Environ. Sci. Technol. **2010,** *44,* 8829–8834

Radioadaptive Response Induced by Alpha-Particle-Induced Stress Communicated in Vivo between Zebrafish Embryos

V. W. Y. CHOI,[†] S. H. CHENG,[‡] AND K. N. YU*,[†]

Department of Physics and Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Hong Kong, and Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon Tong, Hong Kong

Received May 6, 2010. Revised manuscript received September 9, 2010. Accepted November 2, 2010.

We report data demonstrating that zebrafish embryos irradiated by alpha particles can release a stress signal into the water, which can be communicated to the unirradiated zebrafish embryos sharing the same water medium and thereby inducing a radioadaptive response in these unirradiated zebrafish embryos. The effects of radiation on the whole embryos were studied through quantification of apoptotic signals at 24 h post fertilization through staining with the vital dye acridine orange, followed by counting the stained cells under a microscope. In these experiments, dechorionated embryos were irradiated and then partnered with two other groups of unirradiated embryos, namely the bystander group (no more further treatments) and adaptive group (subjected to a further challenging dose) of embryos. The adaptive group of embryos were then separately further irradiated with a challenging dose. The results show that the number of apoptotic signals for the adaptive group is smaller than that for the corresponding control group, while that for the bystander group is larger than that for the corresponding control group. These suggest that the stress communicated in vivo between the irradiated zebrafish embryos and those unirradiated embryos sharing the same medium will induce radioadaptive response in the unirradiated embryos.

Introduction

Recent research have demonstrated the communication of radiation-induced bystander signals between fish in vivo. Mothersill et al. (*1*) reported their pioneering work to show that freshwater rainbow trout (*Oncorhynchus mykiss, W*) irradiated to 0.5 Gy total-body X-ray dose released bystander signals into the water to induce bystander effects in naive (unirradiated) partners, through showing that the media from explants from cultured tissues of the naive partners caused increased cell deaths in reporter HPV-G cells. The work was the first demonstration of radiation-induced bystander signals in vivo between fish, although a similar phenomenon was earlier reported by Surinov et al. (*2*) for unirradiated mice housed with irradiated mice, for which case the signal was found to be transmitted through urine. Mothersill et al. (*3, 4*) further demonstrated radiation-induced stress response communicated in vivo between zebrafish (*Danio rerio*) and Medaka (*Oryzias latipes*). Mothersill et al. (*1*) suggested that the radiation-induced bystander signal was likely an evolutionarily conserved mechanism with a final objective to enable an effective population response. It is interesting to explore how such radiation-induced bystander signals communicated *in vivo* between organisms can benefit the population.

In the present work, the benefit will be studied in terms of the induction of radioadaptive response (RAR) by communication of radiation-induced bystander signals. RAR is a kind of low-dose radiation effect, which occurs when a small preceding priming dose decreases the biological effectiveness of a subsequent large challenging dose. Such an adaptive response in cells (*in vitro* studies) was first reported by Olivieri et al. (*5*), who showed that peripheral blood lymphocytes irradiated with tritiated thymidine had fewer chromosomal aberrations when they were subsequently irradiated with 15 Gy of X-rays. RAR was also shown in mice in vivo (induced within an organism, in contrast to the new results in the present work, which showed RAR in vivo induced between zebrafish embryos sharing the same medium). A whole body exposure of mice by using X-radiation was conducted by Cai et al. (*6*), who showed that mice with pre-exposure to low doses of radiation had significant decreases in chromosome aberrations. Wang et al. (*7*) evaluated the RAR in mice and found a range of dose-rates capable of inducing RAR in mice. Although the phenomenon was shown to occur in vivo, a great variability in the induction of adaptive response was found in mice (*8*). Streffer et al. (*8*) showed that the induction of adaptive response in mice was not always consistent; it might depend on the dose range, developmental stage of the embryos, and the exposure interval.

Research studies have also demonstrated a beneficial adaptive effect having been resulted from the bystander signal sent from irradiated cells through the culture medium (e.g., refs *9*–*11*). Iyer and Lehnert (*9*) studied the induction of RAR from the bystander response in the human lung fibroblast (HFL-1) cell. An increase in the clonogenic survival was observed in the unirradiated HFL-1 cells cultured in the conditioned medium (the supernatant from the HFL-1 cells that were irradiated with 1 cGy of alpha particles) and subsequently exposed to low-fluence alpha particles. Another similar study conducted using γ rays also demonstrated the occurrence of RAR in HFL-1 cells through a bystander manner (*10*). Kadhim et al. (*11*) showed the occurrence of RAR in unirradiated bystander murine hemopoeitic stem cells which subsequently received a 1 Gy alpha-particle challenging exposure by using genomic instability as the end point.

Despite the successful induction of RAR through bystander response using both high-LET and low-LET (LET stands for linear energy transfer) radiations in vitro, no in vivo studies have been reported. It is therefore pertinent to explore the induction of RAR through bystander response in vivo. In their pioneering work to demonstrate the communication of radiation-induced bystander signals between fish in vivo, Mothersill et al. (*1*) also commented that their results showing the reduced survival of the reporter cells did not rule out the possibility of an adaptive response in the naive fish and stressed that their data only showed that a signal could be released from an irradiated fish to induce a response in a naive unirradiated fish.

In the present work, embryos of the zebrafish, *Danio rerio*, were employed as the model for studying the RAR induced

* Corresponding author e-mail: peter.yu@cityu.edu.hk.
[†] Department of Physics and Materials Science.
[‡] Department of Biology and Chemistry.

10.1021/es101535f
Published on Web 11/10/2010

by alpha-particle-induced stress. In recent years, the zebrafish, *Danio rerio*, a small vertebrate from Southeast Asia, has become a preferred model for studying human disease, including carcinogenesis. The most important advantage is that the human and zebrafish genomes share considerable homology, including conservation of most DNA repair-related genes (*12*). Rapid embryonic development is another advantage in that major organ systems become evident within 48 h postfertilization (hpf). Incidentally, Mothersill et al. (*3*) also characterized the radiation-induced stress response communicated in vivo between zebrafish (*Danio rerio*). A number of research works using the zebrafish embryo as an in vivo model to study the DNA damage response to ionizing radiation have emerged. For example, Bladen et al. (*13*) studied the DNA damage response and Ku80 mRNA function in the zebrafish embryos irradiated with ^{137}Cs γ rays. McAleer et al. (*14*) evaluated the effects of 250 kVp X-rays in combination with a known radioprotector (free radical scavenger Amifostine) or radiosensitizing agent (tyrosine kinase inhibitor AG1478) with a view to validate zebrafish embryos as a screen for radiation modifiers. McAleer et al. (*15*) also used zebrafish embryos to study radiosensitizing effects of flavopiridol in normal tissues exposed to ^{137}Cs γ rays or 250 kVp X-rays. Daroczi et al. (*16*) evaluated the radioprotective effect of the nanoparticle DF-1, which was a fullerene with antioxidant properties, in zebrafish embryos exposed to ^{137}Cs γ rays. Geiger et al. (*17*) studied the effects of ^{137}Cs γ rays and concurrent treatment with Amifostine on the development of the zebrafish embryos.

Our group has recently demonstrated that dechorionated embryos of the zebrafish *Danio rerio* at 1.5 hpf irradiated with alpha particles from an ^{241}Am source released bystander signals into the water to induce bystander effects in naive (unirradiated) zebrafish embryos (*18*). This work gave support to the work of Mothersill et al. (*1*) demonstrating radiation-induced bystander signals in vivo between fish. As such, it is feasible to study RAR induced by radiation-induced stress communicated in vivo between live organisms using zebrafish embryos.

We hypothesized that RAR would be developed in unirradiated naive zebrafish embryos exposed in vivo to the water shared by alpha-particle irradiated zebrafish embryos.

Materials and Method

Experimental Animals. The adult zebrafish were kept in tanks with water temperature set at 28 °C with the use of thermostats. A 14/10 h light-dark cycle was adopted in order to maintain a good production of embryos. Synchronization of the zebrafish embryos, i.e., to ensure they were at the same developmental stage, was important in our experiments. Once the 14-h photoperiod began, a specially designed embryo collector was immersed into the fish tanks and rested on the bottom of each tank to collect the embryos for a short period lasting only 15 min to ensure synchronization of the embryos. The embryo collector was a rectangular plastic container opened on the top and with a partition inside to let the embryos but not the adult fish pass through. A layer of plastic fake seaweed on the partition was to attract the adult zebrafish to lay embryos. The collected embryos were then incubated in a 28 °C incubator for development and allowed to develop until 4 hpf. Healthy developing embryos were selected at 4 hpf under a stereomicroscope; they should be at the sphere stage of the blastula period. Those healthy developing embryos were transferred into a Petri dish, which had a layer of agar gel on top of it, for dechorionation (see below).

Setup for Alpha-Particle Irradiation. The experimental setup for alpha-particle irradiation largely followed that devised by Yum et al. (*19*) to study effects of alpha particles on zebrafish embryos. First, all embryos used for experiments were dechorionated, so the alpha particles could reach the cells of those irradiated embryos. Second, the dechorionated embryos were irradiated from the bottom (i.e., from the side of the support substrate) (see Figure 1) to avoid the problems introduced by the varying depths of the medium above the cells of different embryos or even the same embryo. Specially prepared polyallyldiglycol carbonate (PADC) polymer films were used as the support substrates for the embryos during irradiation since they were sufficiently thin to allow the alpha particles to reach the cells with a sufficiently large energy, and their thickness could be conveniently controlled through chemical etching (*20*) with a reasonable accuracy so that the delivered alpha-particle energy and dose could also be controlled. PADC is a solid-state nuclear track detector (SSNTD), which is usually marketed under the name CR-39. A recent review on SSNTDs has been given in ref *21*. CR-39 detectors purchased from the Page Moldings (Pershore) Limited (Worcestershire, England), with original thickness of 100 μm were etched in 0.25 M sodium hydroxide in ethanol (*20*) to 16 μm. The thin PADC films with a thickness of 16 μm were then glued by an epoxy (Araldite Rapid, England) onto the bottom of a Petri dish with a diameter of 35 mm. Alpha-particle irradiations of the dechorionated embryos were performed with a planar ^{241}Am source (with an α particle energy of 5.49 MeV under vacuum and an activity of 0.1151 μCi) (Figure 1). The thin PADC films used as support substrates for the embryos could also be used to record alpha-particle hit positions and to enable calculations of the dose absorbed by the embryos if needed, although this was not carried out in the current work (this was carried out in ref *19*).

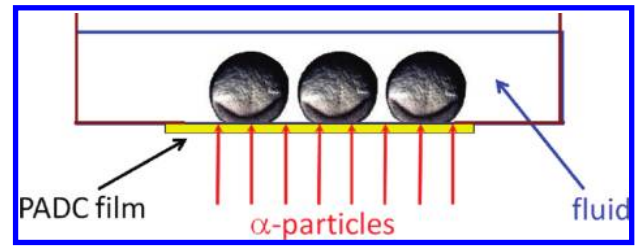

FIGURE 1. Irradiation of zebrafish embryos through the PADC-film based holder.

Priming and Challenging Doses. The time points for applying the priming and challenging doses were important. In the present experiments, as previously suggested by Choi et al. (*22*), a time interval of 5 h was chosen. Although the underlying mechanism for adaptive response in cells is still largely unknown, some research findings suggested that DNA repair might play an important role in inducing adaptive response (*9, 23*−*25*). Ikushima et al. (*23*) showed that the rate of rejoining DNA double-strand breaks was higher in adapted cells than in nonadapted cells; Sasaki et al. (*25*) reported reduction or absence of adaptive-response induction in a repair-deficient cell line. For zebrafish embryos, the DNA repair mechanism starts operating after the cleavage stages (0.7 to 2.2 hpf) (*26*). Hence, the priming exposure in this study was applied to embryos at 5 hpf at the blastula stage (2.2 to 5.2 hpf), at which stage the DNA repair mechanism should have started. In conclusion, the embryos were collected within 15 min when the light photoperiod began, which were then incubated, dechorionated at 4 hpf, and irradiated by alpha particles at 5 hpf (priming dose) and subsequently at 10 hpf (challenging dose). Note that the priming dose and the challenging dose were applied to different groups of embryos, which shared the same water medium (see below).

Irradiation Protocol. The dechorionated zebrafish embryos were divided into six groups each having 10 embryos, with the groups being referred to as the irradiated embryos, sham irradiated embryos, adaptive group, adaptive control

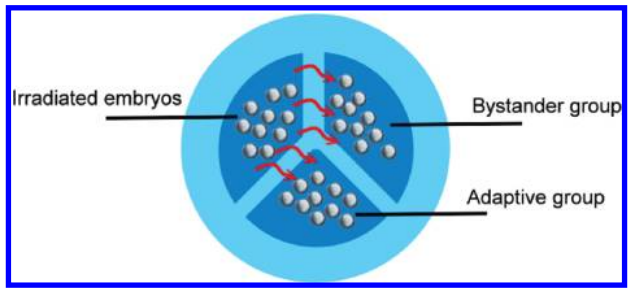

FIGURE 2. A custom-made agarose dish with 3 shallow regions containing the "experimental" embryos which shared the same medium.

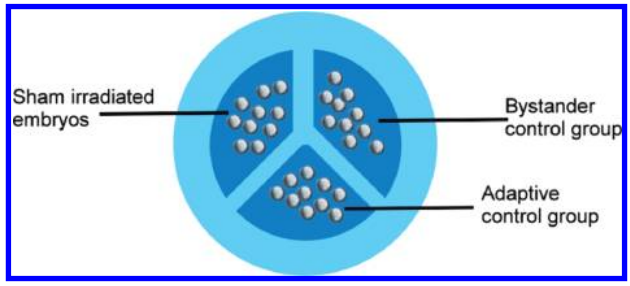

FIGURE 3. A custom-made agarose dish with 3 shallow regions containing the "control" embryos which shared the same medium.

group, bystander group, and the bystander control group. The group of irradiated embryos were irradiated for 4 min at 5 hpf using a planar ^{241}Am source (with an α particle energy of 5.49 MeV under vacuum and an activity of 0.1151 μCi), which corresponds to ~2.3 mGy (*19*). Bystander signals were found to be released into the water to induce bystander effects in naive (unirradiated) zebrafish embryos (*18*). The set up for alpha-particle irradiation was shown in Figure 1. After irradiation, the group of irradiated embryos were transferred immediately into a custom-made agarose plate with 3 shallow regions to accommodate three different groups of embryos (see Figures 2 and 3). The custom-made agarose plates were fabricated in order to allow separation of the three groups of embryos but at the same time allowing them to share the same medium. The group of irradiated embryos were partnered by the bystander group (no more further treatments) and adaptive group (subjected to a further challenging dose) of embryos, which were separately accommodated in the three shallow regions dredged in the agarose, and the three dredged regions were separated by small ridges as shown in Figure 2. The height of ridges was ~3 mm from the bottom, while the depth of water was ~7 mm from the bottom, so the flow over the ridges was not hindered and should be free. The bystander signal schematically represented by the red arrows in Figure 2, if any, could be transferred from the irradiated embryos to the bystander group and the adaptive group of embryos through the medium (with a volume of 3 mL) in the same agarose plate. Another custom-made agarose plate was set up by having sham-irradiated embryos, the bystander control group (no treatments at all) and the adaptive control group (only treated by the challenging dose) of embryos sharing the same medium (also with a volume of 3 mL) as the control experiment (see Figure 3). The two custom-made agarose plates were then incubated in a 28 °C incubator until the embryos developed until 10 hpf. The adaptive group and the adaptive control group of embryos were then transferred to separate Petri dishes with a 16 μm PADC film as support substrates for application of the challenging doses using the same planar ^{241}Am source (see Figure 1). The embryos were irradiated by alpha particles for 4 min (~2.3 mGy) and then transferred to separate Petri dishes coated with agarose gel at the bottom and further incubated to allow the embryos to develop until 24 hpf. Alpha-particle irradiation using the same source for 4 min was also employed to provide a challenging dose in a previous study (*22*). On the other hand, both the bystander group and bystander control group of embryos at 10 hpf were also transferred to separate Petri dishes coated with agarose gel at the bottom for incubation. Figure 4 gives a flow diagram for the dose schedules to different groups of zebrafish embryos.

Vital Dye Staining. Quantification of the apoptotic signals is widely used to investigate the effect of radiation on the whole embryos (*13, 17, 27, 28*). In the present experiments, as previously suggested (*22*), apoptotic signals in the 24 hpf embryos were quantified through staining with the vital dye acridine orange, followed by counting the stained cells under a microscope, which was a common method to quantify the level of apoptosis in zebrafish embryos (*29*–*31*). The 24 hpf embryos were stained for 60 min and washed twice in the culture medium thoroughly. They were then anaesthetized using 0.016 M tricaine (Sigma, St. Louis, MO, USA). For each embryo, two images with focuses on different sections of the embryo were captured under a fluorescent microscope with a magnification of 60×, which were then combined into a single image for quantification of apoptotic signals with the

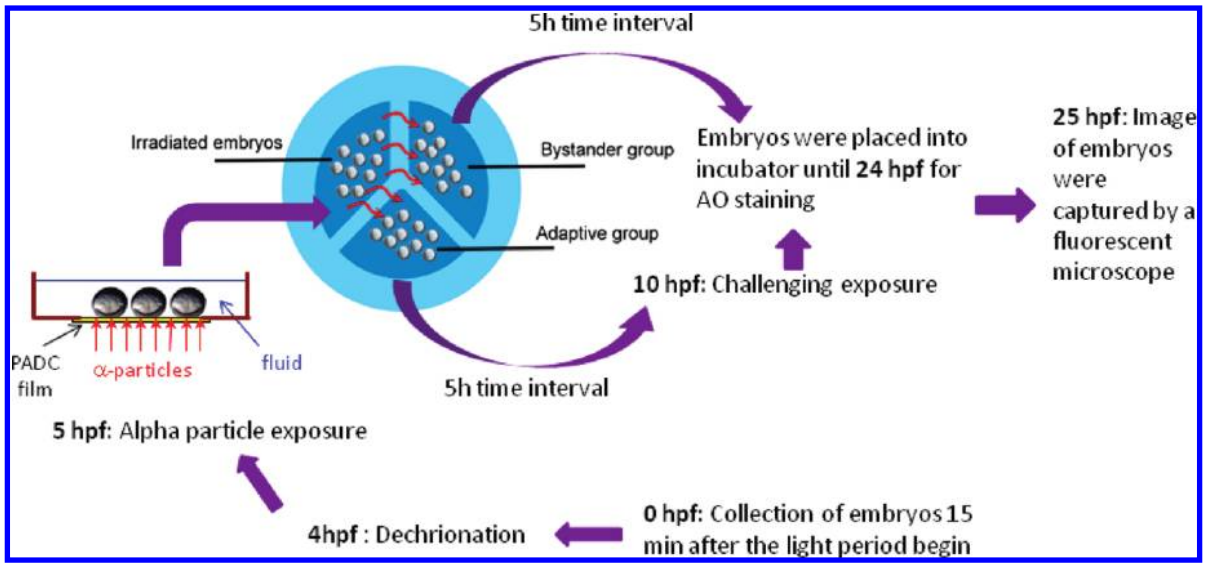

FIGURE 4. A flow diagram of the dose schedules for different groups of zebrafish embryos.

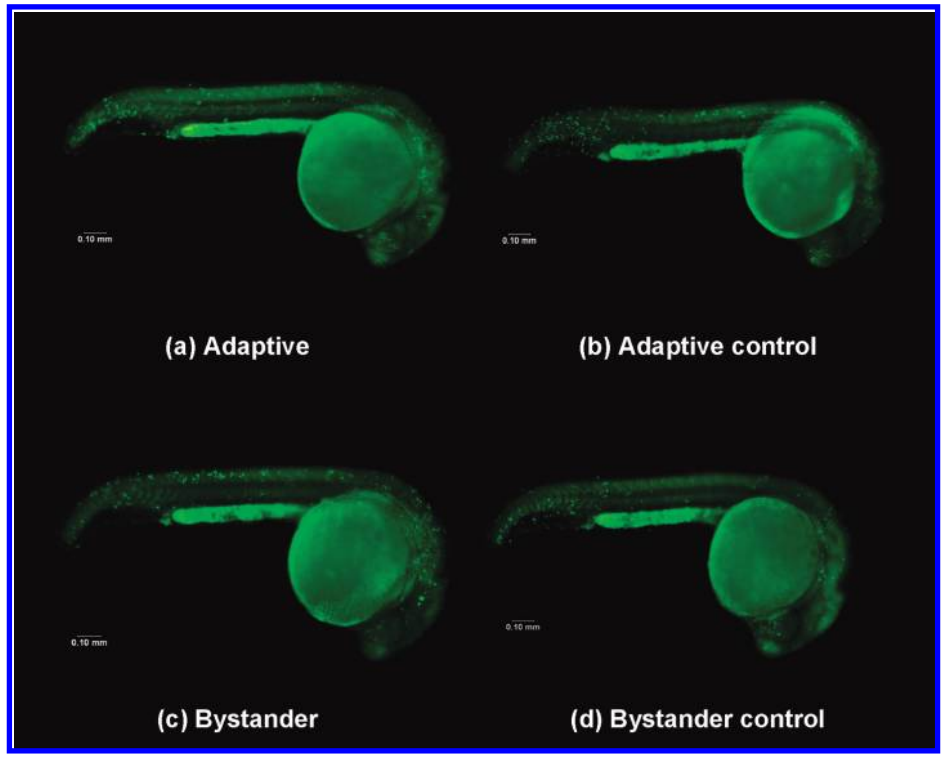

FIGURE 5. Apoptotic signals of typical 25 hpf zebrafish embryos revealed by acridine orange staining (from experiment 5). The magnification was 60×. (a) adaptive group: embryos partnered with irradiated embryos at 5 hpf (priming exposure) and separately irradiated at 10 hpf (challenging exposure); (b) adaptive control group: embryos partnered with sham-irradiated embryos at 5 hpf and separately irradiated at 10 hpf (challenging exposure); (c) bystander group: embryos partnered with irradiated embryos at 5 hpf (priming exposure); (d) bystander control group: embryos partnered with sham-irradiated embryos at 5 hpf.

help of the software MetaMorph Version 7.0r0 (1992−2006 Molecular Devices).

Results

The numbers of apoptotic signals in zebrafish embryos were counted from the combined images. Figure 5 shows the apoptotic signals of typical 25 hpf zebrafish embryos revealed by acridine orange staining (from experiment 5) which had (a) (adaptive group) partnered with irradiated embryos at 5 hpf (priming exposure) and separately irradiated at 10 hpf (challenging exposure); (b) (adaptive control group) embryos partnered with sham-irradiated embryos at 5 hpf and separately irradiated at 10 hpf (challenging exposure); (c) (bystander group) embryos partnered with irradiated embryos at 5 hpf (priming exposure); and (d) (bystander control group) embryos partnered with sham-irradiated embryos at 5 hpf. The number of apoptotic signals is smaller in embryos from the adaptive group when compared to that for the adaptive control group, and the number of apoptotic signals is larger in embryos from the bystander group when compared to that for the bystander control group.

The results for the five sets of experiments are shown in Table 1, which summarized the (means ± standard errors of the mean) for the number of apoptotic signals obtained in the four treatment groups, namely, adaptive group, adaptive control group, bystander group, and the bystander control group. The results are also shown graphically in Figure 6. For all the five sets of experiments, the number of apoptotic signals for the adaptive group is smaller than that for the adaptive control group and that for the bystander group is larger than that for the bystander control group.

The difference in the apoptotic signals between the adaptive group and adaptive control group, D_A, and the difference between the bystander group and the bystander control group, D_B, and the corresponding p values from t-tests are shown in Table 2. For experiments 1, 2, 3 and 5, the differences D_A and D_B are statistically significant (>0), viz., $p < 0.05$. For experiment 4, $D_A > 0$ and $D_B > 0$, with corresponding p values very close to 0.05 (i.e., $p = 0.054$ and 0.057, respectively). These results proved the existence of stress communicated in vivo between the irradiated zebrafish embryos and those naive unirradiated embryos sharing the same medium with the irradiated zebrafish embryos, and the successful induction of radioadaptive response in the naive unirradiated embryos through such communicated stress. If we treat the apoptotic signals for the "bystander control group" as the background signals, we can have an idea on the percentage decrease in the damage (ΔD) with both the priming and challenge dose versus the challenge dose alone. As such, the values for ΔD are 37, 95, 48, 70, and 52%, respectively, for experiments 1 to 5.

TABLE 1. Means ± Standard Errors of the Mean for Number of Apoptotic Signals Obtained in Four Treatment Groups of Zebrafish Embryos from Five Sets of Experiments[a]

experiment	adaptive group	adaptive control	bystander group	bystander control
1	69 ± 7 ($n = 8$)	92 ± 9 ($n = 7$)	70 ± 6 ($n = 7$)	29 ± 3 ($n = 6$)
2	58 ± 10 ($n = 7$)	100 ± 12 ($n = 7$)	120 ± 19 ($n = 9$)	56 ± 7 ($n = 9$)
3	55 ± 8 ($n = 9$)	76 ± 5 ($n = 7$)	61 ± 9 ($n = 7$)	32 ± 7 ($n = 9$)
4	52 ± 7 ($n = 9$)	71 ± 9 ($n = 9$)	63 ± 10 ($n = 9$)	44 ± 2 ($n = 9$)
5	114 ± 26 ($n = 9$)	169 ± 16 ($n = 10$)	99 ± 12 ($n = 8$)	64 ± 6 ($n = 9$)

[a] n is the number of zebrafish embryos in a particular sample. t test p values are presented in Table 2.

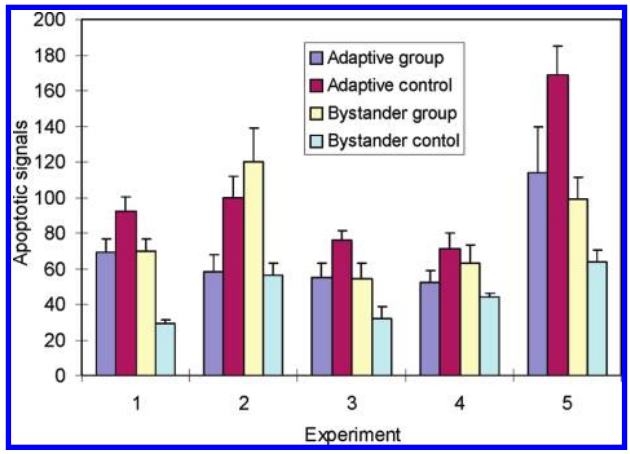

FIGURE 6. Number of apoptotic signals for the adaptive group, adaptive control group, bystander group, and bystander control group of embryos, all revealed by vital dye acridine orange staining. Error bars represent one standard errors. Quantitative analyses of the results have been given in Table 2.

TABLE 2. *p* Values for Comparisons of Zebrafish Groups in Table 1[a]

experiment	D_A	*p*	D_B	*p*
1	23 ± 11	0.039*	41 ± 7	0.00017*
2	42 ± 16	0.0095*	64 ± 20	0.0098*
3	22 ± 9	0.020*	29 ± 11	0.012*
4	19 ± 11	0.054	18 ± 10	0.057
5	55 ± 31	0.047*	35 ± 13	0.013*

[a] D_A = apoptotic signals for adaptive control - apoptotic signals for adaptive group; D_B = apoptotic signals for bystander group - apoptotic signals for bystander control group; *p*: *t* test *p* values (cases with $p < 0.05$ are asterisked).

Discussion

This paper demonstrates that zebrafish embryos, which have been irradiated by alpha particles, communicate stress through the shared medium to their partner zebrafish embryos, and that the communicated stress induces radioadaptive response in the partner zebrafish embryos. The effects of the communicated stress as well as the induced radioadaptive response are all investigated by quantification of apoptotic signals in the 24 hpf embryos through staining with the vital dye acridine orange, followed by counting the stained cells under a microscope. This is the first demonstration of radioadaptive response induced by radiation-induced stress communicated between living organisms.

Out of a total of five sets of independent experiments, four showed the presence of communicated stress as well as the induction of radioadaptive response with statistical significance. For these cases, unirradiated embryos allowed to share the same medium with the irradiated embryos 5 h before receiving a challenging dose of alpha-particle radiation had significantly decreased apoptotic signals compared to embryos exposed only to that challenging dose. The finding suggested that radioresistance was developed in these partnered embryos, which was consistent with the results obtained in in vitro studies using alpha particles as the radiation source (*9*). Iyer and Lehnert (*9*) observed a decreased level of TP53 and CDKN1A and an increased supra-basal level of intracellular reactive oxygen species and suggested the presence of a growth-promoting activity in the supernatants which enhanced DNA repair. The chemical messengers responsible for the radioresistance in the bystander embryos have not yet been confirmed. Further investigations on the chemical factors responsible for enhancing the radioresistance of the bystander embryos in vivo can help elucidate the mechanisms involved in the RAR induced by communicated radiation-induced stress.

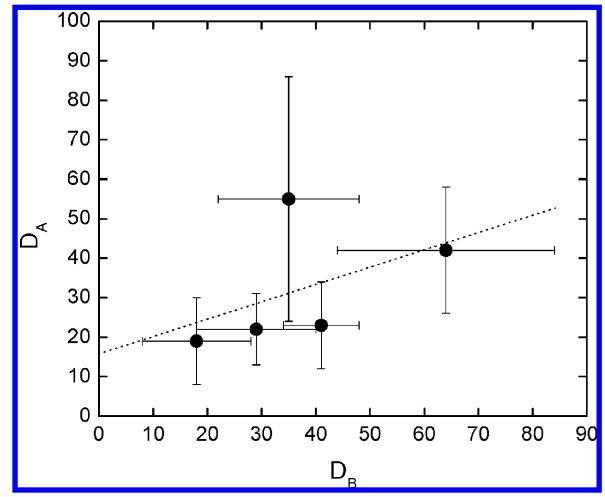

FIGURE 7. Relationship between D_A (apoptotic signals for adaptive control - apoptotic signals for adaptive group) and D_B (apoptotic signals for bystander group - apoptotic signals for bystander control group).

For the remaining sets of experiment, both the presence of communicated stress and induction of radioadaptive response were not demonstrated with statistical significance. Small variations in the statistical significance were in fact expected. As also commented by Tucker and Lardelli (*29*), variability in acridine orange staining results could be observed between embryos even for identical treatment, especially when the apoptosis was diffuse throughout the embryo rather than localized. Mothersill et al. (*3*) also commented that individual bystander fish could have varying levels of sensitivity to signal responses. In our data, the only statistically insignificant result ($p > 0.05$) for D_A corresponds to the only statistically insignificant result for D_B, which are both obtained in experiment 4. The D_A and D_B values in Experiment 4 also were the smallest values we observed. These observations hint that the "magnitude" of adaptive response represented by D_A might be dependent on the "magnitude" of bystander effect represented by D_B, although this conjecture will require more research to prove or disprove. The relationship between D_A and D_B is shown in Figure 7, and a positive correlation between the two parameters is apparent. The concomitant statistical significance or insignificance of D_A and D_B as well as the correlation between the values of D_A and D_B give further support that RAR is induced by the stress communicated in vivo between the irradiated zebrafish embryos and those naive unirradiated embryos sharing the same medium.

The demonstration of RAR induced by radiation-induced stress communicated in vivo between living organisms supports, at least in aquatic species that are close in proximity to one another and sharing the same media, the view that radiation-induced stress communicated in vivo between living organisms were actually an allelopathic effect aimed at coordinating a species-level survival response (*3*). Once an individual living organism is subjected to a radiation exposure, RAR is induced in the entire population in such a way that, in case there is a subsequent large radiation exposure, the damages to other organisms in the population will be decreased.

Literature Cited

(1) Mothersill, C.; Bucking, C.; Smith, R. W.; Agnihotri, N.; O'Neill, A.; Kilemade, M.; Seymour, C. B. Communication of radiation induced stress or bystander signals between fish in vivo. *Environ. Sci. Technol.* **2006**, *40*, 6859–64.

(2) Surinov, B. P.; Isaeva, V. G.; Dukhova, N. N. Post radiation immunosuppressive and attractive volatile secretions: the "bystander effect" or allelopathy in groups of animals. *Dokl. Biol. Sci.* **2005**, *400*, 28–30.

(3) Mothersill, C.; Smith, R. W.; Agnihotri, N.; Seymour, C. B. Characterization of a radiation-induced stress response communicated in vivo between zebrafish. *Environ. Sci. Technol.* **2007**, *41*, 3382–3387.

(4) Mothersill, C.; Smith, R. W.; Hinton, T. G.; Aizawa, K.; Seymour, C. B. Communication of Radiation-Induced Signals in Vivo between DNA Repair Deficient and Proficient Medaka (*Oryzias latipes*). *Environ. Sci. Technol.* **2009**, *43*, 3335–3342.

(5) Olivieri, G.; Bodycote, Y.; Wolff, S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science* **1984**, *223*, 594–597.

(6) Cai, L.; Jiang, J.; Wang, B.; Yao, H.; Wang, X. Induction of an adaptive response to dominant lethality and to chromosome damage of mouse germ cells by low dose radiation. *Mutat. Res.* **2003**, *303*, 157–161.

(7) Wang, B.; Ohyama, H.; Shang, Y.; Tanaka, K.; Aizawa, S.; Yukawa, O.; Hayata, I. Adaptive Response in Embryogenesis: V. Existence of Two Efficient Dose-Rate Ranges for 0.3 Gy of Priming Irradiation to Adapt Mouse Fetuses. *Radiat. Res.* **2004**, *161*, 264–272.

(8) Streffer, C. Bystander effects, adaptive response and genomic instability induced by prenatal irradiation. *Mutat. Res.* **2004**, *568*, 79–87.

(9) Iyer, R.; Lehnert, B. E. Alpha-particle-induced increases in the radioresistance of normal human bystander cells. *Radiat. Res.* **2002**, *157*, 3–7.

(10) Iyer, R.; Lehnert, B. E. Low dose, low-LET ionizing radiation-induced radioadaptation and associated early responses in unirradiated cells. *Mutat. Res.* **2002**, *503*, 1–9.

(11) Kadhim, M. A.; Moore, S. R.; Goodwin, E. H. Interrelationships amongst radiation-induced genomic instability, bystander effects, and the adaptive response. *Mutat. Res.* **2004**, *568*, 21–32.

(12) Barbazuk, W. B.; Korf, I.; Kadavi, C.; Heyen, J.; Tate, S.; Wun, E.; Bedell, J. A.; McPherson, J. D.; Johnson, S. L. The Syntenic Relationship of the Zebrafish and Human Genomes. *Genome Res.* **2000**, *10*, 1351–1358.

(13) Bladen, C. L.; Lam, W. K.; Dynan, W. S.; Kozlowski, D. J. DNA damage response and Ku80 function in the vertebrate embryo. *Nucleic Acids Res.* **2005**, *33*, 3002–3010.

(14) McAleer, M. F.; Davidson, C.; Davidson, W. R.; Yentzer, B.; Farber, S. A.; Rodeck, U.; Dicker, A. P. Novel use of zebrafish as a vertebrate model to screen radiation protectors and sensitizers. *Int. J. Radiat. Oncol. Biol. Phys.* **2004**, *61*, 10–13.

(15) McAleer, M. F.; Duffy, K. T.; Davidson, W. R.; Kari, G.; Dicker, A. P.; Rodeck, U.; Wickstrom, E. Antisense inhibition of cyclin D1 Expression is equivalent to flavopiridol for radiosensitization of zebrafih embryos. *Int. J. Radiat. Oncol. Biol. Phys.* **2006**, *66*, 546–551.

(16) Daroczi, B.; Kari, G.; McAleer, M. F.; Wolf, J. C.; Rodeck, U.; Dicker, A. P. In vivo radioprotection by the fullerene nanoparticle DF-1 as assessed in a zebrafish model. *Clin. Cancer Res.* **2006**, *12*, 7086–7091.

(17) Geiger, G. A.; Parker, S. E.; Beothy, A. P.; Tucker, J. A.; Mullins, M. C.; Kao, G. D. Zebrafish as a "Biosensor"? Effects of ionzing radiation and amifostine on embryonic viability and development. *Cancer Res.* **2006**, *66*, 8172–8181.

(18) Yum, E. H. W.; Choi, V. W. Y.; Nikezic, D.; Li, V. W. T.; Cheng, S. H.; Yu, K. N. Alpha-particle-induced bystander effects between zebrafish embryos in vivo. *Radiat. Meas.* **2009**, *44*, 1077–1080.

(19) Yum, E. H. W.; Ng, C. K. M.; Lin, A. C. C.; Cheng, S. H.; Yu, K. N. Experimental setup for studying the effects of alpha particles on zebrafish embryos. *Nuclear Instrum. Methods B* **2007**, *264*, 171–176.

(20) Chan, K. F.; Lau, B. M. F.; Nikezic, D.; Tse, A. K. W.; Fong, W. F.; Yu, K. N. Simple preparation of thin CR-39 detectors for alpha-particle radiobiological experiments. *Nuclear Instrum. Methods B* **2007**, *263*, 290–293.

(21) Nikezic, D.; Yu, K. N. Formation and Growth of Tracks in Nuclear Track Materials. *Mater. Sci. Eng. R* **2004**, *46*, 51–123.

(22) Choi, V. W. Y.; Lam, R. K. K.; Chong, E. Y. W.; Cheng, S. H.; Yu, K. N. Designing experimental setup and procedures for studying alpha-particle-induced adaptive response in zebrafish embryos *in vivo*. *Nuclear Instrum. Methods B* **2010**, *268*, 651–656.

(23) Ikushima, T.; Aritomi, H.; Morisita, J. Radioadaptive response: Efficient repair of radiation-induced DNA damage in adapted cells. *Mutat. Res.* **1996**, *358*, 193–198.

(24) Yatagai, F.; Umebayashi, Y.; Honma, M.; Sugasawa, K.; Takayama, Y.; Hanaoka, F. Mutagenic radioadaptation in a human lymphoblastoid cell line. *Mutat. Res.* **2008**, *638*, 48–55.

(25) Sasaki, M. S.; Ejima, Y.; Tachibana, A.; Yamada, T.; Ishizaki, K.; Shimizu, T.; Nomura, T. DNA damage response pathway in radioadaptive response. *Mutat. Res.* **2002**, *504*, 101–118.

(26) Miyachi, Y.; Kanao, T.; Okamoto, T. Marked depression of time interval between fertilization period and hatching period following exposure to low dose X-rays in zebrafish. *Environ. Res.* **2003**, *93*, 216–219.

(27) Bladen, C. L.; Navarre, S.; Dynan, W. S.; Kozlowski, D. J. Expression of the Ku70 subunit (XRCC6) and protection from low dose ionizing radiation during zebrafish embryogenesis. *Neurosci. Lett.* **2007**, *422*, 97–102.

(28) Bladen, C. L.; Flowers, M. A.; Miyake, K.; Podolsky, R. H.; Barrett, J. T.; Kozlowski, D. J.; Dynan, W. S. Quantification of ionizing radiation-induced cell death *In Situ* in a vertebrate embryo. *Radiat. Res.* **2007**, *168*, 149–157.

(29) Tucker, B.; Lardelli, M. A Rapid apoptosis assay measuring relative acridine orange fluorescence in zebrafish embryos. *Zebrafish* **2007**, *4*, 113–116.

(30) Mei, J.; Zhang, Q.-Y.; Li, Z.; Lin, S.; Gui, J.-F. C1q-like inhibits p53-mediated apoptosis and controls normal hematopoiesis during zebrafish embryogenesis. *Dev. Biol.* **2008**, *319*, 273–284.

(31) Yasuda, T.; Yoshimoto, M.; Maeda, K.; Matsumoto, A.; Maruyama, K.; Ishikawa, Y. Rapid and simple method for quantitative evaluation of neurocytotoxic effects of radiation on developing Medaka brain. *J. Radiat. Res.* **2008**, *49*, 533–540.

ES101535F