J. Microbiol. Biotechnol. (2008), **18**(2), 263–269

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

# Genome-wide Drug-induced Haploinsufficiency Screening of Fission Yeast for Identification of Hydrazinocurcumin Targets

**Baek, Seung Tae[1,6], Dong-Uk Kim[1], Sangjo Han[2], Im Sun Woo[1], Miyoung Nam[1], Lila Kim[1], Kyung-Sun Heo[1], Hyemi Lee[1], Hye-Rim Hwang[1], Shin-Jung Choi[1], Misun Won[1], Minho Lee[2], Song-Kyu Park[3], Sunghou Lee[4], Ho-Jeong Kwon[5], Pil Jae Maeng[6], Hee-Moon Park[6], Youngwoo Park[7], Dongsup Kim[2], and Kwang-Lae Hoe[1,3]***

[1]*Functional Genomics Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-333, Korea*
[2]*Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Korea*
[3]*Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-333, Korea*
[4]*Department of Biotechnology and Informatics, Sangmyung University, Cheonan 330-720, Korea*
[5]*Department of Biotechnology, Yonsei University, Seoul 120-749, Korea*
[6]*Department of Microbiology, School of Biological Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea*
[7]*Therapeutic Antibody Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-333, Korea*

Received: August 20, 2007 / Accepted: October 31, 2007

**Hydrazinocurcumin (HC), a synthetic derivative of curcumin, has been reported to inhibit angiogenesis *via* unknown mechanisms. Understanding the molecular mechanisms of the drug's action is important for the development of improved compounds with better pharmacological properties. A genome-wide drug-induced haploinsufficiency screening of fission yeast gene deletion mutants has been applied to identify drug targets of HC. As a first step, the 50% inhibition concentration ($IC_{50}$) of HC was determined to be 2.2 μM. The initial screening of 4,158 mutants in 384-well plates using robotics was performed at concentrations of 2, 3, and 4 μM. A second screening was performed to detect sensitivity to HC on the plates. The first screening revealed 178 candidates, and the second screening resulted in 13 candidates, following the elimination of 165 false positives. Final filtering of the condition-dependent haploinsufficient genes gave eight target genes. Analysis of the specific targets of HC has shown that they are related to septum formation and the general transcription processes, which may be related to histone acetyltransferase. The target mutants showed 65% growth inhibition in response to HC compared with wild-type controls, as shown by liquid culture assay.**

**Keywords:** Haploinsufficiency, hydrazinocurcumin, septin, *taf4*, yeast

One of the most critical steps in drug development is to optimize therapeutic efficacy while minimizing undesirable side effects. Ideally, optimization is carried out using knowledge of the drug's mode of action – the molecular targets that mediate its therapeutic effects and side effects. Therefore, advancement of useful technologies for target identification would be important for the drug discovery process. The generation of genome-wide heterozygous diploid and haploid gene deletion strains in the budding yeast *Saccharomyces cerevisiae* has opened a new era in the studies of mechanism of action [9, 16]. The heterozygous gene deletion strains, *i.e.*, where one of the two copies of each locus is replaced by a unique deletion module, have been successfully used to identify drug-susceptible strains and, thus, gene products that play a role in the specific growth-inhibitory mechanism [8, 22]. The screening strategy, referred to as drug-induced haploinsufficiency, is based on the fact that lowering the gene dosage of a drug target increases the susceptibility to the drug. The targets of numerous well-characterized compounds have been verified in this fashion, including the targets of tunicamycin, lovastatin, hydroxyurea, and methotrexate [8, 9, 16]. The genome-wide heterozygous deletion strains of budding yeast have been quantitatively analyzed to discover the mode of action for various compounds in parallel, by hybridization to an oligonucleotide array of unique barcode sequences. However, a high percentage of deletion mutants whose barcode regions contain mutations may result in difficulties of interpretation [16]. The limitations may be circumvented by a separate screening of each strain using direct scoring of growth inhibition, which is demonstrated in this study.

Curcumin and its derivatives have proven to be significant inhibitors of angiogenesis as evidenced by the mouse corneal model [2, 5, 12]. Production of angiogenic growth

*Corresponding author
Phone: 82-42-860-4158; Fax: 82-42-860-4594;
E-mail: kwanghoe@kribb.re.kr

factor has been affected by curcumin in nonmalignant and malignant cells. Another function of curcumin is reportedly related to its inhibitory action on histone acetyl transferase [4]. Hydrazinocurcumin (HC), a synthetic derivative of curcumin, also functions as an inhibitor of angiogenesis *via* unknown mechanisms [26]. However, the identification of HC drug targets has been a difficult task, because curcuminoids are notable for the diversity of their biological actions in preclinical models of carcinogenesis at a very wide range of doses [25].

This study examined whether the haploinsufficiency method could be applied to the screening of HC drug targets using genome-wide *Schizosaccharomyces pombe* heterozygous gene deletion mutants [22]. In addition, the putative drug targets of HC were identified. Moreover, this study showed that fission yeast genome-wide gene deletion mutants can be a useful tool for identifying drug targets, because most of the yeast proteins share a conserved sequence with at least one known or predicted human protein, including several hundred genes implicated in human disease such as cancer and metabolic disease [11, 23, 29].

## Materials and Methods

### General Techniques and HC

Cells were routinely cultivated in complete medium YES (0.5% yeast extract, 3% glucose, and amino acid supplements when required) or Edinburgh minimal medium (EMM2) with appropriate supplements at 30°C, unless specified [13, 20]. Transformation was performed using the lithium acetate procedure as previously described [20]. Calcofluor staining was performed as previously described [27]. Cells were observed using a fluorescent microscope (DM5000B, Leica) equipped with a digital CCD camera (DFC350 FX, Leica). HC was chemically synthesized from curcumin as previously described [26].

### Strains

Genome-wide heterozygous *S. pombe* deletion mutants were constructed by the PCR-based gene deletion method using the kanR marker, as described previously [3, 22]. Diploid *S. pombe* strain SP286 (*$h^+/h^+$ ade6-M210/ade6-M216 ura4-D18/ura4-D18 leu1-32/leu1-32*) was transformed with the PCR deletion cassettes and geneticin-resistant colonies were selected on YES plates containing 100 µg/ml G418. Gene deletion was confirmed by colony check-PCR.

### Inhibitory Concentration 50 ($IC_{50}$) Assay

Exponentially growing SP286 cells were diluted to $OD_{600}$=0.1 (~$2\times10^6$ cells/ml) with YES media containing either serially diluted concentrations of HC or DMSO as a vehicle control and aliquoted into 96-well plates in triplicate. The plates were then incubated for 24 h at 30°C. $IC_{50}$ was calculated using Prizm (GraphPad Software Inc, ver. 2.00) by the sigmoidal dose-response equation; $Y=100/\{1+10\text{^}[\text{Log}(IC_{50}-X)]\}$, where X is the logarithm of concentration and Y is the resulting growth normalized as a percentage.

### Genome-wide Screening of HC Targets

For a genome-wide screening of HC targets, the set of frozen strains in the 96-well plates were thawed at room temperature and activated through a 48-h cultivation in YES medium containing G418 (100 mg/l) at 30°C. The mutant arrays were then transferred to 96-well plates containing fresh medium and cultivated to saturation for an additional 24-h incubation at 30°C. Saturated cultures were necessary to compensate for differences in growth rate among strains and finally to ensure equal amounts of cells. Approximately 5 µl of the cells in the 96-well plates were arrayed to a set of four wells in 384-well plates, which were then filled with 45 µl of YES medium containing one of three different concentrations of HC (2, 3, and 4 µM) or DMSO as a vehicle control (no HC). Cells in the 384-well plates were grown over a period of 36 h at 30°C. Growth inhibition was analyzed and compared with the no-drug control group. As shown in Fig. 1B, a target candidate resulted in growth inhibition in the presence of HC, due to sensitivity to the HC. At the second screening, the 178 strains were robotically arrayed onto agar plates containing either 5 µM of HC or DMSO as a vehicle control (no HC) (Fig. 1C). Cells were spotted onto plates at the initial cell density of $2\times10^4$ cells and serially 7-fold diluted. After a 2-d incubation, growth inhibition of the candidates was analyzed and compared with that of wild-type cells.

### Robotics

Biomek NX (Beckman Coulter) was used to manipulate 96- or 384-well plates with Biomek software (ver 3.2). Optical density ($OD_{620}$) was measured using DTX 600 (Beckman Coulter) with the Multimode detection software (ver 1.0.0.14).

## Results and Discussion

### Determination of $IC_{50}$ of HC

As shown in the schematic drawing of the screening strategy (Fig. 1A), determination of the $IC_{50}$ of HC is a prerequisite to decide which concentration of HC to treat in a genome-wide screening. In general, the drug concentration in a genome-wide screening is similar to the $IC_{50}$ and in the range of half to twice of the $IC_{50}$ [16]. To determine the $IC_{50}$ of HC, the growth inhibitory effect was measured over various concentrations of HC (Fig. 2). Treatment of wild-type cells with HC showed growth inhibition in a dose-dependent manner, and its growth inhibitory effects were saturated to the maximum at a concentration of 10 µM, which showed a 60% decrease in growth. The $IC_{50}$ of HC was then calculated from the growth inhibitory effects over various concentrations of HC (Fig. 2). The $IC_{50}$ of HC was 2.2 µM. As with other curcuminoids [6, 14], HC also showed a mild antifungal activity but no fungicidal effects against *S. pombe* at micromolar (µM) level concentrations.

### Identification of HC Targets Through the Genome-wide Screening of *S. pombe* Heterozygous Deletion Mutants

To identify the mode of action of HC, a set of 4,158 heterozygous diploid deletion strains covering 83% of the *S.*

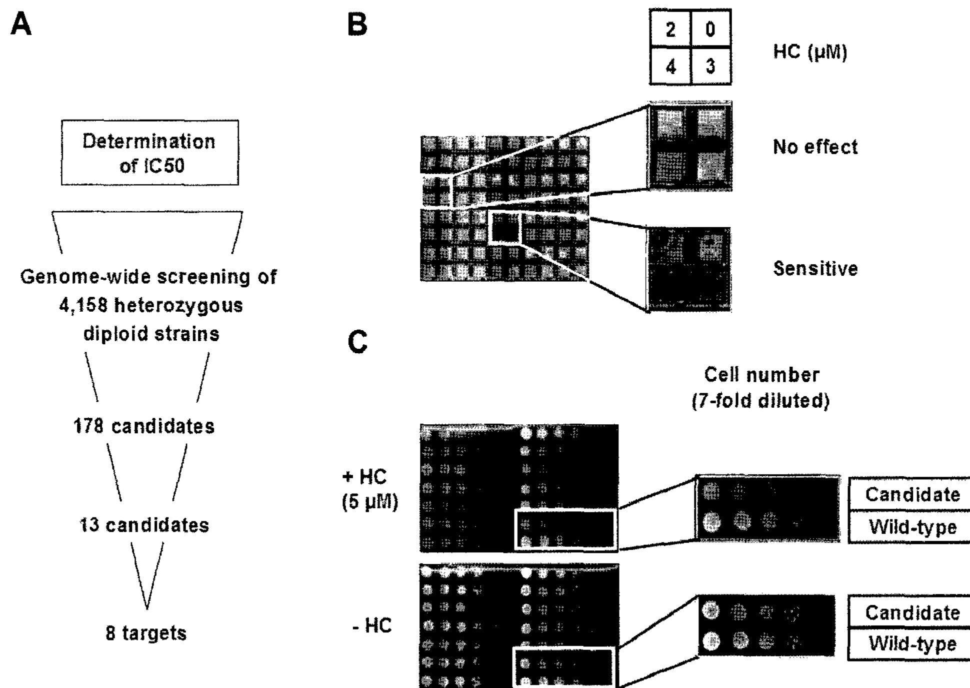


**Fig. 1.** Strategy of genome-wide screening of HC targets.
**A.** Schematic diagram of the whole experiment. **B.** Genome-wide first-screening in 384-well plates. **C.** Second screening on plates. To identify targets of HC, a genome-wide screening of 4,158 heterozygous mutants was initially done using robotics under the principle of drug-induced haploinsufficiency. Through the first screening in 384-well plates using robotics, 178 target candidates were selected and tested for the next screening by assessing their sensitivity on agar plates containing 5 μM HC. Through the second screening, 13 target strains were selected. Finally, 8 targets were identified as specific targets through filtering out nonspecific targets.

*pombe* genome were put into a genome-wide screening as shown in Fig. 1A. Cells were treated with 2, 3, and 4 μM of HC and their growth inhibition was compared with no-drug controls. Although cells were generally treated at lower concentrations than $IC_{50}$ in a genome-wide screening, these cells were treated at a higher concentration to detect tiny growth differences and prevent premature growth saturation due to the 36-h culture time period as opposed to the normal 24-h culture in the determination of $IC_{50}$. As shown in Fig. 1B, 178 candidate strains showing sensitivity to HC were selected and put into the second screening. The 178 strains were then robotically arrayed onto agar plates containing 5 μM HC and their growth inhibition was compared with that of wild-type cells. Among the 178 strains, 13 candidates showed slight sensitivity to HC compared with the wild-type controls, eliminating 165 false positives (Fig. 1C).

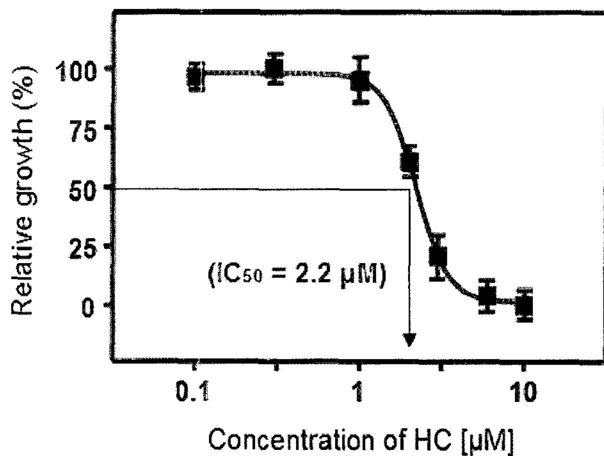


**Fig. 2.** Determination of $IC_{50}$ of HC.
Exponentially growing SP286 wild-type cells were treated with serially 10-fold diluted HC, or 0.01% DMSO as a vehicle control. After an additional 24-h incubation, the growth rate was estimated by measuring $OD_{620}$. It was worthy of note that treatment of SP286 wild-type cells with 10 μM HC caused a 60% decrease in growth rate of SP286 cells. The $IC_{50}$ of HC was calculated from the analysis of the relative growth rate using GraphPad Prism 2.00. The $IC_{50}$ of HC is 2.20±0.07 μM ($p<0.05$). The experiment was performed at least three times ($n \geq 5$).

### Identification of Eight HC Target Genes from Thirteen Candidates

As shown in Table 1, the 13 candidate genes were catagorized into four groups: activation factors for RNA polymerase II transcription, septum formation, unclassified, and condition-induced haploinsufficient genes. However, the fourth class, referred to as condition-induced haploinsufficient genes, is likely to be a nonspecific false positive according to our data (data not shown). The definition of condition-induced haploinsufficient genes is that they are required for normal growth in response to any growth-retardation

**Table 1.** List of 13 candidate and 8 target genes of HC.

| No. | Gene name | Essentiality[a] | Description[b] | Gene ontology[a] | Human ortholog[c] |
|---|---|---|---|---|---|
| 1 | *taf4* | E | Transcription factor TFIID complex subunit | Initiation from RNA pol II promoter | TAF4 |
| 2 | *mcs2* | E | Cyclin, holo TFIIH complex | Regulation of cell cycle | CCNH |
| 3 | *spn3* | V | Septin | Cell separation during cytokinesis | SEPT7 |
| 4 | SPBC19C2.10 | V | BAR adaptor protein | Barrier septum | SH3GL1 |
| 5 | *dic1* | V | Dynein intermediate chain | Horsetail nuclear movement | DYNCC1I2 |
| 6 | SPAC1006.02 | E | WD repeat protein | Disease related, DiGeorge syndrome | GNB1L |
| 7 | *alg9* | V | Mannosyltransferase complex subunit | Protein amino acid N-linked glycosylation | ALG9 |
| 8 | *erg10* | E | Acetyl-CoA C-acetyltransferase | Ergosterol biosynthetic process | MRPL3 |
| 9 | SPAC25B8.17 | V | Peptidase family A22 | D-Alanyl-D-alanine endopeptidase activity | HM13 |
| 10 | *mrpl9* | E | Mitochondrial ribosomal protein subunit L9, peptidyltransferase activity | Ribosomal large subunit assembly and maintenance | RPS4X |
| 11 | *rpl3202* | V | 60S ribosomal protein L32 | Cytosolic large ribosomal subunit | RPL32 |
| 12 | *rpa2* | E | DNA-directed RNA polymerase I complex subunit | Transcription from RNA polymerase I promoter | ACAT1 |
| 13 | *rps401* | V | 40S ribosomal protein S4 | Cytosolic small ribosomal subunit | POLR1B |

[a]Data of essentiality are from the *S. pombe* GeneDB (http://www.genedb.org/genedb/pombe/index.jsp) and tetrad analysis in this study. "E" and "V" represent essential and viable genes, respectively.
[b]Description and gene ontology are from the *S. pombe* GeneDB.
[c]Human orthologs are from the KEGG site (http://www.genome.jp/kegg/).

condition compared with rich YES media, such as minimal EMM2 media or growth-inhibitory drug treatment. Therefore, deletion of any genes in the condition-induced haploinsufficient group causes growth retardation in response to a drug treatment, which makes them look like targets. In addition to the condition-induced haploinsufficient genes, intrinsic haploinsufficient genes, which are required for normal growth without any stimulus, can be represented as nonspecific false positives, as in the case of *S. cerevisiae* [7]. Finally, filtering out the fourth class, condition-induced haploinsufficient genes, gave eight specific target genes of HC.

The first class, activation factors for RNA polymerase II transcription, consists of *taf4* and *mcs2*, which are essential genes in *S. pombe*, as shown in Table 1. In general, specific drug targets are essential for key regulatory genes. The Taf4 protein is one of the evolutionary conserved TBP-associated factors (TAFs) consisting of 13–14 proteins and comprises the RNA polymerase II general transcription factor TFIID with TATA-binding proteins (TBP) [17].

This result raised the possibility of a linkage between the diverse biological action of HC and the role of Taf4 in general transcription. This idea is supported by the observation that inactivation of Taf4 altered fibroblast cell morphology and led to serum-independent autocrine growth accompanied by a positive and negative deregulation of more than 1,000 genes [19]. Among the affected genes are several secreted growth factors including tumor growth factor-β1, -β3, and connective tissue growth factor. In addition to the general role in transcription, Taf4 is also a component of transcription-related complexes, such as the histone acetyltransferase complexes in *Saccharomyces cerevisiae*, humans, and the Polycomb group complex in *Drosophila* [10, 21, 24, 28]. It is well known that curcumin works as a specific inhibitor of acetyltransferase and affects histone acetyltransferase-dependent chromatin transcription [4]. Therefore, the possible influences of HC on Taf4 is through its inhibitory activity of acetyltransferase. The other protein, Mcs2, homologous to metazoan cyclin H, influences transcription through its TFIIH-associated kinase function when complexed with Mcs6 and Pmh1 [15]. However, the molecular mechanism of HC, especially its possible connection to transcription through Taf4 and/or Mcs2, is yet to be elucidated.

The second class is related to septum formation and consists of Spn3 and BAR adaptor proteins. Both genes are dispensible in vegetative growth, perhaps due to gene redundancy. Treatment of *S. pombe* wild-type cells with various concentrations of HC showed abnormal septum formation as judged by calcofluor staining (Fig. 3), which would be a cause of growth retardation. These results are consistent with the previous reports saying that Spn4, a member of the septin classes, is important for the progression of cytokinesis [1]. The other member of the class is BAR,

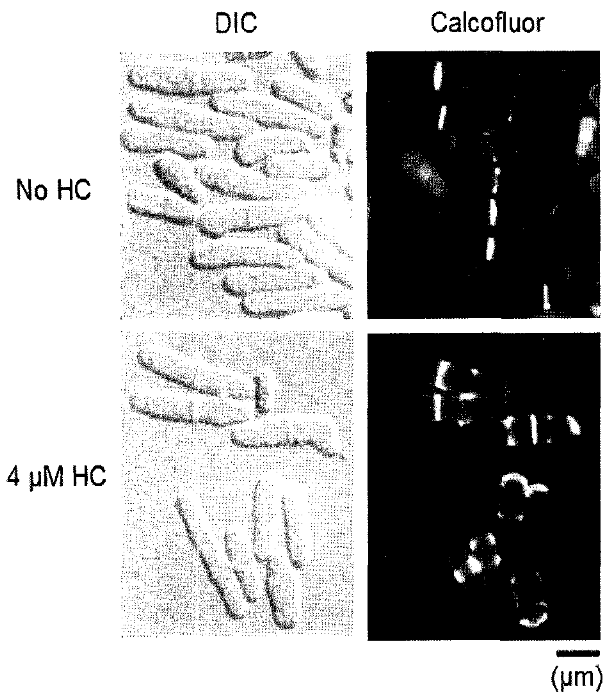


**Fig. 3.** HC-induced abnormality of septum morphology in *S. pombe* cells.

Wild-type cells were treated with 4 μM of HC or 0.01% DMSO. After a 6-h incubation, cells were stained with calcofluor. The scale bar represents 10 μm.

an adaptor protein that reportedly localizes to the barrier septum [18]. Taken together, these results suggest a possible inhibitory role of HC in septation. The third class is unclassified, and consists of two genes related to meiotic chromosome rearrangement and another two genes related to transferase activity. However, speculation of a relationship of HC to these genes will remain unknown until the exact mechanism of the genes is clearly elucidated.

### Confirmation of HC Target Candidates Using Separate Liquid Culture Assay

Among the 13 candidates, targets from different classes were selected and their growth inhibition in response to various concentrations of HC was examined (Fig. 4). Wild-type cells showed a mild growth inhibition in a dose-dependent manner. Treatment with 4 μM of HC showed 80% growth of no-drug controls. However, three strains, *taf4Δ/taf4*$^+$, *rpl3202Δ/rpl3202*$^+$, and *spn3Δ/spn3*$^+$, showed 65%, 67%, and 86% growth of no-drug controls at 3 μM of HC, respectively. The growth inhibition of the *taf4Δ/taf4*$^+$ strain was more sensitive than the others in the specific eight targets.

In conclusion, this technique can be a valuable tool for the study of systematic and genome-wide characterization of a drug's mode of action in combination with the cutting-

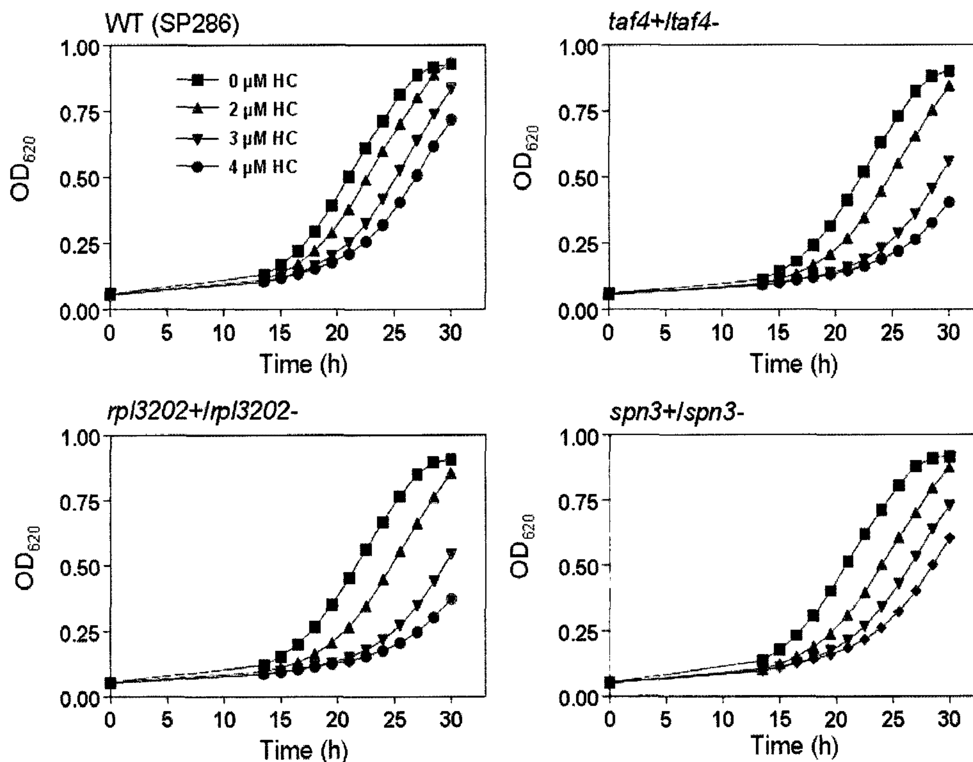


**Fig. 4.** Confirmation of HC target mutants by individual growth analysis.

Actively growing cells, wild-type strain, *taf4Δ/taf4*$^+$, *rpl3202Δ/rpl3202*$^+$, and *spn3Δ/spn3*$^+$, were diluted into $OD_{620}$=0.01 in YE media containing 0.01% DMSO as a vehicle control, and treated with the indicated concentration of HC. Their growth rate was measured at the indicated time points from 13 h to 30 h and the degrees of HC-induced growth inhibion in the mutants were compared with that of wild-type cells. The experiment was performed in triplicate (n≥3) and data of growth curves represent the average of three experiments.

edge oligonucleotide array method. Moreover, it requires no prior knowledge of the drug's mode of action.

## Acknowledgments

This work was supported by the intramural Mission 2007 research program of KRIBB and by the 21st Century Frontier R&D Program and Chemical Genomics Research Program from the Ministry of Science and Technology of Korea.

### References

1. An, H., J. L. Morrell, J. L. Jennings, A. J. Link, and K. L. Gould. 2004. Requirements of fission yeast septins for complex formation, localization, and function. *Mol. Biol. Cell* **15:** 5551–5564.
2. Arbiser, J. L., N. Klauber, R. Rohan, R. van Leeuwen, M. T. Huang, C. Fisher, E. Flynn, and H. R. Byers. 1998. Curcumin is an *in vivo* inhibitor of angiogenesis. *Molec. Med. (Cambridge, Mass.)* **4:** 376–383.
3. Bahler, J., J. Q. Wu, M. S. Longtine, N. G. Shah, A. McKenzie 3rd, A. B. Steever, A. Wach, P. Philippsen, and J. R. Pringle. 1998. Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* **14:** 943–951.
4. Balasubramanyam, K., R. A. Varier, M. Altaf, V. Swaminathan, N. B. Siddappa, U. Ranga, and T. K. Kundu. 2004. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J. Biol. Chem.* **279:** 51163–51171.
5. Choi, I.-K., H. J. Shin, H.-S. Lee, and H. J. Kwon. 2007. Streptochlorin, a marine natural product, inhibits NF-κB activation and suppresses angiogenesis *in vitro*. *J. Microbiol. Biotechnol.* **17:** 1338–1343.
6. Cho, J. Y., G. J. Choi, S. W. Lee, K. S. Jang, H. K. Lim, C. H. Lim, S. O. Lee, K. Y. Cho, and J. C. Kim. 2006. Antifungal activity against *Colletotrichum* spp. of curcuminoids isolated from *Curcuma longa L.* rhizomes. *J. Microbiol. Biotechnol.* **16:** 280–285.
7. Deutschbauer, A. M., D. F. Jaramillo, M. Proctor, J. Kumm, M. E. Hillenmeyer, R. W. Davis, C. Nislow, and G. Giaever. 2005. Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. *Genetics* **169:** 1915–1925.
8. Giaever, G., D. D. Shoemaker, T. W. Jones, H. Liang, E. A. Winzeler, A. Astromoff, and R. W. Davis. 1999. Genomic profiling of drug sensitivities *via* induced haploinsufficiency. *Nat. Genet.* **21:** 278–283.
9. Giaever, G., P. Flaherty, J. Kumm, M. Proctor, C. Nislow, D. F. Jaramillo, A. M. Chu, M. I. Jordan, A. P. Arkin, and R. W. Davis. 2004. Chemogenomic profiling: Identifying the functional interactions of small molecules in yeast. *Proc. Natl. Acad. Sci. USA* **101:** 793–798.
10. Grant, P. A., D. Schieltz, M. G. Pray-Grant, D. J. Steger, J. C. Reese, J. R. Yates 3rd, and J. L. Workman. 1998. A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. *Cell* **94:** 45–53.
11. Hughes, T. R. 2002. Yeast and drug discovery. *Funct. Integr. Genomics* **2:** 199–211.
12. Jung, H. J. and H. J. Kwon. 2006. Chemical genomics with natural products. *J. Microbiol. Biotechnol.* **16:** 651–659.
13. Kim, S., M. Won, N. Sun, J. Jang, S. Lee, H. Shin, and K. B. Song. 2006. Two-dimensional reference map of *Schizosaccharomyces pombe* proteins (update). *J. Microbiol. Biotechnol.* **16:** 1499–1512.
14. Lee, C. H., H. J. Lee, J. H. Jeon, and H. S. Lee. 2005. *In vivo* antifungal effects of *Coptis japonica* root-derived isoquinoline alkaloids against phytopathogenic fungi. *J. Microbiol. Biotechnol.* **15:** 1402–1407.
15. Lee, K. M., I. Miklos, H. Du, S. Watt, Z. Szilagyi, J. E. Saiz, R. Madabhushi, C. J. Penkett, M. Sipiczki, *et al.* 2005. Impairment of the TFIIH-associated CDK-activating kinase selectively affects cell cycle-regulated gene expression in fission yeast. *Mol. Biol. Cell* **16:** 2734–2745.
16. Lum, P. Y., C. D. Armour, S. B. Stepaniants, G. Cavet, M. K. Wolf, J. S. Butler, J. C. Hinshaw, P. Garnier, G. D. Prestwich, *et al.* 2004. Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. *Cell* **116:** 121–137.
17. Matangkasombut, O., R. Auty, and S. Buratowski. 2004. Structure and function of the TFIID complex. *Adv. Protein Chem.* **67:** 67–92.
18. Matsuyama, A., R. Arai, Y. Yashiroda, A. Shirai, A. Kamata, S. Sekido, Y. Kobayashi, A. Hashimoto, M. Hamamoto, Y. Hiraoka, S. Horinouchi, and M. Yoshida. 2006. ORFeome cloning and global analysis of protein localization in the fission yeast *Schizosaccharomyces pombe*. *Nat. Biotechnol.* **24:** 841–847.
19. Mengus, G., A. Fadloun, D. Kobi, C. Thibault, L. Perletti, I. Michel, and I. Davidson. 2005. TAF4 inactivation in embryonic fibroblasts activates TGF beta signalling and autocrine growth. *EMBO J.* **24:** 2753–2767.
20. Moreno, S., A. Klar, and P. Nurse. 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* **194:** 795–823.
21. Ogryzko, V. V., T. Kotani, X. Zhang, R. L. Schiltz, T. Howard, X. J. Yang, B. H. Howard, J. Qin, and Y. Nakatani. 1998. Histone-like TAFs within the PCAF histone acetylase complex. *Cell* **94:** 35–44.
22. Park, J.-Y., Y.-J. Jang, S.-J. You, Y.-S. Kil, E.-J. Kang, J.-H. Ahn, Y.-K. Ryoo, M.-Y. Lee, S.-Y. Park, *et al.* 2003. Drug-induced haploinsufficiency of fission yeast provides a powerful tool for identification of drug targets. *J. Microbiol. Biotechnol.* **13:** 317–320.
23. Parsons, A. B., R. Geyer, T. R. Hughes, and C. Boone. 2003. Yeast genomics and proteomics in drug discovery and target validation. *Prog. Cell Cycle Res.* **5:** 159–166.
24. Saurin, A. J., Z. Shao, H. Erdjument-Bromage, P. Tempst, and R. E. Kingston. 2001. A *Drosophila* Polycomb group complex includes Zeste and dTAFII proteins. *Nature* **412:** 655–660.

25. Sharma, R. A., A. J. Gescher, and W. P. Steward. 2005. Curcumin: The story so far. *Eur. J. Cancer* **41:** 1955–1968.
26. Shim, J. S., D. H. Kim, H. J. Jung, J. H. Kim, D. Lim, S. K. Lee, K. W. Kim, J. W. Ahn, J. S. Yoo, J. R. Rho, J. Shin, and H. J. Kwon. 2002. Hydrazinocurcumin, a novel synthetic curcumin derivative, is a potent inhibitor of endothelial cell proliferation. *Bioorg. Med. Chem.* **10:** 2987–2992.
27. Tang, Z., L. L. Mandel, S. L. Yean, C. X. Lin, T. Chen, M. Yanagida, and R. J. Lin. 2003. The kic1 kinase of *Schizosaccharomyces pombe* is a CLK/STY orthologue that regulates cell-cell separation. *Exp. Cell Res.* **283:** 101–115.
28. Wieczorek, E., M. Brand, X. Jacq, and L. Tora. 1998. Function of TAF(II)-containing complex without TBP in transcription by RNA polymerase II. *Nature* **393:** 187–191.
29. Wood, V., R. Gwilliam, M. A. Rajandream, M. Lyne, R. Lyne, A. Stewart, *et al.* 2002. The genome sequence of *Schizosaccharomyces pombe*. *Nature* **415:** 871–880.