CHAPTER

6

Genomic and Proteomic Analysis of Wine Yeasts

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1. INTRODUCTION

The selection of suitable microorganisms for use in industrial processes is a key issue in food biotechnology. One of the key challenges in this area is to improve the properties of starter cultures, such as the ability to establish reproducible growth. Many of the programs aimed at enhancing the properties of industrial microorganisms, however, are restrained by a lack of sufficient knowledge regarding the metabolic and regulatory processes occurring within the cells. These shortcomings may, however, be short-lived, considering the continuous advances being made in functional genomics and proteomics. Studies in these areas will help, for example, to identify the effects of genetic alterations on final products, generate

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desirable pleiotropic effects through mutations in regulatory genes, predict stress responses in the different environments to which microorganisms are exposed, and identify genomic variations associated with adaptation to the particular conditions of winemaking.

This chapter focuses exclusively on the yeast species *Saccharomyces cerevisiae*. In addition to being the main microorganism involved in wine fermentation, it has been used as a model organism in molecular biology for many years (Miklos & Rubin, 1996) and is the only wine yeast species for which abundant genomic and proteomic information is available. It was the first eukaryote to have its complete genome sequenced (Goffeau et al., 1997), and, since then, numerous functional analysis projects have uncovered enormous amounts of information on the biology of this microorganism (Dujon, 1998). It can safely be said that *S. cerevisiae* is currently the best understood of all eukaryotic organisms. Most of the techniques currently used in functional genomics and proteomics were initially developed in this yeast. DNA chip, or microarray, technology, for example, was primarily developed using *S. cerevisiae* (DeRisi et al., 1997; Schena et al., 1995; Wodicka et al., 1997), and all the latest advances in this field have also been tested using this yeast (see Section 4). Vast amounts of data have thus been compiled on gene expression in *S. cerevisiae*. Indeed, the information on *S. cerevisiae* far exceeds that available for any other prokaryotic or eukaryotic organisms. As a result, it has been possible to propose global models for genetic and metabolic regulation (Gasch et al., 2000).

The fact that *S. cerevisiae* was the first microorganism to be widely used in the development of genome technology allowed other phylogenetically related yeasts to be analyzed subsequently in global sequencing projects, and the use of comparative genomics has since led to important conclusions regarding gene functionality (Butler et al., 2009; Cliften et al., 2003; Kellis et al., 2003; Liti et al., 2009; Souciet et al., 2000). DNA microarray analysis is a very useful tool for comparing genomes from different strains of *S. cerevisiae*, including wine strains (Carro et al., 2003; Hauser et al., 2001; Schacheter et al., 2009) and similar species.

S. cerevisiae has also been used in the development of the more recent field of proteomics. Proteomic studies have generated vast amounts of data on protein expression profiles and variability in laboratory strains of *S. cerevisiae* (Washburn et al., 2001), and these have recently been extended to include wine strains (Rossignol et al., 2009; Trabalzini et al., 2003; Zuzuárregui et al., 2006). Important advances have also been made in metabolomics, a new field in which *S. cerevisiae* is practically the only eukaryote to have been studied to date (Raamsdonk et al., 2001; Rossouw et al., 2008). The integration of different types of "omic" data into predictive models has provided the basis for new research strategies in systems biology (Borneman et al., 2007; Pizarro et al., 2007).

Most of the information that has been gathered in all of the above areas is related to laboratory strains of *S. cerevisiae*, although more recent studies have been extended to other strains (particularly wine strains) and industrial processes (Bisson et al., 2007). Knowledge generated from the analysis of laboratory strains may be helpful in understanding the results of studies conducted with wine strains during industrial fermentation, and it is extremely simple to apply techniques used with laboratory strains to their industrial counterparts. This chapter will therefore also look at the methods used and results obtained for non-wine strains of *S. cerevisiae*.

2. GENOMIC CHARACTERISTICS OF WINE YEASTS

The history of wine yeasts is as old as the earliest civilizations in the Mediterranean

region, with the first references to winemaking dating back to 7400 years ago. Reports of wine production were limited to this geographical area for many centuries, until the practice was spread to other parts of the world with suitable climate conditions, as Europe embarked on its conquest of other continents in the fifteenth century (reviewed in Mortimer, 2000 and Pretorius, 2000). Must fermentation was considered to occur spontaneously until 1863, when Louis Pasteur discovered that yeasts were responsible for the process. Although numerous yeasts and bacteria contribute to must fermentation (see Chapters 2–6 and 9), the principle microorganisms responsible for this biotransformation belong to the genus *Saccharomyces*, principally *S. cerevisiae*. This is why *S. cerevisiae* is often referred to as *the* wine yeast (Pretorius, 2000).

The origin of *S. cerevisiae* has been much debated. While some authors are of the opinion that it is naturally present in fruit (Mortimer & Polsinelli, 1999), others believe that its origin is more recent and that it is the result of hybridization with other natural species and subsequent natural selection in artificial environments (Martini, 1993). This second hypothesis is supported by the fact that *S. cerevisiae* is found only in areas close to human activity. According to this theory, all the modern isolates of *S. cerevisiae* would have been transported by insects from the winery back to the vineyards (Naumov, 1996). While this debate is central to determining the true origin of the *S. cerevisiae* genome, what is known for certain is that the genomic constitution of this species has been molded by the severe fermentation-related stresses to which it has been exposed throughout the centuries. Proof of this are the genomic differences between primary and secondary fermentation wine strains and between brewing strains and bread-making strains, whose genotypes have been unknowingly selected over hundreds of years with the continual improvements made to these biotechnological processes. Another important point is that all of today's laboratory strains are derived from natural isolates. The best-documented case is that of the most popular yeast among molecular biologists: the S288c strain, which was derived from a heterothallic (ho), diploid strain isolated in a rotten fig in California in 1938 (Mortimer & Johnston, 1986). It is very likely that the strain had been transported from a winery by insects.

Most laboratory strains of *S. cerevisiae* are ho, haploid or diploid, and have a set of 16 fixed-length chromosomes (see Figure 6.1). The majority of wine strains, in contrast, are diploid, aneuploid, or polyploid (Bakalinsky & Snow, 1990; Codón et al., 1995). They are also homothallic (HO), variably heterozygous (Barre et al., 1993; Butler et al., 2009; Carreto et al., 2008; Codón et al., 1995), and characterized by a high level of polymorphism in chromosome length (Bidenne et al., 1992; Rachidi et al., 1999). Many strains are trisomic or tetrasomic for certain chromosomes (Guijo et al., 1997; Bakalinsky & Snow, 1990). The above characteristics have numerous practical implications, including highly variable sporulation capacity (0–75%) (Bakalinsky & Snow, 1990; Barre et al., 1993; Mortimer et al., 1994) and spore viability (0–98%) (Barre et al., 1993; Codón et al., 1995; Mortimer et al., 1994). The ability of *S. cerevisiae* to alter its genome is enhanced by the existence of mitotic and meiotic cycles. Genome ploidy and plasticity provide wine yeasts with certain advantages that facilitate their adaptation to changing external environments and perhaps also increase the dosage of genes that have an important role in fermentation (Bakalinsky & Snow, 1990; Salmon, 1997). This genomic plasticity, however, is not restricted to *S. cerevisiae* and even allows stable hybridization with closely related species. Several natural strains, such as S6U and CD1, for example, are hybrids of *S. cerevisiae* and *Saccharomyces bayanus*. S6U is an allotetraploid (Naumov et al., 2000), which probably explains its stability despite having two distinct

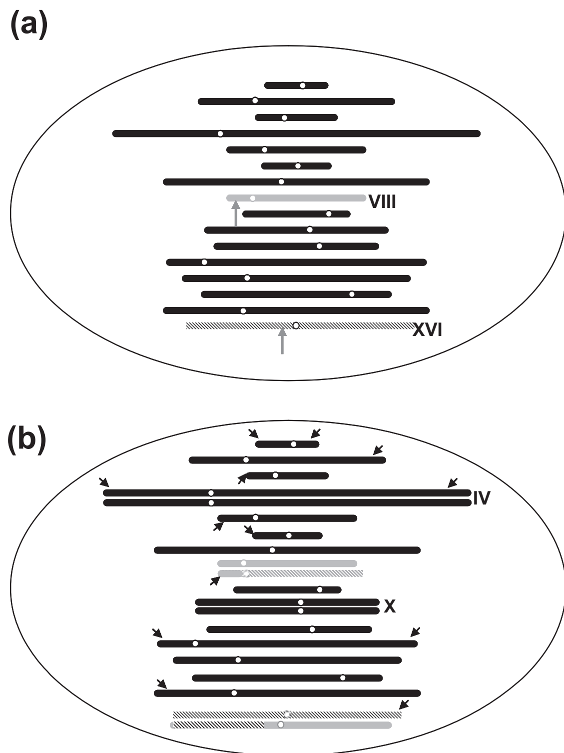

FIGURE 6.1 The genome of the reference *Saccharomyces cerevisiae* laboratory strain has 16 chromosomes whose lengths are shown to scale (a). The centromeres are shown as white dots. The haploid genome is shown in this figure. Diploid strains have two, probably identical, copies of each chromosome. Many variants of this reference genome have been found in wine strains. The T73 strain (b), for example, isolated in musts from the Alicante appellation (Querol et al., 1992) has, at least, the following variations: (1) a reciprocal translocation between chromosomes VIII and XVI, which generates two variants of each chromosome in T73 (Pérez-Ortín et al., 2002a) (the site of the translocation is shown by grey arrows) (a); (2) an additional, presumably identical, copy of chromosomes IV and X (Pérez-Ortín, unpublished results); (3) many variations in the copy number of genes from subtelomeric families, shown by arrowheads (b) (García-Martínez & Pérez-Ortín, unpublished results); and (4) markedly fewer copies of Ty transposons (Hauser et al., 2001). The T73 genome shown probably has two copies of each chromosome except for chromosomes IV and X. For simplicity, we have shown just a single copy of chromosomes with two copies. For chromosomes with three copies, we show the name and just two copies. We have included the two copies of chromosomes VIII and XVI to show the translocation between these chromosomes.

genomes. The same has been observed with brewing strains (Kielland-Brandt et al., 1995). The formation of interspecific hybrids between members of the *Saccharomyces sensu stricto* group appears to be one of the adaptive mechanisms employed by industrial yeasts (Belloch et al., 2009; Querol et al., 2003). This genome plasticity, which is inherent in wine strains, is not a desirable property in model organisms used in genetic studies, and laboratory strains used for such purposes are selected precisely for their lack of plasticity. Laboratory strains are also capable of adapting to changing environmental conditions, normally via point mutations (Ferea et al., 1999), although in certain circumstances large regions or entire chromosomes may also be modified (Hughes et al., 2000b).

Wine strains, unlike laboratory strains, are capable of chromosomal rearrangement during mitosis (Longo & Vézinhet, 1993). In an experiment by Puig et al. (2000), *URA3* was replaced with an exogenous marker gene, *KanMX*, in the natural wine strain T73 and used to monitor genetic variation in a series of consecutive must fermentations. The authors found that *URA3* homozygotes appeared at a rate of 2×10^{-5} per generation in a process they attributed to mitotic recombination or gene conversion. Phenotypically, the Ura^{-} cells were at a selective disadvantage to the Ura^{+} cells (heterozygotes [*URA3*/*ura3*] and homozygotes [*URA3*/*URA3*]). Chromosomal changes were also detected in some cells. Because of their strong tendency towards genomic changes, wine strains do not display the same genetic uniformity as that used to define laboratory strains (Pretorius, 2000; Snow, 1983). This problem is further compounded by the HO nature of these strains. Haploid cells produced by sporulation can change their mating type and conjugate to form new diploid cells. The frequent use of such mechanisms during vinification would lead to the generation of multiple genome combinations and very rapid changes. This

particular evolutionary mechanism has been termed "genome renewal" (Mortimer et al., 1994; Mortimer, 2000). The proponents of this theory suggest that this renewal would give rise to highly homozygous strains and eliminate deleterious mutations by natural selection. Natural strains are known, however, to be typically aneuploid (Bakalinsky & Snow, 1990; Guijo et al., 1997) and heterozygous for many loci (Barre et al., 1993; Kunkee & Bisson, 1993), and such properties are inconsistent with the genome renewal hypothesis (Puig et al., 2000). While the possible influence of meiotic changes cannot be entirely ruled out, there are other mechanisms that might explain the natural variation observed in wine strains. For instance, translocations mediated by Ty transposons (Rachidi et al., 1999), mitotic crossing-over (Aguilera et al., 2000), and gene conversion have all been described as mechanisms capable of causing the most rapid adaptive changes (Puig et al., 2000).

The practice of inoculating must with pure wine yeast cultures to improve the quality and homogeneity of wines produced from one year to the next dates back to the 1970s (Pretorius, 2000). Pure cultures have been obtained from natural strains in wine-producing countries around the world. In the first half of the twentieth century, these strains were selected and modified by more or less empirical methods. The selection techniques were improved in later years, however, with the emergence of classical genetic tools (reviewed in Pretorius, 2000). The end of the twentieth century brought genetic engineering methods that opened up a world of possibilities and further improved the quality of the selection methods used (see Chapter 8). The plasticity of the wine strain genome, however, poses a new challenge, as there is a risk of genetically engineered changes becoming unstable with successive generations. Mutations or insertions in a single locus, for example, could eventually be eliminated by gene conversion, homologous recombination, or even perhaps by meiosis and conjugation. Consequently, all the homologous loci in a particular strain (two or more, depending on the case) must be manipulated in an identical fashion to ensure the phenotypic stability of the strain (Puig et al., 1998, 2000).

3. COMPARATIVE GENOMICS AND THE ORIGIN OF THE *S. CEREVISIAE* GENOME

Although the origin of *S. cerevisiae* is unknown, that of its genome can be investigated by comparing genomes from natural strains of this species with those from other more-or-less-related species. A better understanding of the origin and evolution of the *S. cerevisiae* genome will have a positive impact in numerous areas. It will greatly improve our knowledge of the origin of the species and the ways in which it has adapted to industrial processes over the years, and also shed light on the mechanisms underlying the evolution of its genome, and, by extension, that of other eukaryotic organisms.

Comparative genomics studies in yeasts have been performed by partial or complete sequencing followed by bioinformatic comparison of sequence data and chromosomal organization of genes. The first complete genome sequence for *S. cerevisiae* was published for a laboratory strain in 1997 (Goffeau et al., 1997). The corresponding sequences for natural wine strains were made available about 12 years later (Borneman et al., 2008; Novo et al., 2009). Today, full genome sequences are available for several dozen *S. cerevisiae* strains, including laboratory, wine, and other strains (Liti et al., 2009; Schacherer et al., 2009).

In 1997, it was suggested that the *S. cerevisiae* genome was the result of an ancient duplication, dating back approximately 10^8 years, of an ancestral genome followed by the elimination of duplicated genes and the acquisition of new

functions for other genes (Wolfe & Shields, 1997). This theory would explain the genetic redundancy detected in this species. *S. cerevisiae* has 2458 genes from 722 families containing between two and 108 members (Herrero et al., 2003). Part of the redundancy would be due to ancestral duplication and part to smaller duplications that took place later (Llorente et al., 2000). The existence of large numbers of gene families is a common feature of hemiascomycetous yeasts. In a comparative genomic study of these yeasts, Malpertuy et al. (2000) found a substantial number of genes that do not exist in other organisms. The genes, which are specific to ascomycetes, seem to have evolved more rapidly and are perhaps responsible for the biological differences that characterize this group of yeasts. When this ancient duplication actually took place in *S. cerevisiae* is a subject of debate. Langkjaer et al. (2003) postulated that it was before the divergence of *Saccharomyces* and *Kluyveromyces* but other authors have suggested that it was later (Fares & Wolfe, 2003). In a study of collinearity (synteny) between different hemiascomycete species, Llorente et al. (2000) proposed that the primary evolutionary mechanism (apart from global genome duplication) was the duplication of small regions (the length of a few genes) of the genome followed by specialization or gene loss. In related species, such as *S. cerevisiae* and *S. bayanus*, the duplication sites tend to be located close to copies of Ty transposons or in subtelomeric regions where families of repeated genes are concentrated (Fischer et al., 2001). A genomic comparison of *S. cerevisiae*, *Saccharomyces paradoxus*, *S. bayanus*, and *Saccharomyces mikatae* found the greatest variability in subtelomeric regions, particularly in terms of repeated gene families (Kellis et al., 2003). These regions range in size from 7 to 52 kb and their function might be to facilitate rapid changes via duplication and translocation. While these mechanisms have played a part in the evolution of the *Saccharomyces* genus, they have also had a much more recent role in facilitating adaptation to specific industrial processes. Indeed, various subtelomeric gene families are of immense importance to the biology of these yeast strains. Based on the results of a comparative genomic study of multiple wine and non-wine strains, Carreto et al. (2008) proposed that the diversity observed in the strains analyzed was mainly the result of Ty element insertions and subtelomeric recombination. The fact that the subtelomeric regions of different chromosomes contain many members of gene families involved in hexose transport (Bargues et al., 1996), use of natural carbon sources such as sucrose (Carlson et al., 1989), maltose (Chow et al., 1989), and melibiose (Naumova et al., 1997), flocculation (Teunissen & Steensma, 1995), and resistance to the toxicity of molasses in which industrial yeasts are cultured (Ness & Aigle, 1995) suggests that these regions might act as reservoirs of variability for rapid adaptations to the changing environments to which industrial yeasts are exposed. This mechanism may indeed still be very active in certain strains such as Cava strains, in which high rates of subtelomeric variability have been detected (Carro et al., 2003; Carro & Piña, 2001). Small and large duplications and translocations may also have contributed to speciation due to reproductive isolation in the *Saccharomyces* genus (Delneri et al., 2003; Fischer et al., 2000, 2001). There may be other cases where the selection of one particular chromosomal rearrangement rather than another is random. Nonetheless, it is reasonable to think that many of the combinations produced by the different genomic rearrangement mechanisms discussed above have been selected because they provide the organism with a particular selective advantage. Our group found a case in which reciprocal translocation between chromosomes VIII and XVI gave rise to a new, more efficient promoter for the sulfite resistance gene *SSU1* (Pérez-Ortín et al., 2002a). As sulfite has been used as a treatment in vineyards,

wineries, and wines for thousands of years, resistance to this substance was probably selected by wine strains as a useful survival mechanism. In an extensive study of translocation between various wine and non-wine strains, our group found that the reciprocal translocation between chromosomes VIII and XVI was present in some but not all of the wine strains, but was absent from all the non-wine strains, providing evidence that this translocation is associated with the use of sulfite in winemaking (Pérez-Ortín et al., 2002a). In that study, we also detected a close phylogenetic relationship between wine strains from geographically distant countries such as South Africa, France, Japan, Spain, and the United States, suggesting that strains that had originated in Europe were spread to other parts of the world with the expansion of winemaking.

The recent development of high-resolution genome mapping techniques such as mass sequencing and tiling array analysis (see Section 4) has permitted the genomic sequencing of several dozen *S. cerevisiae* strains and the formulation of hypotheses regarding the origin of this species and that of other strains used for biotechnological purposes (brewing, bread making, sake production) and pathogenic strains isolated in immunosuppressed patients (Liti et al., 2009; Schacherer et al., 2009). Single nucleotide polymorphism (SNP) analysis has shown that the genomes of different strains of *S. cerevisiae* tend to represent a mosaic generated by recombination between lineages with different geographical and/or ecological origins (Liti et al., 2009). What seems clear is that this species has been domesticated on various separate occasions, at least once in the case of wine fermentation and another time in the case of sake fermentation (Liti et al., 2009). Today's strains would thus be derivatives and combinations of those initial domesticated strains. Pathogenic strains, however, seem to have arisen on multiple occasions from wild and domesticated strains opportunistically adapted to the new ecosystem of human tissues (Schacherer et al., 2009).

Another interesting point worth noting is the discovery of hybrid wine yeasts derived from *S. cerevisiae* and other *Saccharomyces* species. It has been known for some time that certain lager brewing strains have genomes derived from more than one species (Rainieri et al., 2006). These strains are partial allotetraploids that arose from a natural hybridization event between *S. cerevisiae* and a yeast similar to *S. bayanus* (Nakao et al., 2009; Rainieri et al., 2006). More recently, however, there have also been descriptions of wine strains with a genome containing chromosomes from more than one species and wine yeast hybrids of *S. bayanus* and *Saccharomyces kudriavzevii* (González et al., 2006). Genomic analysis showed that all the hybrids arose from a single hybridization event. The resulting genome would then have evolved through successive chromosome rearrangements resulting in the generation of hybrid chromosomes and the loss of several chromosome copies (mostly corresponding to *S. kudriavzevii*). Such rearrangements affected not only sequences of transposons (as in the cases described above) but also other conserved regions such as ribosomal DNA (rDNA) and protein-encoding genes (Belloch et al., 2009). The study of these hybrids is of practical interest because they might have useful properties for biotechnological applications. It is known, for example, that *S. bayanus* var. *uvarum* is responsible for the fermentation of must at low temperatures and the production of large quantities of glycerol and β-phenylethanol (Solieri et al., 2008). In an attempt to obtain yeast strains with improved winemaking properties, Solieri et al. (2008) constructed artificial hybrids between *S. cerevisiae* and *Saccharomyces uvarum* by spore conjugation and found that the hybrids contained mitochondria from only one of the two species and that the fermentative properties of the hybrid depended on these mitochondria.

4. THE USE OF *S. CEREVISIAE* AS A MODEL ORGANISM FOR THE DEVELOPMENT OF DNA MICROARRAY TECHNOLOGY

There are a number of reasons why many of the technologies used in the field of genomics were developed using *S. cerevisiae*, but the main one is probably that it was the first organism to be analyzed in a genomic sequencing project that generated numerous functional genomics studies even before the full sequence was published (Goffeau et al., 1997). The fact that *S. cerevisiae* has been used as a model organism for genetics and molecular biology since the 1940s has given rise to an enormous number of very powerful tools for these types of analysis. As a result of these developments, our knowledge of the genetics and biology of this yeast is unparalleled. The only other organism that has been so thoroughly investigated is perhaps *Escherichia coli*. Even before the emergence of DNA microarray technology, *S. cerevisiae* was used in the development of numerous methods for the global analysis of gene expression such as Serial Analysis of Gene Expression (SAGE) technology, which was used to perform the first analysis of the entire messenger RNA (mRNA) complement (baptized transcriptome) of a cell (Velculescu et al., 1997). As with many other technologies, SAGE was later used to analyze other organisms with great success (Velculescu et al., 2000). While SAGE is an extremely powerful tool, capable of accurately quantifying the number of copies of mRNA present in a cell, it has largely been replaced by DNA microarray analysis, which is a much simpler and less costly technology. In recent years, however, the development of high-throughput sequencing techniques (also developed using *S. cerevisiae*) has led to a renewed interest in tag-sequencing technologies. RNA-seq, for example, has been successfully used to characterize the transcriptome of *S. cerevisiae* with considerable improvements over previous techniques in terms of sensitivity, transcript quantification, and, to some degree, resolution (Nagalakshmi et al., 2008).

DNA microarrays have been widely used to investigate many aspects of *S. cerevisiae* metabolism (Figure 6.2). The technology has other uses, however. Apart from providing valuable information on metabolic activity in different conditions and mutants, it has also been used to investigate the effects of many drugs and toxic products on gene expression and to analyze genomic variations in *S. cerevisiae* and related species. All of these uses have also been applied to wine yeast strains.

4.1. Metabolic Studies

Given the vast information already available on yeast regulatory pathways, global expression studies should be able to provide sufficient data to allow individual genes to be linked to one or more phenotypes or metabolic pathways. It should also theoretically be possible to determine the components of each of these pathways, to provide, for the first time, a global view of a eukaryotic cell. The first global gene expression study, performed by Pat Brown's group, used DNA microarray analysis to study gene expression in *S. cerevisiae* during growth in glucose and during the shift from fermentative to respiratory growth (DeRisi et al., 1997). The study has already become a classic in its field and has been cited over 2500 times (as of August 2009). Similar studies have analyzed other processes or situations that involve metabolic changes. Transcriptional changes in *S. cerevisiae*, for example, have been analyzed in the change from a fermentable to a nonfermentable carbon source (Kuhn et al., 2001), in aerobic compared to anaerobic conditions in a continuous-culture study (ter Linde et al., 1999), in the lag phase prior to active culture growth (Brejning et al., 2003), during sporulation (Chu et al., 1998), and during the cell cycle (Cho et al., 1998). Another major research focus is the functional

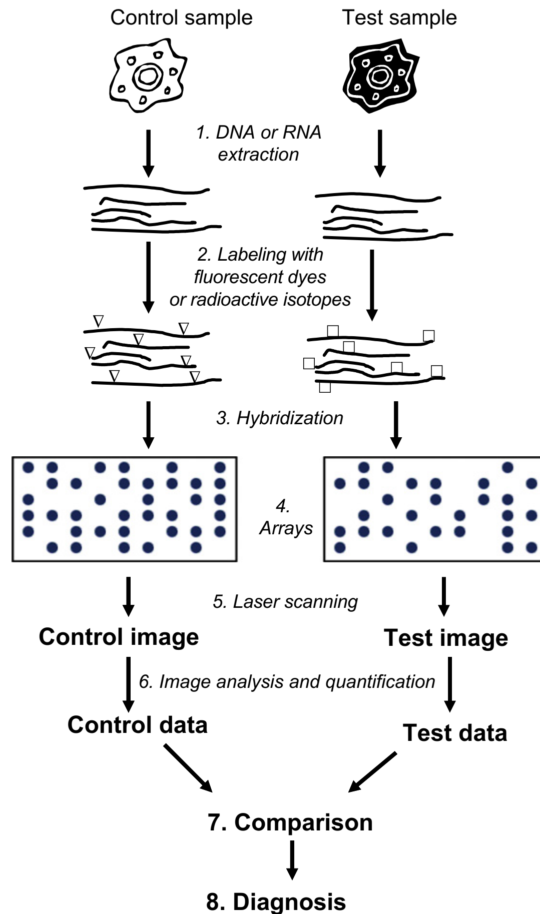

FIGURE 6.2 DNA microarray analysis. 1. RNA or DNA is extracted from a test and a control sample using conventional methods. 2. In the case of microarrays on glass slides, the probes are labeled with fluorescent dyes (using a different fluorophore for the test and control sample). The probes used in macroarrays on nylon filters are labeled with radioactive isotopes. 3. Just one hybridization step is used in glass-slide microarrays as these involve the use of a single array with both the test and control samples mixed together prior to hybridization. Two hybridization steps are required for nylon-filter macroarrays. These steps are preferably performed on the same filter but they need to be sequential as the probes are labeled with radioactive isotopes. 4. Following hybridization, the arrays are washed to allow detection of the different hybridization signals. 5. The hybridization images are captured using a laser scanner. This is done directly using two different lasers (one for each fluorophore) in the case of microarray analysis. In macroarray analysis, however, latent images are generated on special screens and later scanned by laser. 6. The readings generate an image for each sample. The intensity is then quantified using special software that generates hybridization intensity data that allows comparison of the samples. 7. Statistically significant differences are analyzed using purpose-designed programs. 8. The final stage involves the formulation of corresponding hypotheses and conclusions.

analysis of transcription factors via overexpression or analysis of null or conditional mutants (Carmel-Harel et al., 2001; DeRisi et al., 1997; Holstege et al., 1998).

Clusters of genes that display identical or similar expression patterns under the different conditions studied have been used to identify the functions of individual genes based on the

assumption that coregulated genes must be involved in the same metabolic pathways. The most common way to conduct a study of this type is to use clustering algorithms to group genes by expression profiles (reviewed in Hughes & Shoemaker, 2001 and Brazma & Vilo, 2000) in order to identify groups that have putative functional relationships. Another way is to search for transcription-factor-binding sites in gene promoters. Two types of study have been used for this purpose: in silico comparison of promoter sequences (Brazma et al., 1998; Bussemaker et al., 2000; Hampson et al., 2000; Roth et al., 1998) and in vivo studies of genome-wide transcription-factor-binding sites using a technique called Chip-ChIP, which is a combination of DNA microarray analysis (Chip) and chromatin immunoprecipitation (ChIP).

4.2. Effects of Drugs and Other External Factors

DNA microarray technology can be used to measure, in a single experiment, an organism's global transcriptional response to treatment with an external factor such as a drug or environmental agent (Gasch et al., 2000; Hughes et al., 2000a; Jelinsky et al., 2000; Jelinsky & Samson, 1999). Because the response of genes to experimental conditions is a dynamic process characterized by multiple interactions, analyzing responses to external agents can reveal functional relationships within or between metabolic pathways. Such techniques have been used to analyze, for example, the transcriptional response to inhibition of translation or amino acid biosynthesis, or to compounds with antifungal activity (Bammert & Fosel, 2000; Hardwick et al., 1999; Jia et al., 2000). Molecular targets of specific drugs can also be identified by comparing expression profiles induced by a particular drug with those induced in mutants for specific genes (Hughes et al., 2000a). Similar results can be achieved by inducing haploinsufficiency, which consists of studying growth deficiencies caused by the loss of one of the two gene copies in a diploid cell. To perform a systematic, comparative study, it is necessary to have a full collection of a diploid strain in which each gene has been deleted and replaced with a specific sequence tag (Winzeler et al., 1999). In these studies, the full collection of approximately 6000 strains with single deletions is grown together under particular conditions (such as the presence of a drug) and strains that exhibit delayed growth compared to wild-type strains indicate genes that are necessary for resistance to certain drugs or culture conditions (Giaever et al., 1999, 2002). This technique can uncover subtle growth differences that would otherwise remain undetected. Up to 6000 strains can be compared simultaneously thanks to the sequence tags present in each strain, which enable an accurate count to be made of the cells in a strain at any moment using special DNA microarrays containing probes for each sequence tag. These studies open new perspectives not only for pharmacogenomics but also for the study of the effect on wine yeasts of toxic substances such as alcohol, pesticides, and treatments such as copper and sulfite. Although most of the studies to date have been conducted using standard laboratory strains, the results can be easily extrapolated to industrial strains.

The fermentation of sugars by wine yeasts is followed by rapid growth and carbon dioxide production, which can be interrupted with the depletion of carbon or nitrogen sources or the appearance of growth inhibitors (reviewed in Pretorius, 2000). An improved understanding of the metabolic changes that occur in the shift from one carbon source to another (DeRisi et al., 1997; ter Linde et al., 1999) and of metabolic signal transduction pathways (Hardwick et al., 1999; Ogawa et al., 2000) will contribute to improving the technical aspects of fermentation processes in wineries and help to prevent stuck fermentations.

In an extensive large-scale experiment that analyzed response to many of the stress conditions to which yeasts are exposed, Gasch et al. (2000) found that the transcriptional response to almost all of the stress factors tested was practically identical across a large group of genes. The authors termed this the "environmental stress response" (ESR). The experiment provided a basis for further tests with wine strains exposed to general or specific stresses associated with wine fermentation. Indeed, the first experiments of this type have already been performed (see below). The ultimate aim of such studies is to identify the most suitable strains for the various fermentation conditions found in different wineries and wines. Similar experiments involving a limited group of genes have also been performed (Ivorra et al., 1999). A more detailed discussion is given in Chapter 2 of this book. On investigating the effect of ethanol on laboratory yeast strains by DNA microarray analysis, Alexandre et al. (2001) concluded that cells used ionic homeostasis, heat protection, and antioxidant defense, in addition to previously described mechanisms, to respond to stress. In a study of the effect of copper excess and deficiency on laboratory strains, also using DNA microarrays, Gross et al. (2000) found that a small number of genes were differentially expressed and that some of these were involved in the iron uptake system. This finding suggests that the copper and iron uptake systems might be related. Because copper is commonly used to inhibit bacterial and fungal growth in wines, wine yeast strains must be able to endure elevated copper concentrations and it would be useful to determine how they have achieved this capacity.

4.3. Use of DNA Microarrays in the Analysis of Wine Yeasts

S. cerevisiae was also the first microorganism in which genomic tools such as DNA microarray analysis were used to analyze natural and industrial strains. Since this yeast plays a key role in winemaking and has an enormous influence on the final product, it is important to understand the molecular events underlying fermentation and the influence of the winery and vintage, and of the physical, biological, and chemical properties of the must, on this process. Such an understanding would be greatly enhanced by analysis of the gene expression profiles of these yeasts in different growth conditions. Before the emergence of DNA microarray technology, the expression profiles of only a small number of genes at a time could be analyzed in wine yeasts (see Chapter 2). Most of the DNA microarray experiments described so far in this chapter, however, have analyzed laboratory strains, which are incapable of wine fermentation.

Various strategies have been employed in studies using DNA microarrays to analyze expression profiles in wine yeasts. Two studies have been conducted using laboratory media and culture conditions (Cavalieri et al., 2000; Hauser et al., 2001), whereas others have used synthetic musts that reproduce the conditions found in a natural environment but provide the means to accurately determine and reproduce the composition of the must (Backhus et al., 2001; Rossignol et al., 2003). Another strategy has involved the use of grape juice medium sterilized by filtration (Marks et al., 2003; Mendes-Ferreira et al., 2007a, 2007b).

The use of standard laboratory conditions has the advantage of allowing comparison of data from wine strains with those from the more extensively studied laboratory strains. The enormous amounts of information available on reference strains can thus be used to undertake a much more in-depth investigation of the metabolic pathways and molecular mechanisms underlying wine yeast fermentation. Cavalieri et al. (2000), for example, detected at least twofold variability in global expression levels for 6% of the genome between progeny of a natural wine strain isolate. Their findings indicate that

wine strains are highly heterozygous. Because most of the metabolic differences segregated as a suite of traits, the authors concluded that they were the result of changes in a small number of regulatory genes. One specific example would be the genes involved in the biosynthesis of amino acids. There have also been descriptions of other phenotypes caused by changes in structural rather than regulatory genes, explaining why these changes are not associated with other phenotypes. Examples include the *YHB1* gene (Hauser et al., 2001), genes involved in resistance to sulfite (Pérez-Ortín et al., 2002a) and copper, and the filigreed phenotype (Cavalieri et al., 2000).

It is important to conduct experiments in real-life conditions as, although laboratory culture conditions greatly facilitate analysis, they do not fully reproduce the conditions found in natural environments. Given the variability of natural musts, one option is to use synthetic musts, which mimic natural conditions but can be easily reproduced in different laboratories. In a study of this type, using macroarrays and various wine strains with different fermentative capacity, Zuzuárregui and del Olmo (2004) found that the expression levels of certain stress-response genes were similar across the strains. They also found that the mRNA levels of many of these genes remained very high in the strains with weaker fermentative capacity. Their results demonstrated that it is possible to establish a correlation between stress resistance and fermentation capacity.

The amount of available nitrogen is considered to be one of the main limiting factors for yeast growth in musts (reviewed in Pretorius, 2000). Studies performed with wine yeasts have generally found high expression levels for genes linked to amino acid and purine biosynthesis (Backhus et al., 2001; Cavalieri et al., 2000; Hauser et al., 2001), which are indicative of high growth rates. Activation of the methionine biosynthesis pathway and alterations in sulfate and nitrogen assimilation are known markers for metabolic phenotype as they are connected with cell-cycle progression (Patton et al., 2000). The effect of nitrogen availability on the growth of wine yeasts has been analyzed in two recent studies. One of these compared global gene expression profiles in synthetic media containing high and low concentrations of arginine (a source of nitrogen) (Backhus et al., 2001), whereas the other compared expression profiles in a Riesling must with normal concentrations of nitrogen and another to which diammonium phosphate (DAP) was added during the late fermentation phase, when yeast growth is no longer active (Marks et al., 2003). In the first study, it was found that nitrogen limitation induced genes that would normally be repressed by the high concentrations of glucose in the must. This suggests that, in the growth conditions that characterize the fermentation of must containing high concentrations of sugars and nitrogen, the use of glucose might be diverted, at least partly, to a respiratory metabolism (Backhus et al., 2001). This effect would be similar to what is known as the Pasteur effect, which is the inhibition of fermentation in the presence of oxygen. Although this effect has been reported to be irrelevant for yeast in laboratory growth conditions (Lagunas, 1986), it might occur in the fermentation of musts with low levels of nitrogen, and, accordingly, cause sluggish or stuck fermentations. Indeed, it is standard practice in wineries to add DAP in such cases. A study by Marks et al. (2003) found that the addition of DAP affected the expression of 350 genes. The 185 genes that were found to be downregulated encoded small-molecule transporters and nitrogen catabolic enzymes, including enzymes involved in the synthesis of urea, which is a precursor of ethyl carbamate. The other 165 genes affected were all upregulated. These included genes involved in the biosynthesis of amino acids, purines, and ribosomal proteins (suggesting a more active metabolism despite an absence of cell proliferation)

and assimilation of inorganic sulfate (necessary for the elimination of hydrogen sulfide). The results of the study by Marks et al. provide a possible explanation for why the addition of DAP reduces the production of ethyl carbamate and hydrogen sulfide, two undesirable components in wines. They are also consistent with results from a study that analyzed samples taken at different time points during fermentation of a synthetic must with a relatively low level of nitrogen (300 mg/L). The authors reported that the gene expression pattern observed could be explained by entry into the stationary phase (cell proliferation arrest) in response to nitrogen depletion; they also reported that the process was regulated by the *TOR* pathway (Rossignol et al., 2003).

A more comprehensive and realistic study of transcriptional response in *S. cerevisiae* to different nitrogen concentrations during alcoholic fermentation was published more recently (Mendes-Ferreira et al., 2007a, 2007b). The authors, using real grape must, compared 11 samples from different time points of a series of control vinifications, nitrogen-limiting fermentations, and fermentations to which DAP was added. They found alterations in approximately 70% of the yeast transcriptome in at least one of the fermentation stages and also showed a clear association between these changes and nitrogen concentrations. In agreement with earlier findings published by Backhus et al. (2001), their results indicated that early response to nitrogen limitation involved the induction of genes associated with respiratory metabolism and a subsequent general decrease in the levels of genes associated with catabolism. Curiously, they also found a slight increase in the expression level of genes encoding ribosomal proteins and involved in ribosome biogenesis during nitrogen depletion. In total, 36 genes were found to be overexpressed when nitrogen levels were low or absent compared to when DAP was added. These signature genes might be useful for predicting nitrogen deficiency and detecting sluggish or stuck fermentations (Mendes-Ferreira et al., 2007b). The study also demonstrated that the main transcriptional effect of adding nitrogen was an upregulation in genes involved in glycolysis, thiamine metabolism, and energy pathways (Mendes-Ferreira et al., 2007a), findings that are similar to those reported by Marks et al. (2003) following DAP addition. A study performed by Jiménez-Martí and del Olmo (2008) showed that the effect of nitrogen refeeding depended on the source of nitrogen used, as they detected differences in gene expression reprogramming depending on whether ammonia or amino acids were added. The addition of ammonia resulted in higher levels of genes involved in amino acid biosynthesis, whereas that of amino acids directly prepared cells for protein biosynthesis.

Global gene response has also been analyzed in low-temperature winemaking conditions, which are widely considered to improve the sensory quality of wine. In experiments carried out at 13 and 25°C, Beltrán et al. (2006) observed that the lower temperature induced cold stress-response genes at the initial stage of fermentation and increased levels of genes involved in cell cycle, growth control, and maintenance in the middle and late stages of fermentation. Furthermore, several genes involved in mitochondrial short-chain fatty acid synthesis were found to be overexpressed at 13°C compared to 25°C. These transcriptional changes were correlated with higher cell viability, improved ethanol tolerance, and increased production of short-chain fatty acids and associated esters.

The natural environment of *S. cerevisiae* has shaped the evolution of this organism's metabolism to allow it to exploit the anaerobic conditions and high ethanol levels that characterize fermentation and to tolerate high levels of certain compounds that are common during alcoholic fermentation. All these situations, however, are causes of stress for *S. cerevisiae* and are reflected in the yeast's gene expression pattern, even though the organism is capable

of responding effectively to these stresses. As has already been discussed, differential expression of certain stress-response genes has been detected in wine yeasts. The expression levels of genes involved in oxidative metabolism, for example, are low (Backhus et al., 2001). The results of the fermentation monitoring study conducted by Rossignol et al. (2003) indicate that anaerobic stress is a characteristic of wine fermentation and that the absence of ergosterol synthesis, one of the main growth-limiting factors for yeasts in musts with low oxygen and high ethanol levels (see Pretorius, 2000), is due to the continuous decrease in the expression levels of genes involved in ergosterol biosynthesis.

Ethanol stress is another major pressure that *S. cerevisiae* has to deal with during vinification. Ethanol tolerance is not fully understood (Pretorius, 2000) but it is known to partly depend on alterations in the plasma membrane. Genes encoding enzymes involved in the synthesis of fatty acids, phospholipids, and ergosterol are highly expressed (Backhus et al., 2001) in *S. cerevisiae* yeasts but decrease towards the stationary phase (Rossignol et al., 2003). Using microarray analysis to identify target genes and analyze ethanol sensitivity in knockout strains, Hirasawa et al. (2007) found that the biosynthesis of tryptophan can confer ethanol tolerance. Ethanol stress, however, does not appear to be the main pressure in vinification. The greatest effect on gene expression is produced upon entry into the stationary phase (Rossignol et al., 2003). The changes in gene expression seen in this phase, however, appear to differ from those observed under laboratory conditions (Gasch et al., 2000).

In a comprehensive study of the transition from the exponential to the stationary phase in wine fermentation, Marks et al. (2008) discovered 223 genes that were dramatically induced at various points during fermentation. They called this the "fermentation stress response" (FSR). The most interesting point was that the FSR was found to overlap only partially with the ESR (Gasch et al., 2000). Interestingly, 62% of the FSR genes were novel, suggesting that the stress conditions in wine fermentation were rather different from those observed in laboratory conditions. Also of interest was the fact that respiratory and gluconeogenesis genes were expressed even in high glucose concentrations and that ethanol accumulation, at least in the experiment by Gasch et al., was the main reason for entry into the stationary phase.

Because compounds such as copper sulfate and sodium bisulfate have been used for many years to inhibit fungal and bacterial growth on vines and grapes and in wines, wine strains might very well respond more efficiently than other strains to these stresses thanks to the overexpression of certain detoxifying genes. Indeed, wine strains have been found to overexpress genes involved in the transport of sulfur (*SUL1-2*) and sulfite (*SSU1*) (Cavalieri et al., 2000; Hauser et al., 2001). It can be concluded that the pressures to which wine strains have been exposed over thousands of years have led to the selection of strains that are better adapted to the fermentation conditions found in wineries. Strains that have developed resistance to treatments such as copper sulfate and sodium bisulfate are a good example of this adaptation.

Finally, two studies have analyzed the genomic response in a commercial wine yeast strain to rehydration and adaptation to osmotic stress at the beginning of vinification. In the first study, rehydration was carried out in a complete glucose medium to identify events related to re-establishment of fermentation (Rossignol et al., 2006). The authors reported substantial transcriptional changes. The expression profile observed in the dried yeasts was characteristic of cells grown under respiratory conditions and exposed to nitrogen and carbon starvation and considerable stress during rehydration. Furthermore, many genes involved in biosynthetic pathways (transcription or protein synthesis)

were coordinately induced while those subject to glucose repression were downregulated. While expression of general stress-response genes was repressed during rehydration, despite the high sugar levels, that of acid-stress genes was induced, probably in response to the accumulation of organic acids. In the second study, rehydration was carried out in water to separate this process from adaptation to osmotic pressure (Novo et al., 2007). The results of the study showed that rehydration for an additional hour (following an initial period of 30 min) did not induce any relevant changes in global gene expression. The incubation of rehydrated cells in a medium containing fermentable carbon sources activates genes involved in the fermentation pathway, the nonoxidative branch of the pentose phosphate pathway, ribosomal biogenesis, and protein synthesis.

Erasmus et al. (2003) analyzed yeast response to high sugar concentrations by inoculating rehydrated wine yeast in Riesling grape juice containing equimolar amounts of glucose and fructose to a final concentration of 40% (wt/vol) and comparing global gene expression with that observed in yeasts inoculated in the same must containing 22% sugar. Although the sugar concentration used is not generally found in winemaking conditions, some of the results coincided with those reported by Rossignol et al. (2003), with sugar stress resulting in the apparent upregulation of glycolytic and pentose phosphate pathway genes and structural genes involved in the formation of acetic acid from acetaldehyde and succinic acid from glutamate and the downregulation of genes involved in the de novo biosynthesis of purines, pyrimidines, histidine, and lysine. The authors also reported considerable changes in the expression levels of stress-response genes. These changes affected, among others, genes involved in the production of the compatible osmolyte glycerol (*GPD1*) and genes encoding the heat shock proteins HSP104/12/26/30/42/78/82 and SSA3/4.

Gene expression profiling of industrial strains may also help to uncover as-yet-unknown functions of numerous genes in the *S. cerevisiae* genome, as these genes might only have relevant functions in industrial fermentation conditions. For instance, 130 genes from various subtelomeric families of unknown function (*PAU*, *AAD*, *COS*) have been found to be induced during wine fermentation (Rossignol et al., 2003), indicating that they probably have an important role in this process. It should also be noted that 28% of the FSR genes detected in the experiment by Marks et al. (2008) described above had an unknown function.

4.4. Genomic Studies

DNA microarray analysis is also a promising tool for the study of wine strain genomes. This technology forms the basis for various types of study in this area, including Affymetrix oligonucleotide microarray analyses. These microarrays consist of a very large number of short oligonucleotide sequences derived from the reference *S. cerevisiae* laboratory strain S288c. The oligonucleotides represent all the open reading frames (ORFs) distributed throughout the yeast genome. In this method, hybridization is highly dependent on the identity of the sequence, and a single nucleotide change will alter the hybridization signal. Thus, the signals produced by a particular strain can be compared with those from a reference strain to identify sequence changes, including SNPs. The method has been successfully used to study polymorphisms in various strains (Primig et al., 2000; Winzeler et al., 1998). Affymetrix also manufactures tiling arrays, another type of oligonucleotide microarray system that covers the entire sequence of the yeast genome. Tiling arrays are used for transcriptome mapping and to identify transcripts that do not correspond to annotated genes (Royce et al., 2005). These arrays have also been used for detailed genomic analysis. As described in Section 3,

Schacherer et al. (2009) used this method to resequence 63 yeast strains, including 14 wine strains.

There also exist tiling arrays with long oligonucleotides (manufactured by Agilent, for example) and arrays containing probes spotted at a lower density than that seen in tiling arrays (oligonucleotides over 60 bases long or double-strand fragments of 300 or more bases). These tools, however, are not suitable for detecting isolated sequence variations. Microarrays consisting of long oligonucleotides or double-strand fragments are, however, useful for genomic comparisons designed to identify increases or decreases in the number of copies of a particular gene or chromosomal region. The first study of this type was conducted by Hughes et al. (2000b) using laboratory strains. A similar study by Infante et al. (2003) that analyzed *S. cerevisiae* flor yeast strains found that two natural strains had differences in the copy number of 38% of their genes, which illustrates the enormous genomic variability that characterizes yeasts of this type. In many cases, the differences were in regions flanked by Ty transposons and other regions with a high recombination rate, which would explain the amplification or deletion events observed. The authors suggested that such regions were the site of double-strand breaks responsible for free ends capable of recombination with short homologous regions (10−18 base pairs). A similar mechanism has been described for the *SSU1* gene region in wine strains (see Section 4.3). In the case of *flor* yeast strains, the continuous presence of acetaldehyde and ethanol in the medium would increase the frequency of double-strand breaks, conferring a selective advantage on strains that have adapted to this hostile environment.

DNA macroarray analysis has also been used to study gross gene expression profiles in the T73 wine strain (Pérez-Ortín et al., 2002b). The study revealed numerous copy-number variations for genes from subtelomeric families and a number of other genes such as the copper resistance gene *CUP1*. Curiously, *CUP1* has a deletion in the genomic region of the wine strain (Pérez-Ortín et al., 2002b), which reduces its expression levels (Hauser et al., 2001). The study by Hauser et al. found that the number of Ty transposons (Ty1, Ty2, Ty3, and Ty4) was greatly reduced in the T73 wine strain compared to the S288c laboratory strain. This finding was consistent with less-complete previously published results (Jordan & McDonald, 1999), with later results (Carreto et al., 2008), and with results for brewing strains (Codón et al., 1998) and suggests that the colonization of the genome of laboratory strains by these molecular parasites may be recent. The strong selective pressure exerted on wine strains might have prevented the excessive accumulation of sequences of this type (Jordan & McDonald, 1999).

The flexibility of DNA chip technology means that purpose-designed arrays can be created for specific studies. In a study of chromosomal rearrangements in Cava strains (secondary fermentation), Carro et al. (2003) used specially designed and constructed macroarrays containing 14 chromosome I probes and hybridized them with DNA from chromosome I isolated from various Cava strains with length variations in this chromosome. Their results indicated the existence of a subtelomeric region that tends to be deleted in the right arm of chromosome I of this highly variable strain.

5. PROTEOMIC ANALYSIS OF WINE STRAINS

DNA microarray technology allows the expression of all the genes in a particular organism (the transcriptome) to be analyzed. Global analyses can thus be used to assess the effects of physical, chemical, and biological agents, and even specific mutations, on gene expression. Nonetheless, analysis of mRNA levels is not sufficient for a complete description of biological systems. This also requires accurate

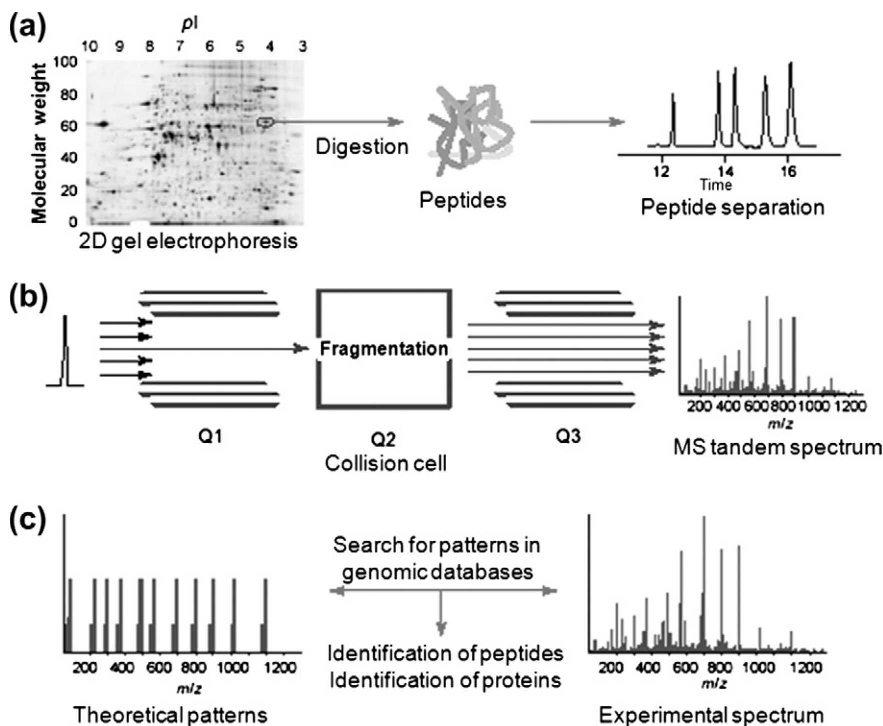

FIGURE 6.3 Standard proteomic analysis by two-dimensional (2D) gel electrophoresis and mass spectrometry (MS). The method consists of three fully integrated steps. In the first step, the proteins are separated on 2D gels, stained, and then individual spots isolated. The protein spots are then digested with trypsin and the resulting peptides are separated by high-performance liquid chromatography (HPLC). In the second step, each eluted peptide is ionized by electrospray ionization. It then enters the mass spectrometer through the first quadrupole mass filter (Q1) and is fragmented in a collision cell (Q2). The resulting spectrum is recorded (Q3). In the third step, the tandem MS spectrum of a selected ionized peptide contains sufficient specific sequencing information to identify the peptide and its associated protein. m/z = mass to charge ratio.

measurement of the expression and activity of the corresponding proteins (the proteome). Furthermore, even though expression levels of different mRNA species and the proteins they encode are correlated, this correlation is not perfect for all genes (Futcher et al., 1999; Ideker et al., 2001). Of even greater importance, however, is the level of correlation between changes in mRNA and protein levels. While changes in the proteome and transcriptome generally occur in parallel (homodirectional changes), the multiple effects caused by post-transcriptional regulation justify the need for proteomic studies (Griffin et al., 2002; Ideker et al., 2001). Thus, proteomics, which is the analysis of the full complement of proteins expressed by a genome (Pennington et al., 1997; see Figure 6.3), is considered to be the best tool for obtaining a quantitative description of the state of a biological system. In other words, proteome analysis provides a better picture of an organism's phenotype than does the analysis of mRNA levels.

While there are vast amounts of genomic data available for yeasts (including sequence and gene expression data obtained by DNA microarray analysis), the yeast proteome is still largely undefined (Fey et al., 1997). This is particularly true for yeasts of industrial and biotechnological interest, as most of the studies to date have analyzed laboratory strains (Link et al., 1999; Washburn et al., 2001). The first comparative study in this area, performed using three haploid strains derived from laboratory strains, led the authors to conclude that differences in protein expression level and post-translational modifications influenced the molecular and biochemical

characteristics of cells and were possibly responsible for the different mutant phenotypes observed in these strains (Rogowska-Wrzesinska et al., 2001).

Several studies have analyzed the effect of environmental stresses on proteome-level responses in laboratory strains. These studies are similar to those conducted in the area of genomics analyzing the influence of environmental factors on global gene expression in laboratory strains. One such proteomic study analyzed oxidative stress caused by hydrogen peroxide (Godon et al., 1998) leading to the expression of batteries of genes referred to by the authors as "stimulons." The expression of 115 proteins with different functional roles was observed. These included proteins linked to antioxidant activity, heat shock response, and protease activity. The expression of 52 proteins, including metabolic enzymes and proteins involved in translation, was repressed. In another study of *S. cerevisiae*, sorbic acid was found to produce slightly different and less drastic effects, although it did reveal expression of stress-response proteins (mainly linked to oxidative stress) and several molecular chaperones (Hsp12, 26, 42, and some isoforms of Hsp70) (de Nobel et al., 2001). Analysis of mRNA levels following the induction of sorbic acid stress showed that these were poorly correlated with protein abundance.

In another proteome analysis, the addition of cadmium (Cd^{+2}) induced expression of 54 proteins and repressed that of a further 43 (Vido et al., 2001). Of these, nine enzymes involved in the sulfur amino acid biosynthesis pathway and glutathione (GSH) synthesis were strongly induced, as were proteins with antioxidant activity. Although Cd^{+2} is not an active redox ion, it can cause oxidative stress and lipid peroxidation and also affect cellular thiol redox balance. These data suggest that the two cellular thiol redox systems—GSH and thioredoxin—are essential protection mechanisms against cadmium stress, a theory later corroborated by Fauchon et al. (2002), who related cadmium stress with sulfur metabolism. As GSH is essential for the detoxification of cadmium, when exposed to this substance, cells convert most sulfur into GSH. The cells change their proteome to reduce the production of sulfur-rich proteins to permit optimal GSH turnover and ensure optimal levels of this essential compound. It has been estimated that this change allows for a 30% reduction in sulfur amino acid incorporation into proteins, which would enable a considerable increase in GSH production and thus ensure cell survival. This is a clear example of the important role of proteome plasticity in yeast cell adaptation to adverse conditions and agents.

Little information is available on the proteomic profiles of industrial yeasts as most of the studies in this area have been carried out using laboratory strains. In two studies involving the analysis and identification of over 200 proteins, Joubert et al. (2000, 2001) concluded that the K11 brewing strain was a hybrid of *S. cerevisiae* and *Saccharomyces pastorianus* (*S. bayanus*). Their work also led them to postulate that the physiological properties required by top-fermenting (ale) strains (flocculation and fermentation at low temperatures) might have been acquired by hybridization. Their reasoning was based on the fact that, unlike bottom-fermenting (lager) strains, which are all hybrids, top-fermenting strains are not hybrids and are very closely related to *S. cerevisiae* laboratory strains. The two types of brewing strain also have very different physiological properties.

Trabalzini et al. (2003) studied the proteomic response in a wine strain of *S. cerevisiae* (k310) isolated during spontaneous wine fermentation. Wine strains are exposed to numerous hostile conditions during fermentation. Unlike other studies, which have analyzed isolated effects of environmental stress on yeasts, the study by Trabalzini et al. investigated physiological response to fermentation stress; in particular, depletion of the main carbon source and glucose,

and increasing ethanol levels. They found that specific proteins, which differed from those observed for other *S. cerevisiae* strains (such as those used in bread making), were either induced or repressed in response to these physiological stresses. The proteomic response also involved the induction of intracellular proteolysis, which appeared to be directed towards certain classes of protein. The main inference from this study is that the proteomic response to fermentation stress in a wine strain of *S. cerevisiae* is largely directed at mitigating the effects of increasing ethanol levels. Ethanol stress has been associated with both oxidative damage (due to an increased production of free radicals) and cytotoxic effects (due to acetaldehyde production). Ethanol also induces the expression of heat shock proteins and proteins involved in trehalose metabolism, whose purpose is to stabilize membranes and proteins and suppress protein aggregation. It is extremely important to further investigate proteomic responses in fermentation yeasts as a good wine strain must be capable of overcoming the hostile conditions it is faced with in industrial processes. Additionally, the cell changes that occur in *S. cerevisiae* during fermentation (autoproteolysis) and aging (autolysis) are responsible for the organoleptic properties of wine. Accordingly, the amount of nitrogen in autolysates together with free amino acid concentrations, which differ greatly depending on the yeast strain, can have a considerable influence on the flavor, composition, and quality of the final product (Martínez-Rodríguez et al., 2001a, 2001b). Proteolytic enzymes might be involved in the turnover of nitrogenous compounds before and during autolysis in winemaking conditions. It has also been proposed that yeasts might use amino acids not only as sources of nitrogen but also to restore the redox balance in critical environmental conditions (Mauricio et al., 2001).

Two recent studies have compared the transcriptome and proteome of wine yeasts. In the first of these, Zuzuárregui et al. (2006) compared two wine strains with different fermentative capacities and found that one of the strains was incapable of completing fermentation. Although the transcriptome and proteome analyses revealed specific differences, they both indicated that the strain with fermentation difficulty had defects, namely excess proton uptake (a sign of ethanol intolerance) and increased oxidative damage due to elevated levels of acetaldehyde. In the second study, Rossignol et al. (2009) compared proteomic changes in a wine strain between the exponential growth phase and the stationary phase during wine fermentation. They found major changes in the abundance of proteins related to glycolysis, ethanol production, and amino acid metabolism. The most interesting finding was that these changes were very poorly correlated with previously observed transcriptional changes (Rossignol et al., 2003), which suggests that post-transcriptional regulatory mechanisms are very important in the late stages of wine fermentation. A recent study involving laboratory strains and laboratory culture conditions with various nutrient deficiencies indicated that the response to nitrogen depletion was fundamentally controlled at a translational and not a transcriptional level (Kolkman et al., 2006).

The importance of gaining a comprehensive understanding of proteomic response in fermentation yeasts is thus clear: it will greatly contribute to improving the organoleptic properties associated with high-quality wines.

6. OTHER GLOBAL STUDIES

One of the aims of large-scale studies is to provide a global view of living systems. Genomics, for example, focuses on the full genome to help understand the relevance of individual genes, while transcriptomics and proteomics analyze the link between physiological changes and changes in transcript and protein expression levels with respect to total RNA or protein

expression levels. Most of the large-scale functional studies conducted to date have been based on transcriptomic and proteomic analyses. A more recent "omic" approach, metabolomics, aims to characterize the physiological state of a cell by determining the concentration of all of the small molecules that comprise the metabolism and identifying metabolic pathways and fluxes. This approach may provide the best and most direct measurement of an organism's physiological activity and bring us a little closer to a true approximation of its phenotype since, as stated by Delneri et al. (2001), "mRNA molecules are not functional entities within the cell, but simply transmitters of the instructions for synthesising proteins… proteins and metabolites [in contrast] represent true functional entities within cells" (p. 87). Furthermore, the use of metabolomic data in the systematic analysis of gene function has the added advantage that there are considerably fewer metabolites than genes or gene products. Nevertheless, unlike proteins, metabolites are not directly related to genes.

Metabolomic studies have emerged in an attempt to assign functions to genes on the basis of metabolic analyses. The primary aim is to discover biochemical reactions catalyzed by enzymes encoded by genes of unknown function (Martzen et al., 1999). The difficulty with such an approach is that it assigns mechanisms rather than biological functions.

An alternative approach would be to study changes in the metabolome induced by the deletion or overexpression of a specific gene and to then assign functions by comparing the changes induced with those observed in similar manipulations of known genes. Such an approach, referred to as metabolic footprinting, was used by Raamsdonk et al. (2001) in *S. cerevisiae*. Measuring concentrations of specific metabolites in a cell, however, is a very costly process. The approach used by Raamsdonk et al. was extended in a subsequent study by the same group (Allen et al., 2003) to permit large-scale analyses by optimizing the experimental conditions and surmounting the technical difficulty of measuring intracellular metabolites, which have a rapid turnover and need to be separated from the extracellular space. The optimization of mass spectrometry has allowed the analysis of extracellular metabolites in spent culture medium.

It is also possible to study and define specific metabolic pathways by integrating and incorporating data obtained using the technologies discussed in this chapter into biological models to predict cell behavior that can then be tested experimentally. Ideker et al. (2001), for example, used a combined genomic and proteomic approach to elucidate the galactose utilization metabolic pathway. They followed a typical strategy used in systems biology. The steps they described are summarized in the following points: (1) definition of all the genes in the pathway of interest; (2) perturbation of each pathway component through a series of genetic or environmental manipulations and quantification of global cellular response; (3) integration of the observed mRNA and protein responses with the current, pathway-specific model; and (4) formulation of new hypotheses to explain observations not predicted by the model. Although metabolomics is a relatively new field, a study by Eglinton et al. (2002), using metabolomic analysis of mutant laboratory strains, showed how genetic modification affects the production of several secondary metabolites of fermentation including acids (such as acetic acid), esters, aldehydes, and higher alcohols. Many of these metabolites make an important contribution to the flavor and aroma of the wine. A recent study by Rossouw et al. (2008) investigating the relationship between the transcriptomes of five wine strains and the aroma profile produced during fermentation found that the expression levels of five genes were related to differences in aroma. They then constructed wine strains overexpressing these genes and found that the

changes in the exo-metabolome corresponded to the predicted changes.

7. FUTURE DIRECTIONS

The use of genomic and proteomic methods to study wine yeasts is still in its infancy. Although the results achieved so far have begun to provide molecular explanations to problems related to wine yeast physiology, we are still far from the level of detail available for laboratory strains. It is important to discover what makes wine strains capable of must fermentation in circumstances in which the much-better-known laboratory strains are not. Laboratory strains of *S. cerevisiae* are indeed nothing more than simplified genomic derivatives of natural strains. Deciphering the genome of wine strains is also interesting from a basic scientific perspective. Gaining a deeper understanding of the genome, transcriptome, and proteome of wine yeasts and integrating this information into mathematical models capable of predicting physiological changes will allow carefully constructed improvements in the characteristics of these strains and the biotechnological processes in which they participate.

Although we have been making wine for over 7000 years, we only very recently discovered, thanks to Louis Pasteur, that *S. cerevisiae* was the main driving force behind the process. Since then, this yeast has been the focus of much basic and applied research. Nowadays, the in-depth information that large-scale studies can provide on the full complement of macromolecules found in this microorganism will help us to fully understand its physiology and elucidate the manner in which it makes wine.

References

Aguilera, A., Chávez, S., & Malagón, F. (2000). Mitotic recombination in yeast: Elements controlling its incidence. *Yeast, 16*, 731–754.

Alexandre, H., Ansanay-Galeote, V., Dequin, S., & Blondin, B. (2001). Global gene expression during short-term ethanol stress in *Saccharomyces cerevisiae*. *FEBS Lett., 498*, 98–103.

Allen, J., Davey, H. M., Broadhurst, D., Heald, J. K., Rowland, J. J., Oliver, S. G., et al. (2003). High-throughput classification of yeast mutants for functional genomics using metabolic footprinting. *Nat. Biotech., 21*, 692–696.

Backhus, L. E., DeRisi, J., Brown, P. O., & Bisson, L. F. (2001). Functional genomic analysis of a commercial wine yeast strain of *Saccharomyces cerevisiae* under differing nitrogen conditions. *FESM Yeast Res., 1*, 1–15.

Bakalinsky, A. T., & Snow, R. (1990). The chromosomal constitution of wine strains of *Saccharomyces cerevisiae*. *Yeast, 6*, 367–382.

Bammert, G. F., & Fostel, J. M. (2000). Genome-wide expression patterns in *Saccharomyces cerevisiae*: Comparison of drug treatments and genetic alterations affecting biosynthesis of ergosterol. *Antimicrob. Agents Chemother, 44*, 1255–1265.

Bargues, M., Salom, D., Gómez, A., Paricio, N., Pérez-Alonso, M., & Pérez-Ortín, J. E. (1996). Sequencing analysis of a 4.1 kb subtelomeric region from yeast chromosome IV identifies *HXT15*, a new member of the hexose transporter family. *Yeast, 12*, 1005–1011.

Barre, P., Vézinhet, F., Dequin, S., & Blondin, B. (1993). Genetic improvement of wine yeast. In G. H. Fleet (Ed.), *Wine Microbiology and Biotechnology* (pp. 421–447). UK: Harwood Academic, Reading.

Belloch, C., Pérez-Torrado, R., González, S. S., Pérez-Ortín, J. E., García-Martínez, J., Querol, A., et al. (2009). Chimeric genomes of natural hybrids of *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*. *Appl. Environ. Microbiol., 75*, 2534–2544.

Beltrán, G., Novo, M., Leberre, V., Sokol, S., Labourdette, D., Guillamón, J. M., et al. (2006). Integration of transcriptomic and metabolic analyses for understanding the global responses to low temperature winemaking fermentations. *FESM Yeast Res., 6*, 1167–1183.

Bidenne, C., Blondin, B., Dequin, S., & Vézinhet, F. (1992). Analysis of the chromosomal DNA polymorphism of wine strains of. *Saccharomyces cerevisiae. Curr. Genet., 22*, 1–7.

Bisson, L. F., Karpel, J. E., Ramakrishnan, V., & Joseph, L. (2007). Functional genomics of wine yeast *Saccharomyces cerevisiae*. *Adv. Food Nutr. Res., 53*, 65–121.

Borneman, A. R., Forgan, A. H., Pretorius, I. S., & Chambers, P. J. (2008). Comparative genome analysis of a *Saccharomyces cerevisiae* wine strain. *FESM Yeast Res., 8*, 1185–1195.

Borneman, A. R., Chambers, P. J., & Pretorius, I. S. (2007). Yeast systems biology: Modelling the winemaker's art. *Trends Biotechnol., 25*, 349–355.

Brazma, A., Jonassen, I., Vilo, J., & Ukkonen, E. (1998). Predicting gene regulatory elements in silico on a genomic scale. *Genome Res., 8*, 1202–1215.

Brazma, A., & Vilo, J. (2000). Gene expression data analysis. *FEBS Lett., 480*, 17–24.

Brejning, J., Jespersen, L., & Arneborg, N. (2003). Genome-wide transcriptional changes during the lag phase of *Saccharomyces cerevisiae*. *Arch. Microbiol., 179*, 278–294.

Bussemaker, H. J., Li, H., & Siggia, E. D. (2000). Building a dictionary for genomes: Identification of presumptive regulatory sites by statistical analysis. *Proc. Natl. Acad. Sci. USA, 97*, 10 096–10 100.

Butler, G., Rasmussen, M. D., Lin, M. F., Santos, M. A., Sakthikumar, S., Munro, C. A., et al. (2009). Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. *Nature, 459*, 657–662.

Carlson, M., Osmond, B. C., & Botstein, D. (1989). *SUC* genes of Yeast: A dispersed gene family. *Cold Spring Harbor Symp. Quant. Biol., 45*, 799–803.

Carmel-Harel, O., Stearman, R., Gasch, A. P., Botstein, D., Brown, P. O., & Storz, G. (2001). Role of thioredoxin reductase in the Yap1p-dependent response to oxidative stress in *Saccharomyces cerevisiae*. *Mol. Microbiol., 39*, 595–605.

Carreto, L., Eiriz, M. F., Gomes, A. C., Pereira, P. M., Schuller, D., & Santos, M. A. (2008). Comparative genomics of wild type yeast strains unveils important genome diversity. *BMC Genomics, 9*, 524.

Carro, D., García-Martínez, J., Pérez-Ortín, J. E., & Piña, B. (2003). Structural characterization of chromosome I size variants from a natural yeast strain. *Yeast, 30*, 171–183.

Carro, D., & Piña, B. (2001). Genetic analysis of the karyotype instability in natural wine yeast strains. *Yeast, 18*, 1457–1470.

Cavalieri, D., Townsend, J. P., & Hartl, D. L. (2000). Manifold anomalies in gene expression in a vineyard isolate of *Saccharomyces cerevisiae* revealed by DNA microarray analysis. *Proc. Natl. Acad. Sci. USA, 97*, 12369–12374.

Cho, R. J., Fromont-Racine, M., Wodicka, L., Feierbach, B., Stearns, T., Legrain, P., et al. (1998). Parallel analysis of genetic selections using whole genome oligonucleotide arrays. *Proc. Natl. Acad. Sci. USA, 95*, 3752–3757.

Chow, T. H. C., Sollitti, P., & Marmur, J. (1989). Structure of the multigene family of *MAL* loci in *Saccaromyces*. *Mol. Gen. Genet., 217*, 60–69.

Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O., et al. (1998). The transcriptional program of sporulation in budding yeast. *Science, 282*, 699–705.

Cliften, P., Sudarsanam, P., Desikan, A., Fulton, L., Fulton, B., Majors, J., et al. (2003). Finding functional features in *Saccharomyces* genomes by phylogenetic footprinting. *Science, 301*, 71–76.

Codón, A. C., Benítez, T., & Korhola, M. (1998). Chromosomal polymorphism and adaptation to specific industrial environments of *Saccharomyces* strains. *Appl. Microbiol. Biotechnol., 49*, 154–163.

Codón, A. C., Gasent-Ramírez, J. M., & Benítez, T. (1995). Factors which affect the frequency of sporulation and tetrad formation in *Saccharomyces cerevisiae* baker's yeasts. *Appl. Environ. Microbiol., 61*, 630–638.

de Nobel, H., Lawrie, L., Brul, S., Klis, F., Davis, M., Alloush, H., et al. (2001). Parallel and comparative analysis of the proteome and transcriptome of sorbic acid-stressed *Saccharomyces cerevisiae*. *Yeast, 18*, 1328–1413.

Delneri, D., Brancia, F. L., & Oliver, S. G. (2001). Towards a truly integrative biology through the functional genomics of yeast. *Curr. Opin. Biotech., 12*, 87–91.

Delneri, D., Colson, I., Grammenoudi, S., Roberts, I. N., Louis, E. J., & Oliver, S. G. (2003). Engineering evolution to study speciation in yeasts. *Nature, 422*, 68–72.

DeRisi, J. L., Iyer, V. R., & Brown, P. O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science, 24*, 680–686.

Dujon, B. (1998). European Functional Analysis Network (EUROFAN) and the functional analysis of the *Saccharomyces cerevisiae* genome. *Electrophoresis, 19*, 617–624.

Eglinton, J. M., Heinrich, A. J., Pollnitz, A. P., Langridge, P., Henschke, P. A., & de Barros Lopes, M. (2002). Decreasing acetic acid accumulation by a glycerol overproducing strain of *Saccharomyces cerevisiae* by deleting the *ALD6* aldehyde dehydrogenase gene. *Yeast, 19*, 295–301.

Erasmus, D. J., van der Merwe, G. K., & van Vuuren, H. J. J. (2003). Genome-wide expression analyses: Metabolic adaptation of *Saccharomyces cerevisiae* to high sugar stress. *FESM Yeast Res., 3*, 375–399.

Fares, M. A., & Wolfe, K. (2003). Accelerated evolution of some gene copies after genome duplication in *Saccharomyces*. *Yeast, 20*, S277.

Fauchon, M., Lagniel, G., Aude, J. C., Lombardia, L., Soularue, P., Petat, C., et al. (2002). Sulfur sparing in the yeast proteome in response to sulfur demand. *Mol. Cell, 9*, 713–723.

Ferea, T. L., Botstein, D., Brown, P. O., & Rosenzweig, R. F. (1999). Systematic changes in gene expression patterns following adaptive evolution in yeast. *Proc. Natl. Acad. Sci. USA, 96*, 9721–9726.

Fey, S. J., Nawrocki, A., Larsen, M. R., Gorg, A., Roepstorff, P., Skews, G. N., et al. (1997). Proteome studies of *Saccharomyces cerevisiae*: A methodological outline. *Electrophoresis, 18*, 1361–1372.

Fischer, G., James, S. A., Roberts, I. N., Oliver, S. G., & Louis, E. J. (2000). Chromosomal evolution in *Saccharomyces*. *Nature, 405*, 451–454.

Fischer, G., Neuveglise, C., Durrens, P., Gaillardin, C., & Dujon, B. (2001). Evolution of gene order in the

genomes of two related yeast species. *Genome Res., 11*, 2009−2019.

Futcher, B., Latter, G. I., Monardo, P., McLaughin, C. S., & Garrels, J. I. (1999). A sampling of the yeast proteome. *Mol. Cell. Biol., 19*, 7357−7368.

Gasch, A. P., Spellman, P. T., Kao, C. M., Carmel-Harel, O., Eisen, M. B., Storz, G., et al. (2000). Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell., 11*, 4241−4257.

Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Véronneau, S., et al. (2002). Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature, 418*, 387−391.

Giaever, G., Shoemaker, D. D., Jones, T. W., Liang, H., Winzeler, E. A., Astromoff, A., et al. (1999). Genomic profiling of drug sensitivities via induced haploinsufficiency. *Nat. Genet., 21*, 278−283.

Godon, C., Lagniel, G., Lee, J., Buhler, J. M., Kieffer, S., Perrot, M., et al. (1998). The H_2O_2 stimulon in *Saccharomyces cerevisiae*. *J. Biol. Chem., 273*, 22 480−22 489.

Goffeau, A., et al. (1997). The yeast genome directory. *Nature, 387*(Suppl. 1), 1−105.

González, S. S., Barrio, E., Gafner, J., & Querol, A. (2006). Natural hybrids from *Saccharomyces cerevisiae, Saccharomyces bayanus*, and *Saccharomyces kudriavzevii* in wine fermentations. *FESM Yeast Res., 6*, 1221−1234.

Griffin, T. J., Gygi, S. P., Ideker, T., Rist, B., Eng, J., Hood, L., et al. (2002). Complementary profiling of gene expression at the transcriptome and proteome levels in *Saccharomyces cerevisiae*. *Mol. Cell Proteomics, 1*, 323−333.

Gross, C., Kelleher, M., Iyer, V. R., Brown, P. O., & Winge, D. R. (2000). Identification of the copper regulon in *Saccharomyces cerevisiae* by DNA microarrays. *J. Biol. Chem., 275*, 32 310−32 316.

Guijo, S., Mauricio, J. C., Salmon, J. M., & Ortega, J. M. (1997). Determination of the relative ploidy in different *Saccharomyces cerevisiae* strains used for fermentation and "flor" film ageing of dry sherry-type wines. *Yeast, 13*, 101−117.

Hampson, S., Baldi, P., Kibler, D., & Sandmeyer, S. B. (2000). Analysis of yeast's ORF upstream regions by parallel processing, microarrays, and computational methods. *Proc. Int. Conf. Intell. Syst. Mol. Biol., 8*, 190−201.

Hardwick, J. S., Kuruvilla, F. G., Tong, J. K., Shamji, A. F., & Schreiber, S. L. (1999). Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins. *Proc. Natl. Acad. Sci. USA, 96*, 14 866−14 870.

Hauser, N. C., Fellenberg, K., Gil, R., Bastuck, S., Hoheisel, J. D., & Pérez-Ortín, J. E. (2001). Whole genome analysis of a wine yeast strain. *Comp. Funct. Genom., 2*, 69−79.

Herrero, E., de la Torre, M. A., & Valentín, E. (2003). Comparative genomics of yeast species: New insights into their biology. *Int. Microbiol., 6*, 183−190.

Hirasawa, T., Yoshikawa, K., Nakakura, Y., Nagahisa, K., Furusawa, C., Katakura, Y., et al. (2007). Identification of target genes conferring ethanol stress tolerance to *Saccharomyces cerevisiae* based on DNA microarray data analysis. *J. Biotechnol., 131*, 34−44.

Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., et al. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. *Cell, 95*, 717−728.

Hughes, T. R., Marton, M. J., Jones, A. R., Roberts, C. J., Stoughton, R., Armour, C. D., et al. (2000a). Functional discovery via a compendium of expression profiles. *Cell, 102*, 109−126.

Hughes, T. R., Roberts, C. J., Dai, H., Jones, A. R., Meyer, M. R., Slade, D., et al. (2000b). Widespread aneuploidy revealed by DNA microarray expression profiling. *Nat. Genet., 25*, 333−337.

Hughes, T. R., & Shoemaker, D. D. (2001). DNA microarrays for expression profiling. *Curr. Opin. Chem. Biol., 5*, 21−25.

Ideker, T., Thorsson, V., Ranish, J. A., Christmas, R., Buhler, J., Eng, J. K., et al. (2001). Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science, 292*, 929−934.

Infante, J. J., Dombek, K. M., Rebordinos, L., Cantoral, J. M., & Young, E. T. (2003). Genome-wide amplifications caused by chromosomal rearrangements play a major role in the adaptive evolution of natural yeast. *Genetics, 165*, 1745−1759.

Ivorra, C., Pérez-Ortín, J. E., & del Olmo, M. (1999). An inverse correlation between stress resistance and stuck fermentations in wine yeasts. A molecular study. *Biotechnol. Bioeng., 64*, 698−708.

Jelinsky, S. A., Estep, P., Church, G. M., & Samson, L. D. (2000). Regulatory networks revealed by transcriptional profiling of damaged *Saccharomyces cerevisiae* cells: Rpn4 links base excision repair with proteasomes. *Mol. Cell. Biol., 20*, 8157−8167.

Jelinsky, S. A., & Samson, L. D. (1999). Global response of *Saccharomyces cerevisiae* to an alkylating agent. *Proc. Natl. Acad. Sci. USA, 96*, 1486−1491.

Jia, M. H., Larossa, R. A., Lee, J. M., Rafalski, A., Derose, E., Gonye, G., et al. (2000). Global expression profiling of yeast treated with an inhibitor of amino acid biosynthesis, sulfometuron methyl. *Physiol. Genomics, 3*, 83−92.

Jiménez-Martí, E., & del Olmo, M. (2008). Addition of ammonia or amino acids to a nitrogen-depleted medium affects gene expression patterns in yeast cells during alcoholic fermentation. *FESM Yeast Res., 8*, 245−256.

Jordan, I. K., & McDonald, F. (1999). Tempo and mode of Ty evolution in *Saccharomyces cerevisieae*. *Genetics, 151*, 1341−1351.

Joubert, R., Brignon, P., Lehmann, C., Monribot, C., Gendre, F., & Boucherie, H. (2000). Two-dimensional gel analysis of the proteome of lager brewing yeasts. *Yeast, 16*, 511–522.

Joubert, R., Strub, J. M., Zugmeyer, S., Kobi, D., Carte, N., van Dorsselaer, A., et al. (2001). Identification by mass spectrometry of two-dimensional gel electrophoresis-separated proteins extracted from lager brewing yeast. *Electrophoresis, 22*, 2269–2282.

Kellis, M., Patterson, N., Endrizzi, M., Birren, B., & Lander, E. S. (2003). Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature, 423*, 241–254.

Kielland-Brandt, M. C., Nilsson-Tillgren, T., Gjermnsen, C., Holmberg, S., & Pedersen, M. B. (1995). Genetics of brewing yeasts. In A. Wheals, A. Rose & J. Harrison (Eds.), *The yeasts* (pp. 223–254). London, UK: Academic Press.

Kolkman, A., Daran-Lapujade, P., Fullaondo, A., Olsthoorn, M. M., Pronk, J. T., Slijper, M., et al. (2006). Proteome analysis of yeast response to various nutrient limitations. *Mol. Syst. Biol., 2*, 2006, 0026.

Kuhn, K. M., DeRisi, J. L., Brown, P. O., & Sarnow, P. (2001). Global and specific translational regulation in the genomic response of *Saccharomyces cerevisiae* to a rapid transfer from a fermentable to a nonfermentable carbon source. *Mol. Cell. Biol., 21*, 916–927.

Kunkee, R. E., & Bisson, L. F. (1993). Wine-making yeasts. In A. H. Rose & J. S. Harrison (Eds.), *The yeasts: Yeast Technology* (pp. 69–125). London, UK: Academic Press.

Lagunas, R. (1986). Misconceptions about the energy metabolism of. *Saccharomyces cerevisiae. Yeast, 2*, 221–228.

Langkjaer, R. B., Cliften, P. F., Johnston, M., & Piskur, J. (2003). Yeast genome duplication was followed by asynchronous differentiation of duplicated genes. *Nature, 421*, 848–852.

Link, A. J., Eng, J., Schieltz, D. M., Carmack, E., Mize, G. J., Morris, D. R., et al. (1999). Direct analysis of protein complexes using mass spectrometry. *Nat. Biotech., 17*, 676–682.

Liti, G., Carter, D. M., Moses, A. M., Warringer, J., Parts, L., James, S. A., et al. (2009). Population genomics of domestic and wild yeasts. *Nature, 458*, 337–341.

Llorente, B., Durrens, P., Malpertuy, A., Aigle, M., Artiguenave, F., Blandin, G., et al. (2000). Genomic exploration of the hemiascomycetous yeasts: 20. Evolution of gene redundancy compared to *Saccharomyces cerevisiae*. *FEBS Lett., 487*, 122–133.

Longo, E., & Vézinhet, F. (1993). Chromosomal rearrangements during vegetative growth of a wild strain of *Saccharomyces cerevisiae. Appl. Environ. Microbiol., 59*, 322–326.

Malpertuy, A., Tekaia, F., Casarégola, S., Aigle, M., Artiguenave, F., Blandin, G., et al. (2000). Genomic exploration of hemiascomycetous yeasts: 19. Ascomycetes-specific genes. *FEBS Lett., 487*, 113–121.

Marks, V. D., Ho Sui, S. J., Erasmus, D., van der Merwe, G. K., Brumm, J., Wasserman, W. W., et al. (2008). Dynamics of the yeast transcriptome during wine fermentation reveals a novel fermentation stress response. *FESM Yeast Res., 8*, 35–52.

Marks, V. M., van der Merwe, G. K., & van Vuuren, H. J. J. (2003). Transcriptional profiling of wine yeast in fermenting grape juice: Regulatory effect of diammonium phosphate. *FESM Yeast Res., 3*, 269–287.

Martínez-Rodríguez, A. J., Carrascosa, A. V., & Polo, M. C. (2001b). Release of nitrogen compounds to the extracellular medium by three strains of Saccharomyces cerevisiae during induced autolysis in a model wine system. *Int. J. Food Microbiol., 68*, 155–160.

Martínez-Rodríguez, A. J., Polo, M. C., & Carrascosa, A. V. (2001a). Structural and ultrastructural changes in yeast cells during autolysis in a model wine system and in sparkling wines. *Trends Biochem. Sci., 15*, 305–309.

Martini, A. (1993). The origin and domestication of the wine yeast *Saccharomyces cerevisiae*. *J. Wine Res., 4*, 165–176.

Martzen, M. R., McCraith, S. M., Spinelli, S. L., Torres, F. M., Fields, S., Grayhack, E. J., et al. (1999). A biochemical genomics approach for identifying genes by the activity of their products. *Science, 286*, 1153–1155.

Mauricio, J. C., Valero, E., Millan, C., & Ortega, J. M. (2001). Changes in nitrogen compounds in must and wine during fermentation and biological aging by flor yeast. *J. Agric. Food Chem., 49*, 3310–3315.

Mendes-Ferreira, A., del Olmo, M., García-Martínez, J., Jiménez-Martí, E., Leão, C., Mendes-Faia, A., et al. (2007b). Signature genes for predicting nitrogen deficiency during alcoholic fermentation. *Appl. Environm. Microbiol., 73*, 5363–5369.

Mendes-Ferreira, A., del Olmo, M., García-Martínez, J., Jiménez-Martí, E., Mendes-Faia, A., Pérez-Ortín, J. E., et al. (2007a). Transcriptional response of *Saccharomyces cerevisiae* to different nitrogen concentrations during alcoholic fermentation. *Appl. Environm. Microbiol., 73*, 3049–3060.

Miklos, G. L. G., & Rubin, G. M. (1996). The role of the genome project in determining gene function: Insight for model organisms. *Cell, 86*, 521–529.

Mortimer, R. K. (2000). Evolution and variation of the yeast (*Saccharomyces*) genome. *Genome Res., 10*, 403–409.

Mortimer, R. K., & Johnston, J. R. (1986). Genealogy of principal strains of the yeast genetic stock center. *Genetics, 113*, 35–43.

Mortimer, R. K., & Polsinelli, M. (1999). On the origins of wine yeast. *Res. Microbiol., 150*, 199–204.

Mortimer, R. K., Romano, P., Suzzi, G., & Polsinelli, M. (1994). Genome renewal: A new phenomenon revealed from a genetic study of 43 strains of *Saccharomyces cerevisiae* derived from natural fermentation of grape musts. *Yeast, 10*, 1543–1552.

Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M., et al. (2008). The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science, 320*, 1344–1349.

Nakao, Y., Kanamori, T., Itoh, T., Kodama, Y., Rainieri, S., Nakamura, N., et al. (2009). Genome sequence of the lager brewing yeast, an interspecies hybrid. *DNA Res., 16*, 115–129.

Naumov, G. I. (1996). Genetic identification of biological species in the *Saccharomyces* sensu stricto complex. *J. Ind. Microbiol., 17*, 295–302.

Naumov, G. I., Naumova, E. S., Masneuf, I., Aigle, M., Kondratieva, V. I., & Dubourdieu, D. (2000). Natural polyploidization of some cultured yeast *Saccharomyces sensu stricto*: Auto- and allotetraploidy. *Syst. Appl. Microbiol., 23*, 429–442.

Naumova, E. S., Turakainen, H., Naumov, G. I., & Korhola, M. (1997). Superfamily of alpha-galactosidase *MEL* genes of the *Saccharomyces sensu stricto* species complex. *Mol. Gen. Genet., 253*, 111–117.

Ness, F., & Aigle, M. (1995). *RTM1* a member of a new family of telomeric genes in yeast. *Genetics, 113*, 35–43.

Novo, M., Beltran, G., Rozes, N., Guillamon, J. M., Sokol, S., Leberre, V., et al. (2007). Early transcriptional response of wine yeast after rehydration: Osmotic shock and metabolic activation. *FESM Yeast Res., 7*, 304–316.

Novo, M., Bigey, F., Beyne, E., Galeote, V., Gavory, F., Mallet, S., et al. (2009). Eukaryote-to-eukaryote gene transfer events revealed by the genome sequence of the wine yeast *Saccharomyces cerevisiae* EC1118. *Proc. Natl. Acad. Sci. USA., 106*, 16 333–16 338.

Ogawa, N., DeRisi, J., & Brown, P. O. (2000). New components of a system for phosphate accumulation and polyphosphate metabolism in *Saccharomyces cerevisiae* revealed by genomic expression analysis. *Mol. Biol. Cell., 11*, 4309–4321.

Patton, E. E., Peyraud, C., Rouillon, A., Surdin-Kerjan, Y., Tyers, M., & Thomas, D. (2000). SCF(Met30)-mediated control of the transcriptional activator Met4 is required for the G(1)-S transition. *EMBO J., 19*, 1613–1624.

Pennington, S. R., Wilking, M. R., Hochstrasser, D. F., & Dunn, M. J. (1997). Proteome analysis: From protein characterization to biological function. *Trends Cell Biol., 7*, 168–173.

Pérez-Ortín, J. E., García-Martínez, J., & Alberola, T. (2002b). DNA chips for yeast biotechnology. The case of wine yeasts. *J. Biotechnol., 98*, 227–241.

Pérez-Ortín, J. E., Querol, A., Puig, S., & Barrio, E. (2002a). Molecular characterization of a chromosomal rearrangement involved in the adaptive evolution of yeast strains. *Genome Res., 12*, 1533–1539.

Pizarro, F., Vargas, F. A., & Agosin, E. (2007). A systems biology perspective of wine fermentations. *Yeast, 24*, 977–991.

Pretorius, I. S. (2000). Tailoring wine yeast for the new millennium: Novel approaches to the ancient art of winemaking. *Yeast, 16*, 675–729.

Primig, M., Williams, R. M., Winzeler, E. A., Tevzadze, G. G., Conway, A. R., Hwang, S. Y., et al. (2000). The core meiotic transcriptome in budding yeasts. *Nat. Genet., 26*, 415–423.

Puig, S., Querol, A., Barrio, E., & Pérez-Ortín, J. E. (2000). Mitotic recombination and genetic changes in *Saccharomyces cerevisiae* during wine fermentation. *Appl. Environ. Microbiol., 66*, 2057–2061.

Puig, S., Ramón, D., & Pérez-Ortín, J. E. (1998). An optimized method to obtain stable food-safe recombinant wine yeast strains. *J. Agr. Food Chem., 46*, 1689–1693.

Querol, A., Barrio, E., Huerta, T., & Ramón, D. (1992). Molecular monitoring of wine fermentations conducted by active dry yeast strains. *Appl. Environ. Microbiol., 58*, 2948–2953.

Querol, A., Belloch, C., Fernández-Espinar, M. T., & Barrio, E. (2003). Molecular evolution in yeast of biotechnological interest. *Int. Microbiol., 6*, 201–205.

Raamsdonk, L. M., Teusink, B., Broadhurst, D., Zhang, N., Hayes, A., Walsh, M. C., et al. (2001). A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat. Biotech., 19*, 45–50.

Rachidi, N., Barre, P., & Blondin, B. (1999). Multiple Ty-mediated chromosomal translocations lead to karyotype changes in a wine strain of *Saccharomyces cerevisiae*. *Mol. Gen. Genet., 261*, 841–850.

Rainieri, S., Kodama, Y., Kaneko, Y., Mikata, K., Nakao, Y., & Ashikari, T. (2006). Pure and mixed genetic lines of *Saccharomyces bayanus* and *Saccharomyces pastorianus* and their contribution to the lager brewing strain genome. *Appl. Environ. Microbiol., 72*, 3968–3974.

Rogowska-Wrzesinska, A., Larsen, P. M., Blomberg, A., Görg, A., Roepstorff, P., Norberk, J., et al. (2001). Comparison of the proteomes of three yeast wild type strains: CEN.PK2, FY1679 and W303. *Comp. Funct. Genom, 2*, 207–225.

Rossignol, T., Dulau, L., Julien, A., & Blondin, B. (2003). Genome-wide monitoring of wine yeast gene expression during alcoholic fermentation. *Yeast, 20*, 1369–1385.

Rossignol, T., Kobi, D., Jacquet-Gutfreund, L., & Blondin, B. (2009). The proteome of a wine yeast strain during fermentation, correlation with the transcriptome. *J. Appl. Microbiol., 107*, 47–55.

Rossignol, T., Postaire, O., Storaï, J., & Blondin, B. (2006). Analysis of the genomic response of a wine yeast to rehydration and inoculation. *Appl. Microbiol. Biotechnol., 71*, 699–712.

Rossouw, D., Naes, T., & Bauer, F. F. (2008). Linking gene regulation and the exo-metabolome: A comparative transcriptomics approach to identify genes that impact on the production of volatile aroma compounds in yeast. *BMC Genomics, 9*, 530.

Roth, F. P., Hughes, J. D., Estep, P. W., & Church, G. M. (1998). Finding DNA regulatory motifs within unaligned non-coding sequences clustered by whole-genome mRNA quantitation. *Nat. Biotech., 16*, 939–945.

Royce, T. E., Rozowsky, J. S., Bertone, P., Samanta, M., Stolc, V., Weissman, S., et al. (2005). Issues in the analysis of oligonucleotide tiling microarrays for transcript mapping. *Trends Genet., 21*, 466–475.

Salmon, J.-M. (1997). Enological fermentation kinetics of an isogenic ploidy series derived form an industrial *Saccharomyces cerevisiae* strain. *J. Ferment. Bioeng, 83*, 253–260.

Schacherer, J., Shapiro, J. A., Ruderfer, D. M., & Kruglyak, L. (2009). Comprehensive polymorphism survey elucidates population structure of *Saccharomyces cerevisiae*. *Nature, 458*, 342–345.

Schena, M., Shalon, D., Davis, R. W., & Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science, 270*, 467–470.

Snow, R. (1983). Genetic improvement of wine yeast. In J. F. T. Spencer, D. M. Spencer & A. R. V. Smith (Eds.), *Yeast genetics – Fundamental and applied aspects* (pp. 439–459). New York, NY: Springer-Verlag.

Solieri, L., Antúnez, O., Pérez-Ortín, J. E., Barrio, E., & Giudici, P. (2008). Mitochondrial inheritance and fermentative: Oxidative balance in hybrids between *Saccharomyces cerevisiae* and *Saccharomyces uvarum*. *Yeast, 25*, 485–500.

Souciet, J., Aigle, M., Artiguenave, F., Blandin, G., Bolotin-Fukuhara, M., Bon, E., et al. (2000). Genomic exploration of the hemiascomycetous yeasts: 1. A set of yeast species for molecular evolution studies. *FEBS Lett., 487*, 3–12.

ter Linde, J. J., Liangm, H., Davism, R. W., Steensma, H. Y., van Dijken, J. P., & Pronk, J. T. (1999). Genome-wide transcriptional analysis of aerobic and anaerobic chemostat cultures of *Saccharomyces cerevisiae*. *J. Bacteriol., 181*, 7409–7413.

Teunissen, A. W., & Steensma, H. Y. (1995). The dominant flocculation genes of *Saccharomyces cerevisiae* constitute a new subtelomeric gene family. *Yeast, 11*, 1001–1013.

Trabalzini, L., Paffetti, A., Scaloni, A., Talamo, F., Ferro, E., Coratza, G., et al. (2003). Proteomic response to physiological fermentation stresses in a wild-type wine strain of *Saccharomyces cerevisiae*. *Biochem. J., 370*, 35–46.

Velculescu, V. E., Vogelstein, B., & Kinzler, K. W. (2000). Uncharted transcriptomes with SAGE. *Trends Genet., 16*, 423–425.

Velculescu, V. E., Zhang, L., Zhou, W., Vogelstein, J., Basrai, M. A., Bassett, D. E., et al. (1997). Characterization of the yeast transcriptome. *Cell, 88*, 243–251.

Vido, K., Spector, D., Lagniel, G., Lopez, S., Toledano, M. B., & Labarre, J. (2001). A proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. *J. Biol. Chem., 276*, 8469–8474.

Washburn, M. P., Wolters, D., & Yates, J. R. (2001). Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat. Biotech., 19*, 242–247.

Winzeler, E. A., Richards, D. R., Conway, A. R., Goldstein, A. L., Kalman, S., McCullough, M. J., et al. (1998). Direct allelic variation scanning of the yeast genome. *Science, 281*, 1194–1197.

Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., et al. (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science, 285*, 901–906.

Wodicka, L., Dong, H., Mittmann, M., Ho, M. H., & Lockhart, D. J. (1997). Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nat. Biotechnol., 15*, 1359–1367.

Wolfe, K. H., & Shields, D. C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nature, 387*, 708–713.

Zuzuárregui, A., & del Olmo, M. L. (2004). Expression of stress response genes in wine strains with different fermentative behavior. *FESM Yeast Res., 4*, 699–710.

Zuzuárregui, A., Monteoliva, L., Gil, C., & del Olmo, M. (2006). Transcriptomic and proteomic approach for understanding the molecular basis of adaptation of *Saccharomyces cerevisiae* to wine fermentation. *Appl. Environ. Microbiol., 72*, 836–847.