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Hans Ris (1914-2004). Genophore, chromosomes and the bacterial origin of chloroplasts

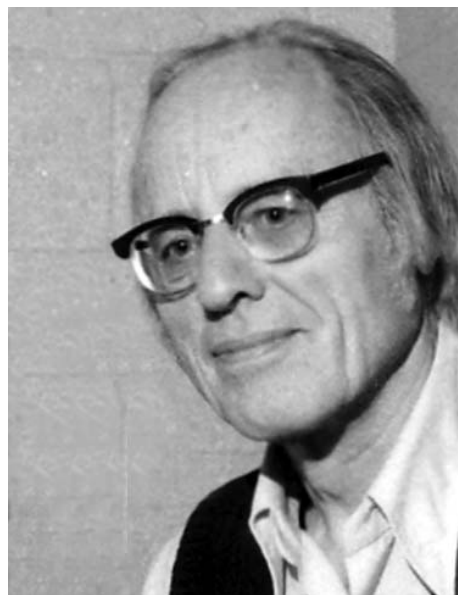
Hans Ris (Fig. 1) professor of biology at the University of Wisconsin in Madison, once described his scientific work saying: "My research interests include levels of organization in chromosomes, [...] evolution of mitotic mechanisms, high voltage and stereoscopic electron microscopy [...] cytoplasmic filament systems, use of low voltage-high-resolution scanning microscopy in the study of nuclear pores, the nuclear pore complex and of cytoplasmic structure [1]." His studies of the structure of genetic material led him to be the first modern scientist to document the similarity, if not identity, between the nucleoids of cyanobacteria (then called "primitive plants", "Cyanophyceae", "cyanophytes" or "blue-green algae") and those of the chloroplasts of algae (e.g. *Chlamydomonas* [4]).

Ris's meticulous life's work to compare genetic material by microscopy analysis included studies of a wide range of organisms: the Rocky Mountain spotted fever and flea-borne typhus bacterium, *Rickettsia*; the kappa particle in the cytoplasm of *Paramecium aurelia* (this particle is now known to be the prokaryote *Caedibacter*); the kinetoplastid-bearing protist associated with certain tropical disease symptoms, *Trypanosoma*; an apicomplexan correlated with fetal abnormalities, *Toxoplasma*. Ris' favorite comparisons of genetic material were of the chromosomes, nuclear membranes and cytoskeleton of eggs and sperm cells of animals including sea urchins, frogs, toads and salamanders. His long experience with comparative chromosomal cytology developed his acute awareness of the difference between the thin (2.5 nm diameter) all-DNA fibrils compris-

ing the nucleoids of bacteria and the much thicker (10–30 nm diameter) protein-studded DNA that makes up the chromatin of animals, plants and nearly all other eukaryotes [2].

Ris was especially interested in the peculiarities and oddities of insect chromosome cytology. With Sally Hughes-Schrader at Columbia University in New York City he studied chromosomes in an aphid species in which the offspring of fertilized eggs develop into females. Males develop parthenogenetically from unfertilized eggs. In the males (which are XO), spermatocytes contain one oversized X chromosome and cell division is very unequal. The chromatids (half-chromosomes) of the large X chromosomes do not separate from each other in the first meiotic cell division in the testes. Rather the distinctive spermatocyte's large X chromosome associates with the membranes, fibrils and microtubules of cytokinesis (cytoplasmic division). The entire whole X chromosome, with both chromatids still attached to each other at the kinetochore, stretches along the axis of the spindle fibers, parallel to them. One resulting offspring sperm cell wins the competition for the X and fertilizes the egg. The other cell product

in this first meiotic cell division is very small and inevitably dies. By study of spermatogenesis in these and other spermatocytes, Ris established that the normal, if unusual, cycle of events in chromosomes in this modified male meiosis determines the life history of these aphids. He was thus reinforced in one of his strongest educational dicta: "The lesson I learned was that, whenever feasible, we must include the



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Fig. 1. Hans Ris (1914-2004).

study of living cells" (p. 3 in H. Ris, *A Life Remembered*, 1994).

He also wrote that "Dr. Dingh in biochemistry at Wisconsin was studying nitrogen fixation in blue green algae [...] We decided to study their cell organization by electron microscopy [...] Electron microscopic analysis showed a striking resemblance between chloroplast structure and cell organization of blue-green algae." He studied, too, "kappa" a "cytoplasmic gene" in *Paramecium*. This "non-nuclear genetic factor" in the cytoplasm (to which Tracy Sonneborn, genetics and zoology Professor at the University of Indiana had drawn his attention) determined the transmission of the killer trait in these ciliates. Ris wrote of the kappa particle: "it had a bacteria-like ultrastructure."

Persistent and dedicated comparative ultrastructural studies such as these, especially in photosynthetic organisms with their distinctive thylakoid membranes, inspired Ris to uncharacteristically generalize, as he was never prone to over-interpretation or generalization. "In 1960, at the International Congress of Cell Biology, I reported these observations, which strongly supported the old idea of endosymbiotic origin of mitochondria and chloroplasts." His work at the time rekindled concepts from Russian and German literature made known in the English-speaking world by E.B. Wilson's book *The Cell in Development and Heredity*. Wilson had collected studies that extended back to the 19th century that photosynthetic organelles of plant cells originated from bacteria.

In his advanced cytology graduate class, Ris used to read aloud to us, a few dedicated students, from Wilson's book. In these classes and lab courses (which he taught himself), he displayed his deep European-style knowledge of zoology, anatomy and of course, cells science. I had studied non-Mendelian inheritance of genetic factors including the maternal inheritance of the green color in *Chlamydomonas*, *Epilobium* and other algae and plants. Thus when Ris read to us: "More recently Wallin (1922) has maintained that chondriosomes [old name for mitochondria] may be regarded as symbiotic bacteria whose associations with other cytoplasmic components may have arisen in the earliest stages of evolution [...] To many, no doubt, such consideration may appear too fantastic to mention in polite scientific society; nevertheless, it is in the range of possibility that they may some day call for more serious consideration..." the course of my professional life was set forever!

Hans Ris was born in Bern, Switzerland, in 1914, the eldest of three sons. His father and his father's older brother, who became a schoolteacher, had lost both their parents at a young age. They grew up in an orphanage near Bern. Ris' father became a businessman who, when Hans was six years

old, bought a coal and wood delivery business. His mother was raised on a mountain village farm where, as a child, Hans often spent his school vacations. She was a photographer who eventually bought and managed an elegant hat shop, and married in her mid-thirties. Hans remembered her as a "warm, caring person, intelligent, adventurous and very independent". She died when he was only 15 years old.

Ris' love of nature, interest in science generally and biology in particular began early. From age ten he spent his free time roaming in the woods around Bern. "Observing, listening, I became fascinated by the diversity and beauty of living things." By the year his mother died he had built his first microscope that magnified about 400 times. He made it of "cigar boxes of my father and cardboard from old school books." Young Hans had obtained lenses and a set of instructions from a German popular science magazine. "And what magnificent Secret Gardens it opened for me! Here was born my passion to explore the world beyond human vision!" In the early 1930s Ris discovered the writings of the artist, scholar and activist Ernst Haeckel, the champion of Darwinism in Germany. "This led to an agonizing revolution in my beliefs about nature, man and universe. In Sunday School I had been taught that God created the world, plants, animals and finally, in his image 'man'; and he told man: go and multiply and exploit my creations for your benefits. Now I learned how scientific investigations revealed a very different concept: the unity of all life. Man originated by the same processes that over millions of years produced the stunning diversity of living forms. Science teaches us that all living creatures are our brothers and sisters. At age 16 I decided I would turn to science when creating my own world view" (p. 1 in: H. Ris, *A Life Remembered*, 1994).

At Bern University Ris earned a "Diploma of High School Science" so that he might be able to teach even though his real goal was to become a *Naturforscher* (a naturalist). Fritz Baltzer, an outstanding teacher of his, did two things to excite his curiosity for experimental biology: Baltzer provided Ris with salamander sperm and eggs, showing him how the eggs might be enucleated and asked him to do experiments to help understand the role of the nucleus in development. "This was my introduction to the designing of experiments to answer significant questions. Secondly, in the spring of 1937 he took me along with the staff of the [Bern University] Zoological Institute to Banyuls, a French marine biological station near the border of Spain and France. [...] During two weeks in [Laboratoire Arago of CNRS, France at] Banyuls [-sur-Mer] we collected and studied marine organisms, but most importantly we obtained sea urchin eggs and sperm and observed fertilization and cleavage in life. This experience later led to my interest in chromosomes and mitosis."

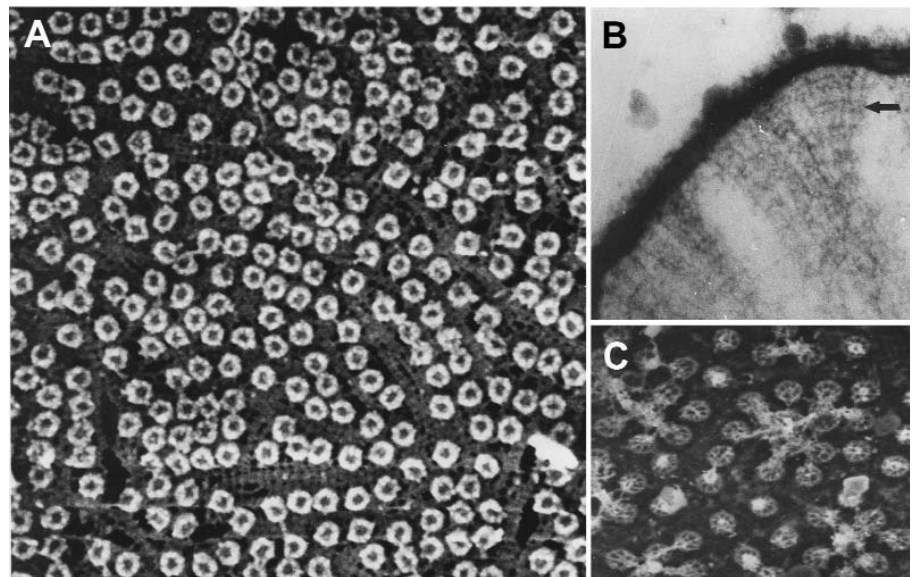


Fig. 2. Nuclear pore complexes and cables. (A) Newt (*Notophthalmus viridescens*) oocyte, external view of the egg cell membrane, low voltage SEM. (B) *Xenopus laevis* hollow nuclear cables of the pore-studded nuclear egg cell membrane, thin section TEM. (C) *Xenopus laevis* egg-cell nucleus. Isolated oocyte membrane shows that tops of the “fish trap” pore-studded structure are connected by the hollow nuclear cable system.

His academic advisor in Bern, Ernst Hadorn, obtained a fellowship for Ris to study at the University of Rochester in upstate New York. In 1939 a young instructor at Rochester (Kenneth Cooper) arranged for Ris to receive a teaching assistantship at Columbia University in New York City with Cooper’s former PhD professor: Franz Schrader. Thus began Ris’ important work with Sally Hughes-Schrader, wife of Franz and fine experimental scientist from whom Ris claims to have learned more than he did from anyone else at Columbia. After he received his PhD in 1942 at Columbia he was hired as an instructor at Johns Hopkins University in Baltimore. When he was 33 he spent a month at the Bermuda Biological Station where he was able to obtain a large variety of tissues, eggs and sperm from marine animals. With the use of cytophotometric methods he measured the DNA content in many species. He found that in members of any given species, the amount of DNA was the same for nearly all cells. Indeed, the amount of DNA per chromatid (half-chromosome) for any animal or plant varied a lot but it was constant in the cells of all members of a given species. Since the amount of DNA per chromosome set was constant and the number of chromosome sets in egg and sperm was half that in the somatic tissues, Ris elegantly demonstrated that sperm and eggs contain half the amount of DNA as do somatic (body) cells. This and much other work he summarized in an important review [3]. This work, together with contemporary studies by Swift and Pollister, strongly reinforced the notion that DNA was the genetic material, 3-4 years before the Hershey-Chase experiment.

From 1969 until 1984 Ris was director of the High Voltage Electron Microscope Facility supported by the National Center for Research Resources–National Institutes

of Health (NCR) at the University of Wisconsin Madison. Many regarded him as the finest microscopist in the world and certainly one of the most innovative. He was a founder and an active member of the American Society for Cell Biology throughout his life.

Ris was hired to the faculty of the Zoology Department in Madison in 1949 and at his death he was Professor Emeritus in that same institution. Although always attending to many other projects his passion was the structure of DNA in living cells. His work in recent years with two collaborators: Soo Siang Lim (now at the National Science Foundation in Washington DC) and Marek Malecki (still at the University of Wisconsin-Madison) involved details of nuclear pores, pore complexes and their associated hollow cables. These electron micrographs that we found in his Madison office filing cabinet drawers (he called them his “treasure-chest”) give some idea of the quality of his work. The definitive manuscript on the three-dimensional structure of the nucleus of the amphibian egg, work Ris began before 1979, will be published posthumously as soon as possible by his co-author, Malecki. The photographs show a stunning pattern of the nuclear pore complexes by low voltage scanning electron microscopy correlated with thin-section transmission electron-micrographs and stereomages of the same pores obtained in high voltage electron micrographic images (Fig. 2). His son found the magnificent manuscript of the joint work with Malecki nearly finished both on Ris’ computer and in a file labeled “manuscript” on a shelf at home [H Ris, M Malecki in *Xenopus* oocyte nuclei, branching hollow cables connect the nuclear interior to the intranuclear components of the pore complexes (fish traps). Manuscript in preparation].

Ris regretted, he told me on several occasions, that his insistence to limit the word “chromosome” to eukaryotes (because no prokaryote has them) tended to be ignored. He strongly urged the use of the word “genophore” which means “gene-holder” instead of “chromosome” to refer to the genetic behavior and correlated DNA fibrils of bacteria. In all prokaryotic cells genophores are inferred from experiments with bacterial genetic recombination by conjugation, plasmid insertion, viral replication and other transmission genetic techniques [5]. Ris always referred to genetic material of prokaryotes, as seen in electron microscopic thin section, as the “nucleoid”, a nucleus-like structure. Nucleoids are never nuclei since they never contain chromatin nor do pore-studded membranes bound them. Nuclear double-membranes with their pore complexes surround at least two *bona fide* histone protein-rich chromatin-chromosomes per eukaryotic cell. Nuclei, pores, microtubules and chromosomes are lacking in bacteria (whether archaeobacteria or eubacteria). For Ris “chromosome” always refers to eukaryotic structures inside nuclei that are present in numbers proportional to cell ploidy. In animal and plant cells chromosomes stain pink with the Feulgen reaction, a Schiff-base forming reaction. Chromosomes comprised of histone and other proteins complexed to the DNA are entirely lacking in all bacteria at all times. In nucleated cells chromosomes attach to microtubules, made of tubulin proteins, at kinetochores. Even in some marine protists, the dinoflagellates, whose peculiar chromosomes (made of bacterial-like 25 nm-small fibrils that are histone-depleted) standard nuclear membrane and microtubules accompany protist mitosis. The French investigator, Marie-Odile Soyer-Gobillard at Banyuls-Sur-Mer, worked out the peculiarities of dinoflagellate chromatin and mitosis in collaboration with Ris. Soyer-Gobillard notes that “without Ris’ visits, his intellectual and electron microscopical aid”, she could not have accomplished her wonderful work on dinoflagellate mitosis. Ris enjoyed an international reputation for his advice and help to women and very young scientists.

Ris was very disappointed when his appropriate name for the “bacterial chromosome, the “genophore”, a term he coined, was not generally known or, if known, not accepted by important microbiologists. Ris, of course, was correct about the profound differences between the proteinaceous chromatin of eukaryotes (chromosomes) and the genophore DNA fibers in the nucleoids of prokaryotes. Kind of tricky as the word chromosome came from their staining by dyes that are specific for the protein component, so bacterial nucleoids/genophores are not typically “chromo-”. I think he was right to use the term “genophore”, esp. since the suffix –some (body) was not apparent as it is in the mitotic or meiotic chromosomes of the *Eukarya*. In his terminology and profound understanding of genetic systems I have always tried to follow his lead. “Graduate students”, he wrote, “were always an important component of my research lab. I chose students who were independent and developed their own projects. I considered them collaborators, not members of a team. Science is not a soccer game.”

At the end of 2004 the world of biology lost a superb scientist, an excellent and dedicated teacher, a scholar of the first order and a true lover of nature. His colleagues and friends, his former students and family sorely miss him.

References

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