A lifelong passion for natural history and an aptitude for chemistry had led me to study for a B.Sc. in biochemistry at University College London (UCL) from 1963 to 1966. It was during this period that the allosteric theory of enzyme regulation was put forward by Jacques Monod, Jean-Pierre Changeux, and Francois Jacob at the Institute Pasteur. This explained how the end products of a biosynthetic pathway exerted feedback control on the enzyme catalyzing the initial and rate-limiting step. Reading these papers sparked an interest in enzyme regulation and led me to become a graduate student in the UCL laboratory of Michael Rosemeyer, with whom I had carried out a short undergraduate project the previous year. Michael had offered a project to study the control of glucose-6-phosphate dehydrogenase, the first enzyme in the pentose phosphate shunt. I purified Glu-6-P dehydrogenase and determined its subunit composition (1, 2) because the "quaternary structure" of an enzyme was thought to be key to how it was regulated by allosteric effectors. The project gave me an excellent grounding in protein chemistry, but Glu-6-P dehydrogenase seemed merely to be regulated by the NADPH/NADP ratio, and it was clear that I needed to find a more challenging problem to work on. The idea of applying to Edmond (Eddy) Fischer for a postdoctoral position arose during a discussion with Prakash Datta, a professor in the department at UCL, and Bill Whelan, the Chairman of Biochemistry at the University of Miami. Bill had just founded FEBS Letters and had come to visit because Prakash had become the first Managing Editor of the journal. I had heard Eddy Fischer give several lectures at a protein chemistry summer school in Venice in 1967, and so Bill, who was a friend of Eddy, agreed to write a letter of support to accompany my application. However, my initial approaches were unsuccessful, and only after I had obtained a UK Science Research Council (subsequently renamed the Biotechnology and Biological Sciences Research Council) postdoctoral fellowship funded by NATO did Eddy agree to let me come. To this day, I therefore always re-evaluate very carefully persistent postdoctoral applicants who will not take no for an answer!

Seattle

In 1955, Eddy Fischer and Edwin (Ed) Krebs had discovered that glycogen phosphorylase was activated by a phosphorylation mechanism and inactivated by dephosphorylation. This was the first example of enzyme regulation by reversible phosphorylation, a finding that was to gain them a Nobel Prize 37 years later (Fig. 1). However, in October 1969, when my wife, Tricia, and I arrived in Seattle (Tricia to carry out postdoctoral research with Arno Motulsky in the Department of Genetics), protein phosphorylation was still considered to be a specialized control mechanism, largely confined to the regulation of glycogen metabolism. Three enzymes involved in this process were known to be regulated by phosphorylation, namely glycogen phosphorylase itself; its activator, phosphorylase kinase; and glycogen synthase. cAMP-dependent protein kinase (PKA) had been identified in 1968 in Ed Krebs’ laboratory and was known to activate phosphorylase kinase and to inhibit glycogen synthase, explaining how adrenalin mobilized glycogen and suppressed its synthesis.

The 2 years working in Eddy Fischer’s laboratory shaped the rest of my career and was a fantastic experience. I was interested in how control by reversible phosphorylation had evolved and
embarked on a study of the regulation of glycogen phosphorylase in a primitive vertebrate (the Pacific dogfish). However, the dogfish enzyme turned out to be disappointingly similar to its mammalian counterpart (in retrospect, not that surprising), and I even found that dogfish phosphorylase kinase could activate mammalian phosphorylase and that mammalian phosphorylase kinase could activate dogfish phosphorylase (3). It was these experiments that started to kindle my interest in phosphorylase kinase.

Eddy’s favorite project at the time was a study of the “glycogen particles” that could be isolated from rabbit muscle with the enzymes involved in glycogen metabolism and their regulation still attached. When glycogen particles were incubated with MgATP, there was a rapid “flash” activation of glycogen phosphorylase, provided that micromolar concentrations of calcium ions were included in the incubations. This was followed by an almost equally rapid dephosphorylation and inactivation once the ATP had been rapidly hydrolyzed by elements of the sarcoplasmic reticulum ATPase that contaminated the preparations. The requirement for calcium was explained when Ludwig Heilmeyer and Richard Haschke, the postdoctoral researchers working on this problem, showed that phosphorylase kinase was reversibly activated by calcium ions. Similar findings were made by Ed Krebs, who had left Seattle in 1968 to become the founding Chairman of the Department of Biological Chemistry at the University of California at Davis. (Ed returned to Seattle in 1978 to become Chairman of Pharmacology.)

Dundee

In October 1971, Tricia and I returned to the United Kingdom to become lecturers (the UK equivalent of assistant professor) in the then embryonic Department of Biochemistry at the University of Dundee. I decided to study phosphorylase kinase initially because its regulation by calcium ions and PKA seemed to be key to understanding how glycogen was mobilized for energy production during muscle contraction and when adrenaline was released. I did not have a research grant (my first application had been rejected), and so I carried out the experimental work myself, the cost of the research consumables being generously provided by Peter Garland, the Chairman of the department, who had hired me. I was therefore the sole author on the first paper I published at Dundee (4), reporting that phosphorylase kinase was composed of three subunits, termed α, β, and γ, and that the α- and β-subunits were phosphorylated by PKA.

In 1978, Tom Vanaman paid a visit to Dundee and presented a seminar on calmodulin, a calcium-binding protein originally identified by Shiro Kakiuchi as an activator of a cAMP phosphodiesterase isofrom. Tom mentioned that calmodulin had recently been found to be the calcium-dependent activator of myosin light chain kinase by both Dave Hartshorne and Koichi Yagi. About halfway through the seminar, I suddenly realized that, being very small, calmodulin would have run off the end of the 5% SDS-polyacrylamide gels I had been using to resolve the very large (>100 kDa) α- and β-subunits! A few simple experiments soon proved that this was indeed the case and revealed that calmodulin was the fourth component (the δ-subunit) of phosphorylase kinase (5). This explained how phosphorylase kinase was activated by calcium ions and is a good illustration of how easy it can be to miss an important result!
From the Multisite Phosphorylation of Phosphorylase Kinase to the Characterization of Protein Phosphatases

The finding that PKA phosphorylated two serine residues on phosphorylase kinase, one on the α-subunit and one on the β-subunit, intrigued me, as glycogen phosphorylase was phosphorylated only at a single site. I found that the reversible phosphorylation of the β-subunit correlated with changes in phosphorylase kinase activity (4, 6), which raised the question of what the role of phosphorylation of the α-subunit might be and which phosphatase(s) dephosphorylated the two sites. John Antoniw, my first graduate student, was assigned to the latter project, which led him to separate two phosphatase activities that dephosphorylated either the α- or β-subunit relatively selectively (7). We then found that the phosphatase specific for the β-subunit copurified with the major phosphatase acting on glycogen phosphorylase and glycogen synthase (8) and later called this enzyme protein phosphatase 1 (PP1) (9). We also found that PP1 was the phosphatase that was potently inhibited by two small thermostable proteins, termed inhibitor-1 (I-1) and inhibitor-2 (I-2) (10, 11), that had been initially described by Walter Glinsmann (12). In contrast, the phosphatase specific for the α-subunit of phosphorylase kinase had little activity toward glycogen phosphorylase and glycogen synthase and was insensitive to I-1 and I-2 (10, 11). We therefore termed it PP2 (9) but later changed the name to PP2B when it became clear that tissue extracts contained three types of protein phosphatase that dephosphorylated the α-subunit of phosphorylase kinase preferentially and were insensitive to I-1 and I-2 (10, 11). By the early 1980s, it had become obvious that the regulation of protein function by reversible phosphorylation was not confined to glycogen metabolism. Tom Ingebritsen (a postdoctoral researcher) and Gordon Foulkes (a graduate student) found that PP2B was a calcium-dependent phosphatase (20, 21). Another problem was our use of 32P-labeled phosphorylase kinase as its substrate, because PP2B was being activated by the calmodulin attached to the substrate! It was only when we identified calmodulin as a subunit of phosphorylase kinase and found alternative substrates for PP2B that the requirement for calmodulin became apparent. Lex Stewart, the graduate student working on this problem, purified PP2B to homogeneity, which revealed that, surprisingly, it had already been isolated by others as an abundant calmodulin-binding protein in the brain of previously unknown function and termed calcineurin by Claude Klee (20). Many years later, Stuart Schreiber identified PP2B/calcineurin as the cellular target of cyclosporin (22), the immunosuppressant drug that has permitted the widespread use of organ transplantation. We now know that PP2B stimulates T-cell proliferation by dephosphorylating members of the NFAT (nuclear factor for activated T-cells) family. This enables these transcription fac-
tors to enter the nucleus and stimulate the production of interleukin-2.

**Regulation of Protein Phosphatases by Targeting Subunits**

In 1985, we finally managed to isolate the form of PP1 associated with glycogen particles in muscle (termed PP1G), the enzyme that Eddy Fischer’s lab had found so difficult to purify many years earlier. Peter Stralfors, the postdoctoral researcher who cracked this problem, discovered that PP1G was a heterodimer composed of the PP1 catalytic subunit complexed with a glycogen-binding (G) subunit (23). (The extreme susceptibility of the G-subunit to proteolysis may explain the earlier difficulties in purifying PP1G.) Later, we found that the G-component enhanced the PP1-catalyzed dephosphorylation of glycogen-bound substrates (24) and that phosphorylation of the G-component by PKA triggered its dissociation from PP1. This releases PP1 from glycogen particles, preventing the phosphatase from dephosphorylating glycogen-bound substrates (25, 26), and provides an additional way in which adrenalin activates glycogen phosphorylase and inhibits glycogen synthase.

The major cytosolic form of PP1 turned out to be a complex between the PP1 catalytic subunit and I-2 (27), and we later discovered a third form of PP1 in muscle associated with myofibrils (28, 29). This species comprised PP1 complexed with a myofibrillar targeting M-subunit, which enhanced the dephosphorylation of myosin. These studies indicated that the subcellular location, substrate specificity, and regulation of PP1 were determined not by the PP1 catalytic subunit itself but by its interaction with different “targeting” subunits (30). Subsequently, a huge number of additional PP1-targeting subunits were identified, many interacting with PP1 through an (Arg/Lys)-(Val/Ile)-Xaa-(Phe/Trp) motif (where Xaa is any amino acid) (31). The control of PP1 by targeting subunits was the first example of what came to be recognized as a general feature of cell regulation. We now know that many protein kinases and other enzymes are also directed to the correct subcellular locations and to their substrates by analogous targeting devices (30).

**From the Multisite Phosphorylation of Glycogen Synthase to an Understanding of How It Is Activated by Insulin**

In the 1960s, Joe Larner reported that two forms of glycogen synthase were present in muscle, a D-form that was dependent on the allosteric activator glucose 6-phosphate for activity and an I-form whose activity was independent of glucose 6-phosphate. Joe had also shown that the proportion of glycogen synthase present as the I-form was increased within minutes of stimulating muscle with insulin. Joe and Ed Krebs both had found that PKA converted the I-form to the D-form *in vitro*, suggesting that insulin might promote the dephosphorylation and activation of glycogen synthase by inhibiting PKA. However, exposure to insulin did not alter the concentration of cAMP in muscle, and so Joe had suggested that insulin might induce the formation of a novel “second messenger” that bound to PKA and prevented its activation by cAMP.

In 1973, I finally managed to obtain my first research grant from the British Diabetic Association (later renamed Diabetes UK) to study this problem, which enabled me to recruit a postdoctoral researcher. Hugh Nimmo, who was appointed to this position, soon showed that glycogen synthase was phosphorylated by at least two protein kinases that he detected as trace contaminants in the purified enzyme (32). One was PKA, but the other was not activated by PKA and was insensitive to a small thermosable protein inhibitor of PKA, termed PKI, identified in Ed Krebs’ lab in the 1960s. We therefore called it glycogen synthase kinase 2 (GSK2) to distinguish it from PKA (GSK1). Excitingly, most of the phosphate incorporated into glycogen synthase by PKA could be released by trypsin as trichloroacetic acid-soluble phosphopeptide(s), but the phosphate incorporated by GSK2 could not. This implied that GSK2 phosphorylated glycogen synthase at a site(s) distinct from that (those) targeted by PKA. In the article reporting these findings, we suggested that insulin might activate glycogen synthase by modulating the activity of GSK2 rather than PKA (32).

The more we studied glycogen synthase, the more complex its regulation seemed to become. (Glycogen synthase eventually turned out to be phosphorylated at nine serine residues by at least six protein kinases.) Noor Embi, a graduate student, identified an additional protein kinase that inhibited glycogen synthase, which we therefore called GSK3 (33). Dennis Rylatt, a postdoctoral researcher, showed that GSK3 phosphorylated serine residues distinct from those targeted by PKA and GSK2 (34), which paved the way for Peter Parker, another postdoctoral researcher, to show that the activation of glycogen synthase by insulin resulted from dephosphorylation of the sites targeted by GSK3 (35). This indicated that insulin must exert its effects by inhibiting GSK3 and/or by activating PP1G, the major glycogen synthase phosphatase.

We wondered how insulin might inhibit GSK3. In 1982, Ron Kahn had shown that the insulin receptor was a protein-tyrosine kinase and, soon after, that it phosphorylated...
IRS1 (insulin receptor substrate 1). By this time, highly purified preparations of GSK3 had been isolated by Brian Hemmings, a postdoctoral researcher, and by Jim Woodgett, a graduate student, so Jim and I took the purified kinase to Joe Avruch at Massachusetts General Hospital to see if Joe’s purified insulin receptor phosphorylated and inactivated GSK3, which it did not. Jim also injected insulin into rabbits to see if the activity of GSK3 decreased. However, no immunoprecipitating antibodies were available, and the synthetic peptide substrate that revolutionized the assay of GSK3 was introduced by Peter Roach some years later. Jim therefore had to take GSK3 through several steps of purification before its activity could be measured, but even then, the assay was not that reliable because the glycogen synthase substrate was contaminated with other glycogen synthase kinases that caused a high “blank” in the control assays. These technical difficulties probably explain Jim’s failure to detect a decrease in the activity of GSK3 at that time.

Jim Woodgett eventually discovered that GSK3 was inhibited by insulin nearly 10 years later in a collaboration with Bill Benjamin. Bill had found that insulin induced the dephosphorylation of a particular threonine residue in ATP-citrate lyase. He had partially purified a protein kinase that phosphorylated this site and noticed that its activity declined by 50% within 5 min of exposing 3T3L1 adipocytes to insulin. By this time, Jim had set up his own laboratory in London, defined the two isoforms of GSK3 (GSK3α and GSK3β), and developed antibodies that recognized them specifically. This enabled Jim and Bill to rapidly establish that Bill’s insulin-inhibited ATP-citrate lyase kinase was GSK3α (36). Independently, Chris Proud, another former graduate student, had identified an insulin-inhibited protein kinase that phosphorylated the protein synthesis initiation factor eIF2B and identified this enzyme as GSK3 (37). Thus, by 1993, it had become clear that GSK3 was inhibited acutely by insulin.

The Class 1 phosphatidylinositol (PI) 3-kinases, as they later came to be called, had been identified by Lew Cantley in the late 1980s through their association with several protein-tyrosine kinases. Later, they were shown to bind to the tyrosine-phosphorylated form of IRS1, and it became clear that their true physiological substrate was not PI but the minor inositol phospholipid PI(4,5)P2 (38). This implied that the function of Class 1 PI 3-kinases was to produce PI(3,4,5)P3. Their crucial role in insulin signal transduction became clear when the PI 3-kinase inhibitor, wortmannin, was identified (39) and shown to suppress many of the metabolic actions of insulin. These findings implied that PI(3,4,5)P3 was the long sought after second messenger for insulin, but how it exerted its effects was still a mystery.

A breakthrough came when Darren Cross, a graduate student in my lab, found that the insulin-induced inhibition of GSK3 was prevented by wortmannin and reversed by treatment with a serine/threonine-specific protein phosphatase (40). Taken together, these experiments implied that GSK3 was inhibited by the phosphorylation of a serine or threonine residue(s) and that this was catalyzed by a protein kinase(s) activated “downstream” of PI(3,4,5)P3. Calum Sutherland, another graduate student, made the important finding that GSK3 could be inactivated in vitro by two different insulin-stimulated protein kinases, namely p90 RSK2 (ribosomal S6 kinase 2) and p70 S6K (ribosomal S6 kinase), which both phosphorylated a particular serine residue in each GSK3 isoform (41, 42). This raised the issue of whether it was an RSK or S6K isoform that mediated the insulin-stimulated, wortmannin-sensitive inhibition of GSK3. Fortunately, pharmacological inhibitors capable of addressing this question had recently become available. The signaling pathway leading to the activation of S6K could be prevented by the immunosuppressant drug rapamycin, an inhibitor of the protein kinase mTOR, whereas the pathway leading to the activation of RSK2 was blocked by PD 98059. The solution as to how insulin inactivated GSK3 now seemed to be within our grasp. It therefore came as a huge shock when we found that neither rapamycin nor PD 98059, either alone or in combination, had any effect on the insulin-stimulated inhibition of GSK3 (43). This meant that yet another insulin-stimulated, wortmannin-sensitive protein kinase that inhibited GSK3 must exist in cells.

To identify the elusive protein kinase, Darren Cross incubated a myoblast cell line with rapamycin and PD 98059 to prevent the activation of RSK and S6K and then stimulated the cells with insulin. Using this trick, he detected and partially purified an additional insulin-stimulated, wortmannin-sensitive protein kinase that inhibited GSK3 (43). At this juncture, Brian Hemmings arrived in Dundee to examine one of Tricia’s graduate students. Darren and I showed him the latest data and asked if he had any thoughts as to what our new insulin-stimulated protein kinase might be. Brian suggested protein kinase B (PKB; also called Akt) because he had heard “on the grapevine” that two papers would soon appear showing that this protein kinase was activated by a PI 3-kinase-dependent pathway (44, 45). As luck would have it, Brian had been the first to clone PKB/Akt a few years earlier and had raised an immunoprecipitating antibody. Using this antibody, Dar-
ren was able to quickly establish that his insulin-stimulated protein kinase was indeed PKB/Akt (43). Dario Alessi, a postdoctoral researcher in my lab, found that the insulin-induced activation of PKB/Akt resulted from its phosphorylation at Thr308 and Ser473 (46). In Brian Hemmings’ laboratory, Mirjana Andjelkovich mutated these residues to Ala, and studies with these constructs revealed that the activation of PKB/Akt was largely determined by the extent of phosphorylation of Thr308, with the phosphorylation of Ser473 playing a lesser role under our assay conditions. Dario then went on to identify and purify the protein kinase that activated PKB/Akt by phosphorylating Thr308. To our delight, he found that it activated PKB/Akt only in the presence of lipid vesicles containing PI(3,4,5)P3 (47), and so we called it 3-phosphoinositide-dependent protein kinase 1 (PDK1). Dario cloned PDK1 (48), revealing that, like PKB/Akt, it contained a PI(3,4,5)P3-binding pleckstrin homology (PH) domain. The binding of PI(3,4,5)P3 to the PH domains of PDK1 and PKB/Akt is thought to co-localize these protein kinases at the plasma membrane, allowing PDK1 to activate PKB/Akt, and also to induce a conformational change that allows PKB to be phosphorylated by PDK1. The protein kinase that phosphorylates Ser473 was later identified by David Sabatini as TORC2, a rapamycin-insensitive form of mTOR (49).

PDK1 was the missing link in the chain of events by which insulin stimulates glycogen synthase, and its identification allowed a complete signaling pathway from the insulin receptor to the activation of glycogen synthase to be formulated for the first time (Fig. 3). The genetic evidence that validated this pathway was obtained several years later. In particular, Ed McManus, a graduate student in Dario Alessi’s lab, created “knock-in” mice in which GSK3α and GSK3β were replaced by mutants in which the sites phosphorylated by PKB/Akt were mutated to Ala. He showed that insulin no longer stimulated the activation of glycogen synthase in the skeletal muscle of these mice, establishing that the proposed pathway was correct (50).

The serine residues in GSK3 phosphorylated by PKB/Akt lie in Arg-Xaa-Arg-Xaa-Xaa-(Ser/Thr) motifs. We found that small synthetic peptides containing this motif were excellent substrates for PKB/Akt but that phosphorylation was abolished by the mutation of either Arg to Lys (51). This finding facilitated the identification of many other physiological substrates for PKB/Akt, and it soon became clear that the PDK1-PKB/Akt signaling pathway mediates many of the actions of insulin, not just the stimulation of glycogen synthesis.

Classical Mitogen-activated Protein Kinase (MAPK) Cascade and the First Small Molecule Inhibitor of This Pathway

Prior to the discovery that insulin inhibits GSK3, we had been investigating the possibility that insulin stimulated glycogen synthase by activating PP1G, the major glycogen synthase phosphatase in muscle. Consistent with this possibility, Paul Dent, a graduate student, had partially purified an insulin-stimulated protein kinase (ISPK) from muscle extracts that activated PP1G in vitro (52). Paul found that, like p90 S6K of Xenopus oocytes (53), the ISPK was activated by MAP2 (microtubule-associated protein 2) kinase, an insulin-stimulated protein kinase identified by Tom Sturgill a few years earlier (54). Later work revealed that ISPK was RSK2 (55), a mammalian isoform of Xenopus p90 S6K. MAP2 kinase was later renamed MAPK, and the two mammalian isoforms are now called ERK1 and ERK2 (extracellular signal-regulated kinases 1 and 2) following the cloning of their DNA (56).

Tom Sturgill had made the surprising observation that the activation of pp42 MAPK (ERK2) required its phosphorylation at both a threonine and tyrosine residue (57). It was therefore assumed that two different protein kinases, one phosphorylating the threonine and the other the tyrosine residue, would be needed to activate ERK1 and ERK2. However, we found that this was not the case and that, surprisingly, a single MAPK kinase (MKK1) with "dual specificity" phosphorylated both sites (58). Chris Marshall, Joe Avruch, and Tom Sturgill then went on to identify Ras and Raf as key "upstream" elements of the pathway (59 – 62). The “Ras-Raf-MAPK pathway” (or the “classical” MAPK cascade as it came to be called) was also dissected by geneticists studying eye development in the...
fruit fly *Drosophila* and vulval development in the nematode *Caenorhabditis elegans*.

Unfortunately, it soon became clear that our efforts to dissect the classical MAPK cascade had been misdirected, at least from the standpoint of understanding insulin signal transduction. John Lawrence showed that, unlike insulin, epidermal growth factor did not activate glycogen synthase in rat adipocytes, despite being a much stronger activator of the classical MAPK cascade (63). This meant that the RSK2-catalyzed activation of PP1G that we had observed in vitro was most unlikely to underlie the activation of glycogen synthase by insulin. Moreover, as discussed above, we later found that PD 98059, a small molecule inhibitor of the classical MAPK cascade, did not block the insulin-induced inhibition of GSK3.

PD 98059 was identified by Alan Saltiel and colleagues at Parke-Davis as a compound that inhibited MKK1(EE), a form of MKK1 in which the serine residues phosphorylated by Raf had been mutated to Glu to generate a weakly active enzyme. It therefore came as a rather surprise when Dario Alessi found that PD 98059 did not inhibit the normal form of MKK1 that had been activated by phosphorylation and that PD 98059 suppressed activation of the classical MAPK cascade in cells by preventing the activation of MKK1 by Raf (64). This was the first example of a compound that is not a protein kinase inhibitor but prevents the activation of one protein kinase by another.

**p38 MAPK-MAPKAP-K2 Cascade and the First Small Molecule Inhibitor of This Pathway**

In 1992, David Stokoe, a graduate student, detected a new protein kinase activity that, like RSK2, could be inactivated by dephosphorylation and reactivated by ERK2 in vitro. However, its substrate preference differed from RSK2, and unlike RSK2, it was not inhibited by the protein kinase inhibitor H7. We therefore termed it MAPK-activated protein kinase 2 (MAPKAP-K2) to distinguish it from RSK2 (MAPKAP-K1) (65).

We expected that MAPKAP-K2 would be activated by ERK1 and ERK2 in cells, but John Rouse, a graduate student, found that instead it became activated if cells were exposed to heat shock or other cell-damaging agents. He went on to show that these stimuli activate a novel MAPK family member, which then activates MAPKAP-K2. A collaboration with Angel Nebreda in Tim Hunt’s lab soon revealed the activator to be the mammalian homolog of a *Xenopus* MAPK (XMpk2) that Angel had recently identified and cloned (66). The same MAPK was identified independently by Jeremy Saklatvala as an interleukin-1-activated enzyme and by Richard Ulevitch as a 38-kDa protein that became tyrosine-phosphorylated when monocytes were stimulated with bacterial lipopolysaccharide. For this reason, it is called p38α MAPK today.

At this juncture, George Poste, the President of Research and Development at SmithKline Beecham, invited me to join the company's Discovery Advisory Board. This was most unexpected because, despite working in the field of protein phosphorylation for 25 years, the pharmaceutical industry had never before shown the slightest interest in my work. At the first board meeting I attended in Philadelphia in early 1994, SmithKline Beecham reviewed its program to treat rheumatoid arthritis, an area of biology that was not remotely on my radar at the time. John Lee described the work that he had been carrying out to identify the cellular target of cytokine synthesis anti-inflammatory drugs (CSAIDs), a class of pyridyl imidazoles that suppressed the production of tumor necrosis factor in lipopolysaccharide-stimulated monocytes. To my astonishment, I learned that John had just identified p38α MAPK as a specific binding protein of these drugs. This coincidence proved to be of mutual benefit. On the one hand, we had the substrate (MAPKAP-K2) that John needed to assay p38α MAPK and an upstream activator of p38α MAPK (later called MAPK kinase 6) required to switch on the inactive p38α MAPK that John had expressed in *Escherichia coli*. On the other hand, John provided me with one of his compounds, SB 203580, which Ana Cuenda, a postdoctoral researcher in my lab, then showed was a potent and relatively specific inhibitor of p38α MAPK in vitro. We also exploited SB 203580 to establish that MAPKAP-K2 was a physiological substrate of p38α MAPK and that heat shock protein 27 (HSP 27) was a physiological substrate for MAPKAP-K2 (67).

**Division of Signal Transduction Therapy**

The compounds PD 98059 and SB 203580 were two of the first relatively selective cell-permeable inhibitors of serine/threonine-specific protein kinases to be developed. The articles that we published with Parke-Davis (64) and SmithKline Beecham (67) have been very highly cited because so many labs later used these compounds to identify novel substrates and physiological roles of the MKK1-ERK1/2 and p38α MAPK-MAPKAP-K2 pathways. It was obvious that small cell-permeable inhibitors of other protein kinases were going to equally valuable and that the only way that I was going to obtain many more was via a collaboration with the pharmaceutical industry. In 1996, my colleague Peter Downes and I therefore started to try to persuade a number of pharmaceutical companies to support a Division of Signal Transduction Therapy (DSTT) at Dundee, in which we would help them to accelerate the development of kinase inhibitors with therapeutic poten-
tial. However, this proved to be far more difficult than we had anticipated because kinase drug discovery was still in its infancy. Many companies we talked to were far from convinced that protein kinases were good drug targets, and the research director of one company (which no longer exists) told us that there was absolutely no future in this area! Persuading the lawyers of five companies to sign a single agreement also turned out to be rather challenging. However, the collaboration on “mammalian kinases and phosphatases” was eventually launched in July 1998 with Astra, NovoNordisk, Pfizer, SmithKline Beecham, and Zeneca as the founding members, later joined by Boehringer Ingelheim.

The way that the DSTT works is relatively simple. The participating companies share the unpublished results, reagents, technology, and “know-how” of the participating laboratories and the first right to license the Intellectual Property that they generate. However, the information that we obtain with any reagent or technology that has been introduced to us by one company (typically a kinase inhibitor) is not shared and is relayed back only to the company that provided the compound. About 60% of the funding that we receive is used to support fundamental research projects chosen by the Dundee-based research teams. The remainder supports a service facility, comprising teams for DNA cloning, protein and antibody production, and assay development; a mass spectrometry facility; and a “kinase profiling service”. (Since July 2008, the kinase profiling service has been supported by the UK Medical Research Council (MRC) and not the companies supporting the DSTT.)

The kinase profiling service originally set up to help the companies assess the specificities of the compounds that they were generating has proved very helpful for our own research. We routinely test the specificities of kinase inhibitors that are available commercially or that we synthesize from patents to decide whether they are sufficiently specific to be worth using in cell-based experiments (the great majority are not!). I thought that this information might be useful to the cell signaling community but was taken aback by the deluge of E-mails that followed the publication of our first “kinase profiling” paper (68) and that far exceeded the reaction to any other paper I have published. The first kinase profiling paper has so far been cited over 2200 times, and I understand that it was downloaded 7600 times from the Biochemical Journal Web site in 2004 alone. This is a reflection of the huge need for pharmacological inhibitors of protein kinases and the impact that they are now having on the study of cell signaling.

The success of the DSTT led to renewal for an additional 5 years in 2003 at a greatly expanded level, this time supported by AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Merck & Co., Merck KGaA, and Pfizer. It was renewed again in July 2008 for another 4 years. One reason why the DSTT continues after so many years is that the 14 participating research groups and their 200 scientific support staff members provide a critical mass of expertise that enables the companies to obtain much of what they need in the kinase/phosphatase arena by interacting with a single center.

The DSTT is an interesting alternative to setting up a new biotechnology company and has taught me the huge benefits that major industrial funding can bring to basic research. One obvious difference between the DSTT and a biotechnology company is that the income and royalties do not go to investors or shareholders but are entirely plowed back to support fundamental research. Over the past 11 years, the DSTT has brought in over £40 million ($65 million) of research funding to the MRC Protein Phosphorylation Unit and College of Life Sciences at Dundee and enabled expansion of our research programs and infrastructure to a level that would have been impossible had we simply relied on funding from government and medical charities. The DSTT has become a model for how academia and industry can work together (Fig. 4), and several articles have been written about it (e.g. ESRC Society Today Web site).

Interplay between Protein Phosphorylation and Protein Ubiquitylation in Regulating the Innate Immune System: Formation of a Protein Ubiquitylation Unit

For me, the beauty of protein phosphorylation is that it is a general control mechanism that regulates almost all
aspects of cell life. This not only allows one to keep a broad perspective in this age of increasing specialization but also means that the same technology can be used to attack a wide range of biological problems. Therefore, once the insulin signal transduction pathway downstream of PI 3-kinase had become clear, at least in outline, I started to look for another problem to work on that was still largely a “black box.”

Six years ago, Peter Cheung, a postdoctoral researcher, identified a feedback control loop in which p38 MAPK suppressed the activity of TAK1 (69), a protein kinase thought to play a central role in controlling the production of inflammatory mediators by the innate immune system. Innate immunity is vital for defense against infection but can be a “double-edged sword” because the overproduction of inflammatory mediators is a cause of chronic inflammatory and autoimmune diseases and sepsis. This was the system that I had first become aware of at the augural meeting of the SmithKline Beecham Discovery Advisory Board in 1994. As I dipped deeper into the background literature, I realized that there were major gaps in our understanding of how the production of inflammatory mediators was controlled and that this was a fascinating system in which complex signaling networks cooperate to combat infection by pathogens. I gradually started to switch my lab over to the study of this problem, a process that was essentially completed a couple of years ago. I am greatly enjoying working in this new field, although coming to grips with the huge literature on innate immunity has been challenging!

The innate immune system is controlled by a remarkable interplay between protein phosphorylation and protein ubiquitylation, and much of my current research is focused on this topic (e.g. Refs. 70–72). In this system, protein kinases switch on E3 ubiquitin ligases, leading to the formation of Lys63-linked polyubiquitylated proteins that act as scaffolds for the assembly and activation of additional protein kinases and other signaling molecules. Understanding how phosphorylation and ubiquitylation “talk” to each other is going to be critical for our comprehension of many biological systems, not just innate immunity. I have therefore recently set up the Protein Ubiquitylation Unit (PUU) with funding from the Scottish Government (see the Scottish Institute for Cell Signaling Web site). The PUU is located next to the MRC Protein Phosphorylation Unit to enable the two units to interact closely. By building up a critical mass of researchers working on the ubiquitin system, I hope that the PUU will be able to capture benefits similar to those that have helped to underpin the research of the Protein Phosphorylation Unit.

The analogies between protein ubiquitylation and protein phosphorylation are striking, although polyubiquitylation adds an extra dimension to control by ubiquitylation. Like phosphorylation, ubiquitylation can occur at a single site or at multiple sites on a protein, and comparable numbers of proteins control these two processes (around 10 E1 enzymes, 40–50 E2 conjugating enzymes, and nearly 600 E3 ligases compared with 500 plus protein kinases; nearly 100 protein deubiquitylases compared with 140 protein phosphatases). The development of drugs that target components of the ubiquitin system is still in its infancy compared with protein kinases, but interest is growing and, when the times comes, the PUU will be in a strong position to play its part in helping to accelerate drug development in this area.

Reflections

Many scientists’ careers are determined by where they carry out postdoctoral research, and mine is no exception. I was exceptionally fortunate to work with Eddy Fischer and would probably never have entered the field of protein phosphorylation had it not been for this experience. After setting up on my own lab, it was simple curiosity about why phosphorylase kinase and glycogen synthase were phosphorylated at more than one site that drove my research, with these findings developing into 25-year programs to characterize the protein phosphatases involved in cell regulation and to understand insulin action. I was also lucky that protein phosphorylation turned out to be a control system of such general significance that it came to be of huge interest to the pharmaceutical industry. The past 15 years of my research career would have been entirely different had it not been for the convergence of two quite separate events: first, the invitation from George Poste to join the Discovery Advisory Board of SmithKline Beecham, and second, my mistaken idea that the classical MAPK cascade mediated the stimulation of glycogen synthase by insulin. It was by studying this pathway that I stumbled across p38α MAPK, the target for SmithKline Beecham’s anti-inflammatory drug. The ensuing collaboration alerted me to the power of protein kinase inhibitors for the study of cell signaling; led to a long-term collaboration with the pharmaceutical industry; and introduced me to signaling in the innate immune system, the area that later became the main focus of my laboratory. As Avram Hershko mentioned in a recent article in this Reflections series, “accidental observations may be the most important ones. Grab your luck!” (73).
I was only 26 when I started to run my own lab in Dundee, and in retrospect, I think that starting so early has been an enormous advantage. These days, with Ph.D. programs taking longer and two periods of postdoctoral training becoming the norm, scientists’ independent careers are starting later and later. I was recently told that the average age at which United States scientists receive their first research grant has risen to over 40. In my opinion, this is surely far too late. Scientists have to get going on their own much earlier, when imagination and energy levels are at their highest and when there is plenty of time to make mistakes and learn from them.

I also think that it is important for younger scientists to continue to work “at the bench” for as long as possible after setting up their own labs and not to be in too much of a hurry to build a “team.” Young independent scientists who know exactly what they want to do can achieve far more at the bench than a handful of students and postdoctoral researchers with little or no experience in the field. I was the sole author on the first major paper that I published as an independent investigator (74) and was still publishing occasional first authored papers 15 years later. My former student John Rouse later followed in my footsteps after rejoining the MRC Protein Phosphorylation Unit at Dundee as a Programme Leader. Single authored papers make a big impact because they are so rare, and I suspect that they were a significant factor in John and I being awarded the Colworth Medal of the British Biochemical Society 30 years apart. (The Colworth Medal is awarded annually by the Biochemical Society for outstanding research carried out in the United Kingdom by a biochemist not more than 35 years of age.) A single authored paper leaves little doubt about who conceived and carried out the experimental work and who wrote the manuscript!

I would also urge young scientists starting their own independent careers to pay less attention to where their papers are published. What really matters is whether a paper is widely read and cited, and being the first to publish is far more important than where a paper appears. As Alan Fersht said in a recent commentary on impact factors, “even the best journals publish papers that are never cited plus some fraudulent papers and excruciatingly bad ones. So, it is ludicrous to judge an individual paper solely on the IF of the journal in which it is published” (74). A few years ago, I was asked by The Scientist (75) to choose my five favorite papers from the scientific literature. None were published in a journal with a particularly high “impact factor,” yet three led to the award of a Nobel Prize. I have never considered the impact factor of a journal when deciding where to publish, and yet according to the Institute for Scientific Information, I was the world’s second most cited scientist in the field of Biology and Biochemistry from 1992 to 2002. Today, everyone finds the papers they want to read by searching the Internet. Where a paper is published will become increasingly irrelevant, and impact factors will be superseded by more reliable indicators of scientific worth, such as the “Eigenfactor” (74).

I also feel that I was lucky in naively deciding in 1971 to join a tiny and then unheralded Department of Biochemistry at Dundee. Although times were tough in the 1970s and the department’s financial position extremely precarious, the opportunity to contribute to the development of life sciences in Dundee has been immensely rewarding. Dundee is now recognized as one of Europe’s leading centers for the life sciences, and since 1971, the number of people employed in the life sciences and biotechnology sectors has risen from 40 to over 4000 and accounts for some 16% of the local economy. (Dundee is Scotland’s fourth largest city with a population of 140,000.)

Finally, if you have been puzzled by the title of this article, I should mention that it stems from a conversation I had with Ed Krebs at a Gordon Conference in New Hampshire many years ago, when Ed said to me, “You know Phil, you’ve just got to keep nibbling at the edges.” In other words, most of the research that we carry out is all about making small incremental advances until eventually we get to the heart of the problem. These hard won advances require persistence and perseverance but are well worth all the effort when they finally lead to significant new insights.

Acknowledgments—My work is currently supported by the UK MRC and the Royal Society and by the companies that support the DSTT (AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Merck-Serono, and Pfizer). I thank the 44 graduate students and 61 postdoctoral researchers who have worked with me over the years and without whose efforts this article could not have been written.

Address correspondence to: p.cohen@dundee.ac.uk.

REFERENCES
1. Cohen, P., and Rosemeyer, M. A. (1969) Eur. J. Biochem. 8, 1–7
2. Cohen, P., and Rosemeyer, M. A. (1969) Eur. J. Biochem. 8, 8–15
3. Cohen, P., Duewer, T., and Fischer, E. H. (1971) Biochemistry 10, 2683–2694
4. Cohen, P. (1973) Eur. J. Biochem. 34, 1–14
5. Cohen, P., Burchell, A., Foulkes, J. G., Cohen, P. T. W., Vanaman, T. C., and Nairn, A. C. (1978) FEBS Lett. 92, 287–293
6. Cohen, P., and Antoniw, J. F. (1975) FEBS Lett. 34, 43–47
7. Antoniw, J. F., and Cohen, P. (1976) Eur. J. Biochem. 68, 45–54
8. Antoniw, J. F., Nimmoh, H. G., Yeaman, S. J., and Cohen, P. (1977) Biochem. J. 162, 423–433
9. Cohen, P. (1978) Current Top. Cell. Regul. 14, 117–196
10. Cohen, P., Nimmoh, G. A., and Antoniw, J. F. (1977) Biochem. J. 162, 435–444
11. Nimmoh, G. A., and Cohen, P. (1978) Eur. J. Biochem. 87, 335–365
12. Huang, F. L., and Glinsmann, W. H. (1976) Eur. J. Biochem. 70, 419–426
13. Ingebritsens, T. S., and Cohen, P. (1983) Eur. J. Biochem. 132, 255–261
14. Ingebritsens, T. S., Foulkes, J. G., and Cohen, P. (1983) Eur. J. Biochem. 132,
