pieces of information about that life have been irretrievably lost, yielding an under-determined landscape. Conjectures about that landscape, although often interesting, might also prove to be, in the end, unfalsifiable.

For a realm as large and complex as the ubiquitin system, it is a given that its malfunctions are both numerous and diverse. Hence, the enormous importance of the ubiquitin field for understanding and treating human diseases, including cancer, neurodegenerative syndromes, immunological abnormalities and a myriad of other illnesses, genetic or otherwise, that can be traced to ubiquitin-dependent processes. Research on regulated protein degradation, from its mechanistic and physiological beginnings in the 1980s, has become a veritable monster of a field. Several pharmaceutical companies are developing compounds that target specific components of the ubiquitin system. The fruits of their labors have already become, or will soon become, clinically useful drugs. Efforts in this area will yield, I hope, not only 'conventional' inhibitors or activators of enzymes but also more sophisticated drugs that will direct the ubiquitin system to target, destroy and, thereby, inhibit functionally any specific protein.

Acknowledgements

Studies in my laboratory are supported by grants from the NIH and the Ellison Medical Foundation. I am most grateful to past and present colleagues for their contributions, some of which are mentioned above. I also thank R.D. Magnuson for his helpful comments on the article.

References

1 Ciechanover, A. *et al*. (1978) A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. *Biochem. Biophys. Res. Comm.* 81, 1100–1105
2 Hershko, A. *et al*. (1980) Proposed role of ATP in protein breakdown: conjugation of protein with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc. Natl. Acad. Sci. U. S. A.* 77, 1783–1786
3 Hershko, A. *et al*. (1983) Components of ubiquitin-protein ligase system. Resolution, affinity purification, and role in protein breakdown. *J. Biol. Chem.* 258, 8206–8214
4 Baumeister, W. *et al*. (1998) The proteasome: paradigm of a self-compartmentalizing protease. *Cell* 92, 367–380
5 Finley, D. *et al*. (1984) Thermolability of ubiquitin-activating enzyme from the mammalian cell cycle mutant ts85. *Cell* 37, 43–55
6 Bachmair, A. *et al*. (1986) *In vivo* half-life of a protein is a function of its amino-terminal residue. *Science* 234, 179–186
7 Bachmair, A. and Varshavsky, A. (1989) The degradation signal in a short-lived protein. *Cell* 56, 1019–1032
8 Jentsch, S. *et al*. (1987) The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. *Nature* 329, 131–134
9 Finley, D. *et al*. (1987) The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell* 48, 1035–1046
10 Goebl, M.G. *et al*. (1988) The yeast cell cycle gene *CDC34* encodes a ubiquitin-conjugating enzyme. *Science* 241, 1331–1335
11 Finley, D. *et al*. (1989) The tails of ubiquitin precursors are ribosomal proteins whose fusion to ubiquitin facilitates ribosome biogenesis. *Nature* 338, 394–401
12 Chau, V. *et al*. (1989) A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science* 243, 1576–1583
13 Hochstrasser, M. and Varshavsky, A. (1990) *In vivo* degradation of a transcriptional regulator: the yeast Matα2 repressor. *Cell* 61, 697–708
14 Johnson, E.S. *et al*. (1990) *Cis–trans* recognition and subunit-specific degradation of short-lived proteins. *Nature* 346, 287–291
15 Bartel, B. *et al*. (1990) The recognition component of the N-end rule pathway. *EMBO J.* 9, 3179–3189
16 Hochstrasser, M. *et al*. (1991) The short-lived Matα2 transcriptional regulator is ubiquitinated *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 88, 4606–4610
17 Varshavsky, A. (1997) The ubiquitin system. *Trends Biochem. Sci.* 22, 383–387
18 Pickart, C. (2004) Back to the future with ubiquitin. *Cell* 116, 181–190
19 Glotzer, M. *et al*. (1991) Cyclin is degraded by the ubiquitin pathway. *Nature* 349, 132–138
20 Hershko, A. *et al*. (1991) Methylated ubiquitin inhibits cyclin degradation in clam embryo extracts. *J. Biol. Chem.* 266, 16376–16379
21 Yuan, J. *et al*. (1993) The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell* 75, 641–652
22 Uhlmann, F. *et al*. (1999) Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature* 400, 37–42
23 Rao, H. *et al*. (2001) Degradation of a cohesin subunit by the N-end rule pathway is essential for chromosome stability. *Nature* 410, 955–960
24 Kenniston, J.A. *et al*. (2005) Partitioning between unfolding and release of native domains during ClpXP degradation determines substrate selectivity and partial processing. *Proc. Natl. Acad. Sci. U. S. A.* 102, 1390–1395
25 Tobias, J.W. *et al*. (1991) The N-end rule in bacteria. *Science* 254, 1374–1377

0968-0004/$ - see front matter
doi:10.1016/j.tibs.2005.04.005

Protein phosphorylation in signaling – 50 years and counting

Tony Pawson[1] and John D. Scott[2]

[1]Samuel Lunenfeld Research Institute, Mt Sinai Hospital, 600 University Avenue, Toronto, Ontario M5G 1X5, Canada
[2]Howard Hughes Medical Institute, Vollum Institute, Oregon Health Science University L-474, 3181 S.W. Sam Jackson Park Road, Portland, OR 97201-3098, USA

A fundamental quest in cell biology is to understand the dynamic nature of cellular organization and behavior. Genomic sequences tell us the coding potential of an organism, whereas transcriptional analysis can reveal the subset of genes expressed in any cell. However, the resulting protein products are in a constant state of flux, with their activity, subcellular localization, molecular interactions and stability being constantly modified in response to external signals, such as growth-factor stimulation, or internal cues, such as DNA damage. The

Corresponding author: Pawson, T. (pawson@mshri.on.ca).
Available online 5 May 2005

study of protein phosphorylation has provided many of the central themes that underlie the regulation of normal cells, and has revealed how signaling pathways can be subverted in human disease. As is often the case in science, advances in this field have been characterized by equal measures of insight and serendipity.

In 1955, the phenomenon of protein phosphorylation had been known for almost 50 years. In 1906, Phoebus A. Levene at the Rockefeller Institute for Medical Research (http://www.rockefeller.edu) identified phosphate in the protein Vitellin [1], and by 1933 had detected phosphoserine in Vitellin, with Fritz Lipmann [2]. However, it took another 20 years before Eugene Kennedy described the first 'enzymatic phosphorylation of proteins' [3].

After this slow start, spurred by a desire to understand the biochemical basis for hormone action, things started to heat up. Fifty years ago, the underlying principles of cell signaling were mysterious. A diverse band of researchers were working on various aspects of 'hormone action' and 'metabolism', guided by a general interest in discovering the mechanisms of insulin and glucagon action, and the basis for the adrenergic response. At Case Western Reserve University (http://www.cwru.edu), Earl Sutherland and Thomas Rall were working on the relationship of epinephrine and glucagon with the enzyme glycogen phosphorylase in the liver. They found that both hormones stimulated the production of 3′,5′-cyclic-adenosine monophosphate (cAMP), which acted inside the cell to propagate changes in phosphorylase activity, indicating that cAMP is an intracellular mediator (or second messenger) of specific hormonal signals [4]. At about the same time, Edwin Krebs, who had trained alongside Sutherland in the Cori lab, was also working on phosphorylase with Edmond Fischer by studying the conversion of inactive phosphorylase *b* into active phosphorylase *a* in muscle extracts [5]. They found that ATP was required for phosphorylase activation and, in a somewhat unusual experiment, discovered that calcium, leaching from filter paper used to clarify the extract, was an important co-factor. By using γ-^{32}P-labeled ATP, they demonstrated that phosphate was incorporated into a specific serine residue of phosphorylase, thereby yielding the activated phosphorylase *a* form [5,6].

Subsequently, Fischer, Krebs and colleagues at the University of Washington (http://www.washington.edu) confirmed that this phosphorylation is mediated by a phosphorylase *b* kinase, which is itself controlled by a cAMP-responsive kinase, leading to the idea of a kinase cascade. In 1968, Krebs purified this cAMP-dependent protein kinase (PKA) [6,7], while others, including Ora Rosen, Paul Greengard, Jackie Corbin and Susan Taylor, went on to find that it exists as tetramer of regulatory and catalytic subunits [8]. In the mid-1970s, Philip Cohen and Bruce Kemp underscored the importance of kinase-mediated protein phosphorylation by defining the structural determinants and functional consequences of numerous PKA phosphorylation events [8]. Furthermore, in 1989, Louise Johnson and David Barford solved the crystal structure of phosphorylase *a*, providing the first 3D view of molecular regulation by protein phosphorylation [9]. This work linked to Sutherland's discovery of cAMP, and led to new ways of thinking about cellular communication. When combined with the discovery of heterotrimeric G proteins by Alfred Gilman and Martin Rodbell [10], and the analysis of G-protein-coupled receptors by Robert Lefkowitz [11], the outline of a canonical signal-transduction pathway was formed, and serine/threonine phosphorylation was established as a key mechanism for rapidly modulating protein function via post-translational modification (Figure 1a).

To this point, the hydroxyamino acids serine and threonine were known to be targets for protein phosphorylation in animal cells. In 1979, Tony Hunter and colleagues identified phosphotyrosine as the product of a protein kinase activity in immunoprecipitates of a viral oncoprotein, the polyomavirus middle T antigen [12]. This revealed a new form of protein phosphorylation, which was implicitly linked to malignant transformation. Indeed, it soon became clear that cytoplasmic retroviral oncoproteins such as v-Src, v-Abl and v-Fps possess intrinsic protein tyrosine kinase activity, which is required for their ability to elicit cellular transformation [13]. Furthermore, the normal counterparts of these corrupted cytoplasmic proteins, in addition to transmembrane receptors for several growth factors and metabolic hormones, were also found to have intrinsic protein tyrosine kinase activity [13]. Notably, aberrant tyrosine kinase activity was ascribed to the products of several human oncogenes, including the chimeric Bcr-Abl protein, which is characteristic of chronic myelogenous leukemia (CML), as demonstrated by Owen Witte and colleagues [14].

The mechanisms by which tyrosine phosphorylation controls protein activities have broad implications for our understanding of cellular control (Figure 1b). First, the phosphorylation of a regulatory tyrosine residue can induce a conformational change in a substrate that stimulates its enzymatic activity, much as phosphorylase *b* undergoes a cooperative allosteric transition to phosphorylase *a* upon serine phosphorylation [9]. Tyrosine kinases themselves typically become autophosphorylated within the activation segment of their kinase domains, inducing conversion to more active state [13,15]. However, a frequent consequence of tyrosine phosphorylation is to create specific binding sites for Src homology 2 (SH2) domains [16], which are common components of an otherwise diverse set of cytoplasmic proteins that mediate intracellular signaling by normal and oncogenic tyrosine kinases [13]. Such phosphotyrosine-dependent protein–protein interactions serve to recruit regulatory proteins to phosphorylated receptors and docking proteins, and thereby activate signaling pathways that control numerous aspects of cellular behavior. They can also regulate catalytic activity, as in the case of the SH2 domain of the Src tyrosine kinase, which engages an inhibitory C-terminal phosphotyrosine site, leading to an auto-inhibited conformation of the kinase domain [13].

Although initially identified in the context of tyrosine phosphorylation, these latter observations are also relevant for understanding signaling by serine/threonine kinases. For example, Andrey Shaw and colleagues showed that

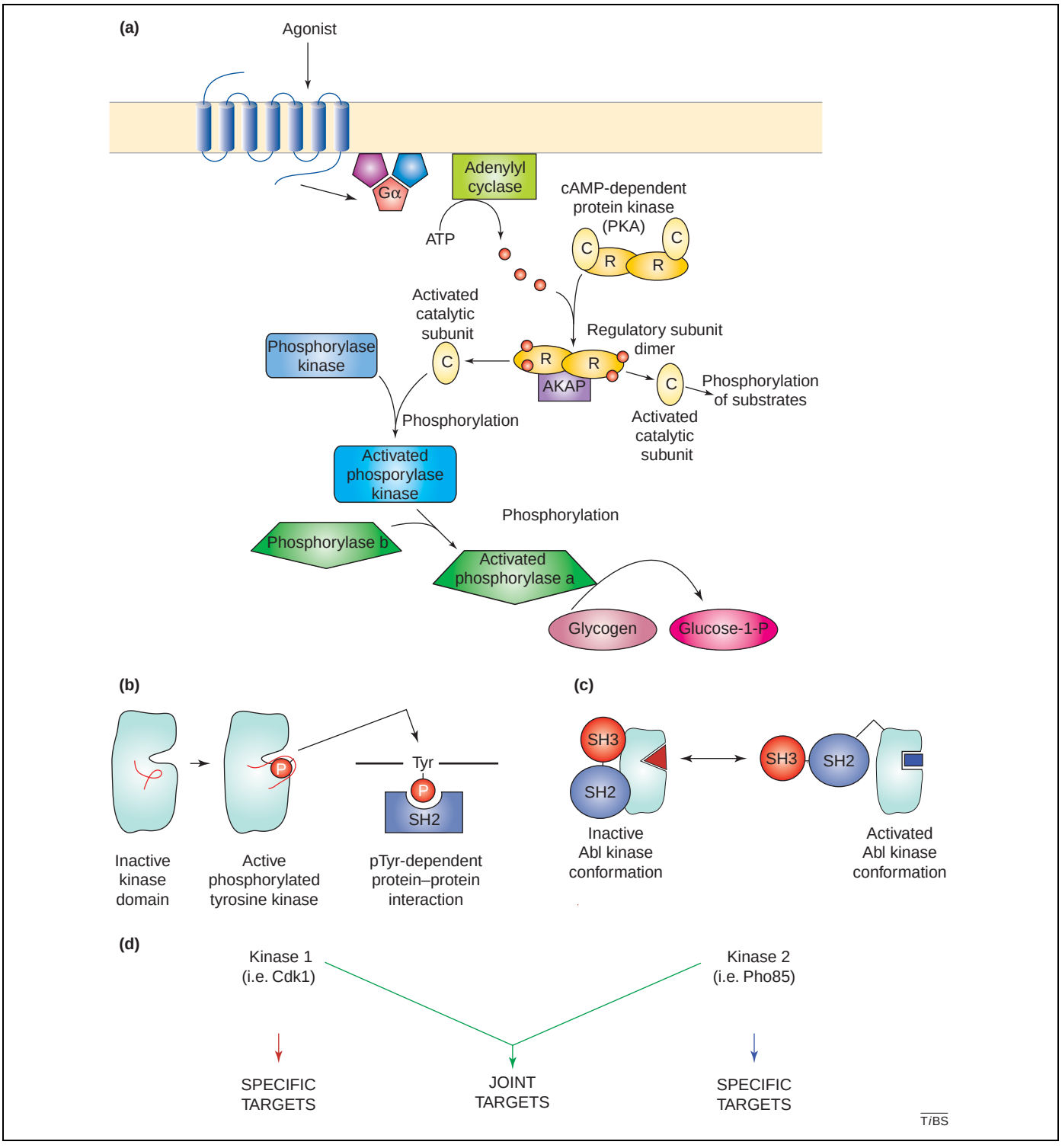

Figure 1. Past, present and future themes in protein phosphorylation. **(a)** A cascade of protein-serine/threonine kinases couple G-protein-coupled receptors (GPCR) to the control of glycogenolysis (see Ref. [6]). **(b)** Two effects of protein phosphorylation, illustrated for protein-tyrosine kinases. Phosphorylation can induce a conformational change in an enzyme (such as a tyrosine kinase itself), thereby regulating its activity, or provide a docking site for an interaction domain. **(c)** Multiple modes of protein-kinase inhibition. The Abl inhibitor Imatinib (red triangle) selectively recognizes the auto-inhibited conformation of the Abl kinase domain, normally imposed by intramolecular interactions with regulatory domains, whereas the compound BMS-354825 (blue square) binds to and inhibits the active conformation of Abl. **(d)** Two kinases can act in conjunction to regulate targets (green arrow) that are not affected by either kinase acting alone.

14–3-3 proteins bind selectively to serine/threonine motifs that are phosphorylated by basophilic kinases such as Akt/PKB and PKA, leading to altered conformation, subcellular localization or protein–protein interactions of the bound substrate [17]. Subsequently, a large family of interaction domains has been found to selectively recognize sites phosphorylated by serine/threonine kinases. Michael Yaffe and others have shown that these modules bind their targets in a manner akin to the SH2-domain-mediated recognition of phosphotyrosine-containing motifs [18]. Furthermore, somewhat like Src, the activity of serine/threonine kinases can be regulated by intramolecular

interactions of the kinase domain with internal phosphorylated sites, as in the case of glycogen synthase kinase-3 [19]. In addition, serine/threonine kinases, like tyrosine kinases, frequently have specific docking sequences through which they are targeted to their substrates. A striking example of such modular protein–protein interactions in the physiological control and selectivity of serine/threonine kinases is provided by A-kinase anchoring proteins (AKAPs). These scaffolding proteins bind to a regulatory subunit of inactive PKA, and also to a specific subcellular anchor that juxtaposes PKA to particular targets [20]. A rise in cAMP then releases the PKA catalytic subunit to phosphorylate local substrates in the vicinity of the AKAP. Thereby, AKAPs impose spatial and biological specificity on PKA (Figure 1a).

The mechanisms by which phosphorylation modifies protein function have also proven useful in understanding the effects of other post-translational modifications, including the acetylation, methylation or ubiquitination of lysine residues, arginine methylation and proline hydroxylation [21]. Frequently, the enzymes that catalyze these modifications are targeted to their substrates via docking interactions, and the modified sites are recognized by specific interaction domains (i.e. bromodomains for acetylated lysines) [21]. It is also apparent that different post-translational modifications can be used sequentially or concurrently to greatly expand the dynamic repertoire of signaling systems. For example, specific phosphotyrosine sites on activated receptor tyrosine kinases (RTKs) are recognized by the SH2 domain of the Cbl E3-protein ubiquitin ligase, which consequently monoubiquitinates the receptor at sites that then bind to the ubiquitin-interaction motifs of endocytic proteins [22]. Similarly, the N-terminal tail of a single histone (i.e. H3) can have multiple sites for acetylation, methylation, phosphorylation, ubiquitylation and sumoylation that can act in synergistic or mutually exclusive modes to control chromatin organization and gene expression [21]. Indeed, the effects of modification at one site can vary dramatically depending on the presence or absence of modification at another site [21]. This capacity of post-translational modifications to act in combination greatly increases the range of their biological activities.

These discoveries lead to the next generation of fundamental questions and practical applications. Protein kinases represent attractive drug targets in several diseases, but developing specific protein kinase inhibitors that are competitive with ATP was initially viewed as a difficult challenge because of the high concentration of ATP in the cell and the rather conserved nature of the ATP-binding pocket. However, Alexander Levitzki and others found not only that it was possible to design such compounds, but also that these molecules could show surprising selectivity [23]. Furthermore, the most recent generation of these inhibitors have significant clinical activity. Imatinib (Gleevec/STI-571), developed by Nicholas Lydon and colleagues at Novartis (http://www.novartis.com), inhibits Bcr-Abl in addition to the Kit RTK and the platelet-derived growth-factor receptor, and has therapeutic effects in CML and gastro-intestinal tumors, as demonstrated by Brian Druker, Charles Sawyers and others [24]. Structural analysis shows that Imatinib specifically binds to an auto-inhibited conformation of the Abl kinase domain, which is normally imposed by intramolecular interactions with its regulatory domains [25]. Thus, Imatinib pulls the oncogenic kinase into an inactive structure. Other inhibitors (e.g. BMS-354825) can bind to Abl kinase in its active state, and directly interfere with catalysis (Figure 1c). The combined use of these different classes of inhibitors might be especially useful in cancer treatment, for example, in limiting the effects of drug-resistant variants [26].

Most individual kinase inhibitors show significant cross-reactivity. Although this trait was initially viewed as undesirable, it is potentially advantageous from a practical point of view. For instance, Imatinib is active against multiple kinases and, thus, at least two very different cancers. Furthermore, recent work from Kevan Shokat and colleagues demonstrates that simultaneously inhibiting the two different cyclin-dependent kinases in *Saccharomyces cerevisiae* (Cdk1 and Pho85) elicits cellular responses that are not induced by blocking either kinase alone [27]. These findings are consistent with the view that cellular behavior is controlled by signaling networks with emergent properties, and that inhibiting multiple nodes in such a network (e.g. by coordinately blocking two or more kinases) can have synergistic effects that might be clinically useful (Figure 1d).

In the realm of protein phosphorylation, we are approaching a point reached several years ago by those pursuing genome sequences. We know most, if not all, of the protein kinases that are encoded by the human genome, and for the subset of tyrosine kinases we can identify the great majority of their binding partners and potential targets. Indeed, new approaches such as mass spectrometry have the capacity to provide a comprehensive inventory of serine/threonine- and tyrosine-phosphorylation sites. If the kinases, their binding partners and substrates can be quite thoroughly enumerated, the challenge becomes to match known kinases and substrates, for which newly developed chemical biology and computational tools will be essential [28]. An even more daunting issue is to determine the biological roles of the large number of phosphorylated sites, and their influence on the dynamics of signaling and protein-interaction networks. Increasing emphasis will be placed on imaging techniques to define the spatial and temporal context of phosphorylation events, and to learn how these differ between different cells, or even at distinct locations in the same cell. For example, does the repertoire of kinases, targets, phosphorylation events and phospho-dependent protein interactions differ between distinct dendritic spines of the same neuron?

Finally, the biological consequences of multi-site phosphorylation and of the combined use of different post-translational modifications are poorly understood, but they might contribute in an important way to biological complexity, potentially eliciting switch-like or graded effects depending on their context. A single polypeptide might be modified at numerous sites by phosphorylation, or other classes of post-translational modifications, yielding many isoforms, each with a different combination of post-translational modifications and, potentially, a distinct biological activity [21]. This diversity of protein isoforms generated by post-translational modifications might ultimately rival or exceed the

contribution of differential RNA splicing and gene expression to the range of biological variation, and is likely to be pivotal for dynamic cellular organization.

The field has come a long way in the past 50 years, but we are just at the start of understanding the intersection of protein phosphorylation and cell biology. It is hard to imagine where we will be at the century of protein kinase-mediated phosphorylation in 2055, but we plan to be around to find out.

Acknowledgements

We are indebted to Edwin Krebs and Edmond Fischer for their historical perspective and personal insights regarding the advent of protein phosphorylation. We thank Tony Hunter for providing valuable information regarding the very early years of protein phosphorylation. We apologize to the many outstanding scientists who have made pioneering contributions, but are not cited here owing to space limitations; additional references to key individuals can be found in Refs [6,13]. Work in the authors' laboratories is supported by the grants from the Canadian Institutes for Health Research to T.P. and the National Institutes of Health to J.D.S. (grant number DK44239).

References

1 Levene, P.A. and Alsberg, C.L. (1906) The cleavage products of vitellin. *J. Biol. Chem.* 2, 127–133
2 Lipmann, F.A. and Levene, P.A. (1932) Serinephosphoric acid obtained on hydrolysis of vitellinic acid. *J. Biol. Chem.* 98, 109–114
3 Burnett, G. and Kennedy, E.P. (1954) The enzymatic phosphorylation of proteins. *J. Biol. Chem.* 211, 969–980
4 Rall, T.W. and Sutherland, E.W. (1958) Formation of a cyclic adenine ribonucleotide by tissue particles. *J. Biol. Chem.* 232, 1065–1076
5 Fischer, E.H. and Krebs, E.G. (1955) Conversion of phosphorylase *b* to phosphorylase *a* in muscle extracts. *J. Biol. Chem.* 216, 121–132
6 Krebs, E.G. (1993) Nobel lecture. Protein phosphorylation and cellular regulation I. *Biosci. Rep.* 13, 127–142
7 Walsh, D.A. *et al*. (1968) An adenosine 3′,5′-monophosphate-dependant protein kinase from rabbit skeletal muscle. *J. Biol. Chem.* 243, 3763–3765
8 Taylor, S.S. *et al*. (1990) cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annu. Rev. Biochem.* 59, 971–1005
9 Barford, D. and Johnson, L.N. (1989) The allosteric transition of glycogen phosphorylase. *Nature* 340, 609–616
10 Gilman, A.G. (1995) Nobel Lecture. G proteins and regulation of adenylyl cyclase. *Biosci. Rep.* 15, 65–97
11 Lefkowitz, R.J. (2004) Historical review: A brief history and personal retrospective of seven-transmembrane receptors. *Trends Pharmacol. Sci.* 25, 413–422
12 Eckhart, W. *et al*. (1979) An activity phosphorylating tyrosine in polyoma T antigen immunoprecipitates. *Cell* 18, 925–933
13 Pawson, T. (2004) Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems. *Cell* 116, 191–203
14 Konopka, J. *et al*. (1984) An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. *Cell* 37, 1035–1042
15 Hubbard, S.R. (1997) Crystal structure of the activated insulin receptor tyrosine kinase in complex with peptide substrate and ATP analog. *EMBO J.* 16, 5572–5581
16 Sadowski, I. *et al*. (1986) A non-catalytic domain conserved among cytoplasmic protein-tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus $P130^{gag\text{-}fps}$. *Mol. Cell. Biol.* 6, 4396–4408
17 Muslin, A.J. *et al*. (1996) Interaction of 14–3-3 with signaling proteins is mediated by the recognition of phosphoserine. *Cell* 84, 889–897
18 Yaffe, M.B. and Elia, A.E. (2001) Phosphoserine/threonine-binding domains. *Curr. Opin. Cell. Biol.* 13, 131–138
19 Frame, S. *et al*. (2001) A common phosphate binding site explains the unique substrate specificity of GSK3 and its interaction by phosphorylation. *Mol. Cell* 7, 1321–1327
20 Wong, W. and Scott, J.D. (2004) AKAP signalling complexes: focal points in space and time. *Nat. Rev. Mol. Cell. Biol.* 5, 959–970
21 Yang, X-J. (2005) Multisite protein modification and intramolecular signaling. *Oncogene* 24, 1653–1662
22 Haglund, K. *et al*. (2003) Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat. Cell. Ciol.* 5, 461–466
23 Anafi, M. *et al*. (1992) Selective interactions of transforming and normal abl proteins with ATP, tyrosine-copolymer substrates, and tyrphostins. *J. Biol. Chem.* 267, 4518–4523
24 Druker, B.J. *et al*. (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N. Engl. J. Med.* 344, 1031–1037
25 Nagar, B. *et al*. (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell* 112, 859–872
26 Burgess, M.R. *et al*. (2005) Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. *Proc. Natl. Acad. Sci. U. S. A.* 102, 3395–4000
27 Kung, C. *et al*. (2005) Chemical genomic profiling to identify intracellular targets of a multiplex kinase inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* 102, 3587–3592
28 Shah, K. *et al*. (1997) Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. *Proc. Natl. Acad. Sci. U. S. A.* 94, 3565–3570

0968-0004/$ - see front matter
doi:10.1016/j.tibs.2005.04.013

RNA silencing

David Baulcombe

The Sainsbury Laboratory, John Innes Centre, Norwich, NR4 7UH, UK

To justify his work on genetic regulation in bacteria, Monod said that 'what is true in *Escherichia coli* is also true for elephants'. In the RNA-regulation field, a modified version of this maxim – 'what is true in petunias is also true for people' – is perhaps more appropriate because discoveries in plants and animals have revealed a novel and common mechanism of RNA-mediated gene silencing.

All RNA-silencing mechanisms involve the cleavage of double-stranded RNA (dsRNA) by an RNase III-like protein, known as Dicer, into 21–28 nucleotide (nt) short RNAs (sRNAs) with 2-nt overhangs at the 3′ ends. The two strands of these sRNAs are then separated, presumably by

Corresponding author: Baulcombe, D. (david.baulcombe@sainsbury-laboratory.ac.uk).
Available online 6 May 2005