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Seven years of yeast microarray analysis

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Abstract

Since the advent of microarray technology in the mid-1990s, and finishing of the complete genome sequence of the yeast *Saccharomyces cerevisiae* in 1996, yeast has been at the forefront of genomic biology. In this article we review the uses of microarrays to investigate yeast biology, and the wide range of applications that they have been used for. While typically people think of microarrays for generating large-scale expression measurements, we discuss several different experimental approaches that have used microarrays to probe almost every facet of a living yeast cell.

Main Text

Introduction

One of the specific goals of the Human Genome Project, defined by the National Research Council study in 1988, was to sequence the genomes of a number of model organisms, with the expectation that an understanding of the genomes of model organisms would guide us in our investigations of the human genome. *Saccharomyces cerevisiae* was the first eukaryote to be fully sequenced, which revealed approximately 6000 simply constructed genes. At the same time, DNA microarrays were invented at both Stanford University and Affymetrix, yielding the ability to simultaneously quantify the abundance of thousands of nucleic acid species in complex mixtures. Easy genetic manipulation, compact gene structures and a complete genome sequence made yeast the center of genomics, and microarrays provided an unprecedented opportunity to assay an entire biological system. This short review examines the development of microarray technology, and the variety of innovative uses to which it has been put in furthering our understanding of yeast biology, illustrating how yeast was, and continues to be, at the forefront of genomic research.

Large-scale studies of Gene Expression

In 1997, Joe DeRisi and colleagues, from Pat Brown's laboratory at Stanford, published a landmark microarray study (DeRisi *et al.*, 1997). Based on the recently published genome sequence, the Brown and Botstein labs PCR amplified 6400 predicted open reading frames from yeast, and spotted each PCR product to create the first whole genome microarray in an 18mm x 18mm square. DeRisi, Iyer, and Brown investigated the diauxic shift, which is the point at which yeast switch to ethanol as a carbon source, after having exhausted the available glucose. Seven samples taken every two hours resulted in more than 43,000 expression measurements,

their analysis of which showed that changes in gene expression during the diauxic shift result in metabolic reprogramming consistent with the environmental changes that were occurring.

The following year, two groups analyzed the transcriptional program of the yeast mitotic cell cycle, using high-density oligonucleotide arrays from Affymetrix (Cho *et al.*, 1998), or homemade microarrays with spotted PCR products (Spellman *et al.*, 1998, which includes the authors of this chapter). Together they used four different methods of whole cell culture synchronization to determine the periodically transcribed genes through the cell cycle. While the two studies were not in exact agreement as to the number and identity of those transcripts, there was a large overlap in their findings. At that time, our cell cycle dataset was (and continued to be for some time) the largest single dataset published with microarray experiments from 62 unique arrays. It should be noted that both the Davis and Brown/Botstein groups made their data available on the internet and that in our cell cycle study we actually reinterpreted the Davis lab data, which was the first time a dataset was reanalyzed. This marked the beginning of the age of microarray data reanalysis, with our study (and a number of the following studies) showing complex reanalysis of yeast gene expression data were possible.

Yeast Gene Deletions and DNA microarrays

Concurrent to the efforts to characterize global gene expression profiles was an effort to generate systematic deletion mutants, a set of 24,000 yeast strains available as homo- and heterozygous diploids and as haploids of each mating type. This project was both driven by, and drove microarray technology because Ron Davis' lab, in conjunction with Affymetrix had developed a system of using a molecular barcode for each deletion, which could be detected by hybridization. In the proof of principle experiment Shoemaker and colleagues (Shoemaker *et al.*, 1996) demonstrated that these bar-coded yeast strains could be grown competitively under a

given condition, and the relative abundance of each strain could be assayed using microarrays to assess if deletion of a given gene resulted in a measurable quantitative trait.

Winzeler (Winzeler *et al.*, 1999b) extended this approach, by assaying complex mixtures of more than 500 homozygous bar-coded deletion mutants, under growth in either rich or minimal medium, to determine whether the deletion of these non-essential genes affected the fitness of the strains. While generation of the mutants initially was an enormous effort, the ability to rapidly assay their phenotypes in competitive mixtures was relatively simple to implement. Thus, after generation of the initial reagents, it is relatively easy to assay a large number of growth conditions to uncover the functional roles of previously uncharacterized genes.

A recently developed method, Synthetic Lethality Analyzed by Microarray (SLAM; Ooi *et al.*, 2003), has taken this concept one step further. Instead of assaying the fitness of individual deletion mutants, a pool of mutants is transformed with a deletion construct for a gene of interest. Synthetic interactions are detected using microarrays to determine if a deletion mutant in the original pool has been depleted. The advantage of this method over traditional screens of this nature, in addition to microarray identification of candidates, is that it is quantitative, and candidates can be ranked. Theoretically, it could also be used to find deletion mutants that enhance the ability of another deletant to grow.

Assaying Drug Modes of Action

At the same time as the first whole genome expression studies were being carried out to characterize biological pathways in *S. cerevisiae*, Marton (Marton *et al.*, 1998) demonstrated that expression signatures from deletion mutants treated with different drugs could be used to identify candidate drug targets. Their logic was that the typical drug induced signature would be largely

absent if the cell were deleted for the protein that was the target of the drug. Additionally, they characterized 'off-target' effects, which remained even in the absence of the target, thus elucidating drug specificity. This result established that drug action could be systematically studied in yeast, which contains many genes for which there exist homologs in human cells.

Hughes (Hughes *et al.*, 2000) built on this idea and created a compendium of yeast gene expression data, generated both from deletion mutants, and from cell treated with compounds with known molecular targets. Their goal was to construct a reference database of gene expression profiles, such that interpreting expression profiles of new mutants, or cell treated with drugs of unknown targets could be done in the context of the preexisting data. If treatment with a drug resulted in a similar expression *signature* as resulted from deletion or mutation of a particular gene, then that drug may target that gene, either directly or indirectly. Hughes *et al.*, as had Eisen *et al.* (Eisen *et al.*, 1998) before them, were among the first to realize the cumulative value of microarray data and that different microarray experiments are not islands, but instead each add to the value of the others, such that the sum of biology that can be derived from a global set is greater than that which can be derived from any single set alone.

Mapping Traits Using Microarrays

In an illustration of the diverse applications of microarrays, Winzeler (Winzeler *et al.*, 1998) used high density oligonucleotide arrays to scan for allelic variation in the genome, based on the premise that polymorphisms in the genomic DNA of a yeast strain would cause decreased hybridization to oligonucleotides that were designed based on the sequenced strain of *S. cerevisiae*, S288C. They were able to identify markers spaced roughly every 3.5 kilobases throughout the genome of a strain unrelated to S288C. Using these markers they were able to map the phenotype of cyclohexamide sensitivity in one of the parental strains unambiguously to

the Pdr5 multidrug resistance pump. Steinmetz (Steinmetz *et al.*, 2002) extended this technique by identifying the causative alleles of Quantitative Trait Loci (QTLs) responsible for a clinically important phenotypic trait in yeast, the ability to grow at high temperature. In an even more far-reaching study, Brem (Brem *et al.*, 2002) used the same technique as Winzeler (Winzeler *et al.*, 1999a) to track genetic markers, but instead of scoring simple measurable traits in plate or growth assays, they measured considered genome-wide transcription as the phenotype. They used whole genome microarrays to measure expression the two parental strains and 40 haploid segregants and were able to link the expression levels of 570 genes to one or more loci. Furthermore, they identified 8 loci that appeared to encode *trans* acting factors responsible for some of the expression variation that they observed. Thus microarrays can be used to track both genome-wide genotypes and genome-wide phenotype, and identify causative linkages between them.

Systems Biology

While there are many applications of microarrays that can be used to probe the state or contents of a cell or biological system, microarray data can be greatly enhanced by the addition of other systematic data. In an attempt to characterize the network of interactions that control galactose metabolism Ideker (Ideker *et al.*, 2001) systematically perturbed the galactose metabolic network, both genetically and environmentally. They used microarrays to assay the gene expression changes, and tandem mass spectrometry of tagged proteins to assay changes in protein abundance for several hundred proteins. These data were then combined with pre-existing 2-hybrid interaction data and protein-DNA interaction data from transcription factor databases to build a model of galactose metabolism within the cell. The approach of combining

microarray data (potentially of different types) with other functional genomics data is likely the way by which we will elucidate how a cell works as a system.

Assessing the State and Content of the Genome

Raghuraman (Raghuraman *et al.*, 2001) used microarrays to define Origins of Replication for the whole genome using microarrays. Using the classic, Heavy/Light isotope trick, they purified EcoRI fragmented DNA whose ratio of Heavy to Light isotopes depended on proximity to an origin. Comparing Heavy-Heavy and Heavy-Light fractions by hybridization to a microarray identified regions of the genome that acted as ARS, and determined when they fired in the cell cycle as well as fork migration rates.

Identification of Protein DNA Binding Sites

One of the most important advances in moving towards a full systems biology approach is the use of DNA microarrays to determine the *in vivo* binding sites for transcription factors using Chromatin immunoprecipitation (ChIP). Rick Young's group at MIT was the first group to microarray-based ChIP data (Ren *et al.*, 2000), characterizing genes whose regulatory regions are bound by Ste12 and Gal4. They followed up this result with a truly impressive body of work, systematically studying 106 yeast transcription factors to determine their *in vivo* specificities (Lee *et al.*, 2002). In this one paper they were able to characterize nearly all of the sequence specific transcription factors and were able to identify network motifs, such as autoregulation, multi-component loops and feedforward loops. These data were used, in conjunction with other existing expression data, to produce network models for various cellular machinery, such as the cell cycle.

Protein Chips

While the majority of microarray experiments have used DNA fragments on the microarray, either oligonucleotides, PCR products, or cDNA clones, protein microarrays have also been developed. Zhu (Zhu *et al.*, 2001) purified proteins encoded by 5800 different yeast ORFs, and then spotted the purified proteins onto a glass substrate to create a protein microarray. The microarray was then used to screen for proteins that interacted with calmodulin or phospholipids. This approach allowed them to identify a putative calmodulin binding site that was present in many of the proteins that interacted with calmodulin. Again, while the initial reagents are time consuming to prepare, once they are in hand, they can rapidly be used to assay various properties of proteins.

Summary and Future

Figure 1 near here

Since their development in 1996, microarrays have become a powerful tool in the biologists' arsenal. While they are most often thought of in connection with microarray expression studies, several landmark studies have demonstrated a wide variety of applications for microarrays, the majority of which were carried out in yeast. Using microarrays, researchers are able to assay almost every aspect of a living cell to characterize it, from the parental origin of genes, to their copy number, to the replicative state of the genome, to many aspects of the transcriptome and it properties, to the proteome itself (Figure 1). Due to space constraints, we have limited the scope of our review but we would like to point out that microarrays have been used to study RNA stability and decay, RNA splicing, transcript length, RNA localization and even the kinetics of protein translation (Holstege *et al.*, 1998; Wang *et al.*, 2002; Grigull *et al.*, 2004; Clark *et al.*, 2002; Hurowitz & Brown, 2003; Marc *et al.*, 2002; Arava *et al.*, 2003). Furthermore,

microarrays can be used as a screening technology, to identify the effect upon the fitness of a cell in the presence of various deletions or mutations.

Great strides have been made in understanding several fundamental aspects of yeast cell biology using microarrays since 1996, yet by no means can we say that yeast is 'solved' as an organism. First, and most surprisingly, we do not yet know with certainty the number and location of all yeast genes nor do we know the set of transcripts produced from these genes. Second, we do not know the functions of almost a third of the genes that are identified. Tiling arrays, in which nearly the entire yeast genome sequence can be interrogated, will enable us to elucidate all the transcripts encoded by the genome, and accurately determine their 5' and 3' ends. It is unclear at this point how many additional, as yet unrecognized genes exist in the genome, and how many of those currently marked as dubious by the community database, SGD (Christie *et al.*, 2004) really have no function, or do not produce a transcript. Comprehensive understanding of yeast biology on an organismal scale requires additional microarray experiments, using more comprehensive microarrays, coupled with the integration of additional non-microarray data types, such as 2-hybrid and other protein-protein interaction data. In addition it will be necessary to integrate these results with a comprehensive analysis of the yeast metabolome. Once we understand, through experimentation, how all the components in the central dogma fit together (the genes in the genome, when and what transcripts are produced, and the functions of the encoded products), and how the metabolites and their abundance and fluxes change during various physiological programs, we will understand, to a first approximation, how yeast works on an organismal level. Application and extension of the fundamental principles that have been learned from such studies will be instrumental in understanding how more complex, multicellular organisms work.

References

Arava, Y., Wang, Y., Storey, J.D., Liu, C.L., Brown, P.O. and Herschlag, D. (2003) Genome-wide analysis of mRNA translation profiles in Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **100,** 3889-3894.

Brem, R.B., Yvert, G., Clinton, R. and Kruglyak, L. (2002) Genetic dissection of transcriptional regulation in budding yeast. *Science* **296,** 752-755.

Cho, R.J., Campbell, M.J., Winzeler, E.A., Steinmetz, L., Conway, A., Wodicka, L., Wolfsberg, T.G., Gabrielian, A.E., Landsman, D., Lockhart, D.J. and Davis, R.W. (1998) A genome-wide transcriptional analysis of the mitotic cell cycle. *Mol Cell* **2,** 65-73.

Christie, K.R., Weng, S., Balakrishnan, R., Costanzo, M.C., Dolinski, K., Dwight, S.S., Engel, S.R., Feierbach, B., Fisk, D.G., Hirschman, J.E., Hong, E.L., Issel-Tarver, L., Nash, R., Sethuraman, A., Starr, B., Theesfeld, C.L., Andrada, R., Binkley, G., Dong, Q., Lane, C., Schroeder, M., Botstein, D. and Cherry, J.M. (2004) Saccharomyces Genome Database (SGD) provides tools to identify and analyze sequences from Saccharomyces cerevisiae and related sequences from other organisms. *Nucleic Acids Res* **32,** D311-314.

Clark, T.A., Sugnet, C.W. and Ares, M., Jr. (2002) Genomewide analysis of mRNA processing in yeast using splicing-specific microarrays. *Science* **296,** 907-910.

DeRisi, J.L., Iyer, V.R. and Brown, P.O. (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278,** 680-686.

Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A* **95,** 14863-14868.

Grigull, J., Mnaimneh, S., Pootoolal, J., Robinson, M.D. and Hughes, T.R. (2004) Genome-wide analysis of mRNA stability using transcription inhibitors and microarrays reveals posttranscriptional control of ribosome biogenesis factors. *Mol Cell Biol* **24,** 5534-5547.

Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S. and Young, R.A. (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., Bennett, H.A., Coffey, E., Dai, H., He, Y.D., Kidd, M.J., King, A.M., Meyer, M.R., Slade, D., Lum, P.Y., Stepaniants, S.B., Shoemaker, D.D., Gachotte, D., Chakraburtty, K., Simon, J., Bard, M. and Friend, S.H. (2000) Functional discovery via a compendium of expression profiles. *Cell* **102,** 109-126.

Hurowitz, E.H. and Brown, P.O. (2003) Genome-wide analysis of mRNA lengths in Saccharomyces cerevisiae. *Genome Biol* **5,** R2.

Ideker, T., Thorsson, V., Ranish, J.A., Christmas, R., Buhler, J., Eng, J.K., Bumgarner, R., Goodlett, D.R., Aebersold, R. and Hood, L. (2001) Integrated genomic and proteomic analyses of a systematically perturbed metabolic network. *Science* **292,** 929-934.

Lee, T.I., Rinaldi, N.J., Robert, F., Odom, D.T., Bar-Joseph, Z., Gerber, G.K., Hannett, N.M., Harbison, C.T., Thompson, C.M., Simon, I., Zeitlinger, J., Jennings, E.G., Murray, H.L., Gordon, D.B., Ren, B., Wyrick, J.J., Tagne, J.B., Volkert, T.L., Fraenkel, E., Gifford, D.K. and Young, R.A. (2002) Transcriptional regulatory networks in Saccharomyces cerevisiae. *Science* **298,** 799-804.

Marc, P., Margeot, A., Devaux, F., Blugeon, C., Corral-Debrinski, M. and Jacq, C. (2002) Genome-wide analysis of mRNAs targeted to yeast mitochondria. *EMBO Rep* **3,** 159-164.

Marton, M.J., DeRisi, J.L., Bennett, H.A., Iyer, V.R., Meyer, M.R., Roberts, C.J., Stoughton, R., Burchard, J., Slade, D., Dai, H., Bassett, D.E., Jr., Hartwell, L.H., Brown, P.O. and Friend, S.H. (1998) Drug target validation and identification of secondary drug target effects using DNA microarrays. *Nat Med* **4,** 1293-1301.

Ooi, S.L., Shoemaker, D.D. and Boeke, J.D. (2003) DNA helicase gene interaction network defined using synthetic lethality analyzed by microarray. *Nat Genet* **35,** 277-286.

Raghuraman, M.K., Winzeler, E.A., Collingwood, D., Hunt, S., Wodicka, L., Conway, A., Lockhart, D.J., Davis, R.W., Brewer, B.J. and Fangman, W.L. (2001) Replication dynamics of the yeast genome. *Science* **294,** 115-121.

Ren, B., Robert, F., Wyrick, J.J., Aparicio, O., Jennings, E.G., Simon, I., Zeitlinger, J., Schreiber, J., Hannett, N., Kanin, E., Volkert, T.L., Wilson, C.J., Bell, S.P. and Young, R.A. (2000) Genome-wide location and function of DNA binding proteins. *Science* **290,** 2306-2309.

Shoemaker, D.D., Lashkari, D.A., Morris, D., Mittmann, M. and Davis, R.W. (1996) Quantitative phenotypic analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy. *Nat Genet* **14,** 450-456.

Spellman, P.T., Sherlock, G., Zhang, M.Q., Iyer, V.R., Anders, K., Eisen, M.B., Brown, P.O., Botstein, D. and Futcher, B. (1998) Comprehensive identification of cell cycle-regulated genes of the yeast Saccharomyces cerevisiae by microarray hybridization. *Mol Biol Cell* **9,** 3273-3297.

Steinmetz, L.M., Sinha, H., Richards, D.R., Spiegelman, J.I., Oefner, P.J., McCusker, J.H. and Davis, R.W. (2002) Dissecting the architecture of a quantitative trait locus in yeast. *Nature* **416,** 326-330.

Wang, Y., Liu, C.L., Storey, J.D., Tibshirani, R.J., Herschlag, D. and Brown, P.O. (2002) Precision and functional specificity in mRNA decay. *Proc Natl Acad Sci U S A* **99,** 5860-5865.

Winzeler, E.A., Richards, D.R., Conway, A.R., Goldstein, A.L., Kalman, S., McCullough, M.J., McCusker, J.H., Stevens, D.A., Wodicka, L., Lockhart, D.J. and Davis, R.W. (1998) Direct allelic variation scanning of the yeast genome. *Science* **281,** 1194-1197.

Winzeler, E.A., Lee, B., McCusker, J.H. and Davis, R.W. (1999a) Whole genome genetic-typing in yeast using high-density oligonucleotide arrays. *Parasitology* **118 Suppl,** S73-80.

Winzeler, E.A., Shoemaker, D.D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J.D., Bussey, H., Chu, A.M., Connelly, C., Davis, K., Dietrich, F., Dow, S.W., El Bakkoury, M., Foury, F., Friend, S.H., Gentalen, E., Giaever, G., Hegemann, J.H., Jones, T., Laub, M., Liao, H., Davis, R.W. and et al. (1999b) Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. *Science* **285,** 901-906.

Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, S., Houfek, T., Mitchell, T., Miller, P., Dean, R.A., Gerstein, M. and Snyder, M. (2001) Global analysis of protein activities using proteome chips. *Science* **293,** 2101-2105.

Further Reading

Cho, R.J., Fromont-Racine, M., Wodicka, L., Feierbach, B., Stearns, T., Legrain, P., Lockhart, D.J. and Davis, R.W. (1998) Parallel analysis of genetic selections using whole genome oligonucleotide arrays. *Proc Natl Acad Sci U S A* **95,** 3752-3757.

Diehn, M., Eisen, M.B., Botstein, D. and Brown, P.O. (2000) Large-scale identification of secreted and membrane-associated gene products using DNA microarrays. *Nat Genet* **25,** 58-62.

Dunham, M.J., Badrane, H., Ferea, T., Adams, J., Brown, P.O., Rosenzweig, F. and Botstein, D. (2002) Characteristic genome rearrangements in experimental evolution of Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **99,** 16144-16149.

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

Gerton, J.L., DeRisi, J., Shroff, R., Lichten, M., Brown, P.O. and Petes, T.D. (2000) Global mapping of meiotic recombination hotspots and coldspots in the yeast Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* **97,** 11383-11390.

Giaever, G., Chu, A.M., Ni, L., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., Andre, B., Arkin, A.P., Astromoff, A., El-Bakkoury, M., Bangham, R., Benito, R., Brachat, S., Campanaro, S., Curtiss, M., Davis, K., Deutschbauer, A., Entian, K.D., Flaherty,

P., Foury, F., Garfinkel, D.J., Gerstein, M., Gotte, D., Guldener, U., Hegemann, J.H., Hempel, S., Herman, Z., Jaramillo, D.F., Kelly, D.E., Kelly, S.L., Kotter, P., LaBonte, D., Lamb, D.C., Lan, N., Liang, H., Liao, H., Liu, L., Luo, C., Lussier, M., Mao, R., Menard, P., Ooi, S.L., Revuelta, J.L., Roberts, C.J., Rose, M., Ross-Macdonald, P., Scherens, B., Schimmack, G., Shafer, B., Shoemaker, D.D., Sookhai-Mahadeo, S., Storms, R.K., Strathern, J.N., Valle, G., Voet, M., Volckaert, G., Wang, C.Y., Ward, T.R., Wilhelmy, J., Winzeler, E.A., Yang, Y., Yen, G., Youngman, E., Yu, K., Bussey, H., Boeke, J.D., Snyder, M., Philippsen, P., Davis, R.W. and Johnston, M. (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. and Davis, R.W. (1999) Genomic profiling of drug sensitivities via induced haploinsufficiency. *Nat Genet* **21,** 278-283.

Hughes, T.R., Mao, M., Jones, A.R., Burchard, J., Marton, M.J., Shannon, K.W., Lefkowitz, S.M., Ziman, M., Schelter, J.M., Meyer, M.R., Kobayashi, S., Davis, C., Dai, H., He, Y.D., Stephaniants, S.B., Cavet, G., Walker, W.L., West, A., Coffey, E., Shoemaker, D.D., Stoughton, R., Blanchard, A.P., Friend, S.H. and Linsley, P.S. (2001) Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nat Biotechnol* **19,** 342-347.

Hughes, T.R., Roberts, C.J., Dai, H., Jones, A.R., Meyer, M.R., Slade, D., Burchard, J., Dow, S., Ward, T.R., Kidd, M.J., Friend, S.H. and Marton, M.J. (2000) Widespread aneuploidy revealed by DNA microarray expression profiling. *Nat Genet* **25,** 333-337.

Iyer, V.R., Horak, C.E., Scafe, C.S., Botstein, D., Snyder, M. and Brown, P.O. (2001) Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF. *Nature* **409,** 533-538.

Lashkari, D.A., DeRisi, J.L., McCusker, J.H., Namath, A.F., Gentile, C., Hwang, S.Y., Brown, P.O. and Davis, R.W. (1997) Yeast microarrays for genome wide parallel genetic and gene expression analysis. *Proc Natl Acad Sci U S A* **94,** 13057-13062.

Nagy, P.L., Cleary, M.L., Brown, P.O. and Lieb, J.D. (2003) Genomewide demarcation of RNA polymerase II transcription units revealed by physical fractionation of chromatin. *Proc Natl Acad Sci U S A* **100,** 6364-6369.

Ooi, S.L., Shoemaker, D.D. and Boeke, J.D. (2001) A DNA microarray-based genetic screen for nonhomologous end-joining mutants in Saccharomyces cerevisiae. *Science* **294,** 2552-2556.

Shalon, D., Smith, S.J. and Brown, P.O. (1996) A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Res* **6,** 639-645.

Wyrick, J.J., Aparicio, J.G., Chen, T., Barnett, J.D., Jennings, E.G., Young, R.A., Bell, S.P. and Aparicio, O.M. (2001) Genome-wide distribution of ORC and MCM proteins in S. cerevisiae: high-resolution mapping of replication origins. *Science* **294,** 2357-2360.

Yvert, G., Brem, R.B., Whittle, J., Akey, J.M., Foss, E., Smith, E.N., Mackelprang, R. and Kruglyak, L. (2003) Trans-acting regulatory variation in Saccharomyces cerevisiae and the role of transcription factors. *Nat Genet* **35,** 57-64.

Zhu, H., Klemic, J.F., Chang, S., Bertone, P., Casamayor, A., Klemic, K.G., Smith, D., Gerstein, M., Reed, M.A. and Snyder, M. (2000) Analysis of yeast protein kinases using protein chips. *Nat Genet* **26,** 283-289.

Figure Legends

Figure 1. Illustration of a yeast cell to show how microarrays can be applied to interrogate all aspects of the Central Dogma of Molecular Biology. In the Central Dogma, DNA (the genome) is used as a template to make RNA (the transcriptome), which in turn is translated into protein (the proteome). Although microarrays are often thought of in connection with assaying transcript abundance, they can be used to assay various properties of each of the aspects of the Central Dogma, as shown.