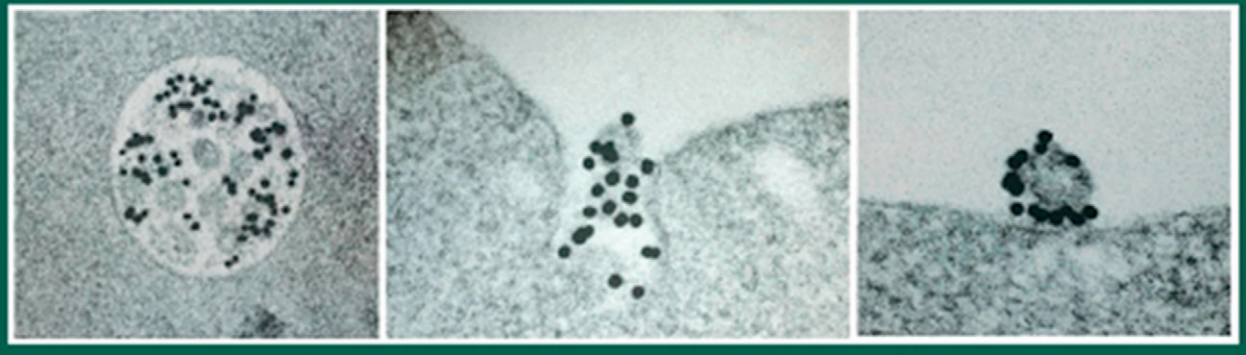
Encyclopedia of Cell Biology



EDITORS IN CHIEF

Ralph A. Bradshaw and Philip D. Stahl

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ENCYCLOPEDIA OF
CELL BIOLOGY

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EDITORS-IN-CHIEF

RALPH A BRADSHAW

University of California, Irvine, CA, USA

PHILIP D STAHL

Washington University School of Medicine, St. Louis, MO, USA

VOLUME 1

MOLECULAR CELL BIOLOGY

VOLUME EDITOR

GERALD W HART

Johns Hopkins University School of Medicine, Baltimore, MD, USA

SECTION EDITORS

KENNETH E NEET

Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

SARAH A WOODSON

Johns Hopkins University School of Medicine, Baltimore, MD, USA

JUDITH S BOND

Penn State University College of Medicine, Hershey, PA, USA

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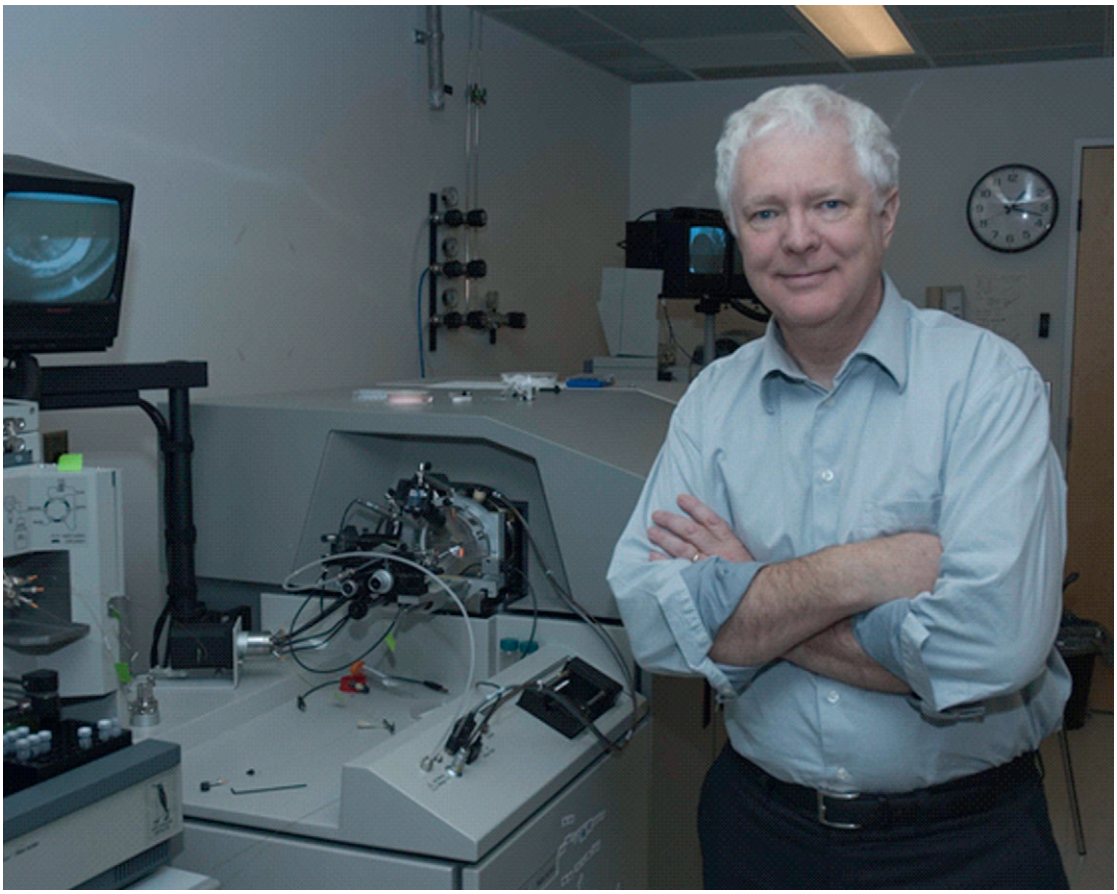
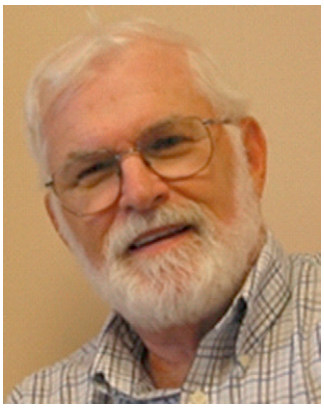
DEDICATION

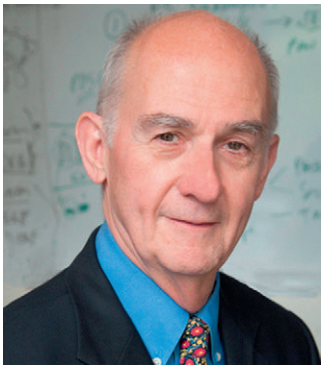
Dedicated to Anthony James (Tony) Pawson (1952–2013), in recognition of his outstanding contributions to cell biology and our understanding of intracellular communication mechanisms…



EDITORS-IN-CHIEF



Ralph A Bradshaw is Professor Emeritus in the Department of Physiology and Biophysics at the University of California, Irvine. Prior to that he was on the faculty of the Department of Biological Chemistry, Washington University School of Medicine in St. Louis, MO and was Professor and Chair of the Department of Biological Chemistry at University of California, Irvine. From 2006 to 2015, he was a member of the Mass Spectrometry Facility and Professor of Pharmaceutical Chemistry at the University of California, San Francisco. He holds degrees from Colby College and Duke University and was a post-doctoral fellow at Indiana University and the University of Washington. He has served as president of FASEB, was the founding president of the Protein Society and was the treasurer of the American Society for Biochemistry and Molecular Biology. His research has focused on protein chemistry and proteomics, with emphasis on the structure and function of growth factors and their receptors, particularly nerve growth factor and fibroblast growth factor, and the involvement of receptor tyrosine kinases in cell signaling. He has also studied the role of proteolytic processing and N-terminal modification in protein stability and turnover.



Philip D Stahl is E. Mallinckrodt Jr. Professor Emeritus at Washington University School of Medicine in St. Louis, MO. He was educated at West Virginia University with post-doctoral work at Vanderbilt University. He served as Head of the Department of Cell Biology and Physiology and Director of the Division of Biology and Biomedical Sciences at Washington University. He has been the recipient of many awards including a MERIT award from the NIH and the WICB Senior Recognition award given by the American Society for Cell Biology in recognition of his work supporting the advancement of women in science. Among StahlLab contributions are the discovery of the lysosomal enzyme clearance pathway now implemented in the treatment of lysosomal storage disease, discovery of the innate immune receptor, the mannose receptor, discovery of the exosome secretion pathway, and the role of Rab5 and Arf6 in endocytosis.

Currently his research focuses on endocytosis, signal transduction, and exosome biogenesis and secretion.

VOLUME EDITORS

Gerald Hart is the Director and DeLamar Professor of Biological Chemistry at Johns Hopkins Medical School. He began his research on glycoconjugates about 40 years ago as a graduate student, and he has been active in the field of Glycobiology ever since. Among his research accomplishments, he determined the minimal sequence requirements for N-linked glycosylation while a postdoc in William Lennarz's lab, and he collaborated with Paul Englund's group at Johns Hopkins to elucidate the biosynthetic pathway for GPI-anchors. In the early 1980s, while probing cells with glycosyltransferases, Hart's laboratory discovered cytoplasmic and nuclear protein glycosylation by O-linked *N*-acetylglucosamine (O-GlcNAc) (e.g., *Journal of Biological Chemistry* 259: 3308; *Journal of Biological Chemistry* 261: 8049). Since that time, the Hart laboratory has published nearly 200 papers on O-GlcNAcylation, identifying and cloning the enzymes controlling cycling, characterizing O-GlcNAcylation and its interplay with phosphorylation on hundreds of proteins, and they have developed many of the tools and methods in use today to study this modification. In 1989, Hart founded the leading journal in the field, *Glycobiology*, serving as Editor-in-Chief until 2001. Hart received the first International Glycoconjuate Organization (IGO) Award in 1997, the Karl Meyer Award from the Society for Glycobiology in 2006, and served as the 2009–2011 president of the IGO. Hart is currently an Associate Editor for *The Journal of Biological Chemistry* and an Associate Editor for *Molecular and Cellular Proteomics*. To date, Hart has published about 263 papers, all in the area of glycosciences. H factor = 84.

Bruno Goud studied biochemistry and immunology at the Ecole Normale Superieure de Cachan and the University of Paris. He received his PhD in 1981 under the mentorship of Jean-Claude Antoine and Stratis Avrameas at Institut Pasteur and did postdoctoral work under the mentorship of Peter Novick at Yale University. In 1995, he established an independent research group in the Department of Cell Biology at the Institut Curie in Paris. The main focus of his studies is the regulation of intracellular transport and membrane trafficking in eukaryotic cells. In particular, his group is working on the functions of Rab GTPases, the mechanisms that sustain the global organization of intracellular compartments and the functions of myosins in membrane traffic. Since 2000, he has also developed original *in vitro* approaches to unravel physical parameters such as membrane tension or membrane curvature that underlie transport processes.

Bruno Goud is a recipient of an ERC (European Research Council) Advanced grant (2013). He is a member of the European Molecular Biology Organization (EMBO). He has been the Head of the Department of Cell Biology of Institut Curie since 2003.

Graça Raposo received her PhD in 1989 in Membrane Biology and Immunology at the University of Paris VII where she specialized in electron microscopy and membrane biology. From 1990 to 1995 she was a postdoctoral fellow at the Immunology Center in Marseille and then in the Department of Cell Biology, Utrecht University, The Netherlands. She is the deputy Director of the Department of Cell Biology at Institut Curie and Director of the Training unit. Her major research interests focus on the biogenesis and functions of exosomes and lysosome related organelles with implications in neurodegenerative disorders, lysosomal diseases, and cancer. Her group have started to unravel the cellular and molecular mechanisms regulating the biogenesis of melanosomes, the lysosome related organelles of epidermal melanocytes, studies that open a new avenue to modulate pigmentation in health and disease. In 2012 she was awarded the CNRS Silver Medal and in 2013 the Descartes Huygens Price from the Royal Dutch Academy of Sciences. She is a member of the European Molecular Biology Organization (EMBO).

Alan Ezekowitz, MBChB, DPhil, FAAP, is Co-Founder, President, and CEO of Abide Therapeutics, a company cofounded by Professors Dale Boger and Ben Cravatt from the Scripps Research Institute. He was previously Senior Vice President and Franchise Head, Bone, Respiratory, Immunology, Endocrine, Dermatology and Urology at Merck Research Laboratories. At Merck, he was responsible for the overall scientific direction of the drug discovery and development process. Prior to Merck, Dr. Ezekowitz was Chief of Pediatric Services at the Massachusetts General Hospital for Children and the Partners Healthcare System and the Charles Wilder Professor of Pediatrics at the Harvard Medical School. He chaired the committee that led to the establishment of the Academy at the Harvard Medical School where he served as a Scholar and Founding member. He served on the Board of Directors of the Partners Healthcare System and Massachusetts General Physicians Organization. In 2008 he was honored with the establishment R. Alan Ezekowitz Professorship in Pediatrics at the Harvard Medical School.

He is a member of the American Society of Clinical Investigation, the Association of American Physicians, the American Pediatric Society, and a Fellow of AAAS. He served on NIH Subcommittees on Biodefense and Vaccine Adjuvants; NAS panels on antibiotic resistance and academic pharmaceutical partnerships; and the ARISE 2 task force of American Academy of Arts & Science that is evaluating the impact of federal and industrial funding of science, engineering, and medicine on American universities.

Dr. Ezekowitz received his MBChB and DPhil from University of Cape Town and Oxford University, respectively. At Children's Hospital in Boston, he was a postdoctoral fellow in the Division of Hematology and Oncology with clinical training in pediatrics. He was on the staff of the Children's Hospital of Boston prior to moving to the Massachusetts General Hospital.

He is a pioneer in the field of innate immunity and has over 150 publications. In particular his group played a major role in defining the structure function of the mannose binding lectin and the macrophage mannose receptor.

Douglas A Lauffenburger is Ford Professor of Bioengineering, and cofounder and Head of the Department of Biological Engineering at MIT, with affiliate appointment in the Department of Biology; he was a founding codirector of the MIT Computational and Systems Biology Initiative in 2002. His major research interests are in cell engineering, with central focus on cell–cell communication and intracellular signal transduction, emphasizing predictive computational models derived from quantitative experiment. Lauffenburger has coauthored a monograph entitled *Receptors: Models for Binding, Trafficking & Signaling* (Oxford University Press, 1993), has coedited the book entitled *Systems Biomedicine: Concepts and Perspectives* (Elsevier, 2010), and has supervised more than 100 doctoral and postdoctoral students. He is a member of the National Academy of Engineering and the American Academy of Arts and Sciences, and has served as President of the Biomedical Engineering Society, Chair of the AIMBE College of Fellows, and on the NIH NIGMS Advisory Council, and coauthored the NRC report on *A New Biology for the 21st Century*.

SECTION EDITORS

Kenneth E Neet received his PhD in Biochemistry in 1965 (with Dr. Frank W. Putnam) from the University of Florida. He was a postdoctoral fellow at the University of California, Berkeley (with Dr. Daniel E. Koshland, Jr.), and joined the faculty of Case Western Reserve University (CWRU) in 1967 as an Assistant Professor of Biochemistry.

Dr. Neet received a Faculty Research Award of the American Cancer Society from 1968 to 1973 and was on sabbatical leave at the National Institute for Medical Research, Mill Hill, England (with Dr. N. Michael Green) 1973–1974. From 1978 to 1990, he was Professor of Biochemistry at CWRU. During 1980–1981, he took a sabbatical year as a Josiah Macy Faculty Scholar in the Department of Neurobiology, Stanford University (with Dr. Eric M. Shooter).

Dr. Neet moved to Rosalind Franklin University of Medicine and Science/The Chicago Medical School in 1990 and was Professor and Chair of Biochemistry & Molecular Biology until 2005. Dr. Neet became Associate Dean for Research of the Chicago Medical School, RFUMS in 2004.

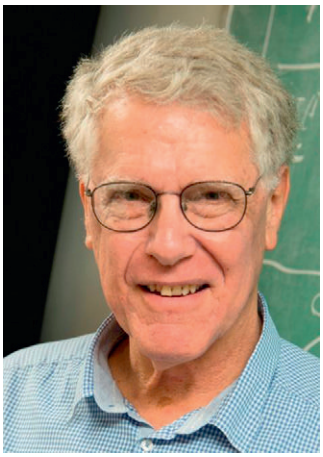
Dr. Neet has served on the Editorial Boards of the *Journal of Biological Chemistry*, *Protein Science*, and *Molecular & Cellular Proteomics* and as a member of study sections for the National Institutes of Health and the National Science Foundation. He was an Associate Editor of the *Journal of Biological Chemistry* (1996–2013).

Dr. Neet's research interests have been in the general areas of protein–protein interactions, allosteric interactions, protein conformational changes, and cell signaling. In his earlier years he studied theoretical and experimental aspects of slow transitions in enzymes, particularly the cooperativity of the monomeric enzyme glucokinase. Later, he studied ligand–receptor interactions of nerve growth factor, establishing a mutational system to study the interactions with its receptors (TrkA and p75), and ultimately the complex signaling emanating from these receptors within neuronal systems.

Sarah A Woodson is a biophysical chemist interested in how RNA molecules fold into specific three-dimensional structures and how they interact with proteins to turn genes on and off in the cell. She was born in Michigan, USA, and attended Kalamazoo College before receiving her PhD in Biophysical Chemistry from Yale University in 1987. After postdoctoral research in the laboratory of Tom Cech at the University of Colorado Boulder from 1987 to 1990, she joined the faculty of the University of Maryland in 1990 and the faculty of Johns Hopkins University in 1999, where she is currently the T.C. Jenkins Professor of Biophysics. Together with Mark Chance and Michael Brenowitz, she pioneered methods for visualizing how RNA molecules change shape in real time. She served on the board of the RNA Society and was elected an AAAS Fellow in 2011 and a Pew Scholar in the Biomedical Sciences in 1993. She is currently a reviewing editor of *Biopolymers* and *Journal of Molecular Biology* and serves on the editorial boards of *Nucleic Acids Research* and *RNA*.

Judith Bond, Evan Pugh Emeritus Professor of Biochemistry and Molecular Biology at the Pennsylvania State University College of Medicine, was President of the Federation of American Societies for Experimental Biology (2012–13) and an Associate Editor of the Journal of Biological Chemistry (1999–2013). She received her BS degree from Bennington College in Vermont, her PhD from Rutgers University, and did postdoctoral research at Vanderbilt University. She rose through the academic ranks at the Medical College of Virginia, Virginia Commonwealth University, became Professor and Head of Biochemistry and Nutrition at Virginia Polytechnic Institute and State University, and served at Penn State University College of Medicine as Professor and Chair of Biochemistry and Molecular Biology from 1992 to 2012. At Penn State, she also served as assistant dean for graduate studies, codirector of the All-University Interdisciplinary Biological Sciences Program and founding director of the Medical Scientist Training Program. She recently directed NIH-funded summer research programs for high school students and teachers and for undergraduate students (particularly underrepresented minority groups). Her work on metalloproteases has been funded continuously by the NIH for over 35 years. She has

trained 5 Master's, 21 PhD students, and 19 postdoctoral trainees. Her professional service included member and chair National Institutes of Health Study Sections, member of the National Institute of Diabetes, Digestive and Kidney Diseases Advisory Council, member of the American Association of Medical Colleges – Howard Hughes Medical Institute Committee on the Scientific Foundations for Future Physicians, and President of the American Society for Biochemistry and Molecular Biology.



Elliot Elson received his AB at Harvard, his PhD at Stanford under the mentorship of R.L. Baldwin and did postdoctoral work at University of California, San Diego, under Bruno Zimm. His first independent faculty position was in the Department of Chemistry, Cornell University, where he stayed for 11 years. He then moved to the Department of Biological Chemistry (now the Department of Biochemistry and Molecular Biophysics) at Washington University in St. Louis, School of Medicine. He has pursued research in several biophysical areas. One of these is the development of Fluorescence Correlation Spectroscopy and Fluorescence Photobleaching Recovery and their application to studies of diffusion in cell membranes and of phase separation in model membrane bilayers as well as of other cellular and noncellular phenomena. He has also worked in the areas of cell mechanics, cell motility, and the mechanical properties of engineered tissues. His laboratory has developed approaches for measuring mechanical properties of cells in monolayer culture and for determining the contributions of cells and extracellular matrix to the mechanics of engineered heart and connective tissue constructs.

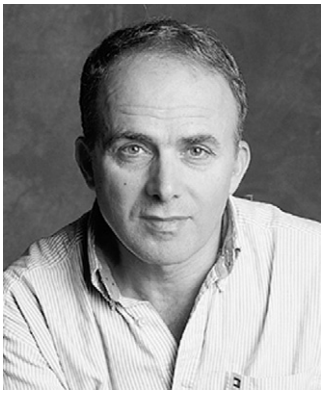
Tamotsu Yoshimori received his PhD degree in 1989 at Osaka University. After working at several places including European Molecular Biology Laboratory (Prof. Kai Simons' lab) and National Institute of Basic Biology (Prof. Yoshinori Ohsumi's lab), he is now a distinguished professor of Osaka University (Graduate School of Medicine and of Frontier Biosciences). His research interests are focused on intracellular membrane trafficking, and especially for the last 18 years, on autophagy. He identified LC3 as an autophagosome-binding protein, which has been widely used as the gold standard in autophagy assays. The paper has been cited over 3000 times. He also provided new insights into membrane biogenesis in autophagy and the role of autophagy in pathogen defense and suppression of various diseases. He authored or coauthored over 220 journal articles and book chapters. He is an editor of *Journal Cell Science*, and on the editorial board of *Journal of Cell Biology*, *Molecular Biology of the Cell*, and so on. He is a member of the Faculty of 1000 and a vice president of Japan Society for Cell Biology. He was awarded Osaka University Presidential Award for 2012 and 2013, Prize for Science and Technology by the Minister of Education, Culture, Sports, Science and Technology in 2013, Kakiuchi Saburo Memorial Prize by the Japanese Biochemical Society in 2014, and selected as Highly Cited Researchers 2014 by Thomson Reuters.

Paul A Gleeson is currently Head of the Department of Biochemistry and Molecular Biology at the University of Melbourne, located at Bio21 Institute. His research interests include the molecular mechanisms of intracellular membrane transport and the molecular basis of organ-specific autoimmune diseases. Paul Gleeson obtained his PhD in 1980 from the University of Melbourne and did postdoctoral research in the biosynthesis and function of glycoproteins at the Hospital for Sick Children, Toronto, National Institute for Medical research, Mill Hill London and Department of Biochemistry, La Trobe University, Melbourne. He established an independent laboratory at Monash University in 1986 where he defined the targeting signals of Golgi glycosyltransferases, identified golgins of the *trans*-Golgi network and along with his colleagues developed mouse models of autoimmune gastritis. In 2001 he moved to Department of Biochemistry and Molecular Biology at the University of Melbourne and has been Head of the Department since 2006. He has a number of international research collaborations and has been a visiting scientist at the EMBL, Heidelberg, and the Institut Curie, Paris.

Anna Akhmanova studied biochemistry and molecular biology at the Moscow State University. She received her PhD in 1997 at the University of Nijmegen, the Netherlands. She worked as a postdoc at the Department of Microbiology and Evolutionary Biology at the University of Nijmegen and at the Department of Cell Biology at the Erasmus Medical Center in Rotterdam, the Netherlands. In 2001, she started her own research group at the Erasmus Medical Center. Since 2011, Anna Akhmanova is professor of Cell Biology at Utrecht University, the Netherlands.

Akhmanova studies cytoskeletal organization and trafficking processes, which contribute to cell polarization, differentiation, vertebrate development, and human disease. The main focus of her studies is the microtubule cytoskeleton. Her group has described key interactions between proteins associated with microtubule ends and determined their role in the regulation of microtubule organization and dynamics. She has also characterized mechanisms of bidirectional microtubule-based motility of membrane organelles such as cell nuclei and exocytotic vesicles. Her research relies on combining high-resolution live cell imaging and quantitative analysis of cytoskeletal remodeling, measurement of protein dynamics using advanced microscopic assays, *in vitro* reconstitution of dynamic cytoskeleton-based processes and different methods of identification of protein–protein interactions.

Anna Akhmanova is a recipient of the ALW Vernieuwingsimpuls VIDI (2001) and VICI awards (2007) (Netherlands Organization for Scientific Research), and an ERC Synergy grant (2013). She is the Chair of the board of the Netherlands Microscopy Society and a member of the European Molecular Biology Organization (EMBO).



Yosef (Yossi) Yarden is a Professor in the Department of Biological Regulation at Weizmann Institute of Science (Rehovot, Israel). Dr. Yarden obtained his PhD from Weizmann Institute (under the mentoring of Dr. Joseph Schlessinger) and later trained at both Genentech Inc., with Dr. Axel Ullrich, and at the Whitehead Institute (MIT), with Dr. Robert A. Weinberg. His group studies the roles played by growth factors and their receptors in cancer progression. They also explore strategies able to intercept growth factor signals in tumors.

Jason D Weber is an Associate Professor in the Departments of Internal Medicine and Cell Biology and Physiology at Washington University and is the Coleader of the Breast Cancer Research Program in the Siteman Comprehensive Cancer Center. Dr. Weber obtained his PhD from Saint Louis University and received postdoctoral training under the mentoring of Dr. Charles J. Sherr at St. Jude Children's Research Hospital in Memphis, TN. His group studies the molecular interplay between oncogenes and tumor suppressors, particularly focused on their role in regulating cellular growth processes.

Michael Dustin received his PhD in 1990 in Cell and Developmental Biology from Harvard University, where he worked in the laboratory of Timothy A. Springer, PhD. During his graduate work he was involved in the identification and cloning of ICAM-1 and ICAM-2, key ligands for the integrin LFA-1. He further demonstrated that LFA-1 dependent adhesion was stimulated by T cell receptor signaling. He did his postdoctoral training with Stuart Kornfeld at Washington University School of Medicine in St. Louis, focusing on mechanisms of lysosome biogenesis. He started his independent lab also at Washington University School of Medicine, and used supported lipid bilayers to define the first protein to modulate organization of the immunological synapse- CD2 associated protein- and the dynamics of immunological synapse formation. He moved to NYU School of Medicine in 2001 where he applied *in vivo* microscopy to tolerance induction and immune responses in effector sites including the liver, brain, and spleen. Continued work with the immunological synapse model resulted in discovery of signaling microclusters, the molecular basis of immunological synapse stability, and the direct budding of extracellular microvesicle enriched in T cell receptors in the immunological synapse. Professor Dustin recently took a position at the University of Oxford with support of a Wellcome Trust Principal Research Fellowship. The focus of his new post is the translation of the immunological synapse. He received a Presidential Early Career Award in Science and Engineering and the DART–NYU Biotechnology Achievement Award.

David Sassoon directs a research team at Paris Sorbonne. He received his PhD from Columbia University (NYC, USA) in 1986 in the biological sciences and was a professor at Boston University Medical School and Mt. Sinai Medical School before relocating to Paris in 2006. His long-standing interests are in developmental and stem cell biology in a number of tissues including skeletal muscle, skin, CNS, and heart. Dr. Sassoon recently concluded the coordination of a EC-funded 15 partner international consortium (Endostem) designed to identify novel therapeutics for mobilizing endogenous stem/progenitor cells (http://www.endostem.eu/) and is a recent recipient as coordinator of a multipartner Transatlantic Network of Excellence grant from the Fondation Leducq focused on cardiac stem cell biology (http://www.fondationleducq.org/nivel2.aspx?idsec=1360).

A major focus of his research is on adult progenitor/stem cell biology and how these cells respond to stress. His team has identified a parentally imprinted gene, PW1/Peg3, which is involved in both p53 and inflammatory responses. PW1 is expressed in adult stem cells in all tissues identified to date. Loss of PW1 function in stem cells results in a loss of stem cell competence including a reduced capacity to undergo self-renewal and respond to hypoxic stress. We are currently evaluating a role for PW1 in postnatal and adult heart with a particular focus on its role in vessel precursors using both myocardial infarction and stress-induced myocardial hypertrophy.

Jason M Haugh is a Professor of Chemical and Biomolecular Engineering at North Carolina State University in Raleigh, NC, USA, where he has been on the faculty since 2000. His laboratory has been among those to pioneer the synthesis of quantitative experiments and modeling to study signal transduction in mammalian cells. Since the lab's inception, their approach has combined biochemical measurements, live-cell fluorescence microscopy, and computational modeling to elucidate signaling mechanisms by analyzing their kinetics and spatial organization in cells. The systems studied by the Haugh Laboratory include: regulation of the phosphoinositide 3-kinase, Ras/extracellular signal-regulated kinase, and phospholipase C pathways mediated by receptor tyrosine kinases, and cross talk between these pathways; dynamic organization of multimolecular complexes at cell membranes; signaling mediated by cytokine and chemokine receptors in immune cells; and integration of adhesion, signaling, and cytoskeletal dynamics that direct cell migration.

After graduating with an MA in Mathematics from the University of Cambridge, Helen Mary Byrne received her Master of Science and DPhil from the University of Oxford. She worked as a postdoctoral researcher at the Universities of Oxford and Bath, before taking up a lectureship at the UMIST in Manchester. She moved to the University of Nottingham in 1998 and was awarded a prestigious Advanced Research Fellowship from the United Kingdom's Engineering and Physical Sciences Research Council (2000–2006). She was promoted to Reader in 2002 and Professor in 2003. While in Nottingham, she established and then led the Centre for Mathematical Medicine and Biology until she returned to Oxford in 2011 where she is now based in the Mathematical Institute at the University of Oxford. She has over 20 years' experience of developing, analyzing, and simulating continuum and multiscale models of biomedical systems, and a particular interest in studying the growth and response to treatment of solid tumors.

Rune Linding completed his PhD at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, followed by postdoctoral training at EMBL. He then jointly trained with professors Tony Pawson and Mike Yaffe at the Lunenfeld at Mount Sinai Hospital in Toronto, Canada, and the Massachusetts Institute of Technology (MIT) in Cambridge, US, respectively. Dr. Linding then established his own laboratory of Cellular and Molecular Logic at the Institute of Cancer Research (ICR) in London, UK, before returning to Denmark to take a position as professor of cellular signal integration at the Technical University of Denmark. In 2014, Dr. Linding moved his laboratory to the Biotech Research and Innovation Centre (BRIC) at University of Copenhagen where he is currently professor of cellular signaling. His research group focuses on big data network biology, exploring biological systems by developing and deploying algorithms aimed to predict cell behavior, in particular looking at cellular signal processing and decision making. A strategic focus is to continue to develop computational tools (such as KinomeXplorer, NetworKIN, and NetPhorest) and to deploy these on genome-scale quantitative data obtained by, for example, mass spectrometry, genomic, and phenotypic screens to understand the principles of how spatio and temporal assembly of mammalian signaling networks transmit and process information at a systems level in order to alter cell behavior. His overarching aim is to advance network medicine by identifying and targeting signaling networks associated with complex diseases. To this end Dr. Linding is currently leading high-level, strategic, multidisciplinary studies of signaling network dynamics driving cancer metastasis in collaboration with other labs at Harvard, Yale, The Jackson Laboratory, Memorial Sloan Kettering Cancer Center, MIT, and BRIC.

CONTRIBUTORS TO VOLUME 1

TM Antalis
University of Maryland School of Medicine, Baltimore, MD, USA

B Antonny
Université de Nice Sophia Antipolis et CNRS, Valbonne, France

SS Apte
Cleveland Clinic, Cleveland, OH, USA

RS Aquino
Harvard Medical School, Boston, MA, USA

BJ Bailus
The Buck Institute for Research on Aging, Novato, CA, USA

SL Beaucage
US Food and Drug Administration, Silver Spring, MD, USA

C Becker-Pauly
University of Kiel, Kiel, Germany

M Blaber
Florida State University, Tallahassee, FL, USA

CP Blobel
Hospital for Special Surgery, New York, NY, USA

JS Bond
Penn State University College of Medicine, Hershey, PA, USA

P Boon Chock
National Institutes of Health, Bethesda, MD, USA

NA Bradbury
Chicago Medical School, North Chicago, IL, USA

SG Bradley
Virginia Commonwealth University, Richmond, VA, USA

RA Bradshaw
University of California, San Francisco, CA, USA

K Brew
Florida Atlantic University, Boca Raton, FL, USA

JL Brodsky
University of Pittsburgh, Pittsburgh, PA, USA

TP Burris
Saint Louis University School of Medicine, St. Louis, MO, USA

MS Buzza
University of Maryland School of Medicine, Baltimore, MD, USA

JJ Caramelo
Fundación Instituto Leloir, Buenos Aires, Argentina

JC Cardenas
University of North Carolina School of Medicine, Chapel Hill, NC, USA and University of Texas Health Science Center at Houston, Houston, TX, USA

TJ Carrocci
University of Wisconsin–Madison, Madison, WI, USA

M Cascio
Duquesne University, Pittsburgh, PA, USA

AM Case
Abbott Molecular Inc., Des Plaines, IL, USA

S Chakraborty
University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Z Chang
Peking University, Beijing, China

JJ-L Chen
Arizona State University, Tempe, AZ, USA

FC Church
University of North Carolina School of Medicine, Chapel Hill, NC, USA

NE Clarke
University of Leeds, Leeds, UK

WA Cramer
Purdue University, West Lafayette, IN, USA

M Datto
Duke University Medical Center, Durham, NC, USA

J Doherty
The Scripps Research Institute, Jupiter, FL, USA

RJ Dohmen
University of Cologne, Köln, Germany

AV Domashevskiy
John Jay College of Criminal Justice, New York, NY, USA

W Dowhan
University of Texas Medical School at Houston, Houston, TX, USA

M Dundr
Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

MJ Edmonds
University of Liverpool, Liverpool, UK

LM Ellerby
The Buck Institute for Research on Aging, Novato, CA, USA

AR Ferré-D′Amaré
National Heart, Lung and Blood Institute, Bethesda, MD, USA

DI Fisher
AstraZeneca, Cambridge, UK

PA Frey
The University of Wisconsin–Madison, Madison, WI, USA

R Fukunaga
Johns Hopkins University School of Medicine, Baltimore, MD, USA

DJ Goss
Hunter College CUNY, New York, NY, USA

GA Grant
Washington University School of Medicine, St. Louis, MO, USA

M Guo
The Scripps Research Institute, Jupiter, FL, USA

M Hafner Česen
Jožef Stefan Institute, Ljubljana, Slovenia

RA Harris
Indiana University School of Medicine, Indianapolis, IN, USA

GW Hart
Johns Hopkins University School of Medicine, Baltimore, MD, USA

VC Hascall
Cleveland Clinic, Cleveland, OH, USA

ML Hastings
Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

K Hayashida
Harvard Medical School, Boston, MA, USA

W-D Heyer
University of California, Davis, CA, USA

DM Hinton
National Institutes of Health, Bethesda, MD, USA

AA Hoskins
University of Wisconsin–Madison, Madison, WI, USA

W Hu
Whitehead Institute for Biomedical Research, Cambridge, MA, USA

PJ Hudson
Avipep Pty Ltd., Parkville, VIC, Australia

JM Huibregtse
The University of Texas at Austin, Austin, TX, USA

HV Jain
US Food and Drug Administration, Silver Spring, MD, USA

M Jaskolski
Adam Mickiewicz University, Poznan, Poland and Polish Academy of Sciences, Poznan, Poland

SS Jenkins
University of California, Davis, CA, USA

JM Jez
Washington University in St. Louis, St. Louis, MO, USA

K Ji
Wayne State University School of Medicine, Detroit, MI, USA

A Jinno
Harvard Medical School, Boston, MA, USA

S Joseph
University of California, San Diego, La Jolla, CA, USA

BM Kenwood
Georgia Institute of Technology, Atlanta, GA, USA

YH Ko
University of Maryland BioPark, Baltimore, MD, USA

AA Komar
Cleveland State University, Cleveland, OH, USA

M Kong
University of Pittsburgh, Pittsburgh, PA, USA

DA Korasick
Washington University in St. Louis, St. Louis, MO, USA

SE Kotermanski
Duquesne University, Pittsburgh, PA, USA

O Kulaeva
Fox Chase Cancer Center, Philadelphia, PA, USA

MWL Lau
National Heart, Lung and Blood Institute, Bethesda, MD, USA

BA Lewis
Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

I Lindberg
University of Maryland-Baltimore, MD, USA

M Lizotte-Waniewski
Florida Atlantic University, Boca Raton, FL, USA

E London
Stony Brook University, Stony Brook, NY, USA

LM Longo
Florida State University, Tallahassee, FL, USA

RL Lundblad
Chapel Hill, NC, USA

Y Luo
National Institute of Infectious Diseases, Tokyo, Japan

G Marius Clore
National Institutes of Health, Bethesda, MD, USA

LM Mayr
AstraZeneca, Cheshire, UK

AA McCracken
University of Nevada, Reno, NV, USA

WC Merrick
Case Western Reserve University, Cleveland, OH, USA

AH Merrill Jr.
Georgia Institute of Technology, Atlanta, GA, USA

AJ Moorhouse
UNSW Australia, Sydney, NSW, Australia

Amitava Mukherjee
University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

S Mukherjee
University of California, Davis, CA, USA

G Murphy
University of Cambridge, Cambridge, UK

KE Neet
Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

IP O'Carroll
National Cancer Institute at Frederick, Frederick, MD, USA

ME O'Donnell
The Rockefeller University, New York, NY, USA

K Okamoto
Nagasaki University, Nagasaki, Japan

H Oubrahim
National Institutes of Health, Bethesda, MD, USA

CM Overall
University of British Columbia, Vancouver, BC, Canada

PW Park
Harvard Medical School, Boston, MA, USA

JL Parsons
University of Liverpool, Liverpool, UK

MS Patel
University at Buffalo, The State University of New York, Buffalo, NY, USA

PL Pedersen
Johns Hopkins University School of Medicine, Baltimore, MD, USA

JR Peinado
University of Castilla–La Mancha, Ciudad Real, Spain

DH Perlmutter
University of Pittsburgh School of Medicine, Pittsburgh, PA, USA and Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA, USA

RN Pike
Monash University, Clayton, VIC, Australia

JD Podlevsky
Arizona State University, Tempe, AZ, USA

DM Raben
Johns Hopkins School of Medicine, Baltimore, MD, USA

SC Raghavan
Indian Institute of Science, Bangalore, India

B Ramos-Molina
University of Maryland-Baltimore, MD, USA

GH Reed
The University of Wisconsin–Madison, Madison, WI, USA

A Rein
National Cancer Institute at Frederick, Frederick, MD, USA

CM Rein-Smith
University of North Carolina School of Medicine, Chapel Hill, NC, USA and Whitsell Innovations Inc., Chapel Hill, NC, USA

PJ Roach
Indiana University School of Medicine, Indianapolis, IN, USA

RG Roth
AstraZeneca, Mölndal, Sweden

BM Ryan
Laboratory of Human Carcinogenesis Center for Cancer Research, Bethesda, MD, USA

IA Sawyer
Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA

M Scheffner
University of Konstanz, Konstanz, Germany

DF Sellitti
Uniformed Services University of the Health Sciences, Bethesda, MD, USA

S Sharma
Indian Institute of Science, Bangalore, India and FIRC Institute of Molecular Oncology, Milan, Italy

SK Singh
Purdue University, West Lafayette, IN, USA

S Sitaula
The Scripps Research Institute, Jupiter, FL, USA

BF Sloane
Wayne State University School of Medicine, Detroit, MI, USA

N Solis
University of British Columbia, Vancouver, BC, Canada

LL Spremulli
University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

SA Stewart
Washington University School of Medicine, Saint Louis, MO, USA

V Stoka
Jožef Stefan Institute, Ljubljana, Slovenia and International Postgraduate School Jozef Stefan, Ljubljana, Slovenia

VM Studitsky
Fox Chase Cancer Center, Philadelphia, PA, USA and Russian Academy of Sciences, Moscow, Russia

K Suzuki
National Institute of Infectious Diseases, Tokyo, Japan

DC Teasley
Washington University School of Medicine, Saint Louis, MO, USA

BP Toole
Medical University of South Carolina, Charleston, SC, USA

C Tredup
University of Kiel, Kiel, Germany

T Tsukuba
Nagasaki University, Nagasaki, Japan

B Turk
Jožef Stefan Institute, Ljubljana, Slovenia and Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Ljubljana, Slovenia

AJ Turner
University of Leeds, Leeds, UK

B Tu-Sekine
Johns Hopkins School of Medicine, Baltimore, MD, USA

B Van Houten
University of Pittsburgh, Pittsburgh, PA, USA

MN Vu
Georgia Institute of Technology, Atlanta, GA, USA

KW Walker
Amgen Inc., Thousand Oaks, CA, USA

PH Weigel
University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

LC Wijeyewickrema
Monash University, Clayton, VIC, Australia

A Wlodawer
National Cancer Institute, Frederick, MD, USA

MS Wolfe
Harvard Medical School, Boston, MA, USA

MJ Wolfgang
Johns Hopkins University School of Medicine, Baltimore, MD, USA

SA Woodson
Johns Hopkins University School of Medicine, Baltimore, MD, USA

K Yamamoto
Kyushu University, Fukuoka, Japan

NY Yao
The Rockefeller University, New York, NY, USA

LF Zacchi
Fundación Instituto Leloir, Buenos Aires, Argentina

SC Zeeman
ETH Zürich, Zürich, Switzerland

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PREFACE

Cell biology is the study of cells, the integral unit that is the basis of all living tissues. In its earliest phases, this area of investigation was largely defined by microscopic-based observations of structure and organization that were, by their nature, largely descriptive. This also served to delineate cell biology from its nearest neighbor, biochemistry, which, dating from its post WWII renaissance, was driven in large part by reductionist approaches, where tissues, cells, and organelles were broken down in order to isolate and then characterize individual components. The melding force that bridged these two areas was molecular biology that allowed the cell biologist to transform pictures into functions and allowed the biochemist to reassemble their components into ensembles and subcellular entities. Not surprisingly the boundary between these two key biological sciences has become blurred beyond recognition. Today a considerable part of what passes as cell biology is by any other name biochemistry (or molecular biology – the distinction between these two areas being equally indistinct) and vice versa. Indeed, one of the first challenges we faced in organizing this Encyclopedia was how much molecular detail to include. It was our conclusion that for the sake of completeness, and to appropriately introduce the volumes Organizational Cell Biology and Functional Cell Biology, we needed a description of the molecular components of the cell, particularly with respect to the synthesis and degradation of proteins and nucleic acids, along with outlines of important chemical principles and key methodology. We were guided to some degree in these decisions by input from undergraduate and graduate teachers and the contents of their introductory courses that they shared with us and by discussions with our volume and section editors. The result was the first volume which we recognize is very 'biochemical,' but certainly lacking the more detailed coverage of the *Encyclopedia of Biological Chemistry* (Lennarz and Lane, 2004) or the many excellent biochemical texts that dot the educational landscape. Readers who find 'gaps' or incomplete treatments in this section will likely be able to find the additional detail they seek from these sources.

The second major decision that we had to confront was whether we would attempt to cover the full range of living organisms or whether we would place some limits on the scope of the material we included. In the final result, it was decided to place the emphasis on higher eukaryotes with only very prescribed treatments of lower eukaryotes and prokaryotes. Volumes 2 and 3 thus deal with material that has perhaps been more traditionally thought of as cell biology. Again we were guided in our decisions about what to include by teaching considerations, brainstorming sessions with our editors, and a perceived need to separate cellular organization from cellular function. However, as even a quick perusal will reveal, this is not a sharp delineation either. Volume 2 begins with a methodological treatment of imaging, which is basically divided between light and electron microscopic applications, and is followed by sections on organelles, interorganellar communication, and the cytoskeleton (including motors). The last part of volume 2 is devoted to intracellular infection and covers a variety of external agents and pathogens that impact cell function as well as human pathology. Volume 3, in contrast, largely deals with major cell functions: signaling, cell-cycle control, and apoptosis. The last two parts are devoted to cells of the immune system and their responses and stem cells – two highly specialized niches characterized by unique cellular involvement.

The last part of the Encyclopedia deals with systems biology. This is really the newest area of cell biology and considers cellular involvement as part of a phenotypic response. As such, it strives to integrate all of the levels of the first three volumes in a consistent manner to produce a seamless description of cellular function. This is clearly a newly evolving area and the most rapidly changing part of cell biology; indeed it is where we expect to see the greatest change in the next few years.

Assembling a treatment of a topic as large as cell biology had multiple challenges. Coverage was a significant problem: on the one hand it was almost impossible to avoid some redundancy in places while, on the other, there were inevitable gaps. Some of these arose from late cancellations; others from oversights on our part. We can only promise to fill these in future editions. We also note that as can be expected for a large multiauthor compilation the individual articles do differ in detail and treatment. We felt it was more important to allow our experts substantial latitude in deciding how to present their topics than to apply rigid guidelines. We did try to limit the number of references (to make it easier for people not familiar with a topic to make the transition to more extensive articles) but there is admittedly some considerable variation in the bibliographic material as well.

When we were well into the project, we were approached by Graham Nisbet (Elsevier) about including the Encyclopedia in a larger collection entitled the Reference Module in Biomedical Sciences. The Reference Module plan was grouped in single integrated work information from several other compendia and it was our view that this would greatly leverage the value of our efforts (and those of our contributors). While we were the 'new kid on the block' in that we were still compiling the Encyclopedia and the other collections to be included were already extant, we felt that it was too good an opportunity to pass up. The Reference Module has since been launched and although it does not yet contain the complete content from the Encyclopedia, this will be achieved in the not too distant future. We consider that this will be a particularly valuable resource for researchers that are just entering (or changing into) this field.

No work of this size could be completed without the help and advice of many people. In this regard we would like to thank a number of people who were instrumental in bringing this project to fruition. First and foremost we would like to recognize our volume editors: Jerry Hart, Graça Raposo, Bruno Goud, Alan Ezekowitz, and Doug Lauffenburger; and our section editors: Ken Neet, Sarah Woodson, Judy Bond,

Elliot Elson, Tamotsu Yoshimori, Paul Gleeson, Anna Akhmanova, Yosef Yarden, Jason Weber, Michael Dustin, David Sassoon, Jason Haugh, Helen Byrne, and Rune Linding; and thank them for their invaluable input, enthusiasm, and hard work. They are truly a global group and have been a great team to work with. We also wish to thank the staff at Elsevier including Janice Audet, Peter Labella, Nicky Carter, Karen Maloney, Erin Hill-Parks, Rashmi Phadnis, Blerina Osmanaj, and Ginny Mills for their many efforts, perseverance (particularly with our idiosyncrasies), and skillful management of every aspect of this project.

In the course of planning this work, we gathered information from a number of sources. At the outset the publishers polled some 167 faculty from both undergraduate and graduate schools to ascertain their perceived interest in such a project, which also garnered considerable input about potential topics. The response was highly favorable. We also talked with many colleagues about experiences at their institutions and would like to particularly thank Catherine Bevier (Colby College), Robert Simoni (Stanford University), Larry Marsh (UC Irvine), and John Cooper (Washington University) for providing syllabi of germane courses taught in their institutions. Special thanks also go to Ruedi Abersold (ETH, Zurich) and Sergio Grinstein (University of Toronto) for their expertise that they readily shared with us, which was invaluable in planning certain sections.

Finally we would like to recognize all of our authors. We have been extraordinarily fortunate in attracting individuals from all around the globe to take time from their busy schedules to prepare this splendid set of contributions. Without these efforts there would be no Encyclopedia. Under ordinary circumstances it would be appropriate to dedicate the work to them and all the colleagues whose works they wrote about. However, during the preparation of this work, we sadly lost one of the most important contributors to cell biology, Tony Pawson. Tony was a true pioneer in elucidating basic mechanisms of how cells transmit information intracellularly, a fundamental knowledge that permeates all of cell biology. In an effort that was initiated by one of the section editors, Rune Linding, it was decided to dedicate the entire work to his memory as recognition of his contributions and a lasting tribute to a man who left us too soon.

We hope that the Encyclopedia will be of value to students and researchers alike and will become a useful tool in the training of future generations of scientists.

Ralph A Bradshaw and Philip D Stahl

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Cell Biology: An Overview

RA Bradshaw, University of California, Irvine, CA, USA
PD Stahl, Washington University School of Medicine, St. Louis, MO, USA

All living organisms can be divided into three principal categories: archaebacteria, eubacteria, and eukaryotes; although they differ in structure and organization, they are all composed of cells as the fundamental life unit. At the molecular level, there is also a great deal of similarity in the basic materials that make up these entities because they use the same kinds of molecules to store and reproduce information, to run the cellular metabolism and machinery and to provide the structural framework. Thus nucleic acids, proteins, lipid membranes, and carbohydrates – alone and in various combinations – are universally present, albeit in distinguishable forms, along with innumerable metabolites and ions. There are components that are apparently essential for life and are found in one form or another in all species and there are many unique moieties and associated activities that are highly specialized and are found in relatively few organisms. Indeed, the similarities have underpinned the development of our understanding of cellular function at a rudimentary level and the differences, basically engendered by evolution, have illustrated and delineated the complexity that speciation has introduced. Perhaps the largest of these differences is that which separates single cell organisms from multicellular organisms. The latter are exclusively eukaryotes while the former are composed of both eukaryotes and prokaryotes. The cellular organization and architecture that distinguishes these two major life forms is striking; although cell biology correctly embraces both, traditionally prokaryotic organisms have been the province of the microbiologists and the majority of cell biology research has been devoted to the eukaryotic world. In practical terms this translates for the most part into the study of human cells and those of easily maintained laboratory animals and selected paradigms, for example, fruit flies, worms, and zebra fish.

Human and animal cell biology is not a tightly proscribed science with well-defined borders. Basically it serves at the interface between biochemistry, molecular biology, and genetics, on the one hand, and anatomy and physiology, on the other. The continuum of these disciplines forms the core of the biomedical sciences, which also include the related but separate fields of pharmacology, microbiology, immunology, and pathology that provide the connections to disease and health. Cell biology has strong connections to all of these. There are also specialized areas, for example, neuroscience, that are of such importance that they warrant their own category and the cell biology associated with them is also highly specialized. Thus, cell biology is as complex as the enormous variety of cells that exist and achieving an accurate description of all of them in terms of their components and functions has long been a major part of the research in this field.

Imaging and Organelle Organization

Among the developments that propelled cell biology into the modern era are the introduction of the ultracentrifuge and the rapid advances in microscopy both electron and light microscopy – the former allowing investigators to fractionate and characterize the components of the cell and the latter to literally see them – either *in situ* or in isolated form. This progress is illustrated by a series of Nobel Prizes starting in the early 1970s that chronicle the grand confluence of classical biochemistry and general physiology that created modern cell biology (Claude *et al.*, 1974) or the discovery and characterization of intracellular organelles – lysosomes and endoplasmic reticulum (ER) among others (Mitchell, 1978), for the chemisomotic hypothesis (Brown and Goldstein, 1985), for endocytosis (Ciechanover *et al.*, 2004), for ubiquitination and protein degradation, and just recently (Rothman *et al.*, 2013), for vesicle trafficking, and (Betzig *et al.*, 2014) for advances in light microscopy. The early cell biologists insisted on quantitative application of these new techniques and laid the groundwork for modern cell biology. As with biochemistry, reductionism has been the keystone for the amazing success over these past several decades. Each organelle has its own research history – the nucleus, mitochondria, peroxisomes, the proteasome, the ER, and cytoskeleton. The Golgi apparatus/secretory pathway and the endosome–lysosome network have all been examined in detail both in isolation and in their relationships with each other. A new member, the exosome (aka extracellular vesicle) has emerged more recently and stimulated the imagination of aspiring young investigators (Harding *et al.*, 2013). The rise of genomics, the development of spectacular imaging modalities, and evolving biochemical techniques (proteomics) have led to the discovery of molecular motors, the identification of macromolecular complexes such as the exocyst, ESCRT, GARP, SNARES among others that choreograph complex intracellular pathways, including cell motility and cytokinesis, have brought cell biology into this new era where the whole is now seen as larger than the individual components. This creates the segue from Parts 1 and 2 of the *Encyclopedia* (*Molecular Cell Biology* and *Organizational Cell Biology*) to Part 3 (*Functional Cell Biology*) where signaling modalities will be seen as an integrative force for regulation and control.

Signaling

While all organisms can sense their environment and respond to cues from it, multicellular organisms must in addition coordinate their responses, which require intercellular communication at a sophisticated level (Bradshaw and Dennis, 2009). The higher the development, the more complex these communication systems become. Thus the cell biologist must focus not only on how molecular function is translated into cell organization and how these functions are coordinated from organelle to organelle but also on the external interactions and signals that control the larger functional responses of organs and ultimately organisms.

Intercellular communication is afforded by stimuli that can be transmitted across the cell membrane, either directly or via membrane bound molecules that in turn pass signals to the intracellular compartment. The origins of these stimuli may be quite diverse. Among other things, they can include cell–cell contacts, soluble factors/messengers, or foreign agents. Lipophilic molecules can cross the membrane by diffusion or by facilitated transport to recognize and bind to intracellular entities but most substances bind to membrane bound proteins and induce their signal by 'activating' them instead. These so-called receptors are capable of producing a variety of responses that invariably involve the generation of posttranslational modifications of preexisting proteins. Protein phosphorylation, which in eukaryotes mostly occurs on tyrosine, serine, and threonine residues, is highly prevalent and appears to be directly or indirectly involved in almost all transmembrane signaling (Gnad *et al.*, 2011). However, it is by no means the only vehicle for transmitting information as acetylation, methylation, monoglycosylation, and ubiquitination, to name a few, are both important and widespread. There are over three hundred different covalent modifications (Khoury *et al.*, 2011) that are introduced into proteins (and many more that have not yet been chemically defined) of both a transient and stable nature and most are likely to be involved to some extent in signal transduction processes. In addition, limited proteolysis can also be an important part of a pathway and this activity is a major player in cellular responses (Turk *et al.*, 2012).

The induction of an intracellular signal is basically perpetrated by the formation of new interactions between the modified proteins and other entities, which can range from small molecules to macromolecular protein complexes. One of the most important advances in understanding how cells process information induced from external stimuli was the appreciation that the newly formed sites produced by the protein modifications were recognized by other proteins with modules specifically designed for this purpose (Pawson, 1995). Thus, for example, phosphotyrosine residues could be recognized by other proteins containing a domain, termed SH2 for its relatedness to a similar domain found in the Src protein kinase, which could then be further modified allowing for additional interactions to occur. By these 'docking' events, signaling complexes could be assembled, often in multiple steps, that ultimately lead to the activation of key effectors. The end point of many of the pathways is the activation of transcription factors that lead to the modulation of gene expression of that cell by ultimately changing its protein expression profile (Bradshaw and Dennis, 2009). However, many molecules are usually modified and activated 'along the way' and these 'new' activities also add to the overall response to the original stimuli. Short term responses indeed require that the protein effectors necessary for it are already present; long term responses per force require new protein synthesis.

Signal transduction is thus dependent on two phenomena: posttranslational modifications, particularly of the readily reversible type, and protein–protein interactions. The extent to which these two activities take place, even in resting, unstimulated cells was greatly underestimated before the advent of proteomics and the introduction of mass spectrometric methodology into cell biological research (Bradshaw and Burlingame, 2005) These high-throughput unbiased analyses of very complex mixtures, basically derived directly from cell lysates, revealed that essentially every protein had multiple interacting partners and that posttranslational modifications affected a very significant proportion of the proteins present. These were profound differences from what had been the prevailing wisdom and amounted to a paradigm shift in thinking about cellular organization and structure. As noted above, these same tools have gone on to cast new light on organelle organization in terms of resident proteins and have helped to disclose the structure of important cellular machines, such as the proteasome (Voges *et al.*, 1999), the nuclear pore (Routa *et al.*, 2000), and transcription complexes (Kornberg, 2007). They have also begun to elucidate the complexity of epigenetic regulation of gene transcription at the histone level (Cosgrove, 2012), which will also be essential in completing our understanding of signaling processes.

Concluding Remarks

Two of the most fundamental aspects of cells are their ability to reproduce themselves and, in higher organisms, to undergo changes that lead to new cell types and functions. These developmental processes leading to differentiation are what allow a single fertilized egg to form a complex adult organism and require the synthesis of all aspects of cell biology to understand. Knowing how cells go from one state to another in a timed and regulated fashion forms the core of developmental biology, which can be viewed as a sub discipline of cell biology. Also of major importance is the maintenance of viability and the associated turnover processes. The control of cell death is not only an essential part of development it is also key to managing situations that have gone awry and underlies many serious disease conditions.

It is clear that science is still a long way from understanding how even simple cells work. There is a long list of functions and structures that remain to be elucidated and integrating the various experimental approaches and the data they produce still lags far behind. This is the 'Era of Big Data' and vast amounts of new information that impact on our understanding of cells and their processes are collected every day. Genomic information is a good example – despite the rapidity that this sort of information can now be obtained, it still remains to be effectively mined in terms of what it can tell us about basic principles of how cells work. There has been an understandable pressure to apply this information to managing disease (Collins and Varmus, 2015), particularly those that are life threatening, but there certainly is information that is yet to be formulated that would apply to fundamental problems as well. The same can be said for transcriptomics, proteomics, and metabolomics. In addition to these powerful new technologies, singular advances in analyzing single cells promises to augment these molecular approaches significantly (Di Palma and Bodenmiller, 2015).

One aspect of cell biology that is looking to address the challenges of integrating these relatively newly minted studies is systems biology (Part 4). It is far too early to assess the value of these efforts in coordinating this flood of information but it certainly looks promising. It will be an elusive goal for some

time to come but also a stimulus for new generations of cell biologists that will be forthcoming.

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Molecular Cell Biology: An Overview

KE Neet, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA
SA Woodson, Johns Hopkins University School of Medicine, Baltimore, MD, USA
JS Bond, Penn State University College of Medicine, Hershey, PA, USA
GW Hart, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Basic Molecular Components and Technology

The history of cell biology is rooted in the related sciences of biochemistry and molecular biology. Biochemistry grew from physiology and chemistry early in the twentieth century and gave fruit to molecular biology in mid-century. The term 'cell biology' first appeared in PubMed in 1917, occurred sparsely for many years, crossed the 10 paper per year mark in 1948, did not reach 1000 papers per year until the mid-1980s, and currently appears in more than 25 000 papers per year. The driving force of this explosion of information is the inherent interest in biological systems, i.e., ourselves, as wellas the intrinsic importance to medicine, i.e., ourselves in a pathological state.

As these sciences moved into the twentieth century, the dividing lines among these disciplines became blurred so that an *Encyclopedia of Cell Biology* must begin with a presentation of the essentials of biochemistry and molecular biology that are needed to understand cell biology. Currently, these disciplines are nearly indistinguishable with a considerable overlapping continuum. Good investigators follow significant research questions regardless of what discipline is involved in the solutions. Biology follows a continuum from atom to molecule to system to organism. Advances in these related scientific areas could not have been made without parallel development of experimental techniques capable of asking and answering the appropriate questions. This first subsection **Basic Molecular Components and Technology** leads to the remainder of the first major section of the Encyclopedia, **Molecular Aspects/Molecular Principles, Components, Technology, and Concepts Important for Understanding Cell Biology** and deals with evolving techniques that have helped elucidate the nature of the molecules, complexes, organelles, cells, and interactions involved in living organisms. The emphasis is not on the techniques themselves, but rather on what the technology reveals about the basic components of biological cells and systems.

The early 'central dogma' of 'DNA makes RNA makes protein' has become outdated since the discovery of reverse transcription in certain RNA viruses and the discovery of catalytic RNA. The concept of one gene for one protein had to be expanded and modified due both to splicing and post-translational modifications. The terminology of noncoding DNA as 'junk' DNA has been made passé by microRNA and other types of noncoding RNA. Thus, the field of biochemistry/molecular biology/cell biology, like with all science, advances. Indeed, as the evolution of the understanding of cell biology has progressed, we have found broader concepts to replace simpler ones resulting in an ever-increasing appreciation of the complexity of structure, function, regulation, and growth of living organisms. Many of these principles and molecules fundamental to cell biology have also been treated from a somewhat different perspective and format in the related Elsevier publication '*Encyclopedia of Biological Chemistry*,' second edition, and should be consulted for additional information.

Since life is based on chemistry, the *Encyclopedia of Cell Biology* starts with certain chemical principles of equilibria, bonding, and catalysis before moving into structural considerations. The biological structures discussed are the four usual major classes of biomolecules: nucleic acids, proteins, lipids, and carbohydrates. More complex molecules/systems encompass more than one of these groups, for example, glycoproteins glycolipids, lipoproteins, and membranes. Several articles are devoted to metabolites and metabolism ('classical' biochemistry and essential for functioning of cells). Certain diseases that are linked to molecular function or dysfunction, for example, cystic fibrosis (*see* Cystic Fibrosis) and Huntington's disease (*see* Diseases of Protein Folding: Huntington's Disease and Amyotrophic Lateral Sclerosis), are specifically presented.

Topics making up the first section, in order, are basic chemical principles (including equilibria) (*see* Chemical and Physical Principles), bonding (*see* Chemical Biology), and catalysis (*see* Biocatalysis); nucleic acids (including chemical properties and sequencing) (*see* DNA, RNA Chemical Properties (Including Sequencing and Next-Generation Sequencing)), chemical synthesis (*see* The Chemical Synthesis of DNA and RNA Oligonucleotides for Drug Development and Synthetic Biology Applications), cloning and expression systems (*see* Expression Systems) and site-directed mutagenesis (*see* Site-Directed Mutagenesis); Proteins (including structure and domains) (*see* Protein Domains: Structure, Function, and Methods), folding and misfolding (*see* Folding, Misfolding, Disordered Proteins, and Related Diseases; Diseases of Protein Folding: Huntington's Disease and Amyotrophic Lateral Sclerosis), posttranslational modifications (*see* Posttranslational Modifications: Key Players in Health and Disease), drug design (*see* Drug Design), antibodies (*see* Antibodies and Improved Engineered Formats (as Reagents)), sequencing (*see* Protein Sequence Determination: Methodology and Evolutionary Implications), purification (*see* Isolation/Purification of Proteins) and spectroscopy (*see* NMR in Structural and Cell Biology); Lipids (including cholesterol) (*see* Cholesterol and Other Steroids), complex lipids (*see* Synthesis and Structure of Glycerolipids; Glycolipids), signaling (*see* Lipid Signaling) and

lipidomics (*see* Lipidomics); Membranes (including properties and biogenesis) (*see* Composition, Physical Properties, and Curvature), rafts (*see* Lipid Rafts/Membrane Rafts), electrochemical potential (*see* Membrane Potential: Concepts), nerves (*see* Neuronal Action Potentials and Ion Channel Allostery) and mitochondria (*see* The Outer Mitochondrial Membrane, a Smooth 'Coat' with Many Holes and Many Roles: Preparation, Protein Components, Interactions with Other Membranes, Involvement in Health, Disease, and as a Drug Target); Complex carbohydrates (including glycogen) (*see* Glycogen and Starch), proteoglycans (*see* Proteoglycans) and hyaluronan (*see* Hyaluronan); and Metabolites (including regulation) (*see* Metabolic Regulation), bioenergetics and organelles (*see* A Structure Perspective on Organelle Bioenergetics).

Nucleic Acid Synthesis/Breakdown

Deoxyribose nucleic acid (DNA) was first discovered in 1869 by the Swiss scientist, Friedrich Miescher. Nucleic acids are biopolymers comprised of nucleotide monomers that are composed of three moieties, a five-carbon sugar, a phosphate group, and a nitrogenous base. DNA contains deoxyribose as the sugar component and RNA contains the sugar ribose. Polynucleotides are formed by covalent linkages between the phosphate of one nucleotide and the sugar of another, resulting in phosphodiester linkages. Nucleic acids are the major information molecules of all known forms of life by encoding, transmitting, and expressing genetic information. Elucidation of the structure of DNA by Watson and Crick in 1953 immediately suggested the semiconservative mechanism by which DNA is accurately reproduced and later demonstrated by Meselson and Stahl. The ability of DNA sequences to be copied into RNA or into copies of DNA with high fidelity in a template-dependent fashion is one of the most fundamentally important processes in living organisms. The transfer of genetic information from DNA to RNA to protein is considered the fundamental process of all living systems. However, as occurs in certain viruses, it is possible to transfer sequence-encoded information from RNA to DNA by reverse transcription. Topics in this section of the *Encyclopedia of Cell Biology* cover all major roles of nucleic acids in information transfer, including the mechanisms by which genetic information is regulated. This section additionally covers how RNA molecules transmit, edit, splice, and regulate the expression of genetic information – processes that greatly increase the number of combinations in which protein-coding sequences can be used.

Topics in this section, in order, are: Comparison of Bacterial and Eukaryotic Replisome Components, which describes the structures and mechanisms of the protein machinery that replicates DNA in bacteria and eukaryotes; Transfer RNA, which not only describes how tRNAs serve as adaptor molecules to translate mRNA sequences into protein sequence but also describes tRNA's roles in many other cellular processes; Messenger RNA (mRNA): The Link between DNA and Protein compares mRNA's structure/functions in bacteria and eukaryotes and how mutations in untranslated regions of mRNAs contribute to human disease; Telomeres and Telomerase describes the end-capping structures, called telomeres, on eukaryotic chromosomes that are critical for chromosome stability, and discusses the importance of telomerase enzymes in maintenance of the telomere; Telomere Biology describes how telomeres maintain genome stability and prevent cellular senescence, and how cancer cells bypass the normal limits on telomere elongation to gain immortality; Small RNAs/Cancer reviews the discovery, biogenesis, and roles of microRNAs in cancer etiology; Eukaryotic Nucleotide Excision Repair describes highly conserved DNA repair mechanisms that remove and repair a wide variety of chemical lesions in DNA structure, which in some cases contribute to the onset of cancer; The Base Excision Repair Pathway reviews a pathway that is critical to genomic stability by correcting small DNA base lesions, first by excising the damaged locus, and then by replacing damaged nucleotides with the correct ones; Nonhomologous DNA End Joining describes and compares how cells repair double-strand breaks in DNA by either nonhomologous DNA end joining or by homologous recombination (HR) mechanisms; DNA Repair by Homologous Recombination reviews mechanisms and proteins involved in repairing complex DNA damage by HR and how specific HR proteins protect stalled replication forks; Prokaryotic Transcription reviews the current understanding of transcription in bacteria from the recognition of DNA to the termination of RNA synthesis; Eukaryotic Transcriptional Regulation describes current models of transcriptional regulation in eukaryotes; Distant Activation of Transcription by Enhancers reviews how distantly-acting DNA enhancers activate transcription by chromatin looping; miRNAs/Small Noncoding RNAs describes how precursor mRNAs in eukaryotes are spliced to remove intervening sequences by a complex ribonucleoprotein complex called the splicesome; Pre-mRNA Splicing: Function and Dysfunction further expands on the process of splicing pre-mRNA and describes human diseases resulting from dysregulation of the splicing machinery; Ribosomal RNAs and Protein Synthesis discusses the structure of ribosomes and how the ribosomal RNA functions in the synthesis of proteins; miRNAs/Small Noncoding RNAs reviews our current knowledge about the biological synthesis and processing of microRNAs, which are typically 22 nucleotides long and function in translational repression and other regulatory processes; The Interplay between Eukaryotic mRNA Degradation and Translation summarizes current views on how eukaryotic mRNA degradation interconnects with mRNA translation to regulate gene expression; Riboswitches and Ribozymes describes the biological roles and potential as tools of riboswitches and ribozymes, which are regions of mRNA that regulate gene expression by binding small molecules, or are RNA that catalyze chemical reactions, respectively; Transgenesis and Gene Replacement presents an overview of the most widely used methods for experimentally manipulating the genomes of mammals to understand gene functions; and *see* Viral Nucleic Acids provides an overview of viral genomes, which can be either RNA or DNA, and how they are replicated in host cells. This collection of articles on nucleic acids not only provide the reader a comprehensive understanding of the state of the science in nucleic acid research, but also provides

a solid foundation for more focused investigations into the topic.

Protein Synthesis and Degradation

This section focuses on molecular mechanisms underlying the regulation of protein concentration and active forms of proteins both inside and outside cells and highlights diseases/pathologies that can result in dysregulation of proteins. Just as the knowledge/understanding of RNA and DNA and other fundamental components of cells and tissues have evolved, so have those of the proteins that make up cells, the extracellular environment, and fluids of living organisms. The complexity of the interactions of proteins has become apparent and the importance of networks of proteins (death pathways, blood coagulation, complement), interacting proteases (the 'protease web'), extracellular matrix, intracellular scaffolds, and membrane, organelle, and factor interactions with intracellular and extracellular proteins are now clearly realized.

Many of the structures involved in the synthesis of proteins have been solved (including the ribosome, translation factors) and there has been considerable progress clarifying the mechanisms that control initiation, elongation, and termination in eukaryotic cells (*see* Components, Initiation, Elongation, Termination, and Regulation). Similarly the components of the mitochondrial protein biosynthetic machinery and mechanisms of transcription and translation have been elucidated (*see* The Protein Biosynthetic Machinery of Mitochondria). Many proteins are synthesized as inactive or latent proteins (proproteins) and have to be activated by enzymes such as proprotein convertases and kallikreins (*see* Regulated Proteolysis of Signaling Molecules: The Proprotein Convertases; Kallikrein). Biosynthesis of secretory proteins that takes place in the endoplasmic reticulum (ER) involves multiple factors (signalases, chaperones, isomerases) to form a mature correctly folded protein (*see* Biogenesis of Secretory Proteins). Factors are in place for quality control in the ER to recognize and shuttle incorrectly folded proteins into the ER-associated degradation pathway (*see* Endoplasmic Reticulum-Associated Degradation and Protein Quality Control).

It was once believed one protease could degrade many proteins in a relatively unregulated manner (somewhat like trypsin in the intestinal tract). It is now known that there are multiple proteases in all cells and fluids that are highly regulated. There are ~600 genes for proteases in the human genome and if there were no regulation there would be widespread necrosis, cell death, and destruction. It is clear that proteolytic systems are highly regulated by localization, activation/inhibition, synthesis of latent proenzymes, interactions with multiple components such as cofactors, carbohydrates, lipids, membranes, organelles, and the pH of the environment. The complement of all proteases and their substrates is known as 'the degradome' and high-throughput methods have recently been developed to study the network of protease and substrate interactions (*see* Mass Spectrometry-based Methodologies for Studying Proteolytic Networks and the Degradome).

Proteolytic systems exist both intracellularly and extracellularly as well as at cell membranes. The mammalian intestinal system serves as a good example of coordinated digestion of food proteins that involves many different types of extracellular proteases (*see* Digestive Proteases: Roles in the Human Alimentary Tract). Blood coagulation represents another extracellular system that is critical to host defense and hemostasis and involves cells (e.g., platelets), a host of plasma proteins (most of which are proteases) and protease inhibitors, which are highly controlled to form and degrade blood clots appropriately (*see* Overview of Blood Coagulation and the Pathophysiology of Blood Coagulation Disorders). The complement system involves highly regulated proteolytic enzymes critical to our immune systems that remove targeted pathogens (*see* Molecular Mechanisms Underlying the Actions of the Complement System). The proteasome is key to intracellular proteolytic systems and works in coordination with ubiquitin-tagged proteins for protein quality control and several signaling systems (*see* Ubiquitin, Ubiquitin-Like Proteins, and Proteasome-Mediated Degradation). In addition, the intracellular lysosomal system containing multiple hydrolases (e.g., proteases, glycosidases) that interact with cellular and extracellular components to degrade and maintain protein/amino acid homeostasis (*see* Role of Lysosomes in Intracellular Degradation). Dysregulation of all of these systems is associated with disease, and there are specific articles on lysosomal storage diseases (*see* Lysosomal Diseases) and the proteases involved in the progression of cancer (*see* Cancer – Proteases in the Progression and Metastasis).

Individual proteases are discussed in many articles and some that are highlighted are matrix metalloproteinases (*see* Matrix Metalloproteinases), calpain (*see* The Calpain Proteolytic System), ADAMS and ADAMTS (*see* ADAMTS Proteases: Mediators of Physiological and Pathogenic Extracellular Proteolysis; ADAMs Regulate Cell–Cell Interactions by Controlling the Function of the EGF-Receptor, TNFα and Notch), membrane-anchored serine proteases (*see* Extracellular: Plasma Membrane Proteases – Serine Proteases), meprins (*see* Metalloproteases Meprin α and Meprin β in Health and Disease), kallikreins (*see* Kallikrein), and aspartic proteases (*see* Aspartic Proteases of Alzheimer's Disease: β- and γ-Secretases; Cathepsin E: An Aspartic Protease with Diverse Functions and Biomedical Implications). Protease inhibitors are also critical to the regulation of protein degradation and disease. Endogenous polypeptide protease inhibitors exist for many of the proteolytic systems (e.g., apoptosis, blood coagulation; Naturally-Occurring Polypeptide Inhibitors: Cystatins/Stefins, Inhibitors of Apoptosis (IAPs), Serpins, and Tissue Inhibitors of Metalloproteinases (TIMPs)). Alpha-1-antitrypsin deficiency is an example of how important endogenous protein inhibitors are for preventing disease, as this deficiency leads to liver damage and emphysema (*see* Alpha-1-Antitrypsin Deficiency: A Misfolded Secretory Glycoprotein Damages the Liver by Proteotoxicity and Its Reduced Secretion Predisposes to Emphysematous Lung Disease Because of Protease-Inhibitor Imbalance). Examples of synthetic protease inhibitors used to control disease process are blood pressure inhibitors (ACE inhibitors; Blood Pressure, Proteases and Inhibitors) and HIV-protease

inhibitors that have been designed to manage AIDS (*see* Inhibitors of HIV Protease).

In summary, the purpose of part I of the Encyclopedia is to describe the chemical principles and the molecular components/organization of the cell and its environment in sufficient detail to allow a clearer understanding of how these components are then assembled, function, and are regulated at a higher level – the themes of the next three parts of the Encyclopedia.

MOLECULAR PRINCIPLES, COMPONENTS, TECHNOLOGY, AND CONCEPTS: BASIC PRINCIPLES

Contents

Chemical and Physical Principles

H Oubrahim and P Boon Chock, National Institutes of Health, Bethesda, MD, USA

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Introduction

Cells of all living organisms contain four classes of macromolecules or polymers consisting mainly of carbon, oxygen, nitrogen, hydrogen, and a small quantity of sulfur and phosphate atoms. Proteins are polymers of amino acids, which serve as enzymes, regulators, structure elements, and receptors; DNA and RNA are polymers of nucleotides, which store and transmit genetic information, and polysaccharides are polymers of simple sugars, which serve as energy-rich fuel stores. To investigate the molecular processes that make life possible, it is essential that investigators are well versed in fundamental chemical and physical principles, since they govern both the thermodynamics and dynamics of biochemical processes.

Living cells must perform work to stay alive, grow, replicate, and evolve, while maintaining themselves in a dynamic steady state, far from equilibrium with their environment. To understand how cells accomplish these processes, within the topic limitation of this article, we focus our discussion on the physical and chemical principles that govern interactions between cellular molecules. They include the energetic aspect that determines whether a molecular process can occur spontaneously, and the kinetic and regulatory aspects of biological processes, as well as effects of the crowded cellular environment.

The Laws of Thermodynamics and Living Cells

Living cells have developed highly efficient mechanisms to utilize the energy obtained from chemical fuels and light to carry out numerous energy-requiring processes in order to maintain themselves in dynamic steady states. When a cell fails to obtain energy, it will die and decay toward equilibrium with its surroundings. To understand how the energy is extracted, stored and channeled into useful work in living cells, we address cellular energy conversions in context of the law of thermodynamics and the quantitative relationships among free energy, enthalpy and entropy.

The laws of thermodynamics are general principles that provide the quantitative description of heat and energy changes and chemical equilibria. These laws apply to all chemical and physical processes, including biochemical reactions (van Holde *et al.*, 1998; Edsall and Gutfreund, 1983; Alberty, 2003). Their importance resides in the fact that they determine the conditions in which a biochemical reaction can proceed.

In thermodynamics, the field of observation is divided into two conceptual regions: the system and the surroundings. The 'system' refers to everything within a defined region of space, including all the constituent reactants, products, solvent of the reaction, and the immediate atmosphere; while the system and its surroundings together constitute the universe. When the system does not exchange either matter or energy with its surroundings, it is considered isolated. If the system exchanges only energy and not matter with its surroundings, it is defined as a closed system. An open system is one that exchanges both matter and energy with its surroundings. The first law of thermodynamics describes the principle of the conservation of energy. In any physical or chemical change, the total energy, E, of a system and its surroundings is constant, although the form of the energy may vary. In other words, the first law states that E can be changed only by the flow of energy as heat or by work. Consequently, energy can neither be created nor destroyed, it can only be changed from one form to another as shown in the mathematical expression below,

$$\Delta E = E_B - E_A = Q - W \qquad [1]$$

where E_A and E_B is the energy of a system at the beginning and the end of the transformation, respectively, Q is the heat absorbed, and W is the work done by the system. Note the change in energy of a system depends only on the initial and final states, independent of the transformation pathway.

When a given chemical reaction occurs under constant pressure, the amount of heat released or absorbed reflects the nature and number of chemical bonds altered during the course of the reaction. This heat of reaction is referred to as enthalpy, H, expressed as joules/mol or calories/mol

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(1 cal = 4.184 J). Because the total enthalpy of a system cannot be measured directly, only the change of enthalpy, ΔH, is evaluated. If heat is being absorbed by the reaction, its ΔH is positive and the reaction is endothermic. On the other hand, if heat is generated by the reaction, the reaction is exothermic and its ΔH is negative. However, the first law of thermodynamics is insufficient to predict whether a reaction can occur spontaneously since some endothermic reactions do occur spontaneously. Thus, a function other than ΔH is necessary to account for this observation. One such function is the entropy, S, expressed in unit of J/mol K. Note that entropy is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction proceeds with a gain in entropy. It is worth mentioning that entropy is a central concept in biochemistry since life requires continual maintenance of order while increased randomness is the natural tendency. The second law of thermodynamics states that a process can occur spontaneously, if and only if, the sum of the entropies of the system and its surroundings is >0. This indicates that the entropy of a system can decrease during a spontaneous reaction, if the entropy of the surroundings increases such that their sum is positive. However, the entropy changes of chemical reactions are not readily determined and the second law indicates that to determine whether the reaction can occur spontaneously requires one to know the value of the entropy changes for both the surroundings and the system of interest. At constant temperature and pressure, a condition fulfilled by most biological systems, this constraint imposed by the second law can be obviated by using a different thermodynamic state function termed free energy (G) or Gibbs' free energy, derived from the combining of the first and second law of thermodynamics by Gibbs (1876–1878, 1878).

The basic equation is:

$$\Delta G = \Delta H - T\Delta S \qquad [2]$$

where ΔG is the change in Gibbs free energy of a reaction under constant pressure, P, and temperature, T, and ΔS and ΔH is the change in entropy and in enthalpy of the reaction, respectively.

Gibbs Free Energy Always Decreases for a Spontaneous Process at Constant Temperature and Pressure

All reactions are generally affected by two forces: The tendency to achieve the most stable chemical bond, indicated by ΔH, and the tendency to achieve the highest degree of randomness, expressed by ΔS. The net effect of these two factors is summed up by the change of Gibbs free energy described by eqn [2]. Thus, ΔG provides a valuable criterion for determining whether a reaction can occur spontaneously. By convention, ΔS is positive when entropy increases and ΔH is negative when heat is released by the system to its surroundings. When either of these conditions is energetically favorable the reaction tends to yield a negative ΔG, a condition for a spontaneous reaction. To determine the actual free energy change, ΔG, for the reaction [3], one needs to take into account the nature of the reactants and products as well as their concentrations as shown in the eqn [4].

$$aA + bB \rightleftharpoons cC + dD \qquad [3]$$

where a, b, c, and d represent the stoichiometry of the indicated components.

$$\Delta G = \Delta G^0 + RT\ln\left([C]^c[D]^d/[A]^a[B]^b\right) \qquad [4]$$

where ΔG^0 is the standard free energy change, a constant that is characteristic of each reaction, R is the gas constant, T is the absolute temperature, and [A], [B], [C] and [D] are molar concentrations of reactants and products. (More precisely, the activity, defined as a thermodynamic function that correlates changes in chemical potential with changes in concentration, through relations formally equivalent to those for ideal systems. For practical reasons, molar concentration is used in biochemical literature.) ΔG^0 is the change in free energy under standard conditions (298 K and 1 atm) when reactants and products are initially present at 1 M, or for gas at 1 atm. Equation [4] indicates that ΔG of a reaction depends on the nature of the reactants, expressed by ΔG^0, and their concentrations, expressed by the second term of the equation.

With this definition, the standard state for reactions involving hydrogen ions is $[H^+] = 1$ M or pH 0. However, most biochemical reactions occur in relatively well buffered aqueous media at a pH around 7, such that both the pH and the concentration of water (55.5 M) are essentially constant. To simplify ΔG^0 calculation, biochemists adopted a convention that in the biochemical standard state, $[H^+]$ is 10^{-7} M (pH7), $[H_2O]$ is 55.5 M, and when the reactions involve Mg^{2+} (when ATP is a reactant), $[Mg^{2+}]$ is 1 mM, a standard state different from that used in chemistry and physics. With this convention, when H_2O, H^+, or Mg^{2+} are reactants or products, their concentrations are not included in the K_{eq} expression, but are incorporated, with a value of 1 for each of their activities, into the constants K'_{eq} and $\Delta G'^0$. These constants are referred to as standard transformed constants written with a prime, to distinguish them from the standard constants used by chemists and physicists. Thus, the standard transformed free energy change at pH 7 is denoted as $\Delta G'^0$, which can be calculated using the equilibrium constant, K'_{eq}, and the equation $\Delta G'^0 = -RT \ln K'_{eq}$, where $K'_{eq} = [C]^c_{eq}[D]^d_{eq}/[A]^a_{eq}[B]^b_{eq}$. Therefore, the criterion for the spontaneity of a biochemical reaction depends on the value of ΔG, expressed as

$$\Delta G = \Delta G'^0 + RT\ \ln([\text{products}]/[\text{reactants}]) \qquad [5]$$

Gibbs Free Energy Changes are Additive

The free energy change of a reaction is independent of its reaction pathway. However, when a reaction consists of two or more successive reactions, and each of the two successive reactions shares a common intermediate, the Gibbs free energy change of the net reaction is equal to the sum of the ΔG of the individual reactions. In other words, the Gibbs free energy changes are additive. This property allows one to determine the free energy of the formation of complex molecules needed to sustain living cells, from ΔG of the individual reaction steps that lead to the formation of the final complex molecules.

Consequently, a thermodynamically unfavorable reaction, with a positive ΔG, can be driven by a thermodynamically favorable reaction to yield a negative ΔG for the sum of free energy changes of the two reactions. Utilizing this energy-coupling strategy, biological systems are able to synthesize and maintain the information-rich polymers required to sustain living cells, and to formulate their metabolic pathways by coupling enzyme-catalyzed reactions such that the overall ΔG of the pathway is negative. In biological systems, this principle is frequently utilized to couple the energy of ATP hydrolysis, a highly exergonic reaction, to otherwise unfavorable reactions, such as those involved in the biosynthetic pathways.

Coupling of ATP Hydrolysis to Drive Thermodynamically Unfavorable Reactions

ATP, maintained at a relatively high and constant level in the cell, has been considered as the molecular currency of intracellular energy transfer. It has been extensively utilized to drive thermodynamically unfavorable biochemical pathways. To illustrate how this principle works, we consider the coupling of ATP hydrolysis to the synthesis of glucose 6-phosphate, an endergonic first reaction step in glucose oxidation pathway:

$$\text{Glucose} + P_i \rightarrow \text{glucose 6-phosphate}$$

$$(\Delta G_1^0 = 3.3 \text{ kcal/mol; endergonic}) \quad [6]$$

where P_i is inorganic phosphate. $K_{eq1} = [\text{Glucose 6-phosphate}]/([\text{Glucose}][P_i]) = 10^{(-\Delta G0/1.36)} = 3.98 \times 10^{-3}\ M^{-1}$ at 25 °C (Note $2.3 \times RT = 1.36$ kcal mol^{-1}). This indicates that there is no net conversion if the molar ratio is equal to or larger than $3.98 \times 10^{-3}\ M^{-1}$. When this reaction is coupled to:

$$\text{ATP} \rightarrow \text{ADP} + P_i\ (\Delta G_2^o = -7.3 \text{ kcal/mol; exergonic}) \quad [7]$$

where $K_{eq2} = ([\text{ADP}][P_i])/[\text{ATP}] = 2.18 \times 10^5$ M. The sum of these two reactions becomes

$$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose 6-phosphate} + \text{ADP}$$

$$(\Delta G_{sum}^0 = -4 \text{ kcal/mol; exergonic}) \quad [8]$$

The equilibrium constant of this coupled reaction becomes

$$K_{eq} = K_{eq1} \times K_{eq2}$$
$$= ([\text{Glucose 6-phosphate}][\text{ADP}])/([\text{Glucose}][\text{ATP}])$$
$$= 10^{(-\Delta G\text{osum}/1.36)} = 8.7 \times 10^2.$$

At equilibrium, the ratio of [Glucose 6-phosphate]/[Glucose] $[P_i] = K_{eq} \times [\text{ATP}]_{eq}/([\text{ADP}]_{eq}[P_i]_{eq})$.

The ATP generating system in cells maintains the $[\text{ATP}]_{eq}/([\text{ADP}]_{eq}[P_i]_{eq})$ at a high level, typically on the order of 500; thus

$$[\text{Glucose 6-phosphate}]/([\text{Glucose}][\text{Pi}]) = 8.7 \times 10^2$$
$$\times 500 = 4.35 \times 10^5 M^{-1}$$

Together, this indicates that by coupling the conversion of glucose to glucose 6-phosphate to ATP hydrolysis in the cell, under standard conditions would shift the equilibrium ratio of [Glucose 6-phosphate] to [Glucose] $\times$ $[P_i]$ by a factor of 10^8.

This example demonstrates the thermodynamic essence of ATP's action as an energy-coupling agent. Since all living cells maintain a concentration of ATP much higher than its equilibrium concentration, coupling of a cellular reaction with the hydrolysis of an ATP molecule can change the equilibrium ratio of products to reactants by a huge factor, for example, by a factor of 10^8. Thus, ATP functions as a major carrier of chemical energy in cells to convert thermodynamically unfavorable reaction sequences into favorable ones.

Reaction Rate and Rate Constant

Note that ΔG of a reaction depends only on the nature of the reaction and the concentration of both reactants and products, but ΔG is independent of the mechanistic pathway. Furthermore, ΔG does not provide information on the rate of a chemical reaction. In fact some thermodynamically favorable reactions fail to take place at measurable rates due to the high activation energy required for the reactions. To overcome this problem, biological systems utilize enzymes to catalyze slow reactions by lowering the activation energy via an alternative reaction pathway to facilitate the formation of the transition state. Like all catalysts, enzymes cannot alter the equilibrium constants of a reaction. They only increase the rate by which a reaction proceeds in the direction governed by thermodynamics. In other words, thermodynamics cannot provide information about intervening states of the system.

While thermodynamics predicts whether a reaction can occur, and how much energy can be derived from them, the concentration of most molecules in living cells is maintained in dynamic steady states, and not by equilibrium constants. Therefore, rates of biochemical reactions, mostly catalyzed by enzymes, play an important role in shaping the metabolic pathways in living cells. The fact that about a quarter of the protein-encoding genes in the human genome encode enzymes and their regulatory proteins points to the importance of understanding the kinetics of biochemical reactions in cellular regulation and metabolism (For further reading on kinetics, see references Moore and Pearson, 1981; Purich, 2010; Connors, 1990).

The rate, d[P]/dt, for the formation of a reaction product, *P*, is determined by the concentration of the reactant(s) and its rate constant, k. In general, a rate equation has the form

$$d[P]/dt = k[S_1]^a[S_2]^b \ldots \quad [9]$$

The value of k is independent of the concentration of S_1, S_2…, but dependent on the nature of reaction investigated, and the environmental conditions of the reaction, such as temperature and chemical properties of the reaction medium. The power variables a and b represent the order of reaction with respect to S_1, and S_2, respectively, and their sum $(a + b + \ldots)$ represents the overall order of the reaction. When eqn [9] is in a form of simple rate equations, it represents the rate expression of elementary (single-step) reactions. Some complex (multiple step) reactions may also possess simple rate equations. Nevertheless, complicated rate equations are required for

kinetic analysis of complex reactions such as those involving parallel or consecutive reactions.

When a reaction is unimolecular, eqn [9] is reduced to $d[P]/dt = k[S_1]$. In this case, the rate of the reaction depends only on k and the concentration of S_1. This reaction is defined as a first-order reaction, and k is a first order-rate constant that has a unit of reciprocal time, for example, s^{-1}. A first-order reaction proceeds via an exponential function and its half-life, $t_{1/2} = \ln 2/k$ or $0.693/k$, can be used to calculate its rate constant. Note that the $t_{1/2}$ of a first-order reaction is constant, independent of the initial time point used for analysis. In the case of a bimolecular reaction, the reaction rate depends on the concentration of two different reactants, or two molecules of the same reactant, such that $d[P]/dt = k[S_1]^2$ or $k[S_1][S_2]$, where k is the second-order rate constant, with an unit of $M^{-1}s^{-1}$. The time course of the second-order reaction is qualitatively similar to that of the first-order reaction, except the consumption of the reactant(s) proceeds faster initially and slower toward the end of the reaction due to the nature of second-order dependency. A third-order reaction, whose rate depends on the product of three concentrations, is relatively rare. In the case of a zero-order reaction, its rate is independent of the concentration of the reactant, and the unit of its rate constant is $M\ s^{-1}$. A zero-order reaction proceeds with a linear time course. This type of reaction is not commonly observed except in heterogeneous systems and in catalyzed reactions when the catalyst is present at a significantly lower concentration than that of the reactant, such that the catalyst is saturated with the reactant. Under this condition, the concentration of the reactant will not change significantly during the course of the initial rate measurement, and the reaction rate depends only on the concentration of the catalyst used.

Reaction Rate-Limiting Step

When the conversion of reactant *S* to product *P* proceeds via formation of a number of intermediates, for example, I_1 and I_2, and if the formation of I_1, I_2, or *P* is very much slower than the rest of the reaction steps, then the rate of *P* formation will be limited by the rate of the slowest step, a reaction that possesses the highest activation energy, $\Delta G^{\ddagger}$. This slowest step is called a rate-limiting or rate-determining step since it is the 'bottleneck' of the overall reaction. Strictly speaking, this flow analogy is valid only for consecutive and irreversible reactions, and it can be misleading if the reverse reaction is significant. In fact even for irreversible reactions, the rate-determining step concept is meaningful only if one of the reactions is much slower than the others. Note when the overall reaction includes more than two elementary steps, the situation may not be easy to analyze, since the product of the nth step is the reactant of the $(n+1)$st step. For these two states to be represented by the same free energy they must have the same composition. This means that the stoichiometric composition must be constant throughout the entire successive reactions (Boyd, 1978).

Rate Constant and Activation Energy

To co-relate the magnitude of a rate constant to Gibbs free energy of activation, $\Delta G^{\ddagger}$, a transition-state theory was derived. This theory assumes that the transition state is in equilibrium with reactants, such that the population of the transition state is governed by Gibbs free energy of activation, $\Delta G^{\ddagger}$. Thus, the magnitude of a rate constant can be expressed as a function of Gibbs free energy of activation and temperature:

$$k = (\mathbf{k}_B T/h)(e^{-\Delta G^{\ddagger}/RT}) \qquad [10]$$

where $\mathbf{k}_B$ is the Boltzmann constant, and h is the Planck's constant. The important point to emphasize here is that the relationship between the rate constant and the activation energy, $\Delta G^{\ddagger}$, is inverse and exponential. In other words, this is the basis for the statement that lower activation energy yields a faster reaction rate.

Enzyme Kinetics

Equation [10] shows that the function of catalysts is to lower the activation energy, $\Delta G^{\ddagger}$, of a reaction and thereby speed up the reaction rate. Most of the catalysts in biological systems are enzymes, and the majority of enzymes are proteins. The majority of enzymes are known to catalyze only one particular type of reaction under very limited chemical and physical conditions, and they are capable of enhancing a reaction rate up to factors of 10^6 or more. Enzyme-catalyzed reactions are characterized by the formation of an enzyme–substrate, ES, complex. The catalytic specificity and capacity of an enzyme are derived mainly from multiple weak interactions between the enzyme and its substrates, mediated by hydrogen bond formation, hydrophobic, and ionic interactions. Furthermore, the enzyme active site tends to be structured such that some of these interactions occurred specifically to stabilize the transition state. The need for multiple interactions could be one of the reasons that an enzyme is a relatively large size molecule. The enzyme-substrate binding energy could be utilized to offset the energy required for activation, as well as to induce protein conformational change at the active site to properly position catalytic functional groups to facilitate the cleavage and formation of chemical bonds by a variety of mechanisms, including general acid–base catalysis, covalent catalysis, and metal ion catalysis. These processes could lead to transient covalent interactions with a substrate or group transfer to or from the enzyme, to provide a new, lower-energy reaction pathway. Once the catalytic action is completed, including the release of the tightly bound product(s), the enzyme reverts to its unbound state.

Multiple techniques have been employed to elucidate the catalytic mechanism of purified enzymes. They include three-dimensional structural analysis of enzymes, coupled with chemical modification and site-directed mutagenesis to examine the role of individual amino acid residues in enzyme structure and action. However, kinetic study of enzyme action remains the most important method for elucidating the mechanistic action of enzymes. Kinetic properties of many enzymes can be described by the Michaelis–Menten model. In this model, an enzyme, E, first forms an ES complex, where S is the substrate, prior to product, P. formation.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad [11]$$

The initial rate of product generation can be described by the Michaelis–Menten equation:

$$v = V_{max}([S]/([S] + K_M)) \quad [12]$$

where V_{max} is the reaction rate when the enzyme is fully saturated with the substrate and K_M is the Michaelis constant, $K_M = (k_{-1} + k_2)/k_1$. When k_2 is rate-limiting such that $k_2 \ll k_{-1}$, K_M is reduced to k_{-1}/k_1, the dissociation constant of ES. It should be pointed out that the validity of the steady-state method does not depend on the assumption that $d[ES]/dt = 0$. This assumption is not valid at the beginning of the reaction when [ES] is being built up, and toward the end of the reaction when [S] is too low to maintain a constant [ES]. This problem was resolved by Wong in 1975 (Wong, 1975) as follow:

Based on the reaction scheme shown in eqn [11],

$$d[ES]/dt = k_1[S]([E]_0 - [ES]) - (k_{-1} + k_2)[ES] \quad [13]$$

The initial rate of product formation is,

$$v = k_2[ES] = k_2\left(k_1[S][E]_0 - d[ES]/dt\right)/(k_1[S] + k_{-1} + k_2) \quad [14]$$

When $d[ES]/dt$ is small relative to $k_1[S][E]_0$ where $[E]_0$ is the total concentration of the enzyme used, then

$$v = k_2[ES] = k_1k_2[S][E]_0/(k_1[S] + k_{-1} + k_2) \quad [15]$$

During the early phase of the reaction, if $[S] \gg [E]_0$, the rate of change in [ES] due to diminishing [S] will be relatively slow. Thus, the validity of steady state is closely tied to the high substrate to enzyme ratio. The maximal rate, $V_{max} = k_2[E]_0$, and k_2, also known as k_{cat}, or turnover number, is the number of substrate molecules being converted into product per unit time at a single catalytic site when the enzyme is fully saturated with substrate. The ratio k_{cat}/K_M provides a good indication of the catalytic efficiency of an enzyme. Note that the Michaelis–Menten equation is also applicable to bisubstrate reactions, which proceed via a ternary complex or double-displacement pathways. A short-hand method of expressing two or more substrates or products has been proposed by Cleland to differentiate order, random, Theorell–Chance, and Ping–Pong mechanisms (Cleland, 1963). It should be pointed out that while the kinetic constants can be accurately determined from *in vitro* study using a high substrate to enzyme ratio, this unique condition required for validating the Michaelis–Menten equation may not be met under cellular conditions. Therefore, one needs to be cautious in drawing conclusions from steady-state kinetic study under such circumstances because it can lead to intolerably high errors if analyzed with the unmodified Michealis–Menten expression (Cha, 1970).

Allosteric Enzymes

All enzymes that exhibit hyperbolic kinetics with respect to increasing substrate concentration follow Michaelis–Menten kinetics, and their K_M represents the [S] required to achieve a rate equal to half V_{max}. However, the Michaelis–Menten model fails to account for the kinetic properties of many enzymes. An important group of enzymes that do not obey Michaelis–Menten kinetics are the allosteric enzymes. An Allosteric enzyme/protein is one in which binding of one substrate or ligand to one site affects the binding affinity of another site in the same molecule. When binding of one ligand impairs the subsequent binding of other ligands, its kinetics exhibits a negative cooperativity. In contrast, the activity of most allosteric enzymes displays a sigmoidal kinetics due to substrate binding at one site that promotes the subsequent binding affinity at a distinctly different site. This group of enzymes is generally, but need not, consists of multiple subunits or multiple active sites. They play a crucial role in many fundamental biological processes, including, but not limited to, cell signaling and metabolism regulation since they are susceptible to be regulated by signaling molecules. With the sigmoidal saturation response, the value of [S] at half-maximal rate cannot be designated as K_M because the enzymatic activity does not follow a hyperbolic function. Thus, the symbol $S_{0.5}$ or $K_{0.5}$ is often used to represent the substrate concentration required to achieve half-maximal velocity of the allosteric enzyme. It should be pointed out that this class of enzyme has important regulatory properties. On one hand, sigmoidal kinetics provides a mean for allosteric enzymes to greatly alter their catalytic output in response to a relatively small change in substrate/effector concentration. On the other hand, an enzyme with a strong negative cooperativity can provide a means for a rapid surge or decrease in enzymatic activity through changes in the concentration of an effector that desensitizes the negative cooperativity (Huang *et al.*, 1982). Interestingly, to date, among the known allosteric proteins, oxygen binding to hemoglobin, an oxygen carrying protein consists of four oxygen binding sites, is the most thoroughly studied. Consequently, hemoglobin is sometimes being referred to as an 'honorary allosteric enzyme.'

From a kinetic standpoint a multisubunit enzyme is not required to achieve a cooperative type of kinetics, since it can be generated by a monomeric enzyme that can exist in several conformations at steady state or utilize alternative pathways in a multisubstrate reaction. The fact that regulatory enzymes, almost without exception, are polymeric, argues strongly for the role of subunit interaction in the cooperativity. The potential for achieving sophisticated control is undoubtedly greater with a polymeric, than with a monomeric enzyme. However, most of prevailing models for explaining the cooperative effects of subunits are based on ligand-promoted conformational equilibria. While they adequately describe the change of enzymatic activity in response to metabolite concentration, they fail to link intersubunit cooperation to the catalytic mechanism per se.

Concerted and Sequential Models

Two major models, the concerted model of Monod *et al.* (1965), and the sequential model of Koshland *et al.* (1966), have been proposed to explain the cooperative binding of ligands to multisubunit proteins. In the concerted model, Monod *et al.* assumed that an enzyme having identical and noninteracting subunits arranged in a symmetrical manner can exist in two conformational states, T and R, with different ligand binding affinities. A key feature of the Monod model is the conservation of symmetry, i.e., the T and R transition is a

concerted one such that all subunits in a given state are equivalent. The cooperative phenomenon, however, arises from the preexisting T⇌R equilibrium in an apparent cooperativity. This two-state Monod model can be extended to accommodate more complicated situations. For instance, the original Monod model based on rapid equilibria cannot generate negative cooperativity. However, a proposed pseudo-conservative transition has been demonstrated as a modified Monod model that allows the two-state model to accommodate both positive and negative cooperativities (Viratelle and Seydoux, 1975). The sequential model of Koshland *et al.* is based on the induced-fit theory. Mathematically, this model is similar to those developed by Adair (Adair, 1925) and Pauling (Pauling, 1935). In this case the subunits are initially identical and binding of a ligand to one subunit affects the subsequent binding of ligands to the remaining subunits. This model is often referred to as the sequential model as opposed to the concerted model of Monod *et al.* The sequential model can be readily adapted to the case where the subunits are initially nonidentical (preexisting asymmetry). Since the binding of a ligand to one subunit can either improve or impair the binding affinities for the remaining subunits, positive, negative, and 'mixed' cooperativities may result. Importantly, both concerted and sequential models have been successfully applied, for example, by Schachman's and Koshland's groups, to the analysis of cooperative systems (Huang *et al.*, 1982).

Water as Life's Aether

Water, representing about 70% of cell's weight, plays a vital role in sustaining all forms of life. Therefore many aspects of life are affected by the physical and chemical properties of water, and many of these properties are derived from different electronegativities of H and O atoms that make water a highly polar molecule. As a consequence, most water molecules are in contact with their neighboring molecules through hydrogen bonding with itself and with solutes (Liu *et al.*, 1996). The hydrogen bonds are rapidly broken and reformed, with an average lifetime of $\sim 10^{-12}$ s. The capability of hydrogen bonding and its polarity make water a highly interacting molecule, as well as an excellent solvent for polar solutes by weakening electrostatic forces and forming hydrogen bonds between polar molecules. However, this property of water also poses a problem for living cells because it weakens interactions between polar molecules. To overcome this problem, biochemical systems generate hydrophobic microenvironments to maintain polar interactions at their maximal strength and specificity where needed. Furthermore, since water binds strongly to itself, it induces self-aggregation of nonpolar molecules such as lipids in an aqueous medium. This capacity of water facilitates the formation of cellular membranes that define the boundaries of cells and their internal components.

Another unique property of water is that it can ionize into H^+ and OH^- where H^+ exists as hydronium ions, H_3O^+, in aqueous medium. For simplicity H^+ is used instead of the actual species present. On average, 1 out of 10^7 water molecules is ionized in its pure liquid. In biochemistry the concentration of H^+ is expressed as pH, defined as $pH = -\log [H^+]$, where $[H^+]$ is in units of molarity. Thus, the greater the acidity of a solution, the lower its pH. A pH 7.0 solution contains $[H^+] = 1.0 \times 10^{-7}$ M. Since the concentration of water is 55.5 M, it does not change much under most biological conditions. Thus, the equilibrium constant for water can be simplified to

$$K_w = [H^+][OH^-] = 1.0 \times 10^{-14} M^2 \quad [16]$$

at 25 °C. This indicates that the ionization of water at 25 °C is highly unfavorable since its ΔG^0 is 19.1 kcal mol^{-1}. However, the extent of water ionization can be altered by the presence of other species that can bind either H^+ or OH^-. These species include proteins, DNA, RNA as well as cellular metal ions. From the K_w expression, one can obtain the $[OH^-]$ in aqueous solution knowing the pH value. For example, if $[H^+]$ is 10^{-3} M, then $[OH^-] = 10^{-14}/10^{-3} = 10^{-11}$ M. In essence, $[H^+]$ and $[OH^-]$ exhibit a reciprocal relationship.

Acid–Base Reactions Play a Central Role in Most Biochemical Processes

The equilibrium constant for the ionization of a weak acid, HA, can be described as $K = ([H^+][A^-])/[HA]$. The pK of this acid is defined as $pK = -\log K$. Thus the pH of a solution can be calculated using the eqn [17], known as Henderson–Hasselbalch equation, if the molar ratio of A- to HA and the pK of HA is known. Conversely, the pK of an acid can be calculated if the molar ratio of A- to HA and the pH of the solution is known.

$$pH = pK + \log([A^-]/[HA]) \quad [17]$$

When a solution contains a weak acid–base conjugate pair, for example, acetic acid and acetate, it can serve as a buffer with the capacity to prevent a significant change in its pH due to the addition of a small quantity of either strong acid or base. In general, the best buffer capacity of a given buffer system occurs in a range of one pH unit on either side of its pK. Interestingly, cells and organisms maintain a specific and constant cytosolic pH to keep biomolecules in their optimal ionic state. Furthermore, a significant change in pH can potentially lead to protonation or deprotonation of key functional groups of biomacromolecules that cause disruption of their molecular structures and lead to harmful biological effects. Thus, nature has evolved to minimize pH changes in biological systems. To this end, biological systems make use of a number of weak acids as their buffer systems to maintain a relatively constant physiological pH, typically around pH 7.4. Since a buffer functions best close to its pK value, among the biological buffers, phosphoric acid, which exists primarily in a near equal mixture of $H_2PO_4^-$ and HPO_4^{2-} at about pH 7.4, plays a major role to maintain physiological pH. Consistent with this notion, inorganic phosphate is present at about 1 mM in blood for maintaining its pH at 7.4.

Noncovalent Interactions Play Key Roles in Mediating Functions of Biomacromolecules

Weak noncovalent interactions exert a decisive role in maintaining the structure and function of macromolecules,

although all biomacromolecules are formed mainly by covalently linked carbon bonds (bond energy ~ 85 kcal mol^{-1}), and covalent interconversion of enzyme cascades, which possess an enormous capacity for signal and rate amplification, play important roles in regulating cell signaling and metabolism (Chock and Stadtman, 1996). Specifically, noncovalent interactions are involved in stabilizing the double helix structure of DNA, in orchestrating RNAs interactions to exert their transcriptional and translational regulation, and in the folding of proteins/enzymes into intricate three-dimensional structures to accommodate their enzymatic activities and substrate specificity. The four extensively utilized noncovalent interactions are electrostatic interactions, hydrogen bonding interactions, van der Waals interaction, and hydrophobic interactions. They differ in their nature of their interactions, bond strength and specificity as follows: (1) Electrostatic interactions, resulting from the Coulombic interaction between two opposite atomic charges located on two molecules. The energy of this interaction is governed by Coulomb's law, namely, $E = kq_1q_2/\mathrm{Dr}$, where E is the energy, q_1 and q_2 are the charges on the two atoms, at r angstroms apart, D is the dielectric constant of the medium and k is a proportionality constant, which has a value of 332 when the energy is expressed in kcal mol^{-1}. By convention, an attractive interaction would have a negative energy. In an aqueous medium, $D = 80$, when $r = 3$ Å between the two ions bear single opposite charges, the electrostatic interaction energy is -1.4 kcal mol^{-1}. However, this interaction is significantly strengthened when it occurs on the protein surface (~ -4.8 kcal mol^{-1}) or in the interior of the protein (~ -60 kcal mol^{-1}) due to changes in the dielectric constant of the reaction medium. (2) Hydrogen bonds, where the hydrogen atom is partially shared by two electronegative atoms such as nitrogen or oxygen. This leads to a favorable dipole–dipole interaction. The strength of this interaction falls off quickly with distance, or when the angle between the dipole is far from linear. Hydrogen bonds between the protein back bone amide nitrogen and carboxyl oxygen play a major role in stabilizing the α-helix and β-sheet structure of proteins, as well as determining the conformation of proteins. The energy for hydrogen bonds is in the range of ~ 1–5 kcal mol^{-1}. (3) van der Waals interactions, derived from weak attractions that occur between atoms in close proximity to each other. The basis of these interactions is the attraction of the positively charged nucleus and negatively charged electron clouds between different atoms. For atoms commonly found in biological molecules, van der Waals attractions are optimal at distances between 3 and 4 Å, and become negligible beyond 5 Å. The van der Waals repulsion prevents atoms getting much closer than ~ 3 Å apart. The energy associated with the van der Waals interaction is ~ 0.5–1 kcal mol^{-1}, depending on the van der Waals distance. (4) Hydrophobic interactions are due to the tendency of nonpolar molecules to stick together in an aqueous medium. Nonpolar molecules do not easily dissolve in water, in part due to their inability to participate in hydrogen bonding or ionic interactions with water and to restructure the hydrogen bonding among water molecules. The poor solubility is governed by a large entropy reduction. A model, generally known as the iceberg model, has been proposed to provide a molecular interpretation for the large entropy loss to the structural enhancement of water molecules near the vicinity of a nonpolar solute. However, the validity of this model is still a matter for debate, in view of recent experimental and theoretical analysis that reveals that water does not form a structure around the nonpolar solute at room temperature and a large part of negative entropy of solvation can be attributed to the small size of water molecules such that the nonpolar solute can interact with high number of water molecules and leads to a large decrease in entropy. For details on this issue, please see reference (Blokzijl and Engberts, 1993; Lee, 1985). Nevertheless, hydrophobic interactions are well accepted as the dominant energetic factor to mediate the formation of protein tertiary structure, enzyme–substrate/effector interaction and the stability of biological membranes.

While each of these noncovalent interactions is relatively weak, collectively they determine the biological structures and functions of proteins, nucleic acids, lipids, and carbohydrates. It is essential to note that every ion in an aqueous medium is surrounded by a shell of oriented water molecules maintained by the attraction of water dipoles to the charged ion. Thus, hydration of ions has a major influence on all aspects of electrostatic interactions for which the strength of acids and bases plays an important role. Since proteins contain multiple acidic and basic groups, it is reasonable to expect both the conformation and activity of enzymes to be altered as a function of the concentration of hydrogen ions.

Effect of Molecular Crowding in Living Cells

The biochemical and biophysical principles discussed in preceding sections were derived mainly from *in vitro* studies using pure reactants, often small molecules, or in relatively low concentrations, for example, less than 1 mg ml^{-1} of total macromolecule such as protein, nucleic acids, or polysaccharides. However, all cells contain various biomacromolecules at high concentration (Hoppert and Mayer, 1999). Biochemical reactions in living cells occur in media crowded with other soluble or structured macromolecules, resulting in nonspecific interactions between individual macromolecules and their immediate surroundings in the cytosol. These background interactions lead to three different phenomena: (1) macromolecular crowding, attributed to volume excluded by one soluble macromolecule to another; (2) macromolecular confinement, attributed to steric-repulsive interactions between the macromolecule of interest and its static boundaries; and (3) macromolecular adsorption, due to reversible association of a macromolecule to the surface of a nearby fiber or membrane (Minton, 2006). Nonspecific interactions may either be repulsive, leading to preferential size and shape dependent exclusion, or attractive, leading to nonspecific binding or adsorption. Predominantly repulsive background interactions tend to enhance the rate and extent of macromolecular association in solution, while predominantly attractive background interactions tend to enhance the tendency of macromolecules to cluster nonspecifically or adsorb onto surfaces. However, in a complex and heterogeneous medium of the cytoplasm, it is a challenge to discern whether the locally dominant background interactions are likely to be

attractive or repulsive and to identify their effects on any specific reaction.

Molecular crowding, in principle, can markedly slow down the diffusion rate. Consequently crowding plays a role in all biological processes mediated by noncovalent associations or conformational changes of the macromocules, such as those involved in the synthesis of nucleic acids and proteins, intermediary metabolism and cell signaling, as well as the functioning of dynamic motile systems. In general, macromolecular crowding nonspecifically enhances reactions leading to the reduction of total excluded volume, independent of hydrogen bondings, van der Waals forces or charges. These reactions include the formation of macromolecular complexes in the medium, binding of macromolecules to surface binding sites, formation of insoluble aggregates, as well as compaction or folding of proteins. Simple statistical-thermodynamic modeling studies reveal that the 'passive crowding macromolecules' could exert order-of-magnitude or greater changes in both the rates and equillibria of numerous reactions tested. To this end, one should also recognize that system studies via simulation are still models instead of the real thing. Not all idiosyncratic details of the model system are of general value for understanding the real cellular system. Biological systems are more complex than theoretical or *in vitro* experimental studies because of enhanced heterogeneity and the presence of nonspecific repulsive and attractive intermolecular interactions in addition to volume exclusion. Model studies also show that the magnitude of the effects is strongly dependent on the relative sizes and shapes of the concentrated crowding species used and on the nature of the macromolecular reactants and products. However, to date, the results obtained via model simulation studies have provided important new insights for understanding the subject. In view of the complexity and heterogeneity of the intracellular fluids, results from simplified model studies can only partially address the complex problems encountered with the *in vivo* system (Zhou *et al.*, 2008; Ellis, 2001).

The densely packed environment in the cytosol appears to impede the folding of relatively large polypeptides since their diffusion rates would be more drastically reduced relative to those of smaller polypeptides. Furthermore, the presence of a large number of crowding macromolecules would increase the probability for a newly synthesized polypeptide to interact with other macromolecules before it can properly fold. To overcome these problems, nature makes use of a class of molecular chaperones as well as a number of protein disulfide isomerases to facilitate proper folding of nascent proteins, including those mediated by cysteine disulfide bond formation, to yield functional proteins. As a result, proteins are found to be folded very efficiently when synthesized inside the cell. Anfinsen showed that it took several hours for ribonuclease-A to fold in the test tube, a rate much slower than the rate at which functional ribonuclease-A is produced in cells (about 2 min.). A similar rationale was adopted in cell signaling. To facilitate cell signaling processes inside crowded cytosols, scaffold or anchorage proteins are adopted to generate signalsomes to process cell signaling. To this end, formation of intracellular Dishevelled-based signalsomes has been demonstrated to occur during the activation of Wnt signaling (Yokoyama *et al.*, 2010).

Concluding Remarks

To understand the molecular mechanisms of biological processes through which living cells stay alive, grow, reproduce, and evolve, we must understand how fundamental chemical and physical principles govern these reactions. In this article, a brief discussion is provided of (1) the chemical nature of cellular macromolecules, (2) the roles of water molecules and noncovalent interactions in stabilizing the reactive conformations of these macromolecules, (3) thermodynamic principles which determine whether reactions can occur spontaneously, and (4) principles of reaction kinetics in maintaining reaction processes at dynamic steady states, away from reaction equilibrium. For a more in-depth understanding of these principles, readers are referred to additional literature (Edsall and Gutfreund, 1983; Alberty, 2003; Moore and Pearson, 1981; Purich, 2010; Connors, 1990; Zhou *et al.*, 2008; Ellis, 2001; Yokoyama *et al.*, 2010; Atkins and de Paula, 2012). Finally, the potential effects of molecular crowding inside the cell were discussed in terms of principles derived from *in vitro* studies with dilute purified macromolecules and high substrate concentrations.

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Biocatalysis

PA Frey and GH Reed, The University of Wisconsin–Madison, Madison, WI, USA

Introduction

Biological Catalysts

Enzymes and ribozymes are essential to life. These macromolecules catalyze a vast array of chemical reactions that, in the absence of the biocatalyst, would take place at very low intrinsic and uncoordinated rates incompatible with life. Biocatalysts control the complex chemistry of thousands of life processes through acceleration and regulation of the rates of virtually every chemical reaction important to life. Prevention of unwanted chemical reactions relies on keeping reactive molecules apart through compartmentalization or on binding of the chemicals to macromolecules that stabilize their desired forms.

The magnitudes of rate enhancement brought about by biocatalysts approach astronomical values. For example, at 25 °C uncatalyzed hydrolysis of phosphodiester linkages between nucleotides in DNA proceeds with a half-life ($t_{1/2}$) of 30 000 000 years (Schroeder *et al.*, 2006). The refractory nature of phosphodiester linkages between nucleotides in DNA permits preservation of genetic information for hundreds of thousands, if not millions, of years. Yet biological processes at times require certain phosphodiester linkages in DNA to be cleaved. Staphylococcal nuclease, one of the enzymes that catalyzes DNA-hydrolysis, functions with a turnover number of 95 s^{-1}, corresponding to a $t_{1/2}$ of 7 ms. This enormous rate difference, or enzymatic enhancement factor, of 1.4×10^{17} corresponds to typical values for enzymes, and is neither the minimum nor the maximum. Nonenzymatic counterparts of certain enzymatic reactions are too slow to measure, so that only lower limits of rate enhancements for those reactions can be estimated.

In life, individual biocatalytic rates must be coordinated, and they must change under varying cellular developmental, metabolic, and environmental conditions. Coordination arises through several mechanisms. In genetic regulation, the relative amounts of enzymes produced by gene transcription and translation can be regulated to produce the appropriate amounts of metabolically or developmentally related enzymes. In metabolic control, metabolites regulate the activities of key enzymes through allosteric effects upon binding to regulatory sites of key enzymes. For example, in end-product inhibition the enzyme catalyzing the first committed step in a metabolic or biosynthetic pathway is often inhibited by the end product of that pathway, thereby shutting it down once the end product rises to an optimal concentration. The intra-organelle microenvironments in cells can activate or inhibit certain enzymes. For example, the acidic environments in lysosomes activate pH-dependent proteolytic enzymes. Posttranslational modifications of enzymes such as reversible phosphorylation of specific amino acid side chains are also important control mechanisms. These regulatory processes orchestrate the best balance of enzymatic activities to support the life of an organism.

Enzymes

Composition

Most biocatalysts are proteins known as enzymes. As proteins, they are linear polypeptides composed of the 20 common α-amino acids (Table 1) linked by peptide amide bonds. They range in molecular weights from $\sim$8000 to $>$150 000. The polypeptide chains of enzymes are folded into definite globular structures, many of which have been determined by X-ray crystallography or by nuclear magnetic resonance spectroscopy. The folded structures include secondary, periodic structures of α-helix and β-sheet segments, together with nonperiodic loop-segments. More than 5000 'different' enzymes are currently covered by the Enzyme Commission, and more are discovered each year.

Figure 1 depicts the protein chain fold of homodimeric enolase, showing the α-helical, β-sheet segments, and loop segments. Enolase catalyzes the dehydration of 2-phosphoglycerate to phosphoenolpyruvate. The chain fold of the core of enolase is widely known as the TIM barrel, named for the first enzyme found to display this fold, triosephosphate isomerase. It is also known as the β-barrel. While many protein chain folds are known, the TIM barrel is highly versatile and serves many purposes in enzymology. About 10% of known enzyme structures are based on the TIM barrel.

Enzymes exist as single polypeptides or as aggregates of identical or different subunits. Multisubunit enzymes can be dimers, trimers, tetramers, pentamers, hexamers, etc. of identical subunits. Some enzymes have nonidentical subunits, in which the subunits are either different enzymes catalyzing related reactions or regulatory entities that control catalytic rates. The largest aggregated enzymes include several enzymes acting in concert to catalyze processive reactions. Examples include α-ketoacid dehydrogenase complexes, which contain three enzymes plus regulatory subunits and are approximately the size of ribosomes. Other examples are the fatty acid synthase complexes, which contain six enzymes and produce fatty acids from acetyl coenzyme A in assembly-line fashion. Polyketide synthases and nonribosomal polypeptide synthases are analogous, aggregated enzymes that produce antibiotics such as erythromycin and tyrocidine.

Kinetics

Activities of typical enzymes can be measured as initial rates under specified conditions. Dependence of initial rates produced by a fixed amount of an enzyme, acting on varying concentrations of the substrate, produce hyperbolic plots of rate against substrate concentrations. The plots are consistent with the Briggs–Haldane revision of the Michaelis–Menten kinetic mechanism and the corresponding rate law, eqns [1] and [2a], where E, S, and P represent enzyme, substrate, and product. In eqn [2a], $K_m=(k_2+k_3)/k_1$ and $V_m=k_3[\mathbf{E_0}]$. Note

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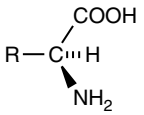
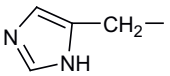
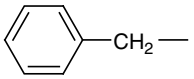
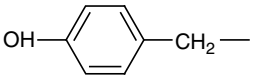
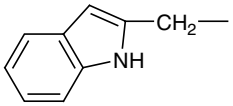
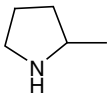
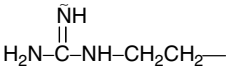
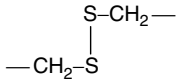
Table 1 The α-amino acid structures



	R–		R–
Glycine	H–	Serine	$HOCH_2$–
Alanine	CH_3–	Cysteine	$HSCH_2$–
Valine	$(CH_3)_2CH$–	Aspartic acid	$HO_2C–CH_2$–
Leucine	$(CH_3)_2CH_2CH$–	Glutamic acid	$HO_2C–CH_2CH_2$–
Isoleucine	$CH_3(CH_3)CHCH_2$–	Histidine	
Phenylalanine		Tyrosine	
Tryptophan		Lysine	$H_2NCH_2CH_2CH_2CH_2$–
Proline		Arginine	
Asparagine	$H_2NCO–CH_2$–	Cystine	
Glutamine	$H_2NCO–CH_2CH_2$–		
Threonine	$CH_3(OH)CH$–		

that the dissociation constant of the substrate, K_d, is defined as k_2/k_1.

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \qquad [1]$$

$$v = \frac{V_m[S]}{K_m + [S]} \qquad [2a]$$

$$\frac{1}{v} = \frac{1}{V_m} + \frac{K_m}{V_m} \cdot \frac{1}{[S]} \qquad [2b]$$

Equation [2a] predicts that when $[S] \ll K_m$, the rate is proportional to [S], and when $[S] \gg K_m$ the rate approaches V_m, the maximum as shown in Figure 2(a). The maximum rate in any experiment is proportional to the enzyme concentration $[E_0]$. The reciprocal form of eqn [2a] (eqn [2b]; Figure 2(b)) predicts a linear relationship between $1/v$ and $1/[S]$ having an intercept on the ordinate of $1/V_m$ and a slope of K_m/V_m. Manual fitting of kinetic data is facilitated by the linear form, whereas computer programs can be used to fit data directly to eqn [2a].

Equation [1] refers to an enzyme with one substrate and one product. Many complex enzymes act on two, three, or more substrates. In such cases, more complicated kinetic mechanisms apply, and the rate laws are correspondingly more complex, with more terms in the numerators and denominators than in eqn [2a]. However, for enzymes not subject to special regulatory effects, the complex equations are reducible to the form of the Michaelis–Menten equation when all co-substrates are held constant and only one is varied. In these cases, the measured parameters are apparent values V_m^{app} and K_m^{app}, from which limiting values can be obtained by varying the concentrations of co-substrates in further analysis.

A full discussion of multisubstrate kinetics lies beyond the scope of this article. However, the complications can be appreciated by considering the general patterns for two-substrate, two-product reactions, the case of substrates A and B reacting to form products C and D. The most common case is that of eqn [3], where the two substrates become associated with the enzyme in stepwise fashion, either in random order or in compulsory order, to form the ternary complex E.A.B in eqn [3]. The substrates react within the ternary complex to form the products C and D, which dissociate in either random or ordered steps. Kinetic mechanisms of this type are known as sequential mechanisms. The rate eqn [4] describes the initial rate kinetics of the rapid equilibrium version of the sequential mechanism.

$$E + A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.A.B \xrightarrow{k_3} E + C + D \qquad [3]$$

$$v = \frac{V_m[A][B]}{K_{iA}K_B + K_B[A] + K_A[B] + [A][B]} \qquad [4]$$

In eqn [4], $K_{iA} = (E)(A)/(EA)$ is the dissociation constant of A, $K_B = (EA)(B)/(EAB)$, and $K_A = (EB)(A)/(EAB)$. In an initial rate

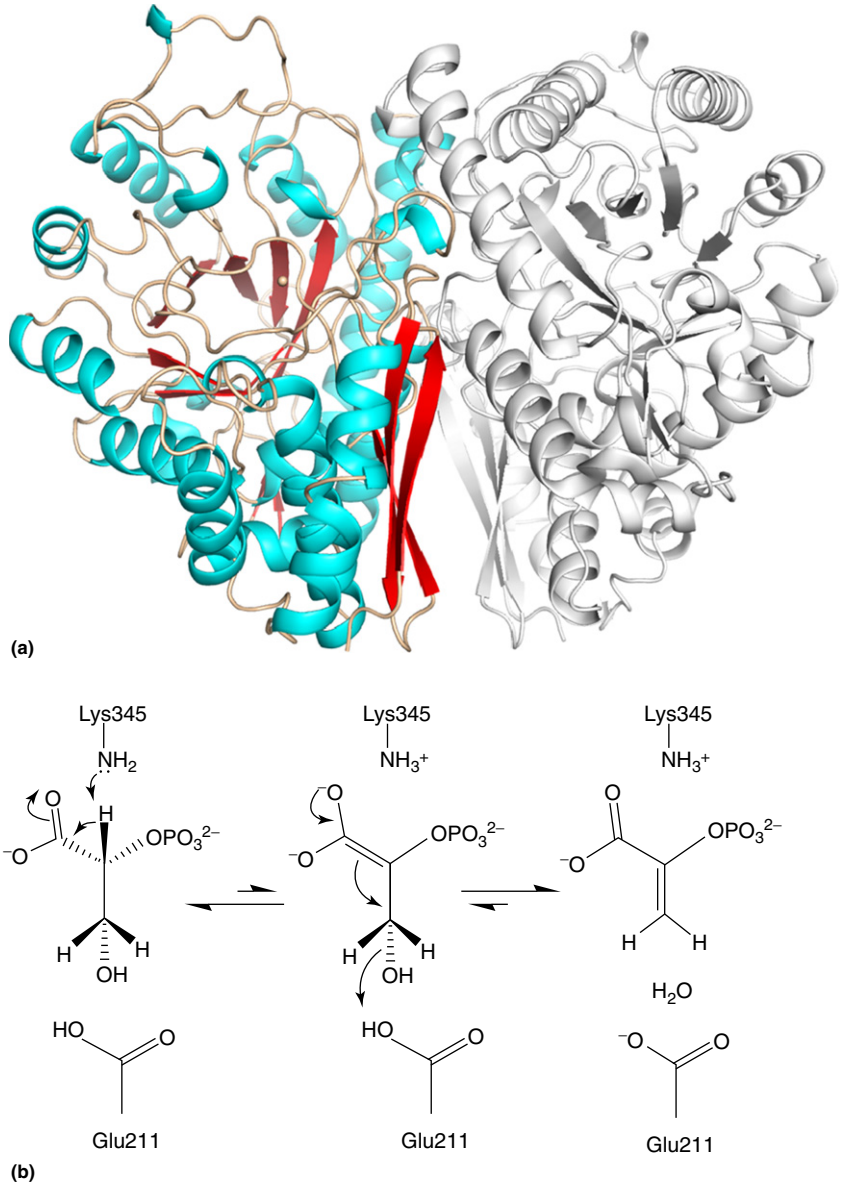


Figure 1 The structure and mechanism of enolase. (a) Shows a cartoon representation of the molecular structure of homodimeric enolase from baker's yeast. Secondary structural elements are color coded in one subunit of the dimer: cyan α-helical; red β-sheet; wheat loops. The tightly bound Mg^{2+} in each subunit appears as a sphere. The eight-stranded barrel is atypical in having one strand pointed in the opposite direction. The structure was obtained from PDB file 1ONE (Larsen *et al.*, 1996). (b) Shows the stepwise mechanism by which enolase catalyzes the dehydration of (*R*)-2-phosphoglycerate, showing the acid–base catalysis by Glu211 and Lys345 and the intermediate formation of the enolate (Poyner *et al.*, 1996).

study with [B] held constant and [A] varied, the data can be treated as in the one-substrate case.

Another kinetic mechanism frequently observed is that described in eqns [5a] and [5b]. In this mechanism substrate A binds and reacts chemically with the active site, transforming it in a distinctive way to a form designated as E-x and releasing product C. The transformation can frequently be ligation of a group (-x) from the substrate to the enzyme. Alternatively, reducing equivalents can be transferred to a cofactor (see below) at the active site. The chemically modified enzyme then reacts with the second substrate B, transferring the group (-x) to form product D. Kinetic schemes of this type are frequently called ping pong mechanisms.

$$\mathbf{E} + \mathrm{A} \rightleftharpoons \mathbf{E}.\mathrm{A} \rightarrow \mathbf{E}\text{-}\mathbf{x} + \mathrm{C} \qquad [5a]$$

$$\mathbf{E}\text{-}\mathbf{x} + \mathrm{B} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathbf{E}\text{-}\mathbf{x}.\mathrm{B} \overset{k_3}{\rightarrow} \mathbf{E} + \mathrm{D} \qquad [5b]$$

$$v = \frac{V_m[\mathrm{A}][\mathrm{B}]}{K_A[\mathrm{B}] + K_B[\mathrm{A}] + [\mathrm{A}][\mathrm{B}]} \qquad [6]$$

The rate equation for the ping pong mechanism (eqn [6]) lacks the constant term, $K_{iA}K_B$, in the denominator of eqn [4].

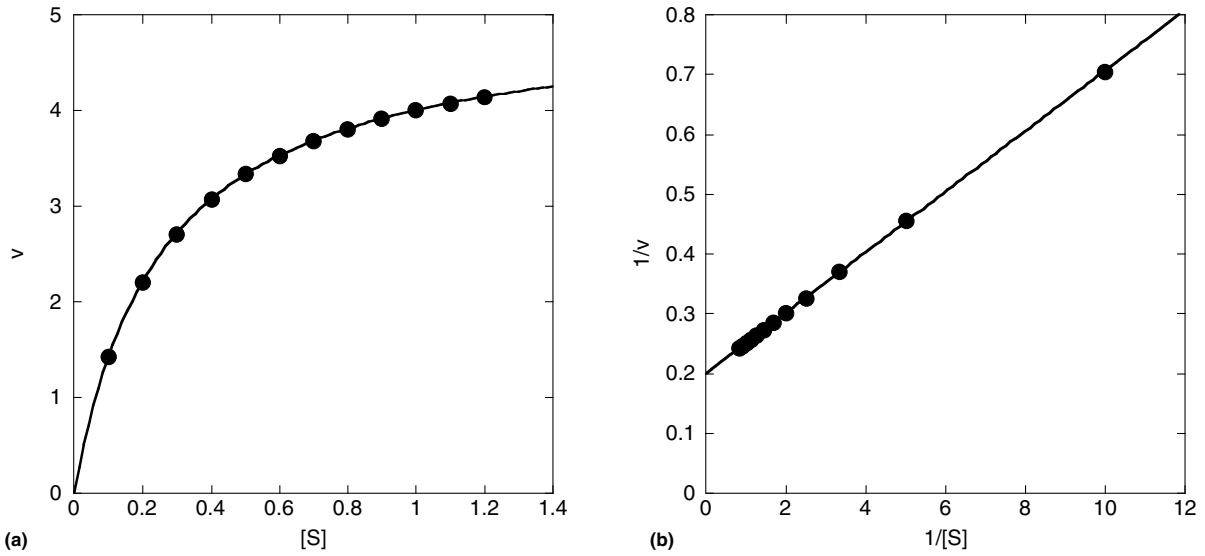


Figure 2 Reaction kinetics for an enzymatic reaction having a single substrate and single product. (a) Shows the direct plot of velocity, *v*, versus substrate concentration, [S], according to eqn [2a] (see text) using V_m of 5 (arbitrary units) and a K_m of 0.25 (arbitrary units). (b) Shows the same data plotted in double reciprocal form according to eqn [2b], where the ordinate intercept is $1/V_m$ and the slope is K_m/V_m.

Double reciprocal plots of data, 1/v versus 1/[B] at different concentrations of A appear as parallel lines, having a common slope and different intercepts. A particular property of this mechanism is that the intermediate **E**-x can be generated, isolated, and chemically characterized by excluding substrate B. This provides chemical information about the reaction mechanism and the function of the active site. A compilation of kinetic expressions and details of their applications are available (Segel, 1993; Cook and Cleland, 2007).

Whether a multisubstrate enzyme functions by a sequential or ping pong mechanism depends on several factors, including the type of chemical reaction. Comparisons of the mechanisms of chemically similar phosphotransfer reaction, where –x is a phosphoryl or nucleotidyl group, have led to the principle of economy in the evolution of binding sites as a governing factor (Frey, 1992). The central fact that phosphotransfering enzymes function by ping pong mechanisms when the phospho-accepting substrates are sterically and electrostatically related inspired the postulation of this principle. The kinetic and chemical mechanisms of adenylate kinase (AK) and nucleoside diphosphate kinase (NucDipK) exemplify this principle.

$$\mathrm{ATP} + \mathrm{AMP} \overset{\mathrm{AK}}{\rightleftharpoons} \mathrm{ADP} + \mathrm{ADP} \qquad [7]$$

$$\mathrm{ATP} + \mathrm{NDP} \overset{\mathrm{NucDipK}}{\rightleftharpoons} \mathrm{ADP} + \mathrm{NTP} \qquad [8]$$

AK functions by a sequential mechanism, whereas NucDipK functions by a ping pong mechanism, in which $-\mathrm{x}$ is the phosphoryl group (PO_3) bonded to a histidine residue. Overall, the reactions are chemically similar, phosphoryl transfer between phosphogroups. In the action of NucDipK, the sterically and electrostatically similar phosphoacceptors, NDP going forward and ADP in reverse, occupy the same binding site. Thus, the enzyme needs just one binding site, incorporating the phospho-accepting histidine residue.

In the reaction of AK two binding sites are required. The phosphoacceptors, AMP going forward and ADP in reverse, are sterically and electrostatically incompatible with a single binding site. Therefore, AK has a phosphodonor site and a phosphoacceptor sites. This allows ternary complex formation (**E**.ATP.AMP) and avoids the need for an E-PO_3 to bind the phosphoryl group during the interchange of phosphocceptors.

The principle of economy in the evolution of binding sites appears to govern a number of other classes of enzymatic reaction mechanisms, where single binding sites function in double-duty fashion (Frey, 1992; Grove *et al.*, 2011).

Substrate Selectivity

The specificity of enzymes for their substrates is frequently overstated. 'Highly selective' is a more apt description of their capacity to discriminate among similar molecules. Most enzymes will act on molecules related to their natural substrates, but at lower rates. An enzyme often functions on alternative substrates at 1/100th or 1/1000th the rate for the natural substrate, and such a rate could still correspond to a rate enhancement of, for example, 10^8–10^{12}. Metabolism of drugs and other xenobiotic compounds typically exploits the capacity of enzymes to function with alternative substrates.

Cooperativity and Allosteric Regulation

In classical behavior, each active site in a multi-subunit enzyme acts independently of neighboring subunits. Multisubunit enzymes may also exhibit cooperative behavior among subunits in binding of substrates and in catalysis such that binding of a substrate to one subunit influences the subsequent binding of substrate to neighboring subunits. This cooperative behavior, either negative or positive, may also be modulated by effector molecules that bind to distinct regulatory sites in the oligomer termed allosteric sites.

Cooperativity and associated allosteric control provide a means to 'fine tuning' of catalytic activity to meet changing demands of cells and organisms.

Chemical Mechanisms

Rates of chemical reactions can be described in terms of transition state theory also known as absolute reaction rate theory. This formalism accounts for the temperature dependence of thermally activated chemical reactions. Descriptions of photochemical and electron transfer reactions are slightly different. For thermally activated reactions, a tiny fraction of the reactant(s) (determined by the Boltzmann distribution law) initially in their ground vibrational state(s), acquire from their surrounding, sufficient excess internal (vibrational) energy to ascend transiently to a high energy state, called the transition state or activated complex. In a reaction coordinate diagram, the transition state is located at the apex – a position at which the molecule or complex can descend either in the reverse direction to reactant(s) or in the forward direction to product(s). Figure 3 illustrates the relevant energies for the reaction of a substrate S to a product P by way of $S^{‡}$, the transition state or activated complex. The activation energy for the spontaneous reaction is E_a^S.

In the corresponding enzymatic reaction, Figure 3 shows the energy of activation E_a^{ES} for the E.S complex. The ground state for the E.S complex is shown as slightly lower than for the spontaneous reaction. The difference $E_a^S - E_a^{ES} = \Delta E_a$ represents the decrease in activation energy for the enzymatic relative to the nonenzymatic reaction. This difference represents the magnitude of enzymatic catalysis, or the rate enhancement. Because activation energies have an inverse exponential influence on rates (Boltzmann distribution of activated complexes) modest changes in ΔE_a translate to large changes in rate. Although kinetic measurements can detect small changes in the activation energy, the general rule is that the smallest free energy difference that can be conceptualized is ~1 kcal, which corresponds to a rate difference of approximately sevenfold. When functioning as catalysts at concentrations much less than those of their respective substrates, enzymes do not alter the thermodynamic equilibria of the reactions that they catalyze but rather alter only the rate at which equilibrium is attained.

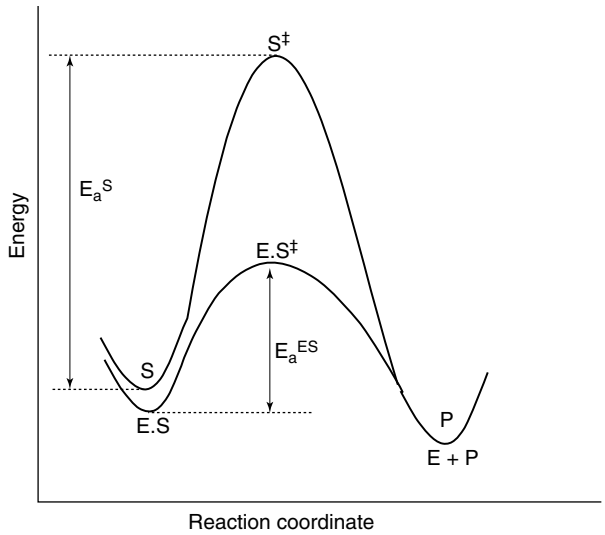


Figure 3 Relative potential energy curves along the reaction path for chemical reactions. The upper curve shows the potential energy along a reaction coordinate for a nonenzymatic reaction, showing the energy of the transition state or activated complex $S^{‡}$ at the maximum. The activation energy is $E_a^{‡}$. The lower curve depicts the potential-reaction coordinate for the same reaction catalyzed by an enzyme. The activation energy is E_a^{ES}.

Rate enhancement by enzymes begins with the binding of substrate(s) at the pre-organized active site, where the substrate(s) is or are bound in close proximity to catalytic groups within the active site. For enzymes that do not require a cofactor or coenzyme, the catalytic groups are one or more of the reactive amino acid side chains in the right-hand column of Table 1. Each of these groups, with the exception of cystine, displays both acid/base and nucleophilic reactivities. In different enzymes, each group functions either as a nucleophilic catalyst or as an acid/base catalyst. For example, in the reaction of enolase (Figure 1) the elimination of water from 2-phosphoglycerate is facilitated by acid–base catalysis by Lys345 and Glu211. In the action of NucDipK, phosphotransfer is facilitated by a histidine residue and nucleophilic catalysis (see above).

Transition State Analogs and Catalytic Antibodies

A prediction of transition state theory is that a substantial part of the catalytic rate enhancement of enzymes is due to a tight binding of the transition state of the respective reaction. Molecules that mimic the transition state of an enzyme-catalyzed reaction (transition state analogs) are expected to bind to the active site with high affinity (Wolfenden, 1972). A substantial fraction of drugs are molecules that bind to enzymes in place of the normal substrates and thereby inhibit the activity of the target enzyme. The transition state analog approach to design of selective tight binding inhibitors of target enzymes has been practiced with substantial success (Schramm, 2007).

Another practical application of transition state mimics is in the generation of antibodies that have catalytic activities for specific reactions. Antibodies arise in the blood serum of animals in the process of immunization, which entails exposure to foreign proteins, antigens, for example, the coat proteins of bacteria or viruses. Antibodies are produced in specialized mammalian cells and released into the blood stream. They recognize and mark antigen molecules for destruction by binding them very tightly ($K_d \sim 10^{-14}$ M). Antibodies are large protein molecules, in which a smaller domain, the Fab fragment, encompasses the antigen-binding site. An antigen-binding site recognizes and binds segments known as epitopes of antigenic proteins. An epitope might be a decapeptidyl unit within a foreign protein. However, antigen-binding sites are not limited to polypeptides as ligands and may bind other molecules as well. Thus, immunization of an animal with a foreign protein chemically linked to a dinitrophenyl (DNP) group, a hapten, will lead to the production of anti-DNP antibodies. The tight binding between an antigen and an antibody can be exploited to create a catalytic antibody. In this method, a molecule embodying a stable,