FEBS Letters 580 (2006) 3013–3017

Evidence for p53-like-mediated stress responses in green algae

Aurora M. Nedelcu*

University of New Brunswick, Department of Biology, Mail Service 45111, Fredericton, NB, Canada E3B 6E1

Received 18 January 2006; revised 21 March 2006; accepted 17 April 2006

Available online 27 April 2006

Edited by Varda Rotter

Abstract The tumor suppressor protein, p53, plays a major role in cellular responses to stress and DNA damage in animals; despite its critical function, p53 homologs have not been identified in any algal or plant lineage. This study employs a functional and evolutionary approach to test for a p53 functional equivalent in green algae. Specifically, the study: (i) investigated the effect of two synthetic compounds known to interfere with p53 activity; (ii) searched for sequences with similarity to known p53-induced genes; and (iii) analyzed the expression pattern of one such sequence. The findings reported here suggest that a p53 functional equivalent is present and mediates cellular responses to stress in green algae.

Keywords: Programmed cell death; p53; Amifostine; Pifithrin-α; *Volvox carteri*; *Chlamydomonas reinhardtii*

1. Introduction

The tumor suppressor protein, p53, is a potent transcription factor that plays a major role in several cellular responses to stress and DNA damage in animals [1]. Despite: (i) p53's critical function in preserving genome integrity; (ii) many similarities between animal and plant cellular responses to stress [2]; (iii) theoretical arguments for the presence of a p53-like protein in land plants [3]; and (iv) the growing number of algal and plant genome sequencing projects (http://www.ncbi.nlm.nih.gov/genomes/PLANTS/PlantList.html), p53 homologs have not been reported in any algal or plant lineage. As the p53-like sequences described to date (including the p63 and p73 homologs) are quite diverged (e.g. [4,5]), standard methods appear inadequate in identifying p53 counterparts in distant evolutionary lineages. This study undertakes a functional and evolutionary approach to test for the presence of a p53 functional equivalent in two green algae: the multicellular *Volvox carteri*, and its unicellular relative, *Chlamydomonas reinhardtii*.

V. carteri is a multicellular green alga with two cell types, somatic and reproductive. An asexual *V. carteri* consists of 2000–4000 bi-flagellated somatic cells and up to 16 asexual reproductive cells (gonidia) (Fig. 1A). *V. carteri* is found in temporary vernal water bodies where it reproduces asexually; however, environmental heat-stress initiates a series of events culminating with the switch to sexual reproduction and the formation of dormant zygospores [6]. Our previous work showed that sexual induction and development in *V. carteri* are in fact responses to oxidative stress and possibly DNA damage [7,8]. Moreover, we found that in *V. carteri* sexual induction, cell-cycle arrest and programmed cell death (PCD) are alternative responses to increased levels of stress [7].

Both oxidative DNA damage [9] and hyperthermia – by mechanisms other than induction of DNA damage [10] are known to induce and activate p53. Therefore, if a p53 counterpart exists in *V. carteri*, it is conceivable that the sexual induction pathway – which: (i) involves chronic hyperthermia, oxidative stress and possibly DNA damage; and (ii) is functionally related to cell-cycle arrest and PCD (which are p53-mediated processes in animals), is also p53-mediated and thus could be affected by agents that interfere with p53 activity. Recently, two synthetic compounds, pifithrin-α (PFTα) and amifostine (AMF), have been reported to interfere with p53 transactivation and p53-dependent apoptosis in animal systems [11,12].

This study: (i) investigated the effect of PFTα on *V. carteri* and *C. reinhardtii* responses to heat-stress; (ii) searched the available genome sequence of *C. reinhardtii* and *V. carteri* for sequences with similarity to known p53-target genes, and (iii) analyzed the expression pattern of one such sequence, namely *pig8*/*ei24*, in the presence of PFTα and AMF. As further functional studies are difficult to be undertaken in the absence of sequence information, it is hoped that this evidence will direct a systematic search for p53 counterparts and other potential p53-target genes when the *C. reinhardtii* and *V. carteri* complete genome sequences become available.

2. Materials and methods

2.1. Strains and culture conditions

The *V. carteri* female strain (Eve) used in this study was kindly provided by Dr. David L. Kirk (Washington University); synchronous cultures were grown in the standard *Volvox* medium, at 28 °C on a 16 h light/8 h dark cycle [6]. A *C. reinhardtii* strain (CC-2454) was obtained from the *Chlamydomonas* Center (www.chlamy.org) and grown in TAP medium [13], on a 12 h light/12 h dark cycle.

2.2. Heat-stress

Cultures of *V. carteri* asexual females (5 individuals/ml) bearing young asexual embryos were subjected to a 42.5 °C heat-stress for 2 h [6]. *C. reinhardtii* cultures were grown up to 2×10^5 cells/ml, then resuspended at 2×10^6 cells/ml in fresh medium and subjected to several combinations of heat-stress (Fig. 2). At the end of the stress, cultures were returned to standard growth conditions. PFTα (Sigma;

*Fax: +1 506 4533583.
E-mail address: anedelcu@unb.ca (A.M. Nedelcu).

Abbreviations: PCD, programmed cell death; PFTα, pifithrin-α; AMF, amifostine

0014-5793/$32.00
doi:10.1016/j.febslet.2006.04.044

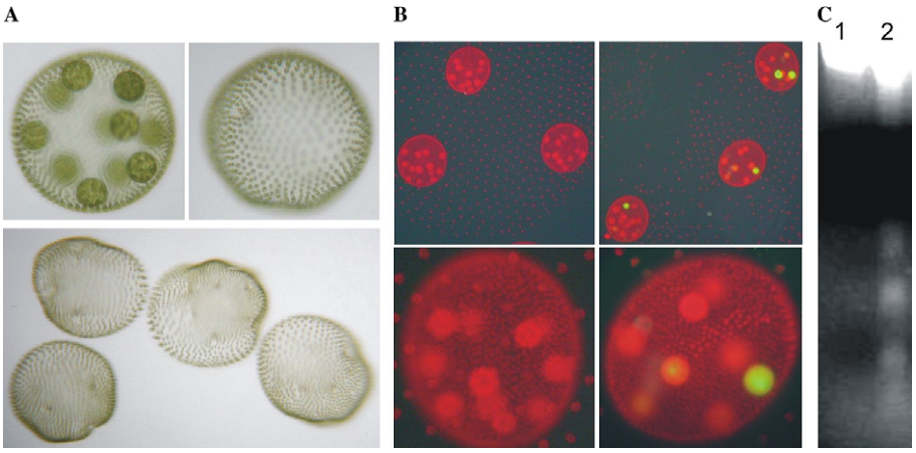

Fig. 1. The effect of PFTα on the response to the sex-inducing heat-stress in *V. carteri*. (A) Top left: Young asexual *V. carteri*; larger cells are gonidia. Top right and bottom: Hatched motile "ghost" colonies, one day after the stress in the presence of PFTα (2 μM); note the marks left in place of gonidia, and the change in overall shape of the colony due to the complete dissolution of gonidia. (B) ROS accumulation during the 2-h stress in the absence (left) and presence (right) of PFTα (red is due to chlorophyll autofluorescence, and green indicates the accumulation of ROS [8]); not all gonidia appear "green" at once, due to stochastic differences in their location and physiological/developmental state; bottom panels show close-ups of the top panels. (C) DNA-laddering effect; DNA was extracted 1 h after the stress in the absence (lane 1) and presence (lane 2) of PFTα.

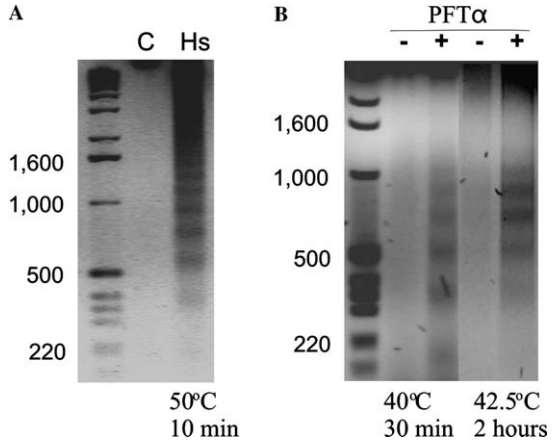

Fig. 2. DNA-laddering in *C. reinhardtii* following (A) an acute heat stress (C, control, Hs, heat-stress), and (B) two types of mild stress in the absence/presence (−/+) of PFTα.

40 mM stock in DMSO) and AMF (Sigma, 1 M stock in water) were added one hour prior to the stress to a final concentration of 2 and 10 μM, respectively. Control cultures were also treated with the same concentration of DMSO as in the PFTα cultures.

2.3. Visualization of reactive oxygen species

Reactive oxygen species (ROS) were detected using the fluorogenic compound 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA; Molecular Probes) as described in [8].

2.4. DNA-laddering assay

Cells were lysed in the presence of SDS (0.5%) and proteinase K (20 μg/ml). DNA was phenol extracted, treated with RNase A (25 μg/ml), and separated on a 1.8% agarose gel.

2.5. Sequence analyses

The *Chlamydomonas* v2.0 database (http://genome.jgi-psf.org/chlre2/chlre2.home.html) – which also includes *Volvox* whole-genome shotgun reads, was searched (tblastn) for sequences with similarity to p53-induced genes. Human (AAC39531), mouse (AAC52483), and *Caenorhabditis elegans* (AAC48294) *pig8/ei24* sequences were retrieved from GenBank. The *C. reinhardtii pig8/ei24*-like sequence (gene model C_1250044 in the *Chlamydomonas* v2.0 database) was blasted against the *Volvox* whole-genome shotgun reads; many sequences with similarity to the *C. reinhardtii pig8/ei24* were retrieved and used to design primers to amplify the entire *V. carteri pig8/ei24*-like genomic sequence (Nedelcu and Li in preparation) and to investigate its gene expression (see below). The predicted PIG8/EI24 sequences were aligned using ClustalW [14]. Pfam (http://pfam.cgb.ki.se), PSORT (http://www.psort.org), and MatInspector (http://www.genomatix.de/) were used for domain identification, cellular localization, and transcription factor binding sites analyses, respectively.

2.6. RT-PCR analyses

RNA was extracted as described in [8]. RT-PCR was performed using the SuperScript One-Step RT-PCR System (Invitrogen) and a Stratagene Robocycler; RNA levels were normalized using actin as a control [15]. Primers were designed across introns to ensure amplification products are from RNA only. Sequence primers and RT-PCR conditions were as follows. For *pig8/ei24*-like –GGCGCTGTGGCTTCTTCCCGT//CGCCCAGCGGCGCTCAAAGTA, and: 55 °C/30 min; 94 °C/2 min; (94 °C/30 s; 63 °C/30 s; 72 °C/1 min) (45 cycles); 72 °C/10 min. For actin – TGGCTGACGAGGGCGAGGTCT//GCGCAGAGTCAGAATACCGCG, and: 50 °C/30 min; 94 °C /2 min; (94 °C /15 s; 56 °C/30 s; 72 °C/1 min) (45 cycles); 72 °C/10 min.

3. Results and discussion

3.1. PFTα induces PCD in both Volvox and Chlamydomonas

To address the possibility that a p53 functional equivalent is present and functions in stress responses in *V. carteri*, asexual females (bearing asexual fully developed embryos) were subjected to the sex-inducing stress (42.5 °C for 2 h) in the presence and absence of an agent known to interfere with p53 activity, PFTα. PFTα was initially reported as a specific inhibitor of p53 transactivation and p53-dependent apoptosis [11]. However, further studies showed that PFTα's activity is cell line-dependent – as PFTα failed to inhibit p53 function in one cell lineage [16], and promoted p53 activation and apoptosis in another cell line [17].

Interestingly, in the presence of 2 μM PFTα, most or all of the reproductive cells inside *V. carteri* embryos underwent rapid shrinkage (visible as early as the end of the two-hour stress) and complete dissolution by next day – when the juveniles

hatched out of the mother colony (Fig. 1A). Noteworthy, in contrast to the dramatic changes that took place in gonidia, somatic cells appeared unaffected as their flagella continued to beat and move about these "ghost" colonies. Protoplast shrinkage is a hallmark feature of PCD, and the same rapid and complete dissolution of gonidia (while somatic cells appeared unaffected) has been previously observed following a more intense stress (43.5 °C for 2 h or 42.5 °C for 3 h) in the absence of PFTα [7].

As during p53-mediated PCD reactive oxygen species (ROS) can be generated [18], the involvement of ROS in the observed PFTα-induced PCD in *V. carteri* was investigated using fluorescence microscopy [8]. Consistent with a ROS-mediated process, gonidia revealed a soaring increase in ROS levels – when compared to the gonidia heat-stressed in the absence of PFTα (Fig. 1B). The fragmentation of DNA – another diagnostic feature of PCD, was also observed in the cultures heat-stressed in the presence of PFTα (Fig. 1C). The low proportion of laddered DNA relative to total DNA (Fig. 1C) reflects the fact that only gonidia undergo PCD (as somatic cells are still alive), and is due to the very low gonidia to somatic cell ratio (ca. 1:200) (Fig. 1A).

The functional significance of PFTα's PCD-inducing effect would be strengthened if a similar effect were also observed in another algal lineage. Thus, the effect of PFTα on the response to heat-stress in *V. carteri*'s unicellular relative, *C. reinhardtii*, was also investigated. In this species, PCD and DNA-laddering can be triggered by an acute heat-stress (e.g., 10 min at 48–50 °C) (Fig. 2A). Interestingly, a milder stress (e.g., a 30-min heat-stress at 40 °C or a 2 h stress at 42.5 °C) – which did not induce a DNA-ladder, did so if the stress was carried out in the presence of 2 μM PFTα (Fig. 2B).

Together, these data indicate that PFTα interferes with pathways that are specifically activated during heat-stress, and this interaction results in the activation of the PCD response in both the unicellular *C. reinhardtii* and the multicellular *V. carteri*. Interestingly, the effect of PFTα in this study is consistent with Gaji et al.'s report [17]; that is, in both *C. reinhardtii* and *V. carteri*, PFTα promotes PCD. Furthermore, the concentration at which PFTα is effective in inducing PCD in green algae (i.e., 2 μM) is similar to the concentration (1–3 μM) reported to induce p53 activation in mouse epidermal cells [17] – which is lower than the concentration level (10–20 μM) reported to inhibit p53-dependent apoptosis [11] or to potentially affect additional p53-independent pathways [19,20].

3.2. A p53-target gene is present in both Chlamydomonas and Volvox

To further implicate a p53 counterpart in stress responses in green algae, the *Chlamydomonas* genome database was searched for sequences with similarity to known p53-induced genes (*pig*) (see Section 2). Remarkably, a sequence with similarity to *pig8* (gene model C_1250044 in *Chlamydomonas* v2.0 database) was found. *pig8*/*ei24* is an important p53-induced gene expressed during DNA damage-induced p53-mediated apoptosis [21,22]. A partial amino acid alignment showing the presence of a conserved region (pfam07264 domain) in the human, mouse, the nematode *Caenorhabditis elegans*, and the two green algal predicted PIG8/EI24 sequences is shown in Fig. 3A.

pig8/*ei24* has been recently described as a candidate tumor suppressor gene coding for an apoptosis factor that is a novel endoplasmic reticulum-localized Bcl-2 binding protein [23]. Noteworthy, the *C. reinhardtii* predicted EI24/PIG8 (Protein ID 154363 in the *Chlamydomonas* v2.0 database) is also an integral membrane protein with potential endoplasmic reticulum localization (as predicted by PSORT). Moreover, MatInspector [24] identified putative p53 DNA-binding consensus sites in the 5′-untranscribed region of the *C. reinhardtii* *pig8*/*ei24*-like sequence (Fig. 3B).

3.3. The expression of the V. carteri pig8/ei24-like sequence is affected by both PFTα and AMF

To provide additional evidence that the *V. carteri pig8*/*ei24*-like sequence is part of a p53-like-mediated cellular response to stress, *pig8*/*ei24* expression was investigated following the sex-inducing heat-stress in the presence of PFTα as well as of another agent known to interfere with p53 activation, amifostine (AMF). Although the functional relationships between p53 and AMF also appear to be dependent on cell type and physiological state (i.e., normal or stressed, normal or cancer cell), in most cases AMF affects p53 activity and that of its target genes (including *pig8*/*ei24*) (see [12] for a discussion). Interestingly, as expected if *pig8*/*ei24* were regulated by a p53 functional equivalent sensitive to these agents, *V. carteri* *pig8*/*ei24*'s expression is altered by both PFTα and AMF;

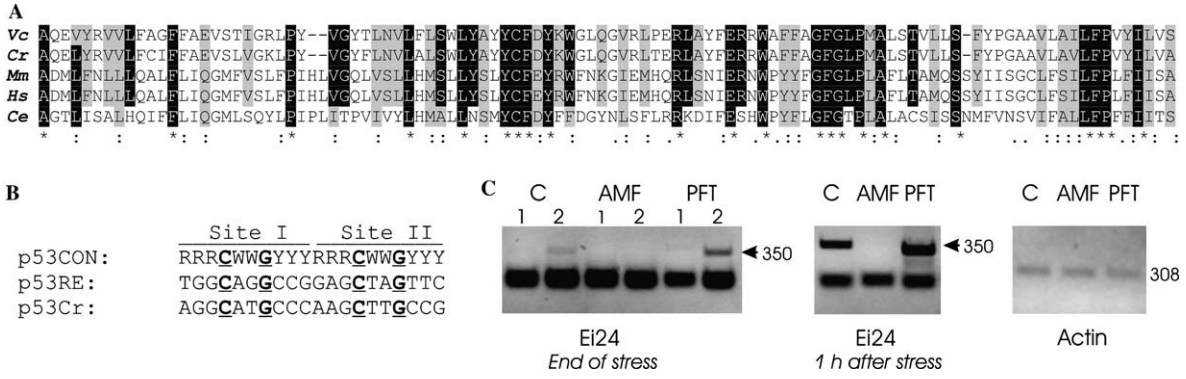

Fig. 3. (A) Partial alignment (position 191–281 in human PIG8/EI24) of *V. carteri* (*Vc*), *C. reinhardtii* (*Cr*), mouse (*Mm*), human (*Hs*), *C. elegans* (*Ce*) predicted PIG8/EI24. (B) p53 DNA binding sites: consensus site (p53CON), murine *ei24* (p53RE; [22]), and putative p53 DNAbinding sites (−275 to −255) in *C. reinhardtii pig8*/*ei24* (p53Cr). (C) *V. carteri pig8*/*ei24*-like gene expression pattern, after a 2 h-stress at 42.5 °C in the absence or presence of AMF/PFTα (1 and 2 indicate two concentrations of RNA used in RT-PCR; actin was used as a control [15]).

specifically, *pig8/ei24* is upregulated in the presence of PFTα (which is consistent with the PFTα's PCD-promoting activity; Fig. 1) and downregulated in the presence of AMF (as previously reported [12]).

3.4. A p53-functional equivalent in green algae

This study provides several lines of evidence suggesting that a p53 functional equivalent is present and mediates cellular responses to stress in green algae. Although: (i) PFTα's and AMF's precise mechanisms of action are not fully understood (and are likely dependent on the nature, type and physiological state of cells); and (ii) PFTα can affect additional pathways, it seems unlikely that in both *C. reinhardtii* and *V. carteri* these agents would interfere nonspecifically with p53-independent pathways and trigger responses that are p53-mediated in animals (PFTα promotes PCD in *C. reinhardtii* and *V. carteri* – Figs. 1 and 2; AMF affects cell cycle progression in *V. carteri* – unpublished data). Furthermore, it seems unlikely that both these agents would nonspecifically alter (in the expected directions) the expression of a gene that possesses putative p53 DNA-binding sites and is known to be a specific p53-target in animals (Fig. 3).

The cell type-specific sensitivity to PFTα in *V. carteri* (Fig. 1) also argues against a nonspecific action of this agent, and is consistent with the response to a more acute stress in the absence of PFTα – that is, PCD is triggered in gonidia but not somatic cells [7]. The observation that the stress-induced PCD in the multicellular *V. carteri* is limited to gonidia (both under acute stress in the absence of PFTα [7] and under milder stress in the presence of PFTα – Fig. 1) parallels the situation in the nematode *C. elegans*, where the pro-apoptotic function of the p53 homolog is also restricted to the germ-line [25]. The adaptive significance of this differential response is twofold: the activation of PCD in germ cells ensures that damaged cells are removed (and thus potential detrimental mutations will not be transmitted to offspring), while the inhibition of PCD in somatic cells (which cannot not be replaced if eliminated) ensures the survival of the individual carrying the surviving gonidia [7].

Noteworthy, p53 can promote PCD via both transcription-dependent and transcription-independent pathways. The latter has been recently described in sensitive organs and involves the rapid (as early as 30 min) translocation of p53 to mitochondria, where p53 initiates a rapid apoptotic response that jump-starts the slower transcription-based response (i.e., 4–8 h after p53 induction) [26]. Remarkably, the PCD response (i.e., ROS production, protoplast shrinking, DNA laddering) in *V. carteri* – following either an acute stress in the absence of PFTα [7] or a milder stress in the presence of PFTα (Fig. 1), is extremely rapid, being observed as soon as the end of stress. This rapid response is also consistent with the recently described heat-induced activation of p53 in human osteosarcoma cells (incidentally, also at 42.5 °C for 1–2 h), which – in contrast to radiation-induced activation, has been shown to be very rapid [10].

The presence of both unicellular and multicellular forms among volvocalen green algae (a monophyletic group of closely related and recently diverged lineages) provides an unprecedented opportunity for the study of PCD in an evolutionary framework. Besides the evolutionary significance, the findings presented here also provide support for *V. carteri*'s suitability as a model-system for PCD research. Although classical model organisms have been useful for studying cell death, it has been recently pointed out [27] that these systems might have also constrained our understanding of PCD; *V. carteri* is among the few taxa suggested as alternative model-systems that could actually improve our understanding of cell death processes. Indeed, its simpler organization and life-cycle on the one hand, coupled with its intriguing similarities in development [28] and PCD responses with the more complex animal systems on the other hand, make *V. carteri* a very attractive system for fundamental and even clinical research.

Acknowledgements: This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada to A.M.N. The *Chlamydomonas reinhardtii* and *Volvox carteri* sequence data were produced by the US Department of Energy Joint Genome Institute (http://www.jgi.doe.gov) and are provided for use in this publication only.

References

[1] Hofseth, L.J., Hussain, S.P. and Harris, C.C. (2004) p53: 25 years after its discovery. Trends in Pharmacological Sciences 25, 177–181.
[2] Hoeberichts, F.A. and Woltering, E.J. (2003) Multiple mediators of plant programmed cell death: interplay of conserved cell death mechanisms and plant-specific regulators. BioEssays 25, 47–57.
[3] Whittle, C.-A., Beardmore, T. and Johnston, M.O. (2001) Is G1 arrest in plant seeds induced by a p53-related pathway? Trends in Plant Science 6, 248–251.
[4] Saccone, C., Barome, P.O., D'Erchia, A.M., D'Errico, I., Pesole, G., Sbisa, E. and Tullo, A. (2002) Molecular strategies in Metazoan genomic evolution. Gene 300, 195–201.
[5] Mendoza, L., Orozco, E., Rodriguez, M.A., Garcia-Rivera, G., Sanchez, T., Garcia, E. and Gariglio, P. (2003) Ehp53, an *Entamoeba histolytica* protein, ancestor of the mammalian tumour suppressor p53. Microbiology-Sgm 149, 885–893.
[6] Kirk, D.L. and Kirk, M. (1986) Heat shock elicits production of sexual inducer in *Volvox*. Science 231, 51–54.
[7] Nedelcu, A.M. and Michod, R.E. (2003) Sex as a response to oxidative stress: The effect of antioxidants on sexual induction in a facultatively sexual lineage. Proceedings of the Royal Society B: Biological Sciences 270, S136–S139.
[8] Nedelcu, A.M., Marcu, O. and Michod, R.E. (2004) Sex as a response to oxidative stress: a two-fold increase in cellular reactive oxygen species activates sex genes. Proceedings of the Royal Society B: Biological Sciences 271, 1591–1596.
[9] Achanta, G. and Huang, P. (2004) Role of p53 in sensing oxidative DNA damage in response to reactive oxygen species-generating agents. Cancer Research 64, 6233–6239.
[10] Guan, J., Stavridi, E., Leeper, D.B. and Iliakis, G. (2002) Effects of hyperthermia on p53 protein expression and activity. Journal of Cellular Physiology 190, 365–374.
[11] Komarov, P.G., Komarova, E.A., Kondratov, R.V., Christov-Tselkov, K., Coon, J.S., Chernov, M.V. and Gudkov, A.V. (1999) A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. Science 285, 1733–1737.
[12] Acosta, J.C., Richard, C., Delgado, M.D., Horita, M., Rizzo, M.G., Fernandez-Luna, J.L. and Leon, J. (2003) Amifostine impairs p53-mediated apoptosis of human myeloid leukemia cells. Molecular Cancer Therapeutics 2, 893–900.
[13] Gorman, D.S. and Levine, R.P. (1965) Cytochrome and plastocyanin: their sequences in photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. Proceedings of the National Academy of Science, USA 54, 1665–1669.
[14] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.

[15] Hallmann, A., Amon, P., Godl, K., Heitzer, M. and Sumper, M. (2001) Transcriptional activation by the sexual pheromone and wounding: a new gene family from *Volvox* encoding modular proteins with (hydroxy)proline-rich and metalloproteinase homology domains. The Plant Journal 26, 583–593.
[16] Walton, M.I., Wilson, S.C., Hardcastle, I.R., Mirza, A.R. and Workman, P. (2005) An evaluation of the ability of pifithrin-alpha and -beta to inhibit p53 function in two wild-type p53 human tumor cell lines. Molecular Cancer Therapeutics 4, 1369–1377.
[17] Kaji, A., Zhang, Y., Nomura, M., Bode, A.M., Ma, W.-Y., She, Q.-B. and Dong, Z. (2003) Pifithrin-alpha promotes p53-mediated apoptosis in JB6 cells. Molecular Carcinogenesis 37, 138–148.
[18] Li, P.F., Dietz, R. and von Harsdorf, R. (1999) p53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome c-independent apoptosis blocked by Bcl-2. EMBO 18, 6027–6036.
[19] Hoagland, M.S., Hoagland, E.M. and Swanson, H.I. (2005) The p53 inhibitor pifithrin-alpha is a potent agonist of the aryl hydrocarbon receptor. Journal of Pharmacology and Experimental Therapeutics 314, 603–610.
[20] Komarova, E.A., Neznanov, N., Komarov, P.G., Chernov, M.V., Wang, K. and Gudkov, A.V. (2003) p53 inhibitor pifithrin α can suppress heat shock and glucocorticoid signaling pathways. The Journal of Biological Chemistry 278, 15465–15468.
[21] Lehar, S.M., Nacht, M., Jacks, T., Vater, C.A., Chittenden, T. and Guild, B.C. (1996) Identification and cloning of EI24, a gene induced by p53 in etoposide-treated cells. Oncogene 12, 1181–1187.
[22] Gu, Z.M., Flemington, C., Chittenden, T. and Zambetti, G.P. (2000) ei24, a p53 response gene involved in growth suppression and apoptosis. Molecular and Cellular Biology 20, 233–241.
[23] Zhao, X.S., Ayer, R.E., Davis, S.L., Ames, S.J., Florence, B., Torchinsky, C., Liou, J.S., Shen, L. and Spanjaard, R.A. (2005) Apoptosis factor E124/PIG8 is a novel endoplasmic reticulum-localized bcl-2-binding protein which is associated with suppression of breast cancer invasiveness. Cancer Research 65, 2125–2129.
[24] Cartharius, K., Frech, K., Grote, K., Klocke, B., Haltmeier, M., Klingenhoff, A., Frisch, M., Bayerlein, M. and Werner, T. (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics 21, 2933–2942.
[25] Derry, B., Putzke, A.P. and Rothman, J. (2001) *Caenorhabditis elegans* p53: role in apoptosis, meiosis, and stress resistance. Science 294, 591–595.
[26] Erster, S., Mihara, M., Kim, R.H., Petrenko, O. and Moll, U.M. (2004) In vivo mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. Molecular and Cellular Biology 24, 6728–6741.
[27] Golstein, P., Aubry, P. and Levraud, J.P. (2003) Cell-death alternative model organisms: Why and which? Nature Reviews Molecular Biology 4, 798–807.
[28] Kirk, D.L. (1998) Volvox. Molecular Genetic Origins of Multicellularity and Cellular Differentiation, Cambridge University Press, New York.