Synopsis

The fact that the genetic material is transmitted from the parent to the daughter cells with great fidelity is suggestive of the existence of precise and efficient DNA repair mechanisms. Much of the understanding of these repair processes has come from the work on bacteria and yeast. Isolation and characterization of mutants sensitive to radiations and/or chemicals have further helped one to gain insight into the genetic control of repair phenomena.

The first radiosensitive mutants of yeast were discovered in 1967. Since then 32 distinct genetic loci conferring sensitivity to radiation have been identified. These loci, having significant effect on radiosensitivity, were designated as rad, followed by locus and allele number. RAD denotes the wild type.

Observations made using radiosensitive mutants of haploid yeast had suggested that the RAD1,2,3,4 loci, constituting the excision repair pathway, are mainly responsible for the repair of UV damage. Also such studies have revealed that the RAD6,2,10 loci (RAD6 group) are associated with the repair of both UV and ionizing radiation damage and that the RAD50 to 57 loci (RAD52 groups) are primarily involved in the repair of X-ray damage.

However, not much is known about the loci controlling the repair of ionizing radiation damage in diploid yeast. That the repair mechanisms and the relative importance of the various loci
may be different in haploid and diploid yeast could be suggested from a consideration of the absence of the following radiobiological phenomena in haploids exposed to ionizing radiations: a) shoulder on the survival curves, b) the ability to recover from potentially lethal damage and c) increasing sensitivity to densely ionising radiation - induced lethality.

A number of diploid rad mutants of yeast *Saccharomyces cerevisiae*, homozygous with respect to the rad loci and known to be deficient in different DNA repair pathways are now available. Using these radiation sensitive mutants the genetic loci associated with the repair of eucaryotic and anoxic radiation damage upon immediate plating (repair of sublethal damage, SLD) and during postirradiation liquid holding (repair of potentially lethal damage, PLD) have been investigated. Stationary phase cultures of wild type strains X2180 and 211 and mutant strains rad2, 6, 9, 18, 50, 51, 52, 53, 54, 55 and 57 were exposed to various doses of $^{60}$Co gamma radiation in the presence and absence of oxygen. After irradiation cell survival was scored upon immediate plating and upon 48 hours of liquid holding. The process of recovery from PLD during liquid holding is termed as liquid holding recovery (LHR).

Log phase cultures of these strains were also exposed to gamma rays to understand the genetic control of budding cell resistance. Furthermore, X2180 and rad53, 54 and 55 strains, in stationary and log phase, were exposed to hyperthermia at $51^\circ$C for different time intervals in order to compare the sensitivity
of these strains to the lethal effects of gamma radiation and hyperthermia. Results obtained in these investigations are presented in the thesis.

Sensitivity of wild type and mutant strains to gamma rays was compared in terms of survival curve parameters: a) shoulder width ($D_q$), b) dose required to reduce the survival to $10\%$ and $\%$ ($D_{10}$ and $D_1$), and c) the mean lethal dose ($D_0$).

Compared to wild type all the mutants showed higher sensitivity to radiation induced lethality both under euoxic and anoxic conditions of irradiation, except rad2. The euoxic sensitivity of rad2 strain was the same as that of wild type. However, the radiosensitivity has been found to vary significantly among the different mutant strains, with rad51, 52 and 54 showing the highest sensitivity.

Oxygen enhancement ratio (OER) is a measure of sensitization by oxygen. When compared to wild type having a OER of 2.9, rad2, 9, 18, 50, 51 and 57 strains showed less OER (1.9 - 2.5). This reduction in OER was found to be due to higher sensitivity of mutants under anoxic condition of irradiation than under euoxic condition. On the basis of this observation it could be suggested that products of RAD2, 9, 18, 50, 51 and 57 loci may be associated with the repair of anoxic damage as well.

Under euoxic conditions of irradiation the sensitivity of rad2 strain was the same as that of wild type indicating that excision
repair pathway may not be involved in the repair of gamma radiation-induced euoxic damage. However, since rad2 strain has shown higher sensitivity under anoxic condition of irradiation it could be suggested that a part of anoxic damage may be similar to that of UV damage.

Shoulder on the survival curves, a measure of the ability of the cells to repair SLD, was reduced in rad6, 9, 18, 50, 53 and 57 and was almost absent in rad51, 52, 54 and 55 strains.

The ability to recover from PLD during postirradiation liquid holding was found to be equal to that of wild type strains in rad2, 6, 9 and 18, reduced in rad52, 55 and 57 and was absent in rad50, 51, 52 and 54 strains. These results have indicated that the function of RAD50 to 57 loci are necessary for the post irradiation cellular recovery. RAD6 group of loci may not be involved in this repair process. Furthermore, in the strains showing LHR, the extent of recovery from PLD produced under euoxic and anoxic conditions was observed to be the same. On the basis of the absence of LHR in rad52 strain which is deficient in the repair of double strand breaks (DSB) in DNA, it was possible to suggest that LHR involves the repair of DSB. Moreover, since rad50, 51 and 54 strains failed to show LHR it could also be indicated that these loci may be involved in the repair of DSB.

Kinetics of recovery from PLD induced under euoxic and anoxic conditions of irradiation have also been studied for all
the strains. This has indicated that the recovery during post-irradiation liquid holding is completed within 48 hours and that the rate and extent of recovery is the same for both types of damage.

Cells of wild type strains in log phase are more resistant than stationary phase cells to gamma radiation. Log phase resistance was reduced in \textit{rad6.9.16} and \textit{57} and was absent in \textit{rad50}, \textit{51.52.53.54} and \textit{55} strains. Response of \textit{rad2} strain in log phase was similar to that of wild type strain. These results suggest that (a) log phase resistance is due to efficient repair of radiation damage, and (b) for the expression of log phase resistance the gene products of \textit{RAD52} group is more essential than that of \textit{RAD6} and \textit{RAD2} groups. Relationship between the repair pathways associated with log phase resistance and IHR has been discussed.

On comparing the present data with that of haploids it could be suggested that: (a) the expression of \textit{RAD6} and \textit{16} loci are more essential for the recovery of haploid than of diploid cells, (b) expression of \textit{RAD50} through \textit{57} loci are essential for the recovery of diploid cells.

Wild type diploid strain X2180, in stationary and log phase, exposed to gamma rays showed sigmoidal survival curves, log phase cells were significantly more resistant than stationary phase cells. When compared to wild type, the gamma radiation response of \textit{rad53.54} and \textit{55} strains indicated that the mutations in these \textit{RAD} loci greatly sensitize the cells in both the phases. The differences in
sensitivity of mutants and wild type strains exposed to heat was significantly less when compared to that of gamma rays. Log phase cells of both wild type and mutants were more sensitive to heat than stationary phase cells. These results suggested that the RAD loci are not involved in the repair of hyperthermic damage. Since it is known that the products of the RAD genes are involved in the repair of DNA damage, wild type response of these rad mutants to hyperthermia has clearly indicated that DNA may not be the principal target for hyperthermic cell killing. Observations implicating proteins and/or membranes as primary targets for heat induced cellular inactivation have been discussed.