Why is the low-energy ion beam fluence to induce cell mutation orders higher than the cell lethal dose? – A puzzle

L.D. Yu[1,2]

[1] Plasma and Beam Physics Research Facility, Department of Physics and Materials Science, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[2] Thailand Center of Excellence in Physics, Commission on Higher Education, 328 Si Ayutthaya Road, Bangkok 10400, Thailand

e-mail address: yuld@thep-center.org

Abstract. Experimental facts showed that in application of low-energy ion beam for biological living materials modification, the ion beam fluence required to induce cell mutation is orders higher than the cell lethal dose. This seems contradictory with common perception that the DNA modification should be proportional to radiation dose so that high dose radiation could cause high-degree damage in DNA to lead to cell death, while limited DNA damage produced by relatively low dose radiation would facilitate cell mutation. The author provides an answer to the puzzle from both physics and biology. Key points include the difference in physics between high-energy ionizing radiation and low-energy ion beam irradiation and the cell's non-linear behavior of responding to exogenous actions in biology.

1. Introduction

Ion beam modification of biological living materials has been developed as the ion beam biotechnology for a few recent decades, particularly applied as a novel mutagen, owing to its advantages in inducing biological effects over conventional radiation mutagens (e.g. [1,2]). There have been many papers elaborating biological effects on cells (in general or particular) for death or mutation and DNA damage from radiations, but no one referring to such a question that which one, cell death or mutation, occurs first as the radiation dose or time increases. In ion beam applications to biology, there is a puzzle. It is well known that the cell lethal dose is in a range of a few Gy to 10^2 Gy, depending on the radiation and cell types. In development of ion beam biotechnology, in parallel to use of high-energy (generally > 10 MeV) ion beams, low-energy (< 10^2 keV) ion beams have also been utilized to induce crop mutation breeding, owing to advantages such as low cost, simple facility and easy operation and maintenance which make the technology more feasible to developing countries. For crop plant cells, different in the ion beam fluence applied to mutation induction from the high-energy ion beams, the low-energy ion beams to induce cell mutation normally require a beam fluence much higher, e.g. in orders of 10^{16} – 10^{17} ions/cm^2. The fluence mentioned above is corresponding to radiation doses in orders of around 10^8 Gy or even more, many orders higher than the cell lethal dose. Common perception is that when a cell is attacked by external actions, the amount of the DNA changes or damage should increase with the increase of the amount of the actions, namely the more the actions, the more the changes or damages. At lower level DNA changes, some

irreversible changes including double strand breaks (DSBs) may lead to mutation, whereas at higher level DNA changes, a large quantity of irreversible DSBs dominates the damage leading to cell nonfunctional and thus to death. This implies a monotonousness of cell's response to the external actions, i.e. the cell mutation first and cell death later when a cell suffers from continuous external actions such as irradiation with an increasing dose. This is virtually in conflict with the experimental facts of low-energy ion beam irradiation of biological living matter (as shown below).

2. Experimental facts

Examples shown below are some results from relevant experiments carried out in our studies of low-energy ion beam irradiations of both plant and mammalian cells and naked DNA.

Irradiation of Thai purple rice seeds with 60-kV accelerated $N+N_2$-ions (mixed atomic and molecular nitrogen ions) to a fluence of 1×10^{16} ions/cm^2 resulted in the majority of seeds to die at about a 60% death rate and very few to mutate at only a 0.5% mutation rate [3], indicating easy death but difficult mutation of the seeds. Irradiation of Thai jasmine rice seeds with N-ions induced potential mutations of the grown rice at the ion energy of 30 – 120 keV and the fluences of 1-15 $\times 10^{16}$ ions/cm^2 [4]. Bud explants of a species of flower chrysanthemum, *Dendranthema morifolium*, were irradiated with 60-keV N-ions to fluences ranging in $1 - 8 \times 10^{16}$ ions/cm^2, resulting the death of the plant starting from the fluence of 4×10^{16} ions/cm^2, whereas flower mutation starting from the fluence of 6×10^{16} ions/cm^2 [5]. Flower *Curcuma* embryo cells were irradiated with 30-keV Ar-ion beams to varied fluences [6], resulting at 4×10^{15} ions/cm^2 most of the cells starting to die, but the flower mutation occurring at 6×10^{16} ions/cm^2. Cells of HEp-2, the human laryngeal epitheloid cancer cell line, were irradiated by nitrogen ions at energy of 14-28 keV to fluences ranging from 1×10^{15} to 1×10^{16} ions/cm^2, resulting the cells irradiated with 14-keV N-ions starting to die at 10^{15} ions/cm^2 [7]. However, subsequent culture of the cells surviving from the irradiation with all applied fluences did not show any mutations induced. Beams of various ion species, including He, C, N, Ar, etc., at very low energy (< 1 keV) irradiate naked DNA samples of plasmid pGFP to study effects on DNA change or damage. The minimum ion energy and fluence of inert ion species to cause DSBs should be around 1 keV and 10^{15} ions/cm^2, respectively [8,9]. Other international groups have also found similar facts that low-energy ion beam irradiation of crop seeds induces comparatively high death rate but low mutation at the ion energy and fluence levels (e.g. [1]) the same as we applied. Ion beam fluence can be converted to radiation dose:

Dose (Gy) = [Fluence(F)×Energy(E)]/[Mass density(ρ)×Range$_{max}$(R_{max})] = $FE/\rho R_{max}$ (J/kg).

For example, in the case of N-ion beam irradiation induced crop mutation, let us take the ion energy as 30 keV, beam fluence as 10^{16} ions/cm^2, and the mass density of the rice seed as approximate 1 g/cm^3. The 30-keV N-ion has a range of about 0.12 μm and the range straggle of about 0.03 μm in biological tissue and so R_{max} = 0.15 μm from SRIM calculation [10]. Therefore, the dose is ~ 10^{11} Gy. For the case of ion irradiation of naked DNA, if the ion species is Ar at the energy 1 keV and the fluence 10^{15} ions/cm^2, which are the threshold to cause DNA DSBs [8], the mass density as about 1 g/cm^3 and the range as 4 nm [11]. Then, the dose is ~ 10^8 Gy. Therefore, in both examples, the converted doses are seen considerably higher than the cell lethal dose, around 10^2 Gy in maximum.

In comparison, high-energy ionizing radiations generally have the doses to induce genetic mutation in the same order as the cell lethal dose. For example, in the case of ^{60}Co-gamma-ray irradiation, the lethal-dose$_{50}$ for rice seeds was a-few-hundreds Gy [12]. To induce mutation of plasmid DNA pUC$_{18}$, the gamma-ray needed doses of only a-few-tens Gy [1]. The effective dose to induce rice mutation in the case of gamma-ray radiation is around the order of 100 Gy [13]. In high-energy particle irradiation of plant seeds to induce mutation, 200-MeV C-ion beam needed a dose in an order of 10^2 Gy, and 2-MeV electron beam needed a dose nearly 10^3 Gy [2]. In 3.8-MeV proton irradiation of Chinese hamster V79 cells with either single-ion or broad beam mode, the lethal dose was a few Gy [14]. These facts indicate important differences in radiation-induced biological effects between high-energy and low-energy ionizing irradiations.

3. Discussion

Critical differences in physics between photon and particle radiations are that photons only input energy, whereas particles input not only energy but also mass (negligible for electrons), charge (no for neutrons) and momentum (negligible for electrons); photons penetrate materials considerably greater than particles (except neutrons) and ions have Bragg peaks whereas others do not. The critical difference in physics between high-energy and low-energy ionizing radiations of biological living materials, in addition to the penetration depth, is that the former process is dominated by electronic interaction while the latter process by nuclear interaction. Electronic interaction leads to significant ionization while the nuclear interaction dominantly brings about atomic displacement. In addition to direct effects, ionization can induce tremendous indirect effects, e.g. productions of secondary electrons, X-rays and free radicals, which could further interact with the target atoms to cause damage. Atomic displacement is a direct effect on damaging the target material structure. High-energy ionizing radiations take the advantage of the long penetration distance to interact with target atoms, while low-energy ion irradiations must take use of a high ion number density in the irradiated area to compete with the former for comparable radiation effects. For irradiation of plant cells, high-energy ionizing radiations can simply pass through the cell envelope which has a thickness around 10^{-1} – 10^{2} μm and then directly interact with DNA inside the cell; while low-energy heavy ions, which have the projectile range around the cell envelope thickness, first interact with the cell envelope to modify the envelope material structure. Thereafter, high-energy radiation causes the cell to die and be mutated at the same dose because the radiation instantly directly damages DNA leading to cell death and mutation simultaneously. But, low-energy heavy ion irradiation modifies or damages the cell envelope to cause the envelope malfunctioning or nonfunctioning which may result in cell death, and thus cell death first occurs. Some ions may pass through the cell envelope to interact with DNA at very low energy. To induce DSBs, there exists a threshold for a combination of the ion energy and fluence [8,9]. The threshold fluence is in an order of 10^{15} ions/cm^2. The ion fluence irradiating cells must be considerably higher so that the DSB threshold fluence can be guaranteed. DSBs may cause both cell death and mutation. But cells may look mutation like virus and so trigger apoptosis. The response of cells to irradiation is not “linear”. The cell likes to die first because dying is actually a self-protection mechanism of cells, whereas the cell dislikes mutation, which has been demonstrated by research on mechanisms involved in UV radiation effect on cell and DNA [15]. In nature, cells prefer unchanged in their all biological structures and functions. When they are forced to change, they prefer dying to build a protection barrier to prevent other living cells from being changed. Therefore, cell dying is the first while mutation follows if the exogenous action continues. Furthermore, the concept of the lethal dose of radiation conventionally refers to photon, light particle and high-energy ion irradiation, but in low-energy ion beam irradiation, nuclear interaction caused atomic displacement is dominant but rarely involved in the dose concept. That is why in low-energy ion beam irradiation the cell lethal dose and the ion beam fluence/dose of inducing mutation differ considerably.

It has been found from studies on low-energy ion beam irradiation of living materials that the survival rate as the function of irradiation fluence or dose is not monotonous, or not exactly logarithmically decreasing as predicted by the radiation effect theory, instead, in a manner of decrease-increase/stable-decrease, namely, a plateau appearing at a certain fluence range as increasing of the fluence (p. 104 and p. 148 in [1]). This abnormal survival-fluence behavior implies something happening in the cells when they are irradiated with the ion fluence that makes the survival rate increasing or stable. Interestingly note that the fluence that makes the survival rate increasing or stable is around 2-3 × 10^{16} ions/cm^2, which is just the fluence found in practice to start to induce mutation of crops. We may now speculate that when cells are irradiated, some of them start to die first because of the destruction of the cell envelope, and as the fluence increases, cell apoptosis is triggered, preferred by the cell itself to protect the cell and prevent it from being mutated, and consequently cells die continuously but mutation is hindered. A note must be made here that actually inductions of mutation and death always coexist but the two processes compete each other while irradiation persists in time or dose/fluence increase. In low-energy ion beam irradiation, at low fluences, some ions may have

occasional chances to hit DNA to possibly induce irreversible changes for potential mutation occurrence, but this occurrence is in a very low probability which cannot compete with a high probability of cell death occurrence. Hence, at low levels of fluence, cell death dominates. When the fluence increases to such a level that adjacent atoms respectively in each strand of the double strands of DNA can be possibly hit, DSBs and thus mutation induction become predominantly possible, and at this level mutation dominates. What should such a fluence level be? A pair of the DNA chains has a radius of 1 nm, or in general, two most adjacent atoms respectively in double strands are 2 nm apart. If the scale of an atom size is 1 angstrom (0.1 nm), to displace both such atoms respectively in double strands to realize a DSB, at least two ions are needed to bombard an area of 0.1 nm × 2 nm = 0.2 nm^2, namely, a fluence of (2 ions)/(0.2 nm^2) = 1 × 10^{15} ions/cm^2. This is the lowest fluence level to ensure DBSs, in an excellent agreement with our experimental result on the ion beam condition threshold to surely produce DSBs when low-energy ions irradiate naked DNA [8,9]. Note that this is only the fluence at naked DNA but not yet at the cell. The fluence irradiating the cell must be considerably higher than the fluence hitting DNA to ensure the threshold. SRIM simulation shows a small portion of the irradiating ions possibly travelling much deeper than the projectile range in target material, due to the range straggling. If this small portion is set to be 10%, to have a fluence of an order of 10^{15} ions/cm^2 at DNA, the fluence irradiating the cell should then be in an order of 10^{16} ions/cm^2. This is just what the experiments have found for low-energy ion beams to induce mutation. At this fluence level, DSBs are greatly produced and hence mutation is overwhelming cell death occurrence, leading to a plateau behavior in the survival rate curve against the fluence/dose. When the ion fluence continues to increase, up to around 3-4 × 10^{16} ions/cm^2, as the cell death also continues, the death gradually dominates over the mutation occurrence and then the survival rate starts to decrease again. Fig. 1 schematically summarizes the process discussed above and illustrates the cell and DNA responses to ion beam irradiation as a function of fluence in the case of 30-keV N-ion beam irradiation of crop seeds.

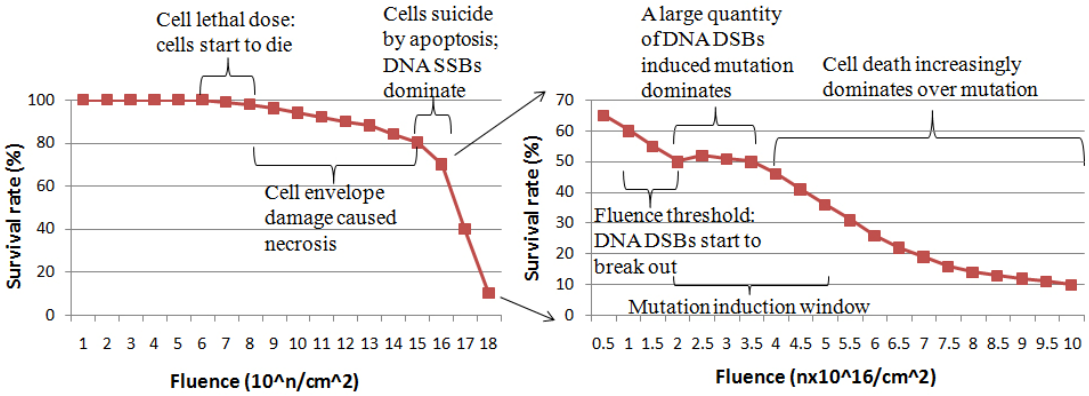

Fig. 1. Schematic illustration of the mechanisms of the cell and DNA behaviors during 30-keV N-ion beam bombardment of crop seeds with increased beam fluence. The plots are presented for the survival rate as a function of the fluence. The right plot is a zoom of the part for the fluence range of 0.5 – 10 × 10^{16}/cm^2 (the survival rate data roughly from p. 104 and p. 148 in [1]) in the left plot.

4. Conclusion

Low-energy ion beam irradiation of biological living materials has important differences in the dose levels to induce cell death and mutation from the high-energy ionizing radiation. Cell response to ionizing irradiation is not “linear” in the quantity of DNA damage as the function of dose. Low-energy ion irradiation needs the dose level to induce mutation orders higher than the lethal dose. This is because of the nuclear interaction domination feature of the low-energy ion irradiation of materials. The nuclear interaction results in dominant direct atomic displacement to induce easily predominant

cell necrosis which was preferred by the cell to prevent the cells from furthermore dying by building a protection barrier surrounding the surviving cells.

Acknowledgements
The work was supported by the Thailand Center of Excellence in Physics, the Chiang Mai University, and the International Atomic Energy Agency.

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