The Modest Beginnings of One Genome Project

David B. Kaback¹

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry of New Jersey
New Jersey Medical School, Newark, New Jersey 07103

ABSTRACT One of the top things on a geneticist's wish list has to be a set of mutants for every gene in their particular organism. Such a set was produced for the yeast, *Saccharomyces cerevisiae* near the end of the 20th century by a consortium of yeast geneticists. However, the functional genomic analysis of one chromosome, its smallest, had already begun more than 25 years earlier as a project that was designed to define most or all of that chromosome's essential genes by temperature-sensitive lethal mutations. When far fewer than expected genes were uncovered, the relatively new field of molecular cloning enabled us and indeed, the entire community of yeast researchers to approach this problem more definitively. These studies ultimately led to cloning, genomic sequencing, and the production and phenotypic analysis of the entire set of knockout mutations for this model organism as well as a better concept of what defines an essential function, a wish fulfilled that enables this model eukaryote to continue at the forefront of research in modern biology.

THE yeast *Saccharomyces cerevisiae* genome project culminated with the first sequenced eukaryotic genome (Goffeau *et al.* 1996) and was followed by the functional analysis of almost all of its ~6000 genes. This project produced a near complete collection of deletion mutants that among other things attempted to define the number of genes that were essential for growth of this organism in the laboratory (Winzeler *et al.* 1999; Giaever *et al.* 2002). These projects had their roots in earlier studies that began with Carl Lindegren's first genetic map (Lindegren 1949; Lindegren *et al.* 1959), continued with the herculean efforts of Robert Mortimer and colleagues who compiled data from hundreds of investigators who had mapped genes (Mortimer and Hawthorne 1975; Mortimer and Schild 1980, 1985; Mortimer *et al.* 1989, 1992), and continues today through the *Saccharomyces* Genome Database led by Mike Cherry (Cherry *et al.* 1997, 2012) that curates genomic and related information.

This is the story of the first functional genomic analysis of yeast, which began with our investigation of chromosome I, the organism's smallest. It is worth telling because it traces its routes to the "phage school" and shows how what was deemed non-hypothesis-driven research and data collection

were actually tied to a fundamental question and indeed to several hypotheses. It is also about how sharing information, unselfish donation of materials, and true collaboration within the yeast community enabled this organism to rise to the forefront of molecular biology. It is told with the idea that it really does take a village to decipher a genome.

Beginning as a Rotation Project

In January 1972 during my first year in graduate school at Brandeis University (Waltham, MA), I began my second rotation in the laboratory of Harlyn Halvorson where I would end up doing my thesis research. Harlyn was the founding director of the Rosenstiel Basic Medical Sciences Research Center and with his overflowing schedule trying to get a research institute off the ground, his approach was to have new students pick a postdoc with whom they would like to work. Having spent the summer of 1970, after my junior year at college as a National Science Foundation-funded Undergraduate Research Participant at Cold Spring Harbor Laboratory (Cold Spring Harbor, NY), I was sufficiently indoctrinated into the dogma that genetics was essential to answer the complex biological problems before us. That particular summer was an incredible time to be introduced to molecular biology because it may have represented when the field came of age in that transcription factors, DNA replicases, reverse transcriptase and even restriction endonucleases all came to the forefront. In addition, it was the first

Copyright © 2013 by the Genetics Society of America
doi: 10.1534/genetics.113.151258

This article is dedicated to the memory of my mentors, Harlyn Halvorson and Norman Davidson.

¹Address for correspondence: Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, PO Box 1709, Newark, NJ 07101-1709. E-mail: kaback@umdnj.edu

time the famous Yeast Genetics course was offered and it became obvious to even a naive undergraduate that yeast was going to be an important model for future study. Yeast was not unfamiliar to me as I had arranged to carry out my senior undergraduate research at Stony Brook University (State University of New York at Stony Brook) with Vincent Cirillo, a biochemist studying membrane transport of sugars in this organism. Indeed, following my summer at Cold Spring Harbor I used yeast mutants in an attempt to find the inducible galactose transport proteins on polyacrylamide gels. I felt that if I had a better understanding of yeast genetics, perhaps the project might have been more successful. Thus, upon entering the Halvorson laboratory I was convinced I needed to learn how to do yeast genetics properly. At that time Susan Henry was a first-year postdoc and was considered the lab geneticist. Accordingly I asked if I could work with her. As she was actually studying the biochemistry of phospholipid biosynthesis during yeast sporulation (Henry and Halvorson 1973), a project that required limited genetics, we had to find a more fitting rotation project for me.

S. cerevisiae grows vegetatively in both haploid and diploid states and tolerates certain aneuploid chromosomes as disomics ($N + 1$) and monosomics ($2N - 1$), respectively. Bruenn and Mortimer (1970) had just published a method to isolate monosomic strains and reported several that were singly monosomic for chromosome *I*. Susan, a superb geneticist, realized that recessive mutations on the single monosomic chromosome would be expressed while those on the other diploid chromosomes would be complemented by their homologous wild-type (WT) allele. Dominant mutations on all chromosomes would also be expressed but are relatively infrequent. Thus, mutagenesis of the monosomic strain could be a good way to define genes in a chromosome-specific manner. We had also heard from Tordis Oyen, a former postdoc of Harlyn's, that DNA from a chromosome *I* disomic strain hybridized more rRNA than DNA from WT strains, suggesting some of the ~140 copies of the rRNA genes (rDNA) might be located on chromosome *I* (Schweizer *et al.* 1969; Goldberg *et al.* 1972). At the time chromosome *I* had only a single mapped gene, *ade1* (*ad1*) located near its centromere (Mortimer and Hawthorne 1969), making it an attractive candidate as a repository for rDNA. Nevertheless, the *S. cerevisiae* genetic map was in its infancy and this chromosome surely had to have some additional genes. Essential genes could be defined by mutations conferring thermosensitive (*ts*) growth on rich medium and there were no known essential genes on chromosome *I*. Thus, we anticipated that we would define a reasonable number of new essential genes, using this easy to assay phenotype. Since redundant rDNA was unlikely to produce a mutant phenotype, we reasoned that we might be able to surmise its location by finding lots of recessive *ts* lethal mutations elsewhere on that chromosome and one or more large regions devoid of mutations. Furthermore, if chromosome *I* disomes contained additional rDNA, the chromosome *I* monosomes

isolated by Bruenn and Mortimer (1970) might be useful for showing that they contained less rDNA if these genes were really located on this chromosome. These assumptions were all logical but some of them would turn out to be untrue.

I was excited by the project for several reasons. In 1970 as a senior at Stony Brook University I attended some lectures given by Bill Studier, where he described his just-published landmark studies on bacteriophage T7. Studier almost single-handedly produced both *ts* and nonsense suppressible mutants that appeared to saturate its genetic map, defining most of its ~30 genes. In addition, using the recently invented slab gel electrophoresis system, he identified the proteins encoded by most of these genes (Studier 1969; Studier and Hausmann 1969; Studier and Maizel 1969). Through the study of these mutants Studier and others quickly advanced some of the biology of this simple bacteriophage and our understanding of its gene regulation to the level of the far more studied but more complicated T-even bacteriophages (Edgar 1969; Summers and Siegel 1969; Studier 1972; Wood and Revel 1976). The summer following my graduation from college I participated in Brookhaven National Laboratory's (Upton, NY) undergraduate research program and got to interact with Bill Studier, so this story was firmly engrained in my mind. I also knew about Lee Hartwell's studies isolating *ts* lethal mutants that set the stage for *S. cerevisiae* being the eukaryotic organism of choice for studies on the cell cycle and macromolecular synthesis (Hartwell 1967; Hartwell and McLaughlin 1968; Hutchison *et al.* 1969; Hartwell *et al.* 1970). Using Susan Henry's idea, I saw myself starting a project that might parallel Studier's but one that involved a free-living real eukaryotic organism. While defining all the essential genes in an organism seemed out of reach, I thought it possible to at least define most of the essential genes on a single chromosome, which we guessed could represent ~5% of the genome. As Mortimer and Hawthorne (1966a,b, 1969) had recently published a genetic map with 16 centromere-associated linkage groups and five unlinked fragments not yet assigned to a specific chromosome, I either boldly or naively reasoned if there were 15 or so other like-minded people who could each take a chromosome, we might be able to do for *S. cerevisiae* what Studier did for bacteriophage T7. At the very least, I would be able to add some genes to one chromosome.

I thus began a project to mutagenize and screen the chromosome *I* monosomic strain for *ts* lethal mutants. I initially isolated 5 *ts* mutants and was able to show that 2 of these were indeed on chromosome *I* and curiously defined a single complementation group, which we called *tsl1* (Kaback and Halvorson 1978). Following this pilot study, I began to get more interested in the molecular biology of rDNA but in my spare time continued to isolate *ts* mutants and by early 1976 when it was time to write my thesis, I had ~100. Anxious to get my degree, I packed up these mutants and went to Pasadena, California, to pursue my postdoctoral

studies in the basement laboratory of Norman Davidson at California Institute of Technology.

Determining Gene Numbers in the Basement

At the time Norm(an) and his laboratory members were labeling genes so they could be either mapped by electron microscopy or enriched or isolated to enable further study. In these very early times of molecular cloning, unless one had a gene or lots of its mRNA in hand, it could not easily be cloned. Colony screening had just been devised but high-quality recombinant DNA libraries were not yet available (Grunstein and Hogness 1975; Maniatis *et al.* 1978). A few clones were available; most contained repeated DNA sequences that could be enriched by centrifugation or were made with cDNA from abundant RNAs. The Davidson laboratory was known for electron microscope (EM) mapping of viral genomes, transposons, and a few structural genes and people in the laboratory were most interested in trying to attach plastic spheres onto nucleic acids so they might float the complementary DNAs on sedimentation gradients or observe them in the EM adjacent to these distinctive spheres (Manning *et al.* 1975, 1977). Norm was particularly interested in studying large DNA molecules and the idea of studying gene arrangement by EM fascinated me so I began coupling spheres to some yeast nucleic acids. Frustrated by the chemistry, I began to putter with a new technique called R-looping. In this technique, RNA displaces a complementary DNA strand to produce a loop at the site of hybridization that is easily observable in the EM (White and Hogness 1977). I noted that most of the R-loops were too short, suggesting instability and that much of the DNA was completely denatured, making it more difficult to find apropos structures. We solved these problems with infrequent psoralen cross-links and the guanine reactive compound glyoxal, which enabled us to produce near full-length R-loops in stoichiometric quantities (Kaback *et al.* 1979, 1981). These modifications enabled us to rapidly map transcribed portions of cloned genes (Early *et al.* 1979; Hereford *et al.* 1979). In addition, I was able to help Eric Fyrberg and Karen Kindle find the first intron in a protein-encoding lower eukaryote (fruit fly) gene (Fyrberg *et al.* 1980).

Nevertheless, my real interests remained in yeast and with Lynn Angerer, another postdoc in the laboratory, I examined gene number and arrangement by directly visualizing poly(A)-containing RNA R-loop saturated yeast genomic DNA. Based on observing ~1 Mb of DNA (approximately one-tenth of a yeast genome equivalent) we estimated that yeast contains ~5000 genes expressed during vegetative growth (Kaback *et al.* 1979). This number was a useful refinement since it was ~40% higher than the RNA renaturation kinetic (R_0t curve) estimates (Hereford and Rosbash 1977) and turned out to be only ~15% below the actual gene number. I was also able to show that rDNA was most likely arranged in a single cluster (Kaback and Davidson 1980), which by then had been properly mapped to chromosome

XII, using one of the first demonstrations of RFLP mapping (Petes and Botstein 1977; Petes 1979). These studies gave us a look at the overall arrangement of genes on the *S. cerevisiae* genome and facilitated later studies using cloned sequences as they became available. Most important to me at the time was that these studies helped me leave the basement and obtain a faculty position near New York City. Since I never unpacked my *ts* mutants, I simply returned them to my luggage and shipped them back East.

No Phenotypes, Too Few Genes, and a Paradox

I started my laboratory with the idea to isolate genes that were preferentially transcribed during yeast meiosis. It was late 1979, and recombinant DNA technology had advanced to the stage where efficient library construction; colony, plaque, and differential filter hybridization; Southern and Northern blotting; S1 mapping; and both Sanger and Maxam–Gilbert sequencing were all available, as was yeast DNA transformation. I wanted to produce gene knockout mutants for the differentially expressed sequences, experiments that could lead to a better understanding of meiosis. Rod Rothstein and I simultaneously took up positions in adjacent laboratories at New Jersey Medical School. While I examined transcription during meiosis and sporulation (Kaback and Feldberg 1985), Rod was working on a scheme for making gene knockout mutations and graciously shared this technique with me as well as with others who would regularly come by the laboratory. Rod's one-step gene disruption (Rothstein 1983) worked so well that we quickly adopted it and used it to delete several sporulation-specific genes. These mutants exhibited no easily recognizable phenotype, then a surprising result that would become commonplace (Gottlin-Ninfa and Kaback 1986). As my real interest was in meiosis, the absence of any meiotic defect led me to abandon this project.

This phase of our research involved almost no “real” genetics and I longed to make mutants, do crosses, and dissect tetrads. Therefore, while people in the laboratory were working on cloning sporulation-specific transcripts and with some help from some undergraduate students, I decided to revive the *ts* mutants that I had isolated in the chromosome I monosomic strain and carried with me across the country twice. By 1980 chromosome I had grown from a short stump to a respectable small chromosome with genes that spanned from an unordered cluster containing *pyk1*, *tsl1*, and *cyc3* on the left arm to *FLO1* on the right arm, a distance of ~90 cM (Mortimer and Schild 1980). Analysis of my *ts* mutants began giving surprising results as all of them that were on chromosome I belonged to the same three complementation groups: *pyk1* (*cdc19*), *cdc15*, and *tsl1*, which turned out to be *cdc24* (Kaback *et al.* 1984; Mortimer and Schild 1985). By the time I decided to stop, of 41 mutants analyzed, 32 fell into these three complementation groups while 9 were on other chromosomes. Additional work using different mutagens in both John Pringle's and my laboratories confirmed

these studies. Indeed, when we were through, only 1 additional essential gene was uncovered and it appeared that chromosome *I* was saturated with mutants that defined only 4 essential genes (Kaback *et al.* 1984; Diehl and Pringle 1991; D. B. Kaback, unpublished results). If chromosome *I* were typical of other chromosomes and represented ~5% of the genome [based on the then available size estimate (Finkelstein *et al.* 1972)], it would suggest that there were <100 essential genes in the *S. cerevisiae* genome, an absurdly low number.

The paucity of *ts* mutants was contrary to what had been found in various bacteriophages in which approximately two-thirds of the genes are essential to produce a plaque. Mutant isolation and analysis of a free-living microbe appeared to be a much more difficult project. Was this gene number an accurate reflection of the number of essential genes or a consequence of gene immutability or gene redundancy, or both? Was temperature sensitivity too restrictive a phenotype or might organisms evolve in such a way that few genes were required for viability or growth? It was not possible to say. Indeed, low gene numbers could have been considered fashionable based on Burke Judd's hypothesis that each densely staining polytene chromosome band (chromomere) in the Zeste-White region of *Drosophila* corresponded to a single gene (Judd *et al.* 1972; Judd and Young 1974; Young and Judd 1978). It became obvious that with gene cloning and the gene knockout technology that were then at hand, we could ask whether the low essential gene number based on *ts* growth was fact or artifact. Thus, we began the functional genomic analysis of chromosome *I*.

Physical Mapping and Beginning the Functional Analysis of Chromosome *I*

The cloning of chromosome *I* began in late 1980 or early 1981. By then, yeast genes could be isolated with relative ease by complementation of mutations, using shuttle vector libraries capable of replicating in both *S. cerevisiae* and *Escherichia coli* (Struhl *et al.* 1976, 1979; Ratzkin and Carbon 1977; Nasmyth and Reed 1980). In addition, Louise Clarke, Craig Chinault, and John Carbon had successfully walked ~75 kb between *LEU* and the *MAT* locus in work that included molecular cloning of a yeast centromere (Chinault and Carbon 1979; Clarke and Carbon 1980). I surmised that we could first isolate a few chromosome *I* genes by complementation, clone the rest of the chromosome by walking (Chinault and Carbon 1979), map almost all of its genes by transcript analysis, and then delete each gene sequentially to determine whether it was essential. If there really were only four essential genes, it would be fascinating and we could at least examine the question of gene redundancy. If there were more essential genes, perhaps we could further investigate why we did not get *ts* mutants for them. At the very least, we would locate all the known genes and be able to add new genes to the genetic repertoire. Furthermore, we

would have a significant part of the yeast genome analyzed and those that needed chromosome *I* genes would have a resource. The idea to sequence the whole chromosome seemed entirely absurd but a year or two into the project, it became an obvious goal as well. Finally, I envisioned as I did when I started the mutant hunt that if there were some other laboratories carrying out similar studies, the whole genome would get analyzed and mutants for every gene would be available. Indeed, at least one other whole chromosome cloning project was started by Carol Newlon, who would later join my department (Newlon *et al.* 1991). Furthermore, the seeds for cloning the whole genome were being sown in Maynard Olsen's laboratory, using a "shotgun" approach (Riles *et al.* 1993).

Joan Crowley, my first graduate student, began the project by cloning the *ADE1* gene from a library made by Kim Naysmith and Kelly Tatchell (Nasmyth and Tatchell 1980; Crowley and Kaback 1984). Soon after, H. Yde Steensma came from the Delft University of Technology (Delft, The Netherlands) and began to clone most of the chromosomal DNA molecule, using the bacteriophage- λ library produced for shotgun cloning in Maynard Olson's laboratory (Riles *et al.* 1993). We started by probing this library with our *ADE1* clone and with *PYK1* (*CDC19*) and *PHO11* clones obtained from Dan Frankel (Kawasaki and Fraenkel 1982) and Rick Kramer (Andersen *et al.* 1983), respectively. Yde obtained several plaques that hybridized to each and further chromosome walking using these λ -clones produced a total of ~175 kb on three *contigs* (Steensma *et al.* 1987, 1989; Kaback *et al.* 1989).

We were joined by two students from John Pringle's laboratory who had cloned *CDC24* by complementation and had come to my laboratory to learn how to map transcripts to more precisely locate their gene. All of the initial complementing clones contained additional transcribed regions that we named *FUN* genes for *F*unction *U*nknown *N*ow with the idea that they would be fun to study but their designations were supposed to be only temporary. Yde realized that one of the *cdc24* complementing clones had a *FUN* gene with restriction fragments equal in size to the *PYK1* clone and its corresponding λ -insert. His observation was followed by genetic complementation and gene knockouts, which confirmed that *CDC24* and *PYK1* (*CDC19*) were much closer to each other physically than the genetic map suggested (Coleman *et al.* 1986). Furthermore, Rod Rothstein who mapped *CYC3* while in Fred Sherman's laboratory noted that this gene must be on our clones as well. Indeed *PYK1* and *CDC24*, which were only 6 kb apart, were >10 cM apart genetically, indicating that we had a *bona fide* hot spot for meiotic crossing over (Coleman *et al.* 1986). Glen Kawasaki and Rod had found that *pyk1* and *cyc3* mutations respectively gave high levels of gene conversion (Rothstein and Sherman 1980) but this was the first time anyone observed such high levels of crossing over in a relatively small physically defined region. This result served as a prelude to our demonstration that this small yeast chromosome undergoes

crossing over at an elevated centimorgan per kilobase level (Kaback *et al.* 1989).

By supplying a chromosome *I* gene probe to David Schwartz, a graduate student in Charles Cantor's laboratory, we soon had a better estimate of the physical size of chromosome *I* based on pulsed-field gel electrophoresis (PFGE) (Schwartz and Cantor 1984). Their estimate of ~300 kb was a bit high but when averaged with cytological estimates (Kuroiwa *et al.* 1984) produced a value very close to its actual size (Carle and Olson 1985; Mortimer and Schild 1985). Based on the R-loop analysis of gene density, it meant that chromosome *I* should contain ~100 genes. It also meant that we had ~80% of the chromosome cloned.

We cloned *MAK16* with the hope of connecting two of the contigs. This gene was defined by the only known *ts* lethal that we were unable to isolate in our mutant screen and was also required for yeast to maintain the virus-like double-stranded "killer" RNA (Wickner and Leibowitz 1979). One of the principles of our project was that it was going to be collaborative because it would not be possible to work on any one gene. The community was small enough so that we could get in touch with anyone interested in any particular gene. For this gene, it was Reed Wickner; while other genes we came across produced similar collaborations (Wickner *et al.* 1987; Barton *et al.* 1992; Malvar *et al.* 1992; Augeri *et al.* 1997). Most important for us was that this DNA narrowed the gap between two of the contigs, enabling them to be connected by a single restriction fragment (Kaback *et al.* 1989).

The physical location of the centromere and several additional genetically mapped genes were determined (Coleman *et al.* 1986; Steensma *et al.* 1987, 1989; Whyte *et al.* 1990). Finally, we produced our first restriction map of chromosome *I*, using rare hitting restriction endonucleases, *NotI*, *SfiI*, and *SmaI*, enabling us to localize all our cloned sequences and most of the genetically mapped loci on the full-length chromosomal DNA molecule. From this map, it was evident that *PHO11* and *FLO1* were close to the right end (Steensma *et al.* 1989) but there were no known markers to the left of *cdc24*. Thus, no genes had been found that mapped on almost one-third of the physical map of the chromosome. We introduced a marker on the most distal cloned region located ~50 kb to the left of *cdc24* and genetically mapped the insert, which increased the length of this chromosome's genetic map by about one-third. Most important was that this longer map indicated that the rate of recombination on the chromosome *I* DNA molecule was greater than the average rate so far found on other chromosomes (Kaback *et al.* 1989). Bob Mortimer and Maynard Olson and their colleagues (Mortimer *et al.* 1989, 1992; Riles *et al.* 1993) were able to corroborate that other short chromosomes also appeared to have higher than average rates of crossing over, supporting our suggestion that enhanced recombination ensured that small homologous chromosomes would recombine during meiosis.

Arnold Barton joined my group in the late 1980s and began systematically mapping transcripts and deleting undefined genes. In total we mapped transcripts on about half the chromosome and made deletions for about one-third of its ORFs. An interesting consequence of this study was that nonessential genes formed large clusters spaced by two or three islands that each had several essential genes. We speculated that essential and nonessential genes might be nonrandomly distributed throughout the genome (Barton and Kaback 1994). These findings were later corroborated by the whole-genome knockout project, which showed that essential genes are nonrandomly distributed in small clusters (Winzeler *et al.* 1999; Giaever *et al.* 2002).

The Yeast Genome Is Sequenced and All Its Genes Are Knocked Out by the Global Village

By the late 1980s Steve Oliver assembled a cadre of European investigators who began to sequence the chromosome *III* DNA molecule (Oliver *et al.* 1992). Soon thereafter, there was a movement to sequence other yeast chromosomes (Feldmann *et al.* 1994; Johnston *et al.* 1994, 1997). As we had most of a chromosome in hand, Jack von Borstel and Howard Bussey asked me to contribute our clones to a chromosome *I* effort. I was more than pleased to oblige since it meant a trip to Alberta to confer about the logistics of the project followed by early spring skiing in Banff. While Jack attempted to obtain funding, Howard Bussey and I agreed (over mojitos at Ernest Hemingway's favorite bar) that we would begin to generate some sequence to increase our chances for funding. Along with Michael Clarke, Theresa Kang, and others, the complete sequence of chromosome *I* was completed over the next couple of years (Bussey *et al.* 1995). During this time Andre Goffeau expanded Steve Oliver's original project and again through a cooperative effort of several dozen laboratories generated the complete sequence of the rest of the yeast genome, making it the first eukaryote sequenced (Goffeau *et al.* 1996). These were exciting times as numerous other model organism genome projects were getting started or reaching various phases of completion (Fleischmann *et al.* 1995; Blattner *et al.* 1997; C. elegans Sequencing Consortium 1998; Adams *et al.* 2000), almost all more or less modeled on the yeast consortium approach. The *S. cerevisiae* sequence was followed by the gene knockout project, another consortium (Winzeler *et al.* 1999; Giaever *et al.* 2002) that finished the work that in essence we started: work that I fantasized about as a first-year graduate student, a set of mutations for virtually every gene in a free-living organism. I have no regrets for not participating in this part of the project as we were now studying what I had originally intended, meiosis, and with my students and collaborators found a few other interesting phenomena that more fully captured our attention (Guacci and Kaback 1991; Kaback *et al.* 1992, 1999; Loidl *et al.* 1994; White *et al.* 2004; Scherthan *et al.* 2007). Coincidentally, some of these projects also started with a diploid yeast

strain containing a monosomic copy of chromosome *I* (Kaback 1989; Guacci and Kaback 1991; Loidl *et al.* 1994).

Examining Essential Gene Numbers

Returning to the gene number paradox, by inserting *URA3* into 200 random sites in a diploid and looking for recessive lethals, Mark Goebel and Tom Petes estimated that 18% or ~1000 *S. cerevisiae* genes were essential for growth on rich medium (Goebel and Petes 1986). This estimate proved to be accurate as the actual tally was 1108 genes (Giaever *et al.* 2002), a conclusion refined by investigations using conditional gene expression or conditional protein degradation (Kanemaki *et al.* 2003; Mnaimneh *et al.* 2004). While all of these studies have some degree of error (Ben-Shitrit *et al.* 2012), the estimate is almost certainly accurate. Nevertheless, an accurate estimate of the number of genes that are nonessential because they are functionally redundant would be useful to enable us to better determine the total number of essential genetic coding units. Some of these redundancies can be discerned by synthetic lethal screens but they are clearly limited to duplicated functions (Tong *et al.* 2001, 2004; Measday *et al.* 2005).

As for chromosome *I*, in the end it appeared to be fairly typical in that there were 14 of ~90 genes (16%) that when deleted produced a recessive lethal phenotype. Interestingly, *ts* alleles have been produced by more powerful *in vitro* mutagenic techniques in some of the genes that we did not find (Harris *et al.* 1992) and our paucity of *ts* lethals was a function of all the previously mentioned possibilities. Nevertheless, the classical genetic study that we began took us into the age of genomics, which, due to the interest of a great many people who realized the usefulness of this type of information, carried out the bulk of the work that I had envisioned as a first-year graduate student. It is again worth acknowledging that the libraries we and others used in our studies were available long before articles describing them were published. This community spirit is what enabled *S. cerevisiae* to be catapulted to the forefront and should serve as an important lesson regarding open science.

Equally important as the essential gene number is raising the question of whether an essential gene actually encodes an essential function. From an evolutionary standpoint, many genes that provide a growth or adaptive advantage are essential to the survival of the organism in the wild. Indeed, second-generation functional analysis began to examine a wide variety of growth phenotypes to define the functions of many more genes (Kelly *et al.* 2001; Giaever *et al.* 2002). Nevertheless, I felt the necessity to have a limited operational definition of an essential gene as one defined by a recessive lethal mutation that prevented growth in a specific medium, yeast extract, peptone, and dextrose (YEPD), at an optimal temperature (30°). It was assumed that the majority of these genes were vital for cell proliferation or survival under most conditions such that a deletion could never be overcome by changing physiological conditions

or genetic background. While these criteria were far more stringent than defining an essential gene by a *ts* growth phenotype, they were not perfect. There are now many examples where such lethality is not indicative of an essential function. Instead, the absence of the gene induces a situation where the cell no longer proliferates for other reasons. These mutations should be classified as “pseudoessential” or perhaps gain-of-function lethals. Recessive lethals that cause a buildup of toxic intermediary metabolites and auxotrophs that cannot grow on YEPD fit this class (Brandriss 1979; Landl *et al.* 1996). In most cases either these mutations can be genetically suppressed by a second mutation or the mutants can be grown in a different kind of medium. Similarly, spore germination mutations appear as recessive lethals in diploids but grow well when introduced directly into haploids. Perhaps even more perplexing are genes encoding essential protein complexes that still function when one or two parts are missing and therefore fail to produce a recessive lethal mutant (Babiano *et al.* 2012). Thus, defining an essential gene using deletions may never lead to a precise determination of which genes are essential and it may still prove useful to determine the percentage of recessive lethal knock-out mutations that can be coaxed into producing colonies. Furthermore, the number of pseudoessential and duplicated essential function genes may partially compensate for each other and, if almost equal, could keep the actual value for the essential gene number static.

Now that we know that there are free-living prokaryotic microbes with <500 genes (Fraser *et al.* 1995), it becomes easier to fathom that a simple eukaryote might be built from so few essential protein components as there are essential genes. Perhaps it might be possible to build a free-living yeast-like eukaryote with only ~1000 different protein parts. While some have contemplated producing a minimal organism, I suspect the essential component made up of not necessarily essential parts is likely to make such an endeavor more difficult than simply assembling genes that encode recessive lethal mutations. Furthermore, all the above studies are at best beset by complications such as neighbor effects, errors in the collection, differences in strain backgrounds, and noncoding and small RNAs. These complications create inaccuracies that can be rectified only by verification on a gene-by-gene basis. Nevertheless for yeast and the great majority of its genes that it shares with other organisms, the mutant phenotypes so far observed provide a proper launching site for further study.

Looking to the future, synthetic genomes bring a new challenge to the concept of essential genes. When there were 4 essential genes, we thought about replacing chromosome *I* with a plasmid containing these few genes. However, by the time we got around to assembling this minimal chromosome, another essential *FUN* gene was uncovered, confounding our efforts. As these experiments predated genomic sequencing and PCR, they were dependent upon the availability of appropriate restriction enzymes, which made the plasmid difficult to design. The advent of synthetic

genomes now makes construction of a minimal single chromosome and perhaps a minimal genome practical if not worthwhile (Dymond *et al.* 2011). Such synthesis of minimal chromosome *I* might first entail construction of a minichromosome with the 14 known essential genes. However, I doubt that this chromosome could replace the WT chromosome to produce a viable cell. Nevertheless, such failure could be followed by adding back large chromosomal fragments that might restore viability (Dymond *et al.* 2011). These chromosomal fragments might then be further explored to find their essential components that could then be added to the minimal constructs. In this way, my original question of the number of essential genes or components might be definitively answered.

One thing that is almost certain is that the high percentage of essential genes found in bacterial viruses is not a good paradigm for free-living organisms. Many of these pared-down viruses have evolved into successful minimalists, taking freely from their hosts to carry out only what is necessary for their own replication and transmission. Nevertheless, it should be realized from a historical perspective that the project described here began when viruses were the only paradigm for a near complete genetic map. During the past 40 years these investigations have shown that the complexity of even simple organisms is such that there is much more to learn and it is still hard to speculate about the real complexity of a living cell, much less of a metazoan. The ability to produce mutants by genomics rather than selection techniques has enabled us to approach this complexity in increasingly incisive ways, foretelling a future still open to great discovery.

Acknowledgments

I thank Mark Johnston, Zache Cande, Amar Klar, Purnima Bhanot, Marjorie Brandriss, and Vincent Cirillo for comments on the manuscript. These are my personal reminiscences and by no means represent a comprehensive review of the field of yeast genomics. The initial funding for the work described here came from the National Science Foundation (NSF) and would not have been possible without the support and encouragement given by my program coordinator, the late DeLill Nasser. Continued funding for these projects came from the NSF and the National Institutes of Health.

Literature Cited

- Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of *Drosophila melanogaster*. *Science* 287: 2185–2195.
- Andersen, N., G. P. Thill, and R. A. Kramer, 1983 RNA and homology mapping of two DNA fragments with repressible acid phosphatase genes from *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 3: 562–569.
- Augeri, L., Y. M. Lee, A. B. Barton, and P. W. Doetsch, 1997 Purification, characterization, gene cloning, and expression of *Saccharomyces cerevisiae* redoxendonuclease, a homolog of *Escherichia coli* endonuclease III. *Biochemistry* 36: 721–729.
- Babiano, R., M. Gamalinda, J. L. Woolford, Jr., and J. de la Cruz, 2012 *Saccharomyces cerevisiae* ribosomal protein L26 is not essential for ribosome assembly and function. *Mol. Cell. Biol.* 32: 3228–3241.
- Barton, A. B., and D. B. Kaback, 1994 Molecular cloning of chromosome *I* DNA from *Saccharomyces cerevisiae*: analysis of the genes in the *FUN38–MAK16–SPO7* region. *J. Bacteriol.* 176: 1872–1880.
- Barton, A. B., C. J. Davies, C. A. Hutchison, 3rd, and D. B. Kaback, 1992 Cloning of chromosome *I* DNA from *Saccharomyces cerevisiae*: analysis of the *FUN52* gene, whose product has homology to protein kinases. *Gene* 117: 137–140.
- Ben-Shitrit, T., N. Yosef, K. Shemesh, R. Sharan, E. Rupp *et al.*, 2012 Systematic identification of gene annotation errors in the widely used yeast mutation collections. *Nat. Methods* 9: 373–378.
- Blattner, F. R., G. Plunkett, C. A. Bloch, N. T. Perna, V. Burland *et al.*, 1997 The complete genome sequence of *Escherichia coli* K-12. *Science* 277: 1453–1462.
- Brandriss, M. C., 1979 Isolation and preliminary characterization of *Saccharomyces cerevisiae* proline auxotrophs. *J. Bacteriol.* 138: 816–822.
- Bruenn, J., and R. K. Mortimer, 1970 Isolation of monosomics in yeast. *J. Bacteriol.* 102: 548–551.
- Bussey, H., D. B. Kaback, W. Zhong, D. T. Vo, M. W. Clark *et al.*, 1995 The nucleotide sequence of chromosome *I* from *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 92: 3809–3813.
- C. elegans* Sequencing Consortium, 1998 Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282: 2012–2018.
- Carle, G. F., and M. V. Olson, 1985 An electrophoretic karyotype for yeast. *Proc. Natl. Acad. Sci. USA* 82: 3756–3760.
- Cherry, J. M., C. Ball, S. Weng, G. Juvik, R. Schmidt *et al.*, 1997 Genetic and physical maps of *Saccharomyces cerevisiae*. *Nature* 387: 67–73.
- Cherry, J. M., E. L. Hong, C. Amundsen, R. Balakrishnan, G. Binkley *et al.*, 2012 *Saccharomyces* Genome Database: the genomics resource of budding yeast. *Nucleic Acids Res.* 40: D700–D705.
- Chinault, A. C., and J. Carbon, 1979 Overlap hybridization screening: isolation and characterization of overlapping DNA fragments surrounding the *leu2* gene on yeast chromosome III. *Gene* 5: 111–126.
- Clarke, L., and J. Carbon, 1980 Isolation of a yeast centromere and construction of functional small circular chromosomes. *Nature* 287: 504–509.
- Coleman, K. G., H. Y. Steensma, D. B. Kaback, and J. R. Pringle, 1986 Molecular cloning of chromosome *I* DNA from *Saccharomyces cerevisiae*: isolation and characterization of the *CDC24* gene and adjacent regions of the chromosome. *Mol. Cell. Biol.* 6: 4516–4525.
- Crowley, J. C., and D. B. Kaback, 1984 Molecular cloning of chromosome *I* DNA from *Saccharomyces cerevisiae*: isolation of the *ADE1* gene. *J. Bacteriol.* 159: 413–417.
- Diehl, B. E., and J. R. Pringle, 1991 Molecular analysis of *Saccharomyces cerevisiae* chromosome *I*: identification of additional transcribed regions and demonstration that some encode essential functions. *Genetics* 127: 287–298.
- Dymond, J. S., S. M. Richardson, C. E. Coombes, T. Babatz, H. Muller *et al.*, 2011 Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. *Nature* 477: 471–476.
- Early, P. W., M. M. Davis, D. B. Kaback, N. Davidson, and L. Hood, 1979 Immunoglobulin heavy chain gene organization in mice: analysis of a myeloma genomic clone containing variable and alpha constant regions. *Proc. Natl. Acad. Sci. USA* 76: 857–861.

- Edgar, R. S., 1969 The genome of bacteriophage T4. *Harvey Lect.* 63: 263–281.
- Feldmann, H., M. Aigle, G. Aljinovic, B. Andre, M. C. Baclet *et al.*, 1994 Complete DNA sequence of yeast chromosome II. *EMBO J.* 13: 5795–5809.
- Finkelstein, D. B., J. Blamire, and J. Marmur, 1972 Location of ribosomal RNA cistrons in yeast. *Nat. New Biol.* 240: 279–281.
- Fleischmann, R., M. Adams, O. White, R. Clayton, E. Kirkness *et al.*, 1995 Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* 269: 496–512.
- Fraser, C. M., J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton *et al.*, 1995 The minimal gene complement of *Mycoplasma genitalium*. *Science* 270: 397–404.
- Fyrberg, E. A., K. L. Kindle, and N. Davidson, 1980 The actin genes of *Drosophila*: a dispersed multigene family. *Cell* 19: 365–378.
- Giaever, G., A. M. Chu, L. Ni, C. Connelly, L. Riles *et al.*, 2002 Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418: 387–391.
- Goebel, M. G., and T. D. Petes, 1986 Most of the yeast genomic sequences are not essential for cell growth and division. *Cell* 46: 983–992.
- Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon *et al.*, 1996 Life with 6000 genes. *Science* 274(546): 563–567.
- Goldberg, S., T. Oyen, J. M. Idriss, and H. O. Halvorson, 1972 Use of disomic strains to study the arrangement of ribosomal cistrons in *Saccharomyces* of ribosomal cistrons in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 116: 139–157.
- Gottlin-Ninfa, E., and D. B. Kaback, 1986 Isolation and functional analysis of sporulation-induced transcribed sequences from *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 6: 2185–2197.
- Grunstein, M., and D. S. Hogness, 1975 Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc. Natl. Acad. Sci. USA* 72: 3961–3965.
- Guacci, V., and D. B. Kaback, 1991 Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. *Genetics* 127: 475–488.
- Harris, S. D., J. Cheng, T. A. Pugh, and J. R. Pringle, 1992 Molecular analysis of *Saccharomyces cerevisiae* chromosome I. On the number of genes and the identification of essential genes using temperature-sensitive-lethal mutations. *J. Mol. Biol.* 225: 53–65.
- Hartwell, L. H., 1967 Macromolecule synthesis in temperature-sensitive mutants of yeast. *J. Bacteriol.* 93: 1662–1670.
- Hartwell, L. H., and C. S. McLaughlin, 1968 Temperature-sensitive mutants of yeast exhibiting a rapid inhibition of protein synthesis. *J. Bacteriol.* 96: 1664–1671.
- Hartwell, L. H., J. Culotti, and B. Reid, 1970 Genetic control of the cell-division cycle in yeast. I. Detection of mutants. *Proc. Natl. Acad. Sci. USA* 66: 352–359.
- Henry, S. A., and H. O. Halvorson, 1973 Lipid synthesis during sporulation of *Saccharomyces cerevisiae*. *J. Bacteriol.* 114: 1158–1163.
- Hereford, L. M., and M. Rosbash, 1977 Number and distribution of polyadenylated RNA sequences in yeast. *Cell* 10: 453–462.
- Hereford, L., K. Fahrner, J. Woolford Jr., M. Rosbash, and D. B. Kaback, 1979 Isolation of yeast histone genes H2A and H2B. *Cell* 18: 1261–1271.
- Hutchison, H. T., L. H. Hartwell, and C. S. McLaughlin, 1969 Temperature-sensitive yeast mutant defective in ribonucleic acid production. *J. Bacteriol.* 99: 807–814.
- Johnston, M., S. Andrews, R. Brinkman, J. Cooper, H. Ding *et al.*, 1994 Complete nucleotide sequence of *Saccharomyces cerevisiae* chromosome VIII. *Science* 265: 2077–2082.
- Johnston, M., L. Hillier, L. Riles, K. Albermann, B. Andre *et al.*, 1997 The nucleotide sequence of *Saccharomyces cerevisiae* chromosome XII. *Nature* 387: 87–90.
- Judd, B. H., and M. W. Young, 1974 An examination of the one cistron: one chromomere concept. *Cold Spring Harb. Symp. Quant. Biol.* 38: 573–579.
- Judd, B. H., M. W. Shen, and T. C. Kaufman, 1972 The anatomy and function of a segment of the X chromosome of *Drosophila melanogaster*. *Genetics* 71: 139–156.
- Kaback, D. B., 1989 Meiotic segregation of circular minichromosomes from intact chromosomes in *Saccharomyces cerevisiae*. *Curr. Genet.* 15: 385–392.
- Kaback, D. B., and N. Davidson, 1980 Organization of the ribosomal RNA gene cluster in the yeast *Saccharomyces cerevisiae*. *J. Mol. Biol.* 138: 745–754.
- Kaback, D. B., and L. R. Feldberg, 1985 *Saccharomyces cerevisiae* exhibits a sporulation-specific temporal pattern of transcript accumulation. *Mol. Cell. Biol.* 5: 751–761.
- Kaback, D. B., and H. O. Halvorson, 1978 Ribosomal DNA magnification in *Saccharomyces cerevisiae*. *J. Bacteriol.* 134: 237–245.
- Kaback, D. B., L. M. Angerer, and N. Davidson, 1979 Improved methods for the formation and stabilization of R-loops. *Nucleic Acids Res.* 6: 2499–2517.
- Kaback, D. B., M. Rosbash, and N. Davidson, 1981 Determination of cellular RNA concentrations by electron microscopy of R loop-containing DNA. *Proc. Natl. Acad. Sci. USA* 78: 2820–2824.
- Kaback, D. B., P. W. Oeller, H. Yde Steensma, J. Hirschman, D. Ruezinsky *et al.*, 1984 Temperature-sensitive lethal mutations on yeast chromosome I appear to define only a small number of genes. *Genetics* 108: 67–90.
- Kaback, D. B., H. Y. Steensma, and P. de Jonge, 1989 Enhanced meiotic recombination on the smallest chromosome of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 86: 3694–3698.
- Kaback, D. B., V. Guacci, D. Barber, and J. W. Mahon, 1992 Chromosome size-dependent control of meiotic recombination. *Science* 256: 228–232.
- Kaback, D. B., D. Barber, J. Mahon, J. Lamb, and J. You, 1999 Chromosome-size dependent control of meiotic reciprocal recombination in *Saccharomyces cerevisiae*: the role of cross-over interference. *Genetics* 152: 1475–1486.
- Kanemaki, M., A. Sanchez-Diaz, A. Gambus, and K. Labib, 2003 Functional proteomic identification of DNA replication proteins by induced proteolysis in vivo. *Nature* 423: 720–724.
- Kawasaki, G., and D. G. Fraenkel, 1982 Cloning of yeast glycolysis genes by complementation. *Biochem. Biophys. Res. Commun.* 108: 1107–1122.
- Kelly, D. E., D. C. Lamb, and S. L. Kelly, 2001 Genome-wide generation of yeast gene deletion strains. *Comp. Funct. Genomics* 2: 236–242.
- Kuroiwa, T., H. Kojima, I. Miyakawa, and N. Sando, 1984 Meiotic karyotype of the yeast *Saccharomyces cerevisiae*. *Exp. Cell Res.* 153: 259–265.
- Landl, K. M., B. Klosch, and F. Turnowsky, 1996 ERG1, encoding squalene epoxidase, is located on the right arm of chromosome VII of *Saccharomyces cerevisiae*. *Yeast* 12: 609–613.
- Lindgren, C. C., 1949 *The Yeast Cell, Its Genetics and Cytology*. Educational Publishers, St. Louis.
- Lindgren, C. C., G. Lindgren, E. E. Shult, and S. Desborough, 1959 Chromosome maps of *Saccharomyces*. *Nature* 183: 800–802.
- Loidl, J., H. Scherthan, and D. B. Kaback, 1994 Physical association between nonhomologous chromosomes precedes distributive disjunction in yeast. *Proc. Natl. Acad. Sci. USA* 91: 331–334.
- Malvar, T., R. W. Biron, D. B. Kaback, and C. L. Denis, 1992 The CCR4 protein from *Saccharomyces cerevisiae* contains a leucine-rich repeat region which is required for its control of ADH2 gene expression. *Genetics* 132: 951–962.
- Maniatis, T., R. C. Hardison, E. Lacy, J. Lauer, C. O'Connell *et al.*, 1978 The isolation of structural genes from libraries of eucaryotic DNA. *Cell* 15: 687–701.

- Manning, J., M. Pellegrini, and N. Davidson, 1977 A method for gene enrichment based on the avidin-biotin interaction. Application to the *Drosophila* ribosomal RNA genes. *Biochemistry* 16: 1364–1370.
- Manning, J. E., N. D. Hershey, T. R. Broker, M. Pellegrini, H. K. Mitchell *et al.*, 1975 A new method of in situ hybridization. *Chromosoma* 53: 107–117.
- Measday, V., K. Baetz, J. Guzzo, K. Yuen, T. Kwok *et al.*, 2005 Systematic yeast synthetic lethal and synthetic dosage lethal screens identify genes required for chromosome segregation. *Proc. Natl. Acad. Sci. USA* 102: 13956–13961.
- Mnaimneh, S., A. P. Davierwala, J. Haynes, J. Moffat, W. T. Peng *et al.*, 2004 Exploration of essential gene functions via titratable promoter alleles. *Cell* 118: 31–44.
- Mortimer, R. K., and D. C. Hawthorne, 1966a Genetic mapping in *Saccharomyces*. *Genetics* 53: 165–173.
- Mortimer, R. K., and D. C. Hawthorne, 1966b Yeast genetics. *Annu. Rev. Microbiol.* 20: 151–168.
- Mortimer, R. K., and D. C. Hawthorne, 1969 *Yeast Genetics*. Academic Press, London.
- Mortimer, R. K., and D. C. Hawthorne, 1975 Genetic mapping in yeast. *Methods Cell Biol.* 11: 221–233.
- Mortimer, R. K., and D. Schild, 1980 Genetic map of *Saccharomyces cerevisiae*. *Microbiol. Rev.* 44: 519–571.
- Mortimer, R. K., and D. Schild, 1985 Genetic map of *Saccharomyces cerevisiae*, edition 9. *Microbiol. Rev.* 49: 181–213.
- Mortimer, R. K., D. Schild, C. R. Contopoulou, and J. A. Kans, 1989 Genetic map of *Saccharomyces cerevisiae*, edition 10. *Yeast* 5: 321–403.
- Mortimer, R. K., C. R. Contopoulou, and J. S. King, 1992 Genetic and physical maps of *Saccharomyces cerevisiae*, edition 11. *Yeast* 8: 817–902.
- Nasmyth, K. A., and S. I. Reed, 1980 Isolation of genes by complementation in yeast: molecular cloning of a cell-cycle gene. *Proc. Natl. Acad. Sci. USA* 77: 2119–2123.
- Nasmyth, K. A., and K. Tatchell, 1980 The structure of transposable yeast mating type loci. *Cell* 19: 753–764.
- Newlon, C. S., L. R. Lipchitz, I. Collins, A. Deshpande, R. J. Devenish *et al.*, 1991 Analysis of a circular derivative of *Saccharomyces cerevisiae* chromosome III: a physical map and identification and location of ARS elements. *Genetics* 129: 343–357.
- Oliver, S. G., Q. J. van der Aart, M. L. Agostoni-Carbone, M. Aigle, L. Alberghina *et al.*, 1992 The complete DNA sequence of yeast chromosome III. *Nature* 357: 38–46.
- Petes, T. D., 1979 Meiotic mapping of yeast ribosomal deoxyribonucleic acid on chromosome XII. *J. Bacteriol.* 138: 185–192.
- Petes, T. D., and D. Botstein, 1977 Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. *Proc. Natl. Acad. Sci. USA* 74: 5091–5095.
- Ratzkin, B., and J. Carbon, 1977 Functional expression of cloned yeast DNA in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 74: 487–491.
- Riles, L., J. E. Dutchik, A. Baktha, and M. V. Olsen, 1993 Physical maps of the six smallest chromosomes of *Saccharomyces cerevisiae* at a resolution of 2.6 kilobase pairs. *Genetics* 134: 81–150.
- Rothstein, R. J., 1983 One-step gene disruption in yeast. *Methods Enzymol.* 101: 202–211.
- Rothstein, R. J., and F. Sherman, 1980 Genes affecting the expression of cytochrome c in yeast: genetic mapping and genetic interactions. *Genetics* 94: 871–889.
- Scherthan, H., H. Wang, C. Adelfalk, E. J. White, C. Cowan *et al.*, 2007 Chromosome mobility during meiotic prophase in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 104: 16934–16939.
- Schwartz, D. C., and C. R. Cantor, 1984 Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* 37: 67–75.
- Schweizer, E., C. MacKechnie, and H. O. Halvorson, 1969 The redundancy of ribosomal and transfer RNA genes in *Saccharomyces cerevisiae*. *J. Mol. Biol.* 40: 261–277.
- Steensma, H. Y., J. C. Crowley, and D. B. Kaback, 1987 Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: isolation and analysis of the CEN1–ADE1–CDC15 region. *Mol. Cell. Biol.* 7: 410–419.
- Steensma, H. Y., P. deJonge, A. Kaptein, and D. B. Kaback, 1989 Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: localization of a repeated sequence containing an acid phosphatase gene near a telomere of chromosome I and chromosome VIII. *Curr. Genet.* 16: 131–137.
- Struhl, K., J. R. Cameron, and R. W. Davis, 1976 Functional genetic expression of eukaryotic DNA in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 73: 1471–1475.
- Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis, 1979 High-frequency transformation of yeast: autonomous replication of hybrid DNA molecules. *Proc. Natl. Acad. Sci. USA* 76: 1035–1039.
- Studier, F. W., 1969 The genetics and physiology of bacteriophage T7. *Virology* 39: 562–574.
- Studier, F. W., 1972 Bacteriophage T7. *Science* 176: 367–376.
- Studier, F. W., and R. Hausmann, 1969 Integration of two sets of T7 mutants. *Virology* 39: 587–588.
- Studier, F. W., and J. V. Maizel, Jr., 1969 T7-directed protein synthesis. *Virology* 39: 575–586.
- Summers, W. C., and R. B. Siegel, 1969 Control of template specificity of *E. coli* RNA polymerase by a phage-coded protein. *Nature* 223: 1111–1113.
- Tong, A. H., M. Evangelista, A. B. Parsons, H. Xu, G. D. Bader *et al.*, 2001 Systematic genetic analysis with ordered arrays of yeast deletion mutants. *Science* 294: 2364–2368.
- Tong, A. H., G. Lesage, G. D. Bader, H. Ding, H. Xu *et al.*, 2004 Global mapping of the yeast genetic interaction network. *Science* 303: 808–813.
- White, E. J., C. Cowan, Z. Cande, and D. B. Kaback, 2004 In vivo analysis of synaptonemal complex formation during yeast meiosis. *Genetics* 167: 51–63.
- White, R. L., and D. S. Hogness, 1977 R loop mapping of the 18S and 28S sequences in the long and short repeating units of *Drosophila melanogaster* rDNA. *Cell* 10: 177–192.
- Whyte, W., L. H. Keopp, J. Lamb, J. C. Crowley, and D. B. Kaback, 1990 Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: isolation, characterization and regulation of the SPO7 sporulation gene. *Gene* 95: 65–72.
- Wickner, R. B., and M. J. Leibowitz, 1979 Mak mutants of yeast: mapping and characterization. *J. Bacteriol.* 140: 154–160.
- Wickner, R. B., T. J. Koh, J. C. Crowley, J. O’Neil, and D. B. Kaback, 1987 Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: isolation of the MAK16 gene and analysis of an adjacent gene essential for growth at low temperatures. *Yeast* 3: 51–57.
- Winzler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson *et al.*, 1999 Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* 285: 901–906.
- Wood, W. B., and H. R. Revel, 1976 The genome of bacteriophage T4. *Bacteriol. Rev.* 40: 847–868.
- Young, M. W., and B. H. Judd, 1978 Nonessential sequences, genes, and the polytene chromosome bands of *Drosophila melanogaster*. *Genetics* 88: 723–742.

Communicating editor: A. S. Wilkins