CURRENT SCIENCE

Volume 95 Number 8 25 October 2008

EDITORIAL

Missing Out on a Nobel Prize

Every year the month of October brings the announcement of the Nobel prizes in the sciences, originally specified in Alfred Nobel's will: physics, chemistry and physiology or medicine. For scientists in these disciplines, the prize remains the pinnacle of academic achievement. In major laboratories across the world there is expectation. When the week is over there is much to celebrate for the chosen few and generally, well hidden disappointment in many, who are passed over. As the number of scientists and subdisciplines has exploded in the last three decades of the 20th century, Nobel choices have become increasingly difficult. There are many specific discoveries to be recognized and many extraordinary lifetime achievements to be honoured. A balance between different areas of the chosen sciences needs to be maintained. When the winners are announced, the postmortems begin. The hopefuls retire into the shadows of their laboratories to await another year. In the immediate aftermath of the Nobel week, there are inevitable discussions on those who might have shared the prize in the chosen field. This year has been no exception. The award of the prize in physiology or medicine to the discoverers of the virus that causes acquired immunodeficiency syndrome (AIDS) to the French scientists Luc Montagnier and Francoise Barre-Sinousi appears to pass a definitive judgement on an old question: 'Who are the discoverers of the AIDS virus?'. Observers of this field will recall the major controversy that provided riveting drama for the biomedical research community, in the 1980s. The French group, now vindicated, was ranged against the American team led by Robert Gallo, in a dispute that involved patent rights and financial returns for an AIDS blood test, based upon the virus. There was a remarkable spectacle, about twenty years ago, when a resolution to the dispute was reached and a settlement announced by Jacques Chirac and Ronald Reagan; a rare example of conflict resolution in science by leaders of two major countries. Echos of this old battle pervade many of the media reports that describe this year's award. In many earlier awards, Nobel watchers have been quick to point out major omissions.

This year's award in chemistry leads us to a strange and somewhat disquieting story; an omission that can hardly be justified on scientific merits. The 2008 award recognizes 'the discovery and development of the green fluorescent protein, GFP'. The laureates named are Osamu Shimomura of the Marine Biological Laboratory, Woods Hole, Martin Chalfie of Columbia University and Roger Tsien of the University of California, San Diego. The GFP is a substance isolated from the jellyfish *Aequorea victoria*, which absorbs light at 400 nm and emits a green fluorescence at about 505 nm. GFP is a protein and hundreds, indeed thousands, of proteins have been studied and dissected by biochemists across the world. Why has the Nobel Committee singled out GFP's discovery for recognition? The answer is evident when one examines the vast and ever expanding literature of molecular and cellular biology. GFP, with its characteristic green glow after excitation, has become an immensely powerful tool for visualizing gene expression in cells and has catalysed an explosive growth of fluorescence imaging as a means by which biologists can directly monitor cellular processes, particularly the 'localization and fate' of cellular proteins. Freeman Dyson's reflective comment that 'science is often driven by new technology rather than by new concepts', is probably more true in the biological sciences than in many other disciplines. GFP has been labelled as 'a guiding star for biochemistry' in the Nobel press release. The scientific background to the Nobel award, produced by the Royal Swedish Academy, notes that 'neither the biochemical nor the genetics revolution provided the experimental tools that would allow for quantitative and experimentally well-defined monitoring at the molecular level of the spatio-temporal intra- and inter-cellular processes that define the dynamic behaviour of all living systems. To obtain such knowledge new experimental and conceptual tools were required. Now, at the beginning of the 21st century we are witnessing the rapid development of such tools based on the GFP from the jellyfish *Aequorea victoria*, its siblings from other organisms and engineered variants of members of the "GFP family" of proteins'.

GFP's history begins with Shimomura's discovery of the protein and its characteristic glow, under UV irradiation, in 1962. This work followed his earlier work on the chemiluminescent protein aequorin, involving the pains-

taking collection of thousands of bioluminescent jellyfish. Shimomura's papers which described the discovery of the protein and the characterization of the chromophore (the small portion of the molecule, which absorbs and emits light) appeared in the period between 1962 and 1979. In a major review published in 1998, one of this year's laureates begins by noting: 'In just three years, the green fluorescent protein has vaulted from obscurity to become one of the most widely studied and exploited proteins in biochemistry and cell biology' (Tsien, R., *Annu. Rev. Biochem.*, 1998, **67**, 509). Clearly, he refers to events that happened in the years 1995–97, when GFP was launched on its career as an indispensable tool in cell biology. The critical breakthrough came in 1992 when the gene that encodes GFP was cloned (Prasher, D. C., *Gene*, 1992, **111**, 229) and the primary sequence of amino acids in the polypeptide established. Cloning genes was still a far from routine task in the late 1980s when Douglas Prasher began to work on the GFP gene. There was no widespread interest in GFP at that time and Prasher must have ploughed a lonely furrow. One of this year's laureates, Martin Chalfie recalls that in 1988, following a seminar, he thought of the wonderful prospect of introducing a fluorescent protein into cells. He found Prasher and after a four-year period did indeed receive the gene. The paper that described the work that followed, entitled 'Green fluorescent protein as a marker for gene expression', appeared quickly thereafter (Chalfie, M. *et al.*, *Science*, 1994, **263**, 802). The remarkable spontaneous generation of the fluorescing group upon GFP production was established and the long awaited cellular marker had arrived. Almost concurrently, Roger Tsien working with Prasher's GFP gene established that fluorescence wavelengths could be tuned by mutations of GFP sequence, setting the stage for engineering the optical properties of the marker protein (Heim, R. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 12501). GFP which had lain dormant in the scientific literature for over a decade after Shimomura's work had been transformed into an indispensable molecular tool in biology. The key breakthrough was undoubtedly the cloning of the GFP gene by Douglas Prasher, who not only provided the gene but also coauthored the seminal papers by Chalfie and Tsien in 1994. Alfred Nobel's will precludes more than three awardees. Prasher, despite his critical contribution, must be content with being mentioned in the 'Scientific Background on the Nobel Prize in Chemistry 2008' produced by the award committee. He is indeed the 'fourth man'.

Who is Douglas Prasher? A search on PubMed reveals 16 publications, the most recent paper appearing over a decade ago in 1997. A short review in *Trends in Genetics* (1995, **11**, 320) details succinctly the 'uses of the green fluorescent protein cDNA' and notes that the accompanying articles 'describing the use of GFP represent only the first of many doors that GFP can potentially open for cell and developmental biology'. Shortly after the Nobel announcement, Prasher was discovered working as a 'shuttle car driver' in Huntsville, Alabama, earning $10 an hour. Browsing the Internet, I came across several sites (including Wikipedia) where the Prasher story is described. It is a tale that scientists who struggle for positions and funding may find haunting. Prasher lost tenure at Woods Hole, having little to show for his struggle with the GFP gene. A move to the US Department of Agriculture and job losses after funding cuts, forced Prasher out of science. In the many interviews that have appeared, Prasher's reactions are stoic: 'Do I feel cheated or left out? No, not at all. I had run out of funds and these guys showed how the protein could be used and that was the key thing'. Could the Nobel Committee have awarded a prize to an ex-scientist, who did not have any institutional address? Was it a pragmatic decision which minimized any controversy that might follow? The answers to these questions must, of course, remain in the realm of speculation.

For over a century, Nobel prizes have been awarded for major conceptual advances, lifetime achievements, although specific contributions are cited, and for discoveries that transform a discipline by providing new tools that permit advance on a broad front. The key steps in the history of GFP must include the discovery of the protein and its properties and the cloning of the gene, which opened the path to biological applications. Douglas Prasher chose his area of research, with an enviable degree of foresight. In retrospect, his choice of GFP satisfied the sage advice that is often given that 'one must work on problems of significance'. He did succeed in the laboratory by producing the clone. All subsequent experiments with GFP expression and fusion proteins worked much better than might have been anticipated in the 1990s. Yet, he failed in every other respect in his quest in science. He lost grants and jobs. The Prasher story is cautionary, emphasizing the imperfections of the peer review process. Unfortunately, like democracy, we have no better system. As competition for grants and positions intensifies, success sometimes depends on abilities to project, market and manage science and to network effectively in a complex community. Prasher, evidently, appears to have fallen by the wayside in an intensely competitive environment. Nevertheless, his research has illuminated the workings of living cells and there is still hope that the warm glow of GFP and the aftermath of missing out on the Nobel prize will light his path in the future.

P. Balaram