PL1

Protein interaction domains and biological complexity

T. Pawson

Mt Sinai Hospital, Samuel Lunenfeld Research Institute, Toronto, Ontario, UK

Signal transduction pathways are typically controlled by regulated protein-protein interactions, mediated by dedicated interaction domains. The prototype for such interactions involves the recognition of phosphotyrosine sites on receptor tyrosine kinases by the SH2 domains of cytoplasmic signaling proteins. Many other types of post-translational modifications are also recognized by specific interaction domains, which therefore provide a general mechanism to couple the dynamic state of the proteome to cellular responses. There are ~100 classes of interaction domains present in human proteins, with each class being represented by up to 300 members. Interaction domains therefore represent a prevalent feature of the proteome. I will argue that they provide a simple mechanism for the evolution of new biological functions, and conversely that aberrant protein-protein interactions are a common basis for disease. Adaptor proteins are composed exclusively of interaction sequences, and serve to couple signaling receptors to specific components of the core cellular machinery, thereby shaping the cellular response to a particular biological input. I will discuss the ability of adaptors to control cellular behaviour, particularly with relation to phosphotyrosine-mediated control of the actin cytoskeleton, and also the mechanisms by which pathogenic proteins can exploit this molecular device to re-wire cellular function.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PL2

Claude Bernard, the first Systems Biologist, and the future of Physiology. The Paton Lecture 2007

D. Noble

Department of Physiology, Anatomy & Genetics, Oxford University, Oxford, UK

Sir William Paton was one of Claude Bernard's great admirers (Paton, 1976). It is easy to see why. His book in 1865 (Bernard, 1865, 1984) was a milestone in defining the nature of physiology, and in establishing its experimental basis. It greatly influenced the foundation of The Physiological Society in 1876 (Noble, 1976). It is much less well-known that Bernard also foresaw the need for theoretical as well as experimental physiology. He wrote "the application of mathematics to natural phenomena is the aim of all science" (Cette application des mathématiques aux phénomènes naturels est le but de toute science), though he did also acknowledge that, in 1865, it was far too early to achieve that in physiology. My lecture will seek to show that computational physiology is now succeeding in establishing the basis for what Claude Bernard anticipated (Crampin et al., 2004), and that it is an essential component of physiology's contribution to the rapidly-growing field of Systems Biology. Physiology has an important contribution to make since it works at all levels of biological organisation. In this lecture I will outline some principles of Systems Biology from a physiologist's perspective (Noble, 2006). These principles will be illustrated with examples of recent physiological modelling.

Bernard, C. (1865, 1984) Introduction à l'étude de la médecine expérimentale (Paris, Flammarion).

Crampin, E.J., Halstead, M., Hunter, P.J., Nielsen, P., Noble, D., Smith, N. & Tawhai, M. (2004) Computational Physiology and the Physiome Project, Exp Physiol, 89(1), pp. 1-26.

Noble, D. (1976) The early minute books of the Physiological Society, Journal of Physiology, 263, pp. 29-37P.

Noble, D. (2006) The Music of Life (Oxford, OUP).

Paton, W.D.M. (1976) An experiment of Claude Bernard on curare: the origins of the Physiological Society, J Physiol, 263, pp. 26-29P.

Work in the author's laboratory is supported by the EU BioSim Consortium, The Wellcome Trust and EPSRC

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PL3

A sympathetic view of the sympathetic nervous system and human blood pressure regulation

M.J. Joyner[1,2], N. Charkoudian[2,1] and B. Wallin[3]

[1]Anesthesiology, Mayo Clinic College of Medicine, Rochester, MN, USA, [2]Physiology & Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN, USA and [3]Neuroscience and Physiology, Sahlgrenska Academy at Goteborg University, Goteborg, Sweden

Ideas about the relative importance of the autonomic nervous system in blood pressure (BP) regulation come and go. Fortunately, the actions of the autonomic nervous system are not so fickle and when this system works well, BP is regulated in a fairly narrow (but not constant) range at rest and is also able to rise and fall "appropriately" to meet the demands of various forms of mental, emotional and physical stress. Both the tight regulation of BP and the ability to reset that regulation to meet variable demands on the system are lost when the autonomic nervous system is absent or when key feedback mechanisms that govern it are destroyed. The 2007 Michael de Burgh Daly lecture will highlight the evolution of our ideas about the autonomic nervous system in the context of BP regulation as general thinking about human normotension emerges from the relatively "renal-centric" era of the last ~30 years. The lecture will focus specifically on the idea that there is a high degree of inter-individual variability in key elements of the autonomic nervous system and the responses they govern. This variability is somehow balanced in normotensive individuals so that it has minimal effects on BP. For example, the vasoconstrictor responses to alpha-adrenergic agonists (including post-junctional alpha-2 receptors) can vary 2- to 3-fold among normotensive young subjects and adrenergic sensitivity declines with healthy ageing. There is even greater variability in sympathetic neural activity in normotensive individuals. Resting muscle sympathetic nerve activity (MSNA) is reproducible in individuals, but can vary as

much as 10-fold among individuals with similar blood pressures. However, MSNA has a reciprocal relationship with both cardiac output (CO) and adrenergic responsiveness that seems to keep BP "normal" in humans who have high sympathetic neural outflow. The relationships among these factors (CO, MSNA and adrenergic sensitivity) and indices of baroreflex function are providing clues and generating questions about how this variability is integrated to regulate BP in a coherent way. Key questions include: 1) What is the biological basis of the variability in key sub-systems? 2) How are the balances among adrenergic responsiveness, MSNA and individual hemodynamic patterns coordinated in normotension? 3) How might dysfunction or imbalance between and among elements of the autonomic nervous system contribute to disease? 4) Finally, are there larger population-based consequences related to variability in the autonomic nervous system that might have implications for public health? By addressing these issues and raising these questions the hope is to be more provocative than correct, emphasize the implicit limits of reductionism, and highlight continuing relevance of studying the alive awake human.

Funding: NIH NS32352; NIH HL73884; AHA 0750036Z; Swedish Medical Research Council Grant 12170.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PL4

New tricks for old dogs: Allosteric modulation of G protein-coupled receptors

A. Christopoulos

Pharmacology, Monash University, Clayton, VIC, Australia

G protein-coupled receptors (GPCRs) account for approx. 2% of the human genome and represent the major targets for around 30% of all medicines on the market. Traditionally, optimising the interaction of lead molecules with the binding site for the endogenous agonist ("orthosteric" site) has been viewed as the best means for obtaining selectivity of receptor action at GPCRs. It is now recognised, however, that GPCRs possess allosteric binding sites, and that ligands can utilise these sites to modulate receptor activity through conformational changes transmitted from the allosteric to the orthosteric site and/or to effector coupling sites. Allosteric modulators may offer therapeutic advantages over orthosteric ligands for some receptors, including a greater potential for receptor selectivity and a higher safety in overdose due to a "ceiling level" to the allosteric effect. With the advent of high throughput screening, the study of GPCR allosterism represents an important new paradigm that has the potential to significantly open up the chemical space associated with novel GPCR ligands.

In order to capitalise on the promise of GPCR allosteric modulators, however, a number of challenges need to be addressed. The first challenge is *conceptual*. The word "allosteric" has been used in a number of ways since it was first coined four decades ago. Much of the confusion arises from the fact that the binding of an allosteric modulator to a GPCR causes a conformational change in the receptor that can manifest a variety of behaviours. In order to describe allosteric drug actions in a quantitative manner that can provide useful information for drug discovery programmes, therefore, a basic (minimal) mechanistic framework is required. Analytically, this has been approached via the application of an allosteric ternary complex model (ATCM) of interaction, and more recent extensions, to quantify not only modulator binding affinity, but also the cooperativity manifested between orthosteric and allosteric sites. At the molecular level, recent studies are beginning to dissect the "topography" of allosteric sites. For example, we have found that the dynamics of the second extracellular loop of the M2 muscarinic acetylcholine are vital for the actions of prototypical muscarinic receptor allosteric modulators.

A second challenge to the study of GPCR allosterism is *practical*. The perceived paucity of allosteric modulators in the known population of biologically active molecules is likely due to the fact that classic high-throughput screens have traditionally been biased towards the detection of orthosteric ligands. Because functional assays have now overtaken radioligand-binding assays as the high-throughput method of choice, allosteric ligands that have minimal effects on orthosteric binding have been discovered through their effects on receptor signalling. However, many allosteric ligands are still likely to appear quiescent in functional assays when tested in the absence of orthosteric probe. In addition, it remains more difficult to validate an allosteric mechanism/site of action in a functional assay than in a binding assay, and thus the optimal detection of novel allosteric ligands requires the combination of both standard functional and modulator-optimised binding assays.

A third challenge for allosteric modulator-based drug discovery arises as a consequence of a combination of the first two challenges and, for want of a better term, can be described as *cultural*. From a "Chemistry" perspective, the structure-activity relationships (SARs) that govern orthosteric effects do not apply to allosteric binding sites. Although this can lead to a greater scope for ligand fishing in the chemical space encompassing biologically active molecules, it can also impose additional complexities/constraints in the application of traditional approaches to rational drug design. From a "Biology" perspective, the ability to not only detect, but to validate and quantify allosteric phenomena in terms of plausible models remains of paramount importance, as it is the parameters derived from such models that can be used to inform the design of refined SAR studies. In turn, the SAR studies can yield more efficient chemical tools with which to further probe the validity of the allosteric GPCR models.

There is now at least one GPCR allosteric modulator on the market, and a number in clinical trials. Provided that drug discovery programmes recognise and accommodate the nuances involved in detecting allosteric effects, the search for allosteric GPCR modulators could yield a significant number of novel therapeutics in the new millennium.

Work in the author's laboratory is funded by the National Health and Medical Research Council of Australia, and by collaborative research agreements with GSK and Pfizer, U.K.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PL5

Smooth muscle excitation

T.B. Bolton

Basic Medical Sciences, St George's University of London, London SW17 0RE, UK

Smooth muscles as a group are defined by the absence of a sarcomeric organisation of their contractile proteins. Great strides have been made in the last 25 years in understanding the functions and physiologies of these relatively small muscle cells. The development of enzyme techniques enabling dispersion of smooth muscle tissues into constituent myocytes has enabled their complement of plasmalemmal ion channels to be studied by single-cell tight-seal voltage-clamp technique. This, combined with high resolution light microscopy of living cells using fluorescent indicators and tags, have enabled us to understand much more about the process of excitation-contraction coupling in smooth muscles. Almost all smooth muscles seem to possess voltage-dependent calcium channels (VDCCs) and calcium-activated potassium (BKCa) channels. However despite this, their electrical activity may be very different. Some readily and often spontaneously discharge action potentials which propagate from cell to cell, travelling limited distances through the muscle tissue: others seldom discharge action potentials but nevertheless they are electrically coupled and calcium entry into the cell through VDCCs is generally very important in controlling tension generation. Because smooth muscles lack sarcomeres, the mechanisms for release of calcium from, and restoration into, the calcium stores (or its extrusion from the cell) cannot resemble striated muscles. SMCs have instead developed a system of preferred sarcoplasmic reticulum (SR) release sites from which calcium is initially released upon the arrival of a stimulus. This may take the form of an action potential, or the activation of a (generally G-protein coupled) receptor, or commonly, a combination of these two mechanisms. The interactions of these two systems provides a basis for our understanding of excitation-contraction coupling in all smooth muscles. There is great interest in the relationship of caveolae to receptors, ion channels and calcium release sites. In addition, many smooth muscles have a process whereby calcium is released in packets from the SR when these become overloaded; the transient high localised calcium concentration created in a subplasmalemmal location acts to cause bursts of BKCa channel openings giving rise to spontaneous transient outward currents (STOCs) which hyperpolarize the membrane but negligibly increase cytoplasmic calcium concentration. In some smooth muscles, similar bursts of openings of calcium-activated chloride channels are believed to be the basis of spontaneous pacemaker potentials. These negative and positive feedback processes contribute substantially to the properties of various smooth muscles.

However, smooth muscles as a group are extremely diverse in their complement of receptor types, their innervation, contractile properties and physiological function. The future holds the promise that we will begin to understand how this diversity arises by tracking the expression of genes which control variations on these common themes, so providing an explanation of the unique physiological properties of each smooth muscle type.

Supported by Wellcome Trust grants 042293, 075112 & 074724 and Britush Heart Foundation grant RG99001.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PL6

BRAF The busy life of a new cancer gene

M. Stratton

Wellcome Trust Sanger Institute, Cambridge, UK

BRAF is a serine threonine kinase that forms a component of the Ras Raf Mek Erk signalling pathway. Somatic point mutations in BRAF were discovered in 2002 in the early phases of a systematic screen for somatic mutations in cancer. Since then a remarkable explosion of research activity has revealed that BRAF has a distinctive spectrum of mutations that are found in several classes of cancer including melanoma, papillary thyroid cancer, and colorectal cancer and that mutations are often present at early stages of neoplastic progression. Indeed, BRAF mutations have become markers of subtypes of some cancers and are associated with particular pathways to oncogenesis. BRAF mutations tend to be found in the classes of cancer that were previously known to carry RAS gene mutations, but samples with BRAF mutations do not usually carry RAS mutations. Germline mutations of BRAF have recently been reported in rare syndromes with developmental abnormalities. BRAF mutations usually result in activation of the kinase activity and direct phosphorylation of MEK, but some do so indirectly through CRAF, another member of the RAF family. Building on previous successes of inhibitors directed at mutated and activated kinases, BRAF and the pathways it regulates have become targets for the development of small molecule drugs which are currently being introduced into clinical trials.

Authors have confirmed where relevant, that experiments on animals and man were conducted in accordance with national and/or local ethical requirements.

PL7

The twin-arginine transport system: moving folded proteins across membranes

F. Sargent

Biological Sciences, University of East Anglia, Norwich, UK

I graduated from the University of Edinburgh in 1992 with a BSc in Biochemistry. I was then offered a place at the University of Dundee and joined David Boxer's group studying metalloenzyme biosynthesis in bacteria. My PhD was awarded in November 1996. I then took up a postdoctoral position studying bacterial protein transport with Tracy Palmer at the John Innes Centre, Norwich. A second postdoc followed in 1998 with Ben Berks (then at the University of East Anglia) before I won a Royal Society University Research Fellowship in 2000.

In prokaryotes, generation of energy by respiratory electron transfer chains involves the plasma membrane. One feature of