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Review

Landmarks in the first hundred years of primary (9 + 0) cilium research

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Abstract

Primary cilia have had a long research history since 1898, but only in the last few years have investigations become intense. Only a few people took an interest in them until recently, when the consequences of their agenesis or dysfunction suddenly became apparent to all. Their function changed overnight from being speculative to being fully "acknowledged" as sensory, and the consequence of their lacking sensory function(s) led to a bonanza for cell and molecular biologists interested in the impact on clinical disease and disorder. Furthermore, once the medical profession had insight not just into the etiology of an extremely rare syndrome (situs inversus), but into one as prevalent as polycystic kidney disease, furious research activity sprang up in numerous centres, symposium were convened, special sessions arranged, and topical reviews written in all sorts of journals. Without an understanding of the history of research about this organelle, many reviews gave little credit to those who had persevered earlier to make key advances in primary cilium research, and most ignore the wealth of information on them that has relevance to fundamental (cell) biology as well as to medicine. Some of the "landmarks" presented here will hopefully provide a little guidance and a better perspective.

Keywords: Primary cilia; Sensory receptors; Agenesis; Dysfunction; Situs inversus; Polycystic kidney disease (PKD)

1. Introduction

1.1. Basic cell biological considerations regarding primary cilia

As a cell biologist, I have frequently stated – and fully stand by this belief – that nothing a cell does should be considered irrelevant and insignificant. If cells produce primary cilia, they do so for some reason, and they probably have a function even if it is only occasionally apparent. My active interest in primary cilia research began in 1965, almost 40 years ago (Currie and Wheatley, 1966). The strong conviction that they were important has been fully vindicated in the interim and especially within the last 6–7 years, which is very gratifying, more so because the implications of ciliary dysfunction or agenesis – predicted to cause major disorders in the body (Wheatley, 1995) – has resulted in some fascinating biology and astonishing advances in the etiology of certain well circumscribed pathological conditions. Furthermore, research on primary cilia throughout those 40 years was relatively steady until the last 7 years. This organelle was never "forgotten" at any time; it had its devotees and casual "visiting" researchers, but most emphatically it has never been overlooked or "ignored" – except, that is, by those very newcomers to the field when the limelight fell on developments in the control of ciliogenesis that were to lead to dramatic medical headlines and a breakthrough in a disorder as prevalent as polycystic kidney disease (PKD; see reviews of Pazour and Witman, 2003; Ong and Wheatley, 2003).

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1.2. Accoutrements of survival

When a local hill-walker takes to the mountains of my chosen domicile, Scotland, he usually knows that a sunny day can become an arctic nightmare within a few minutes. He takes Kendall cake or Mars bars as a reserve of "instant energy", a large thick polyethylene bag (a "survival bag" that doubles on unfortunate occasions as a "body-bag"), extra clothing, warm fluids, and so on. A cell operates in such a way that it can switch to other fuels and supplies when needed. It also carries reserves of many other things and a plethora of molecular gadgets that seem to be an encumbrance, just in case they may be needed, even if it is once in a blue moon. Both cells and hill-walkers adopt sensible survival strategies, even though the strategy requires the humping around of much more baggage than might seem necessary at any moment in time. The primary cilium might often be viewed as an appendage that could sometime be useful, but can give the impression of being vestigial and an encumbrance rather than an asset.

1.3. What cilia normally do

A cilium is a cellular gadget that can help a cell move when free to do so or it can help move the environment passed the cell when the latter is fixed. When stuck in a tissue, a single cilium is not much use on its own, but that self-same structure may be retained because it has another function. Cilia make a huge change in surface membrane for a relatively small volume of cytoplasm. They can also extend far further beyond microvilli, which themselves greatly amplify surface area (Fig. 1). They can probe the environment in many free as well as in some deep-seated tissue. In ciliates (e.g. *Tetrahymena*) some determinants or receptors are on these membranes, such as insulin receptors, which surprises many people – "as if ciliates need to control glucose level in their 'blood'!". Of course, they got there first and we have adopted insulin in our later eukaryotic development for such purposes (yet ironically we still do not know what its purpose is in *Tetrahymena*). So cilia have, and probably always have had, at least two functions. Quite naturally one places sensors and the signaling molecules themselves (ligands) on promonotories, just as we site radio receivers and transmitter on the tops of hills, not in the glens. The lack of a sensor/signaler support device, such as a radio beacon on a promonotory, may quickly spell disaster if it is either not in place or "goes down". Disaster is likely to befall cells that fail to develop similar structures or experience malfunction within them. All cilia can play these roles, not just primary cilia. And since they project from the cell surface, they are inevitably the first organelles that receive signals from the environment, sticking out far further than the microvilli on the cell surface (Fig. 1).

1.4. History, the sadly neglected tool of modern scientific research

Research papers, apparently in the opinion of most present day scientists, are old and only fit to be hidden away in the archives when somewhere between 5 and 10 years old. This does not mean that everything prior to these times is not worth reading. Indeed, without doing so, one may later find that if you had traveled back to a hypothesis propounded 50 years ago, it might be almost mistaken for one much more recently propounded, even though it was not tenable at the time. It behooves every scientist to read *deeply* around his/her chosen topic of research, for it can be a veritable goldmine of ideas, of unfulfilled promises, just as much as it can be, quite appropriately, a mausoleum or resting place for many untenable hypotheses. But most of all, the archive is valuable because it provides the perspective that matters when any piece of scientific work needs to find its rightful context.

Most scientists become seriously interested in the history of their subject after they have retired from the bench, only to bemoan the fact that they should have taken much more notice of it earlier, instead of (seemingly) wasting half their career following false

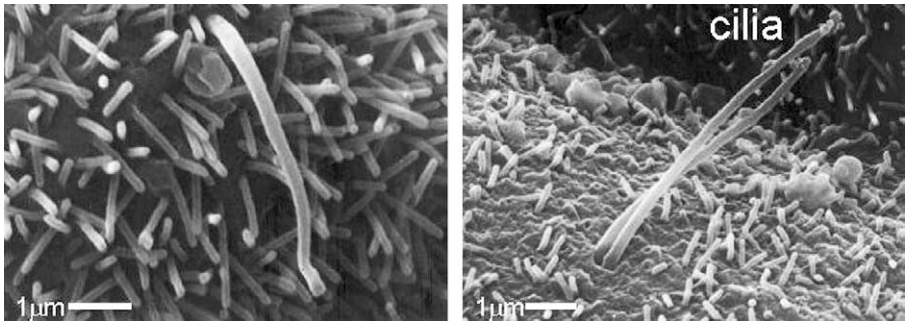

Fig. 1. Serosal cells seen by scanning electron microscope in which their surfaces are covered with microvilli. A single primary cilium protrudes far further into the fluid phase on the left, while on the right a biciliate cell is seen. Ref: cropped figure from Bird et al. (2003; his Fig. 5), with permission.

leads that could have been avoided with this same hindsight. If Newton's paraphrase holds any weight – that one has the advantage of "standing on the shoulders of giants" – then history can only be ignored at one's peril. So now let us consider earlier features of research into primary cilia.

2. Cilia in the early days of microscopy

2.1. Seeing the unseeable: the first glimpse of a primary cilium

Few people give full credit to early microscopists because physics has assured us that some of the minute structures they saw could not have been resolved by the microscopes of the day. True, but Leeuvenhoek (see www.discoveryofthecell.co.uk) accurately described details of organisms to the point where he seemed to be using the "eye of faith". Not so; Wilson (1896) reveals his frustration at the turn of the 20th century by bemoaning the fact that he was in need of a microscope with 1000× better resolution to see in detail the intricate structure of cytoplasmic ground substance. Given the benefit of the doubt that the early microscopists recorded what they saw, not what they wanted to see to fit some hunch, it follows that some microscopists did somehow go beyond the apparent optical resolution of their instruments, for which they should be given credit. One memorable instance with primary cilia occurred when I was photographing interference and fluorescent microscope images of the same primary cilia in PtK1 cells some years back. I also observed an absolutely clear example of a primary cilium under the former optical set-up that should have not been possible without video-enhancement. The reason I did see it with the enhancement being on was that a suitably-sized bubble introduced at mounting of the specimen sat conveniently over the exact position such that the curvature on one side added another little "lens", providing just enough extra magnification to bring it into view. I am not suggesting that Zimmerman (1898) did the same, but if an over-stained specimen was seen through a microscope that was under-focused, he may well have had a similar experience, which allowed him the undoubted privilege of seeing primary cilia poking out into the lumen of a kidney tubule exactly as we would expect them to be found today.

Primary cilium research precedes many other fields of cell biology, being now over 106 years old. Apart from a few similar observations through to the late 1950s (see Wheatley, 1982, for a fuller account), most microscopists had to be content with little advance because a new technique was needed for further advance, the advent of the electron microscope.

2.2. Ultrastructure, the hey-day of the electron microscope

One of the most remarkable structures revealed in the late 1950s and early 1960s was the centriole, with its fascicular bundle of 27 (micro) tubular structures, and its rose-window-like appearance in transverse section. The first fine details were probably from de Harven and Bernhard (1956) in Paris, but many others quickly provided further detail. What is more surprising is that, while the focus was directly on this organelle that so commonly produces cilia, primary cilia were not reported until some years later. The notion was that the centrioles carried specific information about cell division because their duplication (not division) preceded cell duplication. Only now do we appreciate that while it remains a focus for the coming and going of "mitotic" and other proteins during the cell cycle (in the pericentriolar cloud, and a part of the bigger structure, the *centrosome*), the centriole itself is not known to be anything other than acting as a template upon which a cilium can form, although we ought not be surprised if at some future date, other functions became recognised, as clearly foreseen.

3. Selected landmark papers

3.1. Introductory comment

Landmark papers can almost certainly be agreed upon by consensus, but this choice is my own, and colleagues reviewing the literature may well have made different choice from their own perspectives. I make no apology for my own choice since I believe there are moments when a substantial piece of evidence leads to definite progress in this field of research and its impact on biology and medicine in general, and it is as well to be in command of all the happenings that occurred throughout most of that period, of which these ones are disputable "milestones", to use another term. Some of my reasons may seem unusual to others, Section 3.1.6 below being a good example, as explained in the annotation. To ensure some brevity to this editorial, I decided to restrict myself to the 10 papers during the first century of primary cilium research (1898–1998) that should have had major impact, while noting briefly some of the ancillary work of purport to these more seminal ones. I am not going beyond 1998 because that was the start of the period when a major breakthrough led to developments in and understanding of PKD, situs inversus, the Bardet-Beidl syndrome, and a number of other medical disorders. Versions of the recent history (topical reviews) are now legion, but the body of the extant literature prior to this exuberant activity can be easily seen on the primary cilium website <www.primary-cilium.co.uk>.

3.1.1. LM 1 Zimmerman (1898)

This must, of course, be the first documented observation of primary cilia, although not fully verified until the electron microscope (EM) was developed.

3.1.2. LM 2 Barnes (1961)

The next landmark really had to wait until the advent of electron microscopy and improved technology for cutting ultrathin sections. Barbara Barnes provided a lot of ultrastructural details about adenohypophyseal cell cilia, and therefore was one of the first truly detailed studies of a particular type of cell and cilium. This led people to ask the question why do these particular cells have cilia – sometimes not just one, but two (see Fig. 1 for another example in serosal cells). The assumption at that time was that only a few tissues of the human body develop these appendages. Following this, perhaps the most comprehensive description of a primary cilium came from our own work on adrenocortical cells, and through the good fortune that a well-preserved example of a primary cilium with all the attendant structures was cut in almost perfect sagittal section (Currie and Wheatley, 1966). In addition, we (like others) soon began to explore a wider range of tissues, and in our own case cell cultures.[1]

3.1.3. LM 3 Sorokin (1962)

Sorokin's analysis highlights the differences between the development of primary cilia and (9 + 2) cilia. Without the insight to know this, his superb descriptions of the two routes in which cilia may develop hints that the cell makes some sort of choice depending on factors that today remain unknown – the signal to produce 9 + 2 as opposed to 9 + 0 cilia, for we still know of no cell type that possesses both types (the exclusion principle).

3.1.4. LM 4 Dahl (1963)

When more tissues turned out to have cilia than fibroblasts in culture and a few endocrine tissues, one of the surprises was to find that neurons possessed them. I have singled out Dahl's report for two reasons. He used a sensitive silver staining method that Ramon y Cajal himself would have been proud to have done. So Dahl was confident, but could not be 100% so, until Karlsson (1966) had shown ultrastructurally that he was right. The second reason is that in his rat cerebral cortex preparations, it was quite apparent that most – and possibly all – the neurons were ciliated.[2]

3.1.5. LM 5 Dingemans (1969)

There were others making a similar point, but the essence was this. Dingemans, like Fonte et al. (1971), dwelt on the notion that dividing cells might develop cilia to stop centrioles from being so frequently involved in cell divisions. The notion had been widespread during this period that centrioles were intimately in cell division, but it was more *guilt by association* than any direct or convincing evidence. But if centrioles were indeed no more than passengers in the cell duplication cycle and they played no active role in segregation of chromosomes on the spindle into daughter cells during division, then they were merely passengers. Correlation between cilium frequency and division could tell something (Archer and Wheatley, 1971), but only indirectly about mitotic activity (primary cilia disappeared around mitosis but were seen even at late telophase as cells exited division). The crucial evidence was that plant cells do not have centrosomes and centriole/primary cilia, but have homologous mechanisms of cell division. Yet the situation was puzzling because of the next key observation.

3.1.6. LM 6 Albrecht-Buehler (1977)

This is the example where I have chosen for reasons other than the outcome of the reported research being itself a major advance. I draw attention to the first reference and a "companion" paper (Albrecht-Buehler and Bushnell, 1979) because these authors did more

[1] Bowser (www.wadsworth.org/BMS/SCBlinks/cilia1.html) produced two important documents as elements of this comprehensive database – being one of the first cell biological sites on the web – which had major impacts firstly in terms of our understanding of the diversity, range of possible role of primary cilia. And second, it showed that not just a few, but very many different tissue cell types of the body possessed primary cilia. Indeed, latterly it has been easier to record cell types in which cilia have seldom if ever been reported, e.g. mature hepatocytes. The close to ubiquitous nature of the organelle soon became evident. This compilation was done in Albany at the Wadsworth Center, where primary cilium research had already shown cell cycle-related expression (see Section 3.1.7). The current website can now be found at www.primary-cilium.co.uk.

[2] Parenthetically, I have to include – or rather, must not neglect – an additional "neurological" cell type that is also invariably ciliated. For some reason this ciliation had always been seen as something different, perhaps because others might have thought it was the only partly formed 9 + 2 cilium that had been retained as a connecting structure between the inner and outer segments. I have to pause to include – and especially not to neglect – an additional "neurological" cell type that is also well ciliated. For some reason this ciliation has always been seen as something different, perhaps because others might have thought it was an only partially formed cilium retained as a connecting segment between the inner and outer parts of the rods and cones of the retina (Sjostrand, 1953). But this was and always should have been seen as a primary cilium, and ought to have been included much more closely in the development of primary cilium work. Many papers on the cilia of rod and cones were published in the 1960s as EM techniques improved. It is noteworthy that both neurons, like rods and cones, are terminally differentiated, ciliated cells. Could it be that cells which had not reached their ultimate morphogenetic form did not express primary cilia? Work on cells in culture suggested quite the opposite because highly proliferative cells were frequently ciliated, but the next paper (LM 5) reveals this developing train of thought of the rods and cones of the retina.

than just make careful observation of cells; they used cell cultures where they could maintain control over the environment and have the ability to organize material so that *behavioural* properties of cells and their cilia might be followed. In a word, the first-mentioned paper is the start of *experimental* work on primary cilia. The tracking idea studied by the removal of gold particles from surfaces over which cells migrated seemed to indicate that cells make 40° turns, which suggested that the primary cilium sensed the substrate as cells move and relayed information back to an internal "compass" – the centriole – which coincidentally has its vanes set at angles of 40°. Experimentation on primary cilia has been lacking in general, but technology now exists with laser and other tools for more experimentation to become more feasible, although few researchers have progressed this far.

3.1.7. LM 7 Rieder et al. (1979)

Although circumstantial evidence from many studies indicated that dividing cells do not retain primary cilia, the more definitive study was that of the Albany group under Conly Rieder, using PtK1 cells. Fortunately these cells stay quite flattened during division, which made the clearest correlation that cilia were absorbed in quite a short period of time (prior to or during prometaphase of mitosis). Reappearance of cilia could be astonishingly quick in some cases (Archer and Wheatley, 1971), beginning in late telophase; but others (Ho and Tucker, 1989) thought it occurred only in S-phase, which is not the case.

3.1.8. LM 8 Poole et al. (1985)

The work of Poole and co-workers on chondrocytes has never received the credit it deserves. But even more important, the paper I have chosen showed 20 years ago the huge potential of the primary cilium as a chemical and physical sensory device – a veritable "cybernetic probe". This article was clearly speculative, but was based on excellent material which should have attracted more funding at this time, since there has now been ample evidence to validate many of the ideas, some of which are currently being boldly claimed by others with little recognition of Poole's prescient publications, e.g. the recent review of Pazour and Witman (2003). The final paper I have chosen below was one of the first two steps providing the evidence that lead to primary cilium research taking off since 1998, and to a greater understanding of PKD as the first major disorder of what should to be properly called in medical parlance "primary primary (sic) ciliary dyskinesia", to distinguish it from "primary ciliary dyskinesia", which refers mostly to the consequences of malfunction in 9 + 2 cilia (see Section 3.1.11).

3.1.9. LM 9 Schartz et al. (1997)

This work was initiated by Bowser (see Section 3.1.2, with colleagues at a neighbouring university to Albany, Rensselaer Polytechnic in Troy, NY State, USA. It was to conclude that long renal cilia bent in the upper two-thirds of the shaft, the deflection being proportional to the shearing force, i.e. flow rate (Fig. 2). Renal cilia were described as "optimally engineered" sensory probes. The Albany laboratory (Roth et al., 1988) had devised technical skills to see renal epithelial cells on the edges of folded monolayer cultures (Figs. 2 and 3), allowing cilia to protrude into optically clear (fluid) space. This same procedure is now employed with laser treatment to amputate and study the dynamics of ciliary regrowth (Bowser, Cole and Wheatley, unpublished). Two subsequent studies confirmed and extended this work, as was anticipated (Praetorius and Spring, 2001, 2003); Ca^+ transients were released in the cell body as a result of ciliary stimulation, but we still have to find out what the second messenger does and the nature of the important physiological response(s) determining the health or disease of the organism (patient).

3.1.10. LM 10 Nonaka et al. (1998)

This paper provided evidence that suggested a mechanism whereby primary cilium defects in the nodal cilia of developing embryos affected the asymmetry of body organ disposition, a clue in fact to situs inversus that is a cardinal feature of Kartegener's syndrome in man. The defect in this rare disorder in ciliary axonemal motor molecules and the cilia cannot move (Afzelius, 1979). While that may be the case for (9 + 2) cilia, (9 + 0) cilia are normally immotile because they invariably and

Fig. 2. PtK1 cell cilium seen with Smith DIC differential interference microscopy and video-enhancement at approximately 3000× original magnification. The culture is seen along the edge of a folded sheet of monolayered cells (Roth et al., 1988), and the cilium protrudes into the clear, fluid phase. This cilium is about 16 μm in length, shows several irregular lumpiness about mid-shaft region and a varicosity at its tip.

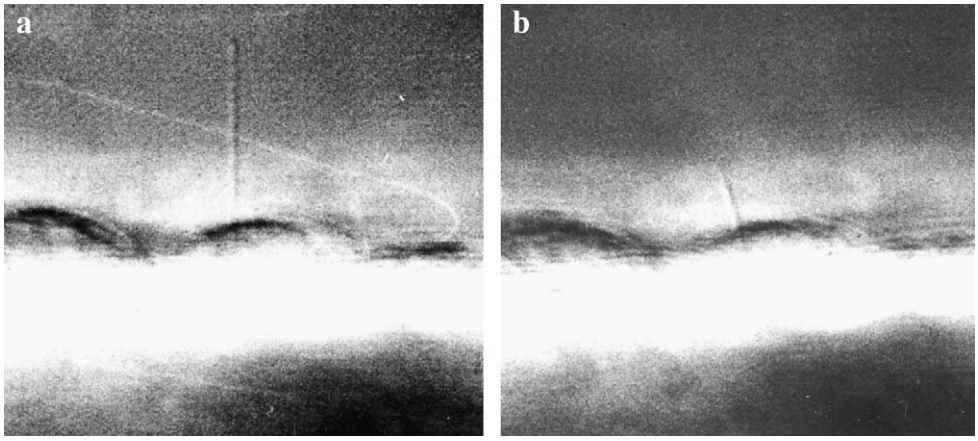

Fig. 3. Same as Fig. 2 but in this case the cilium on the left (a) was ready for laser slicing. The laser beam was moved from right to left across the field at a point about a third the way up the cilium shaft and then retracted. The cilium was cleanly cut, and its stump exuded a small amount of cytoplasm and then sealed off. It remained like this for about 1 h of observation, but its regeneration or fate otherwise was not followed in this case for longer. Approximately 3000× original magnification.

characteristically lack dynein side-arms on the A-tubule. The extraordinary finding of the Japanese group and its follow-up led to much confusion because the primary (9 + 0) cilia of the embryonic node were shown to be motile, but by twirling not beating. If these become immotile through further dysgenesis, then there is no signaling of an important morphogen to the left side of the node, and therefore no defined asymmetry. So we are left having to believe that these nodal (9 + 0) ciliary type constitute a very rare exception among primary cilia; and it also implies a different motor system operates that has yet to be discovered. While this remains a seminal paper, it should have acted as a stimulus for further work on the cell biological side rather than with the medical obsession of the generation of situs inversus. Oddly, the fundamental issues it raised regarding unidirectionality of twirling seem to be of little interest to ciliologist, and the problem clearly remains a mystery worth resolving in its own right.

3.1.11. Final remarks

On another occasion, we might continue the saga of the primary cilium in these pages, but it is definitely worth seeing how things got to the point of take-off in 1998, as shown by the sudden inflection in publications on primary cilia (Fig. 4).

Genetic mutations and deletions that can lead to ciliary dyskinesia or agenesis, first detected by Cole et al. (1998) with regard to shaft assembly processes, means that disorders will occur in any ciliated circumstances, affecting the bronchial linings, sperm motility and so on (the immotile cilium syndrome of Afzelius (1979)). The new developments following discovery of the intraflagellar raft proteins by Cole et al. (1998) apply to (9 + 2) cilia, and mutations of the same genes or of motor proteins on the axonemal microtubules will result in a wide range of disorder that are now unfortunately lumped together in their extensive medical literature under "primary ciliary dyskinesia". Purist devoted to (9 + 0) cilium research can take comfort in their being far more things still to learn about these remarkable organelles than we can imagine, even following the recent surge of interest since 1998. But their pathology should technically be classified as "*primary* ***primary***

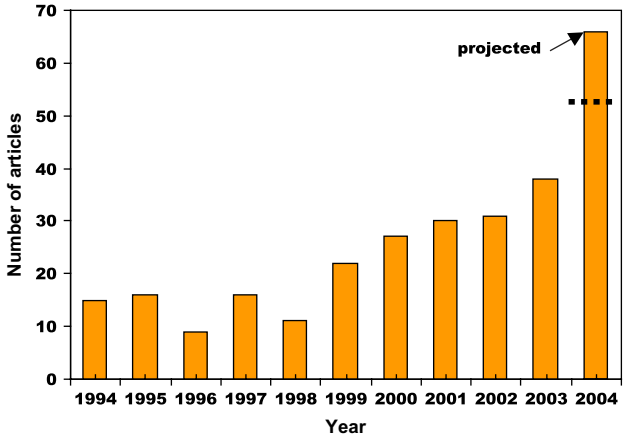

Fig. 4. Graph of publications specifically on primary cilia over the last 10 years. Between 1994 and 1998, a steady average was maintained close to 15 papers (as also throughout the 1980s) per year were recorded until 1998, the graph then rises steadily to 2002, when a sharper acceleration occurs before taking off dramatically as research over the last 2–3 years starts to get reported. Not all the "2004" publications will be listed to date. Dotted line was the number recorded in late December 2004, and more with 2004 dates will be published in the next few months (projected total given). It is almost certain that the rise seen in 2004 will continue through 2005.

ciliary dyskinesia'' (sic). Further research will throw up many other medical correlations arising from defects in primary cilium development and functioning. The future of this research remains exciting.

Acknowledgements

I wish to thank a large number of people, mostly devotees of primary ciliatology for their helpful discussion, their contribution of much rich material, and for many exciting collaborations over 40 years in bringing this field to its rightful place in cell biology. Special thanks must, however, go to Dr. Sam Bowser for initiating so much exciting work as well as the original ''primary cilium resource page'' (website). More recently Dr. Sue McGlashan and Miss Fiona Barker have given valuable assistance in creating and maintaining the current website.

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