Harry K. MacWilliams (1946 - 2011): An Appreciation

Harry was born on July 8th 1946 in Summit, New Jersey. After graduating from Red Bank High School in New Jersey in 1964, Harry entered Harvard University and obtained his Bachelor of Science with high honours in 1968. He stayed on at Harvard to continue his research on *Hydra* under the supervision of Professor Fotis Kafatos, and received his PhD magna cum laude in 1972. After his PhD, Harry obtained a Woodrow Wilson fellowship in 1972, which he used to further his training in developmental biology, under the direction of the leading theoretician Alfred Gierer, at the Max-Planck Institute for Virus Research in Tübingen (1972-75). After a stay with Charles David at the Albert Einstein College of Medicine (1975-76) during which they both started to work on *Dictyostelium*, Harry assumed an assistant professorship of Anatomy at the University of Massachusetts Medical School in Worcester in 1976. When David took up a professorship in Munich, he convinced Harry to join him. From 1983 on Harry was a professor at the Zoology Institute, Ludwig-Maximilians-Universität in Munich, where he taught developmental biology and cell biology, as well as pursuing his research interests.

In the early 1970's, several of the pioneers of molecular biology were fostering a move into developmental biology, aimed at understanding development in molecular terms. Developmental biology already had a firm descriptive basis, and classical grafting and regeneration experiments suggested that embryos are patterned by gradients of diffusible morphogens, but the molecular nature of these morphogens and how they produced embryonic patterns was not known. These problems must have fascinated Harry, for they became a central interest in his scientific life, first with *Hydra*, and then with *Dictyostelium*. In a seminal paper published in Science, Harry and Fotis Kafatos demonstrated the self-regulating potential of basal disk differentiation in *Hydra* [1]. This paper was the result of his undergraduate thesis at Harvard. His doctoral thesis was an elaboration of the mechanism controlling foot differentiation based on simple but carefully interpreted transplantation experiments [2, 3].

These papers brought Harry to the attention of the Gierer lab in Tübingen and he came in early 1972 for a visit and later that year as a postdoctoral fellow. Harry immediately immersed himself in the details of the pattern formation model which Alfred Gierer and Hans Meinhardt had just published [4]. The model provided a specific mechanism for creating a spatial pattern based on the concept of local (autocatalytic) activation and long-range (lateral) inhibition. It could thus, using autocatalysis, start from an essentially homogeneous condition and create a stable spatial pattern de novo. The original formulation of the model was strongly influenced by the facts of head and foot regeneration in *Hydra* and also the ability of reaggregated *Hydra* cells – a homogeneous condition - to regenerate normal head and foot tissue [5]. Harry´s strong theoretical interests combined with his ability to do hundreds of transplantation experiments in various combinations were just the right skills at just the right time in Tübingen. The constant feedback between theory and experiment, which started in Tübingen, ultimately led to two remarkable papers in Developmental Biology [6, 7] and one in the Journal of Theoretical Biology [8]. These defined the basic features of the pattern formation system controlling head formation in *Hydra* [9]. They demonstrated that head activation is, in fact, two phenomena: rapid activation localized to the site of

head regeneration and a stable component graded along the body column and responsible for the polarity of body column tissue. Harry also demonstrated that long-range inhibition was compatible with a diffusion mechanism and determined a diffusion coefficient. Finally, he showed that the results could be quantitatively predicted with a proportion-regulating version of the Gierer-Meinhardt model. These papers are widely viewed as "classics" in the *Hydra* community.

Harry burst onto the *Dictyostelium* scene in 1979 with a review on pattern formation, written with John Bonner [10]. In this they considered how the prestalk-prespore pattern is produced in the slug, using the available experimental evidence in an attempt to discriminate between theoretical models, and coming down guardedly in favour of a reaction/diffusion process. This review is still well worth reading today, for the problem remains unsolved and the ideas are clearly presented and still relevant. Harry followed this with a series of papers and reviews on patterning [11, 12]. His approach in this period was to test theories of patterning using classical grafting and regulation experiments and by isolating patterning mutants [13]. His work included two intricate papers with Barbara Buhl on cell sorting in which they showed that sorting occurred within the prestalk and prespore zones of a slug, as well as between them [14, 15]. Thus, labelled cells taken from the rear of the prespore zone from one slug and mixed with disaggregated cells from unlabelled slugs would sort to the rear of the prespore zones of the reconstituted slugs. But grafting into intact slugs (instead of disaggregation and reaggregation) revealed a more complicated situation, which Harry suggested resulted from extra-cellular negative feedback within the slug that always tended to destabilize the current position of a cell, leading to it changing its sorting preference with time. His general conclusion from this period was that the ratio of prestalk to prespore cells is likely to be set by a combination of intrinsic biases in the cells towards one fate or the other, and two negative feedback loops. He viewed anterior-like (AL) cells – prestalk-like cells which reside in the prespore zone - as an intermediate cell type, with one feedback loop controlling the prestalk to AL cell ratio and the other the AL to prespore cell ratio.

Harry took advantage in his work of the newly developed reporters for prestalk and prespore cells, and in order to increase their usefulness, made a number of technical improvements. One was to develop an efficient method for transforming non-axenic cells, allowing true wild-type strains to be transformed [16]; the other was to construct a series of unstable lacZ and GFP markers that allowed the current gene expression status of cells to be monitored, rather than the historic cumulative expression, which is reported by stable markers [17, 18]. He used these markers to resolve an apparent paradox: the appearance of prespore cells in the prestalk zone of older slugs. In fact, these cells are prespore cells that have changed their status, converting probably to AL cells and then prestalk cells, before sorting into the prestalk zone [17]. This flow of cells probably compensates for the prestalk cells lost from the rear of slugs as they migrate.

The effect of the cell cycle on cell fate was another area that had fascinated Harry since the 1970s. He did not find a good handle to tackle this problem until he noticed the unusual expression pattern of the *rnrB* gene, encoding the small subunit of ribonucleotide reductase. This gene is expressed exclusively in prespore cells and in actively growing cells. A typical eukaryotic cell cycle is decorated with four clear phases (G1, S, G2 and M), *rnrB* is up-regulated immediately after the cell passes the G1/S transition to generate

a pool of deoxyribonucleotides to support DNA synthesis. Lacking a G1 phase, the *Dictyostelium* cell cycle posed an intriguing problem for *rnrB* expression. Harry devised an elegant approach to studying the expression pattern of *rnrB* in growing *Dictyostelium* cells by doubly marking them with the nucleotide analogue BrdU and the unstable lacZ driven by the *rnrB* promoter. Results from both asynchronously and synchronously dividing cells showed that *rnrB* is expressed in two distinct phases: one in mid-G2 and the other after G2/M transition [19]. Expression near G2/M transition can be explained by suggesting the cells are getting prepared for DNA synthesis following the short M phase in a G1-less cell cycle. The mid-G2 expression of *rnrB* was unexpected and it would suggest the existence of a regulatory event in mid-G2 that is normally associated with G1/S transition in most eukaryotes. The possible presence of two controlling points in the cell cycle might provide a framework for switching between stalk and spore preference during the cell cycle.

The game was on, and Harry was on the hunt for the *Dictyostelium* orthologues of G1/S and G2/M controlling proteins of plants and animals. He posited that by altering the function of these key proteins he could manipulate cell fate. While trawling for regulatory genes in the early days of genome sequencing, Harry came across sequences that could encode an orthologue of the mammalian Rb protein. The retinoblastoma tumour suppressor protein Rb is a key protein blocking G1/S transition and is also involved in differentiation in mammals and plants. Based on the plausible existence of an Rb-like protein in *Dictyostelium* cells, Harry invited Adriano Ceccarelli and Adrian Tsang for a meeting to discuss a joint research program. The meeting took place in May 2001 at Adriano's alpine retreat near Turin. Amidst wonderful scenery, endless hiking trails, glorious weather, delectable food, and abundant supply of wine from Orvieto, a plan was hatched to alter the expression and function of the *Dictyostelium* orthologue of the mammalian Rb, RblA, and the key G2/M checkpoint protein cyclin-dependent protein kinase, CdkA. Over-expression of *rblA* leads to G1 arrest, confirming Harry's suspicion that *Dictyostelium* possesses a cryptic G1/S transition checkpoint. *RblA*^{null} cells show reduced cell size, a premature growth-development transition, and a strong preference for the stalk pathway in chimeras with wild-type cells [20]. The behaviour of the mutants suggests that RblA is a credible link between the cell cycle and differentiation. In characterizing the CdkA mutants, Harry was assisted by Kimchi Strasser, a PhD candidate of Concordia University working in Harry's lab. This work, which revealed another cell cycle/differentiation connection, has mostly been completed but is unpublished.

The *rblA* transcript in differentiating spores is some 200-fold higher than in vegetative cells, implying a role in terminal differentiation. To probe this further, Harry roped in Gareth Bloomfield to generate DNA microarrays profiles for the *rblA*^{null} cells during growth and culmination. There was no stopping Harry when Gareth reported that the *rblA*^{null} profiles were the cleanest microarray data that he had analyzed and that the regulation of cell-cycle genes by RblA was unequivocal. Instead of confirming the microarray data using conventional approaches, Harry expanded the study by using the then newly introduced method of whole transcriptome sequencing using the RNA-Seq technology. In addition to confirming the micoarrays results, the RNA-Seq data showed that RblA regulates essentially all genes annotated to be involved in mitosis, DNA replication and DNA repair, as well as genes involved in terminal differentiation. Based

on the data, Harry contributed to the annotation of many cell cycle genes. He wrote and submitted the manuscript describing these results before his untimely death.

The RNA-Seq data represented a candy store for Harry. He devoured every bit of them. The data unleashed his hidden aptitude in statistics and his refined skills in critical analysis. Harry saw the deficiencies in the tools available for analyzing copious amount of short sequence reads. One major problem of existing tools is in assigning sequence reads that are homologous to multiple genes. Typically they are mapped to all of the homologous genes, which could significantly skew the normalized dataset if these sequences are abundant. Harry devised a scheme to bin these common sequences before assigning them to individual homologous genes proportionally based on the abundance of their unique flanking sequences. To make his method accessible to others, Harry recruited his son Asa, an informaticist working for Siemens in Germany, to program the various elements of his method. The manuscript describing this very useful tool has yet to be written up.

Harry's work was invariably original, elegant, thorough and thought-provoking. He was a rare person; always open to discussion, courteous, passionate, generous, and with very little ego. By his example he taught many, colleagues and students alike, the beauty and purity of science. If he had complaints about the politics of science and academia, he kept them unvoiced. Harry left behind many questions unanswered that had stoked his passion, data to be interpreted and manuscripts to be written. He also left behind colleagues, many of whom held Harry as a good friend, who miss his counsel and amity.

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