groove DNA binder adozelesin is capable of inhibiting DNA replication in treated cells through a trans-acting mechanism. The trans-acting replication factor that becomes deficient upon the action of adozelesin has been identified as replication protein A (RPA). Loss of RPA function can be caused by two possible mechanisms: RPA inactivation and/or loss RPA protein. To study the possibility of RPA inactivation, we purified large amount of RPA from adozelesin-treated cells and examined its function in RPA-dependent as says. We found that RPA purified from cells treated with adozelesin has the same single-stranded DNA binding activity and support nucleotide excision repair as normal RPA, but is not able to support SV40 DNA replication in vitro. Using purified proteins in DNA replication initiation assays, we found that RPA purified from cells treated with adozelesin in not able to be involved in the functional interaction with DNA polymerase 0:/primase and SV40 large T antigen.

Bacterial Physiology was inaugurated as a discipline by the seminal research of Maaløe, Schaechter and Kjeldgaard published in 1958. Their work clarified the relationship between cell composition and growth rate and led to unravel the temporal coupling between chromosome replication and the subsequent cell division by Helmstetter et al. a decade later. Now, after half a century this field has become a major research direction that attracts interest of many scientists from different disciplines. The outstanding question how the most basic cellular processes - mass growth, chromosome replication and cell division - are inter-coordinated in both space and time is still unresolved at the molecular level. Several particularly pertinent questions that are intensively studied follow: (a) what is the primary signal to place the Z-ring precisely between the two replicating and segregating nucleoids? (b) Is this coupling related to the structure and position of the nucleoid itself? (c) How does a bacterium determine and maintain its shape and dimensions? Possible answers include gene expression-based mechanisms, self-organization of protein assemblies and physical principles such as micro-phase separations by excluded volume interactions, diffusion ratchets and membrane stress or curvature. The relationships between biochemical reactions and physical forces are yet to be conceived and discovered. This e-book discusses the above mentioned and related questions. The book also serves as an important depository for state-of-the-art technologies, methods, theoretical simulations and innovative ideas and hypotheses for future testing. Integrating the information gained from various angles will likely help decipher how a relatively simple cell such as a bacterium incorporates its multitude of pathways and processes into a highly efficient self-organized system. The knowledge may be helpful in the ambition to artificially reconstruct a simple living system and to develop new antibacterial drugs.

My combined genetic and biochemical strategy has forged novel determinants of RNR regulation and has enhanced our comprehension of the response to replication stress. The ribonucleotide reductase (RNR) inhibitor hydroxyurea (HU) depletes cellular pools of deoxyribonucleotides (dNTPs) and causes replication stress by pausing replication fork progression and blocking DNA synthesis. Stalled or collapsed DNA replication forks can lead to DNA double strand breaks, chromosome rearrangement, and loss of genome stability. The DNA replication checkpoint responds to replication stress by slowing S-phase progression, stabilizing stalled replication forks, and increasing RNR complex activity. In the budding yeast *Saccharomyces cerevisiae*, checkpoint responses hinge on activation of Mec1 (mammalian ATR ortholog) and Rad53 (mammalian Chk2 ortholog) checkpoint kinases. To identify novel gene activities that contribute to tolerance of replication stress, I surveyed the 4,812 strains in the *S. cerevisiae* non-essential haploid gene deletion collection for hypersensitivity to HU. Strains bearing deletions in either CCR4 or CAF1/POP2, which encode components of the major cytoplasmic mRNA deadenylase complex, were amongst 49 gene deletions that confer susceptibility to replication stress. I found that Ccr4 cooperates with the Dun1 branch of the replication checkpoint, such that a *ccr4Delta dun1Delta* strain exhibits irreversible HU sensitivity and persistent Rad53 activation. Mutations in CRT1, which encodes the transcriptional repressor of RNR and DNA damage-induced genes, were uncovered as the major suppressors of *ccr4Delta* HU sensitivity. In addition, expression of RNR genes bypasses HU sensitivity of the *ccr4Delta dun1Delta* strain. These observations implicate coordinated regulation of Crt1 and RNR transcription via Ccr4 and Dun1 as a critical nodal point in the response to DNA replication stress. In parallel, I undertook a biochemical approach to interrogate the molecular function of Hug1, a small protein induced by DNA damage or replication stress. I found that Hug1 interacts with the small RNR proteins Rnr2 and Rnr4. Over-expression of HUG1 is lethal in combination with a *dun1Delta* mutation in the presence of HU; this lethal interaction requires a physical association of Hug1 with RNR subunits. I suggest a model whereby Hug1 is induced by HU and inhibits checkpoint responses via its physical interaction with RNR.

This volume is composed of chapters that review important fundamental aspects of HCV biology and disease pathogenesis including, for example, the discovery and identification of the HCV genome, early virus-cell interactions including identification of various cellular receptors, HCV gene expression studied using the HCV replicon system, identification and characterization of HCV structural- and non-structural HCV proteins, HCV replication in cultured cells, and host factors involved in viral replication. This volume also contains chapters dealing with immunity to HCV infection and pathogenesis. This is particularly important in understanding hepatitis C because HCV infection alone is not cell lytic. Mechanisms underlying the persistent nature of HCV infection are also discussed in these chapters. Many of the authors published articles that were listed among the "top 10 papers" published in the 24 years since HCV was discovered in 1989. Their citations are above 1,000 (Web of Science). The authors describe the background and significance of their contributions to the field in the context of findings from other research groups. This book addresses the innovative themes in characterizing the cellular membrane platforms and intracellular networking, as well as the architectural aspects of cell compartments mediated by the entry and replication cycles of viruses. The instrumentation of modern molecular and cellular biology provides a potent array of wave packets to image, detect and manipulate major dynamics of macromolecular and subviral assemblies as in the host cellular context. The book includes case studies presented with highly coherent and structured illuminations, including microscopy, spectroscopy and scanning probes. The compilation and integration of the methodology provides time-resolved observations on the reactivity of structures from near-atomic resolution to various molecular or cellular levels of descriptors. The book provides a broad introduction to the various fascinating virus systems and may be used as an advanced textbook by graduate students in biomedicine. It provides adequate background material to explore further the research problems of epidemics in the 21st century.

DNA replication and associated processes take place in all living organisms with the same constitutions. The knowledge of the duplication process, chromatin building and repair mechanisms has increased explosively over the last years, but the complex interplay of different proteins and their mechanisms are not conceived properly. During DNA replication, the DNA has to be

unpacked, duplicated and finally repacked into chromatin. These steps require different proteins, e.g. new histone proteins on demand to secure an error-free and undelayed DNA replication. This thesis includes different mathematical models for DNA replication, repair and chromatin formation, which are based on experimental results. Three models of chromatin formation provide a simplified description of histone gene expression and protein synthesis during G1/S/G2 phase and include the contribution of different regulatory elements. Furthermore, all models present two different mechanisms of regulation to test possible scenarios of newly synthesised histones and free DNA binding sites. The basic model presents a single histone gene, which codes for a single histone protein. The stem-loop binding protein (SLBP) acts as a master regulator, which is only present during S phase. Different analyses of early S-phase, over- and underexpressed replication and the down-regulation of SLBP proof the model under extreme conditions. This basic model serves as a template for further scenarios with several genes and different histone families. For this, a second model is realised to simulate imbalances in the histone mRNA synthesis and translation. Additionally, a third model tests a gene knock-out and mRNA silencing. The initial histone model is able to qualitatively reproduce experimental observations and shows basic regulatory principles. The adaptation with several genes and different histone families presents qualitatively different system responses for the discussed regulatory mechanisms and illustrates the ability to compensate the effect of mRNA silencing.

The *Togaviruses: Biology, Structure, Replication* deals with the biology, structure, and replication of rotaviruses. This book covers topics such as the biochemistry of rotaviruses and the biological and medical challenges they pose. It also gives an account of their mechanisms of replication that might lead to perceptions of the capacity to solve biological and epidemiological problems through the concepts and technology of molecular biology. This text is comprised of 21 chapters that explore clinical details, routine procedures for diagnostic virus isolation and identification and for serological tests; immunological host responses; the role of interferons; antiviral chemotherapy; and vaccine development. The discussion begins with a historical overview of arboviruses, followed by a description of all the viruses that belong to *Togaviridae*. These include alpha- and flaviviruses, rubiviruses, pestiviruses, and other "non-arbo" togaviruses. The next chapters focus on the arthropod-vertebrate-arthropod transmission cycle and its experimental equivalents, along with the viruses' structure, composition, and replication. This book concludes with a summary of physicochemical, morphological, and clinical data on non-arbo togaviruses. This reference material will be of interest to physicians, veterinarians, ecologists, entomologists, epidemiologists, cell biologists, immunologists, virologists, physical chemists, biochemists, molecular biologists, and geneticists.

The Dbf4 protein is involved in the initiation of DNA replication, in complex with Cdc7 kinase, and also plays a role in the intra-S-phase checkpoint response via an interaction with Rad53 in *Saccharomyces cerevisiae*. The Dbf4 protein has three highly conserved motifs, called the N, M and C motifs. In view of the fact that a comprehensive analysis of the roles of the three motifs in the initiation of DNA replication and checkpoint response was not previously available, this study was, therefore, conducted. The objectives of the study were: (1) to assess the function of the three conserved motifs, with respect to their essentiality for cell viability, (2) to determine their roles in mediating interactions with other proteins (i.e. Cdc7, Orc2, Mcm2) involved in the initiation of DNA replication and with Rad53 in the intra-S-phase checkpoint response, and (3) to obtain the three-dimensional structure of the Dbf4 N-motif by X-ray crystallography.

Necessary to the survival of cellular life is proper replication and maintenance of the genome. Replication proteins Mcm10 and RecQ4 have well-characterized essential roles in assembly, initiation, proper functioning of the eukaryotic replication machinery, and genome stability. Mcm10 enables efficient assembly of the pre-replication complex while RecQ4 delivers essential proteins forming that complex; both of which are pivotal for origin firing. Recent studies suggest that Mcm10 is not required for RecQ4 chromatin localization or association with the CMG complex, conflicting with a previous report that Mcm10 mediates the interaction between RecQ4 and Mcm2-7 in an S-CDK dependent manner. We have found that a homozygous mutation of RecQ4 is lethal in fruit flies unless they possess a C-terminal domain (CTD) truncation of Mcm10, suggesting that Mcm10 and RecQ4 exhibit a genetic interaction. Here, we investigate how an Mcm10/RecQ4 genetic interaction might be important for organism viability and to facilitate critical replication states – like those found in larval brain development and adult oogenesis. We also investigate the individual and combined roles of Mcm10 and RecQ4 in genome stability. The Mcm10 CTD truncation rescue of lethal RecQ4 phenotypes can reveal much about how the cooperative roles of these proteins affect DNA replication and responses to DNA damage, but this requires further investigation. Our research aims to explore the nature of the Mcm10/RecQ4 genetic interaction by conducting genetic investigations, proliferation assays, and double strand break (DSB) studies in the *Drosophila* larval brain and adult ovary. We propose that Mcm10 and RecQ4 genetically interact to facilitate DNA replication and genome stability in *Drosophila melanogaster*.

This dissertation, "Influenza A Virus Replication and Cytokine Responses in Murine Macrophages in Vitro" by Wan-yi, Chan, ???, was obtained from The University of Hong Kong (Pokfulam, Hong Kong) and is being sold pursuant to Creative Commons: Attribution 3.0 Hong Kong License. The content of this dissertation has not been altered in any way. We have altered the formatting in order to facilitate the ease of printing and reading of the dissertation. All rights not granted by the above license are retained by the author.

Abstract: Abstract of thesis entitled Influenza A virus replication and cytokine responses in murine macrophages in vitro Submitted by Chan Wan Yi for the degree of Master of Philosophy at The University of Hong Kong in October 2005 Abstract Background: Lethal influenza A subtype H5N1 has been documented in Hong Kong, and more recently in Vietnam, Thailand, Cambodia and Indonesia. The ability of the virus to affect the host adversely determines its virulence. H5N1 disease in humans is associated with peculiar severity and high mortality. Patients infected with H5N1 demonstrated unusually high serum levels of interferon-gamma-inducible protein-10 (IP-10). In contrast to human influenza virus subtypes H1N1 and H3N2, the H5N1 viruses of 1997 (A/Hong Kong/483/97) was found to be a more potent inducer of pro-inflammatory cytokines and

chemokines from human macrophages in vitro. It has been suggested that cytokines dysregulation plays a role in the pathogenesis of H5N1 disease. The mechanisms involved in this differential cytokine induction remain unclear. Knock-out mouse models would be useful to delineate the signaling pathways involved. As a prerequisite for such experiments, it is important to characterize the virus-mouse macrophage interaction in vitro. Methods: The ability of the influenza viruses to replicate in the murine peritoneal and bone marrow derived macrophages extracted from ICR, Balb/c and C57bl/6N was determined at the levels of viral gene transcription, protein synthesis and infectious virus production. They were obtained from quantitative RT-PCR of viral matrix (M) gene, immunofluorescence staining of viral nucleoprotein (NP) protein and TCID₅₀ assay of infected macrophage culture supernatants, respectively. The cytokine and chemokine gene expression profiles elicited by viruses A/HK/483/97, A/Vietnam/1194/04 with that of human H1N1 (A/HK/54/98) and mouse adapted A/WSN/33 (H1N1) viruses in murine macrophages in vitro were compared using quantitative RT-PCR. Results and Interpretation: The differential hyper-induction of cytokine and chemokine gene expression by H5N1 viruses in macrophages is well demonstrated in the C57bl/6N peritoneal macrophage and bone marrow derived macrophage supplemented by M-CSF. Macrophage from this inbred strain exhibited a differential cytokine gene induction profile similar to that previously demonstrated in primary human macrophage in vitro study. Therefore, C57bl/6N would be a relevant model for studying the pathogenesis of human H5N1 disease. More investigations on the mechanism underlying this pronounced cytokine upregulation can be delineated in knock-out model constructed with C57W/6N background which is now available. In the process of developing an appropriate model for specific research interest, it was discovered that the recent H5N1 (A/Vietnam/1194/04) appears to be more potent to induce IFN- β and IP-10 than H5N1/97 virus. This finding may be relevant to the understanding of the pathogenesis of human disease caused by the H5N1 viruses recently prevalent in South-East Asian Region. The need to advance knowledge in this area is of paramount importance in view of the global pandemic threat currently posed by this H5N1 influenza virus. Word count: 426 DOI: 10.5353/th_b3382993 Subjects: Macrophages Avian influenza - Cytopathology Cytokines Mice - Immunology

HCV is an enveloped positive-stranded RNA virus that infects 3% of the world population. It has four structural proteins and six non-structural proteins with untranslated regions at the 5' and 3' termini. Functions for all proteins in HCV have been reported except for p7 and non-structural protein (NS5A). NS5A is a phosphoprotein that has two phosphorylated forms (p56 and p58). Although the roles of NS5A are not completely understood, properties have been reported in numerous papers. NS5A has two roles; genomic replication and host cellular protein interactions. And these functions are switched by the level of phosphorylation on NS5A. The hyperphosphorylated form (p58) is suggested to interfere with cellular signaling and the un- or hypophosphorylated form (p56) is involved in genomic replication. In this study, we mapped the minimal domain of NS5A for RNA binding. The N-terminus of NS5A is required for RNA binding. The interface of the dimer has positive electrostatic potential that is sufficient to accommodate an RNA helix. NS5A-RNA binding is dependent on electrostatic interactions and ionic strength. NS5A binds RNA at neutral and low pH, but not at high pH or high salt concentration (300 mM). NS5A is able to form oligomer. Structural feature of dimerized NS5A has enough space for RNA binding and, interestingly, positive charged amino acids in the bottom and negative charged in the side of groove, suggesting that this space can be a putative groove for RNA binding. In addition, glutaraldehyde and UV crosslinking studies reveal that monomeric and dimeric NS5A bind RNA. NS5A is phosphorylated by cellular kinases including cAMP-dependent kinase A (PKA). We identified a PKA phosphorylation site on NS5A and examined the effects of PKA phosphorylation on HCV genomic replication. PKA phosphorylation enhances the accumulation of subgenomic replication during cell divisions and modulates cleaved NS5A subcellular localization. In order to prevent viral spread and expansion, an infected cell recognizes foreign agents through various mechanisms. One of the systems is the PKR pathway, which turns off cellular and viral translation by sensing and binding double-stranded RNA (dsRNA). Herein, we show that single-stranded RNA (ssRNA) is also able to activate PKR and this activation requires a 5'-triphosphate on the RNA. PKR cannot be activated by 7-methyl guanine capped or hydroxylated RNA, suggesting that PKR differentiates between cellular RNA and foreign RNA. HCV NS5A has the ability to prevent PKR autophosphorylation induced by dsRNA and ssRNA. Deciphering the NS5A functions in genomic replication and PKR responses to foreign RNA will provide significant practical applications as well as insight into replication and innate immune response mechanism. Furthermore, this study will contribute to developing strategies for antiviral drug targets.

Maintaining the integrity of any genome is a critical function that requires proteins involved in DNA replication, damage recognition and repair. Particularly deleterious to chromosomal integrity are agents such as ionizing radiation (IR) that cause DNA double strand breaks (DSBs) directly. It has also been suggested that UV-induced DNA damage contributes to the formation of DSBs in dividing cells. UV light does not break DNA directly; it is possible that replication fork arrest at UV-induced lesions leads to the formation of DSBs. Here we examine whether UVC causes DSBs, the role of DNA replication in the formation of breaks, and signal transduction pathways involved in UV-induced DNA damage and repair responses. Both ataxia telangiectasia-mutated (ATM) and a downstream target of ATM, Nijmegen breakage syndrome protein or nibrin (NBS1) play major roles in sensing and responding to DNA damage caused by DSBs mediated through IR. Here we show that A-T cells (cells lacking ATM) accumulated more UVC-induced DSBs. These data suggest that ATM is required for signaling the repair of DSBs generated by replication of UV-damaged templates. By contrast, NBS cells (cells lacking NBS1) showed fewer DSBs, suggesting that NBS1 may be required to induce the initial DSB. The NBS1 protein was bound more tightly in the nucleus after treatment with UVC. The nuclear retention of NBS1 protein was seen as early as 4 h after UVC. We also found that the nuclear retention of NBS1 was increased after UVC radiation in a time-dependent manner. The increased retention of NBS1 after UVC suggests its possible activation after UVC and that this treatment stimulates the association with the NBS1/MRE11/RAD50 DSB repair complex. Our data from the PFGE analysis and neutral comet assay may suggest the NBS1 protein may participate in the generation of the DSBs produced after

replication of UV-damaged templates. Taken together, our results suggest that ATM and NBS1 are involved in response to UVC-induced DSBs.

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Mouse Hepatitis virus (MHV) is a member of the coronavirus family in the order Nidovirales. The 32 kb genome contains cis-acting sequences necessary for replication of the viral genome. Those cis-acting sequences have been shown to bind host proteins, and binding of those proteins is necessary for virus replication. One of the cis-acting elements is the 3' terminal 42 nucleotide host protein binding element. Previous work has demonstrated that mitochondrial aconitase, mitochondrial heat shock protein 70, heat shock protein 60 and heat shock protein 40 bind to the 3' terminal 42 nucleotide host protein binding element. We demonstrated that RNA secondary structure of the 3' terminal 42 nucleotide host protein binding element is necessary for host protein binding in vitro. We also demonstrate that primary structure of the 3' terminal 42 nucleotide host protein binding element is necessary for viral replication by targeted recombination. DI replication assays infer that the 3' terminal 42 nucleotide host protein binding element plays a role in positive strand synthesis from the negative strand template. Current studies involve the infectious cDNA clone, which will provide definitive answers on the role of the 3' terminal 42 nucleotide host protein binding element in MHV replication.

Both replicative stress and DNA damage initiate cellular processes collectively termed the DNA damage response. These processes include activation of appropriate DNA repair mechanisms, cell cycle checkpoints, and in some cases, apoptosis. Accurate and efficient operation of the DNA damage response is essential for preventing mutations that may lead to oncogenic transformation or some types of inherited diseases. The DNA damage response involves sensing the damage, activation of specific kinases that transduce the activation signal via protein phosphorylation, and activation of effector proteins that carry out the functional aspects of the response. Two hallmarks of the DNA damage response are phosphorylation of key regulatory proteins and aggregation of multiprotein complexes into foci at or near the site of damage. The proteins that are phosphorylated and the composition of the foci depend upon the nature of the DNA lesion, and changes as the damage is recognized, processed and then repaired. Although different types of DNA damage activate specific repair proteins and pathways, some proteins respond to multiple types of lesions. Two protein complexes essential for the response to many lesions types are the Mre11/Rad50/Nbs1 (MRN) complex and replication protein A (RPA). Evidence supports the hypothesis that both of these complexes have multiple roles in the DNA damage response, including initial DNA damage recognition, activation of the signal transducing kinases and functional roles in DNA repair pathways. Although the MRN complex and RPA both become phosphorylated and form foci in response to multiple types of DNA lesions, we found that they co-localize to nuclear foci only in response to a subset of lesions. However, depletion of RPA via siRNA abrogates the ability of the MRN complex to form foci. These data suggest that the MRN complex and RPA have functional activities that can be both dependent and independent of each other. Understanding the determinant of whether or not the MRN complex and RPA interact, as well as the functional consequence of this interaction, will help elucidate the cellular responses to different types of DNA lesions and provide crucial information that may allow us to intervene to prevent the negative effects of DNA damage.

This volume aims to describe a variety of techniques that reflects the wide range of research currently performed in the field of coronavirology, and begins with an overview of current understandings of coronavirus replication and pathogenesis to introduce specialists and non-specialists to the field. The rest of the book is divided into several sections of chapters beginning with those that describe

identification, diagnosis, and study of the evolution of coronaviruses. The next few chapters discuss the preparation of cells and organ cultures useful in propagating coronaviruses and titration techniques, as well as techniques for analyzing virus functions that require purification of the viruses. The next chapters describe two commonly used reverse genetics techniques for coronaviruses, and techniques detailing identification of cellular receptors, binding profiles of viral attachment proteins, and virus-cell fusion. The final chapters cover a broad spectrum of techniques to identify virus-host protein-protein interactions, confirm the functional role of these proteins in virus replication, study host cell responses through genome-wide or pathway-specific approaches, and visualize virus replication complexes. Written in the highly successful *Methods in Molecular Biology* series format, the chapters include the kind of detailed description and implementation advice that is crucial for getting optimal results in the laboratory. Authoritative and practical, *Coronaviruses: Methods and Protocols* appeals to a wide variety of scientists because it highlights techniques that are currently used in the coronavirology field, while also discussing practices applicable to other virology fields.

The application to Biology of the methodologies developed in Physics is attracting an increasing interest from the scientific community. It has led to the emergence of a new interdisciplinary field, called Physical Biology, with the aim of reaching a better understanding of the biological mechanisms at molecular and cellular levels. Statistical Mechanics in particular plays an important role in the development of this new field. For this reason, the XXth session of the famous Sitges Conference on Statistical Physics was dedicated to "Physical Biology: from Molecular Interactions to Cellular Behavior". As is by now tradition, a number of lectures were subsequently selected, expanded and updated for publication as lecture notes, so as to provide both a state-of-the-art introduction and overview to a number of subjects of broader interest and to favor the interchange and cross-fertilization of ideas between biologists and physicists. The present volume focuses on three main subtopics (biological water, protein solutions as well as transport and replication), presenting for each of them the on-going debates on recent results. The role of water in biological processes, the mechanisms of protein folding, the phases and cooperative effects in biological solutions, the thermodynamic description of replication, transport and neural activity, all are subjects that are revised in this volume, based on new experiments and new theoretical interpretations.

The field of virology has seen explosive growth in the past few decades. A large amount of effort has gone into successfully delineating virus evolution, genetic diversity, immunology, pathogenesis, structure, vaccine development, viral gene expression and genomic replication strategies. In addition, considerable recent work has been focusing on cellular responses to infection as well as how viruses may induce transformation and oncogenesis. Viruses are obligate intracellular parasites and thus absolutely dependent upon host cells. Not surprisingly, they often cause profound changes in cells, including apoptosis, death and signalling, to name a few perturbations. Thus, the molecular signals for how viruses induce pathophysiological alterations in their hosts have been of growing recent interest. Cellular and organismal responses, such as those induced by virus infection, are invariably mediated by changes in gene and protein expression and modification. Thus, there has been keen interest in understanding how gene and protein expressions and modifications are quantitatively and qualitatively affected by such challenges. From a historical perspective, most early work that examined host protein responses to virus infection employed "biased" approaches, in which investigators targeted a limited number, or only one cellular molecule of interest. Completion of many organisms' genome sequences has allowed the global "non-biased" simultaneous analysis of the entire repertoire of cellular mRNA species, the transcriptome, by gene micro-arrays. This has provided significant information about how cellular gene expressions are altered by virus-induced perturbations, but has not provided as much information about the encoded proteins. This results for several reasons, including, but not limited to the fact that gene expression levels cannot accurately predict protein expression levels, nor the types and extent of post-translational modifications, many genes encode multiple proteins through splice variants, and protein activity may be affected by a large number of conditions, including phosphorylation. Recent technological and bioinformatic approaches make it now possible to begin to extend similar global analyses to probe the cellular proteome, the repertoire of the actual effector molecules. One general strategy has been to take advantage of improved separations technologies, as well as greatly improved mass spectrometry resolution, to quantitatively or comparatively measure hundreds or thousands of proteins. Proteins from multiple conditions (i.e., mock-infected and infected) may be differentially labelled by various techniques, such as 2D-DIGE, ICAT, iTRAQ, SILAC, with 18O during peptide preparation, and/or by various other methods, and then compared to measure comparative alterations in the levels of proteins induced by the virus infection. Such analyses have also been extended by using "label-free" methods for more efficient multiplexing applications, and/or by examining specific protein modifications. In addition, concerted efforts to raise antibodies against all cellular proteins have resulted in the development of "antibody arrays," which are also generally used for quantitative or comparative assays. Finally, while assays, such as the above, are generally limited to delineating the absolute amount of specific proteins, newer technologies have been developed that allow the simultaneous probing of hundreds of proteins' functions. Assays, such as "Activity Based Protein Profiling", are designed to probe enzymatic activity, with current focus on broad-spectrum proteases and other enzymatic classes. This Research Topic will provide an overview of many of these methods, as well as numerous specific examples of each approach, and how they are used to better delineate the ways viruses affect cellular responses during infection.

The ideal foundation of a one-semester course for undergraduate students, Stenesh's Biochemistry presents the basic body of biochemical knowledge and a thorough exposition of fundamental biochemical concepts. Carefully balancing primary and secondary topics, this introductory text covers the essentials in proper depth to establish a firm foundation for further study. Superior to any other first level text available, Stenesh's Biochemistry features: clear writing, thorough explanations, and precise definitions. comprehensive study sections for all chapters, consisting of both review-type questions and calculation-type problems, graded by difficulty and including answers selected reading lists concise chapter summaries two-color text 529 illustrations a separate chapter on bioenergetics, and an extensive index. Four appendixes review acid-base calculations, the principles of organic chemistry, the tools of biochemistry, and oxidation-reduction reactions, and a separate Solutions Manual presents step-by-step answers to problems.

Interactive CD-ROM for biology students allows to participate in landmarks experiments while learning the most important questions and answers in biology. Review DNA Replication, Protein Synthesis, and other biological concepts as viewing and interacting with dynamic animations.

(Cont.) My results demonstrate that the conserved recombination protein, RecA, mediates most of the transcriptional response under the tested conditions. More than 75% of the RecA-mediated transcriptional response is due to the expression of phage and mobile element genes and their indirect effects. Under conditions of replication elongation arrest, there is still a significant recA-independent response, at least part of which is mediated by the replication protein DnaA. The DnaA-mediated response appears to be conserved in other bacteria, as homologues if the affected genes also have DnaA binding sites in their promoter regions. Previously, one of the DnaA regulated genes, *sda*, has been shown to affect cell viability after perturbations in replication. Here I showed that another DnaA-regulated gene, *ftsL*, also affects cell survival after replication arrest by coordinating replication and cell-division. I believe that my results have furthered our understanding of how replication is coordinated with other cell-cycle processes, and have raised interesting questions for future investigation.

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The genome of mammalian cells is under constant attack from DNA-damaging agents. To maintain genomic integrity, cells activate an array of pathways primarily consisting of DNA repair and DNA damage checkpoints. Human replication protein A (RPA), a single-stranded DNA (ssDNA) binding protein, is essential for almost all DNA metabolic pathways. However, the role of RPA in nucleotide excision repair (NER), a DNA repair pathway for removing bulky DNA lesions, remains elusive. In this study, the binding of RPA to a battery of well-defined ssDNA substrates has been systematically examined using fluorescence spectroscopy. The results showed that RPA has a lower binding affinity for damaged ssDNA than for non-damaged ssDNA, and there was no direct contact between RPA residues and the lesion itself. These findings will help define the roles of RPA in DNA damage recognition in NER. In cells, RPA undergoes hyperphosphorylation in the N-terminus of RPA32 subunit after DNA damage. In this study, the hyperphosphorylation-induced conformational changes of RPA have been probed using mass spectrometry-based protein foot-printing, fluorescence spectroscopy and limited proteolysis. The data show that upon hyperphosphorylation RPA undergoes a subtle structural change involving its DNA-binding domain B (DBD-B), reducing its affinity for short ssDNA. These results suggest that hyperphosphorylation may modulate RPA functions by altering DBD-B-mediated RPA-DNA/protein interactions. Cellular accumulation of DNA damage has been widely implicated in premature aging. In Hutchinson-Gilford progeria syndrome (HGPS) and restrictive dermopathy (RD), premature aging is caused by defective maturation of lamin A and linked to accumulation of DNA double-strand breaks (DSBs). However, how lamin A dysfunction leads to genome instability and premature aging is not understood. Here evidence showed that in HGPS and RD fibroblasts DNA damage checkpoints are persistently activated and recruitment of repair factors.

During infection, poxvirus makes host cells conducive for viral replication by causing host shutoff that is marked by global inhibition of host protein synthesis. Host shutoff facilitates the reallocation of cellular resources for viral replication and evasion of host antiviral immune responses. However, it poses a challenge for continuous synthesis of crucial cellular proteins and viral proteins that are important for viral replication. It is unclear whether and how viral and specific cellular proteins are selectively synthesized during poxvirus-induced host shutoff. In this dissertation, we elucidated that vaccinia virus boosts viral post-replicative protein synthesis by using the 5'-poly(A) leader at the 5'-UTR. Vaccinia virus has evolutionarily optimized the length of the poly(A) leader, and uninterrupted poly(A) leader is required for promoting poxvirus protein production. During vaccinia virus-induced shutoff, poly(A) leader stipulates viral post-replicative mRNAs an adaptive mechanism to translate efficiently. The poly(A) leader translation was not mediated by an internal ribosome entry site (IRES) mode, albeit poly(A) leader mediates cap-independent mode of translation. Through further investigation, we uncovered a cellular RNA-binding protein La-Related Protein 4 (LARP4) that was repurposed to augment vaccinia virus post-replicative mRNA translation. During VACV infection, LARP4 is enriched in the virus factory where VACV post-replicative mRNAs are translated. A decrease of LARP4 protein level reduces VACV replication, blocks post-replicative protein synthesis, and decreases 5'-poly(A) leader mediated translational advantage. Further studies showed that LARP4 is vital for the cap-independent mode of translation from poly(A) leader. We also showed that infection of vaccinia virus, the prototypic poxvirus, induced selective synthesis of cellular proteins involved in oxidative phosphorylation. Using simultaneous RNA-seq and ribosome profiling, we determined the mRNAs encoding proteins for oxidative phosphorylation complexes had increased relative translation efficiency. Indeed, vaccinia virus infection increased the activity of oxidative phosphorylation. Inhibition of oxidative phosphorylation function suppressed vaccinia virus replication significantly. Moreover, the mRNAs of oxidative phosphorylation have short 5'-UTRs with a less complex secondary structure that could confer oxidative phosphorylation mRNAs a translational advantage in vaccinia virus-infected cells during host shutoff. Together, these studies advanced our understanding of how vaccinia virus selectively synthesizes viral and cellular proteins for efficient viral replication during host shutoff. The findings may facilitate the development of novel anti-poxvirus strategies and the improvement of poxviruses as vaccine vectors and anti-cancer agents.

In the 1970s James Watson recognized the inability of conventional DNA replication machinery to replicate the extreme termini of chromosomes known as telomeres. This inability is due to the requirement of a building block primer and was termed the end replication problem. Telomerase is nature's answer to the end replication problem. Telomerase is a ribonucleoprotein which extends telomeres through reverse transcriptase activity by reiteratively copying a short intrinsic RNA sequence to generate 3' telomeric extensions. Telomeres protect chromosomes from erosion of coding genes during replication, as well as differentiate native chromosome ends from double stranded breaks. However, controlled erosion of telomeres functions as a naturally occurring molecular clock limiting the replicative capacity of cells. Telomerase is over activated in many cancers, while inactivation leads to multiple lifespan limiting human diseases. In order to further study the interaction between telomerase RNA (TR) and telomerase reverse transcriptase protein (TERT), vertebrate TERT fragments were screened for solubility and purity following bacterial expression. Soluble fragments of medaka TERT including the RNA binding domain (TRBD) were identified. Recombinant medaka TRBD binds specifically to telomerase RNA CR4/CR5 region. Ribonucleotide and amino acid pairs in close proximity within the medaka telomerase RNA-protein complex were identified using photo-activated cross-linking in conjunction with mass spectrometry. The identified cross-linking amino acids were mapped on known crystal structures of TERTs to reveal the RNA interaction interface of TRBD. The identification of this RNA TERT interaction interface furthers the understanding of the telomerase complex at a molecular level and could be used for the targeted interruption of the telomerase complex as a potential cancer treatment.

In bacterial cells, most if not all replication forks encounter some form of DNA damage or roadblock that stall or inactivate the fork during normal cell growth. Numerous pathways exist for repairing and reactivating replication forks and these pathways are crucial for maintaining genome stability and cell viability. Bacterial "Maintenance of Genome Stability Protein A" (MgsA) and related eukaryotic enzymes are implicated in cellular responses to stalled DNA replication processes. MgsA enzymes are members of the clamp loader clade of AAA+ proteins but their structures and biochemical properties are poorly characterized. We describe the first complete crystal structure of Escherichia coli MgsA that reveals a highly intertwined homotetrameric arrangement for the protein that distinguishes it from other clamp-loader clade AAA+ proteins. An extended oligomerization domain relative to the clamp loader proteins accounts for the unique oligomeric state. The structure represents the inactive conformation of MgsA due to displacement of the arginine finger residues from the neighboring active sites. Thus, a conformational rearrangement is required to engage the arginine finger and activate MgsA ATPase activity. Association with double stranded DNA ends appears to be the trigger that induces

the conformational rearrangement and activates ATP hydrolysis. We also describe a potential switch residue, Arginine92, that appears to coordinate DNA binding and ATP hydrolysis within MgsA. MgsA physically interacts with the single-stranded DNA binding protein (SSB). The interaction requires SSB's highly conserved C terminus (SSB Ct) and we define a likely SSB Ct binding site on MgsA. This interaction adds another member to the growing list of SSB interacting proteins and we propose that the interaction is critical for proper MgsA localization to the replisome. Collectively, this thesis presents a structural and biochemical characterization of Escherichia coli MgsA and provides insights into the mechanisms of MgsA-family proteins. Influenza A viruses (IAVs) continue to be a threat to human health. Despite extensive studies, the mechanisms underlying the IAVs-host interactions during IAV infection remain elusive. We employed quantitative proteomic methods to systematically explore the host cell protein expression responses to IAV infection and examine the function of a critical IAV protein called NS1 by identifying its host binding partners. Specifically, we used a 2-dimensional gel electrophoresis (2-DE) based proteomic method to screen host proteins whose expression was substantially altered by IAV. One critical protein named I κ B kinase-gamma (IKK γ) was found to be significantly down-regulated during IAV infection. Functional studies indicated that IKK γ and IAVs were mutually inhibitory and IKK γ might be the target for virus to inhibit IFN production. IAV protein NS1 is known to play critical roles in viral pathogenesis and host immune responses. Through 2-DE proteomic approach and mass spectrometry, we identified several novel host cellular proteins that were associated with NS1. First, we found that heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1) interacted with NS1, affected replication, transcription, expression and nucleo-cytoplasmic translocation of NS1 mRNA, and the eventual whole virus replication. Second, two ATPase proteins, RUVBL1 and RUVBL2, were identified to associate with NS1 for regulation of cell apoptosis in the absence of IFNs. Third, based on previous finding of the interaction between a DEAD family protein designated as DDX100 and NS1 through a more sensitive proteomic approach called SILAC (stable isotope labeling with amino acids in cell culture), we found this interaction promoted virus replication through enhancing viral NS1 gene replication, transcription, and dsRNA unwinding. In summary, through quantitative proteomic, molecular and cell biology studies, we generated the global picture of host cell protein expression responses to IAV infection. For IAV NS1, several host cellular proteins were found to interact with NS1 to regulate the host cell action and virus proliferation.

This 4-hour free course showed how genetic information flows from DNA to RNA to protein. It introduced the concepts of transcription and translation.

Complete and faithful replication of a cell's genetic information is an essential process. Many enzymes are involved in the process of successfully duplicating a cell's genetic information. Helicases, DNA polymerases, ligases, nucleases, and DNA binding proteins all play a role in DNA replication. However, the integrity of these enzymes can be compromised when they encounter DNA damage, which in general could be caused by chemical mutagens, ionizing radiations, or reactive oxidative species. Bacterial cells use a pathway called "DNA replication restart" to resume DNA replication following a disruptive encounter of the DNA replication enzymes with DNA damage. This pathway is catalyzed by primosome proteins, including PriA, PriB, PriC, DnaT, DnaB, DnaC, and DnaG. The importance of DNA replication restart for bacterial cell survival is demonstrated by the inability of strains that carry mutations in key primosome genes to grow and resist DNA damaging agents. Furthermore, this pathway is specific for bacterial cells: human cells don't use the same replication restart pathway and they don't encode genes for the primosome proteins that function in bacteria. Since DNA replication restart pathways are essential for bacterial cell growth and survival and are notably absent in human cells, we seek to answer the following question: can bacterial DNA replication restart pathways be targeted with novel antibacterial compounds? In order to answer this question, we have developed an enzyme based assay for high-throughput inhibitor screening to identify compounds that block the function of the primosome proteins PriA and PriB. Several interesting lead compounds have already been identified from the preliminary screening. In this study, the lead compounds have been validated as legitimate inhibitors and characterized with respect to their potency and mechanism of action.

CAIE A LEVEL Past Year Q & A Series - CAIE A LEVEL Biology Paper 4. All questions are sorted according to the sub chapters of the new A LEVEL syllabus. Questions and sample answers with marking scheme are provided. Please be reminded that the sample solutions are based on the marking scheme collected online. Chapter 1 : Cell Structure 1.1 The microscope in cell studies 1.2 Cells as the basic units of living organisms Chapter 2 : Biological molecules 2.1 Testing for biological molecules 2.2 Carbohydrates and lipids 2.3 Proteins and water Chapter 3 : Enzymes 3.1 Mode of action of enzymes 3.2 Factors that affect enzyme action Chapter 4 : Cell membranes and transport 4.1 Fluid mosaic membranes 4.2 Movement of substances into and out of cells Chapter 5 : The mitotic cell cycle 5.1 Replication and division of nuclei and cells 5.2 Chromosome behaviour in mitosis Chapter 6 : Nucleic acids and protein synthesis 6.1 Structure and replication of DNA 6.2 Protein synthesis Chapter 7 : Transport in plants 7.1 Structure of transport tissues 7.2 Transport mechanisms Chapter 8 : Transport in mammals 8.1 The circulatory system 8.2 The heart Chapter 9 : Gas exchange and smoking 9.1 The gas exchange system 9.2 Smoking Chapter 10 : Infectious disease 10.1 Infectious disease 10.2 Antibiotics Chapter 11 : Immunity 11.1 The immune system 11.2 Antibodies and vaccination Chapter 12 : Energy and respiration 12.1 Energy 12.2 Respiration Chapter 13 : Photosynthesis 13.1 Photosynthesis as an energy transfer process 13.2 Investigation of limiting factors 13.3 Adaptations for photosynthesis Chapter 14 : Homeostasis 14.1 Homeostasis in mammals 14.2 Homeostasis in plants Chapter 15 : Control and co-ordination 15.1 Control and co-ordination in mammals 15.2 Control and co-ordination in plants Chapter 16 : Inherited change 16.1 Passage of information from parent to offspring 16.2 The roles of genes in determining the phenotype 16.3 Gene control Chapter 17 : Selection and evolution 17.1 Variation 17.2 Natural and artificial selection 17.3 Evolution Chapter 18 : Biodiversity, classification and conservation 18.1 Biodiversity 18.2 Classification 18.3 Conservation Chapter 19 : Genetic technology 19.1 Principles of genetic technology 19.2 Genetic technology applied to medicine 19.3 Genetically modified organisms in agriculture

During the summer of 1974 we discussed the state of molecular biology and biochemical developmental biology in plants on a few occasions in Paris and in Strasbourg. The number of laboratories engaged in such research is minute compared with those studying comparable problems in animal and bacterial systems, but by then much interesting work had been done and a great momentum was building. It seemed to us that the summer of 1976 would be a good time to review these areas of plant biology for students as well as advanced workers. We outlined a program for a course to colleagues both in Europe and the United States and asked a few potential lecturers if they would be interested. The response was not just positive; it was overwhelmingly enthusiastic. Those who had some acquaintance with Alsace, and especially with Strasbourg, invariably told us that they had two reasons for being enthusiastic about participating - the subject and the proposed site. The lectures published here* reflect the diversity of current research in plant molecular biology and biochemical developmental biology. Each lecture gives us a glimpse of the depth of questions being asked, and sometimes answered, in segments of this field

of investigation. This research is directed at fundamental biological problems, but answers to these questions will provide knowledge essential for bringing about major changes in the way the world's agricultural enterprise can be improved.

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The field of cellular responses to DNA damage has attained widespread recognition and interest in recent years commensurate with its fundamental role in the maintenance of genomic stability. These responses, which are essential to preventing cellular death or malignant transformation, are organized into a sophisticated system designated the "DNA damage response". This system operates in all living organisms to maintain genomic stability in the face of constant attacks on the DNA from a variety of endogenous by-products of normal metabolism, as well as exogenous agents such as radiation and toxic chemicals in the environment. The response repairs DNA damage via an intricate cellular signal transduction network that coordinates with various processes such as regulation of DNA replication, transcriptional responses, and temporary cell cycle arrest to allow the repair to take place. Defects in this system result in severe genetic disorders involving tissue degeneration, sensitivity to specific damaging agents, immunodeficiency, genomic instability, cancer predisposition and premature aging. The finding that many of the crucial players involved in DNA damage response are structurally and functionally conserved in different species spurred discoveries of new players through similar analyses in yeast and mammals. We now understand the chain of events that leads to instantaneous activation of the massive cellular responses to DNA lesions. This book summarizes several new concepts in this rapidly evolving field, and the advances in our understanding of the complex network of processes that respond to DNA damage.

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