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# Effect of Benzo[a]pyrene on Genes Related to the Cell Cycle and Cytochrome P450 of *Saccharomyces cerevisiae*

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**Abstract** Benzo[a]pyrene (B[a]P) is an environmental pollutant that has been implicated in carcinogenesis. *Saccharomyces cerevisiae* was treated with B[a]P, and the responses of its cytochrome P450 (CYP) enzyme and DNA-damage checkpoint genes were examined through gene expression profiles using a reverse transcription polymerase chain reaction (RT-PCR). The DNA-damage checkpoint genes tested were the *chk1* and *pds1* genes, involved in a metaphase arrest, the swi6 gene targeted by G1 arrest, the *pol2* gene related to S phase arrest, and the *cln2* gene encoding a cyclin protein, all of which are based on *rad9* and *rad24*. Among these genes, no noticeable effect was found when the cells were exposed to various concentrations of B[a]P. However, the transcriptional activity of CYP51 was significantly different when the cells were exposed to B[a]P. Accordingly, the present results indicate that cytochrome P450 plays a more significant role than DNA-damage checkpoint genes in the response of *S. cerevisiae* to B[a]P.

**Key words:** Benzo[a]pyrene, RT-PCR, environmental stress

Benzo[a]pyrene (B[a]P) is one of the very potent carcinogenic polycyclic aromatic hydrocarbons (PAHs). It is commonly generated during incomplete combustion processes, and can be found in wood combustion, diesel engine exhaust, fuel exhaust, coal-tar, and asphalt. Through several B[a]P bioactivation pathways, various electrophilic intermediates are produced, such as 7,8-diol-9,10-epoxide (BPDE), which is an extremely carcinogenic compound and known to cause harmful effects to DNA, protein, and other macromolecules [5]. In addition, in experimental animals and human cells, the formation of DNA adducts is considered as a critical event for the start of neoplasia [1, 2].

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DNA-damage checkpoints are believed to play an important role in carcinogenesis, and are conserved from yeast to human cells. Within budding yeast, genes involved in the recognition of DNA damage have been distinguished primarily in G1 and G2, and classified as the *rad9* gene and *rad24* subclass genes from their functions [9]. The transduction of DNA-damage signals includes three principal protein kinases; Mec1, Rad53, and Dun1. After DNA is damaged, the phosphorylation of another kinase, Chk1, leads to signal transduction to *pds1* with a Rad53 function in parallel, resulting in G2/M arrest. In addition, DNA replication machinery, such as the subunits of DNA polymerase and their accessory factors, senses both DNA damage and the inhibition of DNA synthesis during the S phase [12]. Cell-cycle arrest in the G1 phase is enough to lead to differentiation, while genes encoding three cyclin proteins, CLN1, CLN2 and CLN3, and an associated protein kinase, CDC28, associated with them are required for progression into all phases of the cell cycle in *Saccharomyces cerevisiae*. Finally, the swi4/swi6 heterodimer is needed for the transcription of G1-specific cyclins.

Cytochrome P450 (CYP) enzymes are known to have a significant environmental role in oxidizing procarcinogens and promutagens, such as drugs and toxic chemicals. Only after chemical carcinogens are activated by metabolizing enzymes, including CYP, can they induce tumors from interaction with DNA. The NADPH cytochrome P450 reductase (CPR) is required as an electron donor for the activation of CYPs, and there are three CYP genes which need CPR for functioning in *S. cerevisiae*: CYP51 (sterol 14α-demethylase), CYP61 (sterol 22-desaturase), and CYP56 (dityrosine hydroxylase) [6- 10].

Accordingly, the current study investigated the responses of *S. cerevisiae* to B[a]P-induced damage, including its DNA-damage checkpoint pathways and the expression of its cytochrome P450 based on an analysis of the expression patterns of these stress responsive genes.

**Table 1.** PCR primers used in the current study.

| Name | Sequence |
|---|---|
| ACT1 | 5'-GCGTAAATTGGAACGACGT-3' |
| | 5'-TGACGACGCTCCTCGTGCTGT-3' |
| CHK1 | 5'-TGTTGACTGGTCAAACGCT-3' |
| | 5'-AGCCTCTTGTGTCAAACCTG-3' |
| CLN2 | 5'-TCAGAGAGTGAGCTCATG-3' |
| | 5'-GTGGCAGTACGACTTACT-3' |
| ERG5 | 5'-TAGCTAAGACATCTGGAC-3' |
| | 5'-TAACTACGAGCCCCAGGT-3' |
| ERG11 | 5'-TGGAGTGAAACCCTTATCC-3' |
| | 5'-GTGTATTTAGGACCAAAGG-3' |
| PDS1 | 5'-TGATTCCGAGTGATGCACTC-3' |
| | 5-GTTCCGACGATGATGAAGGT-3' |
| POL2 | 5'-AGCTCCACTTCCAGATCATG-3' |
| | 5'-AGTTGAGCCAAGTGCACT-3' |
| RAD9 | 5'-AGCTAGCGGATGAGTGAAGT-3' |
| | 5'-TCAGTGTTGACCTAAGCGAG-3' |
| RAD24 | 5'-ATGGAGCTCATCTAGACC-3' |
| | 5'-GACAGGTTGGACATCACAG-3' |
| SWI6 | 5'-TCGGTTTGTTCCAGGATGCA-3' |
| | 5'-CAACTGGAGAAGGTAGTGCT-3' |

## Strain, Media, and Chemicals

*Saccharomyces cerevisiae* S288C (ATCC26108) was used in the current study and grown in a YM medium (DIFCO, U.S.A.) in 250-ml flasks in a rotary incubator (Perkin-Elmer Co., U.S.A.) at 30°C and 200 rpm. The benzo[a]pyrene (B[a]P) was purchased from Sigma Chemical Company.

## Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Specific primers were ordered for the corresponding nucleotide sequences, as shown in Table 1. The yeast cultures were grown in a YM medium after being inoculated with 2 ml of an overnight culture. The B[a]P stock solution, prepared in benzene, was diluted into the media so that the final concentration of benzene was 0.1%, which did not result in any significant growth inhibition, and was added to the culture during the mid-exponential growth phase. The total RNA extractions were carried out with a QIAGEN RNAeasy Mini Kit (#74104) for 30 min, 45 min, and 60 min after exposure to 5 μM B[a]P, with which no significant growth inhibition was seen (Fig. 1). The reverse transcription polymerase chain reaction was performed using a STRATAGENE ProSTR™ HF Single-Tube RT-PCR System under the following conditions: for the reverse transcription process, 15 min at 37°C and 1 min at 95°C; and for the PCR, 30 sec at 95°C, 30 sec at 50 or 55°C; and 1 min at 68°C with 25 reaction cycles [4, 8]. The ACT1 gene encoding actin mRNA, a commonly known housekeeping gene, was used as the standard for the RT-PCR.

Yeast, a small unicellular eukaryotic microorganism, is a valuable model because of its similarity to the cellular organization of higher eukaryotes. Thus, the budding yeast, *Saccharomyces cerevisiae*, whose genomic sequence is completely available, was used to evaluate the stress response after exposure to benzo[a]pyrene. B[a]P is a very potent carcinogen present in the environment. As such, the current study focused on the expression patterns of two categorized groups of genes, DNA damage checkpoint genes and cytochrome P450 genes, both of which have been associated with carcinogenesis in experimental higher eukaryotic systems.

## Expression of DNA Damage Checkpoint Genes after Exposure to B[a]P

DNA damage checkpoint genes have critical roles in both sensing DNA damage and the transduction of signals to targeted carcinogenesis genes. Thus, some of these genes were selected and the PCR primers used are listed in Table 1. These genes were also chosen as they are all conserved and critical in arresting each phase of the *S. cerevisiae* cell cycle. To obtain relative transcription patterns, the RT-PCR products of actin mRNA, encoded by the ACT1 gene, were used as the standard, as shown in Fig. 2c.

The expression patterns of the selected genes were not highly altered with the addition of B[a]P at the sublethal concentrations tested, below which no significant growth rate inhibition was apparent. Only the POL2 gene, related to DNA synthesis, was induced. In addition, the decision for the cells to differentiate is known to be directly dependent on the cell-cycle arrest in the G1 phase. However, neither of the genes chosen, because of their relation to a G1 arrest, the SWI6 gene encoding a transcription factor for cyclins, and the CLN2 gene (which is one of the cyclin genes required to start the cell cycle), were induced (Fig.

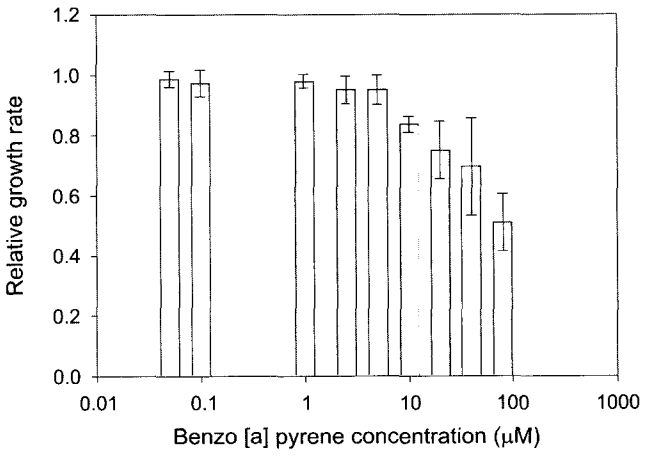


**Fig. 1.** Effect on growth rate after exposure to different B[a]P concentrations.

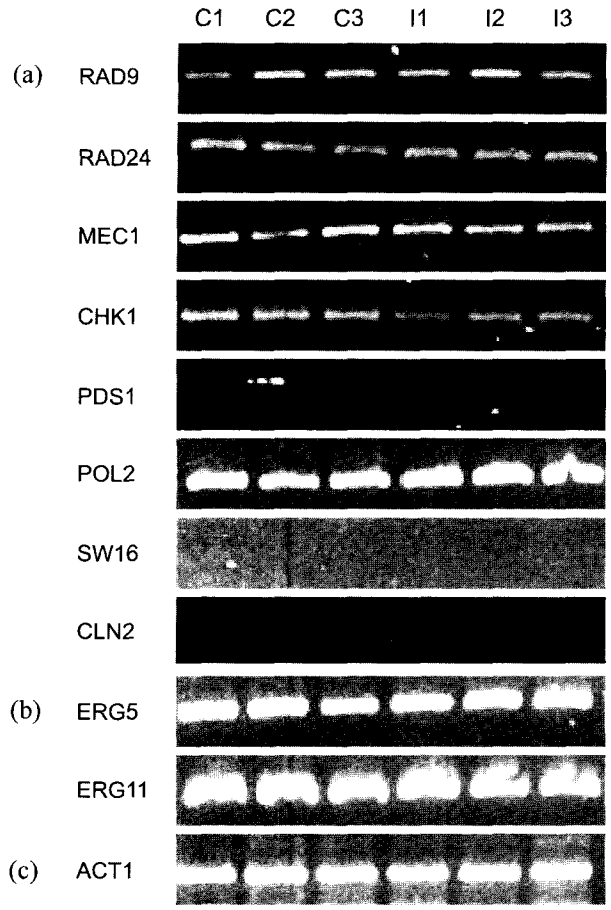


**Fig. 2.** Results of RT-PCR after exposure to 5 μM B[a]P.
(a) DNA damage checkpoint genes and (b) cytochrome P450 genes. In addition, the ACT1 gene was used as the standard (c). C1, C2, and C3 are the results for the unexposed samples, while I1, I2, and I3 are the results for the samples exposed for 30, 45, and 60 min, respectively.

2a), indicating that B[a]P did not cause the G1 arrest of *S. cerevisiae* at the concentrations tested.

### Expression of Cytochrome P450 Genes After Exposure to B[a]P

Benzo[a]pyrene is a ubiquitous carcinogen produced from the incomplete combustion of organic carbons. B[a]P itself is not known to cause any toxicity. Kim *et al.* [3] reported that carcinogenic and mutagenic properties became apparent after its bioactivation processes via cytochrome P450 in experimental rats and mice. Also, some research groups have shown that human CYPs are important enzymes in the activation of various procarcinogens, including B[a]P [2, 3, 9, 11].

Thus, two cytochrome P450s that require CPR for their function, genes, CYP51 (erg5), encoding sterol 14α-demethylase, and CYP61 (erg11), encoding a sterol $\Delta^{22}$-desaturase, were evaluated after the addition of B[a]P to the cultures of *S. cerevisiae*. As shown in Fig. 2b, the expression of CYP51 and CYP61 increased over time, but decreased after 45 min of the initial exposure to B[a]P. The experimental results also revealed that CYP51 was more highly induced than CYP61.

Therefore, the current results demonstrated that, based on their expression patterns, the cytochrome P450 enzymes in *S. cerevisiae* appeared to play a more critical role than the DNA damage checkpoint genes, when the cells were exposed to stress due to sublethal concentrations of B[a]P.

In conclusion, the current study investigated the expression patterns of two categorized groups of genes, DNA damage checkpoint genes and cytochrome P450 genes, both of which are associated with carcinogenesis in higher eukaryotic systems, in the budding yeast, in response to the addition of B[a]P. These genes may also be important when yeast experiences xenobiotic stress, including B[a]P. The results indicate that cytochrome P450 enzymes seem to play a more critical role in yeast than DNA damage checkpoint genes in response to the metabolism of B[a]P.

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