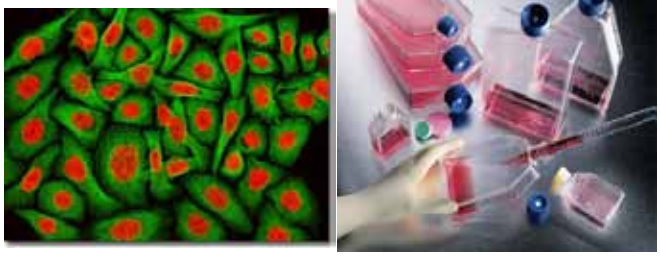


Chapter 12

Cellular radiation damage and repair

The study on radiation effects on biological systems made a significant step forward when it was possible to study human cells cultivated in the laboratory. With a cell culture we have several possibilities for studing basic biological processes.

Wilhelm Roux
(1850 – 1924)

The way towards a cell culture system included a lot of fumbling and unsuccessful attempts. It started with the English physiologist Sydney Ringer that found that salt solutions containing the chlorides of sodium, potassium, calcium and magnesium were suitable for maintaining the beating of an isolated animal heart outside of the body. In 1885 Wilhelm Roux removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days, establishing the principle of tissue culture.

It was early found that the cells could live for a long time, either in solution, or attached to a glas when the right conditions (such as temperature and growth medium) were present in the cell incubator. The growth rate of animal cells is relatively slow compared with bacteria. Whereas bacteria can double every 30 minutes or so, animal cells require anywhere from 18 to 40 hours to double. This makes the animal culture vulnerable to contamination, as a small number of bacteria would soon outgrow a larger population of animal cells. Then the cells died – and this was a mystery for a long time.

Leonard Hayflick

Most cells can be kept alive through a few cell divisions – and then die out. Leonard Hayflick found that the cells are ageing and could only go through a restricted number of divisions (of the order 40 – 60). This puzzle now belongs to the research history due to the work of Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak. They found that the chromosomes are protected in the end by "***telomers***". A telomere is a region of repetitive DNA sequences (such as TTGGGG) at the end of a chromosome, which protects it from deterioration or from fusion with neighboring chromosomes.

Teleomers are indicated by the red caps on the chromosome ends

Each time the cell divide a piece of the chromosome end is not rebuilt, and the chromosome and cell ages. After a number of cell divisions the cell and the culture dies out – unless the cell can rebuild the telomer. The above scintists found that some cells – in particular cancer cells have an enzyme, "***telomerase***", that could rebuild their telomeres. Telomerase is a "reverse transcriptase" consisting of a RNAcomponent and two protein components. The enzyme is usually not present in somatic cells, but is found in embryonal cells, in stem cells and in the sex cells. The activity of telomerase is important for the formation of immortal cell lines. More than 85 % of all cancers have telomerase activity.
The three scientists that pointed out the importance of telomers and telomerase earned the 2009 Nobel prize for this work.

The Nobel Prize in Physiology or Medicine 2009 was awarded jointly to Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak

"***for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase***".

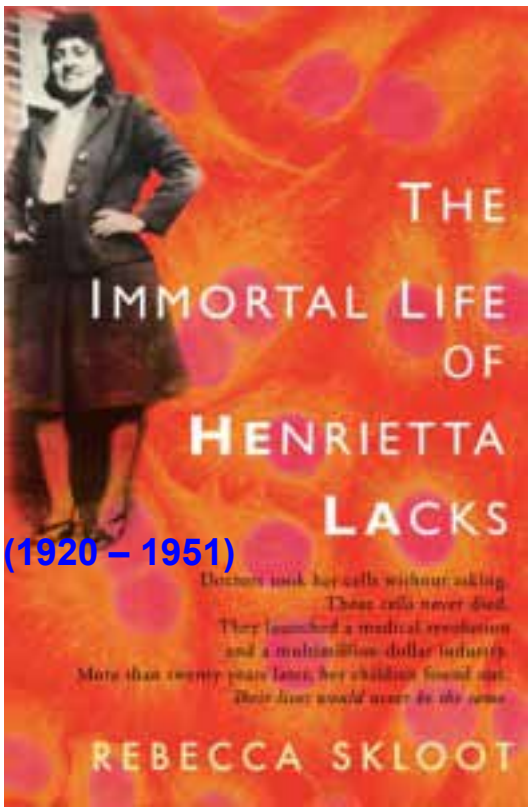
From left; Jack Szostak, Carol Greider and Liz. Blackburn.

Establishment of the HeLa cells

The major step in the direction of an immortal cell line was taken in 1951. George Gey, head of tissue culture research at John Hopkins Hospital in Baltimore was able to cultivate cells from a cervix cancer of Henrietta Lacks. She died from her cancer, but the cancer itself is still alive with the name HeLa cells.

George O. Gey
(1899 –1970)

The HeLa cell line was derived for use in cancer research. These cells proliferate abnormally rapidly, even compared to other cancer cells. In Rebecca Skloot's "***The Immortal Life of Henrietta Lacks***", she explains that HeLa cells have an active version of telomerase during cell division, which prevents the shortening of the telomeres. In this way, HeLa cells circumvent the Hayflick Limit, and the cell line could go on forever, It has been estimated that the total number of HeLa cells that have been propagated in cell culture far exceeds the total number of cells that were in Henrietta Lacks' body.

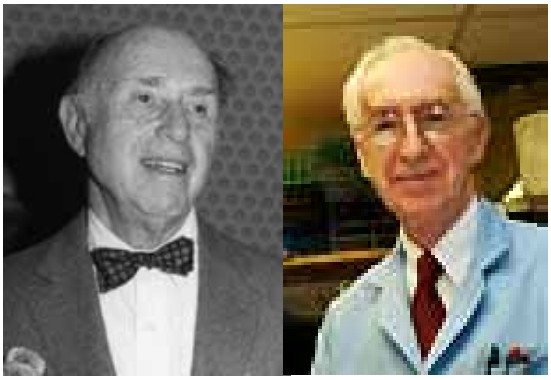


Tissue culture technique is the most convenient way of examining cell behavior so far. A small fragment of tissue (a cell, a population of cells, or a part or complete organ) is sustained in a complex biological (tissue extract) or synthetic nutrient medium with appropriate temperature and pH for the cells being developed. The process has multiple applications in visualizing normal and abnormal cell structure, genetic and biochemical reactions, aging and healing processes, metabolism and radiation biology.

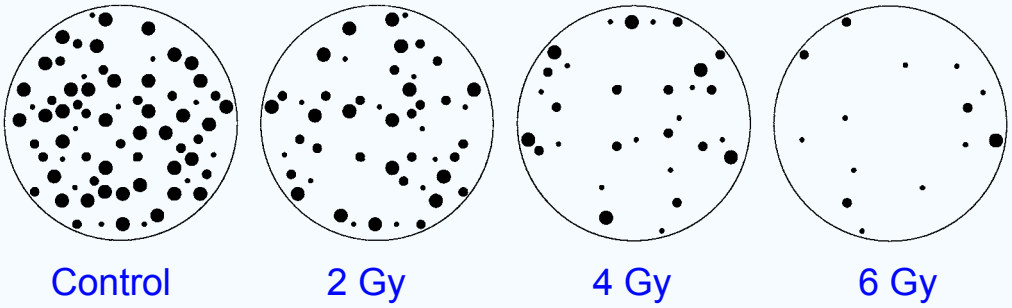
Thedore T. Puck and Philip I. Marcus started to study the effects of x-irradiation on HeLa cells in 1956. They studied the survival of single cells (defined as the ability to form a macroscopic colony within 15 days) as a function of radiation dose.

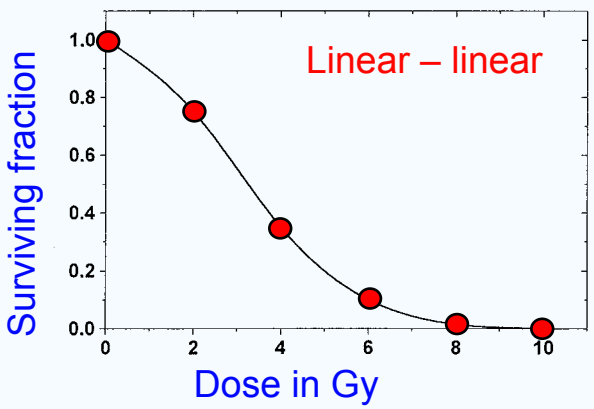
The experiment goes like this:

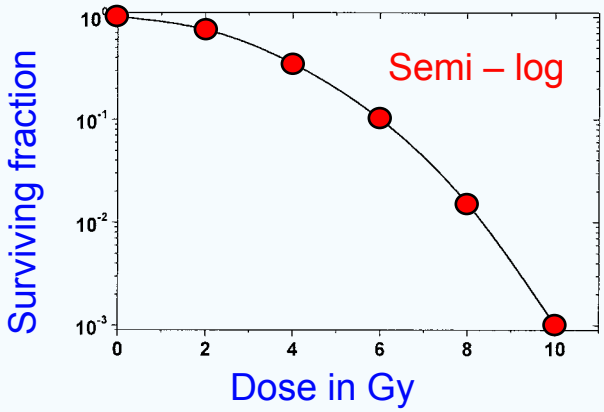
Cells from a dish is suspended. The cells are then counted and a certain number of cells is seeded and incubated for 1 to 2 weeks. The single cells will divide several times and form colonies that can be seen and counted. The control dish give us the "plating efficiency". These cells serves as control.
Other cells are given a radiation dose (before seeding). This procedure give the survival curve. See the figure below.



Theodore Puck Philip Marcus







We have plotted the results in two different ways; in a linear – linear plot (both the dose and the surving fraction are given on a linear scale), and in a semi – log plot. The axis for survival is in a logarithmic scale.

The mathematical expression that gives the best correlation to the experiments is the socalled linear-quadratic curve.

$$S = e^{-\alpha D - \beta D^2}$$

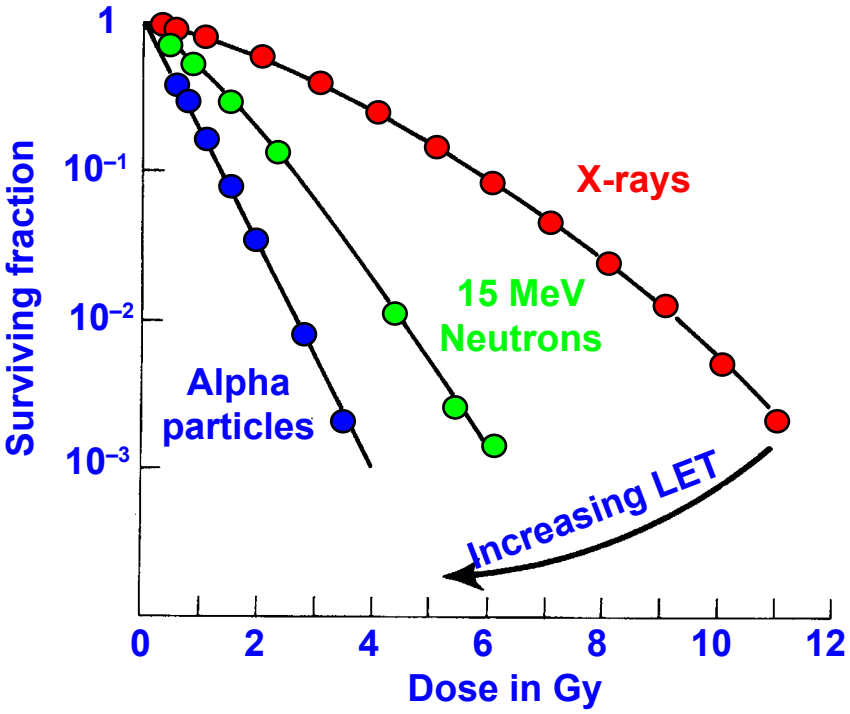
S is the surviving fraction, D is the dose and α and β are constants.
If the radiation is continued to seven and more decades the curve will be more and more linear in the semilog plot.

The cell culture technique gives us opportunities to study the different factors within radiation biology such as the effect of LET (different types of radiation), the oxygen effect, the radiosensitivity through the cell cycle and repair processes. Some of the basic mechanisms within radiation biology can be studied using cell culture.

Examples of properties that can be studied

In the following a couple of examples are given which give information about the effect of radiation on living cells.

LET dependence



In the figure are given some old results which show the effect of increasing LET on the survival curves. With increasing LET the curves becomes steeper and the shoulder seems to disappear.
Steeper survival curves show that the "relative biological effect" (RBE) increases.

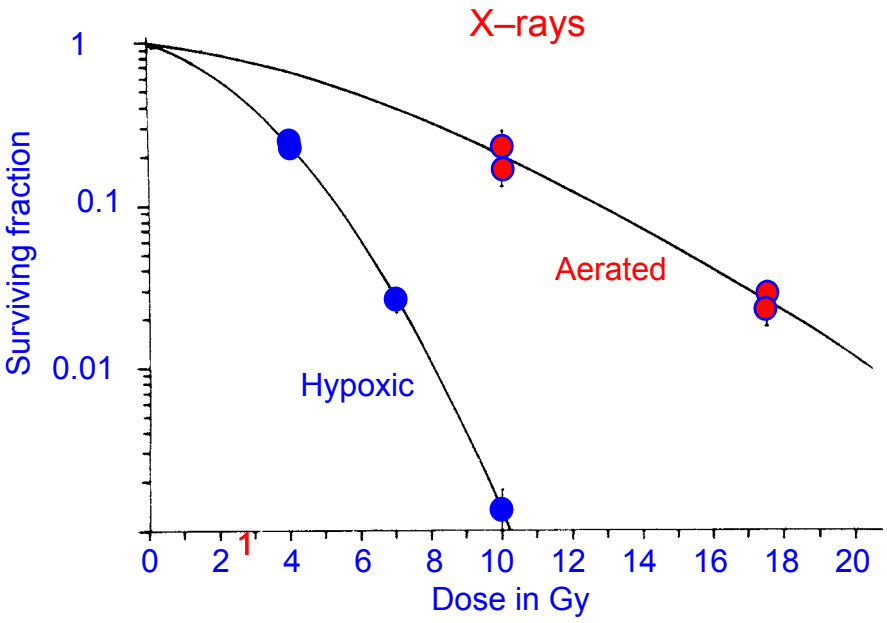
The LET for x-rays is about 2 keV/µm and increases to about 150 keV/µm for α-particles. The LET values that leads to highest kill is approximately 100 keV/µm. With this LET, the relative biological effectiveness may be 4 – 8 times higher than for megavoltage photon radiation. Data like this are very interesting for the discussion we had in connection to the effect of the radiation from radon daughters (see page 97).

The oxygen effect

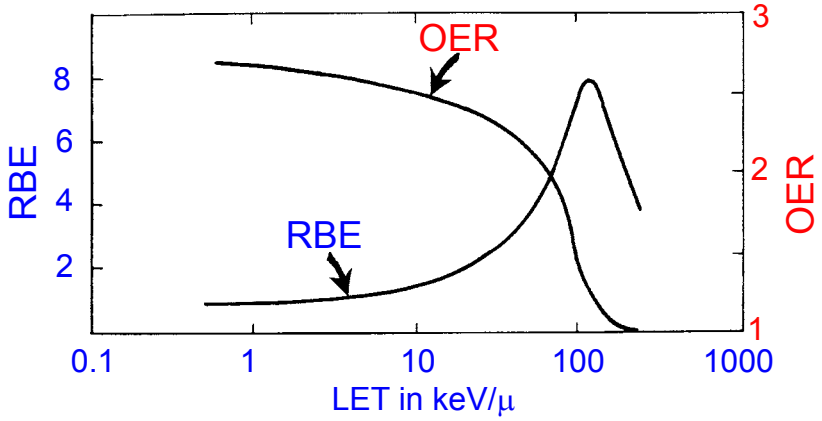
Another research field that is important – particularly within radiation therapy – is what we call the oxygen effect. The early observations (some results are given below) showed that the cells are killed more easily in the presence of oxygen, compared to where the oxygen level is reduced (hypoxic cells). Since solid cancers generally contain areas with abnormally low levels of oxygen, it appears that cancer cells in such hypoxic micro-environments are resistant to treatment.

Recent research has furthermore shown that hypoxia in tumors is one of the major drivers of metastatic spread of cancer, the major cause of death by the disease. Thus, hypoxia is responsible for a double effect of reducing the potential of a successful treatment of the cancer patient: Resistance to treatment and ability to spread. At the same time the very low level of oxygen found in solid tumors are specific to cancer.

Therefore, if one could develop methods that specifically located and inactivated cells in hypoxic areas one might obtain a cancer-specific effect, selective for the most harmful of the cancer cells. This development is the core task of the METOXIA project which is a cornerstone in the biophysics group at the University of Oslo. We shall return to this research, but first we give some of the early results using cell culture.



*From figures like the one to the left it can be calculated that the "**Oxygen Enhancement Ratio**" (OER) is 2.5.*



If we use other types of radiation like neutrons (15 MeV energy) the OER drops to 1.6.
For α-particles with energies in the range 2 – 4 MeV, OER approaches 1.0. If we put the two parameters, OER and RBE, together we obtain the figure to the left.

Radiation damage and repair

We have excellent opportunities with cell cultures to study radiation mechanisms and repair processes. A tissue culture laboratory is also quite central within cancer research.

The cells usually grow asynchronously, that is the cells are in different phases of the cell cycle. Experiments with beams of particles can be directed to different parts of a cell. They demonstrate that the nucleus is more radiation sensitive than the cytoplasm. When asynchronous cell cultures are irradiated with a dose of 5 Gy a decrease in mitosis is observed (mitosis is the division of the cells, and consists of the phases: prophase, metaphase, anaphase and teleophase). The fraction of cells which are in mitosis ("the mitotic index") will decrease. This means that the growth in the number of cells has slowed. After a few hours the mitotic index will again increase, reach a maximum, and then decrease, as the syncronized cell population goes through cell cycle.

Cell death induced by radiation can be divided into two groups:

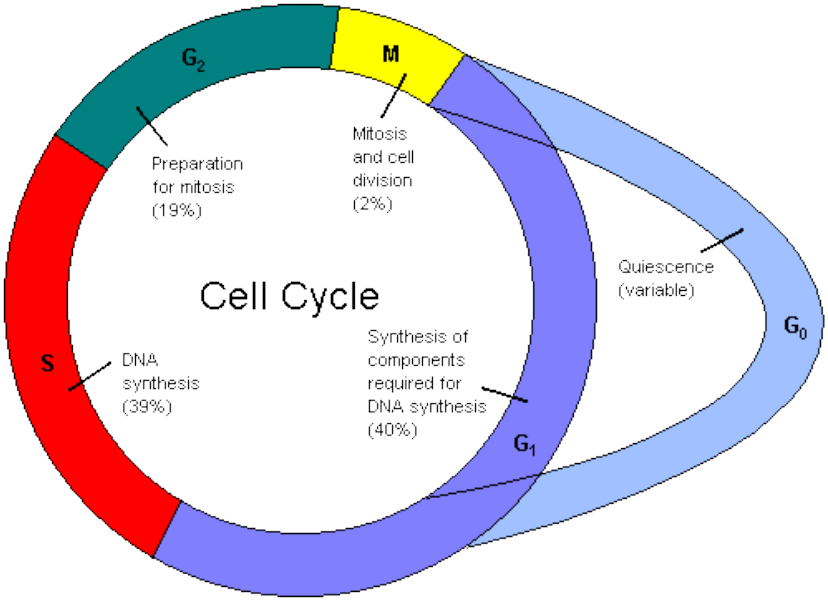
1. If a cell dies after the first mitosis it is called *mitotic death* or *reproductive death.*

2. If the cell dies before reaching the first mitosis it is called *interphase death.*

Cells which survive large doses very often have chromosome abnormalities.

The cell cycle

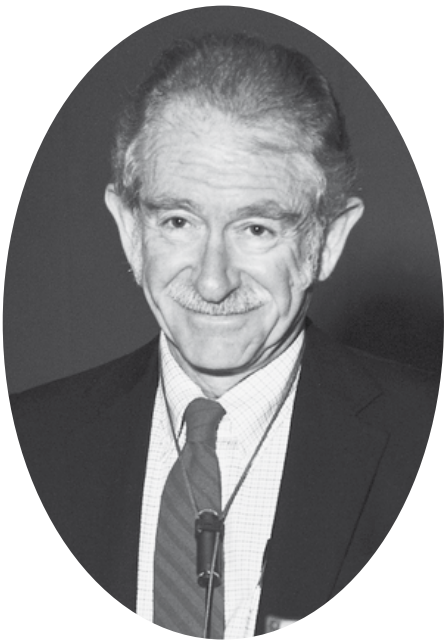
A living cell goes through several stages from the moment it is "born" by cell division until it divides, forming two new cells. Before division takes place the cell content of DNA must double. This cell cycle can be divided into 4 stages (see figure) and the radiation sensitivity differs for each stage.



The cell cycle can be visualized in many different ways – and the illustration to the left is one of them.
During the cell cycle, the first phase includes the creation of enzymes required for DNA synthesis (G_1 phase). The second phase includes the actual DNA synthesis and chromosomal replication (S phase). Protein and RNA synthesis occurs in the next phase (G_2 phase). The last phase is mitosis or cell division (M phase). After this, a cell may either go into the resting phase (G_0 phase) or straight back into the G_1 phase. The cell cycle is regulated by the genes within the cell, signalling molecules, and monitoring biochemical molecules.

Repair Processes

In 1959 M.M. Elkind and Harriet Sutton at the National Institutes of Health published a paper which demonstrated cellular repair processes. Today it seems obvious that living cells must have a repair system since damage occur to DNA all the time, mainly from the life processes in the cell (*endogenous damage*), but also from external sources such as ionizing radiation, UV, and a number of chemical compounds (*exogenous damage*).

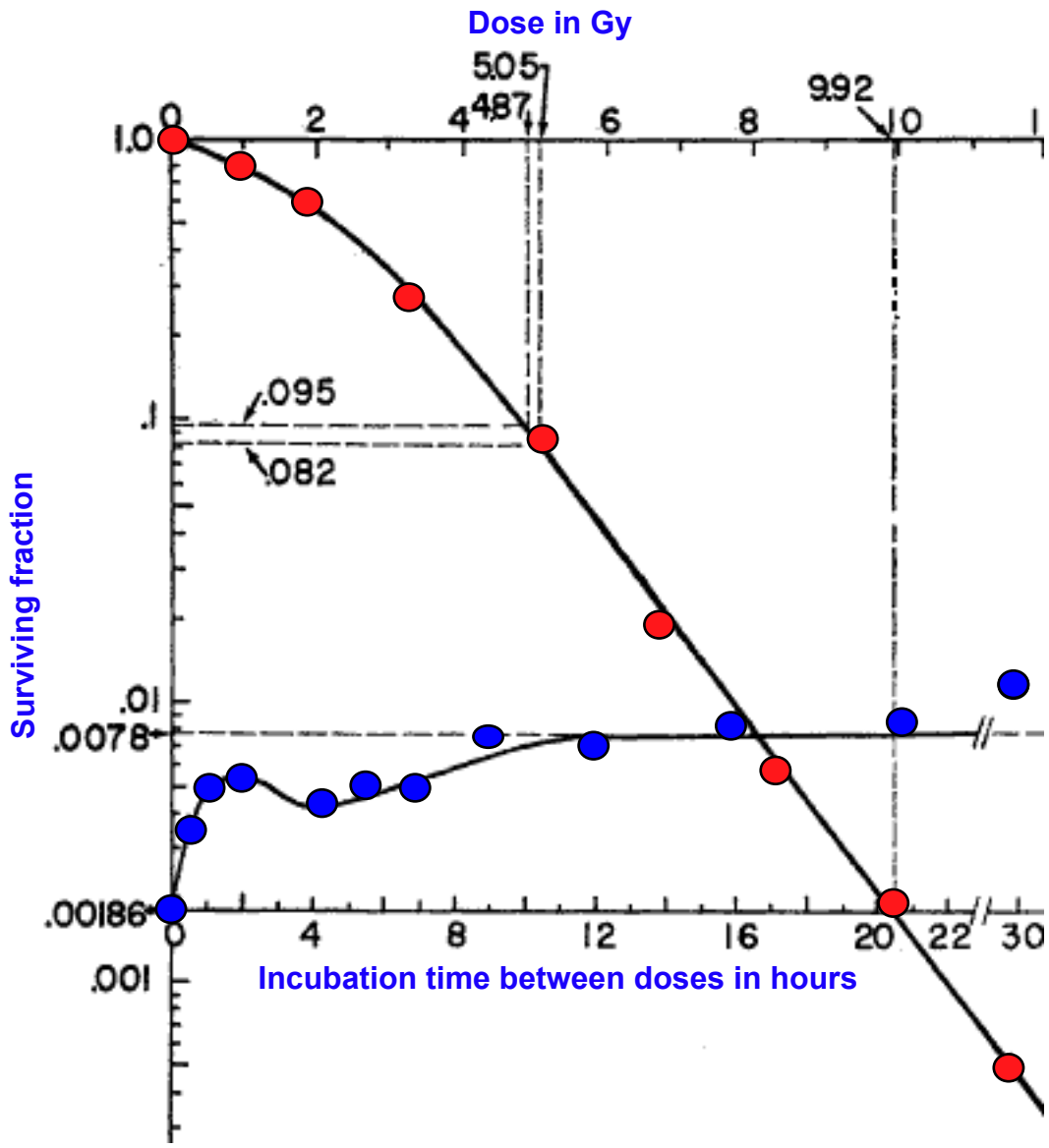


Mortimer M. Elkind
(1922 – 2000)

Since Elkind and Sutton presented the first experiments we shall look in more detail into the experiment. They carried out cell culture work and irradiated Chinese Hamster cells. They obtained survival curves like that in the figure below (red points).
In the next experiment they split up a dose of 10 Gy into two equal doses of 5 Gy with an interval between the exposures. In the interval the cells were incubated at 37 °C. The survival after two 5 Gy doses increased with the time elapsed between the two irradiations. This is given in the figure (next page) by blue points.

Elkind and Sutton interpreted the results in the following way;
the first dose of 5 Gy killed a number of cells whereas other cells attained damage that they called "*sublethal damage*". In the time interval between the two doses the damage could be repaired and the cells were "healthier" when the next dose hit.

This can be compared to a boxing match where one of the boxers sustains a number of hits, but is saved by the bell. Between rounds the crew works in order to get the boxer fit to continue the fight.



*The above figure is from the work of Elkind and Sutton published in Nature, Vol **184**, 1293 (1959). They used Chinese hamster cells. The ordinary survival curve is given by red points. The split dose experiment (two doses of 5 Gy with a time interval between the irradiations is given by the blue points as a function of the time between the irradiations.*

It is clear that the cells have repair systems. This is a necessity for survival. The crew working on repair in our cells are enzymes. It is the job of some enzymes to detect DNA damage while others are called upon to repair the damage. The repair processes can be divided into three types:

• The specific site of damage is repaired. In this case the enzymes work right at the damaged site. The original base sequence is preserved.

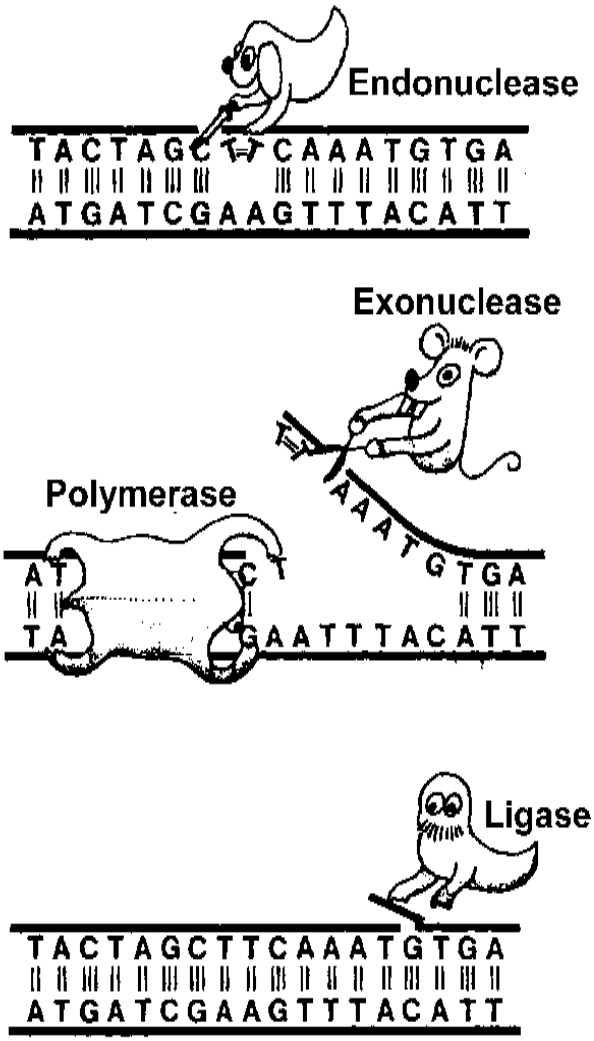
• The whole stretch of DNA containing a damaged site (or sites) is removed and replaced, preserving the native sequence.

• The damage is ignored during replication; it is by-passed. With luck the correct base will be inserted or, if incorrect, it won't matter. Because this type of repair is error prone, it is held in reserve in case the higher fidelity repair systems miss, or cannot cope with, the damage. For this reason, it is aptly called "SOS" repair.

Excision repair

One important repair mechanism is "***excision repair***". This repair mechanism involves enzymes that cut out the damaged part of DNA and replaces it with a new undamaged part.

One of the scientists working on understanding repair processes was Gunnar Ahnstrøm at the University of Stockholm. His imaginative drawing showing the essential elements of DNA repair is given in the figure below.



Courtesy of Gunnar Ahnstrøm

Gunnar Ahnstrøm
(1929 – 2008)

In the figure Gunnar Ahnstrøm has outlined the steps in this repair mechanism. The process includes altogether four enzyme groups. If you look into the details you will find that Prof. Ahnstrøm has included an error in base pairing. Can you find it?

The excision repair mechanism includes the following steps:

1. Recognition. It is important to have enzymes that can recognize the damage and signal for help.

2. Cutting of the DNA-strand. It is a requirement that specific enzymes, like the endonucleases, can cut the DNA-strand in the neighborhood of the damage.

3. The damaged part is removed and rebuilt. Exonuclease and polymerase are key enzymes. The former cuts out the damaged part and polymerase replaces it with a new undamaged part.

4. Joining. The repair is finished when the ligase enzyme joins the cut DNA-strand back together.

The repair system, outlined above, is found in humans and microorganisms. An array of repair mechanisms are used to repair not only radiation-induced damage but also damage stemming from a multitude of other agents, including the routine damage that occurs as part of normal cell function. A repaired cell divides and functions in a normal fashion.

What happens when the repair system fails or is too weak ?

When we are out in the sun and exposed to UV light, adjacent pyrimidine bases (C or T) become fused together and pyrimidine dimers are formed in the skin cells. Normally, our repair mechanisms are intact and can repair the extra damages from the UV-radiation.
However, there is a genetic ailment for which the above described repair system is too weak to repair all of the extra damage caused by the sun. The ailment is called *Xeroderma Pigmentosum*. It has been found that the enzyme endonuclease is weak and often fails to do its job. The result is that people with this genetic defect have a high risk for developing skin cancers that are often lethal.

DNA-damage and defense mechanisms

On page 214 we presented an illustration which showed the many ways for damage to the DNA-molecule. It was pointed out that the DNA-damage may be caused by both endogenous as well as exogenous processes. The endogenous damage is mainly caused by reactive oxygen radicals (called ROS) – whereas the exogenous damage come from different sources including ionizing radiation, UV and chemical substances.

Reactive oxygen species

ROS are produced by the oxygen metabolism. Most of the molecular oxygen is converted into CO_2 whereas a small fraction (about 5 %) is converted into reactive oxygen species. Enzymes and free radical scavengers mainly take care of this endogenous production of ROS. However, some damage is caused in DNA, proteins, lipids, and carbohydrates.

It is assumed that about 50 000 single strand breaks and 8 double strand breaks are produced each day in each cell by ROS. This amount of damage is approximately equivalent to that caused by a daily radiation dose of 200 mGy (or about 8.3 mGy/hour)! (The interested reader can compare this with the dose levels around Chernobyl and Fukushima; page 139 and 147).

A pool of DNA-damaged cells

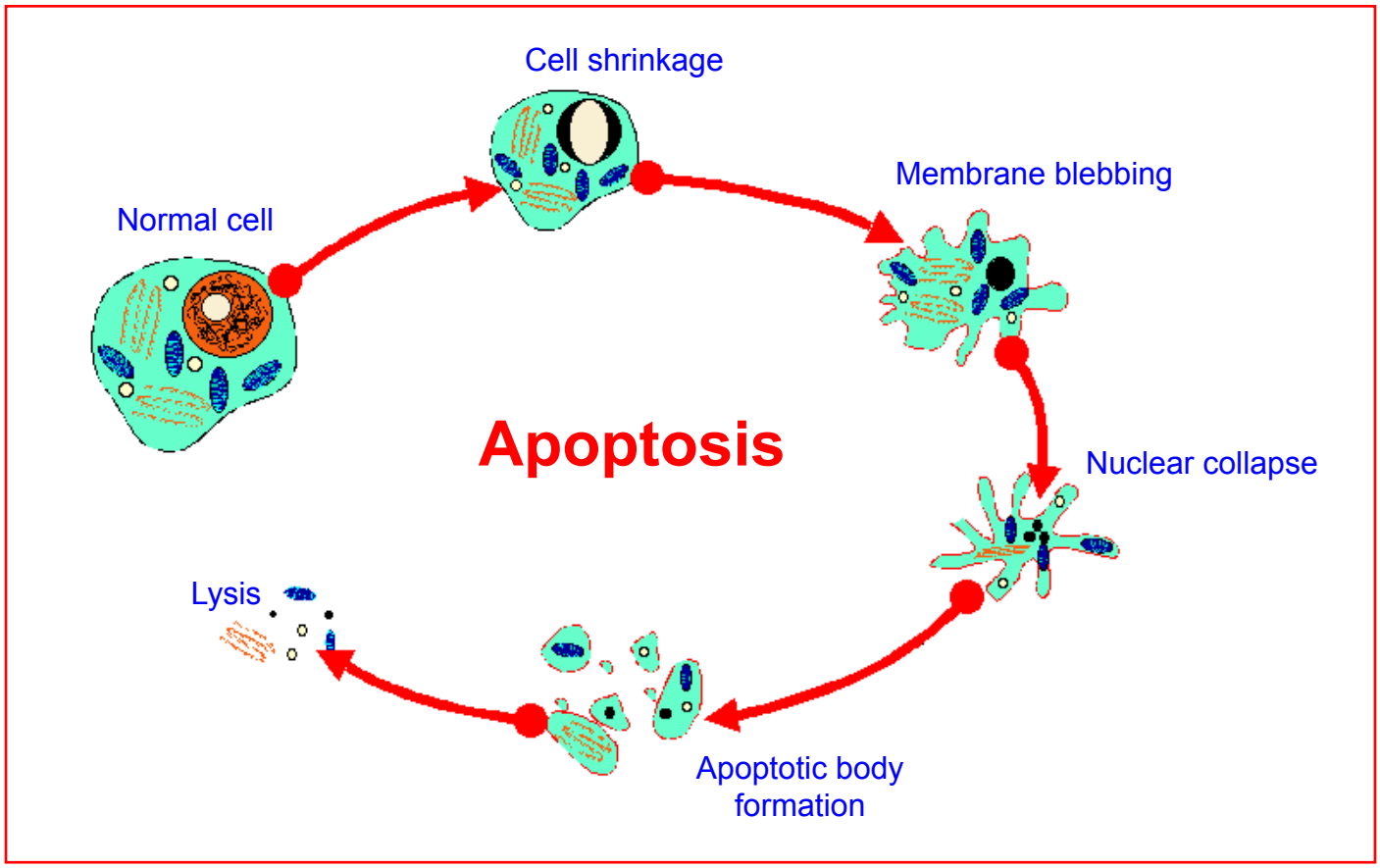
The number of DNA-damage from endogenous and exogenous mechanisms is very large – and we have a pool of damaged cells. As long as these cells are in the resting phase G_0 the situation is under control. However, when damaged cells go into the cell cycle it is important to have a system to handle the damage. It is assumed that the initial step in cancer development is a damaged cell which is promoted into the cell cycle. If the cell passes all the control mechanisms a cancer may develop. Consequently, it is quite clear that life would be impossible without defense mechanisms. The two main routes are:

1. Repair mechanisms. We have previously outline excision repair as an important DNA-repair mechanism, and will not go into more details.

2. Cell death. The other possibility is to kill the damaged cell. (*A dead cell can never give cancer*).
It appears that the cells have a mechanism called "***apoptosis***" or "*programmed cell death*" that can be trigged with the result that the damaged cell is killed whereas the organism survive.

Apoptosis

Apoptosis is a cellular defense system that kill the damaged cell. It consists of a cascade of events which lead to the destruction of the damaged cell – as visualized in the diagram below.



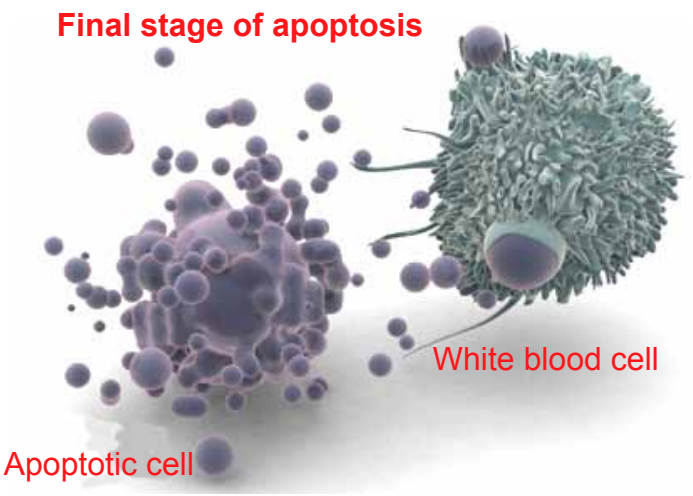
Apoptosis was described more than 100 years ago. However, it was not until 1965 that John Foxton Ross Kerr at University of Queensland discovered what he called "*shrinkage necrosis*".
This discovery resulted in an invitation for Kerr to join Alastair R Currie and Andrew Wyllie at the University of Aberdeen.
In 1972, the three published the famous article in the British Journal of Cancer.

"Apoptosis: A Basic Biological Phenomenon with Wide-ranging Implications in Tissue Kinetics"

Andrew Wyllie

John F. R. Kerr

In the final stage of apoptosis the cell debris is engulfed by white blood cells (see illustration).



Apoptosis, or programmed cell death, is important during development of the embryo, but is also acting to destroy cells that represent a threat to the integrity of the organism. Between 50 and 70 billion cells die each day due to apoptosis in the average human adult.

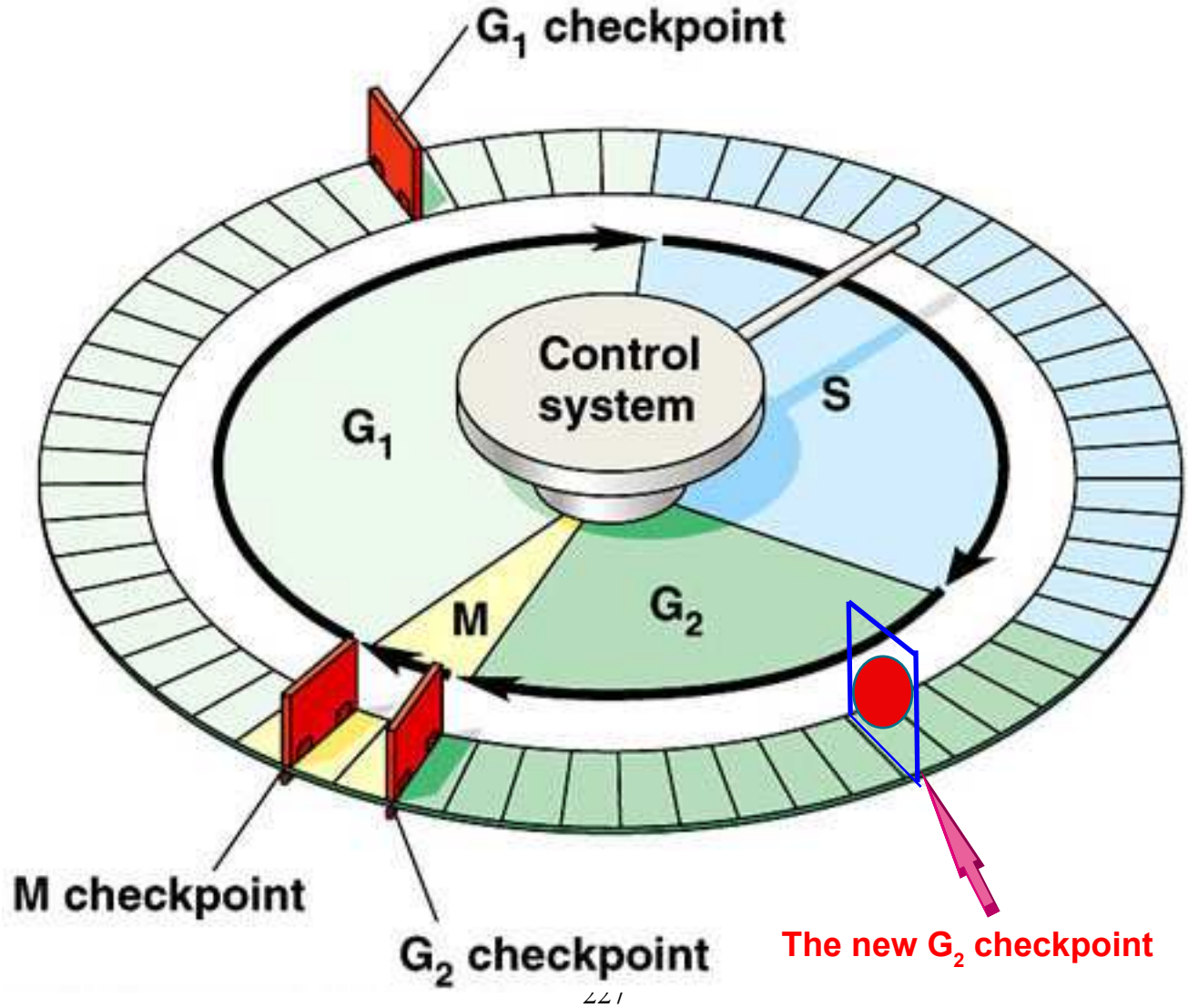
A wide variety of stimuli are capable at trigging apoptosis. Some are universal and can produce apoptosis in almost any cell. The signals usually consist of proteins that serve to protect the organism from cancer by killing cells with DNA-damage that cannot be repaired.

The cell is stopped in the cell cycle at certain checkpoints and proteins like p53 start the apoptotic processes.

Here we embark into an active research field with a number of extremely interesting data. We are not able to go into this research – but would like to give some glimpses of the exiting field.

Cell cycle checkpoints

In the normal cell cycle we find a number of checkpoints through the cycle. The point is that if DNA-damage is present the cell cycle is stopped and time is given to repair. If repair is not the best – cell death such as apoptosis may be triggered.



We have a G_1 and a G_2 checkpoint as well as checkpoints both in the S-phase and in mitosis. Recently, we have learned that radiation may induce checkpoints.

The cell cycle checkpoints are made up of composites of protein kinases and adaptor proteins which all play important roles in the maintenance of the integrity of the division.

All the checkpoints examined require the services of a complex of proteins. Consequently, mutations in the genes encoding these proteins have been associated with cancer; that is, they are oncogenes. This should not be surprising since checkpoint failures allow the cell to continue dividing despite the DNA-damage. We shall return to the newly detected G_2 checkpoint.

One of the proteins engaged should be mentioned in some more detail – namely the protein called p53 and the gene that encodes this protein. The protein is very important and connected to the checkpoints.

p53

The p53 protein was discovered in 1979 by David Lane in England and Arnold Levine in USA. From the beginning it was considered to be very important – and was in 1993 selected as "*molecule of the year*".

The name of the protein is from its molecular weight of approximately 53 kdalton (due to the high number of proline residues the molecular weight may be smaller).
In humans, p53 is 393 amino acids long and is encoded by the TP53 gene located on the short arm of chromosome 17.

Sir David Lane
(1952)

Arnold Levine
(1939)

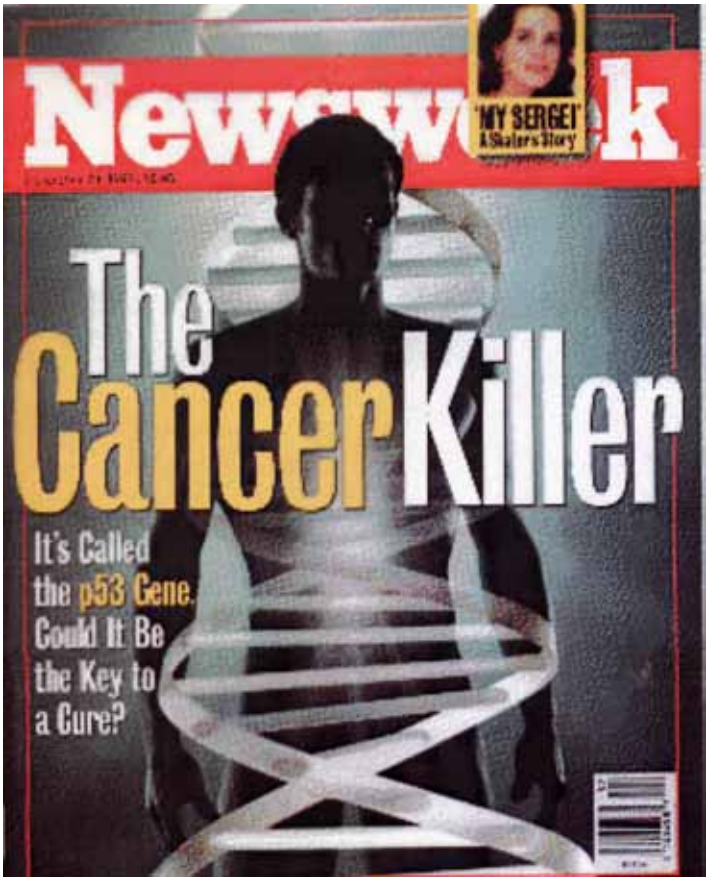
The molecule p53 is important in multicellular organisms, where it regulates the cell cycle. It has been described as "the guardian of the genome", and the "master watchman". The molecule works through several mechanisms:

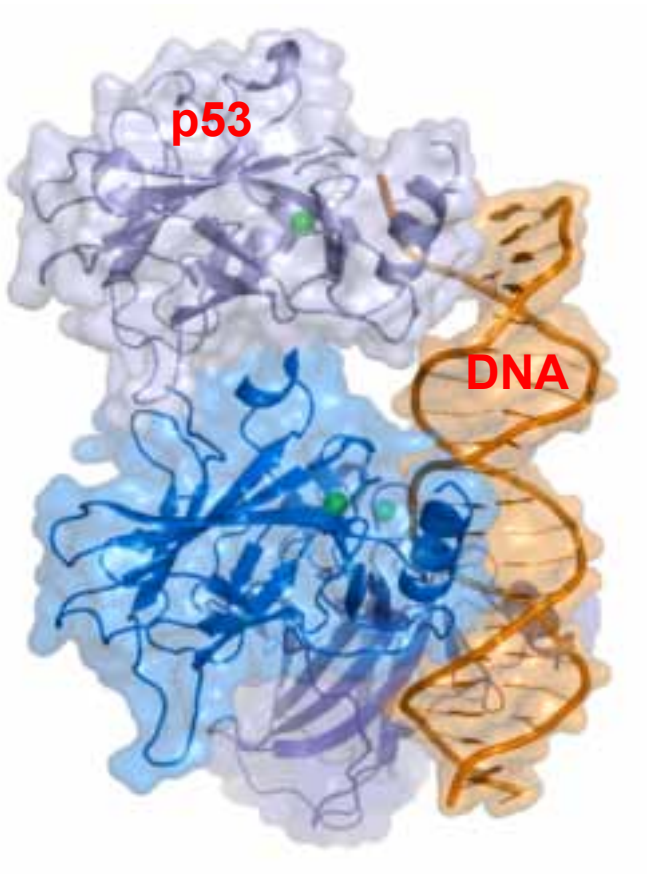
1. It can activate DNA repair proteins.
2. It can induce growth arrest by holding the cell cycle at the G1/S checkpoint and give the cell time for the DNA repair proteins to fix the damage.
3. It can initiate apoptosis, if DNA damage proves to be irreparable.
   The p53 protein is a key player in apoptosis, forcing "bad" cells to commit suicide.

In normal cells, the p53 protein level is low. However, DNA damage and other stress signals may lead to an increase of p53 proteins. The p53 proteins stop the progression of the cell cycle and activate the transcription of proteins involved in DNA repair. If repair is not the best choice, apoptosis is the "last resort" to avoid proliferation of cells containing abnormal DNA.

As you can see from this, cells with a mutant type of protein p53 may develop into a cancer cell. In fact it has been found that more than half of all human cancers do harbour p53 gene mutations and consequently have no functioning p53 protein.

In this discussion it is important to mention that radiation activates the formation of p53. Thus, the defense mechanisms do not follow the LNT-model. Their nature and level of activation depend on dose and dose-rate.





*The above frontpage in Newsweek was presented in connection to the announcement in Science of p53 as "**Molecule of the year**". Below we give some of the arguments for this choice.*

Some molecules are good guys, some are bad guys, and some become bad because they fail in their functions. The molecule p53 is a good guy when it is functioning correctly. It is a tumor suppressor in that it halts abnormal growth in normal cells and thus prevents cancer. Therefore, we have chosen p53 as Molecule of the Year for 1993.

About 50 percent of all human cancers contain a mutation in p53, so hopes are high that the molecule will provide new insights into treating the disease. Curing cancer and curing a bacterial disease are very different problems. The p53 protein is part of a fundamental pathway in human cell growth, and finding p53's function allows scientists to develop strategies for the diagnosis, prevention, and cure of cancers resulting from p53 deficiencies.
The p53 protein is also identified with a process of programmed cell death that may be important in killing cancer cells, and further understanding of this process provides hope for cancer therapy. Thus, p53 and its fellow tumor suppressors are generating an excitement that suggests prevention now and hope for a cure of a terrible killer in the not-too-distant future. In this endeavor the 1993 Molecule of the Year is certain to play a major role.

Tumor suppressor genes

The gene for p53 is called a "***tumor suppressor gene***" since the gene reduces the probability that a cell in a multicellular organism will turn into a tumor cell. It is easy to understand that a mutation in such a gene may increase the probability of tumor formation.

Even though, p53 has got a lot of attention, we have in recent years found a number of other tumor suppression genes. They are connected to different types of cancer. It is important that these genes are working – since damage to them increase the risk for cancer.

Adaptive Response

Research with living cells have demonstrated that cellular resistance to radiation damage can be increased by small stimulating doses. The radiation community has named it *"**adaptive response**"*.

Experiments exhibiting adaptive response started in 1984 with the work on human lymphocytes by G. Olivieri, J. Bodycote and S. Wolff at University of California.

Sheldon Wolff
(1929 – 2008)

The lymphocytes were cultured with ^{3}H-labeled thymidine that was incorporated directly in the DNA-molecule and served as a source of low-level chronic radiation. Tritium (H-3) is radioactive with a half-life of 12.3 years. It emits a β-particle with maximum energy of 18.6 keV. The cells were then irradiated with x-rays to a dose of 1.5 Gy and the yield of chromosome aberrations recorded.

It was found that the number of chromosome aberrations was ***fewer*** after exposure to both sources (tritium β-particles as well as x-rays) than after x-rays alone. These results showed that low levels of radiation can trigger or induce increased repair of radiation induced chromosome breaks.

Throughout the 1990s a large number of experiments were published on different systems that demonstrate an adaptive response. A number of end points have been studied such as cell killing, micronucleus formation, induction of chromosome aberrations, induction of mutations and neoplastic transformations. The adaptive response has been detected when cells have been exposed to a small dose (10 -100 mGy) and then challenged with a much higher dose. Let us briefly mention a few points:

- The amount of chromosome damage in lymphocytes can be reduced by up to 50% if a dose of 10 to 150 mGy is given to the cells before a larger dose of 1.5 Gy is given.

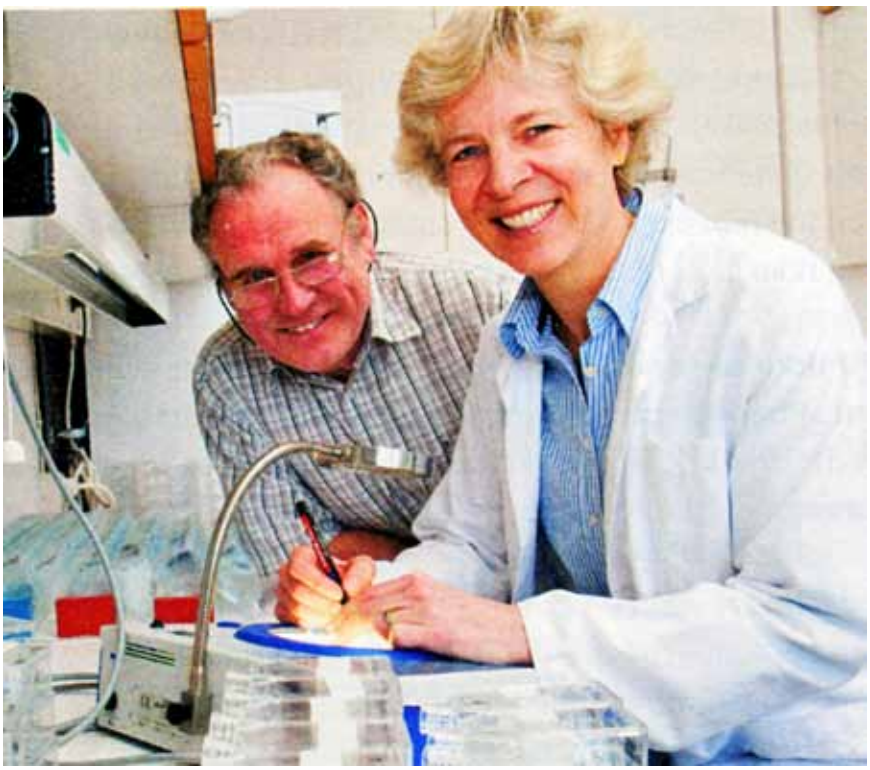
- In order to attain a maximal effect, the stimulating dose must be given 4 to 6 hours ahead of the large dose. The effect seems to last through a couple of cell cycles.

- Adaptive response has also been found in the case of mutations in the fruit fly (drosophila melanogaster). The number of mutations was reduced when a small dose of 200 mGy was given before a large dose of 4 Gy.

In this study with adaptive response a small dose was given before a larger challenge dose was used. The interesting result that a small socalled "***priming dose***" largely influence the effect of the challenge dose has later been used in a number of studies and have given a number of interesting results that largely influences our view of the risk analyses of ionizing radiation. Before we conclude and embark on the relation between radiation and cancer we would present some recent work carried out. The work clearly demonstrate the radiation itself can stimulate and enhance the cellular defense mechanisms.

Some recent research in biophysics at UiO

In the group for biophysics and medical physics at the University of Oslo, experiments are carried out which have a rather strong connection to the field discussed above. Let us therefore try to convey some of the ideas and results here. Those more interested should consult the original work.



Erik Pettersen and Nina Jeppesen Edin in the tissue culture laboratory

Erik Pettersen is in charge of the experiments and Nina Jeppesen Edin is an important coworker. Joe Sandvik as well as a number of students are connected to this group.

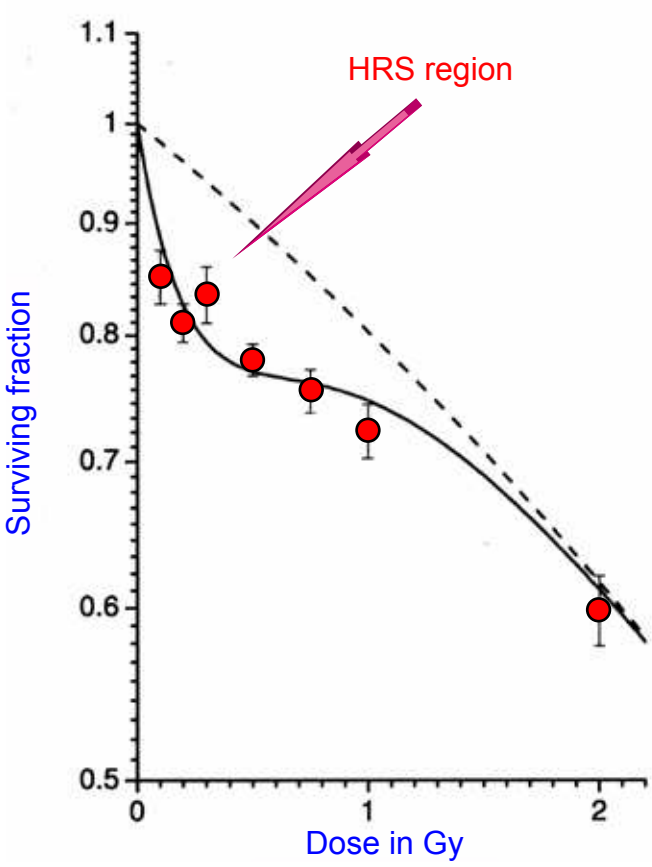
The group is working with different types of cells cultivated in the laboratory. In the figure below T–47D cells (human breast cancer cells), have been irradiated with Co-60 γ-rays and the survival curves have been observed.

If the irradiation takes place at a doserate of 40 Gy per hour (or 0.67 Gy per minute) the curve in the figure is obtained. This very high doserate implied exposure times of less than a minute for the smallest doses in the figure.

The resulting survival curve showed an unexected form that deviates from the normal linear- quadratic form (stippled curve). A rapid decay is observed at low doses – demonstrating what is known as "***low dose hypersensitivity***" (HRS). The rapid decay in survival is followed by a region with increased radioresistance. At about 2 Gy the survival curve follows the usual linear-quadratic form.

We pointed out the high doserate above – and it appears that when the doserate is reduced to 0.3 Gy/h (5 mGy per minute) this peculiar hypersensitivity disappears.

The form of the survival curve depends on the doserate. This suggests that the cells may be in two different forms; either in a high radiosensitive form – or in a more protected form.



In this figure is given the results of Co-60 γ-radiation on asynchronous T–47D cells. The doserate was 40 Gy/h (0.66 Gy per minute).

The dip in the survival curve called HRS ("***low dose hypersensitivity***") was discovered in 1993 by Brian Marples and Michael Joiner for Chinese hamster cells. It is also called the "joiner dip".

It can be noticed that for about 80 % of all human cell lines the survival curves display hyper-radiosensitivity (HRS).

HRS has been shown to be connected to a failure to induce the early G_2 arrest that gives time for repair of DNA-damage before entering mitosis.

Brian Marples

Michael Joiner

The new G_2 checkpoint

On page 228 we outlined the cell cycle including the checkpoints. Both G_1 – S and G_2 – M phase transitions are under constant observations in order to protect the cells from both exogenous and endogenous DNA-damaging agents. The checkpoints are controlled by enzymes.
In 2002 Bo Xu and coworkers discovered a new checkpoint in the G_2 phase of the cell cycle that explained the mechanisms for the low dose hypersensitivity.

Bo Xu

This checkpoint, which comes in addition to the usual checkpoint (also called "Sinclair" checkpoint), is activated shortly after irradiation. Cells that were in G_2-phase at the time of irradiation are arrested. It can be noted that the new G_2-checkpoint is dependent on the protein kinase ATM (Ataxia telangiectasia mutated).
The ATM-molecule is large, consisting of about 3000 amino acids. It is held inactive in unirradiated cells as a dimer or higher-order multimer. Cellular irradiation causes dimer dissociation and initiates cellular ATM kinase activity which seems to be an initiating event in cellular responses.

The new G_2-checkpoint is only activated after a certain amount of ATM has been activated. The threshold dose for activating the G_2 checkpoint depends somewhat on the cell line, but it seems to be above 0,2 Gy. Cells receiving doses below this level will not be arrested, but enter mitosis with unrepaired damage resulting in mitotic death or apoptosis.

Priming doses

In line with the experiments showing adaptive effect, experiments were carried out with priming doses; i.e. a small dose is given some time before the larger challenge dose. The Oslo-group used a priming dose of 0.3 Gy. This dose is not very low, but it had a significant effect. Thus, if the "*priming dose*" was given 6 hours ahead of the challenge dose the HRS could not be observed.
The effect depends on the doserate used for priming. Thus, for a doserate of 40 Gy/h the effect lasted for about 24 hours, however, for a doserate of 0.3 Gy/h the abolition of the HRS seems to last forever (so far it has been observed for 5 years).

Surprising experiments

Experiments were then carried out with cells given a priming dose – both at a high doserate (40 Gy/h) and at a low doserate (0.3 Gy/h). Then the medium from these cells was harvested and transferred to unirradiated T–47D cells. Then the challenge dose was given.

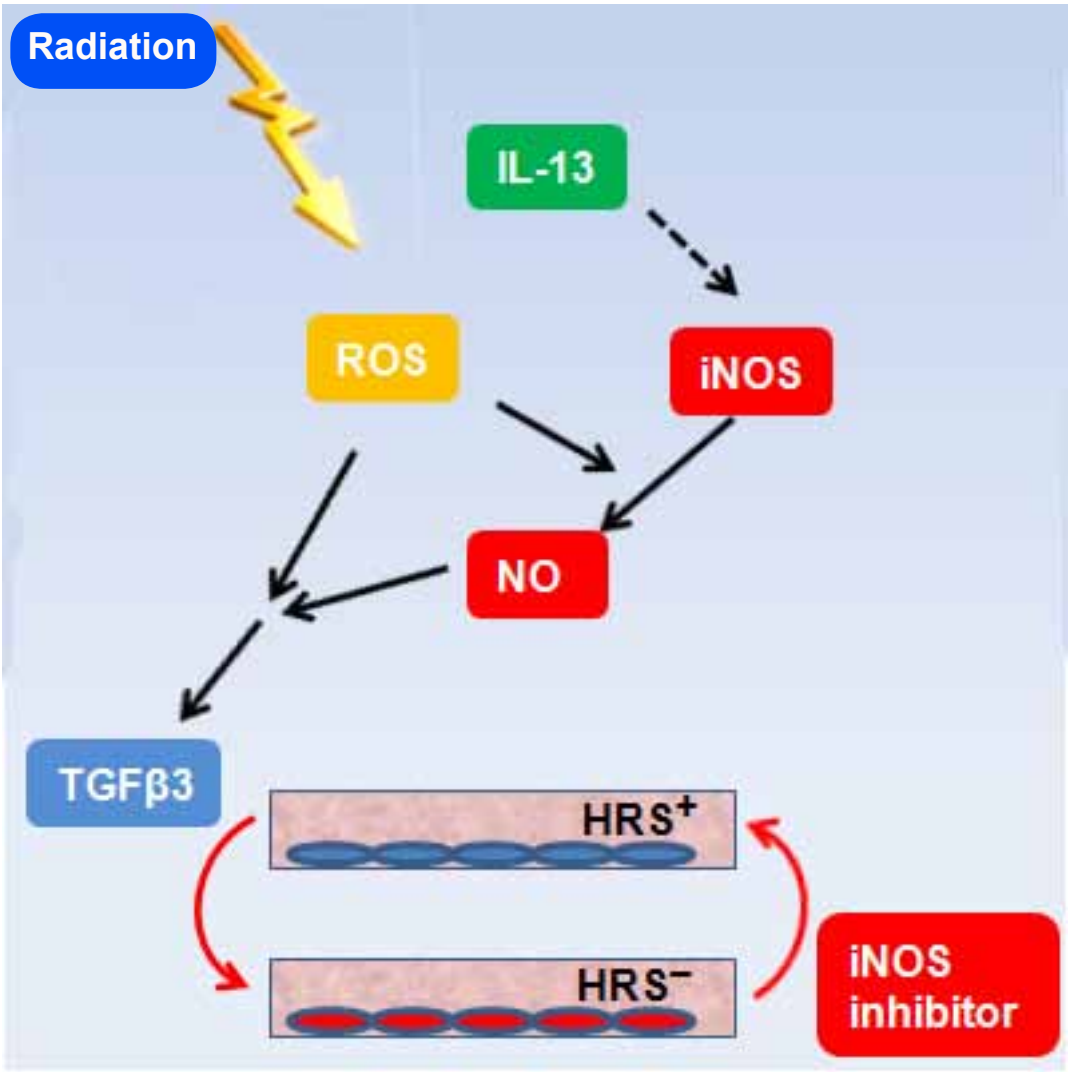
It appeared that the medium from high doserate priming had no effect on HRS (the low dose hypersensitivity) – but the medium from the low doserate priming not only removed the HRS, but the recipient cells that received small doses of challenge irradiation had a higher surviving fraction than the control recipient cells.

These results indicate that low doserate irradiation induced a factor (a protein) to the medium that made the cells more resistant for later radiation.

The Oslo-group have also found that this factor is formed by irradiation of living mice. When serum from these mice are added to the cells and irradiation experiments are performed the cells show no HRS – they are transformed to the radioresistent phase.

The mechanism

The Oslo group have suggested the following mechanism for transfoming cells from HRS to a more radioresistent form and vice versa.



*In this figure **HRS**$^{+}$ implies cells that exhibit high radiation sensitivity and **HRS**$^{-}$ indicates cells that lack this behavior – that is they are more radioresistant.*
The figure indicate that you can go from one side to the other. In the first place you can remove HRS by the factor TGFβ3 – and you can go the other way by iNOS inhibitor.
The TGFβ3 factor is induced by radiation and is formed by priming doses or can be added to the cells from previously irradiated medium or by serum for irradiated mice.

Explanations to the model

TGFβ3 is the short name for "Transforming growth factor β-3". It is a type of protein, known as a cytokine, which is involved in cell differentiation, embryogenesis and development. The figure above indicate that the factor is formed by radiation via ROS (reactive oxygen species – radicals such as hydroxyl OH, superoxide anion O_2^- and others). TGFβ3 is only stabilized in an active form via the NO-radical. The NO radical can be synthesized on demand for short periods of time (seconds to minutes) following enzyme activation. NOS (Nitric oxide syntheases) are a family of enzymes that catalyse the production of nitric oxide (NO) from L-arginine.

An important question is whether radioresistant cells can be transferred to a more sensitive stage? The figure above indicates this possibility may occur using compounds that inhibit the iNOS. This latter possibility may be important in cancer radiation therapy. In the central parts of all solid tumors we find hypoxic cells and these cells have also induced TGFβ3. It would be very important if we could transform these cells into a HRS stage.

Summing up

The model outlined above may have significant importance in cancer research. In the first place the model suggests that a small radiation dose given with a small doserate enhance the defense mechanisms. Thus cells with DNA-damage is stopped at the new G_2 checkpoint and given time for repair or directed into apoptose.

The model also indicate that it is possible to go the other way and force hypoxic cells or cells with previous history of radiation into a more radiosensitive stage.

Conclusions of the chapter

The work with tissue cultures has given a lot of information about the cellular mechanisms and how the cells can cope with errors and damage. We have learned a lot about the mechanisms going on in the cell cycle and that several checkpoints must be passed. The checkpoints are guarded by enzymes and the genes for these enzymes should be free from errors. This is very important in cancer biology. We have seen this in the case of the skin cancer Xeroderma pigmentosum (see page 226). Similar situations are met in the case of leukemia and lymphoma, breast and ovarian cancers, colon cancers and brain tumors.

We can sum up some of the important findings in the following way:

1. The cells have repair systems. Important to the repair system are the enzymes that guard the cellular check-points and carry out the repair.

2. The cells have a programmed death system (apoptosis), that can take out and kill the most damaged cells.

3. Adaptive response. It has been observed that small doses of radiation given before a large challenge dose prepare or trig the repair processes with the result that the radiation damage is reduced.

4. The Oslo group has found that small radiation doses, given at a low doserate, release the TGFβ3 factor that is important with regard to the repair processes. Radiation seems to be a necessity for life!