

## Organotin Compounds Act as Inhibitor of Transcriptional Activation with Human Estrogen Receptor

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In aquatic invertebrates, particularly marine gastropods, organotin compounds induce irreversible sexual abnormality in females, which is termed imposex, at very low concentrations. Organotin compounds are agonists for nuclear receptors such as RXRs and PPAR $\gamma$ . However, the imposex phenomenon has not been reported to act as an antagonist on estrogen receptors in other species, including vertebrates and invertebrates. In order to gain insights into the antagonistic activity of organotin compounds on estrogen receptors (ERs), we examined the inhibitive effect of these compounds on estradiol-dependent  $\beta$ -galactosidase activity using the yeast two-hybrid detection system consisting of a combination of the human estrogen receptor (hER $\beta$ ) ligand-binding domain and the co-activator steroid receptor co-activator-1 (SRC1). Tributyltin-hydroxide (TBT-OH) and triphenyltin-chlorine (TPT-Cl) exhibited an inhibitive effect on E<sub>2</sub>-dependent transcriptional activity, similar to antagonistic chemicals such as 4-hydroxytamoxifen (OHT) or ICI 182,780, at a very low concentration of 10<sup>-14</sup> M TBT or 10<sup>-10</sup> M TPT, respectively. The yeast growth and transcriptional activity with transcriptional factor GAL4 did not exhibit any effect at the tested concentration of TBT or TPT. Moreover, the yeast two-hybrid system using the interaction between p53 and the T antigen of SV40 large did not describe any effect at the tested concentration of OHT or ICI 182,780. However, the interaction between p53 and T antigen was inhibited at a TBT or TPT concentration of 10<sup>-9</sup> M, respectively. These results indicate that TBT and TPT act as inhibitors of ER-dependent reporter gene transcriptional activation and of the interaction

between hER $\beta$  LBD and the co-activator SRC1 in the yeast two-hybrid system. Consequently, our data could partly explain the occurrence of organotin compound-induced imposex on the endocrine system of mammals, including humans.

**Keywords:** Endocrine disruptors, imposex, TBT, TPT, hER, yeast two-hybrid system

Endocrine disruptors (EDs) can cause adverse effects in organisms or their progeny by disrupting the endocrine system. The key targets of EDs are nuclear steroid receptors, which play a role as either agonists or antagonists of endogenous steroids, and are attracting growing interest. A vast number of chemicals are suspected to be EDs, including natural and synthetic hormones excreted by humans, industrial chemicals, pesticides, and their degradation products [7].

Organotin compounds such as tributyltin (TBT) and triphenyltin (TPT) have been widely used as agricultural pesticides, antifouling paints for ship hulls and fishery firm nets, and stabilizers to lessen the effects of heat and light in polyvinyl-chloride plastics. These compounds contaminate human food and water, mainly through contributions from industrial effluents and from leaching of PVC water pipes [1, 8, 30]. When organotin compounds are released into an aquatic environment, they accumulate easily in various marine organisms because of their lipophilic property [11].

Recently, several reports have described that many marine organisms exposed to TBT or TPT exhibit sex transformation. In gastropod mollusks, one of the most sensitive species, organotin compounds induce the superimposition of male sex organs such as a penis and/or vas deferens on females. This phenomenon has been called either pseudohermaphroditism

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or imposex [2, 12, 23]. Organotin-induced imposex is mediated by an increasing androgen level relative to the estrogen level. Many studies have reported organotin compound-induced masculinization in the following marine organism gastropods: (i) TBT inhibits the activity of cytochrome P450 aromatase, which converts androgen to estrogen, thereby leading to an increase in the level of unconverted androgens in the dog-whelk (*Nucella lapillus*) [3, 28, 29, 31], (ii) the possibility for imposex by organotin compounds in gastropods was suggested to be the inhibition of androgen excretion due to a decrease in the sulfur conjugation of androgen being the major cause of TBT-induced accumulation of androgens in gastropod tissues in *Littorina littorea* [27]; (iii) it has recently been reported that TBT and TPT have the ability to androgen-dependent transcription to activate in mammalian cells [33]; (iv) disturbance of the release of penis morphogenetic/retrogressive factor from pedal/cerebropleural ganglia by TBT in the sting winkle (*Ocenebra erinacea*) [9]; (v) activation of retinoid X receptor (RXR) by TBT-induced penis morphogenesis and promoted the development in the rock shell (*T. clavigera*) [26]. Although many studies have reported organotin compound-induced masculinization of females in a variety of marine organisms, the detailed biochemical mechanism of this phenomenon has not been fully understood [13]. Moreover, the organotin compounds mechanism on the affect of estrogen receptor (ER) relative to feminization has not been reported.

We previously constructed a detection system for EDs consisting of a yeast two-hybrid system with a combination of hER $\beta$  ligand-binding domain (LBD) and co-activator SRC1 [19]. The purpose of this study was to clarify the mechanism of the inhibition of feminization on females by organotin compounds. We attempted to investigate whether or not organotin compounds have a directly inhibitive effect on ER activity in the yeast two-hybrid detection system. The results indicated that TBT and TPT compounds show a potential antagonistic effect on hER by competitive binding to E<sub>2</sub>. Furthermore, we found that TBT and TPT compounds exhibit an inhibitive effect on the interaction between the hER $\beta$  LBD and co-activator SRC1. This study demonstrates that the imposex phenomenon by organotin compounds represents indirect evidence for the inhibitive effect of E<sub>2</sub>-induced transcriptional activation on the target gene by the antagonistic function on hER and the interaction between protein–protein in the yeast two-hybrid system.

## MATERIALS AND METHODS

### Chemicals

17 $\beta$ -Estradiol (E<sub>2</sub>) and 4-hydroxytamoxifen (OHT) were purchased from Sigma Chemical Co, tributyltin-hydroxide (TBT-OH) from Aldrich Chemicals, and triphenyltin-chloride (TPT-Cl) from Kanto

Chemical (Tokyo, Japan). ICI 182,780 was kindly supplied by Dr. Kato (Institute of Molecular and Cellular Biosciences, University of Tokyo). All chemicals used were of reagent grade, and used without further purification.

### Plasmids and Yeast Strain

The pGAL4 DBD-hER $\beta$  LBD fusion plasmid was constructed by inserting hER $\beta$  LBD into the pGBT9 vector (included a Gal4p DNA binding domain; Clontech). The co-activator SRC1 cDNA was inserted into pGAD10 (Clontech), which included a Gal4p transactivation domain (GAL4 TAD), to construct pGAL4 TAD-SRC1 [19]. pGBKT7-53, pGADT7-T, and pCL1 were purchased from Clontech as control vectors of a two-hybrid system. pGBKT7-53 is a positive control plasmid that encodes a fusion murine p53 (a.a. 72–390) and the GAL4 DBD (a.a. 1–147). pGADT7-T is a product of recombination that encodes fusion of the SV40 large T antigen (a.a. 84–708) and the GAL4 TAD (a.a. 768–881). pCL1 is a positive control plasmid that encodes the full-length wild-type Gal4 protein. pCL1 was constructed by inserting the *Bam*HI fragment of pMA210, which contains the P<sub>ADHI</sub>-GAL4 gene (a.a. 1–881), into an Ycp50 derivative in which the *LEU2* gene is replaced with the *URA3* gene. These vectors were transformed into *Saccharomyces cerevisiae* YRG-2 (*MATa ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3, 112, gal4-542, GAL80-538, LYS::UAS<sub>GALI</sub>-TATA<sub>GALI</sub>-HIS3, URA3::UAS<sub>GALA 17mers(X3)</sub>-TATA<sub>CYC1</sub>-lacZ*; purchased from Stratagene) by the electroporation method and were selected by growth on SD medium lacking leucine and/or tryptophan.

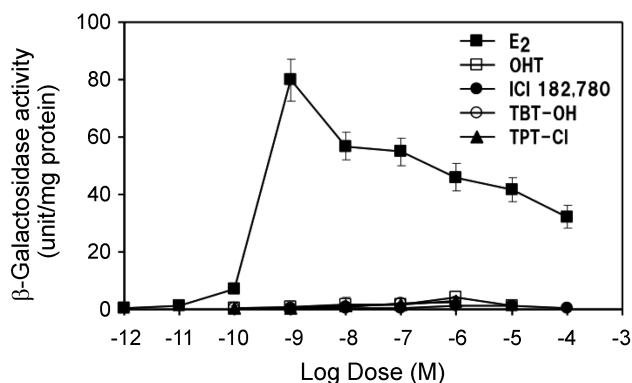
### Growth of Yeast and $\beta$ -Galactosidase Assay

Yeast transformants were grown overnight at 30°C with vigorous orbital shaking in 2 ml of selective medium (SD medium). An aliquot (200  $\mu$ l) of the overnight culture was diluted into 9.8 ml of fresh SD medium containing E<sub>2</sub> or tested compounds, which were added to a DMSO solution. The concentration of DMSO did not exceed 1% of the culture volume. Yeasts were cultured for 11 h at 30°C. After incubation, the yeast cells were collected by centrifugation at 3,500 rpm for 5 min. The  $\beta$ -galactosidase activity was determined by the method of Lee *et al.* [19]. The data are representative of three independent experiments, and respective experiments were triplicated.

## RESULTS

### Ligand-Dependent Activation on the Interaction Between hER $\beta$ LBD and Co-Activator SRC1 in Yeast

We previously demonstrated a yeast two-hybrid system using a combination of the hER $\beta$  LBD and co-activator SRC1 as a sensitive, specific, and reproducible indicator for detecting estrogenic chemicals. Fig. 1 shows the dose-dependent increase in the  $\beta$ -galactosidase activity of yeast treated with the tested chemicals. The combination of hER $\beta$  LBD and SRC1 induced the maximum  $\beta$ -galactosidase activity at 10<sup>-9</sup> M E<sub>2</sub>. Antagonist chemicals such as OHT and ICI 182,780 inhibiting ER activity did not detect the estrogenic activity with this yeast detection system. The additional tested chemicals, TBT-OH and TPT-Cl, showed

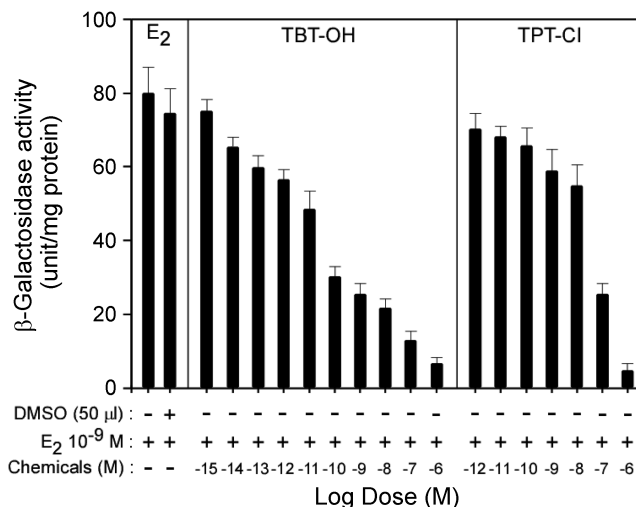


**Fig. 1.** Dose-response curve of the estrogenic activity of steroid hormone ( $E_2$ ), antagonists OHT and ICI 182,780, and two organotin compounds (TBT-OH and TPT-CI) in the yeast two-hybrid system with interaction between hER $\beta$  LBD and SRC1. *S. cerevisiae* strain YRG-2, along with plasmids pGBT9-hER $\beta$  LBD and pGAD10-SRC1, was incubated in the presence of increasing concentrations of the tested compounds. The activity of  $\beta$ -galactosidase was measured after 11 h, calculated in Miller units, as described in the Materials and Methods [15]. Each value is the mean of three independent experiments with three replicates.

a strong effect on the development of imposex in the gastropod but did not induce the expression of a reporter gene in this assay (Fig. 1). Organotin compounds have the ability to induce androgen-dependent transcriptional activation and cell proliferation in human prostate cancer cells [33]. Moreover, organotin compounds indicate the function as a retinoid “X” receptor (RXR) ligand with novel structures that bind to the LBD of RXR with high affinity and stimulate transcription of the receptor [25, 26]. However, we have previously demonstrated that organotin compounds do not bind to hER $\beta$  because the ER-dependent reporter gene transcriptional activation was not induced in the yeast detection system.

### Organotin Compounds Exhibit Antagonistic Activity on hER $\beta$ in Yeast

Some chemicals have been suggested as possible disruptors of the endocrine system through inhibition of estrogen-induced transcriptional activation as antagonists, rather than direct activation of the target genes. We previously confirmed the detection ability of antiestrogenic activities with the medicines OHT and ICI 182,789, which are known as typical antagonists, as determined with the yeast two-hybrid detection system [18]. In the present study, we estimated the antagonistic effect of organotin compounds on inducing sex transformation in the female genital system, with the yeast detection system incubated with  $E_2$  (50  $\mu$ l at a concentration of  $10^{-9}$  M) and organotin chemicals TBT-OH or TPT-OH (50  $\mu$ l at a concentration of  $10^{-15}$  M  $\sim$   $10^{-6}$  M or  $10^{-12}$  M  $\sim$   $10^{-6}$  M, respectively), according to the method of Lee *et al.* [17]. As shown in Fig. 2,  $\beta$ -galactosidase activity decreased from a low concentration



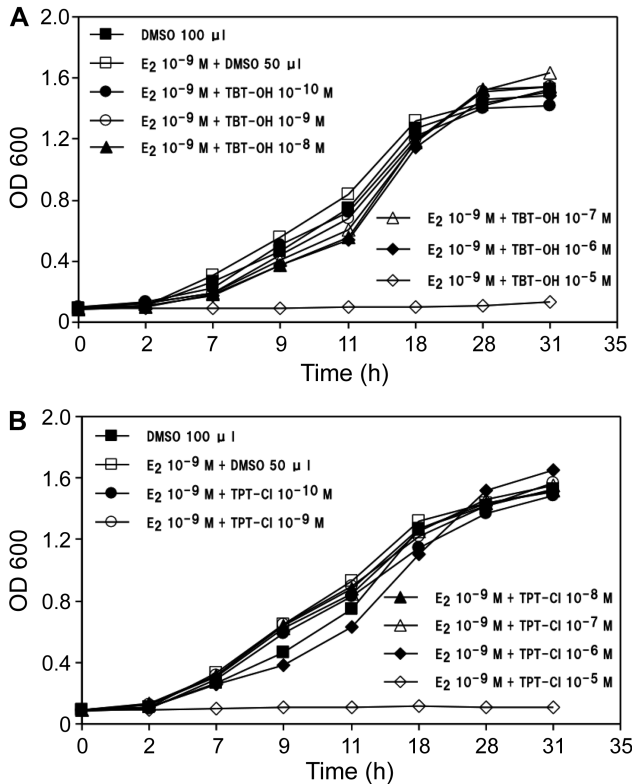
**Fig. 2.** Antiestrogenic activities of organotin compounds (TBT-OH and TPT-CI) determined with the yeast two-hybrid system with interaction between hER $\beta$  LBD and SRC1.

The yeast strain was incubated with DMSO or various concentrations of each chemical in the presence of  $10^{-9}$  M  $E_2$ . The activity of  $\beta$ -galactosidase was measured after 11 h, calculated in Miller units, as described in the Materials and Methods [18]. Each value is the mean of three independent experiments with three replicates.

of  $10^{-14}$  M TBT-OH or  $10^{-10}$  M TPT-CI, respectively, and was certainly reduced according to the increasing concentration of the tested compounds. TBT-OH remarkably decreased  $E_2$ -dependent transcriptional activation at concentrations ranging from  $10^{-13}$  M to  $10^{-6}$  M. Moreover, TBT-OH at a concentration of  $10^{-6}$  M significantly inhibited the binding of  $E_2$  to the estrogen-binding site of hER $\beta$ , and exhibited the same effect at  $10^{-5}$  M OHT [18] as an antagonistic chemical. On the other hand, TPT-CI repressed the reporter activity in a dose-dependent manner at concentrations ranging from  $10^{-9}$  M to  $10^{-6}$  M. In addition,  $E_2$ -induced  $\beta$ -galactosidase activity was significantly inhibited at a concentration of  $10^{-6}$  M TBT-OH. The TPT-CI data corresponded well to the concentration range (0.6  $\sim$  6 nM) that has been reported to induce imposex in female gastropods [4, 10, 12], being 1 nM  $\sim$  1 mM. However, TBT-OH induced imposex at a lower range (0.1 pM  $\sim$  1 mM) than the reported concentration range. These data suggest that organotin compounds such as TBT-OH or TPT-CI exhibit a strong antagonistic effect by competitive binding of  $E_2$  to the estrogen-binding site of hER $\beta$ .

### Influence on Yeast Growth by Organotin Compounds

TBT and TPT are well known to be toxic to several cells or cause apoptosis [5, 22, 32]. We attempted to investigate whether or not reporter gene transactivation decreases because the yeast growth was inhibited by organotin compounds. The results are shown in Fig. 3. The yeast transformant expressing hER $\beta$  LBD and SRC1 was grown for 31 h with  $E_2$  (50 ml was added at a concentration of

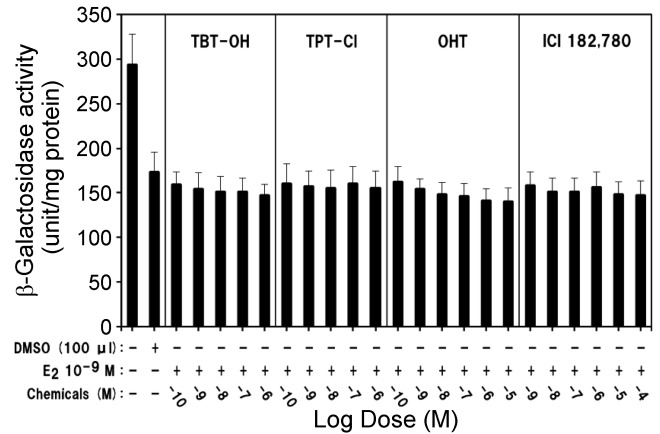


**Fig. 3.** Investigation of the effect on growth of yeast cells expressing hER $\beta$  LBD and SRC1 fusion proteins by organotin compounds TBT-OH (A) and TPT-Cl (B), respectively. The yeast strain was incubated for 31 h at 37°C with DMSO or various concentrations of each chemical in the presence of  $10^{-9}$  M E $_2$ , and then the cell growth (OD 600) was determined by a spectrophotometer. Each value is the mean of three independent experiments with three replicates.

$10^{-9}$  M) and organotin chemicals TBT-Cl or TPT-OH (50  $\mu$ l at a concentration of  $10^{-10}$  M  $\sim$   $10^{-5}$  M). The yeast growth was completely inhibited in the presence of  $10^{-5}$  M TBT-OH or TPT-Cl. However, the yeast growth did not appear to be affected at a concentration lower than  $10^{-6}$  M of the tested compounds TBT-OH (Fig. 3A) or TPT-Cl (Fig. 3B), and was only very weakly inhibited at  $10^{-6}$  M TBT-OH or TPT-Cl. Moreover, the yeast growth did not indicate any effect in comparison with the control (DMSO 100  $\mu$ l or  $10^{-9}$  M E $_2$  and DMSO 50  $\mu$ l) at a concentration above  $10^{-6}$  M TBT-OH or TPT-Cl. Recently, a similar result has been reported where *S. cerevisiae* growth was inhibited by TBT exposure under different concentrations of TBT from 15  $\mu$ M to 60  $\mu$ M [6]. These results indicated that the transactivation of the reporter gene did not decrease because of the yeast growth inhibition by organotin compounds.

#### Influence upon Transcriptional Factor Gal4 by Organotin Compounds in Yeast

The Gal4 protein induced transcriptional activity with interaction between Gal4 protein and basal transcriptional



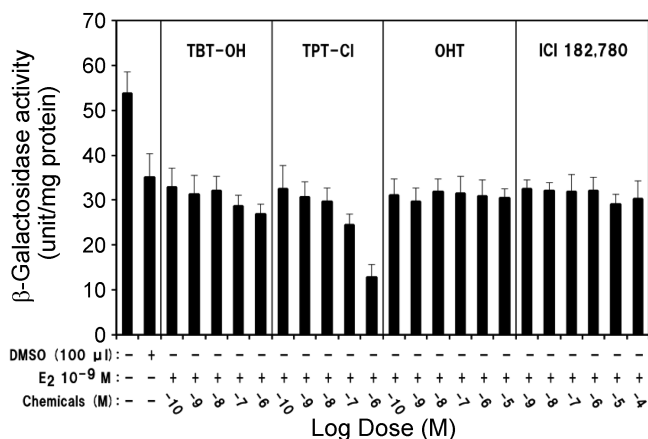
**Fig. 4.** Investigation of the influence of various chemicals on transcriptional activation in the cells expressing wild type Gal4 protein.

pCL1 is a positive control plasmid that encodes the full-length, wild-type Gal4 protein. Vector pCL1 was transformed into *S. cerevisiae* YRG-2 by the electroporation method and selected by the growth on SD medium lacking leucine.

machinery, binding in the GAL4 binding site (GAL4 upstream activating sequence; UAS<sub>GAL4</sub>) on the yeast chromosome. To define whether or not the transcriptional factor GAL4 influenced the transcriptional activity by organotin compounds, we incubated the yeast transformant for 11 h in the presence of  $10^{-9}$  M E $_2$ , and the individual TBT-OH, TPT-Cl, OHT, or ICI 182,780, at the concentrations shown in Fig. 4. The wild-type Gal4 proteins were expressed from pCL1 plasmid in *S. cerevisiae* strain YRG-2, which has a reporter construct, UAS<sub>GAL4</sub> 17mers(X3)-TATA<sub>CYC1</sub>-lacZ, on its chromosome. The  $\beta$ -galactosidase activity did not show any effect according to the increasing concentration of the tested compounds in a dose-dependent manner. However, the transactivation of Gal4 protein by these four chemicals indicated a small decrease in comparison with the control, which is the addition of 100  $\mu$ l of DMSO (Fig. 4). These results suggest that organotin compounds did not exert any effect on the transcriptional activity with transcriptional factor GAL4 in yeast.

#### Influence on the Interaction Between Protein-Protein by Organotin Compounds in Yeast

To estimate the inhibitive effect on the interaction between hER $\beta$  LBD and co-activator SRC1 by organotin compounds in the yeast, a two-hybrid system, we investigated the inhibitive effect of the interaction by organotin compounds using a p53 tumor suppressor and a large T antigen of the SV40 virus, which are well known as a two-hybrid system with interaction between protein-protein [20, 21]. The genes coding for GAL4 DBD-p53 and GAL4 TAD-large T antigen of SV40 fusion proteins were expressed in the *S. cerevisiae* strain YRG-2, which has a reporter gene *lacZ*



**Fig. 5.** Investigation of the influence of various chemicals on the interaction between p53 and large T antigen of SV40.

Vector pGBKT7-53 and pGADT7-T were transformed into *S. cerevisiae* YRG-2 by the electroporation method and selected by growth on SD medium lacking leucine and tryptophan.

on its chromosome. The yeast transformant expressing p53 and T antigen fusion proteins was incubated for 11 h with  $10^{-9}$  M  $E_2$  and the individual tested compounds at the concentrations shown in Fig. 5. Treatment by OHT and ICI 182,780 did not exert any influence on the interaction between p53 and T antigen in comparison with the control, which was the addition of 100 µl of DMSO (Fig. 5). However, β-galactosidase activity was reduced by the addition of organotin compounds at a concentration from  $10^{-9}$  M to  $10^{-6}$  M, in comparison with control. In addition, β-galactosidase activity was further reduced with increasing TPT-Cl concentration. Moreover, β-galactosidase activity was significantly reduced at a concentration of  $10^{-7}$  M or  $10^{-6}$  M TPT-Cl. These data did not correspond to the results of *Kanayama et al.* [15], who reported that TPT showed a strong influence on protein–protein interaction between peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and co-activator TIF2. Furthermore, TBT and TPT strongly enhanced the protein–protein interaction between human RXRs and co-activator TIF2 [15, 26]. Consequently, organotin compounds indicate that the function is a ligand with novel structures, which bind to the LBD of RXR or PPAR $\gamma$ . However, our data indicate that the interactions between protein and protein were significantly inhibited with the addition of organotin compounds. These data suggest that organotin compounds are agonists for nuclear receptors like RXRs, PPAR $\gamma$ , and AR, but that TBT and TPT show no agonist activity to hER $\beta$ .

## DISCUSSION

In the present study, we found that organotin compounds did not induce ER-dependent, reporter gene transcriptional

activation (Fig. 1). However, they did inhibit  $E_2$ -dependent transcriptional activation through the action of antagonistic chemicals such as OHT or ICI 182,780 at a very low concentration of  $10^{-14}$  M TBT-OH or  $10^{-10}$  M TPT-Cl, respectively (Fig. 2). Moreover, the interaction between protein and protein exhibited an adverse effect on the expression of the reporter gene at the concentration treated by the organotin compounds (Fig. 5). Thus, we suggest that the reporter gene expression was inhibited by the organotin compounds because the organotin compounds inhibited the binding of estradiol to ER and the interaction between ER and co-activator/co-repressors, which regulate the expression of estrogen target genes in cooperation with ER. In the present study, we could not confirm whether or not organotin compounds act as an antagonist on ER. Therefore, using the yeast detection system, we could not determine whether or not it binds to ER or blocks the binding of  $E_2$  to ER. Accordingly, further studies are required to clarify the mechanism of imposex with the following supplementary examinations: the effect on ER $\alpha$ -dependent transcriptional activity; the influence on cell proliferation of MCF-7, such as woman breast cancer cells, which have a remarkable feminization function; and the conformational changes of ER or competition binding on ER by organotin compounds.

Recently, organotin compounds including TBT and TPT were identified as nanomolar agonists for RXR and PPAR $\gamma$ , which are members of the nuclear receptor superfamily [24]. In addition, these compounds also induced the transactivation function of RXR and PPAR $\gamma$  in mammalian culture cells [15]. RXR has been reported to play an important role in the development of gastropod imposex, whereas organotin compounds strongly activated RXR and PPAR $\gamma$ , and are high-affinity ligands for RXR and PPAR $\gamma$  [15, 24]. Moreover, TBT and TPT are high-affinity ligands for RXR, whereas 9-*cis* RA, as a natural ligand of RXR, induced the phenomenon of imposex in female rock shells [12, 24]. Consequently, these results indicate that RXR is involved in the development of imposex caused by organotin compounds in female rock shells.

The imposex phenomenon with organotin compounds has not been reported to act against the inhibition of feminization relate to ERs in other species, including mammals. Our data on the imposex mechanism with the inhibition of feminization in the female gastropods indicated that TBT and TPT are potential inhibitors on  $E_2$ -dependent transcriptional activation and the interaction between protein and protein in yeast cells. However, a recent study investigated whether organotin compounds markedly stimulated human chorionic gonadotropin (hCG) production in its mRNA expression concentration ranges, along with its mRNA expression [25]. These results suggest that organotin compounds are potent stimulators of human placental estrogen biosynthesis and hCG production *in*

*vitro* and that the placenta represents a potential target organ in pregnant women for organotin compounds, the endocrine-disrupting effects of which might be the result of local changes in estrogen and hCG concentrations.

Furthermore, the cDNA encoding mollusk ER was isolated from the mollusk *Thais clavigera*. The identified amino acid sequence of *T. clavigera* ER 1 (tcER1) showed a 89% identity in the DNA-binding domain and a 36% identity in the LBD, in comparison with human ER $\alpha$ . The reporter gene assay revealed that tcER1 is constitutively active and unresponsive to estrogen [14]. Based on these results, we conclude that hER was not involved in the development of imposex caused by organotin compounds in gastropods. However, organotin compounds are suspected to cause ED effects in mammals, including humans. Human exposure to organotin compounds may result from the consumption of organotin-contaminated meat and fish products, and from occupational exposure during the manufacture and formulation of organotin compounds [16, 17]. This possible exposure has therefore aroused great concern about potential toxicities. Consequently, our data are useful in investigating the development of imposex with organotin compounds in mammals, including humans. However, further studies are required to clarify the mechanism of imposex with the following supplementary examinations: the effect on ER $\alpha$ -dependent transcriptional activity; the influence on cell proliferation of MCF-7, such as woman breast cancer cells, which have a remarkable feminization function; and the conformational changes of ER or competition binding on ER by organotin compounds.

In summary, we demonstrated that TBT and TPT are potential inhibitors of E<sub>2</sub>-dependent transcriptional activation and of the interaction between hER $\beta$  LBD and the co-activator SRC1 in yeast. This mechanism may, therefore, partly explain the occurrence of organotin compound-induced imposex on the endocrine system of mammals, including humans.

## REFERENCES

1. Antizar-Ladislao, B. 2008. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. *Environ. Int.* **34**: 292–308.
2. Axiak, V., D. Micallef, J. Muscat, A. Vella, and B. Mintoff. 2003. Imposex as a biomonitoring tool for marine pollution by tributyltin: Some further observations. *Environ. Int.* **28**: 743–749.
3. Bettin, C., J. Oehlmann, and E. Stroben. 1996. TBT-induced imposex in marine neogastropods is mediated by an increasing androgen level. *Helgol. Meeresun.* **50**: 299–317.
4. Bryan, G. W., F. E. Gibbs, L. G. Hummerstone, and G. R. Burt. 1986. The decline of the gastropod *Nucella 321 lapillus* around south-west England: Evidence for the effect of tributyltin from anti-fouling paints. *J. Mar. Biol. Assoc. UK* **66**: 611–640.
5. Cai, J., M. Wang, B. Li, C. Wang, Y. Chen, and Z. Zuo. 2009. Apoptotic and necrotic action mechanisms of trimethyltin in human hepatoma G2 (HepG2) cells. *Chem. Res. Toxicol.* **22**: 1582–1587.
6. Chahomchuen, T., K. Akiyama, T. Sekito, N. Sugimoto, M. Okabe, S. Nishimoto, T. Sugahara, and Y. Kakinuma. 2009. Tributyltin induces Yca1p-dependent cell death of yeast *Saccharomyces cerevisiae*. *J. Toxicol. Sci.* **34**: 541–545.
7. Degen, G. H. and H. M. Bolt. 2000. Endocrine disruptors: Update on xenoestrogens. *Int. Arch. Occup. Environ. Health* **73**: 433–441.
8. Fent, K. 1996. Ecotoxicology of organotin compounds. *Crit. Rev. Toxicol.* **26**: 1–117.
9. Feral, C. and S. Le Gall. 1983. The influence of a pollutant factor (TBT) on the neurosecretory mechanism responsible for the occurrence of a penis in the females of *Ocenebra erinacea*, pp.173–175. In J. Lever and H. H. Boer (eds.). *Molluscan Neuro-Endocrinology*. North Holland Publishing, Amsterdam.
10. Gibbs, F. E. and G. W. Bryan. 1986. Reproductive failure in populations of the dog-whelk, *Nucella lapillus*, caused by imposex induced by tributyltin from antifouling paints. *J. Mar. Biol. Assoc. UK* **66**: 767–777.
11. Horiguchi, T. 2006. Masculinization of female gastropod mollusks induced by organotin compounds, focusing on mechanism of actions of tributyltin and triphenyltin for development of imposex. *Environ. Sci.* **13**: 77–87.
12. Horiguchi, T., H. Shiraiishi, M. Shimizu, and M. Morita. 1997. Effects of triphenyltin chloride and five other organotin compounds on the development of imposex in the rock shell. *Thais clavigera*. *Environ. Pollut.* **95**: 85–91.
13. Iguchi, T., Y. Katsu, T. Horiguchi, H. Watanabe, B. Blumberg, and Y. Ohta. 2007. Endocrine disrupting organotin compounds are potent inducers of imposex in gastropods and adipogenesis in vertebrates. *Mol. Cell Toxicol.* **3**: 1–10.
14. Kajiwara, M., S. Kuraku, T. Kurokawa, K. Kato, S. Toda, H. Hirose, et al. 2006. Tissue preferential expression of estrogen receptor gene in the marine snail, *Thais clavigera*. *Gen. Comp. Endocr.* **148**: 315–326.
15. Kanayama, T., N. Kobayashi, S. Mamiya, T. Nakanishi, and J. Nishikawa. 2005. Organotin compounds promote adipocyte differentiation as agonists of the peroxisome proliferator-activated receptor gamma/retinoid X receptor pathway. *Mol. Pharmacol.* **67**: 766–774.
16. Kannan, K., S. Tanabe, H. Iwata, and R. Tatsukawa. 1995. Butyltins in muscle and liver of fish collected from certain asian and oceanian countries. *Environ. Pollut.* **90**: 279–290.
17. Kannan, K., S. Tanabe, and R. Tatsukawa. 1995. Occurrence of butyltin residues in certain foodstuffs. *Bull. Environ. Contam. Toxicol.* **55**: 510–516.
18. Lee, H. S., E. M. Cho, J. H. Jung, and A. Ohta. 2007. Evaluation on antagonist activities of polycyclic aromatic hydrocarbons using the yeast two-hybrid detection system for endocrine disruptors. *Environ. Monit. Assess.* **129**: 87–95.
19. Lee, H. S., K. Miyauchi, Y. Nagata, R. Fukuda, S. I. Sasagawa, H. Endoh, et al. 2002. Employment of the human estrogen receptor  $\beta$  ligand-binding domain and co-activator SRC1 nuclear receptor-binding domain for the construction of a yeast two-hybrid detection system for endocrine disruptors. *J. Biochem.* **131**: 399–405.

20. Li, B. and S. Fields. 1993. Identification of mutations in p53 that affect its binding to SV40 large T antigen by using the yeast two-hybrid system. *FASEB J.* **7**: 957–963.
21. Luker, G. D., V. Sharma, C. M. Pica, J. L. Prior, W. Li, and D. Pivnicka-Worms. 2003. Molecular imaging of protein–protein interactions: Controlled expression of p53 and large T-antigen fusion proteins *in Vivo*. *Cancer Res.* **15**: 1780–1788.
22. Madro, A., A. Korolczuk, G. Czechowska, K. Celiński, M. Słomka, B. Prozorow-Król, and E. Korobowicz. 2008. RAS inhibitors decrease apoptosis of acinar cells and increase elimination of pancreatic stellate cells after in the course of experimental chronic pancreatitis induced by dibutyltin dichloride. *J. Physiol. Pharmacol.* **59**: 239–249.
23. Mensink, B. P., H. Krault, A. Dick Vethaak, C. C. ten Hallers-Tjabbes, J. H. Koernan, B. A. van Hattun, and J. P. Boon. 2002. Imposex induction in laboratory reared juvenile *Buccinum undatum* by tributyltin (TBT). *Environ. Toxicol. Pharmacol.* **11**: 49–65.
24. Nakanishi, T. 2008. Endocrine disruption induced by organotin compounds; organotins function as a powerful agonist for nuclear receptors rather than an aromatase inhibitor. *J. Toxicol. Sci.* **33**: 269–276.
25. Nakanishi, T., J. Nishikawa, Y. Hiromori, H. Yokoyama, M. Koyanagi, S. Takasuga, *et al.* 2005. Trialkyltin compounds bind retinoid X receptor to alter human placental endocrine functions. *Mol. Endocrinol.* **19**: 2502–2516.
26. Nishikawa, J., S. Mamiya, T. Kanayama, T. Nishikawa, F. Shiraishi, and T. Horiguchi. 2004. Involvement of the retinoid X receptor in the development of imposex caused by organotins in gastropods. *Environ. Sci. Technol.* **38**: 6271–6276.
27. Roins, M. J. J. and A. Z. Mason. 1996. The metabolism of testosterone by the periwinkle (*Littorina littorea*) *in vitro* and *in vivo*: Effects of tributyltin. *Mar. Environ. Res.* **42**: 161–166.
28. Saitoh, M., T. Yanase, H. Morinaga, M. Tanabe, Y. M. Mu, Y. Nishi, *et al.* 2001. Tributyltin or triphenyltin inhibits aromatase activity in the human granulosa-like tumor cell line KGN. *Biochem. Biophys. Res. Commun.* **289**: 198–204.
29. Santos, M. M., L. F. Castro, M. N. Vieira, J. Micael, R. Morabito, P. Massanisso, and M. A. Reis-Henriques. 2005. New insights into the mechanism of imposex induction in the dogwhelk *Nucella lapillus*. *Comp. Biochem. Phys. C Toxicol. Pharm.* **141**: 101–109.
30. Snoeij, N. J., A. H. Penninks, and W. Seinen. 1987. Biological activity of organotin compounds: An overview. *Environ. Res.* **44**: 335–353.
31. Spooner, N., P. E. Gibbs, G. W. Bryan, and G. L. Goad. 1991. The effect of tributyltin upon steroid titers in the female dogwhelk, *Nucella lapillus*, and the development of imposex. *Mar. Environ. Res.* **32**: 37–49.
32. Stridh, H., S. Orrenius, and M. B. Hampton. 1999. Caspase involvement in the induction of apoptosis by the environmental toxicants tributyltin and triphenyltin. *Toxicol. Appl. Pharm.* **156**: 141–146.
33. Yamabe, Y., A. Hoshino, N. Imura, T. Suzuki, and S. Himeno. 2000. Enhancement of androgen-dependent transcription and cell proliferation by tributyltin and triphenyltin. *Toxicol. Appl. Pharmacol.* **169**: 177–184.