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REPAIR MECHANISM IN IRRADIATED CELLS

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Many aspects of bacterial mutations which lead to enhanced sensitivity to UV and X radiations have been studied. Radiosensitivity appears to result from a loss of the capacity to repair damaged DNA. The genetic map of these mutations in the chromosome of E. coli is shown.

Sofar the best known DNA repair mechanisms are by excision repair (excision of pyrimidine dimers controlled by nonlinked uvr genes) and by recombination repair (elimination of similar damage as a result of genetic recombination between undamaged regions of the DNA).

The understanding of the lethal and mutagenic effects of radiation on cells rests on the quantitative and qualitative identification of the various lesions in the DNA. Exposure to UV irradiation causes the production of many different types of photoproducts, but pyrimidine dimers appear to be the most important damage causing biological inactivation.

The study of biochemical defects of UV sensitive mutants has allowed a partial understanding of the repair mechanisms. Generally, they consist in removing the UV induced-pyrimidine dimers by cellular enzyme systems (monomerization through photoreactivation, excision or recombination by the action of endo and exonucleases, polymerases and polynucleotides ligases).

The X-ray induced lesions are very heterogenous and not yet as well defined and the mechanisms of recovery are far less understood than in the case of U. V.

1. MAPPING OF LOCI RESPONSIBLE FOR THE RADIORESISTANCE OF CELLS

The most fruitful breakthrough in radiobiology is probably the progress made in the genetical analysis of radiosensitivity. Since *Witkin's* fundamental discovery of the radiosensitive E. Coli B and of the radioresistant E. Coli B/r (1) it had been suspected that response to radiation was genetically controlled. However the greatest advance was made when radiosensitive strains derived from a wild type E. Coli were ana-

lyzed and the loci responsible for this sensitivity were mapped (2). A very good review of the contributions of *Howard-Flanders, Hill, Rörsch, Devoret* can be found in a recent article by *Howard-Flanders* (3). The situation is not yet clear, but we will try and summarize it, and hope that our simplifications will not distort some of the present evidence.

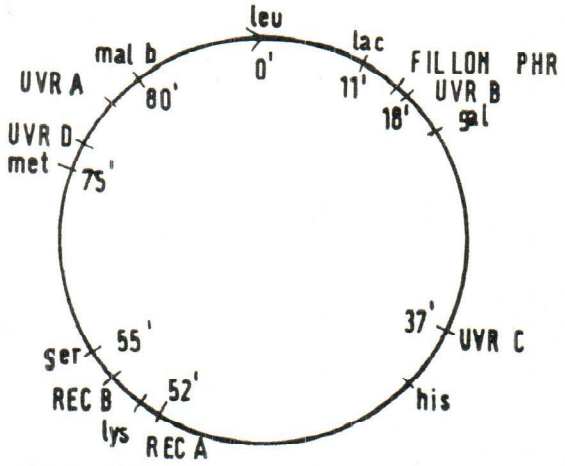

Fig. 1. *Linkage map of Escherichia coli*

Several types of mutants have been isolated and their main properties described by *Howard-Flanders* (2) and by *Rörsch* and their colleagues (4):

a) One class, named LON or FIL is constituted of cells like the E. Coli B of *Witkin* which, after irradiation, grow into long filaments; for instance, one FIL mutant has been mapped in E. Coli between the loci for lactose and galactose.

b) A second class of E. Coli mutants named uvr cannot repair UV lesions made in their own DNA or in the DNA of infecting bacteriophage; in this case the phenotype is called hcr$^-$ (*h*ost *c*ell *r*eactivation defective). Four uvr loci have been mapped: A, B, C and D. Some uvr$^-$ mutants have been found to have an hcr$^+$ phenotype.

c) Exr mutants are both X ray and UV sensitive but do not form filaments after irradiation. *Rörsch* has mapped two of these in or near the uvr A locus.

d) Rec mutants are both UV and X ray sensitive and unable to perform genetic recombination; two loci, A and B, have been mapped, which are located near the lysine locus. Rec A$^-$ breaks down DNA after irradiation, and when irradiated, lysogenic phages, can no more be induced. Rec B$^-$ is more resistant to UV than rec A$^-$, does not break down its DNA after irradiation and when lysogenic for λ, can be induced by UV.

e) Phr$^-$ are mutants usually resistant to UV and X rays, which cannot be photoreactivated after UV. The Phr locus is probably the one respon-

sible for the enzyme capable of monomerizing pyrimidine dimers when activated by visible light.

The understanding of these mechanisms of sensitivity or resistance to radiation therefore rests on the quantitative identification of the molecular lesions which can be induced by radiation and of the various gene products susceptible of repairing this damage. A typical survival curve is given in Fig. 2 of λ phage UV irradiated and plated on different types of mutants.

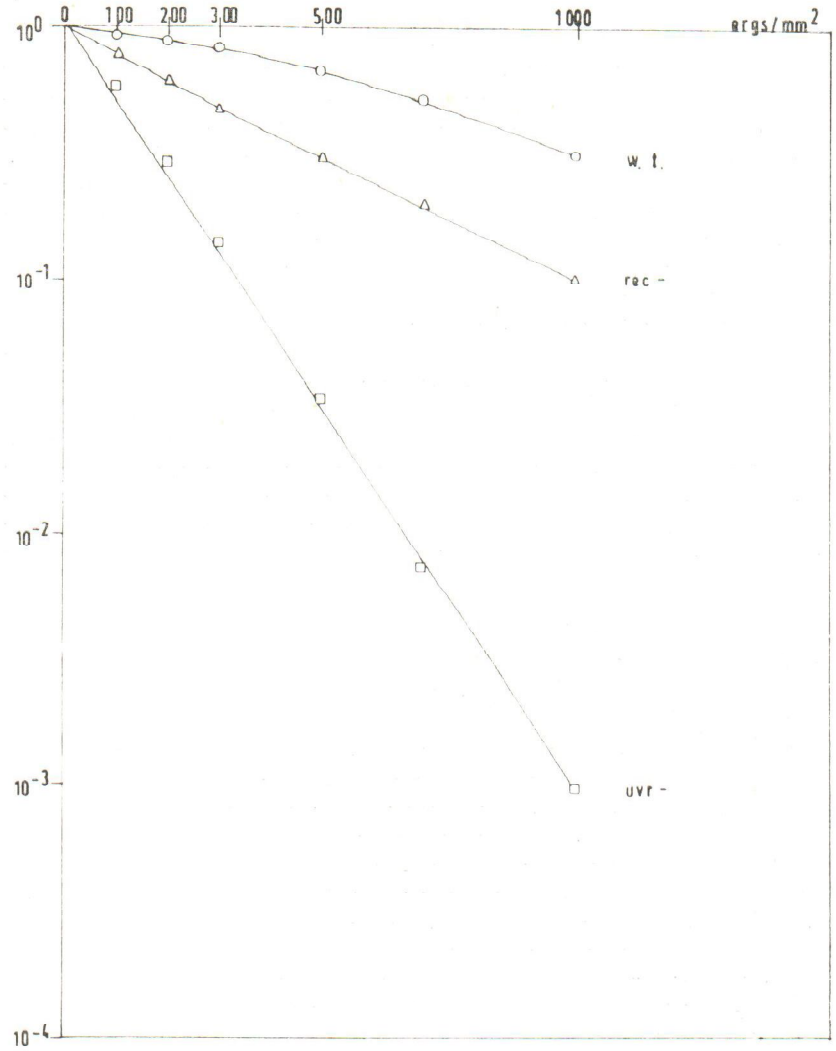

Fig. 2. *U. V. survival curves of phage lambda plated on the various mutants (35)*

2. DAMAGE TO DNA IN IRRADIATED CELLS OR PHAGE

It is obvious that to understand the mechanisms of radiation effects on cells, it is important to evaluate *all* the damage in the DNA extracted from irradiated organisms. Some types of damage to DNA have been induced by in vitro irradiation (Table 1), and although these are presumed to occur also in vivo, this cannot be taken for granted. Our objective is therefore to correlate quantitatively damage to DNA with cell survival or plaque forming ability when phages are studied. To understand lethal processes in irradiated cells, it is thus necessary to obtain data on the relative proportion of each type of damage, its fate within the cell, and its implications on replication and transcription of the genetic information. Table 1 lists qualitatively a number of alterations found in DNA either irradiated in vitro or in bacteria or phage. The advantages of studying phage are that the DNA can both be studied immediately after irradiation with little possibility of further alterations, and in the course of the infective processes during which lesions might be altered or disappear on account of cellular enzymes.

To give a more quantitative evaluation of U. V. effects it has been calculated that a dose of UV of 1 erg/mm^2 will produce about 6 pyrimidine dimers in E. Coli DNA (10^7 nucleotides), but it takes almost one thousand times that amount of U V to kill 90 p. c. of bacteria in the radioresistant strain E. Coli B/r (14). In the case of λ phage UV irradiated in 0,1 M NaCl, 0.01 M Na phosphate, pH 7, 8 (Table 2) (15) gives the various lesions found sofar and the survival of infective activity when these irradiated phages are plated on uvr$^+$ and uvrc$^-$ bacteria. When pure phage DNA is irradiated instead of the virus, the damage found is very similar except for strand breaks which are *not* detected. This poses the problem of the mechanism of production of these breaks when the DNA is irradiated in the presence of its protein coat; it cannot be excluded a priori that these breaks are preparation artefacts and occur as a result of the proteination of DNA molecules crosslinked to their protein or of the breakage of DNA-DNA cross links. It should be emphasized that the nature of the pyrimidine dimers formed depends on the base composition of the phage as indicated in Table 3.

In the case of X rays, the data sofar obtained after in vivo irradiation are less quantitatively known for bacteria or phage where recovery processes have been analyzed by genetical means. There is no quantitative data on damage to deoxyribose or bases; all we know is that microorganisms with the lower A + T ratio appear to be the *most* radiosensitive in spite of the fact that thymine is the most sensitive base in vitro.

Very clear experiments on the decay of ^{32}P introduced in single stranded bacteriophage like OX174 od S_{13} indicate that each decay process is lethal (17, 18). ^{32}P decay can produce single strand breaks in DNA and the simplest interpretation is that when a single stranded DNA is broken, the genetic information is lost and no complete new genome can be formed. Results of target analysis on viruses containing double strand-

Table 1.

Radiation induced damage in DNA

	Type of lesion	U. V.		X	
		in vitro	*in vivo*	*in vitro*	*in vivo*
Deoxyribose:	probable breakage of ring	—	—	+(5)	—
Pyrimidine:	hydration of $C_5 = C_6$	+(6)	+(6)	+(5)	—
	peroxydation	—	—	+(7)	—
	demerization	+(8)	+(8)		—
Purine:	breakage of imidiazole ring	—	—	+(7)	—
Backbone scission:					
Single strand break		—	+(9)	+(5)	+(5)
Double strand break		—	+(9)	+(5)	+(5)
DNA-DNA crosslinking		+(10)	+(11)	+(13)	+(13)
DNA-protein dissociation		+(12)	+(12)		
DNA-protein crosslinking				+(13a)	—

Table 2.

Effect of UV on λ phage

UV Ergs/mm²	Survival		% TT/T	TT/molecule DNA	% molecule with single strand breaks	% molecule with double strand breaks	% molecule cross linked to protein	% molecule cross linked DNA-DNA
	uvrc⁻	uvrc⁺						
3×10^3	$4.5\ 10^{-4}$	2.10^{-2}	0.55	65	5	1	0.2	—
6×10^3	—	$2.2\ 10^{-3}$	1.4	158	13	2.4	0.7	—
15×10^3	—	2.10^{-4}	2.4	290	25—30	6.4	2.2	13
45×10^3	—	$8.5\ 10^{-7}$	5.3	583	—	16.7	6.7	—
90×10^3	—	—	5.8	628	—	37.2	11.0	—

ed DNA indicate that in phage T_7 (19) and in E. Coli B_{s-1} (20) single strand breaks are not necessarily lethal; in the last case there are 7–8 single strand breaks per lethal hit. Similar results have been obtained for ^{32}P decay in phage (21). Interpretation of these results, based on mo-

Table 3.

Number and proportion of pyrimidine dimers produced in DNA after 2.10³ ergs/mm²

	% A + T	Total dimers per nucleoside (× 10^2)	Percentage of dimers			Ref.
			CC	CT + TC	TT	
Hemophilus influenzae	62	2.7	5	24	71	(16)
E. Coli	50	2.0	7	34	59	(16)
Phage λ	49.9	1.87	—	±50	±50	(15)
M. Lysodeiktius	30	1.4	26	55	19	(16)

lecular weight determination of native and denatured DNA (22) are consistant with the idea that 2 single breaks more or less opposite each other on the 2 DNA strands, lead to discontinuity of the molecule and to lethality. However nothing certain is known concerning other types of lesions probably also induced in vivo by ionising radiation.

3. REPAIR PROCESS

We have seen that repair processes exist and in some cases they are partly understood.

Repair of X ray damage

Early leads to the understanding of repair were obtained in Zagreb at the Ruđer Bošković Institute by *Drakulić*, *Miletić* and their colleagues already in 1961 (23) when they found that after X irradiation some deoxyribonucleotides appeared in the acid soluble fraction. Evidence for repair of part of the lesions caused by X rays lead to the conclusion that sofar, the only lesions susceptible of being repaired are single strand breaks in a double stranded DNA. Such repair is possible in irradiated bacteria of wild type like E. Coli B/r but not in the sensitive mutant B_{s-1} (24, 25). Similarly X ray damage caused to the phage can be repaired in a wild type strain of bacteria, but also in various radiation sensitive mutants of E. Coli, including those deficient in recombination (26).

Photoreactivation of UV damage

Photoreactivation of UV damage is the increased survival of UV irradiated cells when they are illuminated with visible light. As previously mentioned, there exists in Phr^+ cells an enzyme which needs light (between 300 and 400 mμ) to become activated and which breaks the cyclobutane ring formed during thymine dimerisation and restitutes the original independent thymine residues (16, 27). Bacteriophage infecting Phr^+ cells can also be photoreactivated (28). Photoreactivation is apparently not limited to nuclear DNA; non nucleated Amoeba fragments have a better chance of survival after UV irradiation when they are cultured in the light as has been shown by *Škreb* (29).

However in this case the mechanism of recovery is not known, nor is it clear that it is cytoplasmic DNA which is involved.

Dark repair of UV damage

We have seen that uvr^+ bacteria are relatively resistant to UV radiation and that they increase the survival of UV irradiated phage (host cell reactivation). It is admitted at present that the 4 uvr loci sofar mapped in E. Coli are genes, the products of which are capable of removing part of the UV damage done to the DNA.

In uvr^+ cells, an acid soluble fraction consisting mainly of small oligodeoxynucleotides containing thymine dimers is released. Uvr^+ strains are thus capable of excising thymine dimers with a number of linked nucleotides. This mechanism necessitates at least *one endonuclease* (perhaps more) acting on a damaged strand of DNA to make an incision near the thymine dimer and then excise the same strand on the other side of the lesion. If two dimers occur in the same region on opposite strands and if both strands were incised near the same base pair, the continuity of the DNA molecule would be lost and if these complementary sequences are of genetic importance, the »repair« mechanism would lead to lethality.

Evidence has been also obtained that the »gaps« made in the nucleotide strand after dimer removal is increased, perhaps by an exonuclease. The reason for this is not known, but may be it is necessary to uncover in the intact strand a sequence capable of binding the repair enzyme or to open up the gap in order that this enzyme can attach to a suitable double stranded sequence. The repair enzyme can attach, at the 3'OH end of the damaged strand, the nucleotides complementary to the intact strand which serves as a template. The last nucleotide remains with its free 3'OH group, but a polynucleotide ligase has been identified, which is capable of making the 3' phosphoester bond with the 5' phosphorous at the other end of the gap.

Several tests have been made which show that polynucleotide ligase is present in all 4 uvr classes of mutants (30). The mutants of uvr bacteria

all possess significant activity for introducing single strand breaks in UV irradiated λ DNA as is clearly shown by the decrease of DNA I molecules (intracellular covalently closed circles) (30, 31). In the uvr D⁻ group, introduction of single-strand breaks leads to degradation of the DNA. These mutants are for these reasons believed to be defective in the process of repair synthesis, but not in the process of excising the damaged regions.

In the uvr A⁻, B⁻, C⁻ mutants excision of the damaged region does not seem to occur.

Repair of chemical damage

There is also evidence that damage done by a certain number of cross linking mutagenic agents (mitomycin C, difunctional alkylating agents) can also be repaired in some strains of bacteria (32) but data is at present too limited to extend these results to the possible repair of X ray or UV induced cross links. A cross link produced by a bifunctional alkylating agent can apparently be removed as a result of excision of a diguanile product (33) in a resistant (B/r) but not in a sensitive (B_{s-1}) strain.

4. POSSIBLE CAUSE OF RADIATION LETHALITY

One can discuss at present as to the possible nature of the lethal hits. Table 2 demonstrates clearly that many pyrimidine dimers are not lethal in spite of the fact that they are thought to distort the DNA backbone: the reason being that most of them can be repaired. Single breaks are probably not lethal, from work on X rays (20) but at present one does not know much on the bonds which are broken during such a process. However when 2 single strand breaks occur opposite to each other, a double strand break occurs and the continuity of the chromosome is lost. One does not yet know how many nucleotides are needed between two opposite single strand breaks to maintain the physical continuity of the double strands, but one may expect that at least 2 or 3 base pairs are needed – and maybe more if one remembers that the »sticky« ends of λ needed for making a closed molecule have about 15 nucleotides each (34).

With UV radiation it is not certain that single strand breaks occur in vivo through photochemical effect, but as »repair« processes remove a certain number of nucleotides together with the pyrimidine dimer, one must expect that if 2 dimers as already mentioned formed near each other on opposite strands they will be removed by »repair« enzymes and a discontinuity will appear in the DNA molecule and be lethal. Non repaired dimers affect DNA or RNA synthesis in vitro and are expected to upset replication and transcription in vivo by producing errors in base pairing leading to mutations. Cross links between 2 DNA strands or between DNA and proteins will also probably be lethal if they are not removed by repair mechanisms. Finally, both after X rays and UV,

there may still be many other types of damage which have escaped detection. It would certainly be extremely important to analyse more thoroughly still the nature and amount of repairable radiation damage. There is still a long way to go before damage done to cells by radiation are thoroughly understood.

5. GENETIC RECOMBINATION, LYSOGENY AND REPAIR OF RADIATION DAMAGE

Genetic recombination is a function common to all cells. The recombination defiscient mutants of E. Coli were found to be highly sensitive to the lethal effects of UV and ionizing radiations. Similar recombination genes undoubtedly also interfere with radiation sensitivity in higher organisms. But biologists are obliged to use more simple material: the genome of an average mammalian cell is about 10^3 times greater than that of E. Coli, and this is the reason for preferring microorganisms. The DNA of a λ phage is one hundred times smaller than in E. Coli, and in Polyoma virus it is even one order of magnitude smaller. Viruses therefore enable one to account for almost all the information contained in their DNA and that is why they are such useful material for the study of fundamental genetics, in addition of course to the clues they can give for the understanding of infectious diseases. There is another type of process which may be simpler than recombination, but probably closely related: lysogeny. The main difference in the two processes is that in lysogeny there is not exchange of DNA regions of 2 genomes but simply integration of the phage genome into the bacterial chromosomes. Both processes undoubtedly rest on:

a. base pairing of complementary DNA strands of the two parental DNA's or of phage DNA with the bacterial DNA.

b. there must follow or precede a step where the base paired polynucleotides are sectioned by an endonuclease in order to allow separation of the recombinant genomes or integration of the viral genome. There exist arguments indicating that the cuts produced in both DNA strands are staggered with respect to each other in order to maintain genetic continuity.

c. gaps may be left between the recombinant strands and these could be filled in by »repair replication« like the one discussed above in which a ligase could finish the process.

The succession of events here crudely described is very similar to what one expects for repair of damaged DNA and these processes as well as DNA replication perhaps need the presence of similar or related enzymes. The mapping of the loci for these various enzymes and their biochemical identification is therefore of primary importance to understand these functions. The DNA has been exposed since the »beginning of

evolution« to innumerable causes of damage – both chemical and physical. It will most probably turn out that these enzymes have a common origin and that natural selection started by favoring organisms which were able of repairing damage. There is in fact a lot of evidence direct or indirect indicating that these mechanisms are not special to micro-organisms and do exist in the higher species including man.

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Sadržaj

MEHANIZMI REPARACIJE KOD OZRAČENIH STANICA

Mnogi aspekti mutacija koje vode povećanoj osjetljivosti na UV i X zračenje izučavani su u Escherichia coli i u velikom broju slučajeva povećanje osjetljivosti je smatrano kao gubitak kapaciteta za reparaciju DNK. Predstavljena je genetska karta ovih mutacija na kromosomu E. coli.

Da bi se shvatio mehanizam biološkog učinka zračenja na živu stanicu, neophodno je dovesti u vezu kvalitativno i kvantitativno oštećenje DNK. Nakon zračenja UV, u zavisnosti od eksperimetalnih uvjeta i fizikalnog stanja DNK, otkriveni su različiti tipovi fotoprodukata. Dokazano je da je među ovim lezijama fotoprodukt pirimidinski dimer značajan u biološkoj inaktivaciji. Mehanizmi reparacije u stanicama koje sadrže enzime potrebne za ovaj proces, dovode do uklanjanja ovog fotoprodukta (»brisanje«) pa uslijed toga i do povećanog preživljavanja. Za razliku od oštećenja induciranih zračenjem UV, oštećenja inducirana jonizirajućim zračenjem su ne samo veoma heterogena već i velikim dijelom neidentificirana. Sudeći po mnogim rezultatima moglo bi se reći da letalno oštećenje nastaje zbog diskontinuiteta genoma prouzrokovanog jonizujućim zračenjem.

Iznijeti su također radovi koji izučavaju biokemijske defekte mutanata koji su osjetljivi na UV zračenje, što dovodi do djelomičnog shvatanja mehanizma reparacije DNK. Ovaj mehanizam uključuje fotorestauraciju (»brisanje« lezije dimer pirimidina, monomerizacijom dimer pirimidina) i restauraciju (rekonstrukcija oštećenih regiona DNK uslijed procesa isijecanja (ekscizije) dimer pirimidina i reparativne sinteze DNK. Reparacija nakon X zračenja samo je djelomično shvaćena i odnosi se na uspostavljanje kontinuiteta DNK u jednom od lanaca dvolančane DNK.

Postoje indikacije da restauracija pomoću isijecanja sinteze DNK (replikacije) i rekombinacije može biti uvjetovana enzimskim kompleksima.

Letalne radiolezije razmatrane su u svjetlosti mehanizma reparacije.

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