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Biochemical and Biophysical Research Communications 338 (2005) 1578–1586
BBRC
www.elsevier.com/locate/ybbrc

Cigarette smoke extract induces changes in growth and gene expression of *Saccharomyces cerevisiae*

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Received 16 October 2005
Available online 2 November 2005

Abstract

The response of *Saccharomyces cerevisiae* cells to an aqueous extract of cigarette smoke was studied. Exposure to cigarette smoke extract inhibits yeast growth and results in global changes in gene expression spanning many functional classes of genes. Genes involved in response to oxidative stress are upregulated after a brief exposure to cigarette smoke extract. The effects of cigarette smoke extract on yeast growth can be reversed by treatment with anti-oxidants. Mutants lacking superoxide dismutase gene were hypersensitive to cigarette smoke exposure. YAP1 is a central transcriptional regulator of oxidative stress in yeast. YAP1 dependent expression of β-galactosidase was enhanced following exposure to cigarette smoke. The overall agreement between our observations and the recently reported effects of cigarette smoke on gene expression in rodent and human cells suggests that yeast can be used as a model system in toxicogenomics studies for monitoring toxic agents and studying the cellular and molecular consequences of exposure to potentially toxic agents.

Keywords: Yeast; Cigarette smoke; Microarray; Oxidative stress

Traditionally, toxicologists have been using bioassays based on rodent models to evaluate the toxic effects of chemical compounds and to study the mechanism of action of toxicants. There is a growing need to develop high-throughput assays for rapid screening of large numbers of toxicants. Baker's yeast or *Saccharomyces cerevisiae* can be a promising model for such assays due to its amenability to genetic studies and the vast amount of genomics knowledge and resources associated with this unicellular fungus [1]. We have, here, used it to study the effect of a well-known toxicant whose mechanism of action is not fully understood, viz. cigarette smoke. We find that as in mammalian cells, exposure to cigarette smoke leads to induction of oxidative stress related genes in yeast.

Saccharomyces cerevisiae shows considerable similarity to higher systems in cellular organisation and function, and has been used as a model system for studying many phenomena of relevance to human biology at the molecular level. Yeast has proved to be a remarkably versatile model system; its applications ranging from the study of cellular mechanisms of cancer to neurological diseases. In the post-genomics era, it has been a valuable tool in every aspect of high-throughput biological research, from gene expression profiling to protein–protein interaction mapping. We and others have previously used gene expression profiling in wild-type yeast cells and knockout mutants after exposure to environmental perturbations and chemical agents using it as a model system to study transcriptional regulation [3–5]. Gene expression profiles generated from these studies provide ready molecular signatures to compare the effects of drugs and toxicants. Such comparisons not only provide clues to the molecular basis for the action of the chemical agent, but also offer assays based on expression of surrogate marker genes for monitoring the chemical as well as high-throughput assays for screening of detoxifying agents. Bioassays based on rodent model

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doi:10.1016/j.bbrc.2005.10.122

in comparison to yeast would take longer, be more expensive, and not be amenable to high-throughput screening.

Exposure to cigarette smoke is amongst the leading causes for many diseases, notably, cancer, cardiovascular disease, and chronic obstructive pulmonary disease. The precise molecular mechanisms by which chemical compounds contained in cigarette smoke manifest its effects are not yet known. Recent studies have focused on the effect of cigarette smoke exposure on the transcriptional profile of respiratory tissues of rats in vivo and Swiss 3T3 mouse cells in vitro [6,7]. The genes induced in rodent cells exposed to cigarette smoke include stress response genes like c-fos, c-jun, heat shock proteins, mediators of inflammatory response like st2, kc, and id3, and oxidative stress related genes like metallothioneins and phase II drug metabolism genes. Gebel and Muller have shown that a rapid depletion of GSH levels in fibroblasts exposed to cigarette smoke extract may be a key step in nuclear factor κ-B mediated regulation of gene expression [6–9].

We report here, the effect of cigarette smoke on the growth and gene expression profile of yeast. In agreement with the studies on rodent cells we see that cigarette smoke extract increases the levels of reactive oxygen species in yeast cells. The growth inhibitory effect of cigarette smoke extract on yeast can be overcome by providing an anti-oxidant during exposure to cigarette smoke. Yeast cells carrying an oxidative stress responsive promoter tagged to a β-galactosidase reporter gene could be used to rapidly assay for exposure to cigarette smoke extract. Amongst the genes most highly induced in response to cigarette smoke extract are the yeast aryl alcohol dehydrogenase genes, whose functional role is not clearly understood. Moreover, a functional superoxide dismutase gene (SOD1) is necessary for allowing yeast cells to survive in the presence of cigarette smoke extract.

Materials and methods

Strains, constructs, media, and growth conditions. Saccharomyces cerevisiae wild-type strain BY4742 (MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) was used for this study. The sod1Δ strain was procured from EUROSCARF and shares the same genetic background. Cells were grown in standard rich media (1% yeast extract, 2% peptone, and 2% dextrose) overnight before all experiments. A fresh culture was allowed to grow at 28 °C until it reached A_{600} 0.6 before treatment. Morin (Sigma 480-16-0) was dissolved in 50% DMSO at 30 mM and used at a final concentration of 300 μM. The YCF1-lacZ reporter construct used has been described before [10].

Preparation of cigarette smoke extract. The mainstream smoke from commercially available standard Indian cigarettes was allowed to bubble through 50 mM sodium phosphate buffer at pH 7.5 by attaching to a vacuum pump. The volume of the buffer was adjusted such that 1 ml of extract corresponds to the smoke from one cigarette [11].

Growth kinetics. Yeast cells were pre-grown in rich medium (1% yeast extract, 2% peptone, and 2% dextrose) and inoculated into fresh media at an A_{600} of 0.3. When the cultures reach an A_{600} of 0.6, corresponding to early log phase, cigarette smoke extract was added. A_{600} was monitored at periodic intervals.

Microarray studies and data analysis. Cells (in 50 ml cultures) exposed to 2 ml cigarette smoke extract for a period of 2 h were harvested by centrifugation and washed. Total RNA was isolated from these cultures by the glass bead lysis method as described before [12]. This total RNA was used for labeling and hybridization onto the microarray slides. Total RNA was labeled by the indirect Micromax NEN TSA labeling system (Perkin-Elmer Life Sciences, USA) according to manufacturer's instructions. Briefly, cDNAs were labeled with biotin or fluorescein, hybridized on the microarray slides, and detected using streptavidin and anti-fluorescein antibody conjugated to horse radish peroxidase which enzymatically converts tyramide Cy3 and Cy5 to Cy3 and Cy5, respectively. Two biological replicates were performed using cigarette smoke extracts prepared from different batches of cigarettes, and cells cultured at different times. The *S. cerevisiae* cDNA microarray slides were procured from The Microarray Center, Clinical Genomics Center, University Health Network, Toronto, Canada. The slides were scanned using an Axon scanner and data were acquired and analyzed using GenepixPro. Data for each spot were corrected for background and the data from treated and reference samples were normalized to the total intensity. The microarray slides contained duplicate spots for each gene resulting in four data points for each gene. The genes that were upregulated or downregulated more than twofold in at least three out of four data points were considered for further analysis. Functional classification of genes was done using the MIPS and CYGD databases [13,14]. Gene Ontology terms associated with differentially expressed genes were retrieved from Saccharomyces genome database [15].

Northern blot analysis. Northern blotting and probing were performed using standard protocols [13]. RNA was extracted by the hot phenol method as described above and blotted onto nylon membranes after separation on a 1% agarose formaldehyde denaturing gel. Subsequently, radioactively labeled probes prepared from PCR products of GTT2, AAD6, and SOD1, and detected by exposure to X ray films.

Detection of reactive oxygen species. Intracellular redox levels were measured by fluorimetry using the fluorescent dye 2,7-dichlorofluorescein diacetate [16]. Cells were grown in YPD medium and exposed to cigarette smoke extract as described above. An aliquot of cells was treated with hydrogen peroxide at a final concentration of 1 mM as a positive control. After an additional incubation for 15 min, cells were collected by centrifugation from 2 ml culture and then washed three times with phosphate-buffered saline (PBS). Cells were resuspended in PBS with 10 mM of 2,7′-dichlorofluorescein diacetate (Sigma Aldrich) and incubated at 28 °C for 1 h. The dye can react specifically with hydrogen peroxide to give a highly fluorescent 2′,7′-dichlorofluorescein (DCF). Cells were collected, washed three times with PBS, and lysed by the glass bead lysis protocol. The cell lysate was used for protein estimation and extracts containing nearly equal amounts of extract were used for fluorimetry. Detection of fluorescence was carried out using a Fluoromax3, SPEX spectrofluorimeter. The dye was excited at 488 nm and emission was monitored from 500 to 700 nm. The increase in emission at 520 nm in lysates of treated cells was compared to that of untreated cells.

β-Galactosidase assay. Yeast cells transformed with YCF1-lacZ were selected and were exposed to cigarette smoke extracts as described above and β-galactosidase activity was monitored as described previously [18]. Briefly, cells were harvested from 3 ml culture by centrifugation at appropriate times, resuspended in 0.8 ml Z buffer, and permeabilized in the presence of one drop of 0.1% SDS and 2 drops of chloroform for 15 min at 30 °C. One hundred sixty microliters of 4 mg/ml ONPG was added and reactions were stopped after development of yellow color by addition of 400 μl of 1 M sodium carbonate solution. The β-galactosidase activity was calculated using the formula, β-gal activity (units) $= (1000 \times A_{420})/(V \times T \times A_{600})$, where V is the volume of the culture and T is the time in minutes.

Results

In order to study the suitability of yeast as a toxicogenomics model for cigarette smoke exposure, we first studied the effect of an aqueous extract of cigarette smoke on yeast growth. The cigarette smoke extract resulted in decreased growth of yeast. Nearly 50% decrease in growth rates was consistently seen at 8 h of exposure to the extract of