CHAPTER I

Introduction
A. BACKGROUND

1. YEAST AS A TEST SYSTEM

Yeasts have been intimately associated with the progress and well being of the human race on two major fronts (Rose and Harrison 1969). The first one, is the capacity of yeasts to bring about a rapid and efficient conversion of sugars into alcohol and carbon dioxide. The elucidation of fermentation processes has led to the development of industries such as brewing, wine making and the large scale production of bakers yeast. The second major contribution has been in the understanding of the fundamental aspects of biochemistry, physiology, genetics, radiation biology and mutagenesis.

Yeasts are widespread in nature. Based on the morphological and physiological features the 350 species of yeast known are grouped into 39 genera (Krager-van Rij 1969). Two species, namely Saccharomyces cerevisiae and Schizosaccharomyces pombe have been used in most of the radiobiological studies with yeast. These are unicellular and constitute one of the simplest eukaryotic organisms (Wallace and Morowitz 1973). By virtue of their characteristics such as clonability, short generation time and adaptability to replica plating, offer several advantages as test systems.

Strains of S. cerevisiae normally exist in stable diploid and haploid states and divide mitotically by budding. Cells of these
strains grow rapidly in appropriate growth medium at 30°C with a generation time of 70 to 90 min. When placed in sporulation medium the diploids yield asci containing 4 haploid spores. Haploids can be easily isolated by micromanipulation (Sherman 1973). If the haploid cells of opposite mating type are brought in contact with each other they form stable diploids. The life cycle of *S. cerevisiae* is shown in Fig. 1. (Mortimer and Hawthorne 1969).

Colonies of *S. cerevisiae* contain uninucleated single cells, ellipsoidal in shape and 5-7 micrometers in size. The DNA content per haploid nucleus is about $1.2 \times 10^{10}$ daltons (Hartwell 1970) which is very small for a eukaryote. The smaller DNA content has facilitated the measurement of intact yeast chromosomal DNA by sedimentation velocity (Petes and Fangman 1972) and electron microscopy (Petes, Eyers and Fangman 1973). Such investigations have indicated that each chromosome contains a single DNA duplex. Furthermore, DNA replication in yeast has also been shown to be similar to the replication in higher eukaryotes (Newlon, Petes, Hereford and Fangman 1974). The DNA in yeast is distributed among 17 chromosomes (Mortimer and Hawthorne 1969) so that, on an average, individual yeast chromosome is one fifth the size of the *E. coli* chromosome and 120 times smaller than a typical human chromosome (Petes and Fangman 1972).

Genetics of yeast is well understood (Kakar 1963a, b; Mortimer and Hawthorne 1966; Mortimer and Hawthorne 1969). About
Fig. 1. Life cycle of *Saccharomyces* species. The diagram illustrates the alternation of haploid and diploid phases and the distinctions between heterothallic and homothallic species.

(Mortimer and Hawthorne 1969)
150 distinct loci controlling a variety of metabolic processes have been mapped (Mortimer and Hawthorne 1973). More than 32 loci are known to control sensitivity to radiation (Cox and Parry 1965; Game and Mortimer 1974).

Strains of *S. cerevisiae* have been extensively used by radiation biologists for understanding several radiobiological aspects such as lethality (Latarjet and Ephrussi 1949), dependence of radiation sensitivity on cell stage (Beam, Mortimer, Wolfe and Tobias 1954; Elkind and Beam 1955), RBE-LET relationship (Mortimer, Brustad and Cormack 1965; Raju et al. 1971), oxygen effect (Averbeck and Ebert 1973), liquid holding recovery (Patrick and Haynes 1964) and mutagenesis (Mortimer and Manney 1971).

The above mentioned studies were carried out at cellular level. The discovery of repair deficient mutants by Nakai and Matsumoto (1967), Resnick (1969) and, Cox and Parry (1968) and strains capable of taking up and incorporating TMP (thymidine 5'-monophosphate) into their DNA (Jannsen, Lochmann, and Mogen 1970; Brendel and Haynes 1972; Wlokner 1974) have made it possible to study the DNA repair, recombination and replication at molecular level.

2. RADIATION BIOLOGY OF YEAST

The effects of radiation on yeast have been studied using several end points such as division delay, stimulation of cell division after irradiation with small doses, radiation induced cell synchrony, morphological changes, membrane damage, genetic changes,
postirradiation cellular recovery under non-growth conditions and lethality. (Magni 1959; James and Werner 1965). The literature on radiation-induced lethality, genetic effects, repair of radiation damage and genetic control of repair relevant to the work reported in this thesis, is briefly reviewed here.

2.1 Radiation Induced Lethality and Modifying Factors

Cell killing as an end point has been used in yeast more often than any other end point to understand radiation damage, since, the techniques for obtaining accurate dose-effect curves are simple and efficient. Furthermore, the influence of pre- and post-treatments can be investigated by subjecting cells uniformly to a vast array of different environments.

Sufficient doses of ionising radiation induce lethality in yeast, as judged by the loss of unlimited proliferative capacity of the cells. Manifestation of different kinds of damage such as gene mutations, chromosomal aberrations, inactivation of various cytoplasmic entities, metabolic disturbances and permeability changes have been implicated in causing radiation death (James and Werner 1965).

Radiation biologists have directed their attention from the beginning to identify the important target for inactivation. Virus, bacteria, yeast and mammalian cell lines were used as test systems. The most convincing evidence for the DNA as the primary target for UV and X-ray lethality has come from the studies on the
sensitizing effect of incorporated halogenated base analogue - 5- bromodeoxyuridine (BrdU) into DNA of bacterial and mammalian cells (Djordjevic and Szybalski 1960; Szybalski and Opera-Kubinska 1965). Other evidence suggesting that DNA is the principal target has been summarized by Haynes (1966) as follows: 1) The X-ray sensitivity of organisms ranging from virus to mammalian cells is correlated with their total DNA content. 2) X-rays, UV and HN₂ (nitrogen mustard) are all mutagenic and capable of producing chromosomal aberrations. 3) Bacterial sensitivity to UV, X-rays and HN₂ is correlated with the DNA base composition. 4) The ploidy of yeast influences its sensitivity to X-rays, UV and HN₂.

Although it is known that the pyrimidine dimers are the molecular lesions responsible for UV inactivation, not much is known about the type of DNA damage responsible for X-ray killing. Base damage, strand breaks, crosslinks and chromosomal aberrations (Freese and Freese 1966) have all been implicated to be responsible. The possible importance of DSB in cell killing by ionizing radiations has been suggested from the work with bacteria (Bonura, Town, Smith and Kaplan 1975; Bonura and Smith 1976), yeast (Ho and Mortimer 1975; Resnick and Martin 1976) and mammalian cells (Resnick 1978; Taylor 1978). On the basis of induction of DSB and their repair in wild type and a mutant strain of yeast, Resnick and Martin (1976) have suggested that 1 to 2 unrepaired DSB per cell may result in the loss of viability. Leenhouts and Chadwick (1978) in their molecular theory developed to explain the action of ionizing radiation on
eukaryotic cells, have implicated the involvement of DSB in all the
different biological effects such as cell death, chromosomal
aberration, somatic mutation and malignancy.

Survival curves indicate the kinetics of induction of
lethality and not the nature of damage. Several physical, chemical
and biological factors such as ploidy, quality of ionizing radiation
(linear energy transfer-LET), oxygen tension, cell stage, chemical
sensitizers and protectors and the repair mechanisms in the cells
are known to modify the lethal response of cells to radiation. An
understanding of the mechanism by which these factors modify the
shape of the survival curves could extend our knowledge on the
nature of the damage.

2.1.1 Ploidy

The effect of ploidy on radiosensitivity has been studied
using haploid to octaploid strains of yeast (James and Werner 1965).
It has been shown that the shape of the survival curve for low LET
radiations is exponential for haploids and sigmoidal for polyploids.
The radiosensitivity was the highest in haploid yeast and the lowest
in diploids. While the diploids show postirradiation liquid holding
recovery (LHR) after X-irradiation, haploids do not show LHR (Patrick,
Haynes and Uretz 1964). Yeast cells of all ploidies are known to
exhibit budding cell resistance to X-rays (Beam 1955).
Another important aspect of ploidy is the radiosensitivity of haploids and diploids to densely ionizing radiations. When lethality is the end point studied, diploids show increasing sensitivity as the LET of the radiation increases to 200 KeV/μm (Mortimer et al. 1965), whereas haploids do not (Raju et al. 1972).

2.1.2 Oxygen

Oxygen is one of the best known modifiers of radiation sensitivity. The biological effects of radiation such as lethality and genetic effects are greater in the presence of oxygen. The sensitizing effect of O₂ has been attributed to the production of more peroxyl radicals and H₂O₂ which react with DNA and increase the amount of damage.

The factor of sensitization by O₂ is expressed as oxygen enhancement ratio (OER); OER is obtained by dividing the dose required to produce a given effect in the absence of O₂ by the dose required to produce the same effect in its presence. For example, the OER for cell survival as the end point can be obtained by taking the ratio of \( D_0 - \text{hypoxic} / D_0 - \text{euoxic} \) (\( D_0 \) = mean lethal dose).

Increase in radiosensitivity of yeast when the irradiation is in the presence of O₂ was first reported by Birge and Tobias (1954). Since then several reports have appeared describing the oxygen effect on different end points. For lethal effects of gamma radiation OER values ranging from 1.4 to 2.5 for haploid yeast (Mortimer 1955; Wood and Taylor 1957; Averbeck and Ebert 1972, 1973) and 1.6 to 3 for
diploid yeast (Mortimer et al. 1965; Averbeck and Ebert 1972, 1973; Subrahmanyam et al. 1979) have been reported. The OER value of 3 for lethality of diploid yeast reported by Subrahmanyam et al. (1979) was obtained under acute hypoxic (anoxic) condition induced by using high cell concentration. OER values reported for genetic end points are similar to those reported for lethality. (Mortimer et al. 1965; Raju et al. 1971; Subrahmanyam, Rao and Madhvanath 1978).

Several factors are known to modify the $O_2$ effect. OER values for both lethality and mutagenesis have been shown to decrease with the increase in the LET of radiation (Mortimer et al. 1965; Raju et al. 1971; Subrahmanyam et al. 1978, 1979) and is absent for very high LET radiation such as neon ions (Raju et al. 1971). Also, radioprotectors such as glycerol (Manney, Brustad and Møoms 1963) and cystiene (Stuart and Stannard 1962) are known to influence the OER in yeast cells. OER is also known to vary with cell stage in haploid yeast, budding population showing less OER (Alper 1959).

In a few radiation sensitive mutants of yeast (Averbeck and Ebert 1972, 1973), bacteria (Alper 1965) and Clamydomonas reinhard (Davies 1967) OER for X-ray induced lethality has been studied. Reduction in OER in some of the mutants has indicated that the OER may be genetically controlled and these mutants may be defective in the repair of damage produced in anoxic stage. Further study of OER using radiation sensitive (rad) mutants should throw more light on the genetic control of oxygen effect.
2.1.3 Cell Stage

The radiation sensitivity of yeast cells is known to be a function of their position in the cell cycle at the time of radiation. Beam et al. (1954) have indicated that budding yeast cells are more resistant to X-rays than non-budding cells. Langguth and Beam (1973a) have shown that the X-ray sensitivity of synchronised diploid yeast cells varies continuously with cell stage, being lowest in the early stages of budding.

Williamson (1965) and Wildenberg (1970) have indicated that yeast cells with small buds undergo DNA synthesis (S-phase). Hatzfeld and Williamson (1974) have observed that maximum radio-resistance occurs at the end of S-phase. Langguth and Beam (1973a) have shown that the survival of log phase cells is reduced to that of stationary phase cells by caffeine present in the postirradiation liquid holding (LH) medium. Moreover, the survival of stationary phase cells upon postirradiation LH is the same as that of budding cells upon immediate plating (Langguth and Beam 1973a). On the basis of these results Langguth and Beam (1973a) and Hatzfeld and Williamson (1974) have suggested that the budding cell resistance is due to the efficient repair of radiation damage and the type of repair occurring in budding cells upon IP and in stationary cells during LH could be similar (Langguth and Beam 1973a).

The repair pathways associated with the budding cell resistance are not well understood. Nakai and Matsumoto (1967),
Hatzfeld and Williamson (1974) and Hama-Inaba and Saeki (1975) have suggested that the recombinational processes may be responsible for the radioresistance of log phase cells. With the availability of radiation sensitive strains known to be deficient in certain repair pathways (Haynes 1975) an examination of the budding cell resistance of these mutants should enable one to understand the nature of damage induced by ionizing radiation and its repair in budding cells.

2.2 Genetic Effects of Radiation

Ionizing radiations are known to induce forward and back mutations, mutations leading to respiratory deficiency (\(\overline{c}\) mutants) and mitotic recombination in yeast (James and Werner 1965; Mortimer and Manney 1971).

Two types of recombinations are known - intergenic and intragenic. Intergenic reciprocal recombination involves exchange of segments of homologous chromosomes. This process in heterozygotes leads to homozygocity and is also known as crossing over. Intragenic non-reciprocal recombination involves replacement of a segment in one homologue with the information contained in the corresponding section of the other. This type is also called gene conversion. In heteroallelic strains this process leads to prototrophy. Breakage and rejoining of DNA duplexes and local DNA synthesis have been suggested to be associated with the recombinational process (Mortimer and Manney 1971).
Rodarte-Ramon and Mortimer (1972), and Brendel and Haynes (1973) have suggested that recombination could be one of the processes for the repair of radiation-induced damage in yeast. Furthermore, the recombinational model for the repair of DSB proposed by Resnick (1976) predicts non-reciprocal and/or reciprocal recombinations. Hence, the induction of recombinants could be taken as evidence for the repair of radiation damage.

Several factors influence the induction of genetic effects by radiation. Induction of mutations (Mortimer et al. 1965) and intragenic recombination (Raju et al. 1971; Subrahmanyan et al. 1978) by low LET radiation was more in the presence of oxygen than in its absence. High LET radiations are more effective than low LET radiations (Mortimer et al. 1965; Murthy et al. 1975). The induction of recombinational events is more in stationary phase diploids than in log phase diploids (Murthy et al. 1976; Perper 1975).

2.3 Repair of Radiation Damage

It is now well recognised that a major part of the radiation damage is repairable. While the process of induction of damage by physicochemical reactions occurring on absorption of the radiation is fast, the repair of the damage is a slow process (Haynes 1966). When the irradiated cells are plated on growth medium to assess the cell survival, repair of radiation damage and replication of DNA occurs. DNA damage has to be repaired prior to the onset of DNA synthesis for the cell to survive.
It is known that several factors such as cell stage, oxygen, postirradiation conditions and the genetic factors modify the repair of radiation damage and thereby the cell survival. From various aspects of postirradiation cellular recovery, radiation damage could be divided into 3 types: 1) sublethal, 2) potentially lethal and 3) lethal damage.

2.3.1 Sublethal Damage (SLD)

The shoulder on the survival curves of diploid yeast and mammalian cells has been taken as an indication of the ability of these cells to accumulate (repair) sublethal damage. Split dose experiments with mammalian and yeast cells (Elkind and Sutton 1960; Bacchetti and Mauro 1965) have confirmed the ability of these cells to repair sublethal damage. Kiefer (1971), and Jain and Pohlit (1973) have shown that the repair of SLD is an energy requiring process. In mammalian and yeast cells the repair of SLD had been shown to decrease with the increase in LET of radiation (Mias, Greene, Fox and Thomas 1967; Barendsen 1962; Bertzsche 1978).

The molecular nature of SLD and its repair is at present not well understood. A radiation sensitive mutant rad51, known to be deficient in mitotic recombination has been shown to be incapable of repairing SLD (Saeki, Machida and Nakai 1974). On the basis of these results Saeki et al. have suggested that the recombinational repair processes may be involved in the repair of X-ray induced sublethal damage.
2.3.2 Potentially Lethal Damage (PLD)

The ionizing radiation damage which is repaired during postirradiation liquid holding (IH) in non-nutrient medium has been called PLD (Phillips and Tolmach 1966). The recovery from PLD during IH is termed as liquid holding recovery (LHR). The repair of PLD has been shown to occur in both yeast (Korogodin 1958; Patrick et al. 1964) and mammalian cells. The extent of LHR for high LET radiations was observed to be less than that of low LET radiations (Bertsche 1978; Reddy et al. 1976; Subrahmanyan et al. 1979). As already noted, stationary phase (G1) haploid yeast cells exposed to ionizing radiation do not show LHR whereas log phase (G2) haploid cells show LHR (Patrick et al. 1964; Moustacchi and Enteric 1970). LHR from the damage caused by UV and chemicals has also been reported (Patrick et al. 1964; Zimmermann 1968a).

On the basis of dependence of LHR on incubation temperature and inhibition of LHR in the presence of certain monovalent and divalent cations and metabolic antagonistic agents Patrick and Haynes (1964) have suggested that LHR is an energy requiring and enzymatic repair process. Furthermore, it has been shown that LHR is an oxygen dependent process and that the role of O2 is to provide energy required for the recovery, through oxidative metabolism in the form of ATP (Patrick and Haynes 1964; Pohlit 1973; Jain and Pohlit 1972, 1973; Jain, Pohlit and Purohit 1973).
Recovery from the damage produced by low and high LET radiations in the presence and absence of $O_2$ has been shown to occur provided oxygen is available during IH (Patrick and Haynes 1964; Reddy et al. 1976; Subrahmanyan et al. 1979). Averbeck and Ebert (1972) have shown that the extent of recovery for hypoxic damage is lower than that for euoxic damage whereas Reddy et al. (1976) have shown that the extent of recovery for both types of damage is the same. IHR data for euoxic and hypoxic damage in a large number of strains would clarify the situation.

The kinetics of recovery during IH has indicated that IHR is complete within 48 h (Patrick and Haynes 1964; Reddy et al. 1976), and that the rate and magnitude of recovery is higher for gamma radiation damage than for alpha ray damage (Reddy et al. 1976). By comparing the recovery kinetics for euoxic and anoxic damage the relative extent of time required to recover from each type could be studied.

The nature of the ionizing radiation damage that is repaired during IH is not well understood. It has been suggested, however, that the PLD may result from the interaction of sublethal lesions (Winans, Dewey and Bettor 1972; Malinovsky 1970) and that during IH the interaction among sublethal damage transforming the lesions to the status of lethal damage is prevented (Rao and Murthy 1978). The study of the IHR phenomena in radiation sensitive mutants could reveal the nature of PLD and the repair processes operating during IH.
2.3.3 Lethal Damage

The damage which the repair proficient cells (wild type) fail to repair under all suitable conditions and leads to cell death may be defined as the actual lethal damage. At a given dose of gamma radiation the highest percent survival of wild type yeast has been observed for log phase cells after immediate plating (Langguth and Beam 1973a; Marthy et al. 1976) and for stationary phase cells after delayed plating (Langguth and Beam 1973a; Reddy et al. 1976). Since immediate plating for budding cells (cells in S-phase) and liquid holding for stationary phase cells are the most favourable conditions for repair, the percent of cells which have suffered lethal damage would be indicated by the dead cells.

1 to 2 unrepaired DSB have been shown to be lethal to cells (Ho 1975; Resnick and Martin 1976). Furthermore, unrepaired SSB would also become lethal since SSB may be developed into DSB by endonuclease attack on the intact strand (Bender, Griggs and Bedford 1974). Although DSB are lethal to haploid cells, all the DSB would not be lethal to diploids. This is so because diploids could repair DSB by using information available on the homologous chromosome as suggested by Resnick (1976). The capacity of a cell to repair any damage is limited. Hence, the damage induced in excess of the repair capacity of the cell would be lethal. Therefore, the percent of cells killed increases with the increase in dose.

As suggested by Resnick and Martin (1976) DSB induced on two homologous chromosomes in the same arms and at the same site would be
lethal since the information is lost on both the homologous chromosomes. Furthermore, at the chromosomal level, aberrations such as asymmetrical translocations and dicentrics would also be lethal to cells.

2.4 Genetic Control of Repair

The first radiation sensitive mutants of yeast were discovered in 1967 by Nakai and Matsumoto. Since then more than 32 distinct genetic loci conferring sensitivity to radiation have been identified (Nakai and Matsumoto 1967; Snow 1967; Cox and Parry 1968; Moustacchi 1969; Resnick 1969; Averbeck, Laskowski, Eckardt and Lehmann - Brauns 1970; Game and Cox 1971). These loci have been designated as RAD followed by locus and allele number. rad denotes the mutant form. Locus numbers from RAD1 to 42 designate genes which primarily control UV sensitivity and locus number from RAD50 onward designate genes which primarily affect sensitivity to ionising radiation. The RAD5, 6, 2 and 18 loci control the sensitivity to both UV and ionizing radiations (Haynes 1975; Game and Mortimer 1974).

The existence of so many loci controlling radiation sensitivity in yeast suggests that the repair of DNA is a complex process. Haynes (1975) has observed that if all the loci are involved in the repair of DNA it could mean that a) the repair may be carried out by multimeric complexes of several enzymes, b) yeast might possess several independent or competing multistep pathways for repair and c) not all the gene products need be repair enzymes—many might be required for the regulation and coordination of repair, normal replication and recombination.
The radiation sensitivity of a mutant as compared to that of wild type is judged by the immediate plating survival. Most of the radiobiological aspects of the rad mutants have been studied using haploid strains (Cox and Parry 1968; Brendel and Haynes 1973; Game and Mortimer 1974). On the basis of cross sensitivity of haploid single and double mutants to UV, chemicals and X-rays the existence of three distinct pathways for the repair of DNA damage has been postulated (Averbeck and Ebert 1972, 1973; Brendel and Haynes 1973; Game and Cox 1973; Cox and Game 1974). Game and Mortimer (1974) have suggested that at least 17 loci are involved in the repair of X-ray damage and that there are two pathways for the repair of X-ray damage.

A useful approach for understanding the relationship among various rad loci and their involvement in repair pathways has been the dose response studies with double and multiple mutants (Brendel and Haynes 1973; Game and Cox 1973; Game and Mortimer 1974). The interaction between two mutant loci could be any one of the types as suggested by Haynes (1975) and is shown in Fig. 2: A) epistasis—where the double mutant is no more sensitive than its more sensitive single mutant parent, B) additivity — where the two loci have an additive effect and C) synergism — where the effect of the two loci is more than additive. It has been suggested that the three types of gene interactions correspond to three distinct situations with regard to the relations among the putative repair processes controlled by the genes in the wild type strains (Haynes 1975). Epistasis implies that
Schematic definition of additive and synergistic interactions between mutant alleles in strains mutant at two different loci controlling radiosensitivity. A double mutant MN in which there was no interaction between the mutant genes (epistasis) would have the same sensitivity as its more sensitive single-mutant parent N. An additive interaction (E) between the two genes would give a survival curve such that all points on it are determined by the relation $AE = AB + BD = AC + BD$. A synergistic interaction ($E'$) between the two genes would give a survival curve of sensitivity undetermined, but greater than that for additivity, i.e. $AE' > AE$ (Haynes 1975).
either the gene products mediate consecutive steps in the same repair pathway or they are parts of a multimeric repair complex; additivity implies that the gene products act on different substrates in independent pathways; and synergism implies that the genes control steps in two pathways that compete for the same substrate.

Haynes (1975) has made a tentative sketch of the possible relations among the ten rad loci, used in radiobiological studies of double mutants, as shown in Fig. 3. RAD1, 2, 3 and 4 loci are epistatic to one another and are known to control the excision of pyrimidine dimers in DNA and constitute the excision repair pathway. Cross sensitivity studies have indicated that these loci also control the excision of HN2 induced defects in DNA (Brendel and Haynes 1973). The rad6 and 18 strains are sensitive to UV, HN2, X-rays and MMS. RAD6 and 18 are epistatic, and have been suggested to be involved in the error prone repair process analogous to the BES system in E.coli. (Brendel and Haynes 1973). The molecular nature of the damage repaired by the RAD6 pathway is not known. The RAD50, 51 and 52 loci are known to affect X-ray and MMS sensitivity. RAD52 has been suggested to control the X-ray induced dominant lethality which arises from gross chromosomal aberrations.

The manner in which DNA damage is repaired not only influences the survival but also the mutagenic effectiveness of the agent. Prakash (1976a, b) has studied the mutagenic effect of 4-NQO (4-nitroquinoline-1-oxide), EMS (ethyl methanesulphonate) and HNO2 (nitrous acid) in wild type and various rad strains.
Tentative relations among the pathways for dark repair of DNA structural defects produced in yeast by UV and X-rays. So far as is known, defects produced by HN2 are repaired via UV pathways, while MMS damage is repaired via X-ray pathways primarily. (Haynes 1975)
She has shown that the reversion (back mutation) at the *cyc1-131* allele induced by EMS and HNO$_2$ was reduced in *rad6* and 2 strains. In case of 4-NQO, the reversion was lower in *rad6*, 9 and 18 strains and was higher in *rad1*, 2, 3 and 4 strains. Similarly for UV, Lawrence and Christensen (1976) have shown that the reversion at *cyc1-2* is much lower in *rad6*, 9 and 18 strains when compared to wild type. Recently, McKee and Lawrence (1979) have shown that the reversion at *cyc1-2* induced by gamma rays is absent in the diploid *rad6* and reduced in the *rad9* and 18 strains. Furthermore, they have shown that the functions of the RAD50 to 57 loci do not appear to play any part in mutagenesis. McKee and Lawrence (1979) have suggested that the RAD6 mutagenic pathway is in fact composed of a set of processes, some of which are concerned with error-prone, and some with error-free, repair activities.

Various repair deficient strains of *E. coli* respond in a similar manner to X-rays and MMS on one hand and UV and 4-NQO on the other (Prakash 1976a). Excision defective strains of *E. coli*, analogous to *rad1*, 2, 3 and 4 strains of yeast, show enhanced UV and 4-NQO mutability whereas *rec* strains of *E. coli* are immutable to X-rays, MMS, UV and NQO (Witkin 1969a, b; Ishii and Kondo 1975). The *rec* strains are known to be incapable of repairing single strand gaps induced by X-rays or generated during repair of chemical and UV damage or during the replication of the damaged DNA (Witkin 1969a, b).

Whereas the induction of back mutations occurs both in haploids and diploids, inter-and intragenic mitotic recombination takes place only in diploids. Recently, Saeki, Machida and Nakai (1980)
have shown that gamma ray induced inter- and intragenic recombination is normal or enhanced in the rad6 and 18 strains, reduced in rad50, 53, 55, 56 and 57 strains and absent in the rad51, 52 and 54 strains. On the basis of these results they have suggested that higher radio-resistance of diploids may be related to the recombinational processes.

3. HYPERThERMIA

Increasing attention is being focussed on the use of hyperthermia in the management of cancer therapy either alone or in combination with radiation. Application of elevated body temperature in the treatment of malignant tumours dates back to 19th century. Hyperthermia was achieved either by streptococcal infection or by the use of bacterial toxins which induce high fever (Coley 1893). Warren (1935) has used a combination of artificial fever and X-ray therapy to cure advanced cases of cancer.

Several investigations have been carried out recently to reassess the potential of hyperthermia in the treatment of cancer. The results obtained so far, with mammalian cell systems, are significant and indicate a definite advantage of heat as an antitumour agent. Temperature in the range of 41-46°C is cytotoxic (Westra and Dewey 1971) and potentiates the lethal effects of radiation by inhibiting the DNA repair processes (Ben-Hur and Elkind 1975; Kim, Kim and Hahn 1976; Corry, Robinson and Getz 1977).
It also can selectively kill radioresistant S-phase or hypoxic or tumour cells (Westra and Dewey 1971; Thrall, Gillette and Dewey 1975; Kim et al. 1976; Connor, Germer, Miller and Boone 1977).

Yeast is another eukaryotic organism on which the effects on elevated temperatures have been studied (Schenberg - Frascino and Moustacchi 1972; Kiefer, Kraft-Weyrather and Alawica 1976; Petin and Berdnikova 1979). Recently Murthy, Deorukhakar and Rao (1979) and Rao, Deorukhakar and Madhvanath (1980) have shown that not only is the response of yeast to hyperthermia, in terms of the shape of the survival curves, similar to that of mammalian cells but also heat can sensitize the cells to radiation and can selectively inactivate log phase (S-phase) and hypoxic cells. The major difference between mammalian and yeast cells is in the order of temperatures required for inactivation viz. 40°C and above for mammalian cells, and, 50°C and above for yeast cells.

While it is known that DNA is the target for radiation induced cell killing (Szybalski and Opera-Kubinska 1965) the nature of the target(s) involved in thermal inactivation is not understood clearly. Various targets such as proteins (Westra and Dewey 1971), Membranes (Mondovi et al. 1969) and lysosomes (Overgaard and Overgaard 1972) have been implicated to be responsible for the cytotoxicity of heat.
By comparing the sensitivity of *rad* mutants and wild type (in stationary and log phase) to heat and *gamma* radiation it is possible to see if heat is inducing damage to DNA similar to that of ionizing radiation. If heat induces such DNA damage, *rad* mutants would be more sensitive to heat compared to the wild type as in the case of gamma radiation. Furthermore, it is known that wild type log phase cells of yeast are rich in several enzymes (Beam and Langguth 1973a) including DNA repair enzymes (Beam and Langguth 1973a; Bokstein et al. 1967), whereas the *rad* mutants are deficient in DNA repair enzymes alone. Hence, by comparing the sensitivity of log phase cells of *rad* mutants and wild type to heat and gamma radiation it would be possible to indicate the role of enzymes in general and of repair enzymes in particular in heat inactivation.
B. SCOPE OF THE THESIS

In order to understand the genetic control of repair of gamma radiation induced damage in diploid yeast and the nature of the target involved in heat induced cell killing the following studies were carried out using wild type and 11 radiation sensitive strains of diploid yeast S. cerevisiae.

1. Survival response of wild type and rad strains in stationary phase, exposed to gamma radiation was studied. Cells were exposed to radiation under euoxic conditions and were plated on YEPD immediately after irradiation.

2. The effect of rad loci on the sensitivity to gamma rays in diploids and haploids was compared.

3. In order to estimate the OER, survival response of wild type and rad strains in stationary phase after exposure to gamma radiation under anoxic conditions was studied. The cells were plated on YEPD immediately after irradiation.

4. After gamma exposure of stationary phase wild type and rad mutants cells, in euoxic and anoxic conditions, were subjected to liquid holding for 48 h. The extent of IHR from euoxic and anoxic damage was estimated in terms of recovery factor.

5. Kinetics of IHR from euoxic and anoxic damage was studied for all the strains.

6. Survival curves of log phase cultures of wild type and rad mutants exposed to gamma rays were obtained.

7. IHR and budding cell resistance in wild type and mutants were compared.
8. Survival response to gamma radiation of wild type disomic strain Z140-51C and its recombination deficient strain rec4 (203) was obtained for both stationary and log phase.

9. Survival of stationary phase wild type and rad53 strains exposed to hyperthermia at 51°C was studied.

10. Survival response of log phase cells exposed to heat at 51°C was also studied.

11. The survival response of wild type and rad strains to gamma radiation and hyperthermia was compared for stationary and log phase.