A Phage Geneticist Turns to Yeast

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MIGRATING FROM PHAGE TO YEAST

I came to Cold Spring Harbor Laboratory in the summer of 1971 to take the Yeast Course taught by Gerry Fink and Fred Sherman. Many (if not quite all) of the early yeast molecular geneticists were, like me, "immigrants," in the sense that we learned molecular genetics in bacterial systems and then chose to apply this way of thinking to *Saccharomyces cerevisiae*. Possibly the best way to convey the spirit and the ideas of the early years of yeast molecular biology is to describe why and how I started to work with yeast.

In 1971, all of my research was focused on the temperate *Salmonella* phage P22. I identified completely with the phage community. Zinder and Lederberg (1952) had discovered generalized transduction in *Salmonella typhimurium*; P22 is the phage vehicle that carries the bacterial DNA from host to host, and it featured a wide variety of interesting biological properties that one could study. Despite this, very few laboratories were working on P22. The opportunity to study such a diversity of phenomena suited perfectly my eclecticism. Then as now, study sections would regularly comment on my "lack of focus." Although small at the time, my laboratory studied DNA replication, transduction and recombination (soon to branch into the study of transposons), morphogenesis of the virion, and of course the complicated genetic regulation of lysogeny. A very interactive and productive research community was interested in these issues, most of them working with the coliphage λ. I had some of the best scientific interactions in my career with this community and I learned from them. My work was interesting, it was challenging, it was productive, and it was great fun.

Why, then, did I even think about doing something different? The reasons derived from a general perception that the end of the road was near for phage molecular genetics. The mechanistic and regulatory paradigms, it seemed, were already all on the table, and many believed that no new principles might be found in these relatively simple systems. Articles appeared in prominent journals trumpeting the "end" of molecular genetics, the more amazing in retrospect because this was well before recombinant DNA technology was conceived. Those of us without tenure were earnestly warned that we might not be perceived as having a future were we to stick with our prokaryotic intellectual game for too long. It was time to get eukaryotic, to look to the future.

I took this advice very seriously. I am glad that I did, even though I believe now as I did then that the intellectual case against phage and bacterial genetics was entirely specious. However, the fact remained that most of my peers and betters thought the end was near, and, unfortunately, it was clear that their

thinking it being so made it so. Tenure committees, deans, and study sections, then as now, were impressed mainly by conventional wisdom. It is now clear that the whole eukaryotic/prokaryotic argument was silly. The intervening 20 years, especially the revolution wrought by recombinant DNA methods, showed prokaryotic biology to be full of new ideas, discoveries, and principles, many of which apply to eukaryotes as well. Nevertheless, there was a big rush of people and money out of the prokaryotic fields. The impressive progress that has changed the face of prokaryotic biology since was accomplished by what can only be called a supremely talented, courageous, and tenacious skeleton crew. Bacterial biology remains underfunded and underpopulated even today, showing the power of conventional wisdom even in the teeth of contravening facts.

Feeling the need to branch out into something eukaryotic, I determined to find a minimalist solution. I sought a field that would allow me to do sophisticated genetics in real time, as with phage; provide access to biochemical tests of genetic insights, as with phage; encompass a breadth of accessible biological phenomena, as with phage; be in an open and interactive community, as with phage; be relatively cheap to set up and bootleg if grants were not immediately forthcoming; and finally, be compatible with continuing work with phage and bacteria in the same laboratory. I considered several alternatives, which I then narrowed down to two: animal virology and yeast. I studied them both seriously, and favored yeast slightly, partly because of the expense associated with animal tissue culture. At about this time I met Gerry Fink, always an effective recruiter for *Saccharomyces*. His arguments and enthusiasm clinched the deal for me. I decided to add yeast to the portfolio of my already perilously "unfocused" phage laboratory and to continue with both. And so I did, although the strictly prokaryotic work has diminished to a low level in the last decade.

I was not alone in my way of thinking. Many other phage and bacterial geneticists traveled the same road. Almost all of us began by taking the Yeast Course at Cold Spring Harbor. In 1971, three bacterial geneticists came to Cold Spring Harbor from the Massachusetts Institute of Technology (MIT); the others were my laboratory partner for the course, Gerry Smith (then a student with Boris Magasanik), and Ira Herskowitz (then just starting a short stint in my laboratory as a postdoc). The Yeast Course, our first introduction to *S. cerevisiae*, was an intellectually wonderful experience. It was also fun. I learned a great deal more than just yeast genetics. By the most direct method, I learned that Ira Herskowitz is a consummate teacher. I am no athlete, yet in 3 weeks Ira taught me enough table tennis to hold my own with all but the most dedicated players. In subsequent years, I even became one of the Ping-Pong set at Gordon Conferences. I got far enough to play at the same table with David Freifelder (who was once national junior champion) and Frank Stahl (who takes his Ping-Pong very seriously indeed). Ira taught me by stressing the fundamentals, so that even though I could not always execute a shot correctly, I knew right from wrong. Genetics is like table tennis in that sense: The many different organisms upon which we practice genetics present diverse difficulties and opportunities in execution, but underneath the fundamentals remain always the same. That is why we geneticists are so easily able to recognize those of our peers who are "real geneticists," regardless of whether they work with phage, worms, flies, mice, or people. At Cold Spring Harbor, I be-

came convinced that Ira, who had then just finished his Ph.D., would become an uncommonly influential teacher and intellectual leader in genetics. I now believe that Ira was the most effective of all of the phage converts to yeast in applying the fundamentals of molecular genetic thinking to the biology of yeast.

The Cold Spring Harbor Yeast Course was central, in my opinion, in making the yeast molecular biology community what it is today. Gerry Fink and Fred Sherman in 1971 were leading yeast researchers who carried a conviction that the future lay with the melding of two schools of thought in genetics: the formally genetic and the molecular. Fred came to this through his long-term interest in cytochrome *c* and suppression (see, e.g., Sherman 1964; Sherman et al. 1973), and Gerry came to this through his devotion to histidine biosynthesis, the study of which he had undertaken both in yeast and in *Salmonella* (see, e.g., Fink 1966; Fink and Martin 1967). Gerry thus had his education divided between the two traditions he and Fred undertook to join and teach at Cold Spring Harbor. We, the prokaryote geneticists, brought with us an already sophisticated molecular thought process, following the intellectual paths illuminated by Jacob and Monod on the one side and Luria, Delbrück, and Hershey on the other. We brought with us an obsession with DNA and the central dogma, especially with the analysis of regulation at the level of transcription and, as we often hoped but rarely found, translation. We worried about messengers, ribosomes, and tRNA. Our favorite genetic tools were the selective cross and the *cis-trans* test.

Fred and Gerry offered their students formal diploid genetics, especially the marvel of tetrad analysis. We, being familiar mainly with bacteria and phage genetics, were impressed by the power of being able to make stable diploids at will and to carry out literally hundreds of complementation tests at once. We revelled in the simplicity with which tetrads and complementation testing on replica plates allows one to construct double mutants, even when the doubles cannot be distinguished from the single mutant by phenotype. We learned that there is genetics beyond the selective cross. We chafed, of course, at the difficulty in doing fine-structure genetics, the phage geneticist's bread and butter. Most of all, however, we were impressed with the garden of phenomena to which little, if any, molecular thinking and analysis had been applied.

All of this was of course already known to Fred and Gerry. But the differences in the styles of genetics between phage and yeast were a constant source of new ideas nevertheless. The prokaryotic geneticists proposed a great variety of schemes, only some of which were crackpot ideas that Fred and Gerry could shoot down at once. Other ideas reflected important insights that led to interesting science, some of which is now quite well-known. We were fascinated with the eukaryotic novelties: diploidy, chromosomes, centromeres, mitosis, mating, meiosis. We were somewhat taken aback at the relatively unsophisticated way (compared to bacteria) in which metabolic and especially regulatory studies were then still being done. The yeast community, beginning with our instructors, were just beginning to learn how truly powerful the molecular genetic tools could be. We immigrants had found, in yeast, a proper eukaryotic system in which we could practice molecular genetics, until then a discipline applicable only to bacteria and their viruses.

It is worth recalling some of the issues and ideas that date from those days.

One that preoccupied me was isogenicity. There was then no way to transfer small segments of the genome from one yeast strain to another. Thus, there was no good way to make isogenic strains. Yeast geneticists used strains of many different backgrounds that were crossed indiscriminately. From our experience with bacteria, we knew that this practice would compromise severely studies of regulation. Gerry Fink, with his hybrid background, already understood this, and following Gerry's lead, many of the immigrants in the early years spent much time backcrossing interesting mutations into our favorite strains. Our concern proved entirely justified when, 15 years later, the first pulsed-field gels showed massive and unpredictable differences in gross sizes of chromosomes among the laboratory strains! Much of the early work in my own laboratory began with programs of backcrossing; having decided upon S288C as our standard wild type, we crossed useful markers (such as Lacroute's uracil auxotrophs and Hartwell's standard cdc alleles) into this background, producing "congenic" derivatives, before working extensively with them. Only with the advent of DNA transformation did it become possible to construct rigorously isogenic sets of yeast strains with which to study subtle differences among mutants.

Many of us were interested in knowing which features of prokaryotic molecular biology are essentially the same in yeast and which are basically different. Sometimes we wanted to know for technical reasons; it seemed important to know, for instance, whether suppression by the dominant allele-specific "supersuppressors" worked via mutant tRNAs that have mutated to read stop codons, as in bacteria. We hoped, in this case, that the mechanism of suppression would be much the same, because then we could exploit nonsense mutations in similar ways. At that time, for example, there was no good way to find null mutations other than nonsense mutations that map near the beginning of a gene. Yet we always had a deeply ambivalent feeling when we found close similarity between bacteria and yeast. After all, if something turned out to work in yeast just as it did in bacteria, it would not be the kind of novel "eukaryotic" principle that conventional wisdom dictated we pursue. Out of this ambivalence, this balance between searching for what was truly new, on the one hand, and what we could understand and manipulate, on the other, we immigrant molecular geneticists were drawn to studies of suppressors, DNA replication, protein synthesis, and, most of all, regulation of enzyme synthesis. We all wanted to find the model regulatory system—the "Lac operon" of yeast. I was quite typical in this way; my earliest work in yeast included work on amber suppressors (Brandriss et al. 1975, 1976) and later the inducible secreted enzyme invertase (Carlson et al. 1981a,b). Ira began to work right away on mating type (Hicks and Herskowitz 1976; Hicks et al. 1977), which already that summer he perceived was really a regulatory system that controls cell type. This meant, as we never tired of telling whomever would listen, that there must be a great hierarchy of morphogenetic and regulatory genes that execute all the functions that differ among cells with the α, a, and a/α phenotypes (see Herskowitz and Oshima [1981] for a definitive statement of this view, first clearly articulated by MacKay and Manney [1974]). I believe that the mating-type system, as a regulatory paradigm, is the closest analog in yeast to the Lac operon.

My major interest, however, lay elsewhere. I was struck with the simplicity with which one could probe essential cellular functions in yeast, taking ad-

vantage of the haploid vegetative state and the simplicity of complementation analysis. The great innovator in this arena was Lee Hartwell, who with Cal McLaughlin had isolated and characterized many hundreds of temperature-sensitive mutants (Hartwell and McLaughlin 1968). Their explicit intention had been to use mutations to investigate any and all aspects of cell function; they would do for yeast what Edgar, Epstein, and colleagues had done for T4 (Epstein et al. 1964). Hartwell and McLaughlin applied very simple approaches lifted from phage and bacterial physiology (Hartwell had studied with Magasanik at MIT) to characterize the mutant phenotypes. They were able to identify genes specifically affecting RNA and protein metabolism and, of course, the progress of the cell cycle per se (for a fine early review, see Hartwell 1974). I recognized that this work was well advanced beyond anything that had been done with bacteria. It was comparable only to the attempts to saturate the genomes of phages (T4, λ, and, of course, P22) with mutations in essential genes. Indeed, this was the work I was engaged in with phage P22; I was already well on the way to identifying, using amber, heat-sensitive, and cold-sensitive mutations, every one of its essential genes (I missed only two or three in the end) and trying to determine what each of their products did for the organism (Botstein et al. 1972; for a comprehensive review of my work during this period, see Susskind and Botstein 1978).

Hartwell and McLaughlin's work was thus the single most important intellectual reason I decided to study yeast. I shared with the phage group the idea that the great use of genetics is to probe biological mechanisms. Hartwell's cell cycle work made it already clear that molecular genetics, properly applied to Saccharomyces, could lay bare the mechanisms underlying phenomena like eukaryotic DNA replication and mitosis, which already were known to be quite different from their prokaryotic counterparts. It was in this direction that I resolved that my laboratory should go with yeast.

GETTING TOGETHER THE MOLECULAR TECHNOLOGY

The arguments for yeast as a eukaryotic system in which one could study molecular genetics in the style of phage and bacteria had some weaknesses. These concerned molecular technology, which was, by bacterial standards, very primitive. During this period, bacterial geneticists had found ways to obtain genes of interest as pieces of DNA, mainly as λ specialized transducing phages. With these tools, it was possible to measure mRNAs by hybridization. Without them, one could not convincingly distinguish regulation at the level of transcription from that at the level of translation. Only by being able to measure mRNA level could we hope to study regulation in a meaningful way. The genes about which we knew a lot were, in the main, those that either were phage genes to begin with (e.g., λcI, encoding the λ repressor) or those that could be manipulated onto specialized transducing phage (notably, the gal, lac, and ara operons; for a good summary of the technology of the time, see Miller and Reznikoff 1978). Gerry Smith, my laboratory partner at Cold Spring Harbor, had produced a significant breakthrough in Boris Magasanik's laboratory just by isolating a λ phage that carried the hut (histidine utilization) operon from Salmonella (Smith 1971), with which he and his successors in Boris' laboratory figured out much about nitrogen regulation in bacteria (cf. Magasanik 1978).

During the summer of 1971, a group of us (including Ira, Gerry Fink, and myself) decided to look for viruses that might infect *S. cerevisiae*. Our idea was that if we could find viruses, some of them might allow us to make the equivalent of λ transducing phages for yeast. We were determined to find a "yeast phage," without which we would find it difficult to bring molecular genetics to full flower in yeast. We called breweries, associations of breweries, yeast taxonomists, and the like in the hope of learning whether batches of beer sometimes went bad because the yeast lysed. In our minds, we had visions of fermenters full of *Escherichia coli* lysing as a result of phage contamination (usually the dreaded T1). This was indeed a scenario that regularly plagued our biochemist friends. We thought that the brewing industry might therefore even be interested in funding our search; after all, had not Pasteur helped the vintners?

All of our respondents told us we were crazy—there were no yeast viruses. Fred Sherman was particularly downbeat. He understood our wish to find the yeast phage; this was by no means a new idea. Indeed, he related that his first project in Bob Mortimer's laboratory was to find yeast viruses! He had looked in all kinds of exotic places (including the zoo, in materials that apparently smelled not so nice) but never found anything. He thought it a waste of our time. The brewers were considerably more emphatic: Not only had they never observed viruses, but they did not want to know about it if one were found. They feared the public relations consequences of the news that Americans might be imbibing viruses along with their beer! In the aftermath of the recombinant DNA debacle, I look back with respect at the brewers' understanding of the American public psyche. In the midst of all this negative feedback, one brewer did answer the call. Jaime Conde, a brewmaster we soon affectionately called the "brewer from Seville," told anecdotes of failed beer batches in Spanish breweries involving lysis. There was correspondence, Jaime sent strains, and he eventually joined the Fink laboratory in Ithaca.

Nobody has yet found a yeast virus, at least not in the sense of something that makes an overtly infectious particle. Interesting science came out of the search, however. Both Gerry and I found quickly that the Seville strains harbored a very efficient killer to which most of the laboratory strains were susceptible. The Fink laboratory used these to define the killer phenomenon more accurately; they learned much about the particle and the RNA, and, probably most significantly, they isolated mutations of yeast that affected the maintenance of the killer (Fink and Styles 1972; Vodkin et al. 1974). In my laboratory, Ira and I found many killers in many different strains of yeast, some from laboratories and many more from the wild, although we stopped well short of the zoo. Jaime spent his time in Ithaca isolating the first karyogamy mutants with Gerry, although he did use the *kar1* mutant to show that killers can be transferred by cytoplasmic mixing (Conde and Fink 1976). After all this effort, we were technologically no better off than before.

In the fall of 1973, I persuaded Gerry Fink and John Roth (a mutual friend and colleague who had worked with Gerry on *Salmonella*) to go on sabbatical (due for all three of us the following year). We would work together on yeast, with an emphasis on genetic and molecular techniques. John suggested and vigorously pursued San Diego as a venue, but this fell through because our hosts were afraid that our yeast would contaminate their tissue culture. Like biochemists, who feared all phage because of their problems with phage T1,

cell culturists to this day fear all yeast because of their contamination problems with *Candida* species. Despite all our learned and perfectly sound arguments that *Saccharomyces* and *Candida* are as different as cats and bats, reason did not prevail, and so we had to look elsewhere. Jim Watson suggested we come to Cold Spring Harbor instead and use the Davenport Laboratory (since renamed in honor of Max Delbrück). Davenport had been used for courses in the summer (including the Yeast Course) but not in the winter. The building had just been renovated, heating had been installed, and, best of all, the equipment we needed would be available from the summer courses. To get fellowships and some modest grant support, we proposed a big inbreeding scheme to get Hartwell's mutants into the S288C background so that we could properly do pseudoreversion genetics, a collaboration with Ray Gesteland (then permanent staff at Cold Spring Harbor) to prove that yeast suppressors are alterations in tRNA, and of course the continued search for yeast viruses. We were fortunate enough to get a small grant that covered supplies and even a technician.

The sabbatical at Cold Spring Harbor in the academic year 1974–1975 was possibly the most important year of my career. Although we did not do all that much useful experimental work, we did think about what we were doing in genetics, what others were doing, the relative importance of genetic, biochemical, and other molecular tools, and quite generally what the future might hold. I think we stopped to question these general issues at a critical moment in the history of genetics. We debated the future of genetics, and the relative virtues of yeast and animal viruses, the great strength of the research program at Cold Spring Harbor. Joe Sambrook was the senior virologist, and his group included as postdocs such embryonic luminaries as Phil Sharp and Mike Botchan. One of the direct consequences of our proximity to Sambrook's laboratory was that we learned about restriction enzyme technology from the pioneers. One discussion that had a particularly powerful effect on me was Mike Botchan's seminar on his then planned use of the gel-transfer hybridization technique (Southern 1975 [then still unpublished]) to determine the site(s) of integration of SV40 (Botchan et al. 1976); we discussed for weeks how one might use Southern's blotting method to study isolated yeast genes someday. Most of these ideas have actually been reduced to practice since. Researchers at Cold Spring Harbor thought hard about each others' work, but we three did it non-stop. John and I talked a lot about transposition in bacteria, discussions that led to several years worth of experiments we later pursued in our own laboratories (cf. Kleckner et al. 1977). We became excited about the usefulness of transposons as genetic tools, which no doubt influenced Gerry to pursue the insertion mutations he encountered several years later in yeast. But most importantly, we recognized that the yeast virus might now not be necessary—there might be much better ways to get at the problem of gene isolation.

FROM YEAST DNA TO RECOMBINANT DNA

The key experiments establishing the recombinant DNA technology had just been done—the papers were already published or in press. I had played a small part in one of the experiments: Dale Kaiser had asked me whether I knew of a simple way to get a large quantity of DNA of uniform size with

flush ends that have a 5' phosphate and a 3' hydroxyl. He wanted this DNA for his student Peter Lobban's experiments. I suggested the P22 genome which Charley Thomas had shown (and I had later confirmed) had such a structure. This was why the seminal Lobban-Kaiser experiment (historically the true beginning of recombinant DNA technology) was done with P22 DNA (Lobban and Kaiser 1973). Gerry Fink and I were particularly impressed with the reports from David Hogness' laboratory at Stanford, where Pieter Wensink, using the Lobban-Kaiser technique, had established "banks" of *E. coli* clones each bearing an insert of randomly sheared genomic DNA from *Drosophila* (Wensink et al. 1974).

Gerry and I decided to make such a bank for yeast, whereas John put most of his effort into the tRNA collaboration (ultimately successful; Gesteland et al. 1976) and the backcrosses. Making recombinant DNA banks was, at that time, a laborious procedure, requiring not only considerable effort, but also expertise not generally available. We made a deal with Pieter Wensink (who had just moved to Brandeis' new Rosenstiel building) to collaborate. All we needed was to bring some yeast DNA, some vector DNA, and an offering of a useful enzyme. This offering was a common practice in the days before commercial enzymes: If you used somebody's reagent, you tried to repay with another scarce reagent. I provided Tom Maniatis (then at Cold Spring Harbor doing his famous cDNA cloning of the globin genes; Maniatis et al. 1976) with some semi-skilled labor for a preparation of the 5' exonuclease of λ, some of which we later gave to Pieter. That was the easy part, now we needed the yeast and vector DNAs.

Nobody had published a good DNA preparation from yeast, let alone one that gave good yields of high-molecular-weight DNA of high purity. I tried the best one and found that it yielded only about 15% of the nuclear DNA, most of it in small fragments on the order of 10 kb. Fortunately, I had some relevant experience. My Ph.D. thesis had involved recovering unsheared DNA preparations from phage-infected bacteria (cf. Botstein 1968). So Gerry and I set about learning how to lyse yeast thoroughly without degrading the DNA and then how to purify that DNA. For the latter step, we needed an ultracentrifuge. Our hosts at Cold Spring Harbor graciously agreed to let us move one of theirs into Davenport. We thought all was lost when the centrifuge fell off the bulldozer that was used to carry it cross-country over the lawn from the Demerec building. Fortunately, Beckman's Spinco division made sturdy machines, and the dented centrifuge worked fine after the Spinco repairman had ministered to it. Beckman didn't even charge us extra, even though it was obvious that the damage was the result of the failure of our exotic transport system. We learned to make DNA, but in pitifully small amounts. The good news about *Saccharomyces*, after all, is its very small genome; the corresponding bad news is that one gets very little DNA per gram of cells. After several months, we pooled three of our best preparations, which yielded barely enough to bother visiting Brandeis.

The vector DNA was not much simpler to obtain. In those days, there was much controversy concerning the plasmid vectors. We chose to work with pSC101, the earliest Cohen-Boyer vector, which we obtained from Stan Cohen (Cohen et al. 1973). There was considerable competitive excitement among different laboratories about how to grow the cells containing various plasmids in order to achieve good yield and, even more important, how best to separate

the plasmid DNA from the chromosomal DNA of the bacteria. Gerry and I were confused by the conflicting advice and claims, so we started on a comprehensive study of parameters such as cell density, presence or absence of chloramphenicol, and the myriad variables in the extraction and purification procedures themselves. We made some progress but were depressed by the reality that we were still making tenfold less DNA per liter of cells than the published claims of some researchers. One day, Gerry called one of these claimants and explained our distress, providing some indication of the results of our study of the problem. After Gerry got to the punch line—that after all this effort we had recovered "only so many micrograms per liter"—there was silence on the line. Finally, our colleague asked "tell me again how much you got per liter?" Gerry repeated the number, and then was astonished to be asked most urgently to send full details of our protocol. It turned out that our claimant friend had published his "best" result, which he could no longer repeat. Our amateur effort was threefold better than his current efforts! We might even, at that moment, have held a world's record for yield and purity of pSC101 DNA! We eventually were able to make adequate, if not yet massive, amounts of pure pSC101 DNA.

The truly wonderful part of our sabbatical year was that we three were equals, thinking and working together on problems of mutual interest. We had all day to think and do experiments. Each of us had a large and active laboratory in Cambridge, Ithaca, and Berkeley, where our postdocs and graduate students were all working hard. Although we were at Cold Spring Harbor also working hard (probably thinking harder about science there than at home), we were not doing nearly as many of the non-science chores involved in running a laboratory and being its guru. We were reminded of this by the almost daily calls from our home laboratories. For example, 1974 was the year of the agar crisis; hard on the heels of the oil crisis, microbiologists were subjected to the unavailability of agar for plates from the normal sources. Each of these august suppliers rationed their customers; like most of the world, they did not understand that genetics uses considerable amounts of plates. One of us discovered that a German company (Fluka) had agar for sale in quantity but that it was not quite pure enough for minimal medium. So we each instructed our laboratories back home to buy some, wash it, and use it for most experiments, saving the rationed Difco agar for critical experiments. One day, one of Gerry's postdocs called Davenport in a panic. They had decided to wash all of their Fluka agar (several kilos) in what they regarded as a large pot. When they had added the water, the agar began to swell and quite quickly to overflow, at which point the calls to Cold Spring Harbor began. What was to be done? What, indeed, could Gerry tell them? New garbage cans were found, the agar was washed in gallons of water with considerable losses, but the day was saved. We got some amusement from the picture of the sorcerer's apprentices (Gerry made a fine sorcerer) dealing with the overflowing agar, but we also were reminded that we were truly enjoying science in a way we could not responsibly do at home. My postdocs were very conscious of this as well; I encouraged them to be self-reliant in my absence and they more than rose to the challenge. In the spring of 1975, the time came for first-year MIT students to choose a thesis advisor. Three of my postdocs (Susan Gottesman, Nancy Kleckner, and Mimi Susskind) conspired to accept one of the students they liked (Fred Winston) without my knowledge. One day they called, each

on an extension, and presented their case to me as a *fait accompli*, complete with a sad tale of the injustice that Fred's entry into my laboratory would avert. What could I do? I went along, thinking all the time how much agonizing had been avoided and hoping that the choice was a good one. Once I got to meet Fred in person, I was of course most happy with their choice. So even in this most important of academic activities I got the year off.

I attended only one meeting that year, the famous Asilomar meeting called with much fanfare to consider the potential dangers of the recombinant DNA technology. This is not the place to recount the history associated with that event. In the present context, however, it is worth noting that I went with the conviction that this technology was the way of the future for yeast and with a real understanding of the magnitude of the opportunity. I will admit that at that time the possibility of danger from recombinant DNA seemed more real to me than it does now. Even then, however, I thought of danger only in terms of accidental inoculation of a laboratory worker with massive numbers of harmful organisms; such an event might occur with an industrial-scale preparation of, let us say, an oncogene-bearing plasmid. I was as surprised as anyone when the process of risk estimation by worst-case scenario began to run amok, as it did during the Asilomar meeting and even more afterward. I never thought yeast could pose a danger and, as a bacterial geneticist, was truly amazed that genes from *Salmonella*, which can cross with *E. coli* naturally, would seriously be considered a potential hazard. It was at Asilomar, with the real possibility of a moratorium that might inhibit our work with yeast, that I realized how much I had come to accept that recombinant DNA was the technical solution we were seeking and that our decision to work with yeast was going to depend on our ability to make that technology work. (A footnote to Asilomar: Much later, when we finally had useful clone banks in microtiter plates sitting in a liquid nitrogen freezer at MIT, a serious effort was undertaken to have them destroyed. This was a consequence of the notorious Cambridge City Council hearings, for at that time our yeast bank was the only existing collection of eukaryotic clones in bacterial hosts in the city. We sent a copy of the bank to Cold Spring Harbor, where it all began, to preserve it. Another copy went to Rochester, to Fred Sherman's laboratory. Whatever Fred may have thought about the wisdom of our search for the yeast phage, he was never in doubt about the usefulness of gene isolation.)

Later in the spring, after Asilomar, Gerry and I went to Pieter Wensink's laboratory where together we made the first yeast recombinants in plasmids. We had too little DNA and had made a minor mess of some of the experiments, but we showed it could be done, and done well enough to saturate the yeast genome. Tom Petes, who joined my laboratory at MIT as a postdoc just as I returned from sabbatical, repeated our DNA preparations and, with Pieter Wensink's help, made the first big plasmid bank (Petes et al. 1978). With it he characterized the ribosomal DNA (the most prominent feature in the DNA of yeast) and used a restriction fragment difference to show genetically that all of the rDNA maps to a single location (Petes and Botstein 1977). This experiment was our first published use of the Southern procedure, and it is the direct precursor of the use of restriction-fragment-length polymorphisms to map the human genome (Botstein et al. 1980). This is a story for another occasion, and I mention it only to reemphasize that genetics is a unitary discipline (cf. Botstein 1990). Yeast had a role very early in the development of a molecular

genetics of the human, just as phage had a role in the development of the molecular genetics of yeast.

MODERN TIMES BEGIN

The beginning of the modern era in yeast molecular genetics for me was the first Molecular Biology of Yeast meeting at Cold Spring Harbor. Gerry Fink and I, both of us still on sabbatical, organized the meeting, which included 167 participants. The program is interesting today both because so many of the major laboratories were already represented and because the influence of the alumni of the Yeast Course (by then 6 years old) was already clear. Every 2 years, the number of people at this meeting doubled, until the meeting outgrew the capacity of the Cold Spring Harbor facilities. In 1975, we adopted the strictly egalitarian phage meeting style—everyone who submitted an abstract gave a talk, and every talk was 15 minutes long. It was very like the phage meetings organized by Luria and Delbrück about 30 years before, and we were proud of that comparison. Indeed, the yeast community was beginning to resemble, in scientific impact as well as egalitarian style, the phage community. Everybody seemed to feel this sense of community, that our science was moving ahead, and that the yeast group was somehow going to make it into the vanguard of molecular biology.

The private discussions at the meeting, of course, concentrated on the possibility of isolating individual genes with recombinant DNA technology. Already many of us had made plans. We knew that our friend Ron Davis (who did not attend the meeting) was in the midst of isolating genes using bacteriophage λ vectors; his student Kevin Struhl would soon isolate the *HIS3* gene on the basis of its ability to complement a bacterial mutation in the gene specifying the corresponding enzyme, and John Carbon would do the same for *LEU2* (Ratzkin and Carbon 1977; Struhl and Davis 1977). I met François Lacroute at this 1975 meeting, and we decided to collaborate on isolating the *URA3* gene in the same way. In the end, we would use these clones to demonstrate transcriptional regulation of the gene in yeast (Bach et al. 1979). Although the really powerful DNA technologies (transformation, cloning by complementation, gene replacement, gene disruption, and gene fusions) were still far in the future, we could see already that the new technology would mean that yeast molecular genetics would flourish.

I remember clearly, at that meeting in 1975, being confident for the first time that my gamble (i.e., investing my effort in yeast instead of animal viruses) was going to pay off. I even fancied that others might also be "coming around" on the possibility that yeast might become a significant model system for eukaryotic molecular biology. The previous winter, Jim Watson had joined Gerry, John, and me for a memorable lunch at Blackford Hall during which he cross-examined us very thoroughly about yeast, especially our claims for its prospects as a model system for eukaryotes. We argued at length, told him about the future we envisioned, and illustrated with examples the actual and potential power of genetic analysis combined with the hoped-for recombinant DNA technology. We came away from the discussion thinking that we had made no impression whatsoever on Jim, who remained openly skeptical. But that summer, Jim came to many of the sessions of that first Molecular Biology of Yeast meeting. For 15 years I have believed that something must have im-

pressed him, taking as proof the famous little essay suggesting that yeast should become the *E. coli* for eukaryotic cell biology that I found in the next (third) edition of *Molecular Biology of the Gene* (Watson 1976). The joke is on me, of course, because the editors of this volume have pointed out to me that the essay first appeared in the *second* edition published in 1970! Of course this makes sense, because it was after all the Cold Spring Harbor Yeast Course that introduced most of us to yeast, and it had to be Jim Watson who invited Fred Sherman and Gerry Fink to teach such a course in the first place. So I no longer have evidence that Jim was impressed by anything that happened in 1975; instead, I have more evidence for Jim's ability to foresee, sometimes even prescribe, the scientific future. No matter, although the concept is clearly much older, I still believe that 1975 was the year in which yeast molecular biology became a reality.

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