Signalling: the remarkable dynasties

The list of *Biochemical Journal* papers selected by the Editorial Board1–12 for the ‘classic’ status in signalling (plus a couple of directly relevant classics from the metabolism/enzymes section that I have included) consists almost entirely of papers with inositol or inosinate in the title. This reflects a truly remarkable relationship between inositides, UK scientists and the *Biochemical Journal*.

The roots of this relationship go back to the 1950s, and could be argued to stem originally from two people. One was the Birmingham biochemist George Hübscher, who, probably as a result of the intense interest in carbohydrate chemistry in the next-door Chemistry Department (the home of Howarth, of ‘Howarth projection’ and vitamin C structure fame), developed an interest in inositol lipids. He fostered this interest through his PhD student, J.N. (Tim) Hawthorne, who, in turn, initiated his own PhD student, Bob Michell, into the wonders of inositol lipids. Bob, of course, spawned a whole army of students and post-docs with a similar inclination, such as Pete Downes, Shamshad Cockcroft, Steve Shears, Phill Hawkins and (indirectly) Len Stephens. The other source was Rex Dawson in the Babraham Institute, Cambridge, whose ‘offspring’ include the author of this article, who in turn has had the pleasure of accommodating such inositide aficionados as Len Stephens (again!), Nal Divecha, Pete Cullen and Kath Hinchcliffe.

The first group of *Biochemical Journal* papers, from the 1950s to 1960s1–3, reflects some of the seminal contributions of the early members of this remarkable dynasty. By 1955 it was known, from the work of Lowell and Mabel Hokin in the USA13, that stimulation of some tissue preparations with agonists led to a specific increase in 32P-labelling of phosphoinositides and phosphatidic acid. Interestingly, the discovery of this specificity was enabled by the pioneering lipid separation technique published in 1954 by Rex Dawson14, who, unusually for such a *Biochemical Journal* stalwart, published this particular seminal work in *Biochimica et Biophysica Acta*! (A more refined version15 later became a *Biochemical Journal* mini-classic.) Although it was much later that it emerged that this ‘PI phenomenon’ might involve a PLC (phospholipase C) came, in part, from the discovery by several groups of just such a PtdIns PLC activity. The classic paper of Kemp et al.1 was one of the first (jointly with Dawson18) to partially purify and unambiguously characterize a PtdIns PLC.

As depicted in Figure 1 (the modern version of this story), the primary substrate for this enzyme in vivo is PtdIns(4,5)P$_2$, and the other two classic papers of the 1960s2,3 focused on this aspect of inositide biochemistry, which at that time was considered to be largely separate. Jordi Folch-Pi had, in the 1940s, discovered a fraction of brain inositol lipid that contained more than one phosphate20. Because his preparation consisted (as we now know) of more-or-less-equal amounts of PtdIns, PtdIns4P and PtdIns(4,5)P$_2$, it contained about twice as much phosphate as inositol, so he called it DPI (diphosphoinositide). The second 1961 *Biochemical Journal* classic2 stemmed from the attempts in Rex Dawson’s laboratory to find out more about this DPI. He and John Dittmer were able to purify from brain tissue, and characterize an inositol lipid with a phosphate/inositol ratio of 3:1, and so they called it TPI (triphosphoinositide). The determination of the distribution of phosphates around the inositol ring needed a different approach, and by a remarkable coincidence, at exactly the same time Clinton Ballou and his colleagues hydrolysed Folch’s DPI and obtained inositol phosphates, which they analysed by classic carbohydrate chemistry. From these analyses, they deduced that ‘DPI’ must actually be a mix of PtdIns, PtdIns4P, and PtdIns(4,5)P$_2$.21 A few years later, Brown and Stewart showed that Dawson’s TPI is indeed Ballou’s PtdIns(4,5)P$_2$.22 Dawson’s group combined their interest in phospholipases and polyphosphoinositides to show that brain contained two different types of enzyme that could hydrolyse TPI: a Ca$^{2+}$-dependent PLC [which would generate Ins(1,4,5)P$_3$ and diacylglycerol], and an
Mg$^{2+}$-dependent phosphatase, which would produce PtdIns(4P). Our current knowledge of the PtdIns-PLC and inositide-phosphatase families is enormous, and encompasses approximately 30 enzymes, so an originating source of this expansion deserves to be a classic.

To get to the next selected *Biochemical Journal* classic^4, we have to jump forward nearly 20 years, so perhaps I should sketch in those years with a few sentences because, as described elsewhere^16,17, a great deal happened during that time! The concepts which emerged (in brief!) were that a receptor-regulated stimulation of a PLC-hydrolysing PtdIns was a widespread phenomenon, that it was central to some aspect of cellular physiology, and that at least one part of that system might involve the generation of diacylglycerol to activate protein kinase C^21. Many of the seminal papers in the long struggle to understand these ideas were published in the *Biochemical Journal*, not least because of the prominent role played in the elucidation of these ideas by the UK 'dynasties’ that I described above. In fact, so much important work on inositides was published in the *Biochemical Journal* during the 1960s and 70s that it is very difficult to choose the classics. As a personal choice of a few other *Biochemical Journal* papers of particular importance, I would highlight the introduction of formate-based anion exchange chromatography for polyphosphoinositol phosphates^34, the discovery of phospholipid exchange proteins^26 and the first (also jointly^27) direct demonstration of an agonist-induced decrease in PtdIns^38.

Berridge and Fain’s 1979 paper^4 represents the beginning of a new phase in inositide signalling. A seminal paper in the inositide field from the 1970s is Bob Michell’s classic review in *Biochimica et Biophysica Acta*^29, in which he proposed that inositide turnover not only was a part of the mechanism of cell activation by some receptors, but that it also was in some way connected with calcium. The paper by Berridge and Fain^4 was, in fact, the second in a series of three *Biochemical Journal* papers focusing on the hydrolysis of inositol lipids in blowfly salivary glands (made feasible by the unique way in which these glands transport inositol^16). John Fain’s sabbatical with Mike Berridge had drawn Mike, with his interest in cAMP and Ca$^{2+}$ signalling in stimulated tissues, into looking at inositol lipids (much against the advice of a number of his ‘Ca$^{2+}$-colleagues’ in Cambridge!). As Mike has himself pointed out^29, his proximity to one of the two sources (see above) later turned out to be a happy chance of geography. What was crucial about this *Biochemical Journal* classic is its simple take-home message: that these glands require inositol to effect a change in Ca$^{2+}$ homoeostasis in response to 5-hydroxytryptamine. This provided the first direct evidence that inositides and Ca$^{2+}$ are indeed linked.

The list of *Biochemical Journal* classics from the 1980s^8–12 is perhaps more difficult to select than any other, because during the early to mid 1980s, when the PtdIns-PLC/InsP$_4$/Ca$^{2+}$ pathway (as we now know and love it) emerged, almost all of the important discoveries were made in the UK. Moreover, with few exceptions, if a UK inositol author couldn’t get his/her paper into *Nature*, then it went straight into the *Biochemical Journal*. I think there are two factors that led to this being such a near-universal trend. Firstly, there were no dreaded Impact Factors, so although everyone agreed then (as now) that a *Nature* paper would be cool, if an observation didn’t make that cut, then there was no agonizing over where next to send it — publishing it was all that mattered, so it was off to the *Biochemical Journal*. Secondly, during the most hectic phase (1982–1986), there was a general agreement within the UK community that we would all keep each other informed about what we were up to, and this unprecedented (and unrepeated?) open policy extended to the refereeing of papers. So we all knew that the *Biochemical Journal* would always give entirely fair and expert reviewing (that has not changed!), and that no one would get scooped/delayed if they sent even crucial and competitive discoveries to the *Biochemical Journal*. If anyone thinks from this paragraph that I am a little nostalgic for what was not only an exciting time, but also a time infused with an extraordinary community spirit based on unselfish sharing of knowledge, then they would be absolutely right.
To return to the selected classics, Berridge et al.\(^3\) (1982) is chosen for two reasons. Firstly, it formulated the idea that the action of Li\(^+\) on bipolar disorder might be due to inositol depletion in hyper-stimulated cells, in turn caused by the known Li\(^+\)-inhibition of Ins\(^P\)\(_4\) phosphatase (still an idea under investigation). Secondly, it introduced a simple combination of (i) inositol labelling of a tissue, (ii) stimulating the tissue with an agonist in the presence of Li\(^+\), and then (iii) analysing the inositol monophosphate by Dowex chromatography, as an extremely easy way of analysing a ‘PI response’. There is no question but that the massive proliferation of groups around the world who began to measure inositide turnover during the 1980s would be Mike Berridge’s 1984 review\(^3\) (quite explicitly put forward the hypothesis that Ins(1,4,5)\(_P\) was the second messenger responsible for mobilizing intracellular Ca\(^{2+}\).

The last two papers from the 1980s\(^{5,6}\) were chosen because they also represent the very start of something that has since grown beyond anything any one of us could have conceived. This is the proliferation of inositides. It began with the discovery that most of the Ins\(^P\)\(_4\) that is produced in parotid slices stimulated by carbachol for a few minutes was not the expected 1,4,5 isomer (although that was also present), but was instead Ins(1,3,4)\(_P\)\(_4\). The probable source of Ins(1,3,4)\(_P\)\(_4\) was then revealed with the discovery of Ins(1,3,4,5)\(_P\)\(_4\) in carbachol-stimulated brain slices\(^7\), combined with the observation that Ins(1,3,4,5)\(_P\)\(_4\) could be converted to Ins(1,3,4)\(_P\)\(_4\) by the 5-phosphatase\(^8\) that had been discovered by Downes et al.\(^6\). The synthesis of Ins(1,3,4,5)\(_P\)\(_4\) was, shortly afterwards, found to be mediated by an Ins(1,4,5)\(_P\), 3-kinase\(^9\), and the proliferation of inositides had begun.

Before we jump 15 years to the two classics from this century, I should, as I did above, mention the rest of the 1980s, during which time the Biochemical Journal remained at the centre of much of the action (and what action!). My choice for some additional minor classics of the 1980s would be Mike Berridge’s 1984 review\(^10\) (quite possibly the highest cited of all Biochemical Journal papers selected by the Biochemical Journal Editorial Board)

References

papers), which first elaborated on the 'dual messenger' concept, two crucial papers33,34 on the 3-phosphorylated inositol lipids (to become, surely, the inositol story of the 1990s), and the discovery of nuclear inositide signalling35.

In the 1990s, cell signalling increasingly became a much broader concept, as, for example, protein kinases cascades, small G-proteins and protein–protein interactions expanded to dominate over classic second messengers. Our last two classic papers11,12 come from this new era, and have been hugely influential in setting a new paradigm for the use (and abuse) of ‘specific’ protein kinase inhibitors. No one uses any of these inhibitors without consulting one of these two epic papers. Some of these protein kinases, such as protein kinase B (also called Akt), PKD1 (phosphoinositide-dependent kinase 1) and CaMKII (Ca2+/calmodulin-dependent protein kinase II), are, appropriately in this context, regulated by inositol-linked pathways. Finally, on the subject of inhibitors, and as we haven’t been given an ‘official’ inositol Biochemical Journal classic from the 1990s, why don’t we include Arcaro and Wymann’s discovery that wortmannin is a specific phosphoinositide 3-kinase inhibitor36? Hardly a 3-phosphorylated-inositol-lipid paper gets published without some exploitation of this amazingly useful compound.

To finish, given the dynamic state of the UK signalling community, and of the current generations of the inositol ‘dynasties’ in particular, it seems to me that if a similar exercise is undertaken for the Biochemical Journal’s 150th anniversary, there will be plenty of new classics to choose from!

Other References

20. Falch, J. (1949) Brain dihexaphosphoinositol, a new phosphoinositide having inositol methylphosphate as a constituent. J. Biol. Chem. 177, 505–519