

The In-flux of Nuclear Science to Radiobiology

G. Taucher-Scholz, G. Kraft (GSI, Biophysik)¹
B. Michael (Gray Lab)²
M. Belli (INFN)³

¹GSI, Biophysik, Planckstr. 1, 64291 Darmstadt, Germany

² Gray Lab., Cancer Research Trust, Mount-Vernon-Hospital, P.O. Box 100, Northwood, Middlesex HA6 2JR, UK

³ Istituto Superiore di Sanita, Laboratorio di Fisica, Via le Regine Elena 299I, 00161 Rome, Italy

In general, radiobiology is mostly concerned with X-rays that are widely used in medical diagnosis like X-ray fluoroscopy or computerized tomography (CT) which both are products of atomic science. Nuclear science entered radiobiology rather late, around 1950, but then most intensely.

At this time, the long-term consequences of the nuclear bombing of Hiroshima and Nagasaki became evident and triggered public discussion. The catastrophe demonstrated that the nuclear bomb was not just a more efficient explosive than dynamite but had also unknown biological consequences that had to be studied in radiobiological experiments (see chapter “The Invisible Threat”). Thus, it was in a rather indirect way that nuclear physics stimulated radiobiological research. A more direct one followed when particle accelerators became available for radiobiological research and - quite more important - for the use in the radiotherapy of tumors in critical regions (see chapter “Ion Beam Therapy”). Finally, the advantageous properties of particle beams as a tool for radiobiological research has been discovered very recently, allowing for a precise research of repair mechanism and signal transduction of biological cells.

The Relative Biological Efficiency - RBE

In all these examples: radioprotection, radiotherapy and basic radiobiological research, a big difference can be observed in the effectiveness of particle radiation compared to X-rays. This different efficiency can potentiate the genotoxic and cancerogenic effects but can also be used for a more efficient tumor therapy. In research practice, the concept of the Relative Biological Efficiency (RBE) is introduced. RBE is defined as the ratio of X-ray dose to particle dose that is necessary to obtain the same biological effect [1]. $RBE = \frac{D_x}{D_p}$ Experimental studies found

RBE values around 3-4 but also higher values have been measured at low doses. It has been demonstrated experimentally that RBE depends on biological reactions as well as on physical

parameters of the applied radiation field, as for instance, particle energy and atomic number, indicating a difference in the structure of the energy deposition at a microscopic level¹.

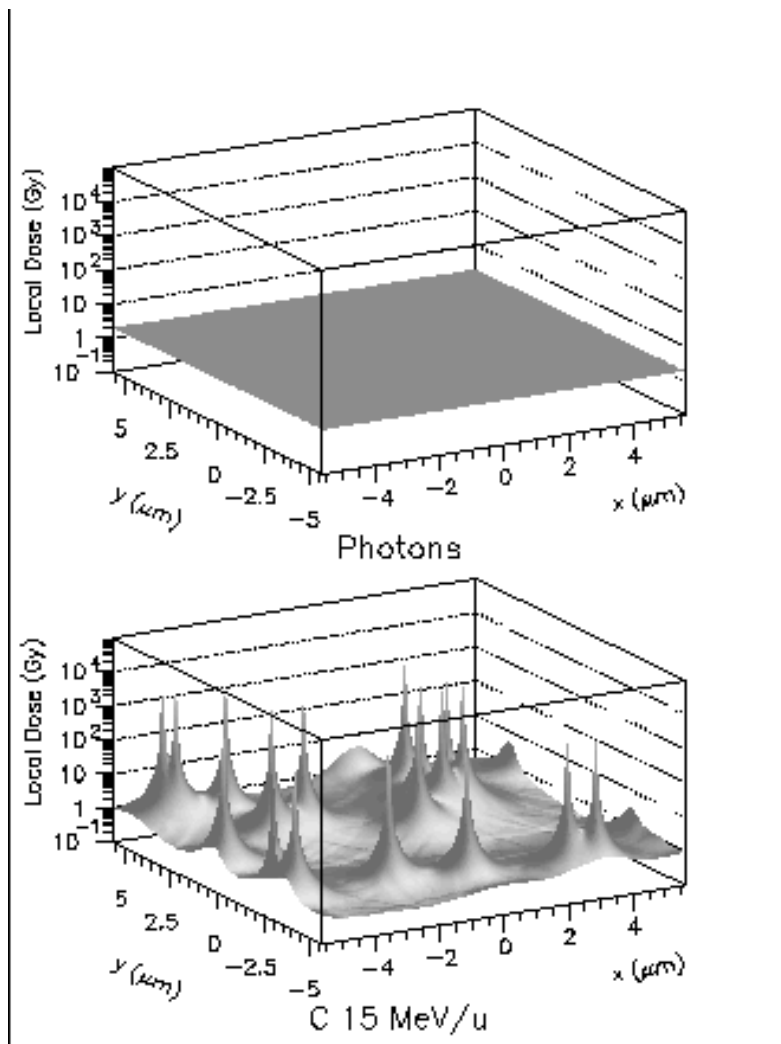


Fig. 1: Dose distribution in a micrometer scale for X-rays and stochastically distributed carbon ion irradiation of 15 MeV/u. For particles exposure the dose is delivered in individual tracks with doses up to Mega Gy in the center of the track and a $1/r^2$ decrease for greater radial distances. X-rays produce a homogeneous dose distribution.

For both, X-rays and particles, the biological effect is due to ionisation events mainly caused by the liberated electrons. This ionisation can take place in the DNA itself and the water molecules around. Once a free electron is produced, there is no difference in its biological action regardless of its origin: X-rays or particles [2]. The main difference between the two radiation types, however, is

the local distribution of the ionisation events as shown in fig. 1. Particles form tracks with high ionisation densities corresponding to local doses of thousands of Gy in the center and a steep decrease in the direction to the maximum radius but outside the tracks, the dose is zero. In contrast, for sparsely ionizing radiation, the dose is more or less homogeneously distributed. Biological experiments on cellular and DNA level, however, have shown that the increase in RBE is most pronounced for biological systems having a large repair capacity while RBE remains nearly constant (equal to one) for repair-deficient systems. This shows that the processing of the damage by cellular repair systems is of utmost importance for the understanding of the increased RBE of particles.

¹ Radioprotection introduced a quality factor different from RBE. The dose D, given as the energy deposited in a mass unit, has to be multiplied by this quality factor Q in order to get the biologically equivalent dose H = H [Sv] = Q x D [Gy]. The dose is given in Gy = Gray = 1 J/kg, the equivalent dose is given in Sv = Sievert and the dimensionless quality factor is defined and fixed by law to values that - for safety reasons - should represent an upper limit [ref 1].

The present understanding of RBE is that high local doses produce clusters of DNA damage that are difficult or almost impossible to repair. For X-rays, these clusters are more frequently produced with increasing dose but for particles, the high local doses occurring in one single track are large enough to produce clustered lesions. For repair-proficient systems, the severity of the damage is potentiated when repair is complicated due to the complexity of particle-induced lesions. For repair-deficient systems, the biological response is not affected by the production of irreparable lesions because repair does not play a major role in this systems.

Induction and repair of DNA damage after heavy-ion radiation.

Among the DNA lesions induced by ionizing radiation, mainly base-pair and strand-break damage, the most deleterious one is the DNA double-strand break (DSB). If left unrepaired, this lesion can result in a loss of genetic information, leading either to cell death or - if misrepaired - to mutations and the induction of cancer. In mammalian cell systems, the repair of DSBs can essentially be observed in two different ways: at the molecular DNA and at the chromosomal level [3]. The DNA in the nucleus is folded and packed with proteins and referred to as chromatin. It gets further compacted and condensed to chromosomes just before cell division. Only in this stage chromosomes are visible under the microscope and DNA damage can be scored as chromosome aberration. Fragmented DNA molecules and incorrect processing of DNA lead to abnormal chromosomes and aberrations. However, many of the most heavily damaged cells are hindered to perform this condensation. As a result, severely damaged cells obtained after exposure to heavy particles are not scored and the ion irradiation was believed to be less dangerous with regard to genetic alterations. Recent experiments, integrating chromosome aberrations expressed over a longer time period, revealed that for particles, a greater RBE can also be observed for chromosomal lesions as it is for cell killing [4].

At the molecular, the DNA level, a similar problem arises. Using conventional electrophoretic separation, no increase in the number of DNA double strand breaks was found that could account for the increased efficiency in cell killing. If the cells are incubated for repair after irradiation the DNA lesions induced by particles are not as well repaired as damage induced by X-rays. In addition, DNA fragment size distributions have recently demonstrated that the correlated production of breaks after exposure to particle irradiation yields a higher proportion of small fragments than an exposure to X-rays. This proximity of DSBs puts an additional

demand on cellular repair systems. Most likely, the processing of DNA damage after irradiation provides the link to the cellular reaction.

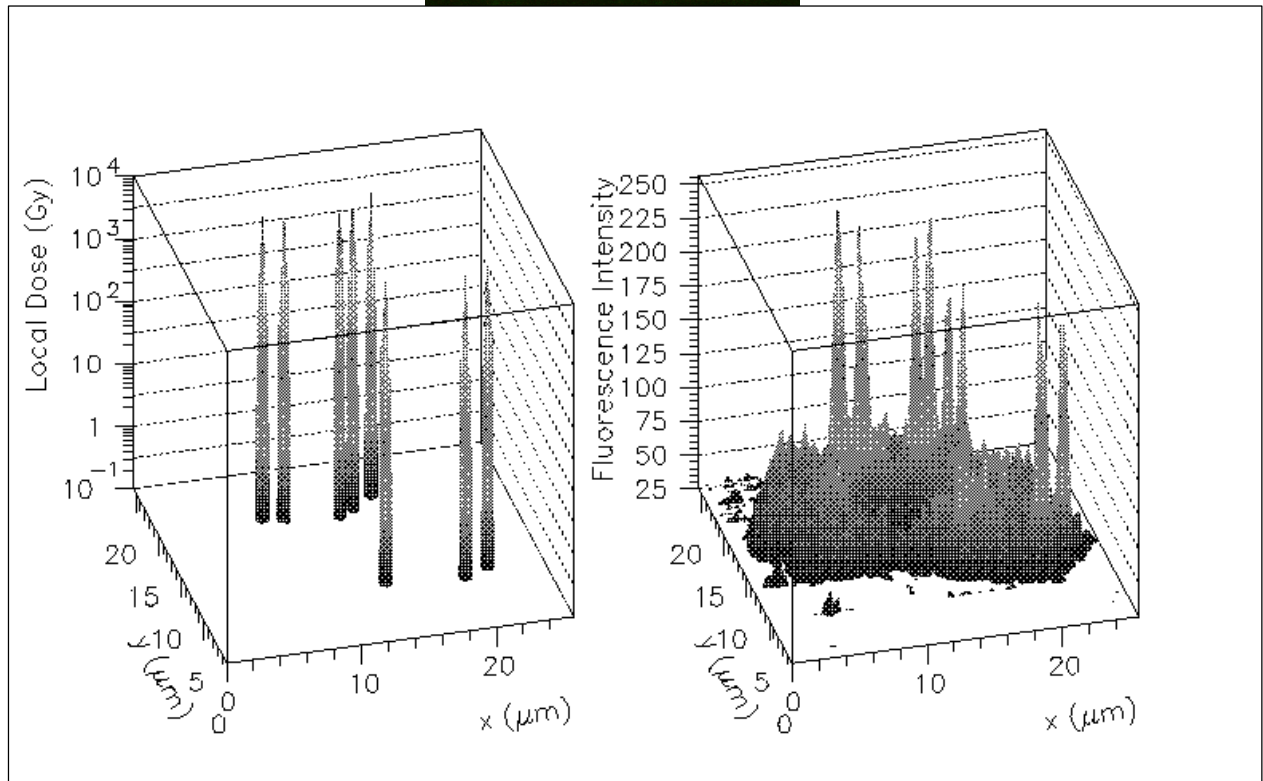
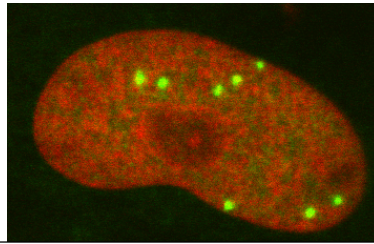


Fig 2: The rapid accumulation of p21 protein at sites of ion-induced DNA damage leads to the formation of protein foci localizing to the sites of particle traversal.

Indeed, there is evidence for a higher fraction of breaks left unrepaired after irradiation with low-energy charged particles showing a maximum of cell inactivation efficiency. This finding helps to explain the observed enhanced RBE for cell inactivation and it has to be concluded that the structure of the particle-produced DNA damage is more complex and less repairable than the damage produced by X-rays. However, the structure of these complex lesions is a subject of research and not known yet. Exposure to ion beams from accelerators can be executed with varying energies and atomic numbers, changing extent and intensity of the damaged sites. Thus, particle accelerators are used to elucidate the structure of DNA damage and its influence on correct repair which is a basic problem of the action of ionizing radiation.

Nuclear dynamics of protein involved in the DNA damage response

The nature of localized dose deposition along the tracks of charged particles is expected to induce complex lesions, representing a challenge to the cellular repair machinery. On the other hand, just this production of DNA damage within well defined regions of the cell nucleus following exposure to particle radiation, provides the means to study the dynamics of protein interactions involved in the response of the cell to this injury. Using fluorescence-labelled antibodies against the various repair proteins, the location of sites of particle-induced damage can be observed under the microscope. In this way, for the first time, the inhomogeneous microscopic dose deposition pattern characteristic to particle radiation could be visualized as a strictly localized, discrete biological response confined to the ion tracks traversing the cell nucleus. The methodology can be used to analyze interactions and functional relationships among proteins involved in the cellular response to DNA damage.

The p21-protein (CDKN1A) is one of the key proteins involved in the inhibition of cell proliferation after exposure to radiation to allow for the repair of DNA damage.

Modelling the particle response

There are many theoretical approaches to correlate this inhomogeneities in the micro-distribution of the dose deposited by particles directly to the biological result [5 a,b]. In a recent theory, the Local Effect Model (LEM), the cell killing by particle exposure is calculated from X-ray efficiency in a way that fully includes repair and microdosimetric dose distribution [6].

In LEM, the increased biological effect of particles is calculated on the basis of three measurable quantities: the size of the cell nucleus, the X-ray dose effect curve and the radial dose distribution. The effect of changes in repair is implicitly contained in the non-linearity of the X-ray dose effect curve. There, the same increment of the dose is more efficient in cell killing at higher than at lower doses if repair is predominant yielding a linearquadratic dose response curve. In case of no or little repair this difference disappears, X-ray dose effect become linear and RBE remains constant. In a Monte-Carlo calculation, tracks are traversing cell nuclei and the dose distribution of the overlapping tracks is folded with the X-ray dose effect curves in order to determine the damage probability (fig 3).

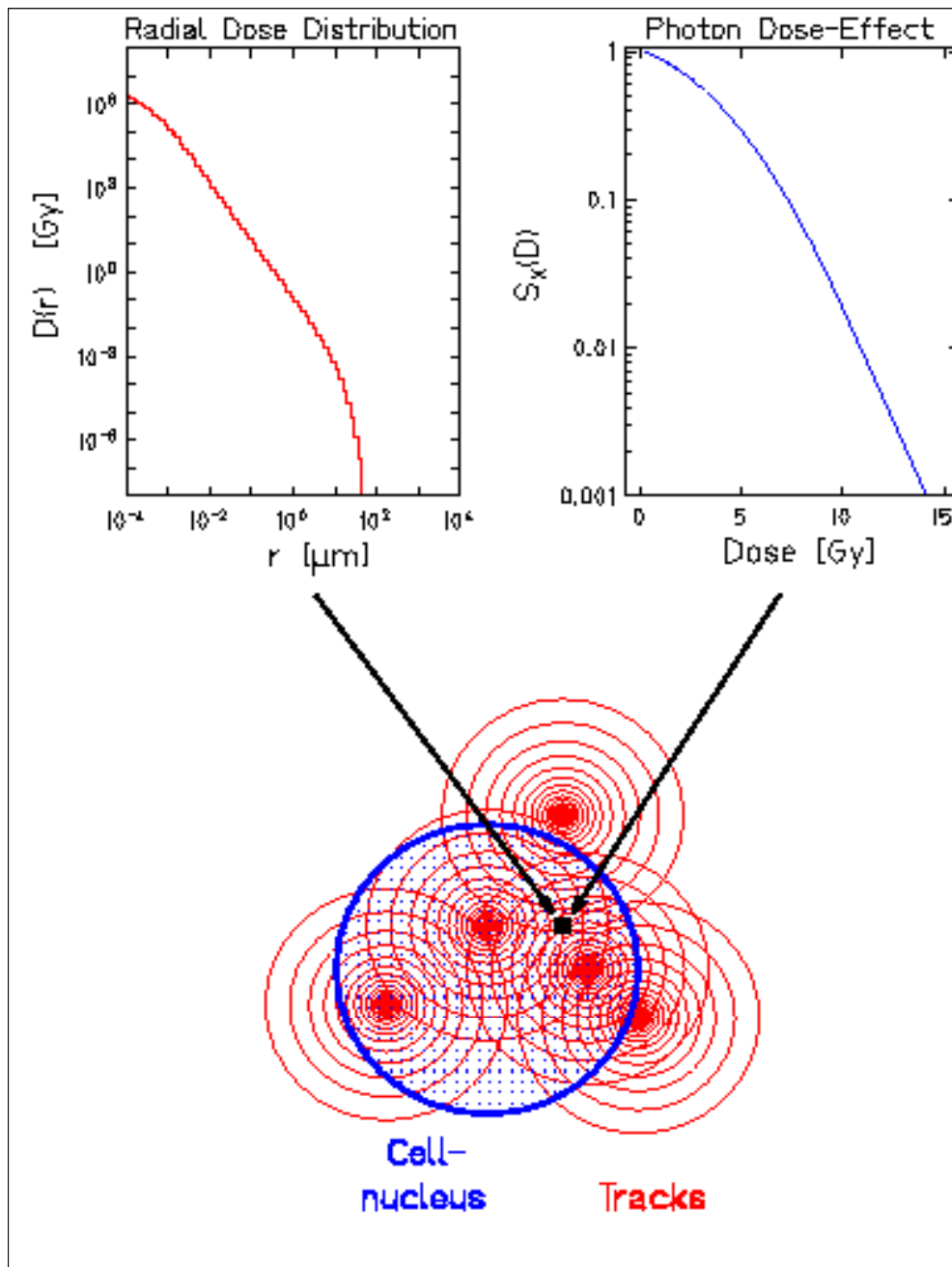


Figure 3. Principle of the Local effect Model (LEM) The cell nucleus as sensitive site is covered with particle tracks and their dose contribution to each pixel of the cell nucleus is calculated. For these pixels, the inactivation probability is calculated according to the measured X-ray dose effect curve. The figure shows the radial dose distribution and X-ray dose effect curve in addition to the operating principles.

Finally, these probabilities are integrated over the complete cell nucleus. Using this approach all the dependencies on dose, energy and atomic number can be reproduced very well. For cell inactivation, LEM has been very successfully used in carbon radiotherapy to calculate the RBE for cell killing over the target volume and for late effects in the normal tissue around. But also numerous tests with cultured cells prior to the therapy confirmed the approach of LEM. Although the model calculation can be used to transfer the X-ray efficiency to the response to particle exposure in a very successful way, LEM does not answer the questions of

the molecular nature of the primary damage or of the pathways of repair or the involved proteins as it was questioned in the earlier models [ref 5 a, b].

Bystander effects of radiation

A new mechanism for radiation damage

The mechanisms and modelling described above relate to what is called the “*classical model*” of radiobiological effects. One of the concepts on which this model is based is that each cell individually presents a target which either responds or does not respond to irradiation independently of its neighbours. In many situations, the classical model continues to serve well as a descriptor and a predictor of the biological actions of various types of radiation field. However, over the past decade it has become clear that does not account for certain types of response, particularly at low doses.

Up until the early 1990’s, it was generally thought that the damaging biological effects of radiation occurred only very close to the tracks along which energy is deposited. Thus it was believed that damage was only induced within nanometre distances of where the primary and secondary charged-particles passed through the cell. It was also believed that all of the significant biological effects of radiation derived from deposition of energy within the nucleus of the cell and not in the cytoplasm and that genomic DNA was much more sensitive than any other constituent. In particular, DSB were considered to be the key initial lesions which, if not correctly repaired, could either lead to cell death, or to permanent changes in the irradiated cell and in its descendents. These changes included chromosomal aberrations, mutations and malignant transformation. There had been some indications of radiation effects that were not consistent with the classical model, for example evidence for the induction of chromosomal damage in cells that had not been exposed to radiation if they were placed in contact with plasma from irradiated individuals. There was also evidence from radiotherapy of changes arising in tissues that were distant from the treated field (“*abscopal effects*”). However, such effects were considered not to be of major importance and, in general, to contribute very little to the overall biological actions of radiation.

In 1992, a report was published by Nagasawa and Little [7] which appeared to challenge the established classical model of direct damage. The authors reported that when Chinese hamster cells in culture were exposed to low doses of α -particles such that on average only 1% of cell nuclei were actually traversed, about 30% of the cells subsequently showed chromosomal damage in the form of sister chromatid exchanges. Thus, their data showed that DNA damage had been induced in many more cells than the fraction that had had energy deposited in their

nuclei. This was a surprising finding and appeared to conflict with the direct damage model. Over the next few years, several further reports from Little's and other laboratories confirmed that cells do indeed incur damage as a consequence of being in the neighbourhood of irradiated cells. The novel concept of a "bystander effect" of radiation gained acceptance. Cellular responses induced via bystander mechanisms have been shown to include the induction of chromosomal aberrations, mutations, cell death, apoptosis (or programmed cell death), malignant transformation and genomic instability. An illustration of the bystander effect is shown in Figure 4.

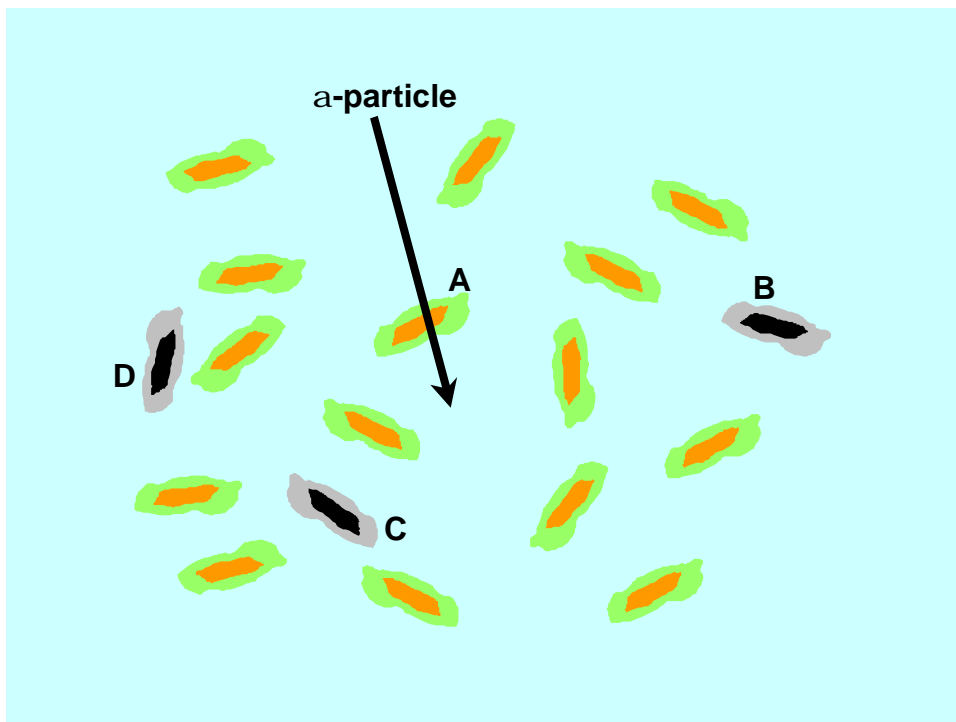


Figure 4. Illustration of the bystander effect of radiation. A single α -particle passes only through cell A, giving it a dose of radiation. This results in damage to cells B, C and D, even though the dose of radiation that these cells have actually received is zero.

What is the mechanism of the bystander effect?

The bystander effect has been found to occur in a number of different cell systems and several types of signalling mechanism appear to be involved. In some systems, where there is direct cell-to-cell contact, there is evidence that signalling occurs via the gap junctions which certain cell types use to communicate. Evidence from other studies, where cells are not in direct contact, points to the involvement of a factor transmitted via the culture medium. There is evidence for the involvement of cytokines, which are small proteins or biological factors that are released by cells and have specific roles in cell-to-cell communication. Other evidence points to a role of ROS (reactive oxygen species). Currently, much of the research on the bystander effect is concerned with identifying the signalling mechanisms. Most of the

information gained so far has come from *in vitro* systems and there is a pressing need to study bystander responses either directly *in vivo* or in model tissue systems.

How do bystander and direct effects compare?

Research has already revealed several important differences between bystander and direct effects of radiation. Perhaps the most striking difference is found in the shapes of the dose-effect relationships and this is illustrated in Figure 5.

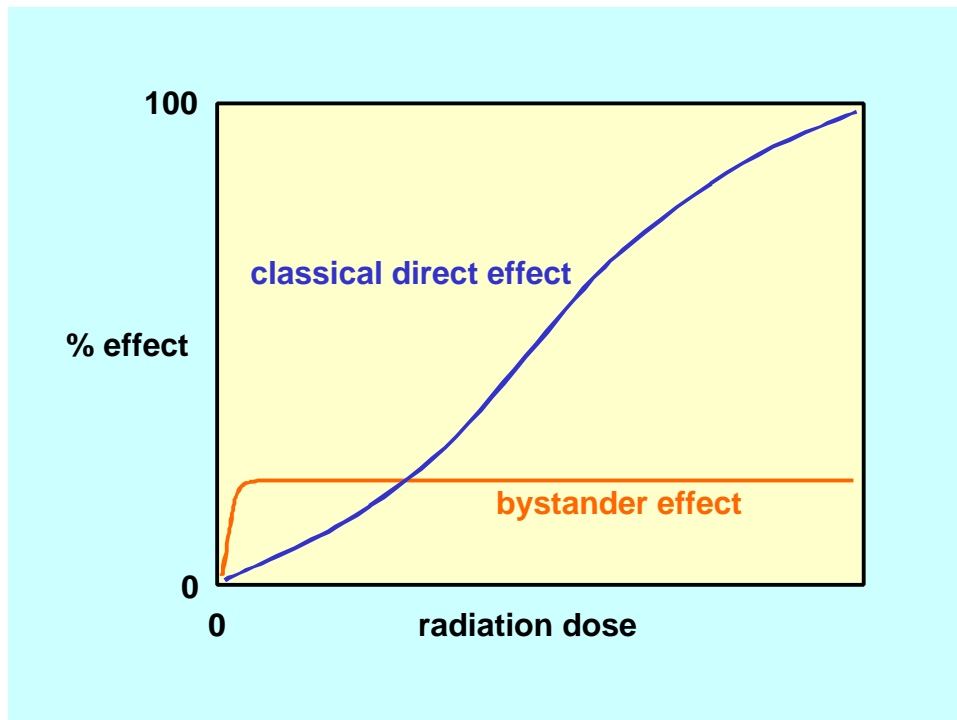


Figure 5. Comparison of general forms of dose-effect relationships observed for bystander and for classical direct effects of radiation on cells. At sufficiently high doses, direct effects damage all cells in an irradiated population. In contrast, bystander effects are observed when only a very small fraction of a cell population is irradiated and damage is found among those cells that have not “seen” any radiation. Typically, between 1 and 30% of the unirradiated fraction of the cell population may show bystander-induced damage, depending on the endpoint observed. In general, bystander responses have been found to reach a plateau above a low threshold dose and there is little or no further increase with dose to the irradiated cells.

With direct effects, for example cell kill, as the dose of radiation is increased the proportion of cells showing a response continues to increase progressively until virtually all cells in the exposed population are affected. This can be explained in terms of the particulate nature of radiation and each cell behaving as a sensitive target with an equal probability of being inactivated if hit by a particle track. Thus the survivors are those that by chance escape a lethal hit. In contrast, bystander effects are characterised by a low threshold dose above which the response exhibits a plateau. Further increases in dose produce little or no increase in the fraction of cells that respond. There are two main theories to explain the plateau. One is that a pre-existing fraction of the cell population is sensitive to the bystander signal. The other is

that the cell population as a whole responds to the bystander signal, but also generates an inhibitory signal which limits the response to a certain fraction.

What is the potential importance of the bystander effect?

There are two main areas in which bystander effects may have a significant role. The first is in relation to the risks associated with low-dose exposures. This is because at low doses the arrival of radiation tracks at the level of cells in human tissues is, on average, well separated both in space and time. For example, natural background exposure amounts to an average of about one electron track per cell per year and one α -particle per cell per century. Conventional estimates of radiation risk indicate that natural background radiation contributes about 3% of all human cancers, but whether this is an over- or underestimate depends in part on assumptions about the biological actions of single tracks.

Bystander effects may also have a role in some forms of radiotherapy. For example, cancer treatments using targeted radionuclides may fail to reach all parts of the tumour and in this case any transmission of lethal effect from hit to non-hit tumour cells would be beneficial. The same consideration may apply in BNCT (boron neutron capture therapy).

Microbeams for research in radiation biology

Over the last decade, there has been a surge of activity in the development of microbeams for research in radiation biology. More than a dozen high-tech microbeams have either been constructed, or are being planned, for research centers around the world. The stimulus for their development stems from many of the questions outlined above. In particular, they promise to help answer the fundamental question, “*What are the effects of single charged-particle tracks delivered to specific regions of the cell?*” This may seem a rather esoteric question, but the answers to it will provide a Rosetta stone which will unlock many of the remaining secrets of the biological actions of radiation.

Modern microbeam developments

The concept of radiobiological microbeams is not new [8], but the current generation of systems [9, 10, 11] have been able to take advantage of modern developments in technology, particularly with image capture and processing and computer control. Today’s microbeams have targeting accuracies ranging from several microns to sub-micron and operate under programmed control to irradiate up to about 10,000 cells per hour. Ions ranging from protons to argon nuclei are available at the various installations. It is standard to count the number of ions and to interrupt the exposure of each cell when it has received the intended number of

traversals. In this way, it is possible to deliver strict numbers of particles to cells and defeat the confounding effects of random (i.e., Poisson-distributed) particle arrivals that occur in experiments using conventional sources. The main elements of a charged-particle microbeam are shown in Figure 6.

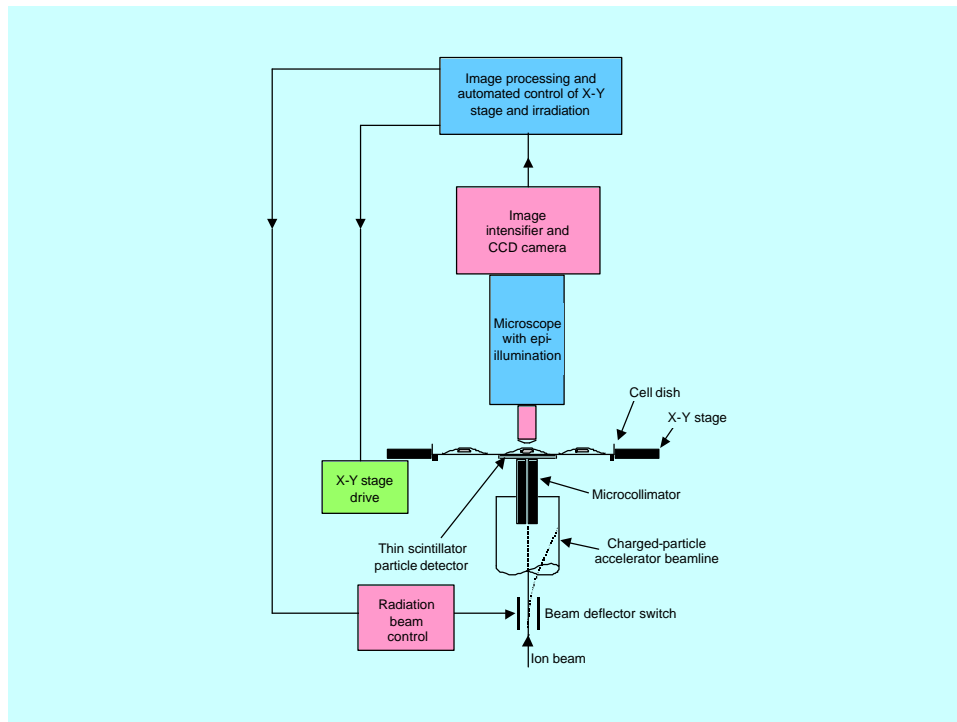


Figure 6. A charged-particle microbeam for individual cell irradiation showing the microcollimator used to shape the beam, the particle detector used to count the ions delivered, the cell dish, microscope and imaging system.

The capability of being able to deliver strictly single particles to individual cells is of direct relevance to the determination of RBE for different types of radiation, as outlined above. For radiation protection purposes, radiation quality factors, which are decided from our knowledge of RBE, need to be estimated for the lowest possible dose level and this corresponds to the biological actions of a single track.

Another important feature of microbeam systems is that they have the capability of revisiting individual cells or their progeny after division. This means that the effects of irradiation on cells that have been targeted and on others that have not can be followed clearly and distinguished, for example in experiments on the bystander effect. It also means that in studies of the nuclear dynamics of proteins involved in the DNA damage response, referred to above, it is possible to visualise the localisation of response proteins in direct microscopic relation to the path of the radiation track that has initiated the damage to which they have responded.

There is also currently much interest in the application of microbeam methods using focused soft x-rays or electrons. The aims here are to exploit the high targeting accuracy achievable with focused x-rays (about 30 nanometres) and to explore low-dose effects of low-LET radiations. Here the aim is ultimately to be able to determine the actions of the passage of a single electron track through the cell, a topic of particular relevance in understanding and predicting low-dose radiation risk.

References

- [1] ICRU, The Quality Factor in Radiation Protection, ICRU-Report 40, Int. Commission on Radiation Units and Measurements, Washington 1986
- [2] Kraft G., Krämer M., Linear Energy Transfer and Track Structure, *Advances in Radiat. Biology* 1993, 17, 1-52
- [3] Goodhead D.T., Thacker J., Cox R., Effects of radiations of different qualities on cells: molecular mechanisms of damage and repair, *Int. J. Radiat. Biol.* 1993, 63, 5, 543-556
- [4] Nasonova E., Ritter S., Gudowska-Nowak, E., Kraft G., High-LET-induced chromosomal damage: time-dependent expression, 1st Intern. Workshop on Space Radiat. Res. and 11th Annual NASA Space Radiat. Health Investigators' Workshop, Arona (Italy), May 27-31, 2000.
- [5a] Lea D. E., *Actions of Radiations on Living Cells*, Cambridge University Press 1956
- [5b] Kellerer A.M., Fundamentals of Microdosimetry, in: *The Dosimetry of Ionizing Radiation*, eds.: H. Kase, B.E. Bjärngard and H. Attix, edition 1985, 1, Academic Press, Orlando, Florida, 78-162
- [6] Scholz M., Kraft G., Calculation of Heavy-Ion Inactivation Probabilities Based on Track Structure, X-ray Sensitivity and Target Size, *Radiat. Prot. Dosimetry* 1994, 52, 1-4, 29-33
- [7] H. Nagasawa and J. B. Little, *Cancer Res.*, **52**, 6394-6396 (1992)
- [8] R.E. Zirkle and W. Bloom, *Science*, **117**, 487-493 (1953)
- [9] L.A. Braby, A.L. Brooks and N.F. Metting, *Radiat Res* **148**, S108-S114 (1997)
- [10] T.K. Hei *et al.*, *Proc Natl Acad Sci U S A*, **94**, 3765-3770 (1997)
- [11] M. Folkard *et al.*, *Radiat Res* (in press)