The Double Entendre

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The double entendre.
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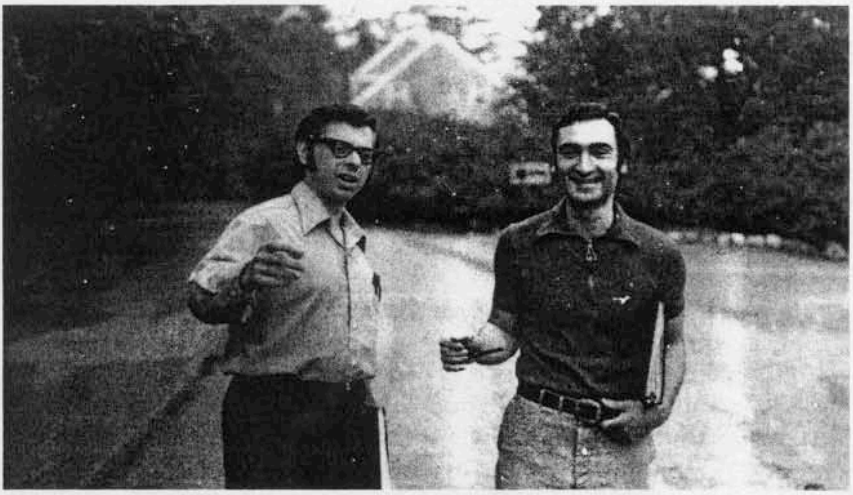
I never saw Fred Sherman in a sober moment. He was always intoxicated by his own slapstick sense of humor. Of course, my only real chance to observe him closely was each summer for the 17 years that we taught the Cold Spring Harbor Yeast Course together. Perhaps in other surroundings, away from the heat and humidity on Long Island, Fred is as solemn and dignified as any other university professor. But at Cold Spring Harbor for the 3 weeks of the Yeast Course, Fred was a one-man show. During those 17 years, the supporting cast changed—in the first year, we were assisted by Bruce Lukins and then by Chris Lawrence, Tom Petes, and in the later years by Jim Hicks. But none of us knowingly provoked Fred's antics. In fact, willingly or unwillingly I often served as his straight man.

To be accurate, Fred could be serious for short periods. These interludes usually occurred early in the morning when, not fully awake, he was presenting a lecture on genetic nomenclature or the arcane aspects of mitochondrial inheritance. His presentation always began, "Briefly,..." but, inevitably, he spoke for many hours. Delivered in a formal, somber academic style, these talks seemed to project from a surgically dehumored Fred. But, as the morning's coffee purged him of the anesthetic residue of sleep, and he became fully alert, all pretense of seriousness vaporized. There were moments of mock sobriety, but these were setups for impending humor.



The yeast course instructors. Fred Sherman (*left*) and Gerry Fink (*right*) in 1974.

Despite his zany antics, Fred was deadly serious about the Yeast Course itself. Throughout the winter months, he pondered logistical and equipment problems. He was constantly inventing inexpensive gadgets for the course to substitute for the costly ones used in research laboratories. His pride and joy was a device designed to examine the cytochrome content in wild-type and *petite* mutants. To replace the top of the line Carey spectrophotometer used in most laboratories, Fred assembled surrogate machinery consisting of a hand-held spectroscope and a cheap high-intensity lamp. The light was focused through a hole drilled in an aluminum cooking pot to illuminate yeast cells frozen in liquid nitrogen. Although the light intensity was difficult to control, this pots and pans contraption had remarkable resolution. Those students who were not temporarily blinded by the intense beam of the lamp were amazed by the clarity of the cytochrome spectra.

Probably his most useful invention was the Sherman micromanipulator. Early in the history of the course, we borrowed micromanipulators from Brooklyn College. They had only a few to lend and these were on their last legs. When we mistakenly left them at Cold Spring Harbor at the end of one summer, none survived winter use by the permanent residents as doorstops or beaker supports. The situation that faced us the next summer was bleak. Cold Spring Harbor could not afford the $5000 needed for even one Mortimer or de Fonbrune manipulator, and we needed eight of them. Faced with a frustrating but definable problem, Fred showed the ingenuity that had gained him celebrity as a scientist. Using elements from microchip assembly devices and various microscope parts, he invented a cheap micromanipulator that is now used throughout the world.

Each summer, we would arrive several days before the course began and work furiously to clean Davenport, usually with the help of graduate students from Fred's laboratory or mine. We would ferret out microscopes and media and set up workstations with a complete set of supplies and materials. These materials included an elaborate course manual (prepared during the winter months), a detailed description of each day's experiments covering the entire 3 weeks, and a set of strains for each pair of students (there were always eight pairs). The strains were updated each year to include new and useful genotypes from our own and other laboratories.

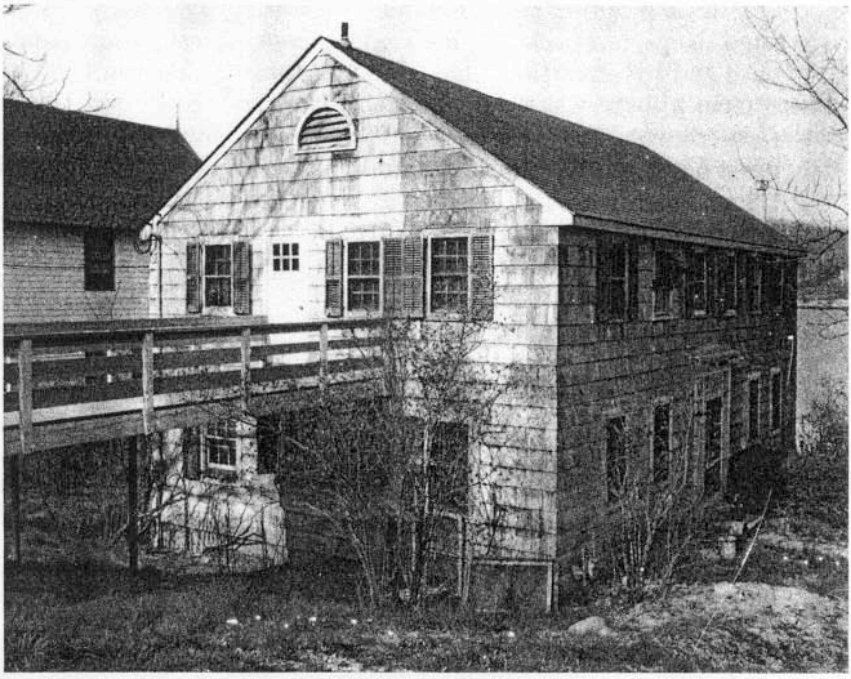
The laboratory manual, *Methods in Yeast Genetics*, assembled in one volume the standard methods and media for growing yeast as well as the latest techniques used in yeast biology. Our colleagues sent us detailed protocols, often before they were published, to be included in the most current version of the manual. This generosity was a sign of the collective trust and support of the yeast community for the course. These techniques as well as reagents and the yeast strains were given to the students with the idea that they could take them back to their own laboratories to expedite their research. The free distribution of strains and other information in the course contributed to the remarkably cooperative atmosphere that has existed in the yeast field.

The students arrived the day before the official opening, surreptitiously observing Fred's pre-course ritual of aligning microscopes, directing the assistants, and checking supplies. The whole scene had a business-like atmosphere portending a no-nonsense course. This serious façade was probably the ballast that steadied many of the students for the weeks ahead. It must have been daunting for some to realize that they were now thrown "back in school"

competing with the likes of Frank Stahl, Julius Marmur, Gottfried Schatz, or Clint Ballou. Even the more seasoned scientists had reason for nervous anticipation. They had given up the comfort of their own laboratories and homes to rough it for 3 weeks, with neither technicians nor students to do their bidding. Moreover, all students regardless of seniority slept in the Page Motel, a Spartan assembly of attached wooden cabins with the appearance of an evangelical campground. Each small room in Page was shared by two students and a cloud of marauding insects driven up the hill by the summer heat. It was rumored that Jim Watson ran the summer courses to feed his pet mosquitos in Page.

The discomfort of the living arrangements and the sense of isolation were intensified by the novelty of the new techniques and the difficulty of executing them in the environment of Davenport Laboratory (now called Delbrück Laboratory). In the first few days, the students struggled to master microdissection (picking up the yeast ascospores and separating them on the agar). They usually worked until 2 a.m. or later the first few nights just learning how to make a microneedle and to manipulate the spores. What they did not know was that the strain Fred brought for this experiment sporulated terribly and never yielded many of the prized four-spored tetrads. So, of course, they had trouble finding them. Although the strain he gave them consistently sporulated poorly, Fred always claimed that "sporulation is better this year than it has ever been, so you should find it easy to dissect."

Even if the frequency of sporulation had been reasonable, dissection would have been difficult because the micromanipulators and microscopes were old and often out of alignment. Moreover, the students' first attempts at making



Davenport Laboratory at Cold Spring Harbor as it appeared in the 1960s and early 1970s.

dissecting needles often resulted in a deformed spike that gouged the agar, entombing the prized spores beneath the surface. Armed with a defective needle and blurry microscope, it could take an hour to find a tetrad and move it up the agar. Davenport Laboratory had only a few window air conditioners to relieve the heat, but the thump of the compressors made the dissecting needles vibrate so wildly that even an expert could not harness the flailing tool.

So unnerving was the vibration of the air conditioners that they were often turned off even on the most stifling summer day. However, this adjustment gave rise to a new terror. The increase in heat and humidity seemed to spawn small aggressive sand fleas that were reborn each morning and mercilessly bit any uncovered appendage. Despite the faulty equipment and voracious insects, many of the students achieved mastery of tetrad dissection. Then they faced a new menace. Each summer, the assistants poured between 10,000 and 15,000 petri dishes, more than half of which became contaminated with a rapidly growing, florid, white mold. Davenport had once been a dusty old boat house and the opportunistic spores seemed to lurk in every crack, ready to sail into a petri dish. Given this atmosphere, it was not surprising that even those students who had weathered the technical difficulties and actually dissected spores would find the next morning that their prize dissection had been overgrown by a vicious mold. Fred's comment "Each student must dissect and analyze at least 10 complete tetrads to 'graduate' from the course. If you don't do so by the time the course is over, you can stay an extra week, or you can come back next year to finish up," seemed to push the weary students to the brink.

I have spent some time discussing the various irritations of a summer course at Cold Spring Harbor because the anxiety evoked by this environment created the perfect backdrop for Fred's humor. The students worked extremely hard and any diversion relieved the pressure and the routine. Each morning at 9 o'clock there was an organizational meeting concerning the progress of the experiments, followed by a 1–2-hour lecture by Fred or me on some area of yeast biology. After lunch, the students would work on an experiment until 2 o'clock when we would trot up to James Laboratory for a talk by a visiting scientist. Fred and I had lined up a lecture series by the most productive yeast biologists. After the lecture was over, the students went back to Davenport to work on experiments until dinner at 6. As the day was filled with lectures and discussion, most of the actual laboratory work was done between 6 p.m. and midnight.

But this prosaic description of a typical day does not convey the spirit of the course. Fred, clad in ragged cutoffs, greeted the students each morning with a deep bow. Then, from a balletic pose that tilted somewhere between first and third position, he would grasp a student's hand and escort him or her into the laboratory in a mock *pas de deux*. Once Fred began, there was no escape from his repartee. Even the most innocent greeting turned into a comedy routine.

Fred: How are you doing?
Student: Fine. How are you?
Fred: Well I think I'm fantastic. But not everyone agrees with me.

The organizational meeting that preceded the lecture at 10 o'clock was

replete with surprises. A typical problem solved in the very first meeting of the course was the choice of laboratory partner. The 16 students were asked to pair off as partners for the duration of the course. In Fred's hands, this decision was treated as an extremely serious endeavor. His prologue pointed out the importance of "choice of partner" as a critical educational experience. In view of this, the students were asked to give a brief explanation of their research interests to provide the others with the basis for making a choice. Just prior to the tense moment when students would make their fateful decision, Fred offered an important criterion for the choice: "Try to pick someone who complements your expertise so that you learn something. For example, if you are a biochemist choose a geneticist for a partner. If you are a geneticist, pick a biochemist. If you think you are smart, pick someone who is stupid."

As the course progressed, the morning sessions provided a forum for discussing both the latest published work and the lore, the unpublished anecdotal observations critical for the success of an experimental scientist. In the early days, as now, there were many alluring papers with provocative claims based on shaky data. In the spirit of protecting our wards, Fred and I liberally debunked these papers. So consistent was one author's trail of unsubstantiated claims that Fred balanced his cynicism with the comment, "Just because X has published it doesn't mean it isn't true."

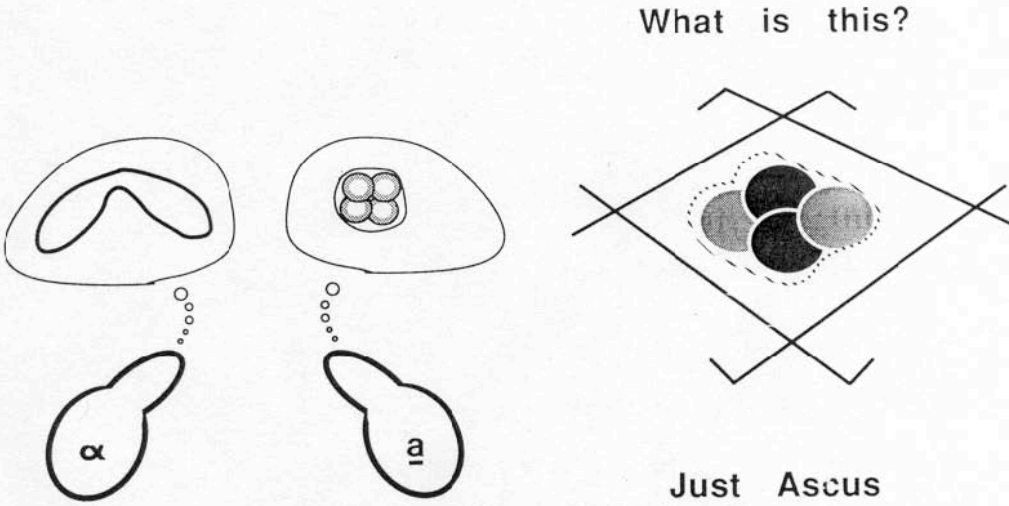
The only part of the day that did not regularly feature a wacky rendition by Fred was the guest lectures at 2 o'clock. But even these were not immune. Fred always wanted to introduce the speaker. His introductions usually began with a biography of the speaker presented in a dry, formal style, but gradually they would wander into saccharine hyperbole (recalling Oscar Wilde's witticism, "Biography poses a new threat to death"). A typical introduction might go something like this:

> This is Lee Hartwell from the University of Washington. He did his degree with Boris Magasanik etc., etc. Hartwell figured out the yeast cell cycle. He is a wonderful geneticist, simply fantastic, a remarkable human being and he is going to give an unforgettable talk. I just can't wait to hear it.

As the hoopla became more outrageous, Fred would appear to become possessed—roll his eyes, heave with rapid short breaths, and stumble off the podium. When Bob Mortimer spoke, Fred would introduce him by reciting a rhyme in a singsong voice:

> *Saccharomyces* on the plate,
> What's the latest thing you ate?
> Was it sugar?
> Was it good?
> Did you do the thing you should?

This doggerel was apparently composed by a student of Mortimer's, Lee Gunther. When asked about its origins, Fred would answer that the "poet" was the most publicized yeast geneticist in the world. This remark was astonishing because no one had ever heard of Gunther. However, Fred was right. Gunther's name (spelled Guenther) appears on every bottle of Difco Yeast



Two of the tee shirt designs from the Cold Spring Harbor Yeast Course.

Nitrogen Base w/o amino acids. Difco originally produced only *Difco Yeast Nitrogen base with three amino acids*. This configuration was extremely inconvenient for use as a minimal medium. Gunther convinced Difco to leave out the three amino acids and has become immortalized on each Difco label, "prepared according to Guenther's modification..."

The lectures were attended not only by the students, but also by Barbara McClintock, Jim Watson, and, in the first few years, Alfred Hershey and Max Delbrück. Delbrück had the habit of getting up in the middle of a lecture and then abruptly bolting out of the room. Max was a tall imposing figure with a shock of white hair, whose exit could not go unnoticed. Rumor had it that Delbrück could not sit through a lecture if the speaker did not get right to the point, or if he considered the point unimportant. The presence of these luminaries, and their apparent interest in this emerging field, seemed to improve the quality of lectures and to encourage even the most secretive speaker to spill out the latest data.

At night, Fred and I would wander about the laboratory discussing experiments with students and making sure that all the necessary reagents were available. Around 10 o'clock, Fred would disappear only to return an hour later washed and dressed for an evening of entertainment. He would usually enter the laboratory with a flourish, make a few balletic turns and announce "Let's dance!" As the students came to understand, this ritual was the prelude to a full night at Chelsey's, a bar in Huntington that provided live music by Little Wilson. At first, only a small coterie of devoted revelers followed the pied piper of yeastdom out of the laboratory, but, as the course proceeded, even the shy and decorous followed him. Chelsey's offered a respite from the commune-like atmosphere at Cold Spring Harbor Laboratory. Those who went seemed to have contact with the outside world and came back imbued with lively tales about the escapades of the previous evening. Each morning, stories of Fred's indefatigable dancing, one time with a biker's girl and another with a bagel, seemed to buoy everyone's spirits and raise expectations for some new and unexpected happening the next night. Although these festive

nights lasted well into the morning hours, all hands were on deck for the 9:00 lecture.

In the 17 years that we taught the Cold Spring Harbor Yeast Course (1970–1987, with a sabbatical in 1980 for a cameo performance in Brazil), Fred and I never had a personal argument. There were, however, many disagreements about scientific issues, often to the delight of the students. One of the disagreements had important consequences for the progress of yeast biology.

In the early 1970s, there was considerable opposition to the notion that yeast had any relevance to other systems. In fact, many scientists felt that yeast was an atypical organism, one that had wandered off the evolutionary tree. This notion was fostered by some yeast geneticists who exaggerated the difficulty of tetrad dissection in order to keep the field to themselves. Both Fred and I had determined that the Yeast Course would be dedicated to eradicating this view.

I was therefore surprised and angry when I heard Fred declare, "You cannot obtain deletions of a yeast gene by in vivo mutagenesis." As deletions

The class of 1982. Gerry Fink is jumping and Fred Sherman is crouching to his left. Jim Hicks is behind Fink's left shoulder.

were commonly obtained in bacteria and *Drosophila*, I thought that this was a preposterous statement. Furthermore, it seemed a violation of our pact to get yeast into the mainstream of science. I was piqued by Fred's assertion that yeast was different from the other "good genetic organisms." Fred regaled me with the details of extraordinary experiments involving the use of exotic high-energy particles to produce deletions in *CYC1*, at that time the best-studied gene in yeast. All had failed to produce deletions. I countered with speculations about the possible inviability of *cyc1* deletions and other arguments that seemed plausible at the time. The liveliness of the debate began to involve the students and they took sides. After realizing how heated the discussion was getting, Fred seemed to concede that maybe I was right. He concluded with the pronouncement: "It may be possible to get deletions in yeast, ...but not on this planet."

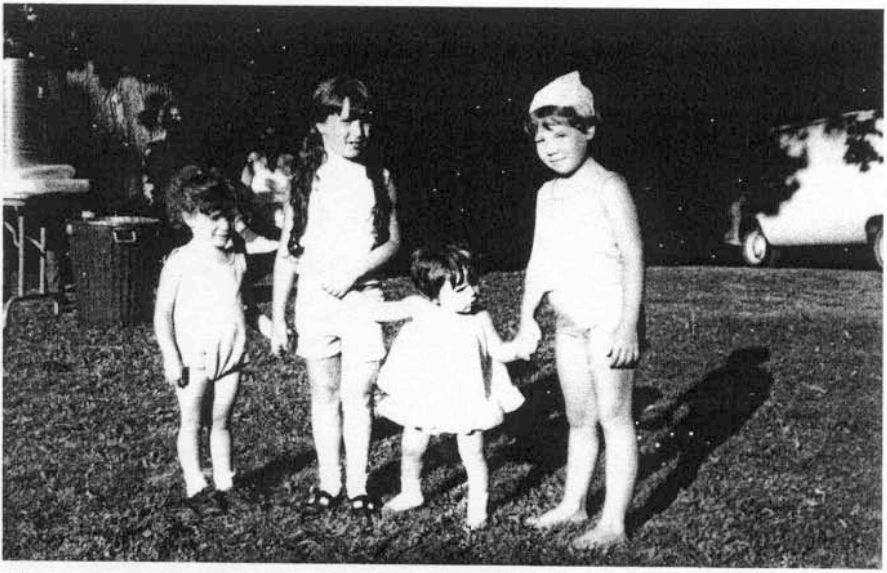
Determined to prove him wrong, I set out to find deletions at *HIS4* the moment I returned to my laboratory at Cornell. After isolating and characterizing several thousand EMS, UV, and spontaneous mutants, I came up empty-handed; not a single deletion was uncovered. It seemed that Fred was right after all. I was about to abandon these experiments when I heard that John Carbon had cloned the first yeast gene, *LEU2*. The availability of this gene seemed to provide an opportunity to set up a transformation system in yeast; the one hitch was that there were no *leu2* deletion mutants that could serve as recipients for the *LEU2* DNA that Carbon had cloned. Without a stable *leu2* mutant, it would be impossible to tell Leu^+ transformants from Leu^+ revertants. I was about to abandon the transformation idea when it occurred to me that it might be possible to construct an ersatz deletion by making a double mutant. In practice, a *leu2* double mutant would be as stable as a deletion and make a perfectly acceptable recipient. When a new postdoctoral fellow, Albert Hinnen, arrived from Switzerland, he agreed to try to construct such a double-mutant strain. The only thing going for us in this experiment was the abundance of *leu* mutants that I had collected in my abortive quest for deletions. The idea was to cross two of them, *leu2-3* and *leu2-112*, and to search through the meiotic recombinants to find the rare *leu2-3 leu2-112* double mutant. The key to the search was a recombination test in which each of the meiotic progeny was crossed by the single *leu2-3* and *leu2-112* strains. The vast majority of the population, the single mutant progeny, would recombine with one or another of the parents, whereas the desired double mutant would recombine with neither. Although there was nothing novel in the method, it had the tedious aspect that every meiotic spore had to be crossed by four strains to determine whether it was one of the parents or the desired double mutant. I warned Albert that he would have to screen lots of progeny because the double mutants were likely to be rare, perhaps as infrequent as 1 in 20,000 progeny. If so, this would mean 80,000 tests.

Albert was a thorough scientist and laboriously collected the large number of progeny I had told him were required to produce a winner. When I inquired about his progress, he replied curtly that although he needed only one double mutant, he had isolated a bushel full. It turned out that Albert was not only thorough, but very orderly as well. He had first collected what I had opined were the requisite number of progeny and only subsequently tested their genotype. My underestimate of the frequency of recombination at *LEU2* meant that he had needlessly processed tens of thousands of progeny. Once in

possession of this double mutant, however, Albert was able to proceed in his quest for transformation without the confusion of revertants and, also, without the benefit of further statistical projections from me. Indeed, Albert appeared at Cold Spring Harbor one day in August 1977 at the end of the course to announce that he and Jim Hicks, another postdoc, had achieved transformation of yeast. In the midst of my elation, I asked how frequent it was. Albert, with a rather deadpan expression, said he did not know because he had obtained only one transformant. One "transformant"! How could he be sure that this one colony was not a contaminant? Unperturbed by my obvious agitation, Albert replied that DNA obtained from this single transformant (and not from the untransformed strain) hybridized to the bacterial ColE1 sequences of the vector. This simple experiment showed beyond a doubt that he had achieved transformation and convinced me of the power of the new technology.

As I reflect on my years at Cold Spring Harbor, I wonder why I continued to teach the course for so long. Someone recently asked me why I was willing to sacrifice a month each summer away from my family. Actually, my family always accompanied me to Cold Spring Harbor. It was a wonderful vacation for them, and in a sense, they became part of the course. Walking down Bungtown Road from the beach, blond, tanned, and carefree, Rosalie, with my daughters Jennifer and Julia strutting behind, radiated a sense of calm domesticity; they provided a reminder to the students inside Davenport that there was another world on the outside.

Actually, the longevity of the course required considerable political skill on our part. Every year for as long as I can remember, Jim Watson would call Fred and me into his office and declare: "This is the last year of the course. Too few applicants means there is no interest in yeast. We can't afford it." This pronouncement was Jim's method of quality control. In the psychodrama that followed, Fred and I would plead the case for yeast to an ever more skeptical



The younger generation of yeast geneticists conferring at a Cold Spring Harbor course picnic: (*left* to *right*) Jennifer Fink, Julia Fink, Rhea Sherman, Joanna Sambrook.

grand inquisitor. Inevitably, these sessions ended with Watson's comment: "Well, we'll try it for one more year. But, if the number of applicants doesn't go up, next year will be the last year."

It strikes me that what drew me back each year was the allure and excitement of starting something new and the prospect of remarkable discoveries. There was the shared sense that we, both the students and the teachers, were standing at the threshold of a gold lode—we knew that yeast had great potential and we were eager to set about mining it. In the early years, the techniques we were teaching could not be learned at most universities. As a consequence, we teachers had the best of all possible situations: We had students desperate to learn. We in turn had to stay a step ahead of them. Wonderful students asked questions that sent me straight to the library—Randy Schekman wanted to know about yeast membranes, Ira Herskowitz about mating-type switching, David Botstein about sugar catabolism, Gottfried Schatz about mitochondrial biogenesis. The isolated environment of Cold Spring Harbor contributed a mystical quality that seemed simultaneously to expect and portend great discoveries. Although the description of the course advertised by Cold Spring Harbor was virtually the same for 17 years, each year was in reality a new course. Only Fred and I were a constant.

Each summer when the course began, I would wonder whether Fred was still enthusiastic. I never had to ask him. I could tell from the Cheshire grin that squeaked out as he partnered one of the students into the laboratory that he was up for another year.