Through the looking glass

Joseph G Gall

After much urging, my parents bought me a microscope when I was 14 years old, not one of the toys I had struggled with up to that time, but the real thing, with crystal-sharp images and an oil immersion objective (**Fig. 1**). Once I was hooked on the vast world that lay beyond what I could see with my unaided eyes, there was no turning back, and I have never been far from a microscope since then. At that time, my family had just moved to a farm in northern Virginia. Because there was no local public high school, I was sent off to boarding school near Charlottesville. I took my microscope with me, and when I was wasn't parsing Latin sentences or memorizing euclidean geometry, I was looking at anything that I could get under my lenses. At home in the summertime, I turned my room into a laboratory and read everything about cells I could lay my hands on. My older brother gave me a copy of E. B. Wilson's *The Cell in Development and Heredity*[1], a book that inspired more than one of my later research projects.

After I had spent three years at boarding school, the headmaster decided I was a sufficiently serious student that I should go north to one of the 'elite' colleges. Because of the military draft at that time, colleges were sparsely populated and happy to have students. How Yale was chosen I am not sure, but I arrived in New Haven in June 1945, just as the Second World War was coming to a close.

Most of my fellow zoology majors were premeds, and for three years I thought I had to attend medical school if I wanted to follow my interests in the cell. Career counseling was nonexistent in those days, so I learned only in my senior year that there was such a thing as graduate school. With the help of Donald Poulson, my future Ph.D. advisor, I was admitted for graduate work in the Yale Zoology Department and began to think seriously about research.

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Chromosome structure and function

Poulson was a *Drosophila* geneticist who had trained with Alfred Sturtevant at Caltech in the 1930s. His main interest was in how mutations, particularly lethal mutations, affect early development. His study of the *Notch* gene was the first of its kind, laying the foundation for the future field of developmental genetics[2]. However, I wasn't interested in studying lethal mutations. My fascination with *Drosophila* lay in its giant polytene chromosomes and their enigmatic banding pattern. I felt that the secret of genes somehow lay in those bands, but unfortunately, I couldn't think of anything to do with them that hadn't already been done. One day I happened to look at C. H. Waddington's book *An Introduction to Modern Genetics*[3] where I saw a photograph of a giant 'lampbrush' chromosome (LBC) from a newt oocyte, taken by a scientist named William Duryee. I thought the stated magnification must have been wrong by an order of magnitude, so I looked up the original paper and found to my surprise that newts indeed had chromosomes as large as those of *Drosophila*. Even better, I found that Duryee was the only person who had studied these chromosomes in living oocytes. The phase-contrast microscope was not available when Duryee did his work, but the Yale Zoology Department had just acquired one of the first commercial instruments, which I could use. Poulson let his students work on whatever interested them, so I knew then and there that I had a thesis problem. Three-and-a-half years later, I presented my thesis on the structure of LBCs in the newt. Forgoing postdoctoral training, I got my first academic position in 1952 as an instructor in the Zoology Department at the University of Minnesota.

For the next years I worked on a variety of problems—the structure of the nuclear pore complex, centrosome replication and sperm structure—all of which required an electron microscope, the newest tool in the cell biologist's armamentarium. But I kept coming back to the LBCs. While still a graduate student, I learned that an investigator in Scotland, Mick Callan, was also interested in LBCs. We began to correspond by airmail, eventually developing a close scientific collaboration and personal friendship that lasted for 40 years. We shared whatever new findings we made, and everything each of us wrote first went through the filter of the other's thinking. Before we could do anything else, we had to get the morphology of the LBCs correct, which I had had wrong in my thesis. We found that the brush analogy was not quite right. The bristles of the brushes were, in fact, loops: more precisely, pairs of loops formed by the lateral separation of the two chromatids in each chromosome. Along the chromosomal axis, the two chromatids were intimately paired (**Fig. 2a**,**b**).

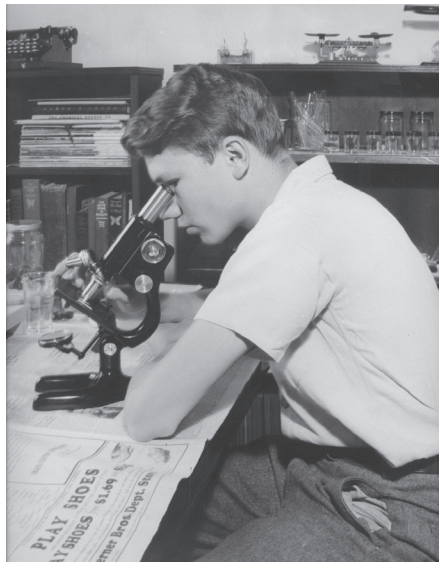

Figure 1 Joe Gall as a teenager, about 1943.

Ultimately, we learned two major things from the lampbrush chromosomes, one structural and one physiological. Structurally, we found that the chromatid is a single long DNA molecule. Callan's student Herbert Macgregor showed that lampbrush chromosomes break into thousands of small fragments when treated with DNase, whereas they simply lose mass when subjected to RNase or various proteases[4] (**Fig. 2c–e**).

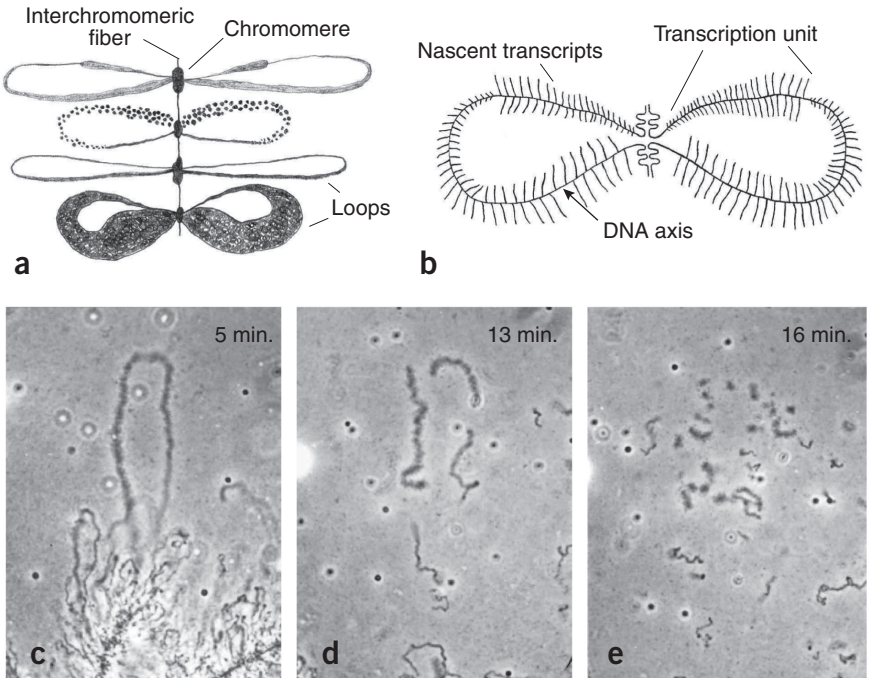

Figure 2 Lampbrush chromosome structure. (**a**) Short region of a lampbrush chromosome showing the characteristic pairs of lateral loops. (**b**) The loops are regions where sister chromatids are completely separated from each other. The loop axis consists of DNA, RNA polymerase and other factors required for transcription. The loop matrix consists of nascent RNA chains and associated proteins. (**c**–**e**) Stages in the digestion of a single loop by the enzyme DNase. The kinetics of breakage are consistent with the loop axis consisting of one DNA double helix.

I followed up Macgregor's study by showing that the kinetics of DNase breakage was two-hit for the lateral loops and four-hit for the main axis[5]. In other words, each chromatid consisted of two units, which were the halves of the DNA double helix. This finding corroborated the elegant experiments of Herbert Taylor, who had just shown that chromatids, like the DNA molecule itself, replicate in a semiconservative fashion[6].

The physiological finding concerned the synthetic activity of the chromosomes. By autoradiographic experiments with tritium-labeled RNA precursors, Callan and I showed that LBC loops were the sites of nuclear RNA synthesis[7]. Similar experiments by Beermann and his collaborators in Germany showed essentially the same thing for the 'puffs' and 'Balbiani rings' of polytene chromosomes of *Drosophila* and the midge *Chironomus*. It took much longer to establish the details of this synthesis and to show that each loop consists of one or more transcription units for a specific pre–messenger RNA. This idea was given a major boost by the elegant studies of Oscar Miller, who discovered how to examine the transcription units by electron microscopy[8]. Oscar was one of my first postdoctoral students (while I was still at the University of Minnesota), but he invented 'Miller spreads' completely on his own after leaving my lab.

Gene amplification

In 1963, I took a sabbatical leave from the University of Minnesota and returned to Yale for what I thought would be one year. Before that year was out, I was invited to stay as a full professor and to plan a laboratory for the new Kline Biology Tower, the construction of which had just begun. At that time, molecular biology was a fledgling science that clearly held the key to future studies in cell biology. But I had almost no training in biochemistry and felt woefully inadequate to make any useful contribution. After much soul-searching, I decided that self-education was the only hope, so I spent the next several years teaching myself how to handle RNA and DNA.

Because of my earlier interest in LBCs, I chose to study the amphibian oocyte nucleus, also called the germinal vesicle. It took only a few experiments to show that the major RNA being synthesized by the germinal vesicle was ribosomal RNA. At about that time, the mid-1960s, several groups had shown that ribosomal RNA was synthesized in the nucleolus. The template for this synthesis was the ribosomal DNA (rDNA), located in the nucleolus organizer, a specific chromosomal locus discovered in maize some 30 years earlier by Barbara McClintock[9]. The germinal vesicle didn't fit this pattern at all; since the nineteenth century it had been known that the germinal vesicle contained over 1,000 nucleoli that were physically independent of the LBCs. How could ribosomal RNA be synthesized in these nucleoli if they weren't anywhere near the rDNA of the chromosomal nucleolus organizer? The answer was quite simple: they contained rDNA of their own. This conclusion was arrived at independently by Oscar Miller, by Don Brown and Igor Dawid, and by me. Oscar produced elegant electron micrographs showing that nucleoli from the germinal vesicle contained active transcription units[8]. His electron micrographs of nucleolar 'Christmas trees' are among the most widely reproduced images in cell biology. Don and Igor isolated about 10,000 germinal vesicles by hand and showed that they contained far more rDNA than they should; that is, far more rDNA than the same number of somatic nuclei[10]. I took a more cytological approach, tracing the extra rDNA back to the time of its synthesis in the smallest oocytes of recently metamorphosed tadpoles[11]. During a short period in mid-prophase, the oocyte nucleus amplifies its rDNA genes about 1,000 times. As a result, about two-thirds of all the DNA in the early oocyte nucleus consists of extrachromosomal copies of rDNA genes.

In situ hybridization

The discovery of amplified rDNA led directly to the development of *in situ* hybridization as a technique for showing specific DNA and RNA sequences at the cellular level. From the first time I saw polytene chromosomes and LBCs, I wondered if there wasn't some way to demonstrate specific genes in the bands and loops of these giant chromosomes. After all, Coons and Kaplan had shown how to detect specific proteins within cells by fluorescent antibodies[12]. Why not some similar method for nucleic acids? When nucleic acid hybridization was discovered as a test tube phenomenon—several years before our work on amplified rDNA—I tried to hybridize RNA to the DNA of cytological preparations. Ribosomal RNA was the only available RNA because at that time there was no molecular cloning, and the only way to label and detect nucleic acids at the cellular level was by tritium autoradiography. So I hybridized tritium-labeled rRNA to cytological preparations, hoping to detect label at the nucleolus organizers of the chromosomes. The experiments were a total failure. A few years later, when I discovered how much rDNA there was in an early oocyte nucleus, I knew I had the material for a critical test of *in situ* hybridization. This time the experiment worked. When I hybridized tritium-labeled rRNA to cytological preparations of young oocytes, I saw specific label over the amplified rDNA (**Fig. 3**). The earlier experiments with somatic cells had failed

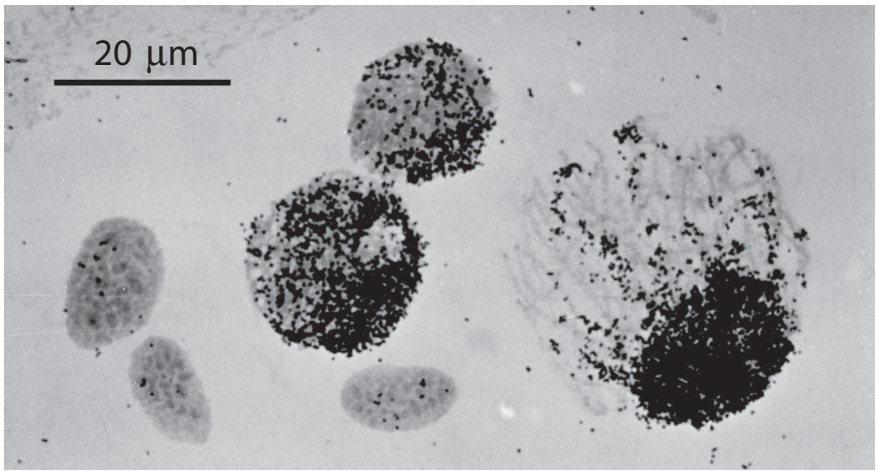

Figure 3 Autoradiograph of *Xenopus* ovary cells after *in situ* hybridization with ^{3}H-labeled ribosomal RNA. Strong labeling occurs over the amplified rDNA in three large oocyte nuclei (the cytoplasm is not visible). Only a few silver grains are present above the three smaller somatic nuclei. This photograph was taken in 1968 from one of the first successful *in situ* hybridization experiments.

simply because the target, the rDNA in a single nucleolus organizer, was too small. With positive results on oocytes, it was easy to optimize the technique and apply it to other cell types. Many of the original *in situ* hybridization studies were carried out with my graduate students Mary Lou Pardue[13] and Susan Gerbi[14].

In the ensuing years *in situ* hybridization became the standard method for localizing DNA and RNA sequences in cells and tissues. It has proven especially valuable for determining the time of expression of various genes during embryonic development. The use of fluorescent tags on the probe molecule[15] has greatly increased both the sensitivity of the technique and the precision of localization, permitting detection of individual genes and their RNA products within single cells and nuclei.

Satellite DNA and heterochromatin

In the early days of *in situ* hybridization, before gene cloning, there were few nucleic acid probes aside from rDNA. One that particularly piqued our interest was the so-called 'satellite' DNA of the mouse. This DNA, which constituted about 10% of all mouse DNA, could be isolated on a CsCl gradient as a pure fraction with a short (but unknown) sequence. It proved to be an excellent probe for *in situ* hybridization, and we were delighted to see that it hybridized to a specific region on each chromosome next to the centromere[16] (**Fig. 4**). We carried out similar experiments with satellite DNAs from *Drosophila melanogaster* and *Drosophila virilis*, again finding simple-sequence DNA near the centromeres[17]. These pericentromeric regions had long been recognized as special because of their unusual staining properties. They were called heterochromatin to distinguish them from 'normal' chromosomal regions, the euchromatin. In *Drosophila*, it was known that the heterochromatin contained very few genes. By linking satellite or simple-sequence DNA with heterochromatin, our experiments provided the first biochemical explanation for the lack of genes in these regions.

Tetrahymena and telomeres

At about this time, I turned my attention to the rDNA in the ciliated protozoan *Tetrahymena thermophila*. *Tetrahymena*, like all ciliates, has two nuclei. The small micronucleus has normal chromosomes and undergoes a more-or-less typical mitosis. By contrast, the giant macronucleus simply pinches in half at the time of cell division. For several reasons, I suspected that the macronucleus might contain extrachromosomal copies of rDNA, akin to the amplified rDNA in the amphibian oocyte. This proved to be true. When I isolated the rDNA as a biochemical fraction on a CsCl gradient, I found that it consisted of discrete molecules, some linear and some circular, but all of the same size[18]. Ultimately, these turned out to be large palindromic molecules, each of which contained two back-to-back copies of the rDNA sequence[19]. Because there were some circular molecules, as well as complicated star-shaped clusters, we suspected that the ends might contain a special sequence that permitted self-association. Elizabeth Blackburn had recently arrived in the laboratory as a postdoctoral fellow, after receiving her degree at the UK Medical Research Council laboratory in Cambridge with Fred Sanger. She was familiar with the emerging techniques of nucleic acid sequencing—her work at Cambridge consisted of sequencing the RNA transcribed from a 48-nucleotide fragment of the φX174 bacteriophage molecule. So, Liz took on the task of sequencing the ends of the *Tetrahymena* rDNA. She found that the ends consisted of multiple repeats of the hexanucleotide CCCCAA (or GGGGTT on the complementary strand)[20]. At that time, we did not suspect that this sequence, or minor variations on it, were found at the ends of chromosomes—the telomeres—in virtually all eukaryotic organisms. Later Liz and her student Carol Greider, still working

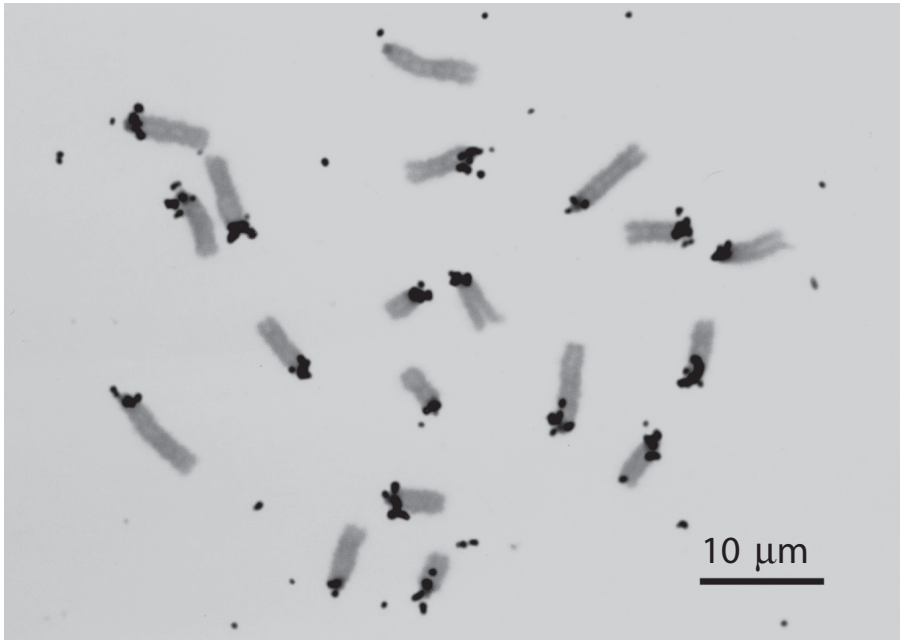

Figure 4 Autoradiograph of mouse chromosomes hybridized *in situ* with ^{3}H-labeled mouse satellite DNA. Label occurs over the heterochromatic region at one end of each chromosome.

with *Tetrahymena*, discovered telomerase, the remarkable enzyme that adds hexanucleotide repeats to preexisting telomeres. From this humble beginning in *Tetrahymena*, the telomere field developed into a major area of research, with important implications for cell division, cell senescence and cancer[21].

Cajal bodies

In 1983, I moved from Yale to the Department of Embryology of the Carnegie Institution in Baltimore, where I continued studies on the oocytes and LBCs of the frog *Xenopus*. For the past 15 years or so I have concentrated on a fascinating nuclear organelle originally discovered by the famous Spanish neurobiologist Santiago Ramón y Cajal. Ramón y Cajal and the Italian Camillo Golgi shared the 1906 Nobel Prize in Physiology or Medicine "in recognition of their work on the structure of the nervous system." In the course of his studies on neurons, Golgi identified a cytoplasmic organelle that attracted a great deal of attention and was immediately dubbed the Golgi reticular apparatus. Ramón y Cajal was not so lucky. He discovered a nuclear organelle in vertebrate neurons, which he named the accessory body because it was closely associated with the nucleolus[22]. Few people paid attention to Ramón y Cajal's accessory body, although it was independently discovered at least five times during the twentieth century in organisms as diverse as plants, insects, amphibians and mammals. Each time it received a new name. In 1999, I suggested that we adopt the name Cajal body to honor the discoverer. Fortunately, there was near consensus among the small number of workers in the field, and the name was readily accepted. More importantly, scientific order was brought to the subject in the early 1990s with the discovery of antisera that cross-react with a marker protein in Cajal bodies from a wide variety of organisms. Since then, it has become reasonably clear that Cajal bodies are sites for assembly and modification of the RNA processing machinery, particularly the splicing machinery. Currently, we are studying Cajal bodies in *Drosophila*[23], which affords many more opportunities for genetic manipulation than frogs and mammals, until now the favorite organisms in which to study these bodies.

ACKNOWLEDGMENTS

Although much of this account has been written in the first person, I hasten to say that very little of what has been accomplished in my laboratory would have been possible without the contributions of the wonderful graduate students, postdoctoral fellows, visiting scientists and technicians who have worked with me over the years. For a complete listing of their names and their current positions, see my web site: http://www.ciwemb.edu/labs/gall/index.php. I also deeply appreciate the extraordinary role played by the US National Institutes of Health (NIH) in funding basic research in the United States, beginning in the years immediately after the Second World War. I received my first NIH grant in the early 1950s and have had continuous funding since that time for our work on the cell nucleus. Most of the *Tetrahymena* studies were supported by the American Cancer Society, which also appointed me American Cancer Society Professor in 1984. One only has to travel to other countries to understand the importance of both governmental and private support in catalyzing the phenomenal growth of biological research in the United States in the latter half of the twentieth century.

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