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Review

Radiation Biology of *Caenorhabditis elegans*: Germ Cell Response, Aging and Behavior

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Nematode/Germ line/Oxidative stress/Nervous system/Model organism.

The study of radiation effect in *Caenorhabditis (C.) elegans* has been carried out over three decades and now allow for understanding at the molecular, cellular and individual levels. This review describes the current knowledge of the biological effects of ionizing irradiation with a scope of the germ line, aging and behavior. In germ cells, ionizing radiation induces apoptosis, cell cycle arrest and DNA repair. Lots of molecules involved in these responses and functions have been identified in *C. elegans*, which are highly conserved throughout eukaryotes. Radiosensitivity and the effect of heavy-ion microbeam irradiation on germ cells with relationship between initiation of meiotic recombination and DNA lesions are discussed. In addition to DNA damage, ionizing radiation produces free radicals, and the free radical theory is the most popular aging theory. A first signal transduction pathway of aging has been discovered in *C. elegans*, and radiation-induced metabolic oxidative stress is recently noted for an inducible factor of hormetic response and genetic instability. The hormetic response in *C. elegans* exposed to oxidative stress is discussed with genetic pathways of aging. Moreover, *C. elegans* is well known as a model organism for behavior. The recent work reported the radiation effects via specific neurons on learning behavior, and radiation and hydrogen peroxide affect the locomotory rate similarly. These findings are discussed in relation to the evidence obtained with other organisms. Altogether, *C. elegans* may be a good "*in vivo*" model system in the field of radiation biology.

INTRODUCTION

Caenorhabditis (C.) elegans is a free-living, non-parasitic soil nematode (Fig. 1). It can be easily manipulated, observed and cultivated in the laboratory, because of its small size (adult worm is approximately 1 mm in length), transparence and feeding on bacteria (e.g., *Escherichia coli*).[1)] *C. elegans* is a well-known unique model organism in the biological research for complete cell lineage,[2)] neuronal networks[3)] and genome sequence.[4)] These advantages make *C. elegans* as a good "*in vivo*" model system in the field of radiation biology. The first study on the effects of ionizing radiation (IR) in *C. elegans* was performed by Herman *et al.* in 1976,[5)] who analyzed chromosome rearrangements induced by X-rays to produce a balancing lethal system using chromosome balancers such as *Drosophila*. Then, radiosensitive mutants, *rad-1* and *rad-2*, were obtained by Hartman in 1985.[6)] In the early phase (1976–1985), radiation-induced mutations were analyzed and various mutants were obtained. In the next phase (1986–1995), the interest was expanded to various fields of research such

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Fig. 1. Photograph of the *Caenorhabditis elegans* adult hermaphrodite. Scale bar, 100 µm.

as radiation-induced DNA damage, repair and the radiation quality effect.[7,8] Recently (1996 – at present), the trend of the interest continued, and these findings broadened. This paper reviews briefly the current knowledge of radiation effects in *C. elegans* with emphasis on the phenomenon and proposed mechanism for the following three topics: (*i*) apoptosis, cell cycle arrest and DNA repair in germ cells, (*ii*) aging that might be interesting because persistent oxidative stress occurs not merely during aging but also after irradiation,[9] and (*iii*) behavioral response. It is hoped that the radiation biology of *C. elegans* provides important clues to mechanisms of the radiation response in the biological system from cell to tissue or individual level.

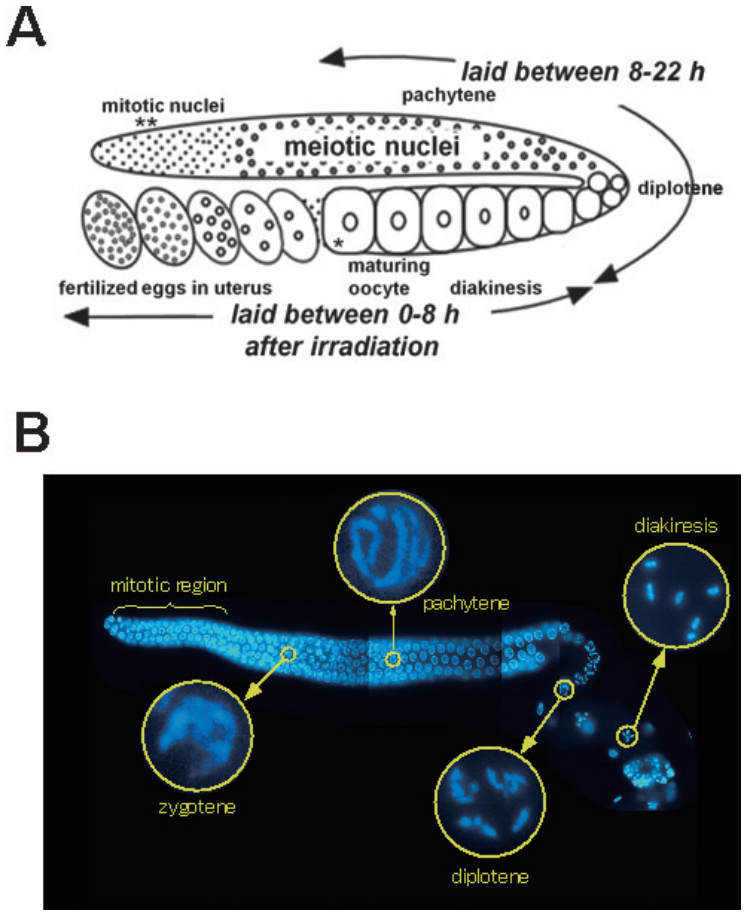

Fig. 2. Oogenesis in the *C. elegans* hermaphrodite. **A)** Schematic illustration of gametogenesis and early embryogenesis in a young gravid harmaphrodites. Mitotic nuclei in the gonad tip (**) and maturing oocyte at the diakinesis stage (*) are indicated. Arrows indicate the area laid between 0–8 h or 8–22 h after irradiation. **B)** Meiotic chromosome structures are visualized by DAPI staining. The distal-most germ cells proliferate mitotically and serve as a stem cell population. During their passage through a transition zone where crescent-shaped zygotene nuclei are observed, germ cells cease division and enter meiotic prophase. Tight attachments between parental chromosomes are observed in the pachytene nuclei. Chiasmata can be visualized in the nuclei at diplotene region. In the proximal arm of gonad, a mature oocyte contains condensed six chromosome bivalents.

GERM LINE

A convenient model to study radiation responses in germ cells

The nematode *C. elegans* has the two sexes, hermaphrodites and males. Hermaphrodites produce both sperm and oocytes, and can reproduce by self-fertilization. After producing approximately 150 sperm in each of two gonadal arms at the L4 larval stage, germ cell differentiation switches to oogenesis in hermaphrodite.[10] Within the adult hermaphrodite, germ line contains oocytes at the various stages of differentiation progression (Fig. 2). The distal-most germ cells proliferate mitotically and serve as a stem cell population. During their passage through a transition zone, germ cells cease division and enter meiotic prophase. Meiotic recombination is accomplished through meiotic prophase I, and tight attachments between parental chromosomes are observed in the pachytene nuclei. In the proximal arm of gonad, mature oocytes containing condensed six chromosome bivalents fertilize through a spermatheca.[11] The spatial and temporal organization of the female germ line is useful to analyze radiation effects in various meiotic progression. Through a reverse time course analysis, we can evaluate sensitivity of germ cells that are at progressively earlier stages of meiotic prophase at the time of acute IR exposure (Fig. 2A).[12,13]

IR induces several responses in *C. elegans* germ cells, including apoptosis, cell cycle arrest, and DNA double-strand break (DSB) repair to maintain genome integrity, which are regulated by conserved components throughout eukaryotes. Completion of *C. elegans* genome sequencing and development of microarray technique have made it possible to survey IR-induced transcriptional alterations.[14] Recently RNA-mediated interference (RNAi) that efficiently silences gene expression in *C. elegans* has been performed to screen the genes required for IR response in the genome-wide scale.[15] Nematode provides a valuable model for understanding IR responses of germ cells.

IR-induced germ cell apoptosis and cell cycle arrest

Within the adult hermaphrodite germ line, physiological germ cell apoptosis invariably occurs in the pachytene region.[16] Dying cells can be observed uniformly as highly refractile corpses under a phase-contrast microscope. DNA damage from genotoxic stress including IR also induces germ cell death to maintain genome integrity.[17] IR-induced germ cell corpses are restricted in the pachytene region and are not distinguished morphologically from physiological apoptosis. Apoptosis increases significantly 2–3 h after irradiation in a dose-dependent manner, and is continuously observed up to 24–36 h.[17] Core apoptotic pathway regulated by caspase homolog CED-3 (cell death abnormality 3) and Apaf-1 (apoptotic protease-activating factor 1) homolog CED-4 which regulates CED-3 caspase activity is indispensable for IR-induced and physiological germ cell apoptosis as well as physiological somatic cell apoptosis during embryonic development.[18] CED-4 activity is repressed by binding of CED-9 which encodes a single worm homolog of mammalian Bcl-2 cell death inhibitor.[19,20] Two BH-3 (Bcl-2 homology 3) domain containing proteins, EGL-1 (egg laying defective 1) and CED-13 function to dissociate CED-4 from heterotetrameric CED-4/CED-9 complex as an inducer for germ cell apoptosis caused by DNA damage.[17,21,22] In wild-type young adults, *egl-1* and *ced-13* mRNA expression in the germ line is highly induced after exposure to IR,[14,21,23,24] in a manner dependent on accumulation of CEP-1, *C. elegans* p53 homolog (Fig. 3).[25,26] In *cep-1* mutant, physiological germ cell apoptosis and somatic cell apoptosis in embryos

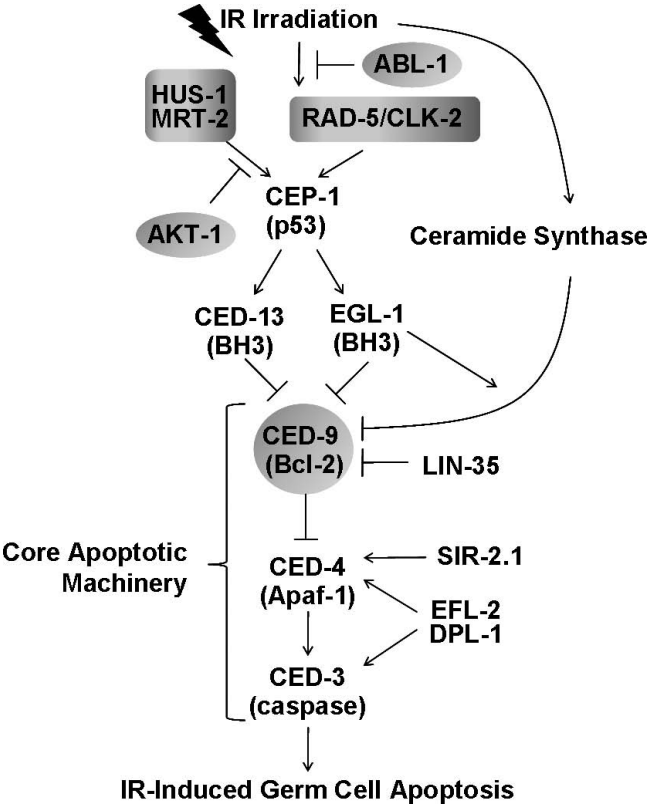

Fig. 3. Regulation of IR-induced germ cell apoptosis. IR activates checkpoint proteins HUS-1, MRT-2 and RAD-5/CLK-2, allowing for activation of CEP-1 (p53). CEP-1 upregulates transcription of *ced-13* and *egl-1*. CED-13 and EGL-1 inhibit CED-9 (Bcl-2), allowing CED-4 (Apaf-1) to activate the CED-3 (caspase), leading to apoptosis. ABL-1, AKT-1 and CED-9 work as apoptosis inhibitors. Ceramide-induced apoptosis pathway functions in parallel with CEP-1 pathway and requires base line level of EGL-1 protein. LIN-35 promotes germ cell apoptosis by blocking the transcription of *ced-9*. EFL-2 and DPL-1 promote germ cell apoptosis by inducing *ced-4* and *ced-3* transcription. SIR2.1 genetically acts in parallel to, or downstream of, *cep-1* transcription.

are executed normally.[26] Therefore IR-induced germ cell death machinery is initiated through a different pathway from physiological apoptosis.

Recently, components for apoptosis induction that function in downstream of CEP-1 or its parallel pathways have been reported. *C. elegans* SIR-2.1 is a member of the sirtuin family related to *Saccharomyces* (*S.*) *cerevisiae* Sir2p, and is localized in the nuclei of most germ cells. SIR-2.1 is essential for the execution of apoptosis in response to IR.[27] In *sir-2.1* mutants, cell cycle arrest and induction of *egl-1* and *ced-13* occur at the same level as in wild-type animals after IR exposure, but DNA damage-induced perinuclear hyper-accumulation of CED-4 is disrupted as is in *cep-1* mutants. This suggests that *sir-2.1* genetically acts, in parallel to or downstream of, *cep-1*-dependent transcription that affects DNA damage-induced apoptosis (Fig. 3). Direct interaction between SIR-2.1 and CED-4 failed to be detected, but its co-localization can be observed. In addition, nuclear disappearance of SIR-2.1 in dying cells occurs at early apoptotic event depending on checkpoint proteins. This suggests that SIR-2.1 is involved in triggering germ cell apoptosis, for which mechanisms should be elucidated in future. Also, IR-induced ceramide biogenesis functions to promote germ cell apoptosis in *C. elegans*.[28] Ceramide accumulation to mitochondrial membrane is required for EGL-1-mediated displacement of CED-4 from the CED-9 /CED-4 complex,[29] which occurs in parallel with CEP-1 pathway (Fig. 3).[28] In mammalian cultured cells and murine tissues, ceramide acts as a second messenger in activating IR-induced apoptosis.[30] Moreover, several environmental stresses, including IR, UV-C, heat shock and oxidative stress rapidly induce ceramide generation.[31–33]

In addition to apoptosis, DNA damage-induced checkpoint control causes proliferation arrest of germ cells of *C. elegans* after exposure to IR, but not in embryonic cells because of rapid cell proliferation without G_1 and G_2 phases during embryonic development.[17,34] Key kinases for checkpoint control, ataxia-telangiectasia protein family, are highly conserved in all eukaryotes including *C. elegans*. *C. elegans* ATL-1 (mammalian ATR ortholog) is needed for checkpoint control, germ cell cycle arrest and apoptosis, in response to IR, UV, and DNA replication stress.[35–38] ATM-1 (mammalian ATM ortholog) is thought to play a minor role in the DNA damage checkpoint response to IR.[35,37,38] HUS-1, MRT-2 and RAD-5/CLK-2 have also been identified as checkpoint proteins, for which mutants prevent IR-induced germ cell cycle arrest and apoptosis but have no effect on physiological apoptosis in *C. elegans*.[17,23,39] HUS-1 and MRT-2 are orthologs of *S. pombe*/human Hus-1 and Rad-1 respectively, which function as sliding clamp components and associate with DSBs *in vivo*. RAD-5/CLK-2 is an ortholog of the *S. cerevisiae* Tel2p that is an essential DNA binding protein to regulate telomere length.[39] Transcription of *egl-1* elevated by IR is abrogated in *hus-1* mutant.[23] Checkpoint mutants, *hus-1*, *mrt-2* and *clk-2* exhibit spontaneous mutator phenotype, whereas *ced-3* and *ced-4* mutants that are exclusively deficient in DNA damage-induced apoptosis do not.[40] These results suggest that DNA damage checkpoint proteins have a critical role in accurate repair of DNA lesion.

Regulators of germ cell apoptosis

Several anti-apoptotic proteins after exposure to IR have been identified recently. ABL-1, a *C. elegans* homolog of conserved nonreceptor tyrosine kinase c-Abl, acts upstream of CEP-1 and checkpoint proteins as a negative regulator of germ cell apoptosis after exposure to IR (Fig. 3).[41] In *abl-1* defective mutant, germ cell apoptosis increases both at baseline and post-irradiation.[41] Hyper-radiosensitive phenotype in *abl-1* mutant is mediated by *cep-1*, *clk-2*, *hus-1* and *mrt-2*.[41] AKT-1 and AKT-2, which are orthologs of mammalian serine/threonine kinase Akt/PKB, also regulate IR-induced apoptosis negatively by acting downstream of, or in parallel to, the HUS-1/MRT-2 branch of the checkpoint signaling pathway.[42] AKT-1 regulates *egl-1* transcription through posttranscriptional modification of CEP-1 (Fig. 3).[42]

Regulation of germ cell apoptosis through transcription of core apoptotic components has been reported. *C. elegans* retinoblastoma gene homolog *lin-35* (abnormal cell lineage 35) and the mammalian E2F-like transcription factor components *efl-2* and *dpl-1* (vertebrate transcription factor DP-like) are required for DNA damage-induced germ cell apoptosis as well as physiological germ cell apoptosis through *ced-9* and *ced-4* transcriptional regulation (Fig. 3).[43] Recently identified ING-3 (inhibitor of growth family of type II tumor suppressors) that promotes IR-induced apoptosis in a *cep-1*-associated pathway may act downstream of CEP-1 mediated transcription, because *ing-3* mutant does not reduce the level of the *egl-1* transcription.[44] ING-3 is suggested to localize in chromatin in the newly fertilized embryo and germ cells. Although it is unknown how ING-3 promotes germ cell apoptosis in the *cep-1* pathway, ING-3 may silence or augment transcription of components associated with germ cell apoptosis, as ING proteins interact with histone acetyltransferase or deacetylase complexes both in mammals and yeast.[45]

CEP-1-dependent and -independent regulators of germ cell apoptosis have been identified by genome-wide RNAi screening.[46] Knockdown each of 21 genes results in a moderate-to-strong increase in germ cell apoptosis in wild-type background. Of these, 16 genes require CEP-1 to activate germ cell apoptosis including DNA repair proteins, *rad-51/rdh-1* and *rad-50,* and *bmk-1* which encodes a *C. elegans* homolog of the BimC kinesin-like motor protein involved in spindle formation.[46] Knockdown of T02E1.3 (zinc finger domain, TIS11 family), *cpb-3* (RNA-binding protein CPEB family), R05D3.4 (RING domain), and *pmk-3* (p38 MAPK) in addition to *ced-9* (Bcl-2 homolog) increases CEP-1-

independent germ cell apoptosis.[46)]

In mammals, three p53 family members, p53, p63 and p73 have been identified.[47)] p53 acts as a tumor suppressor in somatic cells. Although p63 and p73 are implicated in developmental control in epithelial stem cells and neurogenic processes, p53 does not have an obvious developmental role in mammals.[48,49)] p63 isoform, TAp63 is induced in oocytes after exposure to IR to promote germ cell apoptosis in mouse germ line.[50)] *cep-1* which is a unique p53-like gene in *C. elegans* is considered as a primordial p53 family member. CEP-1 is not required for IR-induced cell cycle arrest.[25,26)] DNA damage-induced germ cell apoptosis by CEP-1 may be an ancestral function of p53 family members.

Initiation of meiotic recombination and DNA lesions by IR

Meiotic recombination is strictly controlled by a conserved mechanism throughout eukaryotes. During meiosis, homologous recombination is initiated by DSBs that are created by SPO-11, a topoisomerase II-like enzyme. IR-induced DSBs can bypass the requirement of SPO-11 in initiating meiotic recombination, leading to formation of crossovers and functional chiasmata in *spo-11* mutants (Fig. 4).[51)] *C. elegans* MRE-11 and RAD-51/RDH-1, components required for DSB repair are also involved in meiotic recombination (Fig. 4).[12,52,53)] Rad-51/RDH-1 depletion by RNAi or deletion causes uncondensed chromosomal morphology in diakinesis oocyte and consequently embryonic lethality due to incomplete meiotic homologous recombination.[12,54,55)] Because of the additional unrepaired DSB generation, IR accelerates

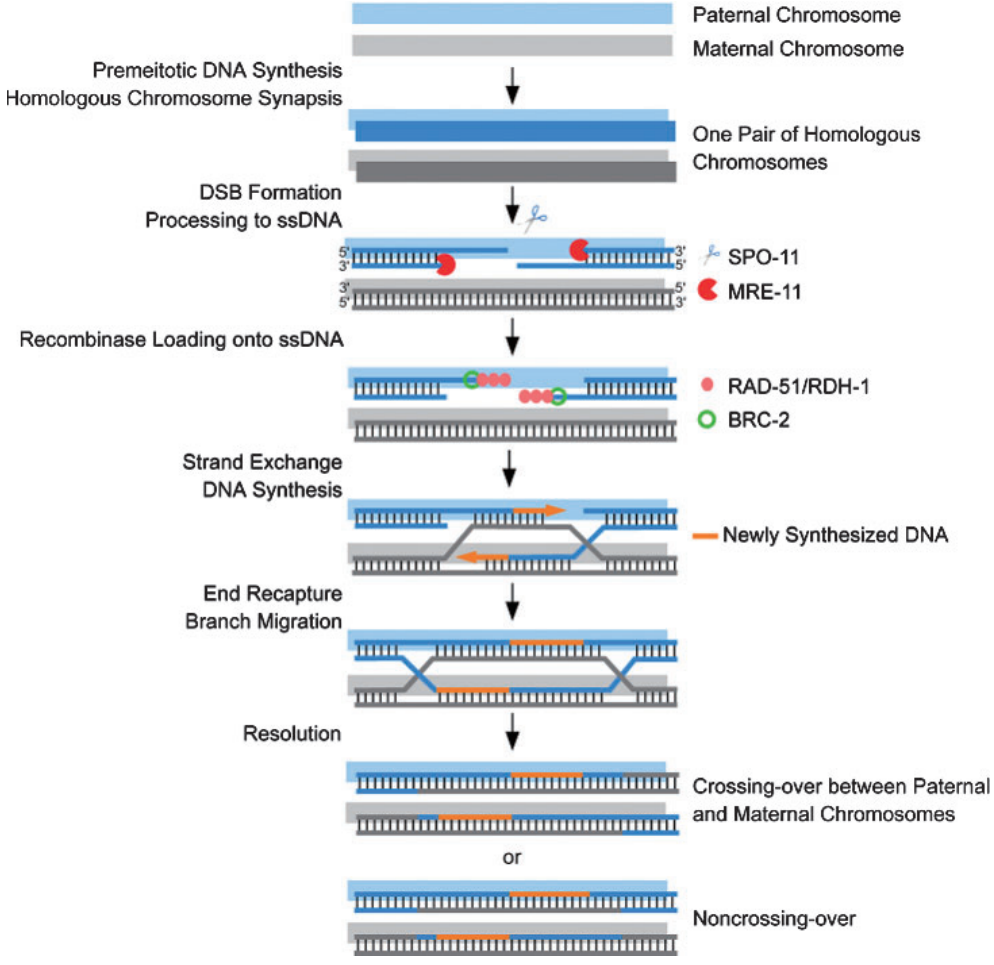

Fig. 4. Molecular mechanism of meiotic recombination in *C. elegans.* Meiotic recombination begins with a DSB made by SPO-11. The 5'end of this break is resected by MRE-11, producing stretches of 3' single-strand DNA (ssDNA). Monomers of RAD-51/RDH-1 polymerize to form a nucleoprotein filament onto the processed ssDNA. BRC-2 interacts directly with RAD-51 through its single BRC domain, facilitating RAD-51 loading onto the ssDNA. The RAD-51-DNA filament searches for the homologous template and invades into an intact homologous sequence with the formation of heteroduplex DNA. This is followed by the DNA synthesis that extends the end of the invaded strand, and then by recapture of this strand. The chromosome then can be resolved into a cross-over or a noncross-over.

chromosomal abnormality in the oocyte of RAD-51/RDH-1 depleted animals.[12] *mre-11* homozygote exhibits twelve univalent chromosomes in the oocyte and shows loss of chiasmata formation.[53] At 18 h after gamma irradiation in L4 larval stage, abnormal chromosomes that tend to be clumped or aggregated are observed in the oocytes of *mre-11* mutant. In contrast, oocyte chromosomes in the wild-type animals irradiated with the same dose are morphologically normal, and embryos derived from these oocytes can hatch. Examined oocytes in these experiments were derived from pachytene nuclei when the worm had been irradiated (Fig. 2A). Table 1 summarizes the effect of IR on chromosomal morphology in diakinesis oocytes of *spo-11, mre-11* and *rad-51/rdh-1* mutants. The abundant localization of RAD-51/RDH-1 protein has been observed from the late zygotene to the midpachytene stages under normal conditions in the wild-type animals.[56] Germ line nuclei accumulate RAD-51/RDH-1 foci at DSB sites following IR in a BRC-2 dependent manner.[56] BRC-2 is an ortholog of mammalian BRCA2 (BREAST-OVARIAN CANCER, FAMILIAL, SUSCEPTIBILITY TO, 2) and indispensable for normal meiotic recombination pathway through direct interaction with RAD-51/RDH-1. Therefore IR-induced DSBs can be repaired using meiotic homologous recombination machinery. Our previous report shows that pachytene nuclei in wild-type animals are hyper-resistant to IR compared to embryos.[12] Hatching rate of eggs laid by irradiated *ced-3* mutant which is deficient in apoptotic pathway are the same level as wild-type animals.[13] Hyper-radioresistance of the pachytene nuclei are due to strong expression of enzymes involved in homologous recombination rather than to apoptotic exclusion.

Greiss *et al.*[14] and we[24] have reported that 83 and 136 genes are up-regulated more than two fold after exposure to IR using whole genome Affymetrix GeneChip microarray, respectively. Out of induced genes, only three, *ced-13* and *egl-1*, and *pme-5* (an ortholog of mammalian tankyrase 1) are involved in apoptosis induction and DNA repair, respectively. The other induced genes following IR are involved in genes in response to abiotic and biotic stresses, small heat shock protein genes, glutathione S transferase-like genes, cytochrome P450 family, cadmium responsive genes, and innate immunity genes.[14,24] It suggests that many genes required for checkpoint control, apoptosis, and DNA repair are not transcriptionally controlled in *C. elegans* adult hermaphrodites. Therefore IR induced foci formation of DSB repair proteins in germ cells does not depend on transcriptional activation but might depend on subcellular relocalization because of their abundant expression throughout meiotic prophase.

Table 1. Effect of IR on chromosomal morphology in diakinesis oocytes of mutants defective in homologous recombination.

Genotype	Unirradiation	18–24 h after IR irradiation
WT	6 bivalents	6 bivalents
spo-11$^{-/-}$	12 univalents	6 bivalents
mre-11$^{-/-}$	12 univalents	clumped, aggregated
rad-51/rdh-1$^{-/-}$	uncondensed	clumped, aggregated

The genome-wide RNAi screening of *C. elegans* gene for increasing radiosensitivity of germ cells has identified 45 genes, including cell cycle, apoptosis and DNA repair related genes.[15] All DNA repair genes found in the screening are related to homologous recombination repair. The error-free homologous recombination pathway could be the predominant way in which *C. elegans* germ cells deal with DSBs.

Chemotherapy and radiotherapy against young cancer patients have increased long-term survival, but side effects of these treatments are ovarian failure and infertility.[57] Mammalian germ cells are sensitive to genotoxic stress, but recent paper shows that mammalian follicles change their radiosensitivity during ovarian development. Irradiation during oogonia proliferation (12.5 dpc; days post conception) and diplotene/diakinesis stage of meiotic prophase I (1 dpp; day postpartum) induces almost total disappearance of the follicular reserve, whereas primordial follicles remain when irradiated at 14.5 dpc (zygotene) and 18.5 dpc (pachytene), respectively.[58] IR sensitivity of follicles is related to induction of caspase-2 activation to promote apoptosis.[58] Together with the results obtained from *C. elegnas,* the expression of meiotic recombination proteins in 14.5 and 18.5 dpc follicles may also contribute to radioresistance at these stages in mammalian oogenesis.

Application of heavy-ion microbeam irradiation

In mammals, the biological effectiveness is known to vary with the linear energy transfer (LET) of radiation. High-LET heavy ions induce DNA deletions, generally greater than 1,000 base pairs in size, whereas low-LET X- or gamma-rays induce base substitutions and deletions of less than 100 base pairs.[59] Because of its strong biological effects, heavy ions have been applied in cancer therapy.[60] Our previous study shows that argon- and carbon-ion irradiation to the adult hermaphrodites of *C. elegans* caused mitotic germ cell proliferation arrest, and that swollen nuclei at distal tip of gonad appeared at 24 h after irradiation (Fig. 5).[36] Similar to the effects of IR, pachytene nuclei are able to repair heavy ion-induced DNA lesions, whereas mature oocyte failed (Fig. 5).[36,61] This fact suggests that large DNA deletion can be repaired by meiotic homologous recombination.

Additionally, a novel concept for radiation effect, the bystander effect, in which cells that have received no irradiation show biological consequences from their neighboring irradiated cells, has been established with microbeam irradiation system.[60,62–64] We also show that the microbeam irradiation is useful in characterizing a tissue-specific, local

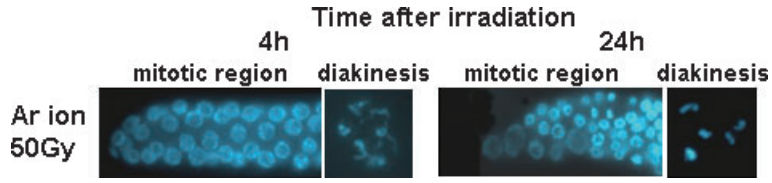

Fig. 5. Chromosomal structure in the mitotic nuclei of the gonad tip and in the diakinesis oocytes just before entrance into the spermatheca at 4 and 24 h after argon particle irradiation, as observed by staining with DAPI. At 4 h after irradiation, mitotic nuclei are morphologically normal, but nuclei in the diakinesis oocyte are abnormally uncondensed. In contrast, at 24 h after irradiation, swollen nuclei are observed in the mitotic region, but diakinesis oocyte contains morphologically normal six bivalents.

biological response to radiation in *C. elegans* germ line cells.[61] Following local irradiation of the tip region of the gonad arm with carbon-ion microbeam, inhibition of germ cell proliferation and swollen cells are revealed in the targeted gonad arm. As well as the irradiation, induction of germ cell apoptosis is observed following irradiation of the pachytene region. On the other hand, in the non-irradiated neighboring region or the non-irradiated opposite gonad, there is little, if any, bystander effect related to cell cycle arrest and apoptosis in germ line cells of *C. elegans.*[61]

C. elegans has contributed as a useful model system to investigate effects of IR on germ cells, especially cell cycle checkpoint, apoptosis and DNA repair. In general, dysfunction of molecules involved in these pathways causes genomic instability, apoptosis resistance and consequently cancer development. The molecular mechanisms elucidated from studies in *C. elegans* can provide a new aspect of human system for radiotherapy against cancer. Although genetic pathways required for these responses have been revealed vigorously, direct regulatory mechanisms between molecules remain unclear. Further understanding of regulatory mechanisms may help understand mechanisms underlying genomic instability in cancer and discover putative anticancer drug targets.

AGING (OXIDATIVE STRESS)

Over the past century, many theories of aging have been proposed and demonstrated experimentally using various cells and model organisms. Particularly, the free radical theory[65] that a cause of aging is the accumulation of oxidative damage in intracellular constitutive molecules appears to be the most popular one to explain normal aging in various organisms. Namely, it had been primarily proposed that free radicals formed at random in a biological system from both enzymatic and non-enzymatic sources would be assumed to produce various deleterious changes same as radiation effects. In the presence of oxygen, the IR-induced free radicals including the hydroxy radical ($OH^{\cdot}$), superoxide radical ($^{\cdot}O_2^-$), and organic radicals (R) are thought to be formed in the cells. Subsequently, these free radicals become a trigger to produce other reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2) and organic hydroperoxides (ROOH).[66] Unlike the case for IR, free radicals produced during aging are induced through reactions that involve molecular oxygen catalyzed in cells by oxidative enzymes;[65] however, the free radicals may share common features with IR-induced radicals, such as for induction of cell death, mutation and cancer.[65,67] The organismic influences in aging are commonly characterized by progressive degenerative changes in tissue organization and function that increase the probability of mortality.[68]

The general correlation linking the levels of a major intracellular protective enzyme against the oxygen-derived toxicity such as superoxide dismutase (SOD) with the maximum lifespan potential was found in various mammalian species in 1980.[69] Similar correlations were observed for several other free radical scavengers including ascorbic acid, carotenoids, and vitamin E.[70] These correlations show that longer-lived species have a higher degree of protection against ROS generated as by-products of oxygen metabolism. In addition, it was established that the longer-lived species have generally low levels of specific metabolic rate. Therefore, it is suggested that the accumulation of oxidative damage due to intracellular ROS production in aerobic metabolism is closely associated with normal aging and lifespan determination in the organisms. Further evidence for the oxygen-derived free radical theory to understand normal aging was obtained by using short- and long-lived mutants in invertebrate and vertebrate model including *C. elegans*, fruit fly, and mouse. The logic of this reasoning was confirmed through experiments conducted on the fruit fly *Drosophila melanogaster*, and a significant lifespan extension was observed in transgenic animals overexpressing both SOD and catalase genes.[71] There was an objection about the effects on lifespan in transgenic flies carrying only Cu/Zn SOD gene; however, it seems that this phenomenon is explicable considering the higher reactivity of H_2O_2 produced by catalysis of intracellular SOD.[72] Alternatively, a defect in the mitochondrial Mn SOD, Sod2 in mice exhibited the phenotypes of neonatal lethal dilated cardiomyopathy and accelerated apoptosis in aging.[73]

Recent investigations suggest that the evolutionary theories stating the previously proposed mechanisms of aging are correct at least in *C. elegans*. Genetic studies of age-related mutants in *C. elegans* have particularly focused on the existence of genes and intracellular pathways taking parts in the aging process. *C. elegans* normally reproduces as a self-fertilizing hermaphrodite, and the mean lifespan of the wild-type animals is around 20 days at 20°C. The *age-1* mutant, which lives significantly averaging 40–60% longer than wild-type and is resistant to oxidative stress, is a most primary case for the genetic isolation and analysis related to aging (Fig. 6).[74,75] Other long-lived mutants, *daf-2*,[76] *eat-2*,[77] and *clk-1*[78] have also resistance against environmental stress including oxidative stress. In addition, the lifespan of transgenic animals overexpressing co-chaperone such as HSP70 is also prolonged compared with noncarrying wild-type.[79] Conversely, short-lived mutants, *mev-1*[80] and *daf-16*[81] have low levels of antioxidant enzymes such as SOD and catalase. Recently, it has been identified that some of these genes play a role as a member of the intracellular insulin/insulin-like growth factor-1 (Ins/IGF-1) signaling pathway associated with aging (Fig. 7). In the pathway, a mutation of a transcription factor *daf-16* is known to suppress the long-lived phenotypes of *age-1* and *daf-2* mutants.[82–84] A phosphatidylinositide 3-kinase encoded by *age-1* gene is located upstream of the DAF-16 forkhead transcription factor, which is homologous to the human forkhead members of the O class.[85,86] Under stress conditions, numerous downstream targets of DAF-16, which control the antioxidant and mitochondrial respiratory chain systems, are serially activated in *C. elegans*. In particular, the evidence that the Ins/IGF-1 signaling controls the mitochondrial bioenergetics by adjusting the rate of ATP synthesis and by regulating the heat-producing proton leak pathway had been established.[87] In addition, the *clk-1* gene encodes the *C. elegans* homolog to a mitochondrial inner membrane protein in coenzyme Q synthetase,[88] and the *eat-3* gene encodes a mitochondrial dynamin family member homologous to Opa1 in humans.[89] Thus, production of ROS, which are by-products of the energy production system in the mitochon-

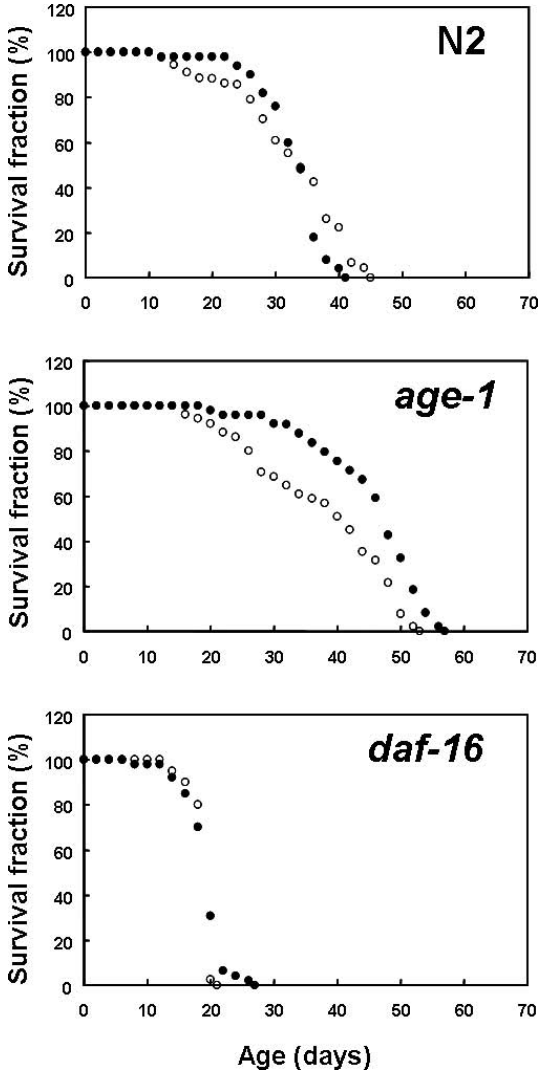

Fig. 6. Survival curves at 20°C in several age-related mutants with hyperoxia-induced hormetic effect. Open circles, without pre-exposure to hyperoxia; closed circles with pre-exposure.[94]

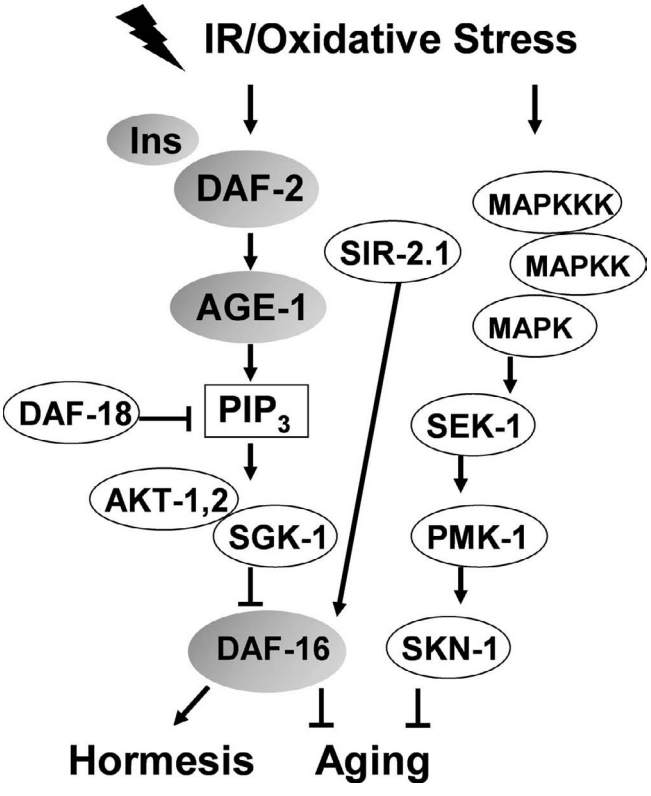

Fig. 7. Ionizing irradiation- and oxidative stress-induced signaling pathways and their modulators.

drial respiratory chain, occurs mainly as a result of trade-off with cellular energy-coupling ATP synthesis in aerobic organisms. Consequently, it is assumed that organisms cannot avoid being exposed to ROS as far as they acquire energy via the mitochondrial respiration. It is possible that the balance between ROS accumulation (via mitochondrial respiratory chain system) and elimination (via antioxidant systems) determines the intrinsic aging rate and lifespan of the mutants. The intracellular ROS balance is likely to be regulated (and finely adjusted?) through the Ins/IGF-1 signaling as an aging pathway. In addition, it seems that ROS affect the longevity of organisms through the activation or inactivation of other various intracellular pathways (e.g., for dietary restriction).[90]

The existence of intracellular ROS is equally important for hormesis or adaptive response to the biological effects of low-level exposure to chemical agents and radioactivity. At first, human lymphocytes were given large acute doses of X-rays after exposure to weak IR.[91] The results are consistent with the concept that exposure to chronic low levels of radiation can trigger or induce increased repair of radiation-induced chromosome breaks. Likewise, similar adaptive response related to low levels of environmental stress was found in *C. elegans*, and it was assumed that the moderate lifespan extensions sometimes observed after irradiation were mediated by any means other than the induction of DNA repair enzymes.[92] Furthermore, similar lifespan extensions in worms exposed to small doses of another environmental stressors such as X-ray irradiation, thermal stress or hyperoxia were recognized as a hormetic effect.[92–95] In fact, the intracellular ROS levels increased in *C. elegans* continuously exposed to hyperoxia, and subsequently the SOD activities were induced.[96,97] Thus, an increase in oxidative stress-related hormetic effects would be expected if the intracellular ROS levels were temporarily amplified in *C. elegans*. For example, an intermittent hyperoxia exposure induced the hormetic effect such as lifespan extensions in the wild-type and long-lived *age-1* mutant (Fig. 6).[94,95] It has been deduced from a recent study that the hyperoxia-dependent hormesis may lower the mitochondrial $\cdot O_2^-$ levels and increase the enzymatic antioxidant such as SODs and catalases by the activation of the Ins/IGF-1 signaling pathway.[81] However, the explanation about the hyperoxia-dependent induction of the gene expression, which is not regulated by the pathway, is inadequate. In this respect, other evolutionarily conserved signaling systems that are also associated with normal aging, stress resistance and host defenses such as the p38 signal transduction pathway may play important roles in the hormesis-inducible lifespan extension.[98]

Thus, an aging process has been understood as the common phenomenon, which is intrinsically hard to resist for most organisms, and the genetic and environmental elements intertwine with each other complicatedly in it. As an intracellular signal transduction system of aging and oxidative stress have been discovered at first in nematode *C. elegans*, the investigation using these model organisms are sequentially expected to contribute to new data for the understanding of the mechanism of normal aging in higher organisms. Furthermore, they would help develop various compounds and dietary supplements to regulate the aging of more higher organisms.

BEHAVIOR

Mammalian studies have revealed that irradiation with the sublethal dose declines behaviors such as shock avoidance,[99] motor performance,[100] spatial navigation,[101] taste aversion[102] and learning.[103] It has been thought that the depression of shock avoidance and motor performance arises from the reduced activity of neurotransmitters such as dopamine and sodium channel, which is temporarily altered in the rat brain after irradiation.[104,105] In addition, IR impaired synaptic plasticity.[106,107] Spatial navigation and learning impairments are thought to be caused by radiation-induced suppression of an adult neurogenesis in hippocampus, which is important for new learning and memory.[103,108] Neurotransmitter and adult neurogenesis studies have contributed much to our knowledge of radiation-induced effects on behaviors. Moreover, the radiation response of the central nervous system and IR-induced redox changes were reviewed elsewhere.[109,110] However, it is still largely unknown about integrations of signals on a neuronal network following radiation exposure.

C. elegans shows a variety of behaviors, e.g., learning-behavior[111,112] motor-behavior,[113] and social-behavior,[114] when it detects and responds to environmental conditions. All of 302 neurons and these connections with synapses and gap junctions have been positionally identified.[3] In addition, we can investigate the behaviors at the gene level because pioneering studies revealed that the mutation of the specific gene can defect the specific behavior.[115] Therefore, *C. elegans* is an attractive model organism for the study of the behaviors based on the paradigms, neuronal circuits and genes.

Although the evidence is limited, several studies have recently investigated the effects of IR on learning- and motor-behaviors. We reported the radiation effects on the salt (NaCl) chemotaxis learning behavior (Fig. 8), which was classified to the associative learning.[116] Associative learning is the learning process through association with separate, or pre-occurring cues.[111,112,117–119] The well-known learning paradigm of "Pavlov's dog" is a kind of associative learning. In the salt chemotaxis learning, a taste aversion to NaCl occurred in *C. elegans* simultaneously experienced exposure to NaCl and starvation (Fig. 8A). It was found that the salt-chemotaxis learning behavior was modulated by radiation exposure only during learning (Fig. 8B).[116] The radiation-induced modulation was the enhancement of learn-

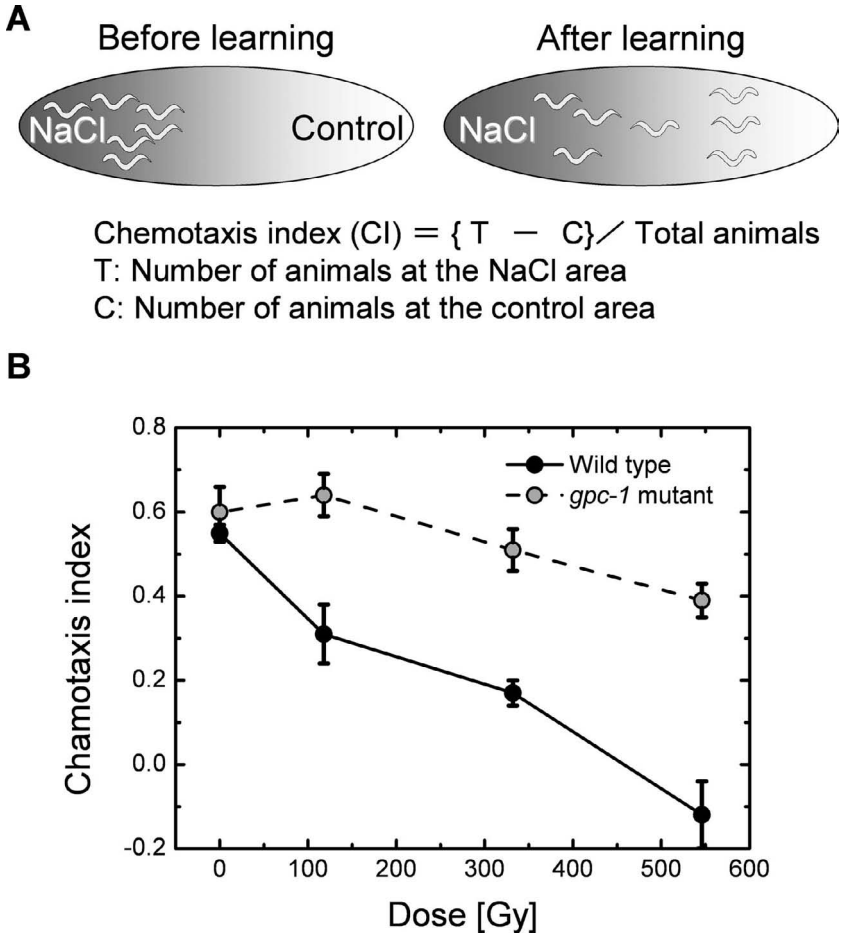

Fig. 8. Salt chemotaxis learning and radiation effects. **A)** Before the salt chemotaxis learning, *C. elegans* animals are attracted to the spot of the salt (NaCl). However, after learning, chemotactic behavior to NaCl of *C. elegans* is suppressed. This behavioral change is evaluated using the chamotaxis index. "T" and "C" indicate the number of animals in the NaCl and control areas, respectively. **B)** Chemotactic change in the salt chemotaxis learning is enhanced by IR irradiation in wild-type animals, but not in the *gpc-1* mutants. The error bars represent the SEM.

ing behavior, and promoted taste aversion to NaCl. It is well known that IR induces a taste aversion in rodents: for example, rats exposed to IR subsequently avoid ingestion of a tasting sweet-favorite solution.[102,120] One may imagine that the radiation-induced enhancement of the salt chemotaxis learning in *C. elegans* is the same phenomenon as the radiation-induced taste aversion learning in rodents. However, there is a fundamental difference between the phenomena observed in *C. elegans* and rodents, such that because IR is an unconditioned stimulus of an associative learning paradigm in the taste aversion learning in rodents, but is not that in the salt chemotaxis learning (i.e., as a modulator for taste aversion) in *C. elegans*.[116] Radiation-induced modulation of the salt chemotaxis learning in *C. elegans* is a fully novel radiation response on behavior.

The effect of IR on the salt-chemotaxis learning might reflect the effects on specific circuits of the nervous system in *C. elegans*. *C. elegans* senses water-soluble attractants of cAMP and lysine via ASE sensory neurons that sense NaCl,[121] and volatile organic compound, benzaldehyde via AWC neurons connected to ASE neurons.[122] Chemotaxis to cAMP, but not to lysine and benzaldehyde, was influenced by IR during the salt chemotaxis learning.[116] Therefore, there are the effective sites of IR in the nervous system of *C. elegans*, suggesting that the sensitive targets of IR for the salt chemotaxis learning are not whole neurons. Moreover, the radiation-induced modulation of the salt chemotaxis learning was significantly suppressed in the *gpc-1* mutant, which was defective in GPC-1 (one of the two gamma subunits of the heterotrimeric G-protein expressed only in the several sensory neurons).[116] These findings suggest that IR affects the nervous system via specific sensory neurons, altering learning behavior (Fig. 9). In other model organisms, it is reported that electrophysiological activity of a

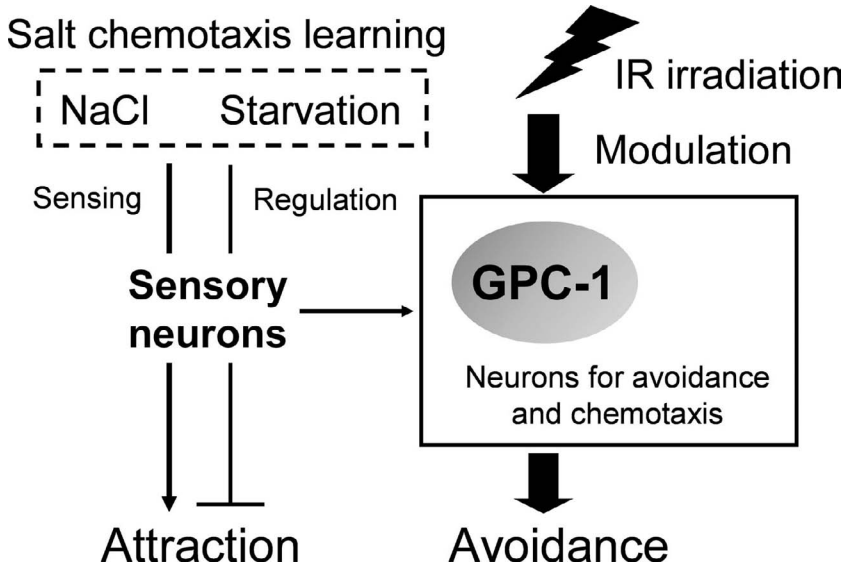

Fig. 9. Possible model of the IR-induced modulatory effects on the salt chemotaxis learning of *C. elegans*. The sensing and regulation pathways for attraction to NaCl is based on the findings of a previous report.[111,134] IR behaves as a modulator in the salt chemotaxis learning via *C. elegans* GPC-1 and a specific neuronal network.

neuronal population in the Guinea Pig hippocampus following radiation exposure (5–10 Gy) was complicatedly altered in a dose and dose-rate dependent manner,[106,123] and spikes of *Aplysia* pacemaker and sensory neurons were significantly increased after irradiation (5–10 Gy).[124,125] Also, the researchers proposed a hypothesis that IR could directly affect the integrated functional activity of neurons. The radiation-induced GPC-1 mediated pathway in *C. elegans* may support this hypothesis.

The effects of IR on the motor-behavior have also been studied. We reported that the motor performance of *C. elegans*, locomotory rate, was reduced by radiation exposure.[126] We further investigated the relation between radiation-induced reduction and bacterial mechanosensation-mediated reduction of the locomotory rate using *cat-2* mutants which defect the mechanosensation-mediated basal slowing response due to a mutation in *cat-2* gene for dopamine synthesis.[127] The locomotory rate of *cat-2* mutants in the absence of bacteria, i.e. without bacterial mechanosensation, was reduced after irradiation. This suggests that the radiation-induced reduction of the locomotory rate is mediated by the different pathway from the CAT-2 related dopaminergic pathway for bacterial mechanosensation. On the other hand, exposure to hydrogen peroxide (one of ROS produced by IR) resulted in the reduction of the locomotory rate.[127] Jones *et al*. reported that the isothermal-tracking learning behavior of the *mev-1* mutant,[128] which has an intrinsic oxidative stress, was improved by a pretreatment with a metabolic antioxidant.[129] From these findings, ROS may be an important key molecule of the radiation-induced behavioral change in *C. elegans*.

Altogether, recent works propose the following important insights into the radiation-induced effects on the nervous system of *C. elegans*: *1)* there are several specific sites affected by radiation exposure, *2)* IR behaves as a cue for behavioral change, *3)* ROS may be one of mediators on the behavioral change, and *4)* radiation-induced stimuli are modified by the other stimuli. To address these issues, it might be important to establish the novel method to understand the signal transmission and integration on the neuronal circuits of *C. elegans*, e.g. systems neurobiological approach with mathematical models,[130,131] in addition to the biological measurement methods.

To investigate the potential risks with interplanetary manned space missions, the study of neuronal plasticity is in progress in the field of space radiation biology.[132] The neuronal integration or plasticity as a consequence of radiation-induced alteration on the electrophysiological, synaptic and neurotransmitter activity might be an important key to understand the human radiation effects on behavior. *C. elegans*'s works of radiation effects on behaviors provided the important insights into the integrations of signals on the nervous system, whereas mammalian studies did not approach to them. Therefore, future radiation biology on behavior will need a large number of studies using model organisms such as *C. elegans*. In addition to the types of behavior that have been analyzed so far, further studies may need to characterize other types of the behavioral response to IR (e.g., odorant associative learning, defecation reflex, mating, egg laying, osmotic avoidance, pharyngeal pumping cycle).

CONCLUDING REMARKS

This review presented some of the background and the recent works on the radiation-induced effects in *C. elegans*. These effects are divergent, and range from deleterious effects to radiation-induced signaling and hormetic responses. The biological mechanism is highly different between mammals and *C. elegans*, but regulatory and basic radiation-induced mechanisms appear to be more conserved across animals. Bertucci *et al*. recently reported the increase of HSP-4 protein in the non-targeted area of *C. elegans* 24 h after microbeam irradiation.[133] This may suggest the radiation-induced metabolic oxidative stress in addition to the bystander effect derived from radiation damage to DNA and membrane. In addition, the study of radiation-induced behavioral changes has a potential impact to provide how to affect the oxidative stress in behavior. Moreover, recent studies have reported the common players for radiation response and aging (AKT-1 and SIR-2.1 in Figs 3 and 7),[14] as well as for aging and the salt chemotaxis learning (Ins/IGF-1 signaling pathway).[134] These findings may suggest a common or cross-talk mechanism that underpins environmental stress responses (IR, oxidative stress, starvation, etc). Finally, *C. elegans* might be engaged as an attractive "*in vivo*" model system for radiation biology, and the study of radiation effects in this organism may also be relevant to the basic biology.

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