S Y N O P S I S

The study of radiation actions on living systems has become an active area of research in life sciences in recent years. Besides its importance in the assessment of magnitude of risk to life on account of natural radiations, nuclear weapons and nuclear industries, radiation research has shown promise in gaining insights into several intriguing phenomena of life sciences. Among the diverse effects produced by radiations on living organisms, the most important are perhaps the cell death and mutations. These two insidious consequences of radiations stem from injury to cellular DNA. The damage to cellular DNA may, in addition, be the underlying factor for a variety of metabolic and physiological perturbations triggered by radiation exposure. A body of information has been built up on the chemistry of various types of DNA damage caused by non-ionizing radiation such as ultraviolet (UV) radiation and ionizing radiation like x- or gamma-radiations. Though DNA is the main target for radiation injury, living cells display a wide range of susceptibility to radiations. Such variations in radiation susceptibility seem to be only marginally related to the composition of cellular DNA or to cellular environment which may protect the DNA from primary injury. The differences in the sensitivity of cells to radiations appear to be predominantly due to the efficiency of enzymatic apparatus responsible for restituting radiation-injured portions of DNA, or bypassing these lesions.

Much attention is now being focussed on certain radiation resistant bacteria such as Micrococcus radiodurans and Micrococcus radiophilus which are about 20 times more resistant than E.coli. Studies on the factors governing their high radiation resistance could unravel some, as yet unknown, facets of DNA repair. Moreover the radiation resistant microbe is also a unique system for investigating effects of higher doses of radiations on various cellular processes, especially those which are generally resistant to low doses of radiation.

The investigations reported in the thesis were aimed at analysing the perturbations in and responses by M.radiophilus when subjected to radiations and other physical and chemical insults.

The introductory chapter (Chapter I) of the thesis surveys literature pertaining to various types of injuries inflicted on the cells by radiations and radiomimetic agents and the knowledge accumulated hitherto on recovery and DNA repair mechanisms.

The experimental part of the thesis forms the subject-matter of the subsequent chapters, namely, Chapter II, III and IV.

M.radiophilus was isolated in this laboratory from Bombay duck (Harpodon nehereus) during studies on

radiation preservation of this fish (Lewis, N.F. J. Gen. Microbiol. 66, 29, 1971). The organism is orange-coloured owing to the presence of carotenoids. It grows mainly in the form of tetrads and occasionally in pairs. The survival curve of the organism suspended in phosphate buffer, in response to the exposure to gamma radiation exhibited a broad shoulder up to 400 krad and a D_{10} of 500 krad. The magnitude of radiation resistance of this organism can be gauged by comparing it with that of E.coli B/r - a comparatively radiation-resistant strain of E.coli - survival curve of which has a negligible shoulder and a D_{10} of only 30 krad. The broad shoulder of M.radiophilus was further extended up to 700 krad when the dose of 700 krad was split as 400 krad + 300 krad with an intermediate incubation in TGYM broth for 2 hr. The survival of M.radiophilus to gamma radiation was however significantly reduced if cells were irradiated in the presence of 0.025 M EDTA. The result indicated that EDTA might either enhance the intensity of damage by modifying the damaged sites, or interfere with the DNA repair machinery.

M.radiophilus also exhibited a very high resistance to UV radiations, the survival curve showing a shoulder up to 800 J/m^2 and a D_{10} value of 1120 J/m^2. As compared to this response, UV radiation survival curve of E.coli B/r exhibited no shoulder and a D_{10} value of 45 J/m^2.

Ionizing radiations such as gamma rays mainly cause strand breaks and the base or sugar damage in cellular DNA whereas UV radiation principally forms dimers between adjacent pyrimidines on a DNA strand. The high resistance of M.radiophilus to both UV and gamma radiations might imply that there could be some common machinery in M.radiophilus to correct different types of lesions formed in cellular DNA. This contention was borne out from the experiment with an alkylating agent methyl methane sulphonate (MMS). MMS is known to alkylate mainly at the N_7 position of guanine of DNA, eventually resulting into a DNA strand break. When held for 2 hrs in TGYM medium containing 5×10^{-2} M MMS, the survival of M.radiophilus was reduced to 35% while that of E.coli cells given a similar treatment decreased to 1.7%.

Because of high resistance to physical and chemical agents damaging DNA it was thought important to ascertain the cellular responses of this organism to other types of injury which may not necessarily arise from a damage to DNA. No significant difference could be discerned between the sensitivity of M.radiophilus and E.coli B/r in respect of sensitivity to heat. The organism however displayed a remarkably high resistance to repeated freezing and thawing. Thus 20 cycles of freezing and thawing were ineffective in inactivating the cells of M.radiophilus whereas only 7 such cycles of freezing and thawing could bring about the

reduction in survival of E.coli to 1.7%. The cells were likewise refractory to sonication up to 20 min at 20 kc/sec, with or without the pretreatment of lysozyme. On the other hand, similar treatment lasting for only 2/3 min was sufficient to solubilize E.coli cells. The results were indicative of the rigidity of the cell wall of M.radiophilus and this presumption was supported by electron microscopy which has revealed a unique three layered cell wall structure. It may be mentioned here that while the thickness of the cell wall of M.radiodurans is 150 nm that of E.coli is 30 nm. The exceptionally high resistance of M.radiophilus to radiations and to other treatments cannot be solely attributed to its rigid cell wall or to its property of growing in the form of tetrads since Staphylococci growing in the form of clusters or B.megatherium growing in the form of chains or Sarcina growing in the form of packets of eight cells are not resistant to radiations and were also quite fragile to various physical and chemical assaults. These studies relating to the effects of radiations and other physical and chemical agents on M.radiophilus with respect to its survival responses form the subject-matter of Chapter II.

In order to elucidate the mechanisms underlying higher radiation resistance of M.radiophilus in detail, a variety of cellular processes were studied in irradiated cells. Phenomenanlly high radiation resistance of this

organism also offers an ideal system to study the effects of high doses of radiations on several biochemical processes. Such study is impossible in sensitive microbial strain as they may succumb to radiation exposure at much lower doses.

As described earlier M.radiophilus cells are orange-coloured due to the presence of carotenes with characteristic absorption maximum at 480 nm. It was found that there was a progressive decrease in the cellular level of the pigment with increase in gamma radiation dose from 200 krad onwards and at 1000 krad, the cells were completely devoid of it. On incubation of the irradiated cells in the fresh TGYM medium, there was a steady reappearance of carotenes which could be noted as early as 30 min. Further experiments involving inhibitors of protein synthesis during post-irradiation incubation indicated that the reappearance of carotenes does not depend on the de novo formation of carotene-synthesizing enzymes. The regeneration of carotenes could be due either to their continuous turnover within the cell or to the reversal of the feedback inhibition of carotene synthetic pathways brought about by the destruction of the end products, i.e. carotenes, by irradiation. The possibility of continuous turnover of cellular pigments was ruled out since it was found that in the cells incubated in TGYM medium containing nicotine (which specifically inhibits the final cyclisation step in the

carotene synthesizing pathway), there was no reduction in the cellular levels of the pigment already present. But only the subsequent cell divisions in the presence of nicotine resulted in the gradual dilution of cellular pigment with an eventual development of colourless cell population.

Studies were also carried out to investigate the effects of high doses of irradiation on macromolecular syntheses in M.radiophilus. As compared to the macromolecular syntheses in E.coli, those in M.radiophilus showed reduction only at very high radiation doses. The lag periods in the syntheses of DNA, RNA and protein were extended as the dose of radiation was increased from 200 to 1000 krad. The rates of syntheses of macromolecules were also found to be progressively reduced with the increment in the dose. It was however found that the inhibition in macromolecular syntheses at a given reduction in survival was more in the case of M.radiophilus compared to that in E.coli. This would indicate that at high radiation doses of 200 krad and above certain non-DNA cellular components may be affected. Other studies revealed that neither E.coli DNA polymerase nor M.radiophilus DNA polymerase is inactivated by 200 krad gamma radiation dose.

Gamma irradiation of bacterial cells is known to be associated with the degradation of DNA. This can be interpreted as the consequence of degradation of DNA from

cells rendered non-viable due to irradiation or due to the removal of damaged portion of DNA in the viable cells. DNA degradation has been shown to be an early step in DNA repair and hence can be a manifestation of cellular repair processes. It was therefore of interest to investigate the degradation of DNA in a radiation resistant organism like M.radiophilus. At non-lethal doses of 200 krad (i.e. 100% survival) degradation of DNA (as assessed by following the loss of radioactivity from pre-labelled DNA) was negligible. However, at 500 krad (10% survival), DNA was degraded to the extent of 20 - 30% within the first generation time. Cellular DNA degradation in M.radiophilus following exposure to UV irradiation exhibited a loss of radioactivity up to 20% even at the non-lethal dose of 630 J/m^2. Thus the results suggest that although M.radiophilus cells are highly resistant to both UV and gamma radiations, there may be some subtle differences in the repair of the lesions introduced by the two types of radiations presumably in the initial steps of the repair processes. The foregoing results of biochemical responses by the cells of M.radiophilus after exposure to radiations are embodied in Chapter III.

Molecular damage suffered by cellular DNA after exposure to radiation and radiomimetic agents is predominantly due to single- and double-strand breaks. The strand breaks could be either the primary lesions of radiations or

formed as a consequence of enzymatic action on the primary damage to a base or a sugar in DNA. Rejoining of strand breaks in the DNA has come to be known as an important parameter of the operation of the DNA repair processes - especially different types of excision repair.

Studies were therefore designed to assess the number of strand breaks formed in M.radiophilus after exposure to radiations and radiomimetic agents and the ability of these cells to eliminate the lesions. These studies form the part of Chapter IV. The well-known technique of McGrath and Williams based on lysing bacterial cells directly on top of a sucrose density gradient was employed for the purpose. The gradients were subjected to ultracentrifugation for the assessment of molecular sizes of DNA fragments and the strand breaks in the DNA. Using this technique, no strand breaks in M.radiophilus exposed up to 200 krad could be discerned. However, at higher doses of radiation up to 1000 krad, a progressive increase in the number of single-strand breaks with the increase in radiation dose was observable. It is reported that a great number of strand breaks undergo repair by a process called fast repair. This process is inhibited if the cells are irradiated in the presence of EDTA. Irradiation (200 krad) in the presence of EDTA indeed caused strand breaks suggesting that M.radiophilus might be endowed with an efficient fast repair machinery.

Sucrose density gradient analysis of DNA of M.radiophilus cells exposed to UV radiation or treated with MMS revealed that, unlike in the case of gamma-irradiation, non-lethal doses of UV radiation and MMS could induce strand breaks. The strand breaks could conceivably arise from an endonuclease action on the lesions like pyrimidine dimers and alkylated bases formed by UV-irradiation and MMS respectively. Further experiments on UV-irradiated cells showed that even the dose falling within the shoulder region results in the induction of strand breaks.

Post-gamma irradiation incubation of M.radiophilus in TGYM broth revealed that strand breaks produced in the presence of EDTA disappear completely within as little as 30 min period. Also the rejoining of strand breaks could not be inhibited by the presence of chloramphenicol in TGYM broth indicating the non-requirement of de novo synthesis of proteins. Repair of strand breaks could also occur in the case of the damage suffered by cellular DNA following UV irradiation and MMS treatment. Evidence was obtained to show that the repairs required a long post-irradiation incubation up to 2 - 4 hr in TGYM broth.

In summary, studies on radiation effects on a highly radiation resistant strain like M.radiophilus

presented in the thesis revealed some new facets of radiation injury and repair mechanisms. The exceptionally high radiation resistance of this organism can be largely ascribed to the highly efficient machinery for fast repair operative during the course of irradiation, thereby exhibiting a long shoulder and to a certain extent on the moderately high efficiency of the slow DNA repair. The rigid cell wall structure may contribute to the general resistance displayed by this organism. However, the role played by the rigid cell wall is far less important compared to that by cellular DNA repair machinery.