

IN MEMORIAM

Joseph G. Gall (1928–2024): Cell biologist, naturalist, and mentor extraordinaire

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Joseph Grafton Gall (1928–2024), a founder of modern cell biology, made foundational discoveries on eukaryotic chromosomes and RNA biogenesis. His major contributions include the development of in situ hybridization (later called FISH), demonstration of one DNA double helix/chromosome, isolation of the first eukaryote gene, localization of satellite DNA to centromeric heterochromatin, determination of the first telomeric DNA sequence, and elucidating the structure and functions of Cajal bodies. He was an expert microscopist, a scholar of science history, and an avid naturalist. These attributes, together with his ready embrace of new technologies, contributed to his remarkable success. He was also an early and strong supporter of women in science. His contributions to science and mentoring were recognized by numerous awards including the American Society for Cell Biology's E.B. Wilson Medal, the Society for Developmental Biology's Lifetime Achievement Award, the Albert Lasker Special Achievement Award in Medical Research, and the AAAS Mentor Award for Lifetime Achievement.

Introduction

Joseph Grafton Gall (Fig. 1) was born on April 14, 1928, in Washington, D.C., and died on September 12, 2024, in Baltimore, MD, at age 96. Joe was a founder of modern cell biology and ranks among the most distinguished biologists of our time. In his words: "I realize that most of my knowledge of the outside world was coming through my eyes, and that has been the story of my life" (Gall, 2009). Joe was a microscopist and much more. His multiple, high-impact discoveries include the following: (1) the invention of in situ hybridization (now called FISH), revolutionizing biology by allowing DNA and RNA to be directly localized on chromosomes and throughout the cell; (2) establishing that each eukaryotic chromosome has a single DNA double helix; (3) isolating the first eukaryotic gene (amphibian ribosomal DNA) before the advent of DNA cloning; (4) demonstrating that centromeric heterochromatin in flies and mice comprises simple sequence satellite DNAs; (5) using ciliated protozoans to determine the first telomeric DNA sequence; (6) elucidation of the Cajal body and stable introns after splicing. In addition to his many scientific achievements, Joe was an early and strong supporter of women in science. He was widely honored for both his science and his mentorship, including

being elected member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the American Philosophical Society; and receiving the American Society for Cell Biology (ASCB)'s highest honor, the E.B. Wilson Medal, the Society for Developmental Biology's Lifetime Achievement Award, and the Albert Lasker Special Achievement Award that honors exceptional contributions to medical research.

Early years and education: Development as a naturalist and scholar

When Joe was 14, he moved with his parents and two brothers to a 500-acre farm in Virginia. There, his mother fostered his interest in the natural world, supplying him with homemade butterfly nets and encouraging his collection of various animals. As a teenager, he convinced his parents to buy him a microscope and a microtome (to make tissue slices for microscopic viewing); Joe set them up in a laboratory in his bedroom (Fig. 2).

Joe always remained a naturalist, conveying his love for the natural world to his trainees and family through field trips. Perhaps his children's career choices were influenced by these excursions. His son, Larry F. Gall, PhD, is the collections manager for entomology at the Yale Peabody Museum, and his

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S.A. Gerbi, V.A. Zakian, and E.H. Blackburn were J.G. Gall trainees.

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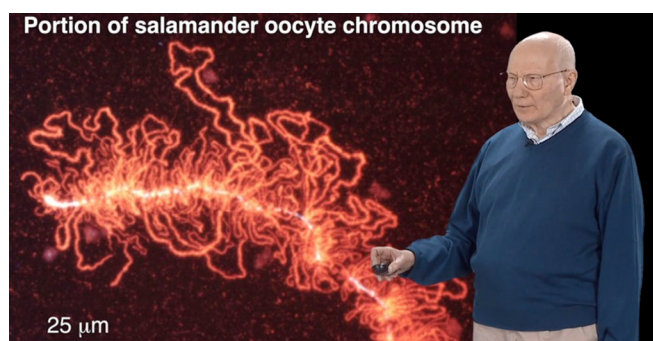


Figure 1. **Joe Gall standing before a picture of a newt lampbrush chromosome.** The central axis of each chromosome is stained white using the DNA-specific dye DAPI. The lateral loops of DNA that extend from the central axis bear RNA-protein transcripts visualized in red using a fluorescent antibody. Picture is from Roth and Gall (1987). The composite image of Gall with the lampbrush chromosome is from <https://www.ibiology.org/genetics-and-gene-regulation/in-situ-hybridization/>.

daughter, Barbara G. Eidel, DVM, is a Maryland veterinarian. On one memorable night, lab members joined Joe on an excursion to collect specimens near New Haven. Amazingly, Joe could identify every animal sound and would shine his flashlight unerringly on its source. Joe's naturalist side was reflected in his lab's animal room that at various times housed insects (the fruitfly *Drosophila*, the fungus fly *Sciara*, and the water beetle *Dytiscus*), amphibia (the toad *Xenopus*, the newt *Notophthalmus* a.k.a. *Triturus*), and protozoa (*Tetrahymena*). He even housed animals not used for experiments (guinea pig, zebrafish, and an albino axolotl).

Joe's expertise as a historian of science also began early. As a teenager, he acquired E.B. Wilson's 1925 classic *The Cell in Development and Heredity*. This introduction to cells inspired his life-long interest in the history of science (Fig. 3) and his extensive collection of historical scientific books. Joe used this collection to generate *Views of the Cell: A Pictorial History*, a picture book of historic micrographs intended by Joe to "delight the mind and the eye."

In 1945, Joe entered Yale, where he obtained a BS in zoology. Learning that a PhD would allow him to pursue a career in research, he entered the Yale zoology graduate program, obtaining his PhD in 3 years. When he started graduate school, he was already fascinated by chromosomes. From Waddington's 1939 book *Introduction to Modern Genetics*, he discovered that salamander oocytes contained huge lampbrush chromosomes and decided to study them for his PhD. *Drosophila* geneticist Don Poulson served as his mentor. Joe needed an inverted microscope for this research, and since they were not commercially available, Joe built his own.

Academic appointments

At age 24, Joe joined the zoology faculty at the University of Minnesota (1952–1964). Using his new electron microscope, Joe made several observations that advanced the nascent field of cell biology. He studied the nuclear pore complexes in isolated nuclear envelopes from amphibian oocytes and demonstrated their octagonal symmetry (Gall, 1954, 1967). His studies on centriole



Figure 2. **Teenage Joe Gall viewing specimens with his Bausch and Lomb microscope at home ~1943.** Photo by Lynn Charles Skeels, with permission from Diane Dwyer.

replication revealed that a newly formed centriole arises from a small procentriole situated at right angles to the mother (Gall, 1961). Joe also continued his studies on lampbrush chromosomes and began corresponding with H.G. "Mick" Callan and his student Herbert Macgregor in Scotland, who also studied them. The three became life-long friends, sharing their results prior to

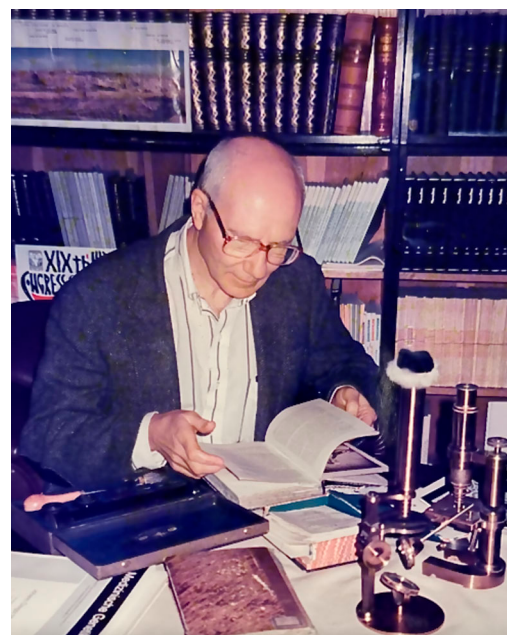


Figure 3. **Joe Gall at the Mendel Museum in Brno, Czechoslovakia, looking through Mendel's copy of Darwin's *Origin of Species*.** Mendel's microscopes are on the table. Photograph taken by and with permission from Diane Dwyer.

publication, even though they were competitors. Among their findings, both labs showed that RNA synthesis occurred on the loops radiating from the main axis of each lampbrush chromosome.

In 1963, during his sabbatical at Yale, they offered him a named professorship. Here (1964–1983), he became immersed in the new field of molecular biology and trained himself in biochemical methods for working with DNA and RNA (Gall, 2006). Throughout his career, Joe's greatest satisfaction came from working at the bench. As expectations for administrative duties at Yale increased, he realized they would limit his time for experiments. Thus, Joe accepted an offer from his friend, Don Brown, for a position at the Carnegie Institution in Baltimore, MD, and moved there in 1983 as a lifetime American Cancer Society Professor of Developmental Genetics. At the Carnegie, the focus of his research shifted from DNA to RNA biogenesis. Joe worked in his lab until a few years before his death.

Foundational discoveries: Gall's greatest hits

Inspired by Herbert Macgregor's demonstration that lampbrush loops are broken by DNase, Joe measured the kinetics of DNase cleavage using microscopy. The result was the first demonstration that each chromatid consists of a single DNA double helix (Gall, 1963).

Years before DNA cloning, Joe and, independently, Don Brown were the first to isolate a eukaryotic gene (Brown and Dawid, 1968; Gall, 1968). Ribosomal DNA (rDNA, encoding ribosomal RNA [rRNA]) could be isolated by CsCl density gradient centrifugation, as its high guanine plus cytosine (G+C) content positioned it apart from bulk DNA. This work showed that rDNA was relatively enriched in oocytes compared with diploid cells and supported the idea that the extrachromosomal nucleoli of amphibian oocytes contained amplified rDNA. Later in Joe's lab, three postdocs showed that this rDNA amplification occurred by a novel rolling circle mechanism (Rochaix et al., 1974).

Using microscopy to locate specific genes on chromosomes was one of Joe's longtime dreams, and the time was ripe with the recent advent of biochemical molecular hybridization (Gillespie and Spiegelman, 1965). Success in developing the method depended on a biological system in which the suspected answer could be verified experimentally; Joe wisely picked *Xenopus* oocytes whose extrachromosomal nucleoli contained amplified rDNA. Moreover, the rRNA that was used as the probe was the only RNA that could be purified at the time. This led to his development of in situ hybridization (ISH) with his graduate student Mary-Lou Pardue (Gall and Pardue, 1969; Pardue and Gall, 1969). Chromosomes were bound to slides, their DNA denatured, and then hybridized to a radioactive RNA probe whose position was visualized by autoradiography and microscopy (Fig. 4, upper). The next step was to demonstrate that ISH could be used to map genes directly on chromosomes. The Gall lab astutely chose giant insect polytene chromosomes to determine the position of chromosomal rDNA as endoreduplication increased the radioactive signal (Pardue et al., 1970).

The next goal was to extend ISH beyond rRNA. In the pre-cloning world, another DNA was needed that could be isolated by physical methods. Satellite DNAs were chosen as they too

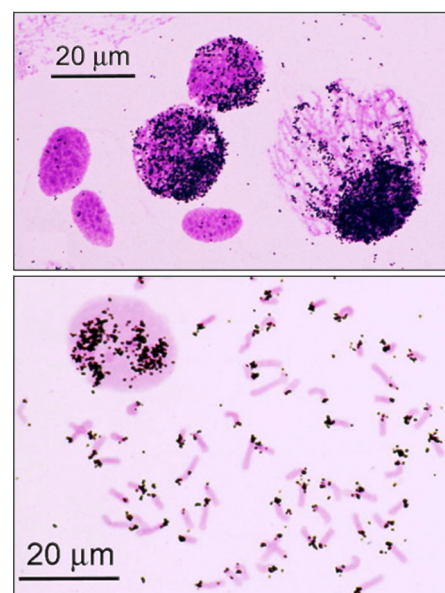


Figure 4. ISH in amphibian oocytes (upper panel) and mouse mitotic chromosomes (lower panel). Upper panel: Autoradiograph of nuclei from a *Xenopus* ovary after ISH with ^3H -labeled rRNA showing the progressive increase in amplified rDNA as the nucleus enlarges. Reproduced from Fig. 2 in Gall (2016). Lower panel: Autoradiograph of an interphase nucleus and mitotic chromosomes of the mouse after ISH. The probe is ^3H -labeled RNA that was transcribed in vitro from CsCl gradient-isolated mouse satellite DNA. Reproduced from Fig. 3 in Gall (2016).

form discrete bands in CsCl gradients. Again with his student Mary-Lou Pardue, they found that mouse satellite DNA localized to the centromeres of all the mitotic chromosomes (Pardue and Gall, 1970) (Fig. 4, lower). To extend the link between satellite DNA and centromeric heterochromatin, Joe chose *Drosophila* since the amount of centromeric heterochromatin is much lower in polytene compared with mitotic chromosomes, especially in *Drosophila virilis*, where Joe knew that about half of each diploid chromosome arm was heterochromatic. The Gall lab found that satellite DNA localized to centromeric heterochromatin, but its amount per chromosome was much lower in polytene compared with mitotic chromosomes (Gall et al., 1971). The localization and non-coding sequence of satellite DNAs in mice and *Drosophila* and their under-representation in non-mitotic polytene chromosomes suggested that they have a structural function in chromosome distribution during mitosis.

ISH was renamed FISH when fluorescent probes replaced their radioactive counterparts. This method has been incredibly impactful for addressing basic biological questions as well as for its medical applications such as identifying disease-associated changes in chromosome structure, as an assay for gene amplification in cancer, and identification of microorganisms in the gut.

Next, *Tetrahymena* entered Joe's zoo of model organisms. Like other ciliates, *Tetrahymena* has both a diploid micronucleus and a highly polyploid macronucleus. To test whether ciliate macronuclei had extrachromosomal rDNA, similar to amphibian oocyte nuclei, Joe isolated *Tetrahymena* rDNA by CsCl centrifugation and examined it by electron microscopy. Indeed, the

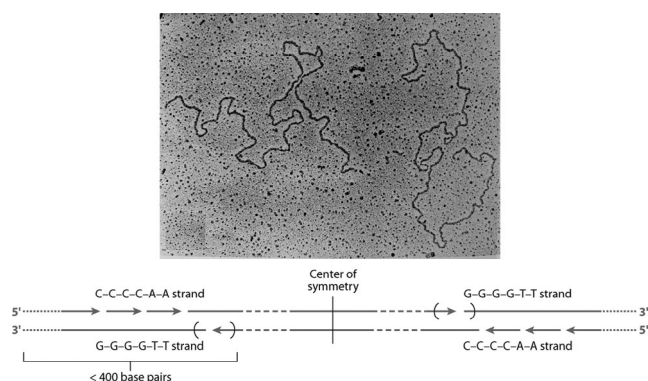


Figure 5. **rDNA molecules from *Tetrahymena* macronuclei.** Upper panel: Electron micrograph of individual rDNA molecules from *Tetrahymena* macronuclei. Shown are two linear molecules, each ~22 kb in length. Lower panel: Map of the deduced structure of 22-kb rDNA molecules showing the CCCCCA/GGGGTT telomeric repeats at both ends. Reproduced from Gall (2009), Fig. 13 a (upper panel) and Fig. 14 (lower panel).

isolated rDNA was extrachromosomal, consisting of 22 kb, mainly linear, molecules (Gall, 1974) (Fig. 5, upper). A similar conclusion was reached independently by Meng-Chao Yao (Yao et al., 1974). Yao joined the Gall lab as a postdoc and showed that the *Tetrahymena* micronucleus contained a single rDNA copy (Yao and Gall, 1977).

Next, Joe's graduate student Kathy Karrer determined the structure of *Tetrahymena* extrachromosomal rDNA. Electron microscopy and reassociation kinetics revealed that each amplified rDNA molecule was a palindrome, containing two head-to-head copies of rDNA (Karrer and Gall, 1976). Joe was in England, so Kathy with the help of postdoc Rob Grainger telegraphed this result to Joe in the form of a cryptic palindrome. Joe kept the fun going by replying: "Dyad is no ciliated detail I considayd" ("PS - Sorry about the spelling"). Similar results were obtained by Jan Engberg (Engberg et al., 1976). Typical of Joe's collegiality, the Engberg and Gall labs shared their data prior to publication and published their papers back to back.

Tetrahymena continued to yield unexpected results. After her PhD in Fred Sanger's lab, in 1975, Liz Blackburn was one of the few people who knew how to sequence DNA. As a postdoc in Joe's lab, she wanted to sequence a eukaryotic DNA terminus because (prior to DNA cloning) ends could be sequenced directly. With Joe's knowledgeable input, she picked the ends of the amplified *Tetrahymena* rDNA. Liz found that each terminus bore a variable number of simple CCCCCA/GGGGTT repeats (Blackburn and Gall, 1978) (Fig. 5, lower). These novel findings were generalizable to most eukaryotes, which have similar telomeric DNA, and were the start of the molecular age of telomere research. Later, in her own lab at University of California Berkeley, Blackburn, with her graduate student Carol Greider, discovered the ribonucleoprotein enzyme telomerase that elongates telomeric DNA using a totally novel mechanism for cellular DNA replication. Blackburn and Greider (together with Jack Szostak) shared the 2009 Nobel Prize in Medicine for these discoveries. Joe and his wife, Diane Dwyer, MD, were Liz's guests at the Nobel ceremony.

After moving to Carnegie, Joe's research focused on RNA (a review of this work is in preparation for RNA). For several years, he studied RNA bodies in the nucleus, especially the spheres in amphibian oocyte nuclei that were renamed coiled bodies because they contain the protein coilin. Later, with the findings that coiled bodies were present in many organisms, Joe proposed renaming them Cajal bodies, honoring their 1903 discovery by the Spanish Nobel laureate Ramón y Cajal (Gall, 2006). Joe's final, highly productive research years focused on stable intronic sequences (sisRNA).

Support for women in science: A lab in which all trainees thrived

In addition to his scientific achievements, Joe was renowned for his strong and early support of female scientists. Contrary to what some thought, Joe didn't favor women. Rather, he treated males and females similarly. This trait made his lab a haven for female trainees. The atypically high proportion of females in his lab intrigued some male scientists. At a 1973 scientific meeting, a prominent scientist asked a group of Gall lab attendees, "What does Joe have that so many 'gals' go to his lab." One female student replied, "When Joe shuts his office door to discuss your data, he discusses your data." This comment reflects another valued feature of the Gall lab: its total lack of sexual harassment. When the authors were in Joe's lab, women were hardly visible in academia. Given this paucity, it is remarkable that virtually all of the female trainees in the Gall lab saw themselves as destined for faculty positions, and many achieved this goal. Female trainees from the Gall lab have succeeded at the highest levels, including four members of the National Academy of Sciences, three ASCB Presidents, a co-founder of the RNA Society, president of the Genetics Society of America, president of the American Association of Cancer Research, and a Nobel laureate. The importance of Gall's mentorship was recognized by his receipt of the ASCB Sandra K. Masur Senior Leadership Award for support of female scientists and the American Academy for the Advancement of Science Mentor Award for Lifetime Achievement. His Lasker award citation included praise for his being a "long-standing champion of women in science."

Joe had a unique mentoring style that he applied to both males and females. He was not preachy or controlling but rather led by example. He loved working at the lab bench. When his students asked what he liked to do on weekends, he said, "Go to the lab and look at my favorite slides." He told another graduate student when they encountered each other at the Yale ice-skating rink that "the only time he didn't think about science was when he was ice-skating!" He seemed totally uninterested in personal power, probably because it would keep him from the bench. In his own lab, he allowed each member to pursue their own interests and to plan, conduct, and interpret their own experiments, as he had done during his graduate training with Don Poulson.

Joe had strong principles about scientific conduct, and again his trainees learned these principles from his example. Science should be collegial, interactive, and honest. Joe was always generous in crediting those who laid the groundwork for his own work as well as those who carried out similar studies at

about the same time. In fact, some of his closest friends in science were also his competitors.

Joe was admired and trusted by all who knew him. His instinct for important biological problems was phenomenal. He was a role model, an amazing mentor, and a giant in the field of cell biology. He was kind, deeply thoughtful, and modest. The authors are proud to have been his trainees.

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