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RESEARCH ARTICLE

RADIATION BIOLOGY OF CULTIVATED PLANT CELLS

***Mandaloju Venkateshwarlu**

Department of Botany, Kakatiya University, Warangal – 506 009

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ABSTRACT

The potentials of these and other ap¬proaches have been presented elsewhere in this volume. This discussion is focused on the radiation2 biology of cultured plant cells. Where information is incomplete or absent for cultured plant cells, we have drawn upon the microbial and animal cell literature for reference. In animals the lethal effects of ionizing radiation are reflected in killing of specific cell types (e.g. crypt cells of the intestine and the stem cells of the bone marrow), and this determines the survival frequency of the irradiated animal population (HALL, 1973). King P J (1984) and Venkateshwarlu M (2020).Nevertheless, studies on cultured mammalian cells have contributed much to our present understanding of the biochemical, biophysical, and genetic aspects of radiation damage and recovery in animals (see ELKIND and WHITMORE, 1967; CLEAVER, 1974). Rajendra Prasad et al (2018), Venkateshwarlu M (2019). In contrast, irradiation of plants, while produc¬ing some differential cell killing, does not appear to induce death of the organism by affecting a single cell type. Thus, studies with plant cells in culture may reflect more the effects of penetrating radiation on the organism as a whole than those with animal cell cultures. Venkateshwarlu M (2019 & 2008)

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INTRODUCTION

Plant tissues can be isolated and cultured in vitro by the application of relatively simple techniques and their use offers great advantages:

- To the biochemist, who can manipulate the cellular environment with added effectors (e.g. hormones, metabolic precursors, inhibitors) while avoiding contributions by contaminating microorganisms.
- To the morphologist, who can explore the developmental potential of single somatic cells.
- To the geneticist, who can treat millions of isolated cells as individual mutable units and select for a rare variant in a few petri dishes.
- To the cell biologist, who can study the basic physiology of a population of essentially identical cells.

The potentials of these and other approaches have been presented elsewhere in this volume. This discussion is focused on the radiation[2] biology of cultured plant cells. Where information is incomplete or absent for cultured plant cells, we have drawn upon the microbial and animal cell literature for reference.

***Corresponding author: Mandaloju Venkateshwarlu,**
Department of Botany, Kakatiya University, Warangal – 506 009.

In animals the lethal effects of ionizing radiation are reflected in killing of specific cell types (e.g. crypt cells of the intestine and the stem cells of the bone marrow), and this determines the survival frequency of the irradiated animal population (HALL, 1973). King P J (1984) and Venkateshwarlu M (2020).Nevertheless, studies on cultured mammalian cells have contributed much to our present understanding of the biochemical, biophysical, and genetic aspects of radiation damage and recovery in animals (see ELKIND and WHITMORE, 1967; CLEAVER, 1974). Rajendra Prasad *et al* (2018), Venkateshwarlu M (2019). In contrast, irradiation of plants, while producing some differential cell killing, does not appear to induce death of the organism by affecting a single cell type. Thus, studies with plant cells in culture may reflect more the effects of penetrating radiation on the organism as a whole than those with animal cell cultures.Venkateshwarlu M (2019 & 2008). The effects of ionizing radiation have been studied in many plant species (e.g. Sparrow *et al*, 1958, 1965; DAVIDSON, 1960; ROMANI, 1966; HABER, 1968, 1972; VERMA, 1974), but the complexity of the; intact organism has left many basic questions unanswered regarding aspects of the molecular and cellular recovery processes following damaging doses of ionizing radiations. Earlier work on radiation effects in cultured plant cells focused primarily on the evaluation of growth inhibitions resulting from UV or ionizing radiation exposure. Questions relating to molecular radiation effects in cultured plant cells have been reported more recently. Venkateshwarlu M *et al* (2010), Odelu *et al* (2016).We will consider these aspects in detail.

We will also discuss the various criteria which have been employed in evaluating the radiation sensitivity of cultured cells, and will emphasize the need to measure single-cell colony formation in radiation survival experiments. Skirvin (1978), Sharma *et al* (1988). Data on the UV sensitivity of plant cells is needed especially in view of the predicted effect of current technology in partially destroying the UV-filtering ozone layer in the stratosphere (e.g. JOHNSON, 1971; ROWLAND, 1974). Rao *et al* (1975), Meins (1983) & Kochba & Roy (1978).Plants are naturally exposed to significant quantities of UV in sunlight in the range of 280-315 nm ("UV-B"), and the damaging effects of solar UV have been demonstrated in plants grown under conditions which excluded the longer-wavelength radiation responsible for photoreactivation of UV damage (CALDWELL, 1971). Jain *et al* (1984), George *et al* (1980) & Deganin (1973).Some important questions which can be answered using cultured cells are: how will natural and cultivated plants respond to increased amounts of solar UV? Can plant cells recover from UV damage? What is the extent of species variation in UV recovery capacity? Are plants now operating, close to the limit of their recovery capacity? Do plants accumulate UV-induced damage in their genetic "material". Botino (1975), Bassam *et al* (1990).

IRRADIATION OF CELLS AND PROTOPLASTS

Plant cell cultures, while less studied than animal cells, offer some unique advantages. Especially significant are the availability of completely defined media for the growth of plant cells, haploid cell cultures, and technique which allow the recovery of the whole plant from tissue cultures. Novak & Micke (1987), Murashig (1974). Cultured plant cells provide excellent material for radiation studies since the use of single cells obtained from liquid suspension cultures or of isolated protoplasts allows quantitative radiation dosimetry and analysis of single-cell survivals. In addition, the absence of a cell wall makes protoplasts ideal for the isolation and analysis of very high molecular weight DNA after radiation treatment (HOWLAND, 1975; HOWLAND *et al*, 1975). Nucleic acids can be labeled with radioisotopes for studies of radiation-induced damage and repair without interference from contaminating microorganisms often encountered in whole-plant or organ experiments (LONBERG-HOLM, 1967). Caution must be exercised, however, to avoid or inhibit catabolism of the radio-active pyrimidines supplied to the plant cells (HOWLAND and YETTE, 1975). When degradation is serious, labelling can be very inefficient and sometimes nonspecific (TAKATS and SMELLIE, 1963).

A unique advantage of plant cells is the availability of haploid material and aneuploid stocks of several different species. By use of monosomic and trisomic lines for radiation studies, it should be possible to test directly the contributions of separate chromosomes to radiation recovery processes and thus to map genetically the location of specific recovery functions (CARLSON, 1972). The utility of haploid cells for mutation studies has been discussed by others (CHALEFF and CARLSON, 1974). The two most useful aspects of haploid mutagenesis are the immediate expression of recessive mutations and the ease with which homozygous diploids can be obtained by colchicines doubling of haploids. Since some cultured plant tissues can be induced to differentiate the intact plant, the breeder can also conduct formal genetic analysis of mutants induced and isolated in vitro, with the possibility of bringing a new genotype into agricultural use (CARLSON, 1973).

These characteristics of cultured plant cells are especially appealing for basic research in genetics and development since there are no available mammalian cell systems which are either haploid or able to totally re differentiate in vitro. Many problems in mutagenesis, gene expression, and differentiation can be readily studied with plant cell cultures. However, there are still some significant limitations on the use of plant cultures for radiation studies. Single-cell preparations are essential for strictly quantitative assay of cell survival by cloning in petri dishes, but many plant suspension cultures do not yield adequate numbers off free cells which can be separated from cellular aggregates. As a convenient recourse, protoplasts can be enzymatically isolated in large quantities, thereby providing the required starting material for survival assays. The plating efficiency of most isolated plant cells and protoplasts tends to be rather poor, especially at low cell densities (below 10^4/ml). This density dependence can be partially overcome by the addition of "conditioned" medium (ERIKSSON, 1967a) or by a "feeder layer" of radiation-killed cells (RAVEH *et al*, 1973).

Recent work indicates that improved culture media can greatly enhance plating efficiency (LOGEMANN and BERGMANN, 1974; KAO and MICHAYLUK, 1975). We fully expect that plant culture techniques will be developed to increase the plating efficiency of plant cells in the same way that animal cell plating efficiency has been improved over the past 15-20 years. In mutation studies, physical agents such as ionizing and UV radiation offer some advantage in that dosage can be precisely determined and quantitatively delivered, whereas chemical mutagens must be taken up by the cells and subsequently washed out, making dosimetry more difficult to control. In addition, many chemical mutagens are spontaneously or metabolically degraded in the cellular environment, or must be metabolically activated in order to produce genetic effects, further complicating the dosimetry.

Ionizing Radiation: There are many different types of X-ray machines ranging in size from the smaller dental units to more powerful therapeutic and research units. The availability of these units accounts for their widespread use in radiation studies. X-rays are generated by the interaction of an electron beam with a tungsten or molybdenum target. A number of factors (i.e. operating voltage, nature of the target, filtration, tube current, and distance to sample) determine the X-ray beam energy spectrum and the dose rate. In general, operating voltages around 100 kV are sufficient for irradiation of cells dispersed in a petri dish. At lower operating voltages, the penetrating power of the beam is correspondingly reduced, so that care must be taken in selection of the appropriate voltage, beam filtration, and sample geometry. The dose rate at the sample position must be measured. This is accomplished with a calibrated ionization meter, or less conveniently by chemical dosimetry. [Refer to ARENA (1971) for an introduction to these considerations]. Gamma rays for biological experiments are generally obtained from isotope sources (e.g. ^{60}Co or ^{137}Cs) which are often housed *within* shielded units; and samples are introduced via a timer-operated elevator. The radiation is more homogeneous and of higher energy than an X-ray beam. Consequently, there is no need to filter out less penetrating "soft" radiation. The dose rate is dependent upon the source-sample geometry and the mass of the isotope. A resulting disadvantage of y-ray sources is that experiments evaluating dose-rate effects are often not possible since many instruments do not permit changes in the source-to-sample distance.

Shielding between the source and sample has been employed to attenuate the y-rays for dose rate studies (e.g. SPARROW, 1966). Since the dose rate changes with time according to the isotopic decay function (the half-life for ^{60}Co is 5.27 years and for ^{137}Cs, 30 years), this rate must be measured or recalculated frequently. SPARROW (1961) has described the various types, characteristics, and sources of ionizing radiation, including neutrons, β particles, α particles, and protons. The text by ELKIND and WHITMORE (1967) provides a discussion of practical and theoretical aspects of radio-biological experimentation with cultured cells. The high energy of X and y-rays reduces problems of differential absorption and penetration of the radiation in the sample. Cells or protoplasts can be conveniently irradiated in a small, sterile, disposable plastic centrifuge tube, thus simplifying subsequent washing of the irradiated material with fresh medium. For irradiation in the: absence of oxygen the sample can be equilibrated with a sterilized stream of nitrogen gas. Since temperature can seriously alter the biological functions related to radiation effects, it is often advisable to eliminate variation in temperature, for example, by cooling the sample on ice during irradiation.

Ultraviolet Radiation: The brief text by JAGGER (1967) provides an excellent introduction to the equipment, methods, and concepts in UV photobiology; workers planning to do UV studies with plant cells will profit from the suggestions and cautions offered therein. Note especially the potential hazard of eye exposure to UV radiation. The most commonly used source of far-UV radiation is the germicidal lamp. These lamps are very effective in inducing mutation or cell killing because the emitted energy (primarily 254-nm radiation) is strongly absorbed by DNA. A desk lamp fitted with two 15 W lamps (e.g. General Electric Germicidal Lamp) provides an inexpensive and convenient source of far-UV radiation.

For uniform UV exposure, single cells or small aggregates of 2 or 3 cells are absolutely essential and should be dispersed in a thin layer of medium in a petri dish or other suitable container. Constant agitation will further improve the uniformity and reproducibility of irradiation. Of course, the petri dish cover and any Other UV-screening material must be removed during irradiation. The high absorbance, scattering, and reflectance of various components (e.g. DNA, RNA, proteins, flavonoids, cuticle) make multicellular organs or large cell aggregates unsuitable for most UV studies. Isolated protoplasts are most nearly ideal; but even so, the concentration and relative localization of organelles and other UV-absorbing components can significantly reduce the actual dose of UV to the nuclear DNA. The UV transmittance of the sample (medium plus cells) should be determined spectrophotometrically and the measured fluences multiplied by a correction factor (MOROWITZ, 1950) to give the incident dose (i.e. fluence) at the level of the cells.

When possible, the cells should be irradiated through a UV-transparent medium, such as distilled water or a dilute salt solution, of the minimum thickness to cover the cells. (Of course, this is not possible with protoplasts, which must be maintained in media of high osmotic strength.) Alternatively, cells settled at the bottom of a UV-transmitting culture dish can be irradiated from below (e.g. ERIKSSON, 1967a). These considerations are necessary for accurate dose determinations and will permit more reasonable comparisons to be made on the sensitivities of cells irradiated under conditions employed in different laboratories. Refer to JAGGER (1967) for a further discussion of dosimetry and dose measurement. Sunlight can be used directly to study effects on cultured cells [e.g. yeast (RESNICK, 1970) and human cells (TROSKO *et al*, 1970)]. The fluence can be determined with a "sunburn meter" (BILLEN and GREEN, 1975) or with a biological assay utilizing UV-sensitive bacteria (HARM, 1969; BILLEN and FLETCHER, 1974).

Factors modifying the recovery of irradiated cells: Factors which modify post irradiation recovery include those which suggest that recovery requires metabolic activity (e.g. oxygen, and temperature), those which suggest a role (or lack of role) for a specific metabolic pathway such as protein synthesis, those which may modify the repair of DNA damage (e.g. certain chemical inhibitors), and those which involve physical modification of irradiations (e.g. dose rate and dose fractionation). In addition, radiolysis products produced in the suspending medium can exert effects on the subsequent growth of both irradiated and unirradiated cells.

Temperature: Low-temperature incubation following irradiation suppresses repair of chromosomal damage in root tip cells (see WOLFF, 1961) and reduces DNA strand-break rejoining in murine lymphoma cells (ORMEROD and STEVENS, 1971). The latter authors report that increasing the temperature from 37° C (standard for these cells) to 43° C did not affect the initial rate of repair, but did lead to DNA degradation. In addition, recent work on survival modification of X-irradiated Chinese hamster fibroblast cells at increased temperatures indicates a definite temperature optimum for recovery from sublethal doses in these cells (BEN-HUR *etal*, 1972).

Oxygen: Cells irradiated with ionizing radiation in the absence of oxygen generally exhibit a 2/3 reduction in damage or lethality. This "oxygen effect" may be due to the formation of peroxides and their subsequent secondary damaging interactions with cellular constituents (BLOK and LoMAN, 1973). Oxygen can be eliminated by equilibrating the cells with a stream of nitrogen gas before and during irradiation, thus allowing evaluation of the "oxygen effect" on radiation-induced lethality or on some of the molecular aspects of radiation damage (e.g. HOWLAND *et ah*, 1975).

Factors Showing Significance of Specific Metabolic Pathways: The requirement for oxygen during recovery and the enhancement of survival, as measured by the ability of cells to proliferate, with increasing time of split-dose X irradiation in *Oedogonium* (HOWARD, 1968) led to the suggestion that cellular energy metabolism is required for recovery. Experiments with yeast (KIEFER, 1971) offer the strongest evidence that metabolism is the critical requirement for split-dose recovery as measured by colony-forming ability glucose, which the cells metabolize either aerobically or anaerobically, supports repair at similar rates whether the cells are in an oxygen or nitrogen environment. ORMEROD and STEVENS (1971) found that inhibition of DNA synthesis, RNA synthesis, or protein synthesis was ineffective in reducing DNA strand-break repair in X-irradiated murine lymphoma cells, while inhibition of oxidative phosphorylation reduced single-strand-break repair after high X-ray doses. TROSKO and HART (1976) have reviewed some effects which inhibit the normal repair of induced DNA damage in animal cells. Survival can be reduced and/or mutation frequency increased. Among the chemicals effective in this regard are caffeine, which inhibits post replication repair and lowers the mutation

frequency, and phorbol myristate acetate (a component of croton oil) which inhibits excision-repair, decreases survival, and increases mutation frequency.

Physical Factors Modifying Recovery: The rate at which a dose is delivered to, cells can modify the effect of the radiation when a recovery mechanism is operating. The distinction can be made between *acute* and *chronic* doses on the basis of the cells opportunity to metabolize the radiation damage being induced. SPIEGEL-ROY and KOCHBA (1973) have observed a dose-rate effect on growth and embryo differentiation in y-irradiated *Citrus* callus. At rates of 3.1 or 50 Krad/h embryo development was stimulated by a 16-Krad dose, while at 100 Krad/h both growth and embryo differentiation were inhibited. It is possible by cooling the cells on ice to reduce any recovery activity further during an acute irradiation, even at relatively low dose rates.

Dose fractionation is a special case of chronic irradiation in that an interval for recovery intervenes between two or mo *e acute exposures. Dose fractionation can be used to assess the capacity for recovery from radiation damage. If no recovery occurs during the interval between the doses fractions, the cumulative dose will have an effect equivalent to that of a single acute dose of the same total magnitude. High-LET (linear energy transfer) radiations (e.g. protons and neutrons) are presumed to cause so much damage to any molecule with which they interact that repair seems unlikely. Furthermore, survival curves from such radiations usually are exponential, supporting the observed lack of sparing action by dose splitting**.** Many other examples of radio sensitivity modification in plants by physical chemical and biological factors have been cited by SPARROW (1961).

Media Effects: Radiation can produce chemical changes in culture media in addition to the direct effects produced in the irradiated cells. These "indirect" effects on the growth and differentiation of cultured plant cells have usually been observed only after massive (2000-5000 Krad) doses of ionizing radiation to the sugar component of the media. Indirected effects of lower radiation doses have also been reported. VERMA and VAN HUYSTEE (1971a) observed increased growth of irradiated peanut cells when the culture medium was replaced with fresh medium after γ-ray doses of 5-1000 Krad. However, GALUN and RAVEH (1975) observed no effect of γ-irradiated medium on the plating efficiency of irradiated (up to 1.5 Krad) tobacco protoplasts. Some enhanced morphogenetic effects; have also been ascribed to radiolysis products induced in culture media. Embryo formation in *Citrus* ovular callus was equally stimulated by 16 Krad γ-rays to the cells plus medium or to the medium alone, while irradiation of the callus alone was ineffective (SPIEGEL-ROY and KOCHBA, 1973).

DEGANI and PICKHOLZ (1973) found that medium irradiated with as low as 0.5 Krad γ-rays permitted shoot development in unirradiated, dark-grown tobacco callus. ZEEVAART and LEE (1968) reported that *Haplopappus* callus tissue failed to grow on medium which had been exposed to about 10^6 J/m^2 of near-UV from fluorescent "black light" lamps, but these workers did not identify the component(s) of the medium responsible for the inhibitory effect. WANG and his associates (1975 and references cited therein): have demonstrated that mammalian cells are killed (ca. 1% survival) by toxic photoproducts produced in the culture medium as a result of moderate levels of near-UV irradiation (2xl0^4 J/m^2 "black light" or 4 x 10^4 J/m^2 "daylight" fluorescent lamps). These toxic products appear to result from a riboflavin-sensitized photooxidation of tryptophan or tyrosine. Some complex plant-cell culture media contain these amino acids, but riboflavin is generally not included. KLEIN (1963) observed no effect of UV-irradiated medium on the growth of *Ginkgo* cells.

Evaluating the effects of radiation on cultured cells: Many end points are available for assessing the cellular effect of a particular dose of radiation. These include evaluations at the molecular level (e.g. damage to enzymes or nucleic acids), at the chromosome level (aberrations), at the physiological level (respiration, macromolecular synthesis, hormone synthesis, ion regulation), and at the cellular level (cell growth, cell division, differentiation). Since each involves measurement of a different end point, a direct comparison among the results from these analyses is not possible. Although it appears that some radiation effects may be transient physiological perturbations, the most profound biological effects appear to result, from damage to the cell's genetic material— DNA. The nucleus (specifically the DNA) is the primary radiation-sensitive site in the cell. This conclusion is supported by a large body of experimental evidence including the following:

- Chromosome aberrations are efficiently induced by ionizing and UV irradiations.
- Irradiation of the cytoplasm is far less effective in cell killing than irradiation of the nucleus.
- Radiation that is attenuated by passage through the cytoplasm before reaching the nucleus is less efficient in cell killing.
- DNA absorbs UV very strongly, and its absorbance spectrum reflects the action spectra for cell killing and mutation.
- The large size of the native DNA molecule renders it most susceptible to damage arising from ionizing irradiation.
- In bacteria, radiation damage to purified DNA is expressed in DNA transformation experiments.
- Organisms which are deficient in repair of DNA damage are much more susceptible to radiation-induced killing.

Having acknowledged that the DNA is the primary radiosensitive site in the cell, we must also recognize that other effects can be observed as expressions of damaged DNA (e.g. induced mutations) or as expressions of direct damage to secondary sites in the cell (e.g. RNA damage, enzyme inactivation).

Radiation-induced DNA Damage and Repair: Both ionizing and UV irradiations result in certain more or less well-defined damage in DNA. Ionizing radiation causes single-strand breaks, base damage, double-strand breaks, and, to a lesser extent, interstrand cross-links (see KANA-ZIR, 1969). UV damage to DNA is primarily in the form of intrastrand dimerization of adjacent pyrimidines, the lesion shown to be important in cell killing (Setlow, R.B., 1968). Other DNA lesions produced by UV are hydrates of cytosine, cross-links between DNA strands, chain breaks, and DNA-protein cross-links. It is convenient to discuss repair of ionizing radiation damage and UV damage separately since the respective DNA repair systems appear to be different in certain aspects.

A number of comprehensive reviews on DNA damage and repair in microbes and animal cells have been published

Repair of Ionizing-radiation-induced DNA Damage: MCGRATH and WILLIAMS (1966), examining DNA damage and repair in bacteria, developed a technique which eliminates the need to extract and purify the cellular DNA, thus avoiding mechanical shearing of the DNA. This technique has since been adapted for use with mammalian cells (see SETLOW and SETLOW, 1972; ORMEROD, 1973), but its application to the analysis of DNA in intact plant cells is precluded by the presence of a cellulose cell wall. However, we have applied this method to higher plant cells by utilizing isolated protoplasts of cultured wild carrot cells (HOWLAND *et al*, 1975). Cells containing radioisotope-labeled DNA are enzymatically converted to protoplasts, irradiated, and placed directly on the surface of an alkaline sucrose gradient. The protoplasts lyse, the DNA is dissociated from the chromosomal proteins and RNA, and the DNA is denatured by the alkaline conditions. The gradient is then centrifuged, and the DNA sedimentation velocity is determined by the distribution of radioactive DNA in the gradient.

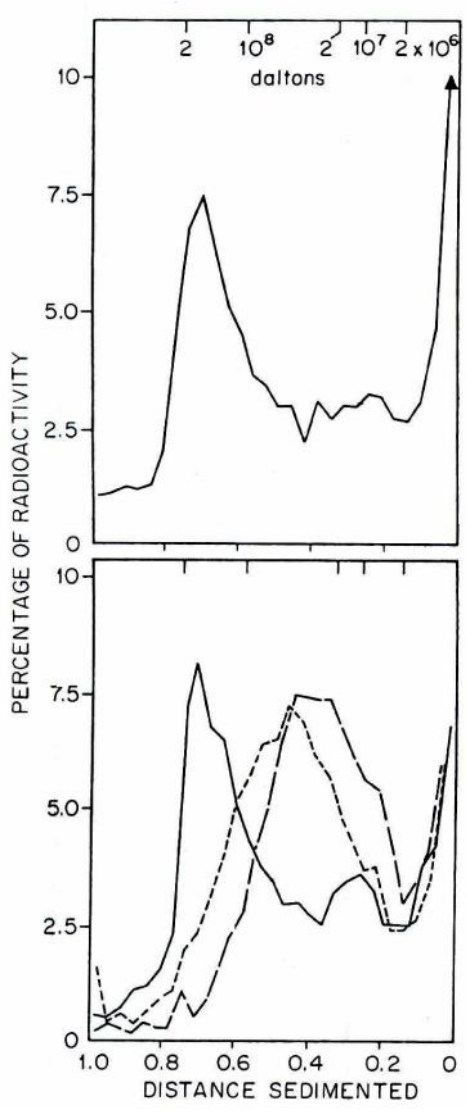

Figure 21.1. Sedimentation profiles in alkaline sucrose gradients of wild carrot protoplast DNA before irradiation *(upper panel);* immediately after 20 Krad γ-rays at 0° C (-----); and after post irradiation incubation at 28° C for 5 min (-----) or 47 min (-----) *(lower panel).*

The quantitative determination of induced strand breaks is based on the reduction in single-strand molecular weight with increasing dose of ionizing radiation. Likewise, repair of strand breaks is seen as the recovery of higher-molecular-weight DNA with post irradiation incubation of the cells prior to lysis on the gradient. For *γ* irradiation of wild carrot protoplasts, the induction of single-strand breaks is linear with increasing doses and amounts to 1.2 x 10^{-12} breaks/ dalton DNA/rad. In the absence of oxygen, the yield of strand breaks is reduced by 2/3 to 0.4 x 10^{-12}/dalton DNA/rad (oxygen effect). It should be noted that single-strand breaks detected on alkaline sucrose gradients after exposure of cells to ionizing radiation are of two types: actual interruptions in the polynucleotide chains and alkali-labile sites such as those which result from base loss (see TOWN *et al*, 1973). If the irradiated (20 K rad) wild carrot protoplasts are allowed to incubate at 28° C, the single-strand breaks are rapidly repaired (Figs. 21.1 and 21.2), so that by 60 min none of the original lesions are detected.

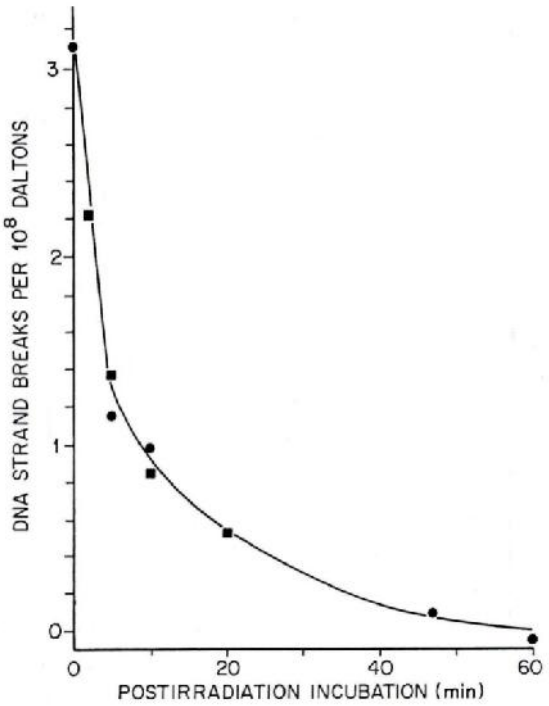

Figure 21.2. Kinetics of repair at 28° C of strand breaks (plus alkali-labile bonds) in DNA of wild carrot protoplasts after 20 Krad of γ irradiation at 0° C. The data from two separate experiments are plotted (● and ■).

All organisms which have been examined thus far, including a higher plant, normally have the capacity to repair strand breaks in DNA. However, recent data on nucleated non dividing chick erythrocytes indicate that these cells have a decreased ability to rejoin DNA strand breaks and appear to accumulate breaks in their DNA with age. Only one higher plant system other than wild carrot has been examined *Vicia faba* root tips. In that system there is an apparent absence of ionizing-radiation induced DNA repair synthesis. Although these negative results may result from technical difficulties in conducting the experiments it may be that wild carrot and *Vicia* represent extremes in the range of ionizing-radiation-damage repair capacities to be found in plants. Since most cells efficiently repair single-strand breaks in DNA, ORMEROD and STEVENS (1971) suggest that lesions other than single-strand breaks (e.g. double-strand breaks, base damage) are responsible for radiation-induced cell killing. Another major class of DNA damage induced by ionizing radiation is "base damage".

CEkRRUTTI and his associates have characterized some specific types of base damage and have developed methods to assay for their excision from the DNA of mammalian cells (see MATTERN *et al*, 1975). These assays should be directly applicable to the characterization of ionizing radiation damage and repair in plant cell DNA. WILKINS (1973) and WILKINS and HART (1974) have described an assay for the repair of lesions in DNA utilizing a DNA endonuclease that acts on ionizing-radiation-induced lesions in DNA -so-called endonuclease-sensitive sites. These sites can be detected as strand breaks after nuclease treatment when the DNA is sedimented in a gradient of alkaline sucrose. A reduction in the number of endonuclease-sensitive sites with time is indicative of repair of these lesions.

Repair of UV-induced DNA Damage: The biologically most important UV-induced lesion in DNA is the "pyrimidine dimer". Pyrimidine dimers in DNA are induced linearly with increasing UV fluence until very high doses are reached. In isolated protoplasts of are reached. In isolated protoplasts of wild carrot cells this amounts to about 2 x 10^4 dimers/cell per J/m^2 UV dose (HOWLAND, 1975). The number of dimers per cell resulting from a specific UV exposure can be larger or smaller depending on the cellular DNA content and base composition and the degree of attenuation imposed by the optical properties of the irradiated cells and by the irradiation conditions. Cells can recover from pyrimidine dimer damage in at least three different ways: (1) photoreactivation; (2) excision repair; and (3) postreplication repair.

Photoreactivation of Pyrimidine Dimers: Pyrimidine dimers in DNA can be monomerized in situ by the action of photo-reactivating enzyme and visible light. The natures of this repair activity and its phylogenetic distribution have been reviewed (COOK, 1970; RUPERT, 1975). Photo reactivation (PR) of pyrimidine dimers has been demonstrated in cultured cells of *Nicotiana tabacum* (TROSKO and MANSOUR, 1968), *Ginkgo biloba* (TROSKO and MANSOUR, 1969 a), and *Daucus carota* (wild carrot) (HOWLAND, 1975), but not in *Haplopappus gracilis* cells (TROSKO and MANSOUR, 1968). However, the negative results obtained with *Haplopappus* may be a function of the very high UV doses employed.

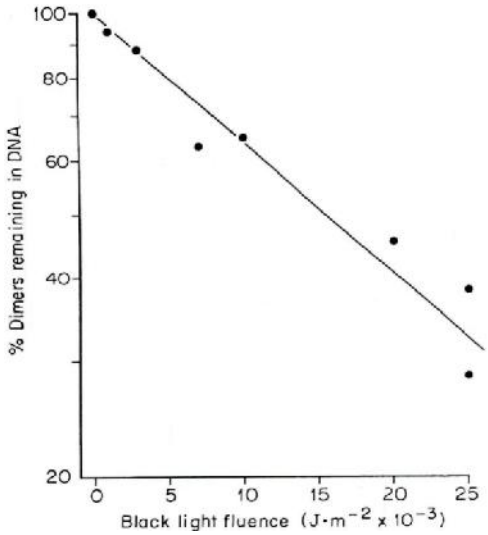

Figure 21.3. Photoreactivation of UV-induced (fluence = 70 J/m^2) pyrimidine dimers in the DNA of wild carrot protoplasts. Black light (1 = 300-400 nm) was obtained from General Electric BLB lamps at a fluence rate of 126 J/m^2/min after passing through 1-cm plate glass. Conditions for dimer analysis were as described previously

The action spectrum for PR of UV-induced lethality in cultured *Ginkgo* cells (KLEIN, 1963) shows maximum efficiency at about 420 nm, similar to PR in a fungus [*Streptomyces griseus* (KELNER, 1949; JAGGER *et al.*, 1970)], and in a blue-green alga [*Agmenellum quadruplicatum*]. In contrast, PR in *Escherichia coli* is maximum in the region of 380 nm (JAGGER, 1967; RUPERT, 1975). This difference should be taken into account when PR is attempted with higher plant cells, since a common source which has been used for PR studies is the fluorescent "black light" (e.g. General Electric BLB, *X* = ca. 300-400 nm, A_{max} = 360 nm). PR of UV-induced, pyrimidine dimers in the DNA of wild carrot protoplasts is illustrated in Figure 21.3. PR enzyme in wild carrot cells can utilize "black light" even though the optimally effective spectral region may be at longer than the maximum wavelength for these lamps. Complete repair (i.e. excision plus PR) of a moderate level of UV damage has been shown for wild carrot protoplasts exposed to cool-white fluorescent light for 24 h, whereas after this UV dose, dark-repair alone gavel only about 60% dimer removal from the DNA (HOWLAND, 1975).

Monomerization of dimers in DNA constitutes a demonstration of "direct" photoenzymatic repair. "Indirect PR" has been observed for many other biological effects after UV irradiation, but can be distinguished on the basis of several criteria (SETLOW, R.B., 1968; COOK, 1970). Most clearly, in order to be considered "direct", the PR light is effective only when administered *after* the damaging UV dose. PR which is effective prior to the UV irradiation is necessarily acting indirectly, and is termed photoprotection. Photoprotection by blue and red light has been reported for growth of UV-irradiated *Ginkgo* tissue (KLEIN, 1963). As a result of photoprotection it is possible that cells propagated in the dark will display a higher UV sensitivity than those which have been grown with illumination. Since PR is apparently specific for the monomerization of pyrimidine dimers in polynucleotides (COOK, 1970), direct PR can be employed as a diagnostic tool for evaluating the possible role of pyrimidine dimers in producing various UV-induced effects. If direct PR reduces or eliminates the UV effect, then the DNA is implicated as the target molecule and the pyrimidine dimer as the important lesion in producing the effect.

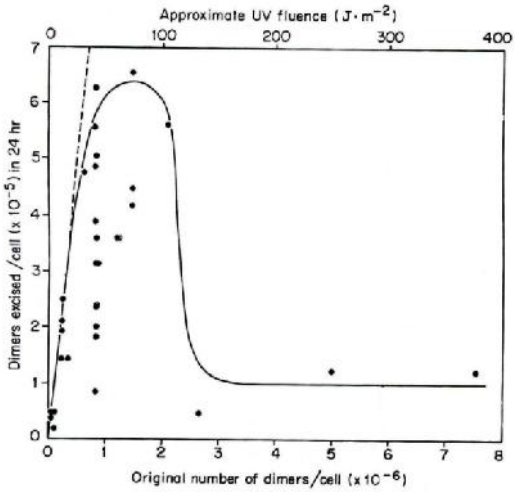

Figure 21.4. Number of UV-induced pyrimidine dimers in wild carrot protoplast DNA excised during 24-h postirradiation incubation (28° C, dark). Values for individual analyses (#) were determined as before (HOWLAND, 1975). Complete dimer excision *(broken line)* and maximum dirrier excision observed *(solid line)* are indicated

Excision-Repair of Pyrimidine Dirmrs: The second major mechanism for repair of dimer damage in DNA is excision-repair. The pathway for this repair activity has been studied in most detail in bacteria, but the essential features appear to apply to eukaryotic cells as well. The first step involves an endonuclease which incises the DNA strand near the lesion. Then exonucleolytic action removes the dimer as well as a number of adjacent nucleotides. Polymerase resynthesizes the DNA which has been excised, using the opposite strand as a template. Finally, DNA ligase seals the remaining nick. Assays for this repair activity include (1) chromatographic analysis of dimers in DNA (2) autoradiographic detection of unscheduled (i.e. repair) DNA synthesis (see CLEAVER, 1974), (3) measurement of DNA repair replication by [^{3}H] bromodeoxyuridine (BrdUrd) incorporation and subsequent equilibrium centrifugation of the DNA (see CLEAVER,, 1974), and (4) incorporation of BrdUrd during repair replication followed by) photolysis of the repaired (i.e. BrdUrd-substituted) regions with 313-nm radiation and analysis of the resulting reduction in DNA single-strand molecular weight on alakaline sucrose gradients (REGAN *et al.*, 1971).

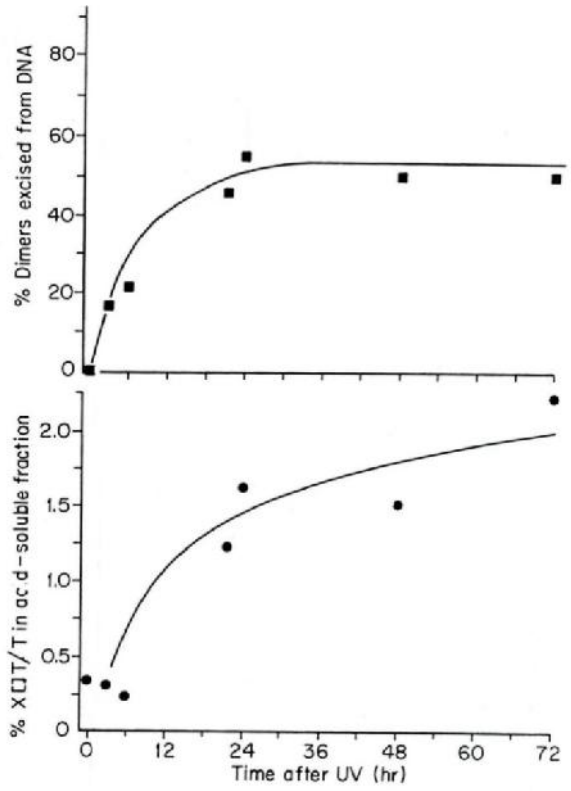

Figure 21.5. Kinetics of pyrimidine dimer excision from the DNA of wild carrot protoplasts (upper panel) and appearance of excised dimers in the cold-acid-soluble cell fraction (lower panel). The UV fluence applied at time = 0 was 42J/m^2. Conditions for dimer analysis were as reported previously

Like all known chemically induced DNA damage, some types of UV-induced DNA damage are not photoreactivable. Consequently, excision-repair competence becomes important to the cell in dealing with a variety of DNA lesions, including dimers. Although several earlier attempts to find dimer excision in cultured plant cells gave negative results recently has been demonstrated in isolated protoplasts of cultured wild carrot cells. Figure 21.4 illustrates that after low UV doses, dimers are efficiently excised (~100%), but that after higher doses, excision is drastically reduced. Since the excision-repair process removes intact pyrimidine dimers from DNA, the appearance of dimers in the liacid-soluble cell fraction confirms that repair is occurring via excision and not by, PR (Fig. 21.5). These data also indicate that in cultured wild carrot protoplasts the rate of dimer excision is initially very rapid, but is essentially zero after 24 h.

$$\% \text{ X } [\,]\frac{T}{T} = \frac{\text{Radioactivity in thymine containing dimers}}{\text{Radioactivity in thymine}} \text{ X } 100$$

Among the placental mammals a wide range in excision-repair capacity has been found, with mouse cells having the least active and human cells exhibiting the most active excision-repair (HART and SETLOW, 1974 a). Work has just begun to determine if a similar variation exists among the higher plants. Excision-repair has also been found in *Haplopappus gracilis* and *Nicotiana tabacum* cells and in *Petunia hybrida* protoplasts. Mutant cells unable to excise pyrimidine dimers display increased sensitivity to UV. Such mutants are known in bacteria and yeast and also in human cells [i.e. xeroderma pigmentosum (CLEAVER, 1968)]; but as yet none has been observed in higher plants. Haploid cell lines derived from anther culture provide the material to select induced mutations in this important function. These mutants would be valuable in advancing our understanding of excision-repair in higher eukaryotes, as well as providing a tool for examining other pathways for DNA repair (e.g. postreplication repair).

Postreplication Repair: Dimers which are not photoreactivated or repaired by excision constitute blocks to normal semi conservative DNA synthesis. In mammalian cells this has been shown to result in the synthesis of smaller-than-normal DNA molecules. At later times the block is by-passed and. the DNA is joined to form the normal high-molecular-weight DNA (see LEHMANN, 1974). The details of post replication repair in mammalian cells are currently under investigation in several laboratories, and some of the concepts already put forth have been questioned (PAINTER, 1974). Evidence of post replication repair activity has recently been observed in protoplasts of cultured wild carrot cells.

Mitotic Delay and Reduced Rate of DNA Synthesis: UV-induced dimers inhibit normal DNA synthesis in many cells. TROSKO and MANSOUR (1969b), following the rate of [^{3}H] thymidine incorporation into cultured tobacco cells, found that DNA synthesis is reduced after irradiation, but that PR could (partially) reverse this inhibition. These data implicate pyrimidine dimers as being the important lesion in UV inhibition of DNA synthesis, and are consistent with more extensive data from bacterial and mammalian systems. Ohyama *et al.* (1974) have observed UV inhibition of DNA synthesis which displays kinetics similar to the UV inactivation of colony-forming ability in isolated soybean protoplasts. In cells that are not undergoing normal semiconservative DNA synthesis and are able to excise pyrimidine dimers from their DNA, there is a UV-stimulated increase in [^{3}H] thymidine incorporation. At low to moderate UV doses, the UV-stimulated repair synthesis may obscure any UV inhibition of normal DNA synthesis. This is true in cultured wild carrot protoplasts where normal DNA replication is much reduced during the first day after enzymatic isolation (HOWLAND, unpublished). ERIKSSON (1967b) observed a reduction in mitotic index in *Haplopappus* cells following X-ray or UV exposure. Division delay per se has not been described in samples of irradiated plant cells in vitro, but observations on cultured mammalian cells and on

algae indicate that some cells can recover from radiation damage after exhibiting a delay in cell division. In these cases the delay period (in cells that can recover) amounts to up to one cell cycle length. ERIKSSON'S (1967a) results with survival of irradiated *Haplopappus* cells suggest that the radiation-induced mitotic inhibition is overcome since many cells can recover to form colonies.

Radiation-induced Chromosome Aberrations: SUNDERLAND (1973) has discussed the high degree of chromosomal variation usually observed in cultured plant cells. A high frequency of "spontaneous" chromosome aberrations makes cultured plant cells less valuable as potential cytogenetic material in which one could evaluate the induced rate of chromosomal aberrations following experimental treatment (e.g. WOLFF, 1961). This spontaneous aberration level can be affected by the culture conditions. SiNGH and HARVEY (1975) have reported strong selection against polyploid and aneuploid *Haplopappus* cells under conditions which favor rapid, homogeneous growth (i.e. suspension culture frequent transfers). It is possible that under the appropriate culture conditions, cultured cells of many other plant species could be maintained with a stable diploid or haploid karyotype**.** ERIKSSON (1967a) evaluated the effects of X-rays and UV on production of chromosome aberrations in *Haplopappus* cells and demonstrated the potential application of cultured plant cells to cytogenetic analyses (ERIKSSON, 1967 b).

Induction of Giant Cells by Irradiation: Cells irradiated with doses high enough to block cell division often retain the capacity for continued growth. After massive *y* irradiation of dry wheat seeds, the embryo can germinate without DNA synthesis or cell division and grow by exaggerated cell enlargement to form a seedling that is surprisingly normal morphologically, biochemically, and physiologically. Giant cell formation has been observed in cultured bacterial, animal, and plant cells. Massive *y* irradiation (550 Krad) of cultured peanut cells increased the frequency of giant cells from 10 to 60% of the population (VERMA and VANHUYSTEE, 1971a); these giant cells had grown to 10-15 times their normal size. Giant cell formation has also been observed in γ-irradiated *Phaseolus vulgaris* culture and in X-irradiated Jerusalem artichoke tissue. Even though no such radiation-induced increases in cell size of microcultured tobacco cells or grape stem callus have been observed, giant cell formation may still, at least in part, account for the observed increases in fresh or dry weight of cultures exposed to high doses of ionizing radiation.

Other Physiological Effects of Radiation: In addition to the previously discussed inhibition of normal DNA synthesis, radiation can also depress the rates of RNA and protein synthesis. UV irradiation of soybean protoplasts results in draniatic reductions in the incorporation (into acid-insoluble material) of [^{14}C]uridind and L-[^{14}C]alanine (OHYAMA *et al* 1974). These effects were observed in the range of UV fluences which also inhibit colony-forming ability. In *Nicotiana* suspension cultures, D_37 (i.e. dose required to reduce the measured parameter to 37% of the control level) for inhibition of protein synthesis is reached at an incident fluence of 388 J/m^2 UV (MURPHY *et al*, 1975). The samples used in these experiments were present as aggregates of 1-35 cells, with about one-half larger than five cells in diameter. The rapid UV inhibition of amino acid incorporation does not appear to result from inhibition of respiration, reduction in endogenous ATP levels, reduced uptake of labeled amino acids, or inhibition of messenger RNA synthesis. Direct UV damage to polysomes, as assayed in a cell-free system, accounts for less than one-half of the inhibition observed with intact cells (MURPHY *et al*, 1975), suggesting that some other, as yet unidentified mechanism(s) is responsible. Additionally, it is likely that inhibition of amino acid incorporation occurs primarily in those cells which are unshielded from the UV radiation (i.e. free cells and cells at the exterior of aggregates). Autoradiographic analysis could be used to investigate this latter possibility. VERMA and VAN HUYSTEE (1971b) observed that the initial 50% depression of protein synthesis in massively irradiated (500 K rad) peanut cells disappeared after one week of postirradiation incubation. By two weeks protein synthesis exhibited a transient increase to ca. 150% of control level, falling to the control level at 3 weeks. However, chromatographic and electrophoretic analyses indicated that the distribution of proteins was clearly abnormal in the cells which had been irradiated. These authors termed this "aberrant recovery" of protein synthesis. Ionizing-radiation has also been employed to reduce the endogenous level of indoleacetic acid in plant cells. Crown-gall tumor tissue, which grows on culture media lacking auxin, can be made auxin-dependent by a 1-Krad dose of X-rays. BAJAJ *et al*.(1910b) reported that the growth of bean callus, as measured by increase in dry weight, was slightly (5%) stimulated by a 0.5 K rad γ-ray dose; but they did not determine whether this effect was due to stimulation of cell division, cell expansion or both. Stimulation of differentiation in cultured plant tissues has been observed after ionizing radiation doses up to 20 Krad. But this appears to be an indirect effect of the irradiated medium on the plant cells.

Lethality as an End Point for Radiation Damage: The ultimate radiation effect is, of course cell killing. A lethal event is usually assayed as the loss of cellular reproductive capacity (i.e. colony-forming ability) in microbial and mammalian cell systems. In the absence of a reliable cell-plating assay other, less-satisfactory measures have been employed in evaluating the radiosensitivity of cultured plant cells.

Measurement of Survival by Mass Increase: Many workers have measured increase in fresh and/or dry weight or sedimented cell volume as a parameter of survival. Although it is certainly true that an absence of fresh- or dry-weight increase in an irradiated culture (compared with control cultures) is indicative of (near) zero survival, it is not possible to interpret intermediate growth increases. Among the confounding factors are: division delay; giant cell formation; transient effects on DNA, RNA, and protein synthesis; and possible temporary rescue by cross-feeding among adjacent cells. Unfortunately, this combination of undefined elements precludes the interpretation of such data in terms of cell survival. VENKETESWARAN and PARTANEN (1966), examining the growth response of γ-irradiated tobacco suspension cultures, observed a significant depression in growth attained after doses beyond 2. Krad. The apparent D_{37} for growth of this tissue is about 18 Krad. BAJAJ *et al.* (1970 b) obtained similar results with *Phaseolus* callus (apparent D_{37} = ca. 19 Krad). A marked contrast exists between these data and those obtained for single-cell survival of cultured mammalian cells, where D_{37} of less than 1 Krad are observed for ionizing radiation.

Attempts to quantitatively interpret radiosensitivity data obtained via weight-increase measurements of cultured plant tissue have led to the (probably erroneous) conclusion that cultured cells are more radioresistant than those in the intact plant. KLEIN (1963) irradiated *Ginkgo* cells with UV and assayed for effects on growth of the cultured tissue by dry-weight increase. The apparent D_{37} in the absence of PR was reached at an incident fluence of ca. 40 J/m^2. TROSKO and MANSOUR (1968) demonstrated a UV-induced growth inhibition, as measured by reduction in fresh weight increase, which could be partially reversed in suspension-cultured tobacco (but not *Haplopappus)* by PR.

Cellular Parameters Used in Survival Assays: We have acknowledged that quantitative, survival assays must reflect the reproductive potential of single cells. Although the most direct and generally accepted method is to measure colony-forming ability of single cells, there are other parameters which can be estimated without; requiring strictly single-cell preparation or high plating efficiency; but such assays do not necessarily reflect the reproductive potential of the irradiated cells. In almost every case they tend to overestimate the true cellular survival. These assays include cytoplasmic streaming and vital staining. CRUZ and HILDEBRANDT (1968) observed that the D_{37} for cytoplasmic streaming in microcultured tobacco cells exposed to γ-rays was 250-500 Krad at two weeks postirradiation. Cells infected with tobacco mosaic virus showed an increased sensitivity for this parameter (D_{37} = ca. 100 Krad), but even this is far beyond the radiosensitivity observed in a cell-plating assay.

ARYA and HILDEBRANDT (1969) used the cytoplasmic streaming assay to compare the radiosensitivities of cultured normal grape stem cells and leaf gall cells. The gall cells appeared to be somewhat more radiosensitive than the normal cells, although the differential was slight (D_{37}'s = 0.5-1.0 Krad). In this case the radiosen sitivities were similar to those found with single-cell-plating assays. Viability of individual cultured plant cells or protoplasts can be assayed using dye exclusion fluorescence or dye reduction. Dead cells are unable to exclude colloid dyes (e.g. phenosafranine, trypan blue, Evan's blue); they do not enzymatically cleave fluorescein diacetate to fluorescein (which fluoresces); nor do they reduce 2,3,5-triphenyltetrazolium chloride to a colored product (red formazan). Vital staining offers the advantages of being rapid and quantitative, but serious questions must be raised about the relationship of these assays to survival as measured by cell proliferation. TOWILL and IVIAZUR (1975) have found a good correlation between tetrazolium dye reduction and plating efficiency *(Haplopappus* cells) or regrowth *(Acer saccharum* cultures) when frozen samples are compared. However, vital stains do not adequately discriminate between viable and lethally irradiated wild carrot or soybean protoplasts [UV and y-rays (HOWLAND, unpublished data)]. In agreement with these observations is the comment of OHYAMA *et al.* (1974) that soybean protoplasts irradiated with supralethal UV doses (based on colony-forming ability) retain the ability to exclude trypan blue. Since cell survival is to be based on reproductive capacity, it is not surprising that vital stains fail to distinguish between cells which are merely physiologically active and normal cells which are capable of proliferation.

Survival Measurement by Single-cell Proliferation: ERIKSSON (1967 b) utilized a plating assay to evaluate the lethal effects of X-rays and UV on a suspension culture of *Haplopappus* which had been filtered (60 um) to remove aggregates of more than four cells. His data indicate apparent D_{37}s of ca. 3 Krad (X rays) and 300 J/m^2 (UV, growth in white light), but ERIKSSON suggests that even these estimates may be somewhat inflated since the plated individuals consisted of mostly 2-4 cell aggregates. In addition, the presence of anthocyanin in the cells would reduce UV sensitivity by screening the radiation reaching the DNA. Collection of the 20- to 53-um filter fraction of a highly disaggregated *Haplo-pappus* suspension culture yielded the following distribution: 70% single cells, 25% doubles, 5% three-celled aggregates, and less than 1% four-celled or larger aggregates. Survival after UV irradiation was determined microscopically as the proportion of plated cells (plus aggregates) that had grown to form colonies of at least 10 cells (Fig. 21.6). When corrected for the probability of totally inactivating the multi celled aggregates as well as the single cells, these data indicate D_{37}s of approximately 80 and 260 J/m^2 without and with PR, respectively (HOWLAND, unpublished).

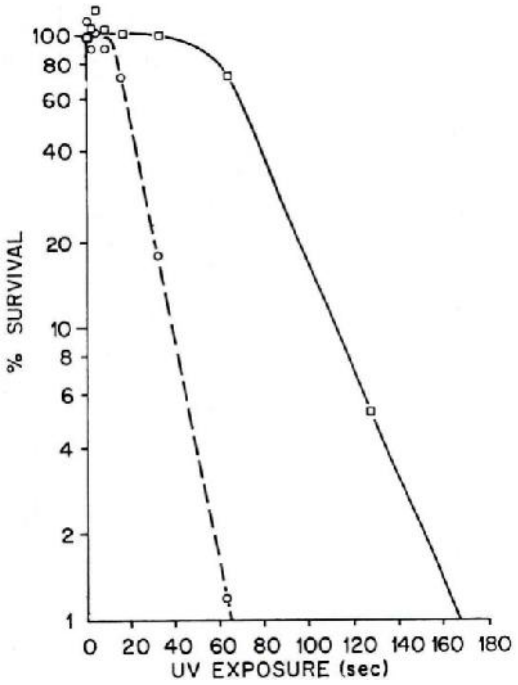

Figure 21.6. Dose survival for colony formation of UV radiation *haplopappus* cells grown without photoreactivation (O) or with continuous white light (□). The incident UV fluence rate was 3.4 J/m^2/sec

Corrections were made by applying the function:
$I_0 = Pf_1 + P^2f_2 + P^3f_3$

Where: I_0 is the observed fractional inactivation of colony formation at a UV dose; f_1,f_2, and f_3 are the proportions of initially irradiated single cells (0.70), doubles (0.25), and three-celled aggregates (0.05), respectively; and p is the actual probability of inactivation for a single cell a, t that UV dose. The value of p is assumed to be the same for isolated cells and for each cell in an aggregate. The development of techniques for protoplast isolation and plating has provided a system in which to evaluate the reproductive potential (i.e. colony-forming ability) of truly isolated cells. GALUN and RAVEH(1975*;* Fig. 21.7) have determined the dose survival relationship for X-irradiated tobacco mesophyll protoplasts. D_{37}s of between 0.5 and 1.0 Krad were observed, comparable to the radiosensitivities of cultured mammalian cells.

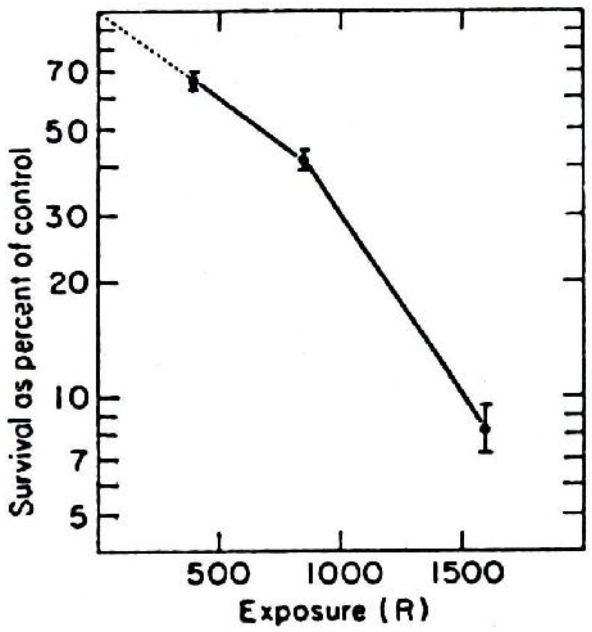

Figure 21.7. Survival of diploid tobacco cells exposed to different X-ray doses on the third day. after protoplast isolation. Plating density was 3 x 101 protoplasts/ml. (From GALUN and RAVEH, 1975^ with permission from the authors and Per-gamon Press)

Haploid protoplasts were found to be somewhat more radiosensitive than their diploid counterparts, but the major determinant of radiosensitivity in this study was apparently the cell cycle stage at the time of irradiation. Mesophyll cells of mature tobacco leaves do not normally divide, but when protoplasts are isolated and cultured they begin to progress through the cell cycle with some degree of synchrony, commencing a wave of nuclear division on the third day after isolation. GALUN and RAVEH (1975) also cite unpublished data of ZELCER indicating that DNA synthesis begins in these cultures at about 24 h and reaches a maximum after 40 h. Radiosensitivity of both haploid and diploid cells was highest on the second day after initiation of the cultures; this peak was presumably correlated with the DNA synthetic period in these cells.

In contrast, BuRHOLTand VAN'THOF (1974) found that initiation of proliferation was correlated with a decreased radiosensitivity, while ploidy difference (diploid vs diploid-plus-polyploid) gave no indication of differential radiosensitivity. In this work, pea root segments were cultured on media which selected for proliferation of diploid pericycle cells only (no kinetin) or for proliferation of both the diploid pericycle cells and polyploid cortical cells (kinetin added). The degree of cell proliferation was determined by macerating explants in 20% chromic acid at 37° C for 24 h, resuspendingin saline, and passing the sample through a 23-guage needle. The separated single cells were then counted using an electronic particle counter. Although this technique does eliminate; some of the possible confusion encountered with measurements of fresh- or dry-weight increase (e.g. giant cell formation) there are still questions as to the possible contributions by

- A radioresistant subpopulation of cells (e.g. cell-cycle-stage-related radiosensitivity),
- Cells that recover from radiation exposure and initiate proliferation at a later time,
- Limited proliferation of lethally irradiated cells.

BURHOLT and VAN'T HOF (1974) were able to distinguish among these possibilities regarding their observation of a limited but constant percent increase in cell number observed at doses above 3 Krad in all their experiments. Since at the time of sampling (7 days postirradiation) the mitotic index in this tissue was at or near zero for the doses over 3 Krad, it follows that pea cells can temporarily divide after being lethally irradiated, and that the extent of this temporary proliferation is independent of dose over the range tested (3-6 Krad). Since lethally irradiated cells can undergo at least 1 or 2 cell divisions before dying after ionizing radiationit is necessary to ignore colonies which have not reached a predetermined minimum size. GALUN and RAVEH (1975) acknowledged this effect and employed the criterion of 20 cells/colony for survival. VARDI *et al.* (1975) have also determined the radiosensitivity of cultured citrus protoplasts, observing a D_{37} of ca. 4 Krad for loss of colony-forming ability. OHYAMA *et al.* (1974; Fig. 21.8) determined the colony-forming ability of UV irradiated soybean protoplasts cultured in the dark (i.e. no photoreactivation). An incident fluence of ca. 4.4 J/m^2 yielded at 37% reduction in survival. The UV fluencies at the level of the cells were actually even less than those reported, due to UV absorbance and scattering properties of the suspending medium and of cytoplasmic constituents. Applying this correction indicates a D_{37} of ca. 3 J/m^2, comparable to the UV sensitivity of UV-sensitive mutants in yeast. OHYAMA *et al.* (1974) also observed that colony formation by cell aggregates of this tissue gives an impression of lower UV sensitivity, probably due to shielding effects and the cell multiplicity in the plated clusters. However, neither survival curve displays a shoulder in the low-dose range as is characteristic of DNA repair-competent cells.

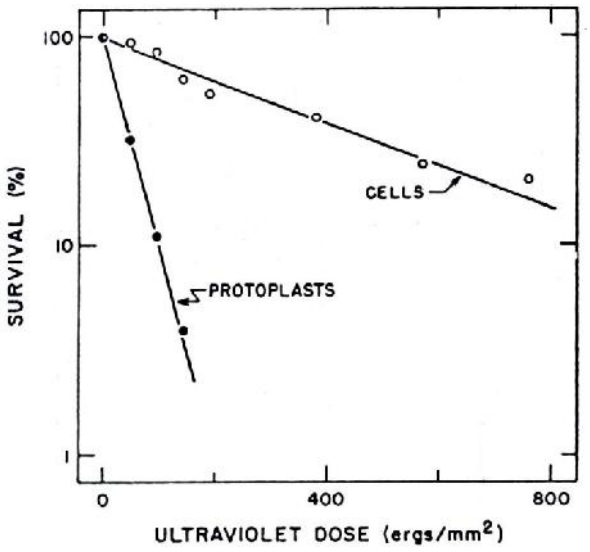

Figure 21.8. Survival of soybean protoplasts and cultured cells as measured by colony forming ability after exposure to increasing UV doses.

Radiation-induced Mutations in Cultured Plant Cells: Cultured plant cells are increasingly being utilized in genetic studies at both the basic and applied levels. First, the plant tissue must sis: the dosimetry can be more easily determined, it is often safer to carry out the mutagenic treatment, and there is no residual mutagen left in the irradiated sample after exposure. Chemical mutagens, on the other hand, offer their own advantages, especially their ability to induce certain relatively specific types of DNA alterations.

Recent reviews have pointed out the potentials and the current limitations on the use of cell cultures in plant improvement research. First, the plant tissue must be established in culture with a high degree of chromosomal stability. Second, the culture of haploid cells is required in order to select directly for recessive mutations in vitro. Third, single cells (or protoplasts) must be isolated and cultured under conditions which permit the induction of mutations and the application of the appropriate selective screen to identify and isolate the (rare) desired mutant cells as clones.

A high degree of genetic resolution can readily be achieved by screening a population of 10^6-10^8 individual plant cells. Finally, to allow parallel formal genetic analysis and to be useful in a plant breeding program, a plant cell culture must give the option of regeneraling shoots or plants from the mutant clones selected in vitro. Each of these goals has already been attained in the case of tobacco, but much more work is needed so that this powerful genetic approach may be effectively applied toward the improvement of other important crop plants. Although chemical mutagenesis has been employed to induce mutant plant cells in culture, little use has been made of radiation mutagenesis. NITSCH and his associates demonstrated that mutant plants can be obtained by 1.5-3 Krad doses of *y* irradiation to haploid microspores or plantlets in cultured tobacco anthers. Mutant characteristics observed included leaf variegation, albinism, and flower color and petal shape variations. DEVREUX and SACCARDO (1971) recovered a high frequency of presumably induced mutations from irradiated (1 Krad X-rays) anther cultures. J. M. WIDHOLM (pers. comm.) has employed UV to induce mutations to amino-acid-analog resistance in cultured wild carrot cells. A mutagenic exposure was selected so as to reduce cell survival by about one-half, as evaluated by vital staining. This treatment increased the mutation frequency about 10-fold over the spontaneous rate. ERIKSSON (1967 b) reported the recovery, from a UV-irradiated *Haplopappus* culture, of a stable variant exhibiting an altered karyotype and a high propensity for anthocyanin production. UV mutagenesis, because of its specificity, availability, convenience, and relative safety, should find wide application in studies with isolated plant cells. More is known about the molecular effects of UV on DNA (i.e. pyrimidine dimer induction) and about the repair of these DNA lesions than for any other agent. Mutation frequencies may be enhanced or suppressed by careful selection of the experimental conditions.

Current prospects for radiation studies on cultured plant cells: From the foregoing discussion it is apparent that much more fundamental work is needed to define the radio sensitivities of cultured plant cells. Recent progress in culturing isolated protoplasts has provided the opportunity to critically evaluate the survival of single plant cells and to relate this information to the known radiosensitivities of the intact plants. Table 1 summarizes data on various assays which have been employed to evaluate radiosensitivity of cultured plant cells. As emphasized throughout the present discussion, the most sensitive (and most significant) parameter is the reproductive potential of individual cells. This becomes especially apparent when the results from assays of cytoplasmic streaming, growth by weight increase, and growth by colony formation in irradiated tobacco cells are compared (Table 21.1). Since cells of different animals vary greatly in their ability to excise DNA lesions, it is possible that different plants also vary in DNA repair capacity.

A survey of DNA repair in our important crops would point out any individual species which may be more sensitive to environmentally induced DNA damage. Measurements of plant growth may well be incapable of detecting the accumulation of sub lethal levels of DNA damage, even though such damage might exert subtle effects on productivity. Increases in solar UV resulting from degradation of the UV-screening stratospheric ozone by pollutants have been predicted. The potential biological effects on plants of increased solar UV and other increases in environmental mutagens are largely unknown at present. Radiation studies on plant cells offer a sensitive method with which to obtain this vital information.

Since some plant species can be regenerated from cultured cells, somatic genetics can become an important complement to the standard techniques of the plant breeder. Radiation, as well as chemical, mutagenesis can be employed to broaden the relatively limited base of genetic variability in many crops. Both UV and ionizing radiations have been shown to reduce the effective auxin concentration in plant tissues. This approach might be used to advantages in experiments aimed at achieving regeneration of certain cultured plant tissues which do not respond to manipulation of exogenous hormone levels. Of the several known systems which can repair DNA lesions, PR (which is specific for pyrimidine dimers) and excision-repair appear to be "accurate", while postreplication repair is "error-prone," resulting in a higher frequency of mutations. Induced mutation frequency may be controlled to some extent by application of our knowledge of these repair systems. For example, cells undergoing rapid proliferation would be expected to display higher induced mutation frequencies than cells synthesizing DNA at a lower rate. Likewise, agents which inhibit excision-repair will result in enhanced mutation yields [e.g. phorbol myristate acetate]. In addition, radiation mutagenesis can be used to dissect normal developmental processes, as for example, photomorphogenesis in fern gametophytes.

An efficient system has recently been developed for studying the process of virus infection and development in cultured plant protoplasts, thus providing a convenient approach to the study of plant virus radiobiology. These are but a few of the potential applications of radiation studies on cultured plant cells. It is surely true that in coming years we shall see a continued and expanding interest in these powerful approaches to the solution of problems in plant genetics, development, and physiology, and applied problems in agriculture.

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