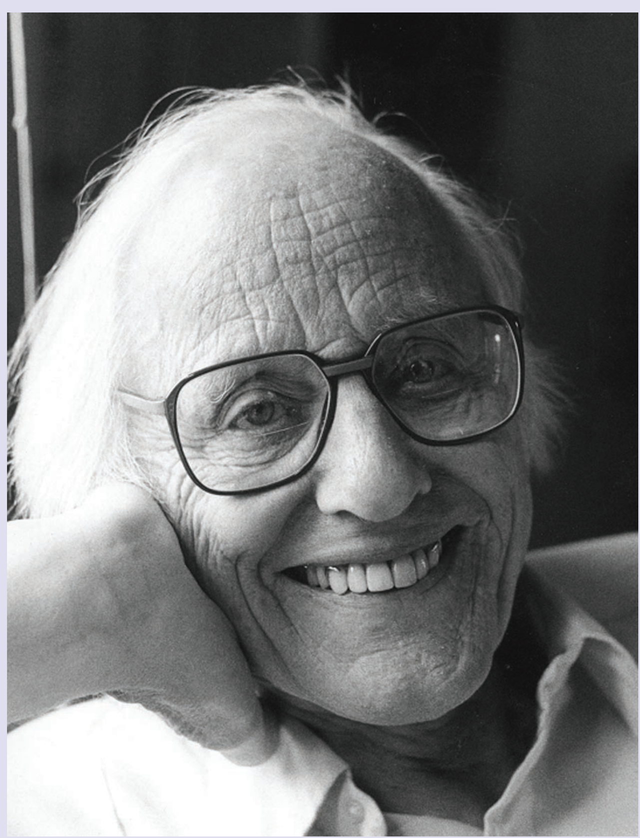
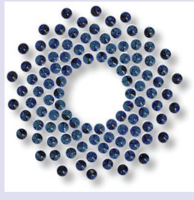
# Hans Ris 1914-2004



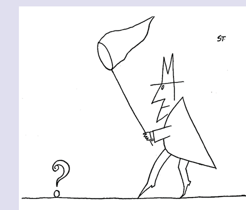
Hans Ris, professor Emeritus of Zoology at the University of Wisconsin at Madison died on November 19, 2004, at the age of 90. Ris was born and grew up in Bern, Switzerland, and came to the US in 1938. He was a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and a founding member of the American Society for Cell Biology. He held an NIH Research Career Award from 1964-1984, and was the director of the Madison HVEM facility from 1970 to 1984. He recieved EMSA's Distinguished Scientist Award in 1983, MSA's Burton award in 1991.

He is survived by his wife, Theron Ris; daughter, Anet Kelman-Ris; and his son, Christopher Ris. A memorial gathering was held on April 9, 2005 at the Alumni Lounge, Pyle Center, University of Wisconsin at Madison. As a prelude to remembrances by colleagues and students, the Mozart g-minor string quartet was played by Ris' colleague Heide Schatten and friends. Following this, a 3-D slide presentation of Ris' work was shown at the home of James Pawley. Hans' inspiration, his many qualities as a role model, his decency, integrity, his impact on science and his students, and commitment to providing serious scientific training to all are remembered with great affection and appreciation.

### A founding father of the American Society of Cell Biology



Hans Ris served as a member of the first NIH Study Section on Cell Biology, founded in 1958. A grant from that Study Section was used to fund meetings of a group of interested biologists at Rockefeller University on January 9 and May 28, 1960. The provisional council for the formation of the American Society for Cell Biology consisted of Keith Porter (chairman), Montrose Moses (secretary), Hans Ris, Don Fawcett, Morgan Harris, Hewson Swift, and Herbert Taylor. At a further meeting in July 18, 1960, it was agreed that the new society should have its first meeting at Chicago. Hans Ris became the first Treasurer of the society, later served on the Council, and at the Society's Annual Meeting in December, 1993 in New Orleans, he was recipient of the ASCB's E.B. Wilson Award Medal.



Excerpt from "My life remembered" as reproduced on the flyer for the memorial gathering.

"My interest in science and biology was deeply rooted in childhood experiences. Since I was ten I spent all my free time exploring the woods covering the hills around Bern. Observing, listening, I became fascinated by the diversity and beauty of living things. I wanted to know about origins and meanings, our relation to other living things, the planets and stars.

Our house was built on land where for centuries blocks of sandstone from nearby quarries were shaped into stones for the houses of old Bern and its cathedral. The leftovers had accumulated perhaps up to forty feet. Our basement on one side was open to this landscape of irregular stones, a great place for day dreaming. I enjoyed making up stories about squeezing through the dark spaces to discover doors that opened into different worlds: secret gardens. And this became a guiding motive of my life, the search for doors to new secret gardens, either in the fantasy world or in the real world.

At the age of 15 I saw an advertisement in a German popular science magazine: make your own microscope; write for instructions and a set of lenses. Thus I built a microscope out of the cigar boxes of my Here was born my passion to explore the world beyond human vision!

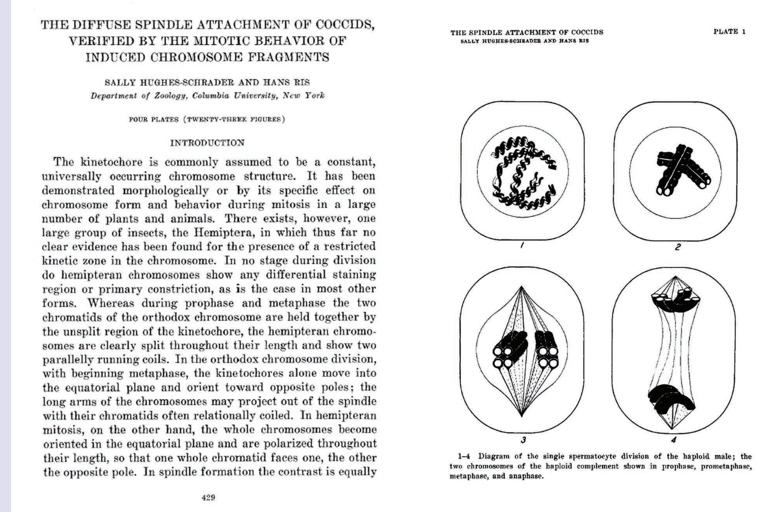
At this time I also discovered the writings of Ernst Haeckel, the champion of Darwinism in Germany. This lead to an agonizing revolution in my beliefs about nature, man and universe. In Sunday School I had been taught that God created the world; plants, animals, and finally, in his image, "MAN", and he told man: go and multiply and exploit my creation for your benefit. 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 that I would turn to science when creating my own world view."

*From "My Life Remembered", Hans' 1994 written reflection on his life*

## Important early contributions

### Early work in mitosis

Ris' interest in mitosis already started as an undergraduate at Bern University, under the influence of Fritz Baltzer, from whom he first learned the scientific method. Baltzer was studying the role of the nucleus and cytoplasm in early development of salamander and frog, and took Hans along to the marine station at Banyuls in southern France to study mitosis in sea urchin eggs. This experience was a major influence on Ris' subsequent work. After graduating with a diploma as a high-school science teacher, Ris continued at Bern, where his theses adviser, Ernst Hadorn, obtained a fellowship for him in 1937 to come to the US in 1938 to work in the lab of B.H. Willier in Rochester, NY. At Rochester, Hans worked on the developmental origin of pigment cells in birds. Working in that lab with Mary Rawles, he became sensitive to the under-appreciation of women scientists, a situation he strove to rectify throughout his career.



THE DIFFUSE SPINDLE ATTACHMENT OF COCCIDS, VERIFIED BY THE MITOTIC BEHAVIOR OF INDUCED CHROMOSOME FRAGMENTS

SALLY HUGHES-SCHRADER AND HANS RIS

*Department of Zoology, Columbia University, New York*

FOUR PLATES (TWENTY-THREE FIGURES)

INTRODUCTION

The kinetochore is commonly assumed to be a constant, universally occurring chromosome structure.

In 1939, a teaching assistantship in the cytology lab of Franz Schrader at Columbia University enabled him to support himself and complete his thesis. At the suggestion of his primary mentor, Sally Hughes-Schrader, his thesis investigated unconventional meiotic divisions in the male bearberry aphid (Hughes-Schrader and Ris, 1941) and formed a solid foundation for his subsequent studies of mitosis, with an appreciation of the importance of *in vivo* studies.

THE ANAPHASE MOVEMENT OF CHROMOSOMES IN THE SPERMATOCYTES OF THE GRASSHOPPER

HANS RIS

*From the Laboratories of The Rockefeller Institute for Medical Research, New York 21, N. Y.*[1]

[1] Part of the work for this paper was done in the Department of Biology, Johns Hopkins University.

SUMMARY

The movement of chromosomes and the changes in spindle size have been recorded in living spermatocytes of the grasshopper during the meiotic divisions. Anaphase movement consists of two separate processes which are related to the action of distinct cellular organelles: (1) The shortening of chromosomal fibers moves the chromosomes to the poles. (2) The elongation of the spindle further separates the daughter plates. The two processes act simultaneously in the grasshopper. With chloralhydrate, spindle elongation can be inhibited without affecting the action of the chromosomal fibers. This demonstrates the independence of these two factors.

After obtaining his PhD from Columbia in 1942, Ris moved on to Johns Hopkins University as an instructor in biology. There, his important contribution came from studies of anaphase movement in insect spermatocytes (Ris, 1949). He showed that separation of chromosomes took place first by movement to the pole (anaphase A), and then by separation of the poles (anaphase B).

### Work on chromosome chemistry at Rockefeller Institute

Hans realized that a proper study of chromosome structure would require better knowledge of chromosome chemistry. A leading laboratory for chromosome chemistry was run by Alfred Mirsky at Rockefeller Institute. A phone call led to an interview, and in 1944 Hans joined the lab at Rockefeller. He stayed five years at Rockefeller, learning how to isolate chromosomes (Mirsky and Ris, 1947) and measure their DNA content (Ris and Mirsky, 1949). These studies showed that that diploid somatic cells of an organism contain identical amounts of DNA, and twice that of haploid germ cells (Mirsky and Ris, 1949). This lent strong support to the hypothesis that DNA was the genetic material. This was followed by quantification of the DNA content in a wide variety of organisms (Mirsky and Ris, 1951), most of which Ris collected himself. Hans' first contact with electron microscopy was in 1947 when he took an air-dried preparation of isolated interphase chromosomes to Keith Porter's early RCA EM. However, little was known at the time about appropriate specimen preparation, and results were not impressive.

**A**

ISOLATED CHROMOSOMES

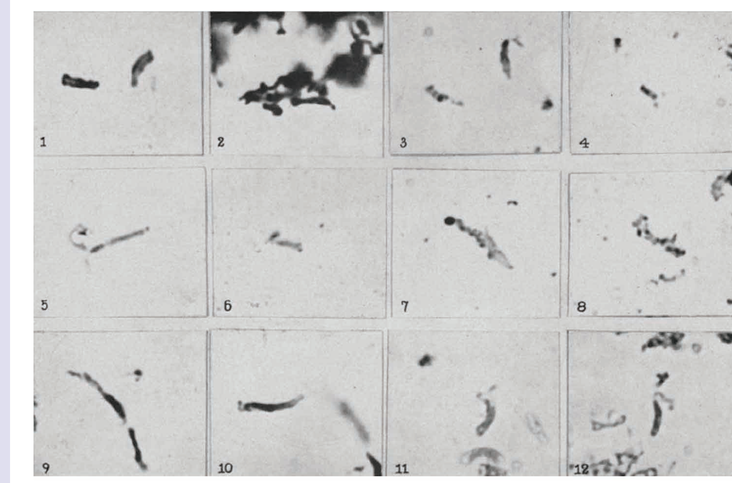
By A. E. MIRSKY AND HANS RIS

*(From the Laboratories of The Rockefeller Institute for Medical Research)*

PLATES 1 AND 2

(Received for publication, April 28, 1947)

The chromatin threads, which we described briefly several years ago (1), have since then been studied carefully both morphologically and chemically. We now consider these microscopic threads of chromatin to be isolated chromosomes (2). From certain animal cells such masses of isolated chromosomes can be prepared that, with respect to quantity of material available, no special methods are required for chemical investigation. It need hardly be said that interest in the chemical properties of this material is greatly enhanced by the knowledge that the material consists of chromosomes, for this provides the chemical studies with a background acquired by several generations of cytological investigation of chromosomes. In this paper the preparation of isolated chromosomes is described and evidence is presented that the bodies isolated are indeed chromosomes.



**B**

VARIABLE AND CONSTANT COMPONENTS OF CHROMOSOMES

By Dr. A. E. MIRSKY and HANS RIS

Rockefeller Institute for Medical Research, New York

Table 3

| Nucleus of: | Desoxyribonucleic acid in 1 nucleus × $10^{-9}$ g | Multiple of value found in sperm |
|---|---|---|
| Bull sperm | 2·82 | |
| Calf thymus | 7·15 | 2·54 |
| Calf lymph node | 7·03 | 2·49 |
| Calf kidney | 6·25 | 2·21 |
| Beef kidney | 6·81 | 2·41 |
| Calf liver | 6·22 | 2·20 |
| Beef liver | 8·4 | 3·0 |

**C**

THE DESOXYRIBONUCLEIC ACID CONTENT OF ANIMAL CELLS AND ITS EVOLUTIONARY SIGNIFICANCE

By A. E. MIRSKY AND HANS RIS*

*(From the Laboratories of The Rockefeller Institute for Medical Research)*

(Received for publication, August 7, 1950)

The DNA (desoxyribonucleic acid) content per cell, according to some recent investigations, is a constant for the various somatic cells of an organism, and sperm cells contain one-half this amount per cell (1, 2). The quantity of DNA per cell is a characteristic of each organism. In this work, done independently by two groups of investigators, the DNA per cell was found by determining the quantity of DNA in a suspension containing a known number of cells and then dividing the total DNA by the number of cells. For sperm and erythrocytes the cells themselves were counted; for tissue cells isolated nuclei were prepared, analyzed, and counted.

TABLE IV

*DNA Content and Mass of Erythrocytes of Various Vertebrates. DNA Expressed as Mg. × $10^{-9}$ per Cell and Mass as Mg. × $10^{-8}$ per Cell*

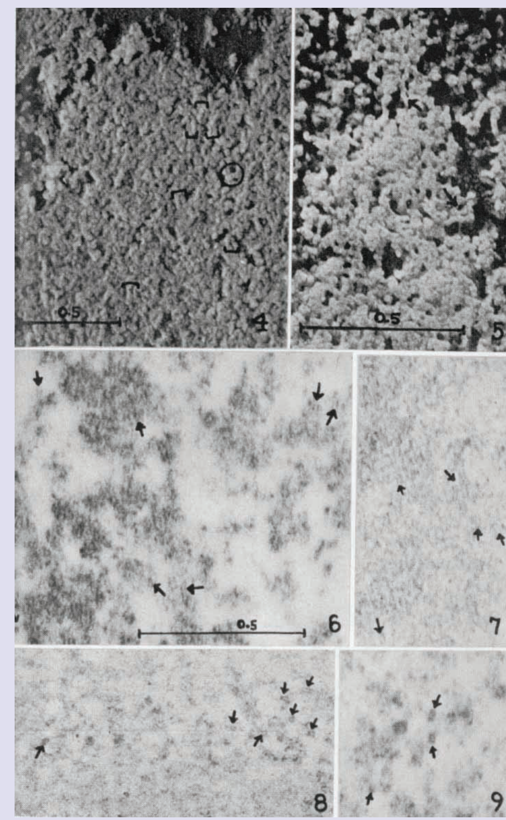
| Animal | DNA | Mass |
|---|---|---|
| Dipnoan: | | |
| African lungfish, *Protopterus* | 100 | 161 |
| Amphibians: | | |
| Amphiuma | 168 | 368 |
| *Necturus* | 48.4 | 40.5 |
| Frog | 15.0 | 27 |
| Toad | 7.33 | 13.7 |
| Reptiles: | | |
| Green turtle | 5.27 | 18.4 |
| Wood turtle | 4.92 | 14.1 |
| Snapping turtle | 4.97 | |
| Alligator | 4.98 | 14.9 |
| Water snake | 5.02 | 13.7 |
| Pilot snake | 4.28 | 13.3 |
| Black racer snake | 2.85 | 10.2 |
| Birds: | | |
| Domestic fowl | 2.34 | 4.39 |
| Guinea hen | 2.27 | 4.58 |
| Duck | 2.65 | 5.44 |
| Goose | 2.92 | 7.37 |
| Mammals: | | |
| Man—Lymphocytes | 5.84 | |
| Granulocytes | 6.25 | |
| (Data of Davison and Osgood) | | |
| Rat—lymphocytes | 6.1 | |
| (Data of Cunningham, Griffin, and Murray) | | |

Sample of the work at Rockefeller Institute. (A) From Mirsky and Ris, 1947: Isolated chromosomes from calf thymus (1,2) calf liver (3,4), and carp erythrocytes (5,6); uncoiled calf thymus (7,8); the same liver (9,10) and thymus (11,12) chromosomes by two different preparative techniques. (B) From Mirsky and Ris, 1949: Demonstration of diploid and haploid DNA content. (C) From Mirsky and Ris, 1951: DNA content in various species.

## First years in Madison

### Early EM work at Madison

Hans Ris' long career in electron microscopy started with his move to the Zoology Department of the University of Wisconsin at Madison in 1949; he recorded his first electron micrographs in 1950. At first, he used an RCA EMU-2B in the soils department, a decidedly unfriendly environment for an electron microscope. The EM was being used to study viruses using the shadowing technique. At first, Ris felt himself an outsider from the EM community, and had to learn EM on his own, but he soon became known as one of the great masters of biological electron microscopy. A big improvement came in 1956 when he acquired a Siemens Elmiskop I for the University. He had encountered this state-of-the-art instrument at the 1954 ICEM in London, and when the 1956 EMSA meeting was held in Madison, he arranged to have the demonstration microscope left at the University. In 1958 he had his own microscope, a Siemens Elmiskop II. This was followed in 1964 with an Elmiskop Ia, which was in operation until 1984.



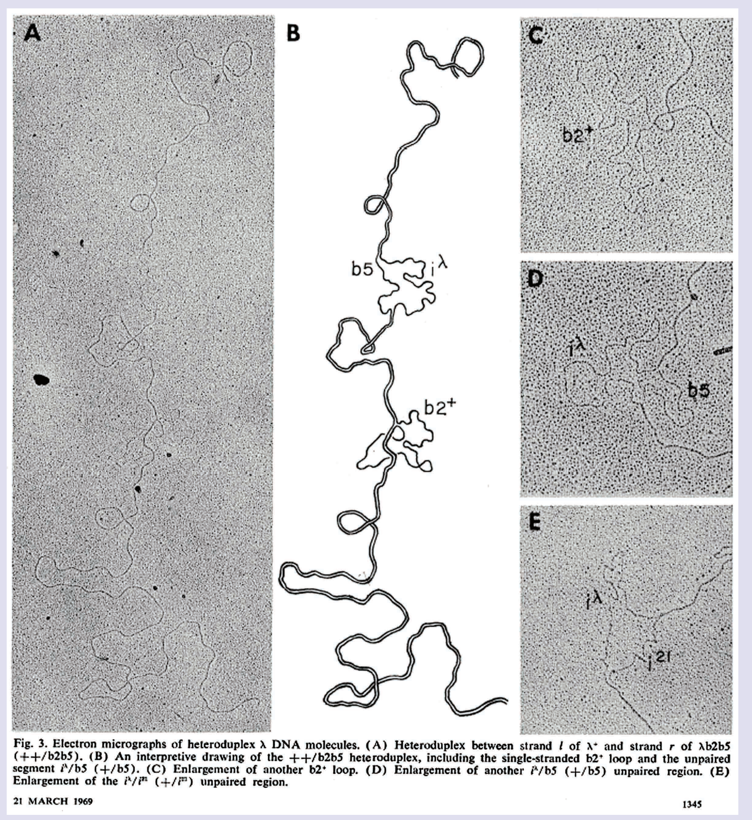
Ris was the first to image mitotic chromosomes in plant cells, using the EMU-2B. The thin sections of lampbrush chromosomes required use of an objective aperture, purchased from a third-party vendor, Canalco. The aperture kit came equipped with a mallet that was used to center the aperture by tapping on the column! This figure, (from Ris, 1956) shows a late prophase chromosome of a *Lilium* microspore in a resin-removed, uranium-shadowed section, with 20-nm fibrils at brackets and circle (4); twisted fibrils (arrows) in *Tradescantia* anther, using the same preparation method (5); a leptotene chromosome of *Lilium* microspore in a conventional section, with double lines and circles at arrows outlining 20-nm microfibrils in (6); fibrils appearing as double lines and circles in a meiotic metaphase *Lilium* chromosome (7), and fibers appearing as dense circles in *Triturus* oocyte nucleus (8) and rat spermatid (9).

FIGURE 6

Part of the chloroplast of *Chlamydomonas moewusii* with the Feulgen-positive area. *R* = ribosomes in the cytoplasm. *CL* = chloroplast lamellae. The arrows point to the 25 A fibrils which are taken to represent the DNA. *CM* = external chloroplast membrane. × 95,000.

Based on reports of DNA in cytoplasmic organelles, Ris became interested in the concept that chloroplasts and mitochondria originated from endosymbiotic microorganisms. He presented ultrastructural evidence supporting the similarity of chloroplasts and blue-green algae (Ris and Plaut, 1962), and mitochondria and bacteria. This work became the project of his graduate student, Lynn Margulis, who promoted the idea, which is now generally accepted.

Using a modification of the Kleinschmidt technique, Ris and collaborators developed a method of making complete high-precision physical maps of viral genomes (Westmoreland et al., 1969).



## The NIH microscopy Resource

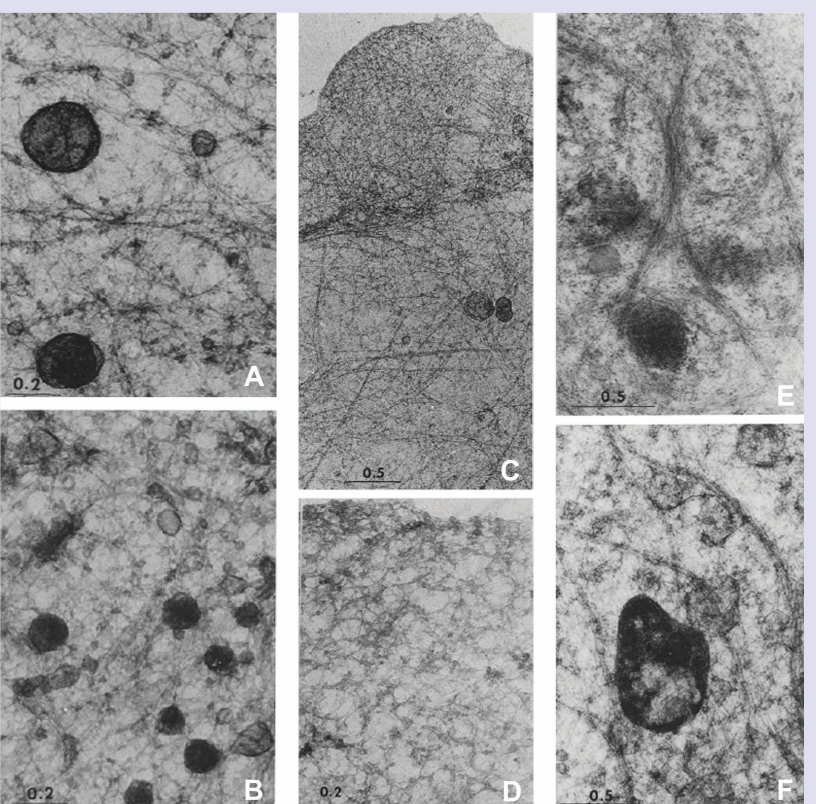
### The Madison HVEM lab

Ris found it frustrating that it was difficult to appreciate the three-dimensional structure of chromosomes when a chromatid is 1 µm thick, but specimens for electron microscopy need to be one-tenth that thickness. At the banquet during the 1958 ICEM in Berlin, Ris happened to be seated next to Gaston Dupouy. Taking advantage of his fluency in French, Ris learned that Dupouy was planning to build a million-volt electron microscope so that he could study live cells and bacteria. When his HVEM was operational, Dupouy invited Ris to Toulouse, where Ris was able to record images of dinoflagellate chromosomes in 1-µm thick sections, in which 2-nm DNA fibers could be seen. Ris applied to NIH for his own HVEM, and the study section was impressed enough with the pictures from Toulouse that the grant was funded in 1970. The Madison HVEM Resource opened in 1971. The first physicist-in-charge was Dale Johnson (later President of EMSA and organizer of the Seattle ICEM), followed by Paul Lin, both students of Albert Crewe. Finally, a tenure-track position for the physicist was created, and in 1978 it was filled by James Pawley from Berkeley, an SEM specialist. Pawley learned HVEM in the few weeks before the NIH site visit, the Resource received continued funding, and Pawley went on to make several important improvements to the HVEM.

Hans Ris at the Madison AEI EM-7 million-volt electron microscope.

### Cyotplasmic filaments

Based on HVEM images of critical-point-dried whole mounts of cultured cells, Keith Porter proposed a new cellular component that organized the cell's cytoplasm, in the form of a branching network of fibers called the "microtrabecular lattice". The structure was not seen in plastic-embedded cells, presumably because of contrast-matching with the resin. In 1985, Ris reported on his findings regarding this structure. Ris noticed a similarity between the microtrabecular lattice and fibers in chromosome preparations that were flawed by introduction of humidity during critical-point drying. By taking care that the $CO_2$ used for critical-point drying was free of water, Ris was able to show that the appearance of the cytoplasm was the same in critical-point dried and plastic-embedded cells, and that the microtrabecular lattice was an artifact (Ris, 1985).



HVEM of cytoplasmic filament preparations. (A, B) Whole cell mounts of critical point dried PtK-1 cells, with (A) and without (B) drying of the $CO_2$. Note that filaments have a uniform thickness in (A), while filaments in (B) are indistinct, non-uniform, and fused. (C,D) The same experiment repeated with whole cell mounts of sea urchin coelomocyte, (C) with and (D) without dried $CO_2$. (E,F) Comparison of PtK-1 cell cytoplasm in a 0.25-µm-thick plastic section (E) and a critical point dried whole mount (F). Except for the difference in contrast, the cytoplasmic structure is identical. From Ris, 1985.

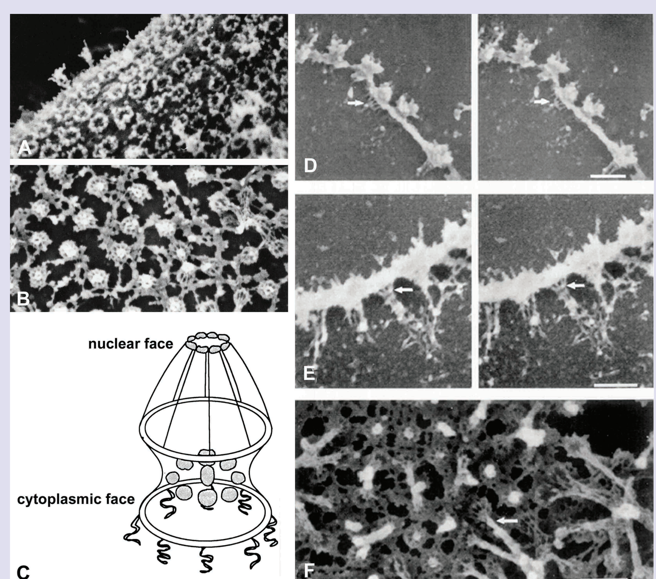
### The Integrated Micoscopy Resource

In response to NIH NCRR's requirement for continued technological development at a Resource, in 1984, Ris and Pawley decided to expand the scope of the facility by adding video-enhanced light microscopy, confocal light microscopy (starting with a prototype from Biorad), and low-voltage SEM. Pawley realized the necessity for use of low acceleration voltage and a field-emission source for high-resolution biological SEM, and the Resource, now re-named the Integrated Microscopy Resource (IMR), received the first Hitachi S-900 FESEM outside Japan in 1986. With the 1984 renewal and the name change, Hans Ris retired as director and the IMR leadership passed to Gerald Schatten. However, Hans stayed on and continued his research, talking full advantage of integrating different imaging techniques in studying a biological system.

## Recent contributions

### Nuclear pores

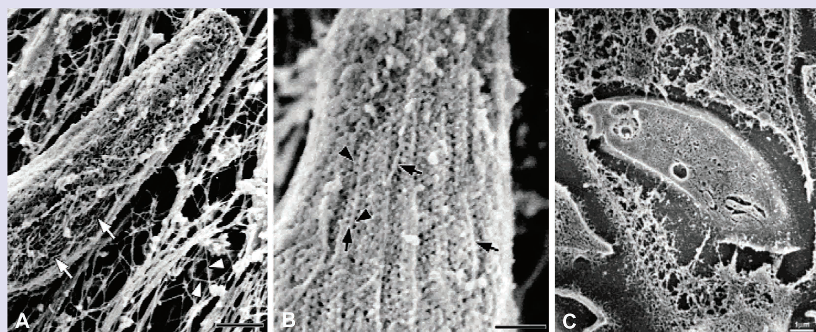
Ris again engendered initial controversy in his work on the nuclear pore complex, using the new FESEM. With it, he obtained a view of the nuclear side of the nuclear pore complex in Xenopus oocytes, which revealed a structure he called a "fishtrap" (Ris, 1991) after the form of baskets used by Chinese fishermen. By a combination of HVEM and FESEM, he was able to demonstrate this structure more clearly in freeze-substituted material after removal of the embedding medium (Ris and Malecki, 1993). A network of cables attached to the nuclear pore complexes, likely involved in nuclear export, was also revealed.




FESEM imaging of the nuclear pore complex. (A) Examples of the cytoplasmic face of the nuclear pore complex on an isolated oocyte nucleus prepared by critical point drying. (B) The nuclear face of the complex, showing the fishtrap structure in plan view. (C) Drawing of the fishtrap structure of the nuclear pore complex. (D,E) Side views of the fishtrap structure (arrows) on isolated nuclear envelopes, prepared by freeze-substitution, microtomy, and Epon de-embeddeding. (F) Nuclear face of nuclear envelope showing cable-like structures attached to the ring of the fishtrap. The tops of adjacent fishtraps are connected to each other through a complex cable system. A,B,C from Ris, 1991; D,E,F from Ris and Malecki, 1993

### Toxoplasma

Hans Ris' last work was done in collaboration with Heide Schatten, a colleague from Madison now at the University of Missouri at Columbia. This work investigated the motility of the parasite Toxoplasma gondii relating to its invasion in human cells (Schatten and Ris, 2002, 2004; Schatten et al., 2003). Using the FESEM at the IMR in Madison, and the techniques developed during the work on the nuclear pore complex, it was found that fibrous material connects the parasite with the host during invasion, and actin filaments, as well as intermediate filaments and microtubules, exist just below the cell membrane of the parasite.



Cytoskeletal studies of toxoplasma by FESEM. (A) Extracted, de-membranated, critical-point-dried preparation showing a parasite invading a human fibroblast. Actin-sized (7-nm) filaments are seen in both host cell (arrowheads) and parasite (arrows). Bar = 200 nm. (B) De-membranated parasite after treatment with cytochalasin-D to remove actin. The 7-nm filaments are now not seen. Longitudinally arranged 20-nm fibers (arrows) are seen in the position where the 22 subpellicular microtubules are known to exist; associated with the microtubules are fibers about 10 nm thick (arrowheads). Bar = 200 nm. (C) Epon de-embedded section showing fibrous connections between the parasite and host cell membranes. Bar = 1 µm. A and B from Schatten et al., 2003; C from Shatten and Ris, 2004

### The Chinese connection

Ris had long had an interest in China, but waited until he had personal contacts to pursue it. The opportunity came in 1984 when a woman researcher visited Madison for six months. He continued the research by correspondence for a time before starting his series of visits to China. He was involved in the EMSA China exchange program, and when he gave lectures at the Beijing Agriculture Institute in 1986 his colleague acted as interpreter. In 1990 he established a formal scientific exchange program between the ASCB and the Chinese Society for Cell Biology. In 1995, he received an honorary professorship of life sciences from Beijing University. Ris learned Chinese in his 70's, and commented that he felt at home in China, where the priorities and attitudes seemed more similar to the Switzerland he grew up in than those in contemporary America. He believed that exchange with China would benefit America.

Hans Ris speaking enthusiastically in 1990 about his interaction with young Chinese scientists.

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Many thanks to Prof. Heide Schatten for providing and suggesting material, and consulting on this poster.


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