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REVIEWS

Structural Perspective on Mutations Affecting the Function of Multisubunit RNA Polymerases. Vincent Trinh, Marie-France Langelier, Jacques Archambault, and Benoit Coulombe..... 12–36

Summary: High-resolution crystallographic structures of multisubunit RNA polymerases (RNAPs) have increased our understanding of transcriptional mechanisms. Based on a thorough review of the literature, we have compiled the mutations affecting the function of multisubunit RNA polymerases, many of which having been generated and studied prior to the publication of the first high-resolution structure, and highlighted the positions of the altered amino acids in the structures of both the prokaryotic and eukaryotic enzymes. The observations support many previous hypotheses on the transcriptional process, including the implication of the bridge helix and the trigger loop in the processivity of RNAP, the importance of contacts between the RNAP jaw-lobe module and the downstream DNA in the establishment of a transcription bubble and selection of the transcription start site, the destabilizing effects of ppGpp on the open promoter complex, and the link between RNAP processivity and termination. This study also revealed novel, remarkable features of the RNA polymerase catalytic mechanisms that will require additional investigation, including the putative roles of fork loop 2 in the establishment of a transcription bubble, the trigger loop in start site selection, and the uncharacterized funnel domain in RNAP processivity.

The BAR Domain Proteins: Molding Membranes in Fission, Fusion, and Phagy. Gang Ren, Parimala Vajjhala, Janet S. Lee, Barbara Winsor, and Alan L. Munn 37–120

Summary: The Bin1/amphiphysin/Rvs167 (BAR) domain proteins are a ubiquitous protein family. Genes encoding members of this family have not yet been found in the genomes of prokaryotes, but within eukaryotes, BAR domain proteins are found universally from unicellular eukaryotes such as yeast through to plants, insects, and vertebrates. BAR domain proteins share an N-terminal BAR domain with a high propensity to adopt α-helical structure and engage in

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coiled-coil interactions with other proteins. BAR domain proteins are implicated in processes as fundamental and diverse as fission of synaptic vesicles, cell polarity, endocytosis, regulation of the actin cytoskeleton, transcriptional repression, cell-cell fusion, signal transduction, apoptosis, secretory vesicle fusion, excitation-contraction coupling, learning and memory, tissue differentiation, ion flux across membranes, and tumor suppression. What has been lacking is a molecular understanding of the role of the BAR domain protein in each process. The three-dimensional structure of the BAR domain has now been determined and valuable insight has been gained in understanding the interactions of BAR domains with membranes. The cellular roles of BAR domain proteins, characterized over the past decade in cells as distinct as yeasts, neurons, and myocytes, can now be understood in terms of a fundamental molecular function of all BAR domain proteins: to sense membrane curvature, to bind GTPases, and to mold a diversity of cellular membranes.

Chemoenzymatic and Template-Directed Synthesis of Bioactive Macrocyclic Peptides. Jan Grünewald and Mohamed A. Marahiel 121–146

Summary: Non-ribosomally synthesized peptides have compelling biological activities ranging from antimicrobial to immunosuppressive and from cytostatic to antitumor. The broad spectrum of applications in modern medicine is reflected in the great structural diversity of these natural products. They contain unique building blocks, such as D-amino acids, fatty acids, sugar moieties, and heterocyclic elements, as well as halogenated, methylated, and formylated residues. In the past decades, significant progress has been made toward the understanding of the biosynthesis of these secondary metabolites by nonribosomal peptide synthetases (NRPSs) and their associated tailoring enzymes. Guided by this knowledge, researchers genetically redesigned the NRPS template to synthesize new peptide products. Moreover, chemoenzymatic strategies were developed to rationally engineer nonribosomal peptides products in order to increase or alter their bioactivities. Specifically, chemical synthesis combined with peptide cyclization mediated by nonribosomal thioesterase domains enabled the synthesis of glycosylated cyclopeptides, inhibitors of integrin receptors, peptide/polyketide hybrids, lipopeptide antibiotics, and streptogramin B antibiotics. In addition to the synthetic potential of these cyclization catalysts, which is the main focus of this review, different enzymes for tailoring of peptide scaffolds as well as the manipulation of carrier proteins with reporter-labeled coenzyme A analogs are discussed.

Characterization of a Novel Virus Causing a Lethal Disease in Carp and Koi. Maya Ilouze, Arnon Dishon, and Moshe Kotler 147–156

Summary: Since 1998 a lethal disease of carp and ornamental koi (Cyprinus carpio) has afflicted fisheries in North America, Europe, and Asia, causing severe economic losses to the fish farming industry. This review summarizes the isolation and identification of the disease-causing agent and describes the currently known molecular characteristics of this newly isolated virus, distinguishing it from other known large DNA viruses. In addition, we summarize the clinical and histopathological manifestations of the disease. Providing information on the immune response to this virus and evaluating the available means of diagnosis and protection should help to reduce the damage induced by this disease. This review does not discuss the economic aspects of the disease or the debate on whether the disease should be registered; both of these issues were recently reviewed in detail (O. L. M. Haenen, K. Way, S. M. Bergmann, and E. Ariel, Bull. Eur. Assoc. Fish Pathol. ***24:****293–307, 2004; D. Pokorova, T. Vesely, V. Piackova, S. Reschova, and J. Hulova, Vet. Med. Czech.* ***50:****139–147, 2005).*

Structure-Function Relationships of Glucansucrase and Fructansucrase Enzymes from Lactic Acid Bacteria. Sacha A. F. T. van Hijum, Slavko Kralj, Lukasz K. Ozimek, Lubbert Dijkhuizen, and Ineke G. H. van Geel-Schutten. 157–176

Summary: Lactic acid bacteria (LAB) employ sucrase-type enzymes to convert sucrose into homopolysaccharides consisting of either glucosyl units (glucans) or fructosyl units (fructans). The enzymes involved are labeled glucansucrases (GS) and fructansucrases (FS), respectively. The available molecular, biochemical, and structural information on sucrase genes and en-

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zymes from various LAB and their fructan and α-glucan products is reviewed. The GS and FS enzymes are both glycoside hydrolase enzymes that act on the same substrate (sucrose) and catalyze (retaining) transglycosylation reactions that result in polysaccharide formation, but they possess completely different protein structures. GS enzymes (family GH70) are large multidomain proteins that occur exclusively in LAB. Their catalytic domain displays clear secondary-structure similarity with α-amylase enzymes (family GH13), with a predicted permuted $(\beta/\alpha)_8$ barrel structure for which detailed structural and mechanistic information is available. Emphasis now is on identification of residues and regions important for GS enzyme activity and product specificity (synthesis of α-glucans differing in glycosidic linkage type, degree and type of branching, glucan molecular mass, and solubility). FS enzymes (family GH68) occur in both gram-negative and gram-positive bacteria and synthesize β-fructan polymers with either β-(2→6) (inulin) or β-(2→1) (levan) glycosidic bonds. Recently, the first high-resolution three-dimensional structures have become available for FS (levansucrase) proteins, revealing a rare five-bladed β-propeller structure with a deep, negatively charged central pocket. Although these structures have provided detailed mechanistic insights, the structural features in FS enzymes dictating the synthesis of either β-(2→6) or β-(2→1) linkages, degree and type of branching, and fructan molecular mass remain to be identified.

The Where, When, and How of Organelle Acidification by the Yeast Vacuolar H^+-ATPase. Patricia M. Kane 177–191

Summary: All eukaryotic cells contain multiple acidic organelles, and V-ATPases are central players in organelle acidification. Not only is the structure of V-ATPases highly conserved among eukaryotes, but there are also many regulatory mechanisms that are similar between fungi and higher eukaryotes. These mechanisms allow cells both to regulate the pHs of different compartments and to respond to changing extracellular conditions. The Saccharomyces cerevisiae V-ATPase has emerged as an important model for V-ATPase structure and function in all eukaryotic cells. This review discusses current knowledge of the structure, function, and regulation of the V-ATPase in S. cerevisiae and also examines the relationship between biosynthesis and transport of V-ATPase and compartment-specific regulation of acidification.

Sortases and the Art of Anchoring Proteins to the Envelopes of Gram-Positive Bacteria. Luciano A. Marraffini, Andrea C. DeDent, and Olaf Schneewind 192–221

Summary: The cell wall envelopes of gram-positive bacteria represent a surface organelle that not only functions as a cytoskeletal element but also promotes interactions between bacteria and their environment. Cell wall peptidoglycan is covalently and noncovalently decorated with teichoic acids, polysaccharides, and proteins. The sum of these molecular decorations provides bacterial envelopes with species- and strain-specific properties that are ultimately responsible for bacterial virulence, interactions with host immune systems, and the development of disease symptoms or successful outcomes of infections. Surface proteins typically carry two topogenic sequences, i.e., N-terminal signal peptides and C-terminal sorting signals. Sortases catalyze a transpeptidation reaction by first cleaving a surface protein substrate at the cell wall sorting signal. The resulting acyl enzyme intermediates between sortases and their substrates are then resolved by the nucleophilic attack of amino groups, typically provided by the cell wall cross bridges of peptidoglycan precursors. The surface protein linked to peptidoglycan is then incorporated into the envelope and displayed on the microbial surface. This review focuses on the mechanisms of surface protein anchoring to the cell wall envelope by sortases and the role that these enzymes play in bacterial physiology and pathogenesis.

Adaptation and Acclimation of Photosynthetic Microorganisms to Permanently Cold Environments. Rachael M. Morgan-Kiss, John C. Priscu, Tessa Pocock, Loreta Gudynaite-Savitch, and Norman P. A. Huner 222–252

Summary: Persistently cold environments constitute one of our world's largest ecosystems, and microorganisms dominate the biomass and metabolic activity in these extreme environments.

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The stress of low temperatures on life is exacerbated in organisms that rely on photoautrophic production of organic carbon and energy sources. Phototrophic organisms must coordinate temperature-independent reactions of light absorption and photochemistry with temperature-dependent processes of electron transport and utilization of energy sources through growth and metabolism. Despite this conundrum, phototrophic microorganisms thrive in all cold ecosystems described and (together with chemoautrophs) provide the base of autotrophic production in low-temperature food webs. Psychrophilic (organisms with a requirement for low growth temperatures) and psychrotolerant (organisms tolerant of low growth temperatures) photoautotrophs rely on low-temperature acclimative and adaptive strategies that have been described for other low-temperature-adapted heterotrophic organisms, such as cold-active proteins and maintenance of membrane fluidity. In addition, photoautrophic organisms possess other strategies to balance the absorption of light and the transduction of light energy to stored chemical energy products (NADPH and ATP) with downstream consumption of photosynthetically derived energy products at low temperatures. Lastly, differential adaptive and acclimative mechanisms exist in phototrophic microorganisms residing in low-temperature environments that are exposed to constant low-light environments versus high-light- and high-UV-exposed phototrophic assemblages.

Glucose Signaling in *Saccharomyces cerevisiae*. George M. Santangelo . . . 253–282

Summary: Eukaryotic cells possess an exquisitely interwoven and fine-tuned series of signal transduction mechanisms with which to sense and respond to the ubiquitous fermentable carbon source glucose. The budding yeast Saccharomyces cerevisiae has proven to be a fertile model system with which to identify glucose signaling factors, determine the relevant functional and physical interrelationships, and characterize the corresponding metabolic, transcriptomic, and proteomic readouts. The early events in glucose signaling appear to require both extracellular sensing by transmembrane proteins and intracellular sensing by G proteins. Intermediate steps involve cAMP-dependent stimulation of protein kinase A (PKA) as well as one or more redundant PKA-independent pathways. The final steps are mediated by a relatively small collection of transcriptional regulators that collaborate closely to maximize the cellular rates of energy generation and growth. Understanding the nuclear events in this process may necessitate the further elaboration of a new model for eukaryotic gene regulation, called "reverse recruitment." An essential feature of this idea is that fine-structure mapping of nuclear architecture will be required to understand the reception of regulatory signals that emanate from the plasma membrane and cytoplasm. Completion of this task should result in a much improved understanding of eukaryotic growth, differentiation, and carcinogenesis.