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PERSPECTIVES

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One hundred years of centrioles: the Henneguy–Lenhossek theory, meeting report

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Over three sun-dappled days last summer (June 24–26, 1998), an international conference took place in the Lederle Graduate Research Tower of the University of Massachusetts at Amherst, with funding provided by the Richard Lounsbery Foundation, New York City. Its title marked the centennial of centriole-kinetosome research and commemorated the two founders of the field: Mihaly Lenhossek (1863–1937), the Hungarian neurobiologist and relative of conference attendee Andrew Szent-Györgyi, and prominent French histologist Louis Félix Henneguy (1850–1928).

The enigmatic centriole, a barrel-shaped structure that sits at the mitotic poles in nearly all animal cells, presents a wide variety of form and function in different taxa. Well known as the site of astral microtubule emergence, the same [9(3)+0] microtubule structure, the kinetosome (or basal body), subtends all undulipodia (eukaryotic flagella and cilia). This polyfunctional organelle has even been shown to confer cell polarity throughout the cell cycle, by determining the number, orientation and polarity of mitotic and interphase microtubules, even in breast cancer cells [8]. Because of the near-ubiquity and identical ultrastructure of centriole-kinetosomes (C-Ks) across eukaryotic taxa, together with the highly conserved nature of the C-K proteins centrin and tubulin, evolutionists believe that C-Ks originated in the earliest eukaryotes, perhaps as long as 2000 million years ago. Subsequent divergence has led to a myriad of C-K-associated elaborations, from the calcium-modulated spasmoneme of *Vorticella* to the paraxonemal rod (transverse undulipodium) of *Peridinium* and the cochlear kinocilium of our inner ear sensory epithelium. As mitotic structures, centrioles are altogether absent from some major taxa (e.g. plants, yeasts, rhodophytes [red algae], and cellular slime molds). As basal bodies of undulipodia, kinetosomes occur in the vast majority of eukaryotic phyla. Amoebomastigotes such as *Naegleria*, however, produce and resorb kinetosomes with every life history transition between amoeboid and mastigote phases [7]. *Uni* mutants in *Chlamydomonas* [5] and "monster" ciliates with inverted orientation of the infraciliature [13], respectively, have shown that a nuclear mutation can delay kinetosomal reproduction in one system, while in another, kinetosome reproduction is partially independent of nuclear control. In short, C-K-related controversy has mushroomed over the past 100 years in concert with our growing appreciation of the bewildering number of variations on the theme.

In April 1898, Lenhossek published a paper entitled "Über Flimmerzellen" wherein he showed that kinetosomes, the bodies at the bases of the rat sperm tails, were identical to those of cilia lining the epididymis. He further proposed that these kinetosomes were identical to centrioles, and that from the centrioles originated the spindle. In the same month Henneguy, working on silkworm spermatocytes, independently published the same interpretation in *Archives d'Anatomie Microscopique*, Tome I. The two founders of C-K theory courteously agreed to equally share the credit. Their contribution, that mitotic centrioles and ciliary basal kinetosomes are essentially the same structure, descends to us today as Henneguy-Lenhossek theory. As anticipated by these 19th century scientists, we can now say that centriole-kinetosomes give rise to all undulipodial shafts (i.e., axonemes) as well as to microtubular elements of the cytoskeleton.

The conference began on the evening of Wednesday, June 24, with open discussion entitled "The centriole's new clothes: a century of controversy at the cell center," moderated by Chandler Fulton of Brandeis University. Discussion centered around the perplexing questions that still remain after many lifetimes of research: How did centriole-kinetosomes first evolve? Why are they absent in many taxa? What kinds of selection may have driven their secondary loss in plants, yeasts

and slime molds? How do they reproduce in the living cell, and to what degree are they controlled by nuclear genes? Do C-K's have their own genes or any remnant of a genetic system? The fewer than two dozen attendees, with few exceptions, were all participants. They constituted an international quorum of scientific writers and historians, cell motility researchers and evolutionists from Spain, Canada and the United States. Over the next two days, this remarkable interdisciplinary milieu imparted a unique atmosphere to the proceedings. It is hard to imagine another organelle which could unite the interest of such diverse scholars from such far-flung home universities.

The morning of Thursday, June 25 was devoted to History and Philosophy, a session chaired by Michael Dolan of the University of Massachusetts. The talk of Andrew Szent-Györgyi (Brandeis University, Department of Biochemistry) was entitled "My cousin Albert and his uncle". He traced the history of biological and political achievement in the Szent-Györgyi and Lenhossek families back over four generations. Andrew's cousin, Nobel prize winner Albert Szent-Györgyi (1893–1986), discoverer of vitamin C and metabolic intermediates of the Krebs cycle, was the nephew of Mihaly Lenhossek. Owing to Albert's poor showing in grammar school, the uncle (a dominant family man in a newly illustrious Austro-Hungarian family and prominent neurohistologist) predicted no future for the nephew in science, though conceded he might make a good proctologist or cosmetologist! Dr. Szent-Györgyi devoted most of his lecture to a chronicle of scientific achievement on the Lenhossek side of the family, which included key roles in bridging Buda and Pest and in founding the Hungarian Academy of Sciences. The C-K theory of Mihaly Lenhossek, as mentioned above, is only one of many significant achievements of the family.

Following Dr. Szent-Györgyi's vivid historical background, historian of science Jan Sapp (York University, Toronto) presented an overview of "History of Henneguy-Lenhossek centriole-kinetosome theory" from a broader perspective. Dr. Sapp outlined two main periods of centriole research, the first from 1887–1954, when these structures were iron hematoxylin-staining dots whose visualization was limited by the light microscope. The second period dates from 1954 to the present, as the electron microscope and molecular genetics enabled new and more fundamental research problems to be addressed. For example, in the 1920s it was suggested that perhaps plant centrioles were present, but too small to resolve. Recent in situ hybridization studies [7] coupled with years of electron microscopic observations in which glutaraldehyde was fixative have confirmed their absence. Electron microscopic studies support and refine Henneguy-Lenhossek theory. Centriole-kinetosome can be structurally and uniquely defined: 3 sets of 24 nm-sized tubules surround a lumen, the outside diameter is 250 nm. But central questions about centriole–kinetosome replication and function have never been answered. Of particular interest was Dr. Sapp's elucidation of the definitional controversy of the 1970s, during which Jeremy Pickett-Heaps challenged the conventional zoocentric view that centrioles are essential to mitosis [14]. Pickett-Heaps introduced the concept of the microtubule organizing center (MTOC), and proposed that centrioles were simply variant MTOCs.

Lynn Margulis (conference host, University of Massachusetts) next spoke on "Symbiogenesis" [15, 16], reviewing the frontiers of symbiogenetic theory of organelles whose evolutionary origin is in question, such as hydrogenosomes, peroxisomes and C-Ks. Dr. Margulis began with quotes from early symbiosis researchers such as "for there is no middle ground between symbiosis and nonsymbiosis. Either symbiosis with cyanophyceae exists, and then one has plants, or it does not exist, and then we have animals" (Konstantin Sergeivich Mereschkowsky in [6]). She next reviewed empirical evidence for nuclear-independent division and function of organelles, and posed the question, "are archaeprotists products (amitochondriate eukaryotes) of symbiogenesis?" [9, 10].

The afternoon session on June 25, entitled "Protist Genetics," was chaired by Dennis Searcy (University of Massachusetts). The two speakers, Michael Adams (Eastern Connecticut State University) and John Hall (The Rockefeller University) respectively discussed the molecular and transmission genetics perspectives on *Chlamydomonas* and C-Ks. Dr. Adams described *Chlamydomonas* as an excellent system for study of organellar genetics, since it is easily induced to enter the haploid (+/–) life cycle stage in which an individual inherits 100% of its organellar and nuclear genetic material from the same parent, i.e. the + and – strains are uniparental. The *uni* mutant, whose genetic defect on chromosome XIX gives rise to a cell with only one undulipodium, and others such as *vfl* (variable flagella) and *fla* (defect in flagellar assembly), have become workhorses of C-K genetics. A Mendelizing *uni* linkage group has been identified based on several sets of characters useful in transmission studies.

Dr. Hall enlarged on the characterization of the *uni* and *fla* defects, tracing structural defects in the undulipodial assembly units which prevent the mutant cell from swimming or from replicating its one "parent" undulipodium (in 95% of cases, *uni* mutants' one undulipodium is *cis* to the eyespot). Dr. Hall also discussed his DNA fluorescence studies, conceived as part of the search for autonomous C-K DNA but more recently interpreted as staining elements of the nucleus which adhere to the kinetosome during replication. That the "parent" kinetosome certainly can not always serve as a template is suggested by the complete disappearance of C-Ks after fertilization, then de novo reappearance in the zygote [4].

Chandler Fulton (Brandeis University) next delivered a commentary entitled, "*Naegleria* makes new centrioles and counts—but won't tell us how!" This common freshwater amoebomastigote forms and resorbs C-Ks with every life history shift between vegetative amoeboid and temporary mastigote forms. Dr. Fulton has been able to isolate mutants with zero or

with extra undulipodia, instead of the usual two; heat shock at 37°C produced cells with up to 17. During differentiation, kinetosomes form first as a single unit, and then two; using inhibitors, cells can be arrested at the one kinetosome stage. Thus the cells somehow count from zero to one to two. Dr. Fulton discussed roles of motility proteins centrin and tubulin in C-K development [7]. The afternoon closed with commentary by Dr. Sapp on the historical perspective.

The morning session on June 26, chaired by Luis Vidali (University of Massachusetts), was entitled "Protists: Morphogenesis and the Fossil Record." Joseph Frankel (University of Iowa) spoke on "Kinetosomes of ciliates: masters or slaves?" Ciliate kinetosomes are arranged in regular rows called kineties, whose collective arrangement forms a lattice with left- or right-handedness (polarity). A fact of great interest to general biological thought is that orientation of the ciliary lattice is independent of nuclear control in ciliates, either micro- or macronuclear. Kineties of offspring cells generally have the same polarity as those of the parent. When conjugating ciliates divide prematurely, i.e. before separation of the conjugants, sometimes the partner cell's kineties are mistakenly used as templates (*structural guidance*, [1]). Such a cell divides and retains the inverted kinety, producing a de facto cortical graft without surgery, which demonstrates the DNA-independent nature of ciliate cortical inheritance. Dr. Frankel proposes that an additional global control system, independent of the nuclei, determines the positions of cortical landmarks in offspring cells.

Commentary followed by John Hall on the ciliate genetic perspective, David Nanney (University of Illinois, Urbana) on "Ciliate cortical behavior" [12] and Mark McMenamin (Mt. Holyoke College) on "Eukaryote origins and the Proterozoic fossil record" [11].

The afternoon session on June 26, chaired by Michael Chapman (Clark University), was entitled "Development as Community Ecology." The first speaker was Ricardo Guerrero (University of Barcelona) who spoke on "Modern Microbial Communities." Dr. Guerrero studies microbial mats and sulfureous lakes such as Lake Cisó, where different species of bacteria (e.g. *Chromatium*, *Chlorobium* spp.) perform coordinated interdependent metabolic tasks in a diurnal photoperiodic cycle, using sunlight for energy and hydrogen sulfide as an electron source. Mat communities, which can consist of up to seven multicolored layers of different bacterial species, are comparable to those of the earliest living organisms, as demonstrated by fossilized microbial mat formations (stromatolites) of the ancient cyanobacteria which gave rise to the oxygen atmosphere [2].

The next speaker, Michael Dolan (University of Massachusetts) discussed his research in termite hindgut protists in a talk entitled, "Calonymphid evolution and differential centriole-kinetosome numbers per cell." Termite symbionts such as *Calonympha* and *Snyderella* are interesting subjects for C-K research, since they have variable numbers of kinetosomes and nuclei per cell. Some kinetosomes are parts of collective structures called "karyomastigonts," which include kinetosomes, nuclear connectors, parabasal bodies (Golgi) and nuclei; other such structures, the "akaryomastigonts" are the same in all respects (same numbered configuration of kinetosomes, Golgi, etc.) except that they lack nuclei. In calonymphids, C-K reproduction occurs and a paradesmose (thin spindle) links offspring nuclei as all the nuclei—up to hundreds—in a single cell divide. Michael Dolan has treated calonymphids with various DNA-specific stains. He has also demonstrated accumulation of DAPI in the Golgi apparatus. No conclusive akaryomastigont signal attributable to C-K-specific DNA has been found, in fact Dolan suspects the genetic determinants of the C-Ks are in the nucleus even in these amitochondriate cells. However, the fact that organellar gene sequences of prokaryotes are present in the nucleus does not preclude a symbiotic model for C-K origins.

The next speaker, Radhey Gupta (McMaster University), presented molecular sequence data interpreted as evidence against the three-domain model of life (Archaea, Bacteria and Eukarya, [17]). Through sequence comparisons of several highly conserved proteins (e.g., Hsp70, glutamine synthase I, asparaginyl tRNA synthase, diaminopimelate epimerase), Dr. Gupta has shown a close evolutionary relationship between archaebacteria and Gram-positive bacteria, i.e., those bacteria bounded by a single membrane. In contrast, all true Gram-negative bacteria (those bounded by two different membranes, with an enclosed periplasmic space) form a distinct clade. His new proposal recognizes only two domains of life, Prokarya and Eukarya, with a subdivision of Prokarya into Monoderm (single-membrane) and Diderm subdomains [3].

The final speaker of the conference was Dorion Sagan of Sciencewriters, who used a pack of playing cards to illustrate the relationship between parts and the whole.

Just as symbiogenesis theory has shown the genetically conglomerate nature of eukaryotes, our concept of the individual must now be expanded to include technology. The Internet, satellite links and other global communication devices unite our planet in a living phenomenon that transcends life science. As Dorion Sagan terms the phenomenon, "superordination" has made one large de facto organism of us all.

In summary, "One hundred years of centrioles" marked the centennial of research into a puzzling organelle, whose function and evolutionary origins are still fertile ground for new studies in cytology and molecular genetics.

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