# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

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# Robert C. King: An Appreciation of His Work

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THE advance of scientific knowledge is a combined product of both the contributions of the individual scientist and the acceptance and authentication of his or her work over time by the larger scientific community. Robert C. King is one of the most distinguished contributors to advancements in genetics, cell biology, and developmental biology. In honor of Bob's seventy-fifth birthday, it seems appropriate to highlight some of his abundant accomplishments.

Among geneticists, Bob King is widely known as a distinguished author and editor of genetics books, but cell and developmental biologists also know him as an accomplished, often ground-breaking researcher. As a researcher, Bob is perhaps most widely recognized for his definitive characterization of the 14 developmental stages of Drosophila oogenesis (see King 1970), his beloved experimental system. It is largely through his early cytological work that we know about the origin and development of the *egg chamber*, the basic structural and functional unit of the Drosophila ovary. Briefly, the egg chamber is composed of 16 interconnected germline cells, the *cystocytes*, 15 of which differentiate as *nurse cells* and support the growth of the sixteenth cell, the *oocyte*, through cytoplasmic transfer via intercellular bridges, or *ring canals* (King 1970). Such oocyte-nurse cell syncytia are present in a majority of orders of higher insects. In Drosophila (and presumably in other insects possessing polytrophic meroistic ovaries), a cytoskeletal, membranous structure known as the *fusome* extends through the ring canals during the early stages of oogenesis and is thought to regulate the division patterns and differentiation of the germline cells (reviewed by De Cuevas *et al.* 1997; McKearin 1997). Complex interactions between these cells and the somatic *follicle cells* that surround them result in the formation of a mature oocyte (reviewed by Lasko 1994).

Bob and co-workers described the formation and early differentiation of the Drosophila egg chamber in the 1960s. Through painstaking three-dimensional (3D) reconstructions of serially sectioned egg chambers, they deciphered the pattern of cystocyte divisions and the formation of the ring canal system (Brown and King 1964; Koch and King 1966, 1969; Koch *et al.* 1967). During the following two decades, the formation of the fusome was unraveled (King 1979; King and Storto 1988; Storto and King 1989). The determination of the spatial relationship between the cells of the egg chamber and their interconnecting organelles was an enormous undertaking involving multiple arduous steps: plastic embedded egg chambers were serially sectioned and photographed at the light and electron microscope levels, composites were made from overlapping micrographs of each section, morphological information from serial composites was meticulously traced by hand on diffusion paper, and information from stacked tracings was then used to construct 3D plastic models of cell clusters.

In electron microscope studies involving painstakingly reconstructed *germaria*, the regions of germline cyst formation, Bob and colleagues identified the developmental stages in the formation of the 16-cell cluster (Koch and King 1966; Koch *et al.* 1967). They deduced from their observations that in the anterior region of the germarium reside a small number of single cells, the *stem cells*. Each of these divides asymmetrically to form two daughter cells, one of which proliferates indefinitely as another stem cell, while the other functions as a *cystoblast*, which undergoes four synchronous incomplete cytokineses to produce a complex, branched pattern of 16 interconnected *cystocytes* (Koch and King 1966; Koch *et al.* 1967).

In a previous ovarian reconstruction study, Bob King had coined the term *ring canals* to describe the "cytoplasmic pores" that interconnect sister cystocytes (Brown and King 1964). Subsequently, he described the formation of the ring canal system at the ultrastructural level in several insect species (Cassidy and King

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1969; Koch and King 1969; King and Akai 1971a; Kinderman and King 1973). Koch and King (1969) observed that in *D. melanogaster* ring canals formed around remnants of mitotic spindles and proposed that interconnected cells arose as a result of incomplete cytokinesis. They also showed that the precise pattern of cystocyte interconnections was linked to the lineage of the cells (Koch and King 1969). This study was the first to describe ring canal rim differentiation and to demonstrate that the oocyte was always one of two cells, the *pro-oocytes,* with four ring canals (Koch and King 1969). Diagrams of a sectioned germarium subdivided into morphologically and functionally distinct subregions (Koch and King 1966, Figure 1) and of a model showing steps in the formation of the 16-cell cluster (Brown and King 1964, Figure 7) are among the most frequently reproduced figures in insect oogenesis-related literature.

The genetic control of Drosophila egg-chamber formation and differentiation is currently the focus of genetic and molecular biological investigations in a number of labs, and Bob's observations have found strong support in recent studies. For example, the existence of germline stem cells and the asymmetric divisions of these cells and their daughters have been confirmed by laser ablation of stem cells (Lin and Spradling 1993) and by direct observations using recently identified molecular markers (Lin *et al.* 1994; Lin and Spradling 1995, 1997; Deng and Lin 1997; De Cuevas and Spradling 1998). Genetic mutations have been identified that affect stem cell division (Lin and Spradling 1997), that abolish the stem cell-to-cystoblast switch (McKearin and Ohlstein 1995), and that affect synchrony of cystocyte divisions (De Cuevas *et al.* 1996; Deng and Lin 1997). And two decades after Bob's cytological descriptions (Koch and King 1969), the molecular components of ring canals had started to be identified by using genetic and molecular biological approaches (Yue and Spradling 1992; Xue and Cooley 1993). Today the development of a functional ring canal is known to require the sequential assembly of several cytoskeletal proteins in the arrested cleavage furrow, and the directional transport of materials through these organelles is recognized as being integral to the formation of a normal oocyte (Robinson and Cooley 1996).

Bob King's contribution to the field of cell biology deserves special attention. Long before confocal microscopy and staining with multiple fluorescent antibodies, ovaries were stained in Bob's lab with a multitude of vibrantly colored dyes—Schiff reagent, fast green, orange G, azure B bromide, and various combinations of these. At specific concentrations and pHs, these dyes identified the distribution of macromolecules such as proteins, lipids, nucleic acids, or polysaccharides in the developing egg chamber and other tissues (King *et al.* 1957; King 1960; King and Koch 1963; Brown and King 1964; Butterworth *et al.* 1965; Cummings and King 1969). Many a student was "hooked" on Drosophila research after witnessing the aesthetic beauty of the colorful specimens under the microscope. Instruction in cytology and cytochemistry would follow later in both the lab and the classroom.

Cytochemical investigation was coupled to ultrastructural analysis of cytoplasmic and nuclear components (King and Koch 1963; Koch and King 1966, 1969; King and Akai 1971a,b; Johnson and King 1972, 1974; King *et al.* 1978; Bishop and King 1984; Storto and King 1989). Procedures for fixation, staining, and embedding were developed and perfected for optimum preservation of submicroscopic organelles (*e.g.*, spindle microtubules, synaptonemal complexes, ring canals, and fusomes), and electron micrographs were generated by the thousands with state-of-the art equipment. Through such work, Bob was actually taking part in creating and cultivating new domains in cell and developmental biology. The detailed morphological descriptions of the 14 developmental stages of Drosophila oogenesis (see King 1970), which are now widely accepted and serve as a foundation for all Drosophila oogenesis-related investigations, are an impressive example of the culmination of such endeavors.

This cytological work was not purely descriptive. What is remarkable about Bob's work is the creative, speculative intelligence with which he interpreted his observations to form hypotheses. Hypotheses were developed to explain why stem-line oogonia are restricted to the anterior region of the ovary, how the branching pattern of the cystocyte cluster arises, or why only one of the two pro-oocytes becomes the oocyte (Brown and King 1964; Koch and King 1966, 1969; Koch *et al.* 1967; King 1975, 1979; King *et al.* 1982). Furthermore, beginning as early as 1957 (King and Burnett 1957; King *et al.* 1957), Bob had the foresight to combine his cytological studies with analyses of genetic mutations for a powerful approach to understanding the genetic mechanisms underlying fundamental cellular processes. In doing so, he helped advance the field of developmental genetics. With the precise classification of the developmental stages of oogenesis (see King 1970) and the isolation of a large number of female-sterile mutations (reviewed by King and Mohler 1975; Spradling 1993; Lasko 1994), oogenesis in Drosophila is now one of the most thoroughly investigated model developmental systems in which complex cellular functions and interactions are deciphered through the proficient use of genetic mutations and cell and molecular biological techniques.

For insights into the genetic control of oogenesis, Bob studied numerous female sterile mutations. Particularly noteworthy is his work on some mutants belonging to the *ovarian tumor* class (*e.g., otu, fs(1)1621, fu,* and *fes*), in which cystocytes appeared to undergo complete, rather than incomplete, cytokinesis and in which nurse cell and oocyte differentiation were abnormal (King and Burnett 1957; King *et al.* 1957, 1961; Koch and King 1964; Smith and King 1966; King 1969; Johnson and King 1972; Gollin and King 1981). His interest in these mutants reflected both his desire to understand the genetic control of germline cytokinesis, as well as his belief that the underlying cause of aberrant germ cell division and differentiation in some ovarian tumor mutants was abnormal fusome formation.

One of the most extensive genetic and cytological characterizations involved the *otu* gene (King *et al.* 1978, 1986; King 1979; Dabbs and King 1980; King and Riley 1982; Bishop and King 1984; Rasch *et al.* 1984; Storto and King 1987, 1988, 1989). Bob and colleagues mapped the *otu* gene genetically and subdivided the variety of ovarian phenotypes exhibited by *otu* alleles into three classes on the basis of morphological criteria (King and Riley 1982; King *et al.* 1986). Careful and systematic analyses of >100 different heterozygous combinations of *otu* alleles with each other and with a deficiency led Bob to propose that the *otu* gene product is made early during oogenesis, acts at several subsequent stages, and is required at a higher concentration at each successive developmental period. The concentration of the gene product determines the stage at which oogenesis is disrupted in the mutants (King and Riley 1982; King *et al.* 1986; Storto and King 1987, 1988; King and Storto 1988). His genetic analyses also suggested that *otu* produces two gene products, which combine to yield fertile flies in some heteroallelic combinations (King *et al.* 1986; Storto and King 1987, 1988). These conclusions were later verified by molecular studies, particularly those of Laura Kalfayan and co-workers, who cloned the *otu* gene and characterized it molecularly (Mulligan *et al.* 1988; Steinhauer *et al.* 1989; Comer *et al.* 1992; Steinhauer and Kalfayan 1992; Sass *et al.* 1993, 1995). In fact, in an article that identified two *otu* protein isoforms and analyzed their expression patterns, Steinhauer and Kalfayan (1992, p. 240) observed, "It is remarkable that the hypothesis developed by King and his colleagues from genetic and morphological analyses should, for the most part, predict observed molecular data so closely."

It was also during the 1970s and 1980s that Bob focused his attention on the formation of the *polyfusome* (a term he applied to the mature, branched fusome) and made deductions regarding the role of this organelle in the formation of the cystocyte cluster and its differentiation (King 1979; King *et al.* 1982; King and Storto 1988; Storto and King 1989). Bob's recognition of the developmental significance of the polyfusome epitomizes his exceptional insight and ability to ask pertinent questions, to form hypotheses, and to combine his expertise in genetics and cytology to seek answers to developmental questions.

The fusome had been observed in a variety of insects and was described as cellular material that contained spindle residue and that extended through the ring canals during cystocyte divisions (see Telfer 1975). Bob built on these observations through electron microscope 3D reconstructions and analysis of genetic mutations and proposed that in Drosophila, fusomes serve to arrest cystocyte cytokinesis, synchronize and restrict the number of mitotic divisions, and affect the orientation of the mitotic spindle (King 1979; King *et al.* 1982; Storto and King 1989). He also had the insight to suggest that this organelle may function as an oocyte determinant (King *et al.* 1982).

In a widely cited article, Storto and King (1989) used electron microscope 3D reconstructions to analyze fusome structure in wild-type and *otu* mutant ovaries. They showed that most germ cells in *otu* ovarian tumors either were single or occurred in clusters of two to three interconnected cells. These cells contained structurally aberrant fusomes, and cystocytes never differentiated into pro-oocytes or nurse cells. These observations supported Bob's belief that an intact polyfusome system was necessary for the production of a branched chain of cystocytes and for their subsequent differentiation. These findings were later confirmed by Rodesch *et al.* (1997), who showed that fusomes in *otu* null mutants were aberrant both structurally and in their molecular composition.

While the precise function of the polyfusome still remains to be identified, the quest for this function is now the focus of research by a new generation of scientists. Recent genetic and molecular biological analyses have identified numerous molecular components of the fusome (reviewed by De Cuevas *et al.* 1997; McKearin 1997), and some of Bob's conclusions have been corroborated by immunocytochemical and genetic analyses. For example, his observation that one pole of the spindle lies embedded in the polyfusome during cystocyte divisions has been confirmed by simultaneous staining with microtubule- and fusome-specific antibodies (Lin *et al.* 1994; Lin and Spradling 1995, 1997; Deng and Lin 1997; McGrail and Hays 1997; De Cuevas and

Spradling 1998) and his proposal that fusomes synchronize cystocyte divisions is supported by the findings that cells without fusomes divide asynchronously (De Cuevas *et al.* 1996; Deng and Lin 1997) and that various proteins involved in cell cycle regulation associate with the fusome (reviewed by De Cuevas *et al.* 1997). His suggestion that "nonrandom distribution" of fusomal material is related to oocyte determination has found partial support in direct observations of fusome asymmetry during cystoblast and cystocyte divisions (Lin *et al.* 1994; Lin and Spradling 1995; De Cuevas *et al.* 1996; Deng and Lin 1997; De Cuevas and Spradling 1998).

Bob King was born in New York City in 1928 and received his Ph.D. in Zoology from Yale University in 1952 when he was just 24 years old. After a few years at Brookhaven National Laboratory in Upton, New York, he accepted an assistant professorship in 1956 in the Department of Biology at Northwestern University in Evanston, Illinois. At Northwestern he taught undergraduate and graduate courses in genetics, developmental genetics, cell biology, and cytology and established his long and distinguished research and writing career. He became a full professor in 1964 and is currently an emeritus professor in the Department of Biochemistry, Molecular Biology, and Cell Biology at Northwestern.

Bob has published 117 articles and review articles related to the genetic control of insect oogenesis and is the author, co-author, and editor of several books, including *Genetics* (Oxford University Press); *Handbook of Genetics* (Volumes 1–5, Plenum Publishing); six editions of *A Dictionary of Genetics* (Oxford University Press); *Ovarian Development in Drosophila melanogaster* (Academic Press); and *Insect Ultrastructure* (Volumes 1 and 2, Plenum Publishing). He is currently working on the seventh edition of his *Dictionary of Genetics*, an interdisciplinary reference work that has become the standard supplementary text for students and researchers in classical and molecular genetics. The *Dictionary* also includes one of the most thorough chronologies of genetic discoveries available in the literature. Bob's varied interests and contributions to interdisciplinary fields are also reflected in his long list of society memberships, among them the American Association for the Advancement of Science (Fellow), the Genetics Society of America, the American Society for Cell Biology (a founding member), the Society for Developmental Biology, and the Histochemical Society.

Bob King is a distinguished scientist who has enhanced scientific knowledge through sheer hard work, as well as a humanist who has embraced people from diverse backgrounds and encouraged and supported those he knows to reach their full potential. Many of his former students have established successful careers of their own—William Klug, Michael Cummings, Elizabeth Koch, Francis Butterworth, and Susanne Gollin among them. His boundless energy, regard for others, and enthusiasm for his work and life in general took him to countries around the world at a time when the world was not quite "a global village." He presented papers at international symposia in Australia, Czechoslovakia, France, and Canada and worked with international scientists in Edinburgh, Tokyo, and Seoul. Students, postdocs, and colleagues were inevitably the beneficiaries of lessons in geography and foreign cultures as an added bonus of his trips abroad. Bob is endowed with a self-effacing sense of humor and great wit. Frank Butterworth recalls a "fly party" where all dressed up as various mutants, but Bob arrived wearing a business suit. Just as his lab thought he had forgotten his costume, Bob took off his jacket to reveal that he had his pants on backward. He was *rotated abdomen*.

For his vital contributions to the fields of genetics, cell biology, and developmental biology, and for his selfless devotion to students and fellow scientists, Bob King has earned our profound gratitude, our congratulations, and our best wishes.

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