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WHOLE YEAST GENOME ANALYSIS USING DNA CHIPS

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In order to assign functional information to the genome sequence, yeast was used as a model organism to efficiently identify the expression pattern of the genes discovered by genome project using cDNA microarray chips. We have developed high-density yeast cDNA microarray chips which contain 2,479 of yeast open reading frames (ORFs) on a 1.5 cm² glass slide. The procedure was as follows (1) Software scripts scanned annotated sequence information from public databases for predicted ORFs. (2) The data was used to automatically select PCR primers that would amplify the ORF. (3) The primer sequences were automatically input into the automated multiplex oligonucleotide synthesizer. (4) The oligonucleotides were synthesized in 96-well format, and used in 96-well format to amplify the desired ORFs from a genomic DNA template. (5) The product were arrayed using a high-density DNA microarrayer. (6) The hybridization of fluorescently labeled samples to the array were detected and quantitated with a laser confocal scanning microscope. These cDNA microarray chips were used to analyze differential gene expression in yeast grown under a variety of different conditions.

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DISTINCT MODULES OF MEDIATOR ARE REQUIRED FOR DEVELOPMENTAL-SPECIFIC TRANSCRIPTIONAL REGULATION.

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The multisubunit Mediator complex of *Saccharomyces cerevisiae* is required for most RNA polymerase II (Pol II) transcription. In order to determine the precise composition of the Mediator complex and to explore the specific role of each Mediator protein, our goal was to identify all of the Mediator components. To this end, we cloned three previously unidentified Mediator subunits, Med9/Cse2, Med10/Nut2, and Med11, and isolated mutant forms of each of them to analyze their transcriptional defects. Differential display and Northern analyses of mRNAs from wild type and Mediator mutant cells demonstrated an activator-specific requirement for each Mediator subunit. Med9/Cse2 and Med10/Nut2 were required, respectively, for Bas1/Bas2- and Gcn4-mediated transcription of amino acid biosynthetic genes. Gal11 was required for Gal4- and Rap1-mediated transcriptional activation. Based on these and other results we demonstrated that the Gal11 module of the Rgr1 subcomplex is required for the efficient recruitment of Pol II holoenzyme to a promoter via activator-specific interactions, while the Srb4 subcomplex functions in the modulation of general polymerase activity.