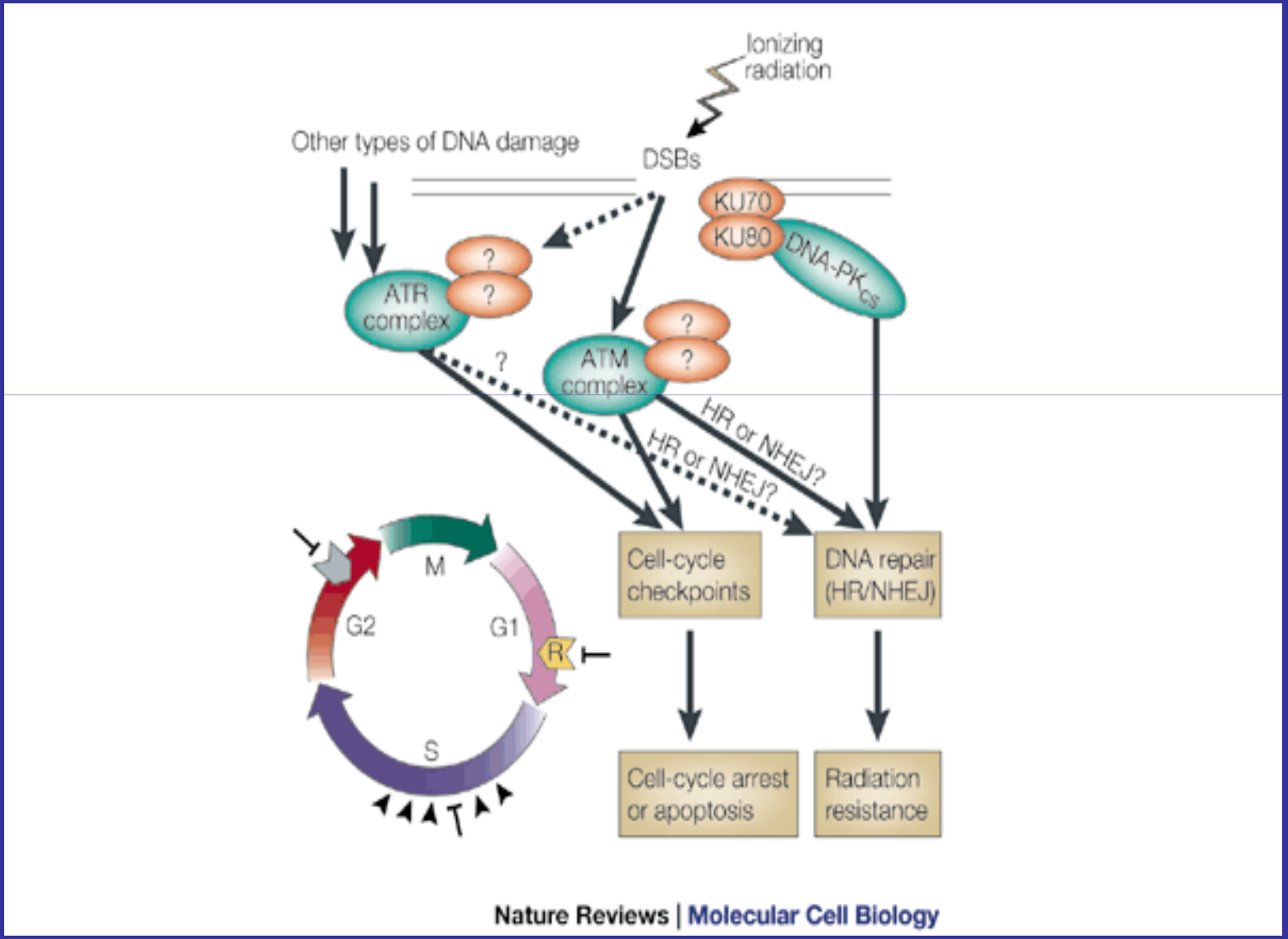
Sugárzás indukálta DNS károsodások javítása

DNS károsodások kijavítása



Homológ rekombináció és NHEJ repair folyamatok

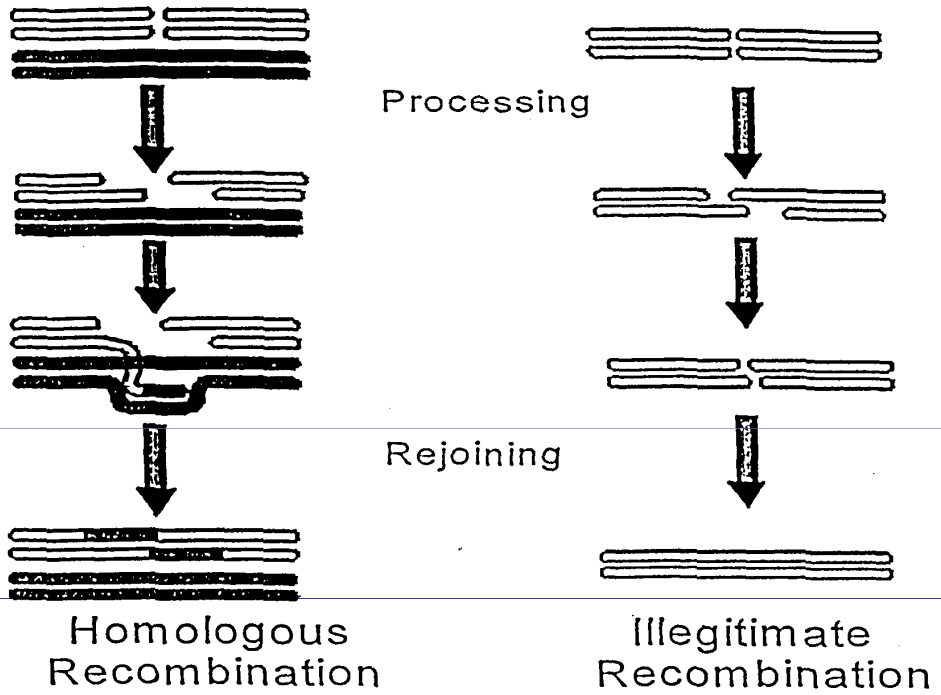


Figure 2.3. Double-strand break repair *via* homologous and nonhomologous (illegitimate) recombination. The lefthand side of the figure shows a double-strand break that has occurred after replication, so that identical sister chromatids are available. In homologous recombination the exposed 3′ end invades the homologous duplex, so that the complementary strand acts as a template for gap filling. The breakage of the other strand and subsequent exchanges are not shown. The righthand side of the figures also shows a double-strand break, but in this case no template exists to guide gap filling. Consequently, errors can occur and for this reason it is called illegitimate recombination. (Adapted from Petrini JHJ, Bressan DA, Yao MS: The rad52 epistasis group in mammalian double strand break repair. Semin Immunol 9:181–188, 1997, with permission.)

NHEJ repair

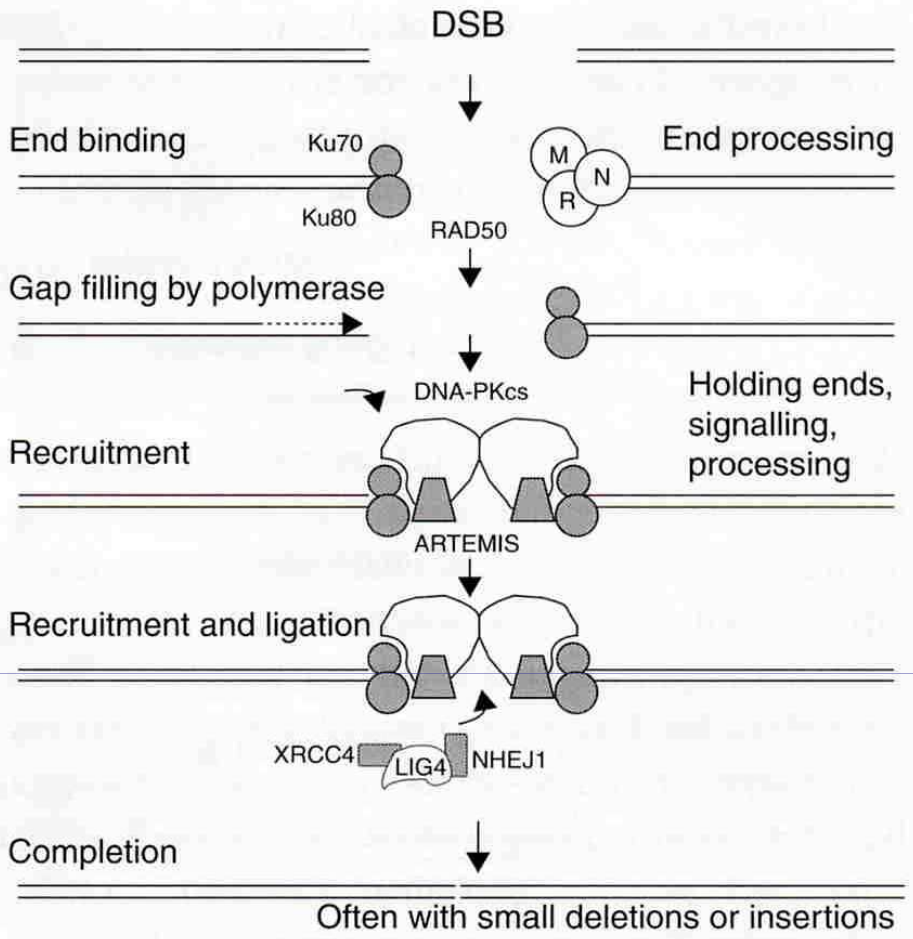
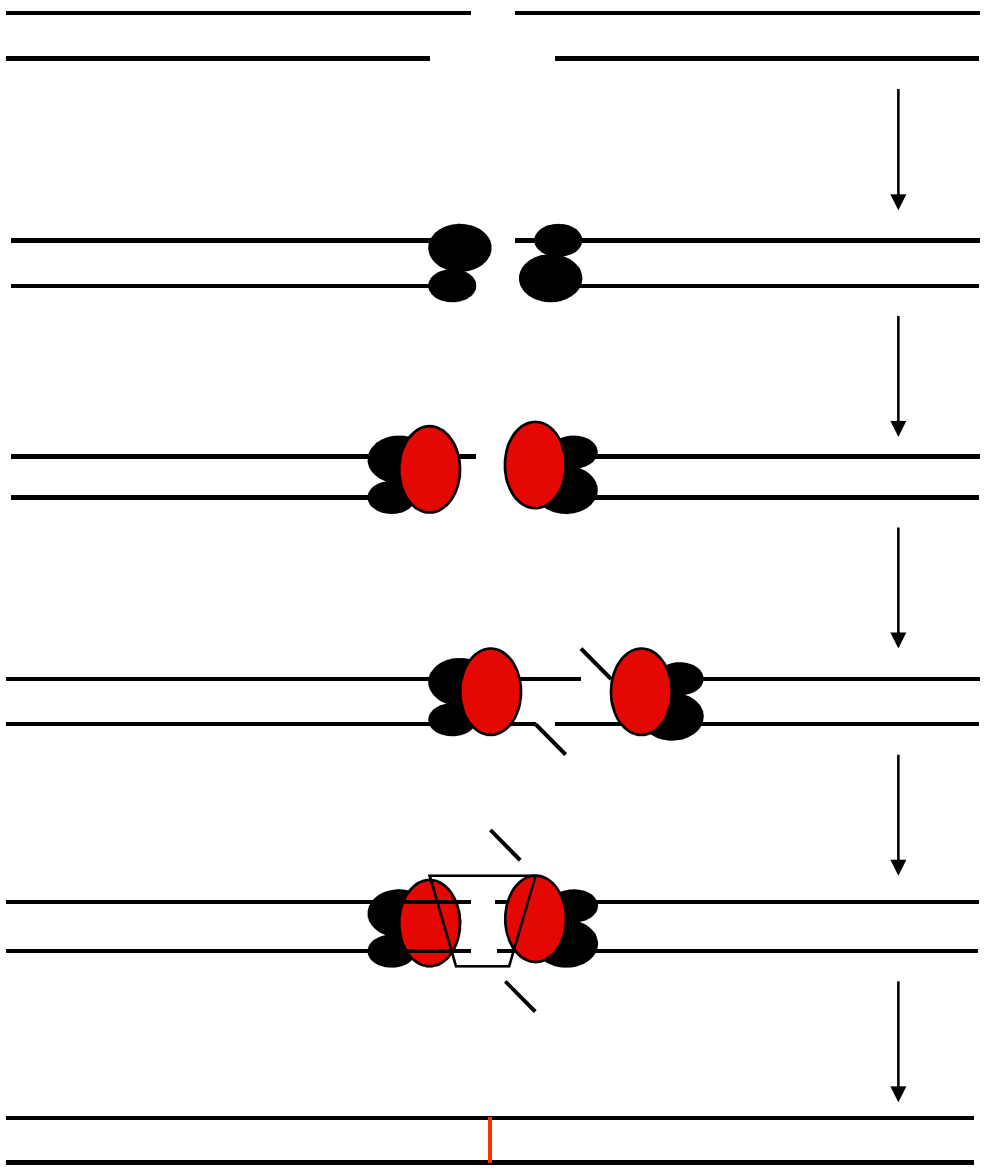


Figure 2.8 Schematic of double-strand DNA break (DSB) repair by non-homologous end-joining (NHEJ). The principal genes known to be involved are shown, although there are others not shown which are also involved in NHEJ. Chromatin remodelling genes are not shown. For clarity, processes such as end-binding have been shown on one side of the break only.


összetett szerkezetű DNS törés
Ku komplex kötődése
DNS-PK$_{CS}$ kapcsolódása
mikrohomológia keresése
tompa vég kialakítása (DNS Polimeráz μ, λ, polinukleotid kináz, Artemis)
lánc zárása (DNS ligáz IV, XRCC4, XFL)
a javítás során néhány nukleotid kieshet

NHEJ repair

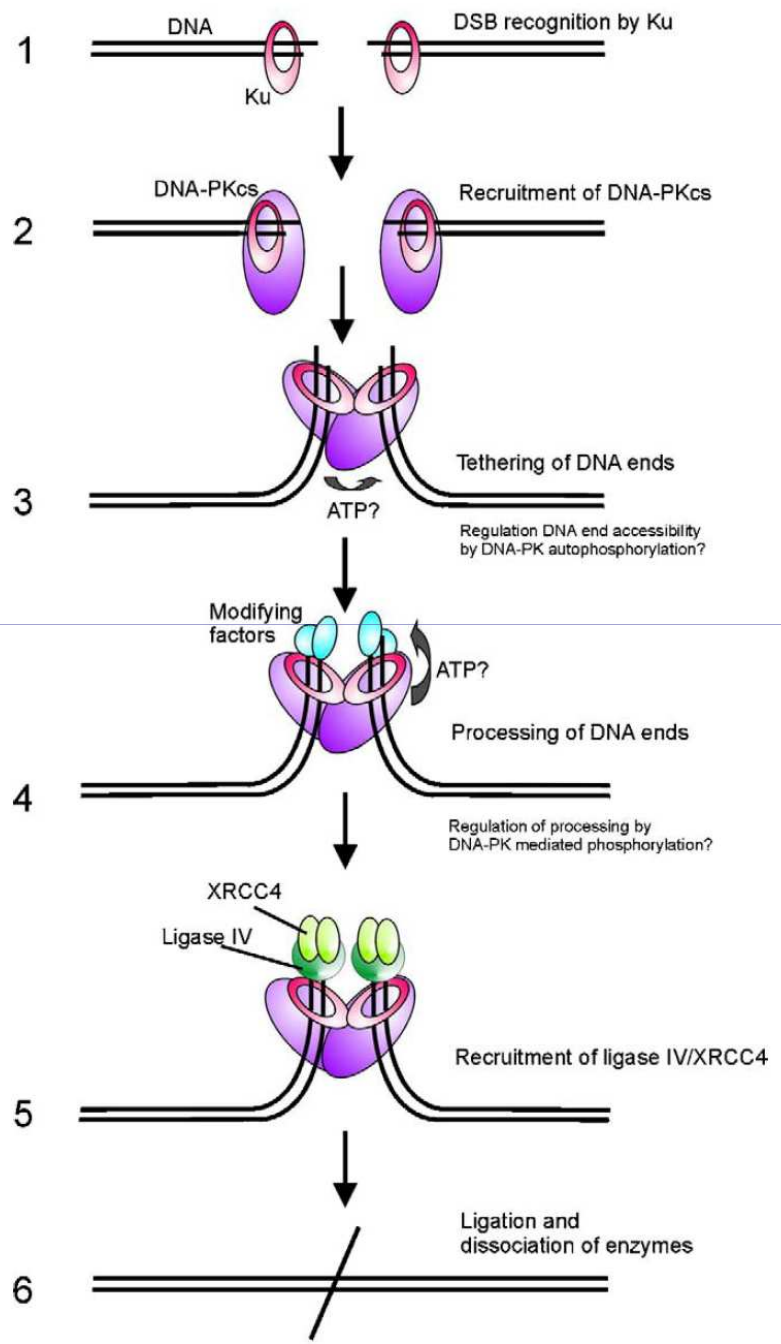


Fig. 3. A contemporary model for NHEJ. Taken all biochemical data on the core enzymatic machinery of NHEJ together, an increasingly more transparent picture of NHEJ mechanistics is arising. Most data can be reconciled in a model along the following lines: Recognition of a DSB by the Ku 70/80 heterodimer (1), which recruits DNA-PK$_{CS}$ (2). Subsequently, the ends are tethered (3) and complex, non-complementary breaks are processed (4). Ligase IV/XRCC4 is recruited to the DSB (5). The presence of Ku and possibly the complete DNA-PK holo-enzyme facilitates the binding of ligase IV/XRCC4 to the DNA ends. Note that the configuration with Ku rings at a more inward position in the DNA would allow for a strong tether between the DNA ends without covering the extreme termini. Finally, the processed DSB is ligated (6).

NHEJ repair alapvető szerepet játszik az az aktív immunrendszer kifejlődésében, a V(D)J rekombinációban

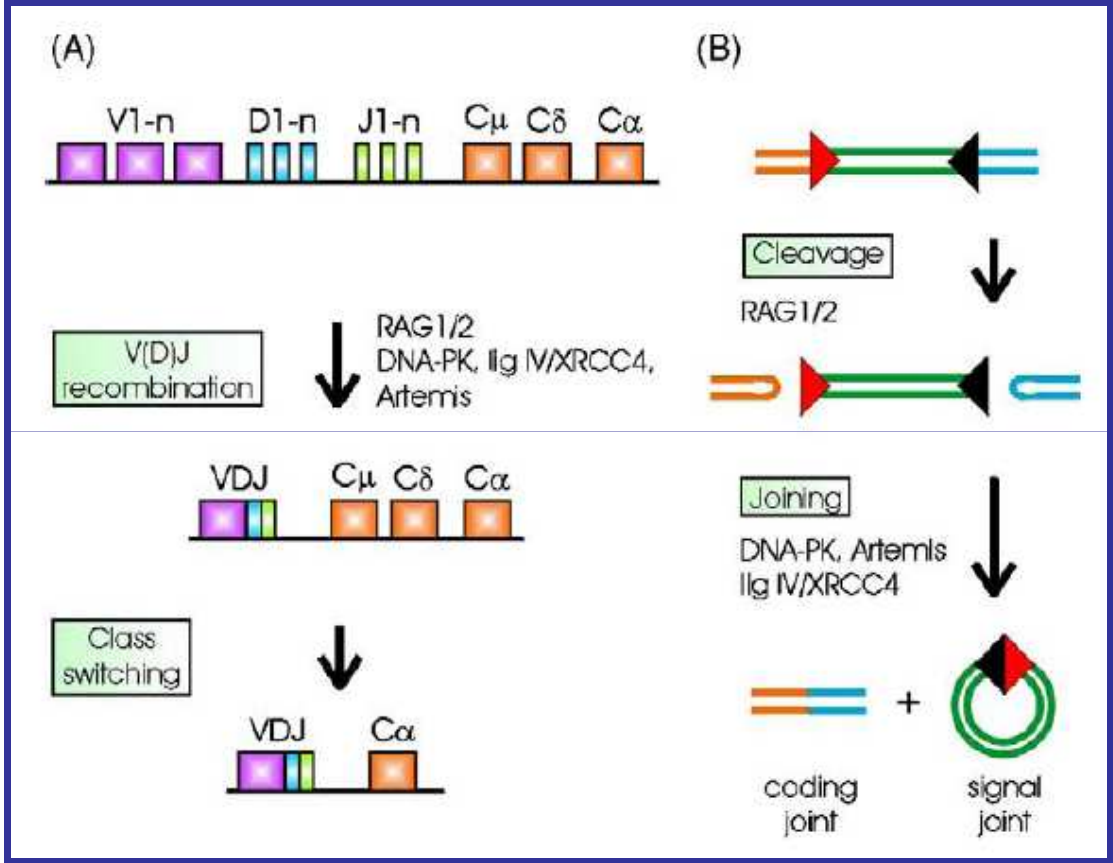
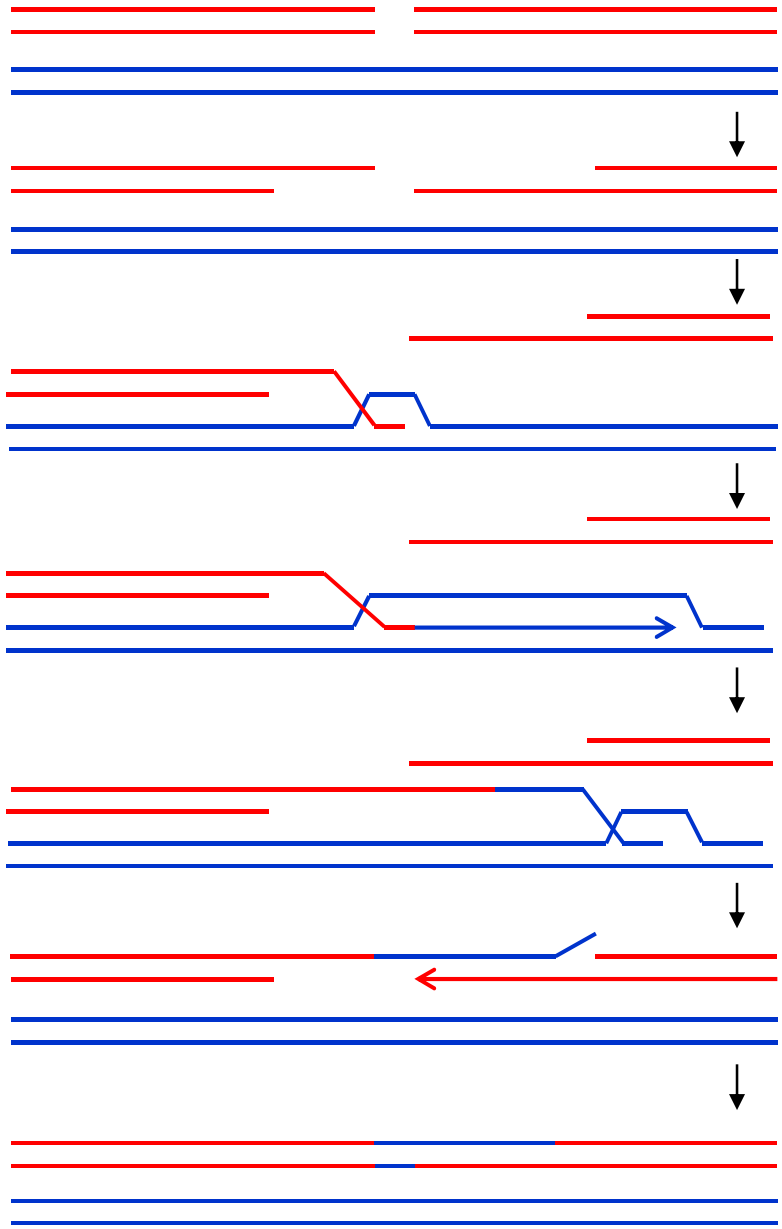


Fig. 2. DNA recombination in the immune system. (A) V(D)J recombination is a process that takes place in developing B- and T-cells. The genes that encode immunoglobulins (Ig) or T-cell receptors (Tcr) are not present in an active form in these cell. In fact, the loci contain gene fragments that have to be combined to form a mature Ig or Tcr gene. The great number of combinations that can be made with these building blocks, forms the basis for diversity in Ig and Tcr gene products. This assembly process is called V(D)J recombination. Segments are classified in several groups, referred to as variable (V), diversity (D) and joining (J) segments. The situation at the IgH locus is schematically depicted in the figure. D and J segments are first coupled. Subsequently, the DJ assembly is joined with a V segment, to form the mature IgH gene. B-cells can change the Ig isotype via a process called class switching, which selects for a constant (C) segment. (B) Ig or Tcr gene segments are assembled by introduction of DNA breaks at the edges of gene segments, removal of intervening DNA and ligation of selected segments. DSBs are made by the RAG1 (recombination-activating gene) and RAG2 proteins at specific sites, the recombination signal sequences (depicted as triangles). Ends of the fragments that contain the recombination signal sequences are called signal ends, whereas ends of the fragments that will form the V(D)J exon are referred to as coding ends. Coding ends have a hairpin structure, whereas signal ends are blunt. Joining of coding ends requires opening and processing of hairpin structures by the Artemis protein, as well as the action of NHEJ enzymes: DNA-PK$_{CS}$, Ku70/80, XRCC4 and DNA ligase IV.

NHEJ repair genetikai károsodása miatt kialakuló betegségek

Table 1 Consequences of NHEJ gene defects in men and mice

Gene	*Human clinical phenotype*	*Knock-out mouse phenotype*	*Ref*
DNA-PK_{CS}	—	Radiosensitive SCID T-cell tumors	(Gao *et al.* (1998a); Taccioli *et al.* (1998)
Ku70	—	Radiosensitive SCID Growth retardation T-cell tumors	Gu *et al.* (1997); Ouyang *et al.* (1997)
Ku80	—	Radiosensitive SCID Growth retardation	Nussenzweig *et al.* (1996); Zhu *et al.* (1996)
XRCC4	—	Embryonic lethal, apoptosis of post-mitotic neurons	Gao *et al.* (1998b)
LIG4	Radiosensitive leukemia or LIG4 syndrome characterized by growth retardation, microcephaly and immunodeficiency or radiosensitive SCID	Embryonic lethal, apoptosis of post-mitotic neurons	Barnes *et al.* (1998); Frank *et al.* (1998); O'Driscoll *et al.* (2001); Riballo *et al.* (1999); van der Burg *et al.* (2006)
Artemis	Radiosensitive SCID	Radiosensitive SCID	(Moshous *et al.* (2001); Rooney *et al.* (2002)
XLF/Cer-nunnos	Growth retardation, microcephaly, and immunodeficiency due to profound T and B-cell lymphocytopenia	ES cells: radiosensitive, impaired V(D)J recombination	(Buck *et al.* (2006); Zha *et al.* (2007)



kétláncú DNS törés

egyláncú DNS szakasz kialakítása (Mre11/Rad50/Nbs1)

Rad51 kötődés

BRCA2, Rad52, Rad54, XRCC2, XRCC3

lánc-invázió, D-hurok kialakulás

DNS szintézis (DNS polimeráz η), Holliday kereszt létrejötte

szálvándorlás (WRN, BLM, p53, Rad54)

újonnan szintetizált és az eredeti lánc kapcsolódása (Rad52), DNS szintézis (DNS polimeráz δ és ε)

a lánc zárása (DNS ligáz I)

Homológ rekombináció

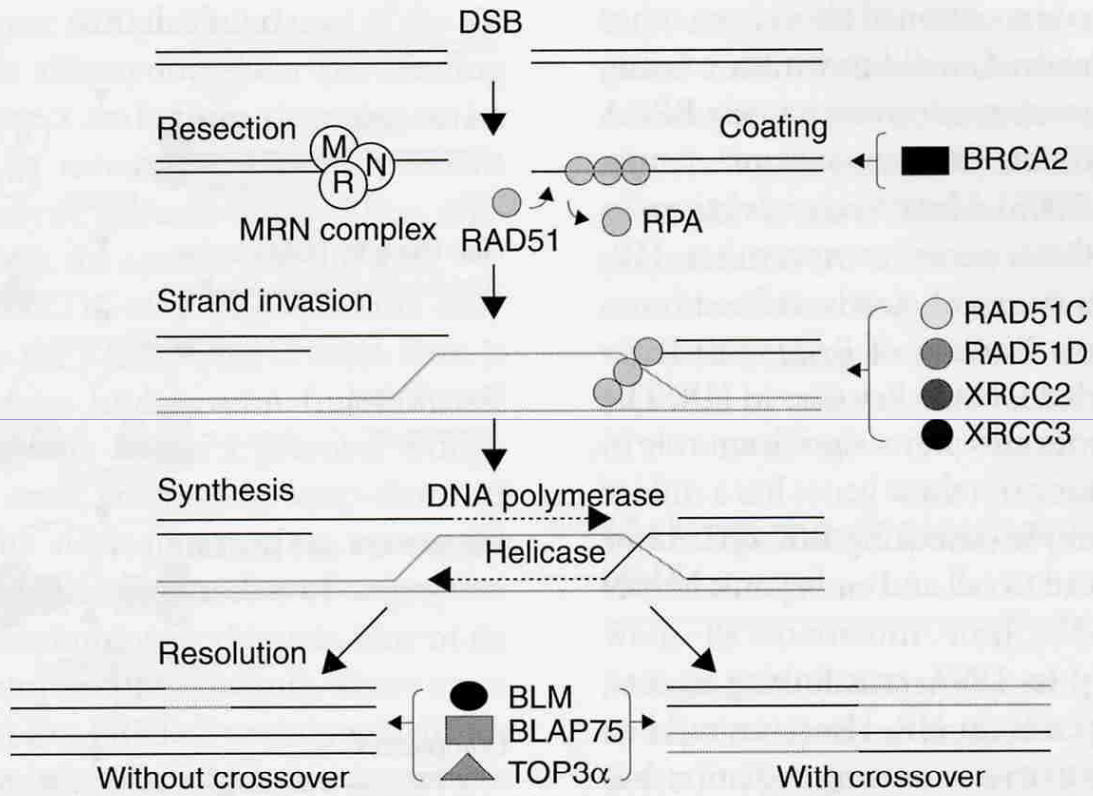


Figure 2.7 Schematic of double-strand DNA break (DSB) repair by homologous recombination (HR). The principal genes known to be involved are shown, although there are others not shown which are also involved in HR. Chromatin remodelling genes are not shown. The main feature is the use of an undamaged sister chromatid sequence (light coloured lines) as template for repair. The groups of genes (right and bottom centre) are involved with the processes indicated by the horizontal arrows.

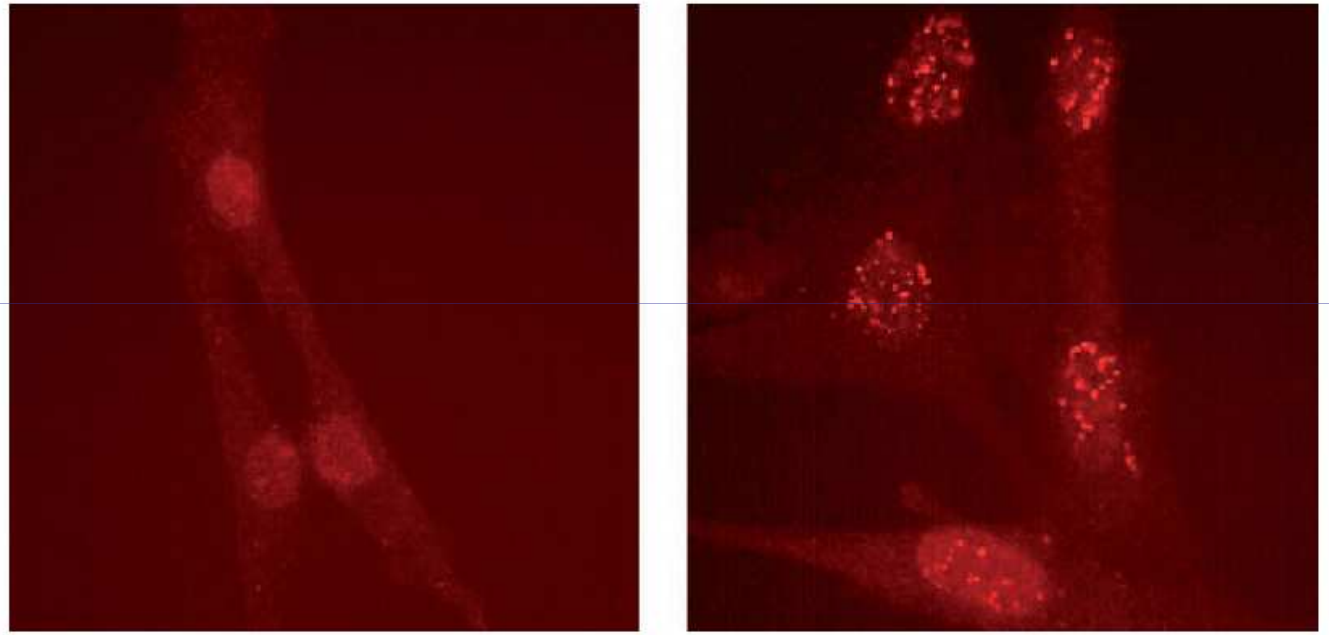
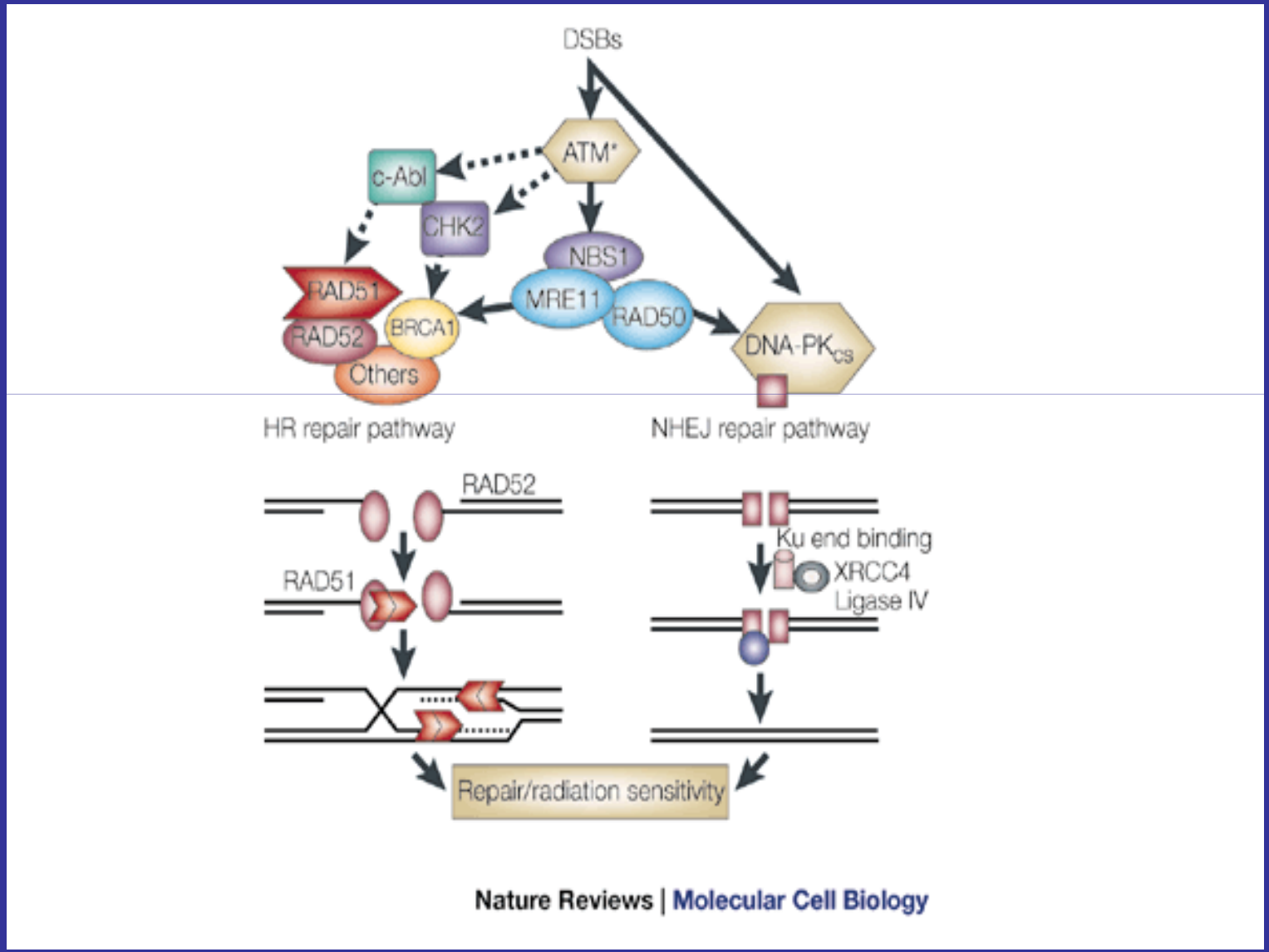


Fig. 6. Ionizing-radiation-induced foci. Immunohistochemical staining for Rad51 (protein involved in HR) in a vascular smooth muscle cell before and 8 h after irradiation with 12 Gy of γ-radiation. Clearly visible are the bright nuclear foci appearing, which are believed to be "DNA damage repair factories" in which accumulation of many of the proteins discussed in this review (i.e., γ-H2AX, Rad54, Rad52, and MRN complex) takes place.

NHEJ vagy homológ rekombináció???



Bázis-kimetszéses és egyláncú-töréseket javító repair

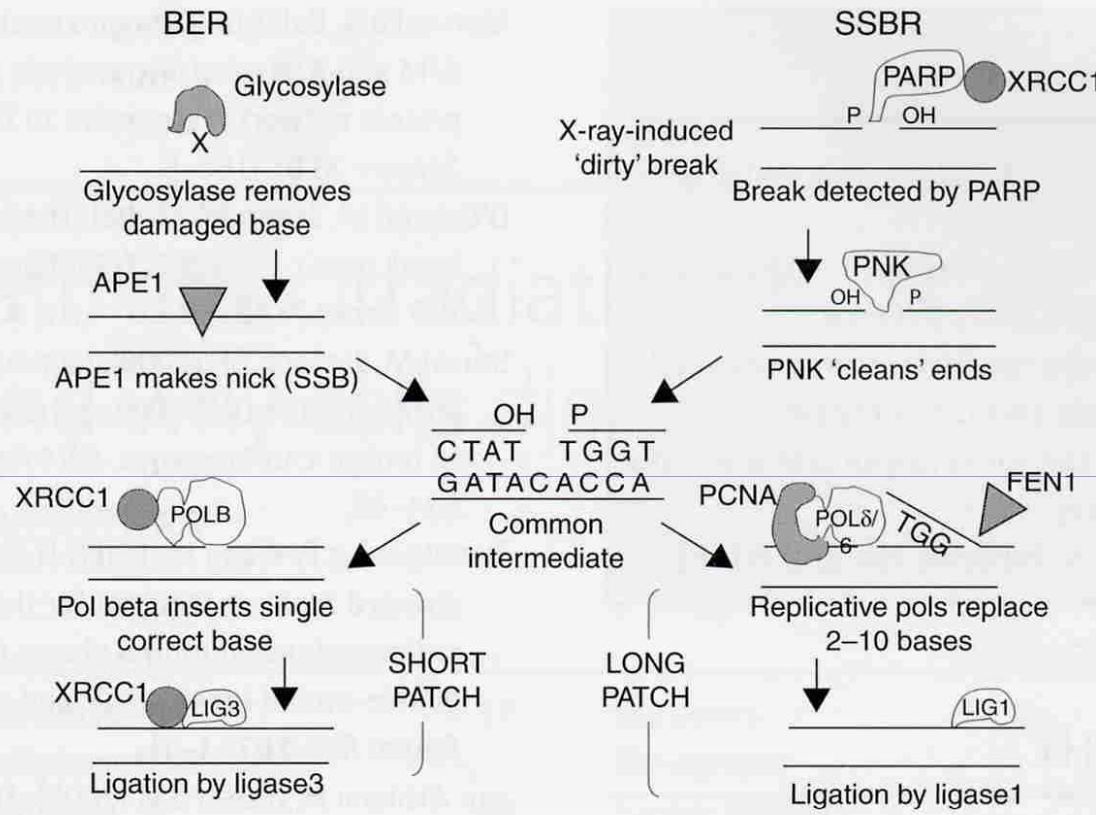
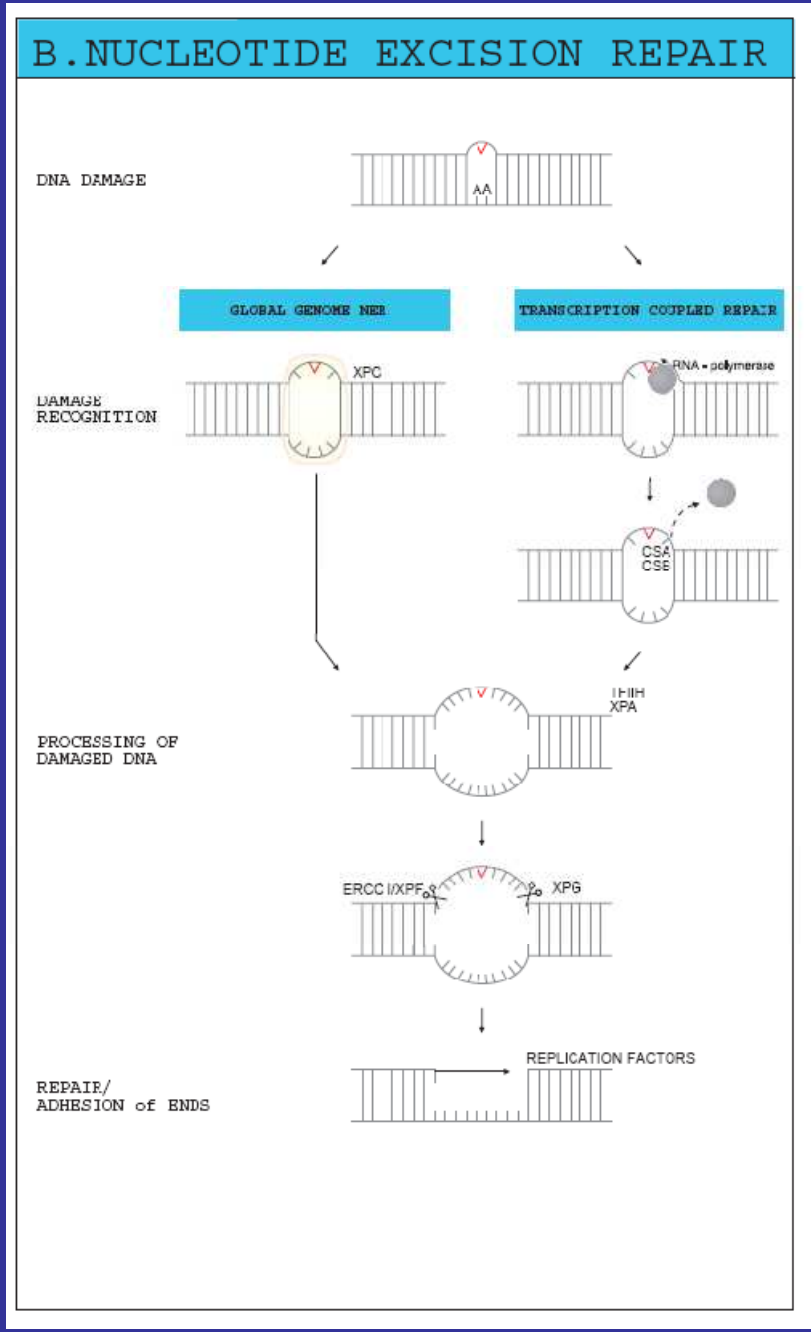
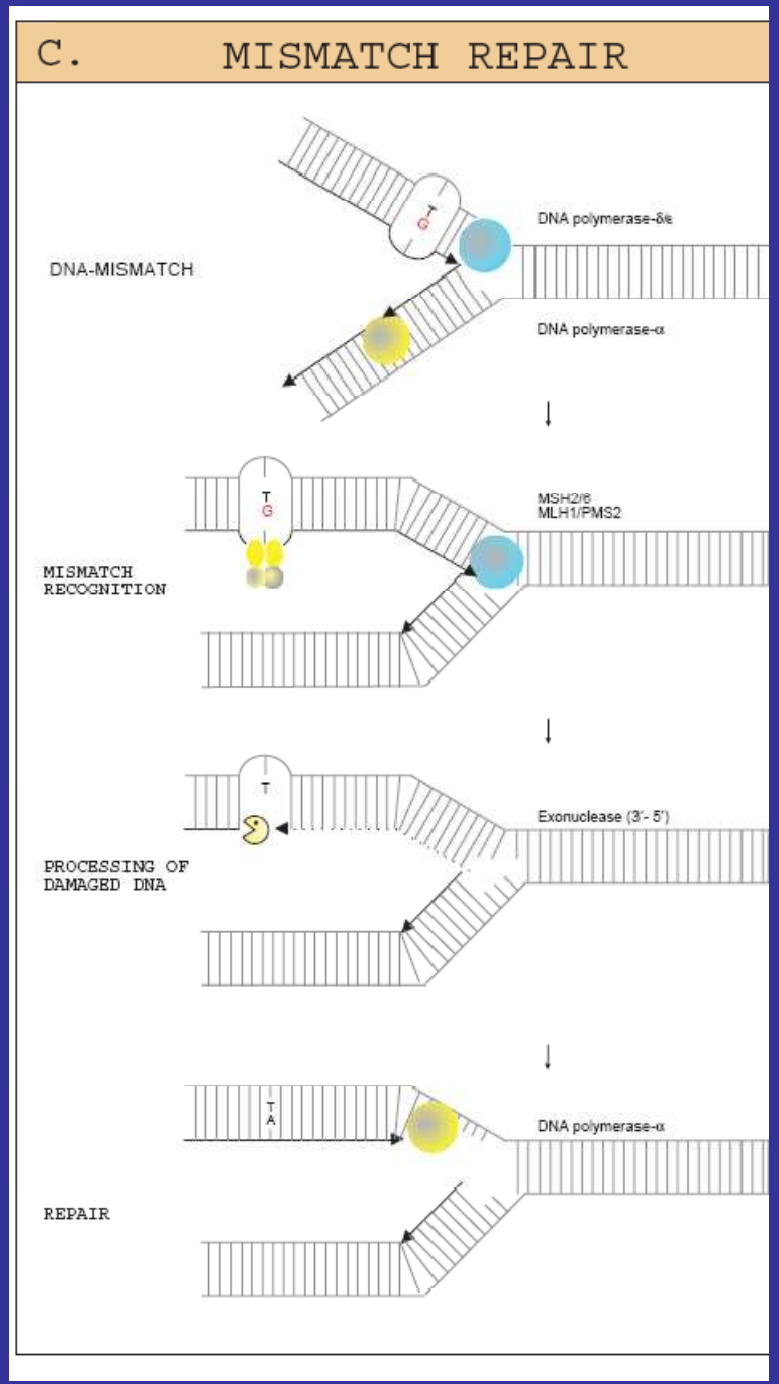


Figure 2.9 Schematic of the related pathways of base excision repair (BER) and single-strand break repair (SSBR). The X (top left) represents a damaged base. Different base damages are recognized and removed by different glycosylases as the first step in BER. Both pathways result in a common nicked intermediate, which is processed by one of two subpathways (short or long patch repair). APE1 apurinic/apyrimidinic endonuclease-1; PARP, poly (ADP-ribose) polymerase; PNK, polynucleotide kinase; POL, polymerase.

UV és kémiai ágensek hatására




C. MISMATCH REPAIR
DNA-MISMATCH
T
G
DNA polymerase-δ/ε
DNA polymerase-α
MISMATCH
RECOGNITION
T
G
MSH2/6
MLH1/PMS2
PROCESSING OF
DAMAGED DNA
T
Exonuclease (3'- 5')
REPAIR
T
A
DNA polymerase-α

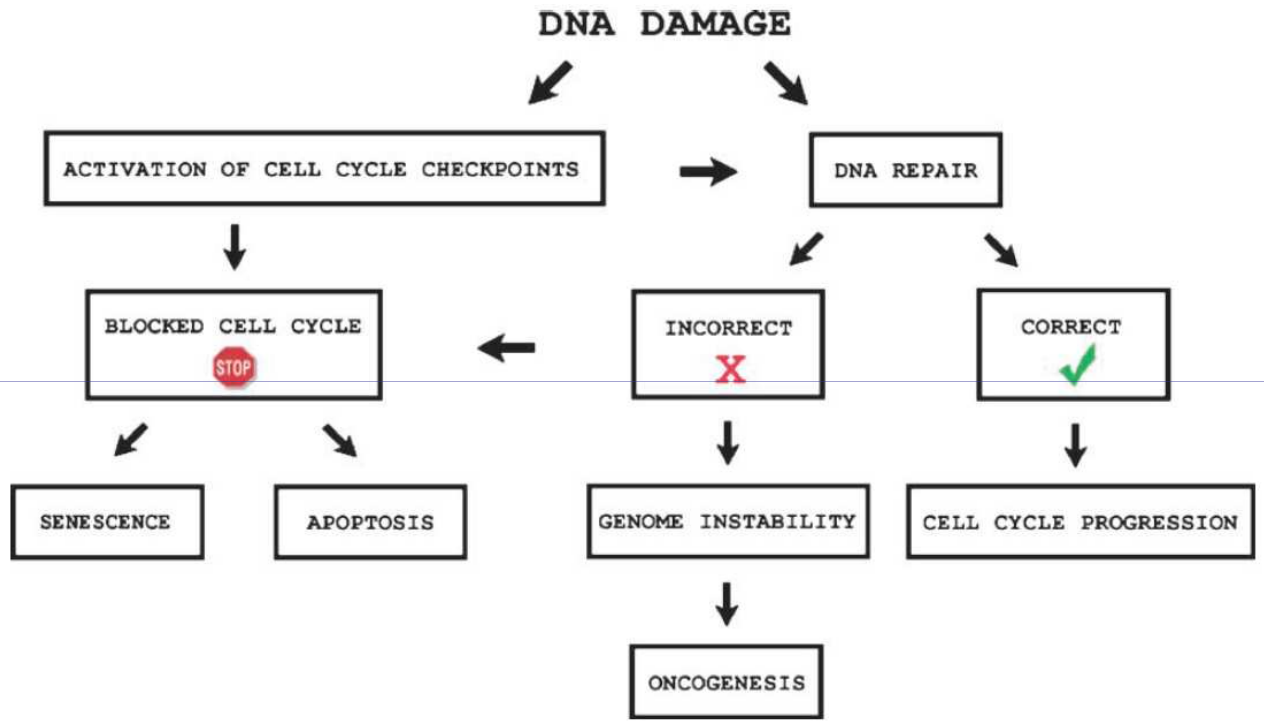


Fig. 3. Flowchart of the cellular response to any kind of DNA damage. In proliferating cells, cell cycle checkpoints will be activated, leading to a cell cycle arrest and providing time to the activated DNA damage repair machinery to repair the DNA damage. In resting/terminally differentiated cells, DNA repair will be initiated directly. When repair is complete, the cell may proceed in its cell cycle. If the damage cannot be repaired or if there is too much damage for the DNA repair machinery to overcome, then the cell cycle can be blocked permanently, leading to a senescent state of the cell, or apoptosis may be induced. If unrepaired damages remain undetected, then this may lead to mutations and genomic instability that ultimately can lead to oncogenesis.

Sugárkárosodások fajtái

- Letális károsodás
- Potenciálisan letális károsodás
- Szubletális károsodás

Potenciálisan letális károsodás

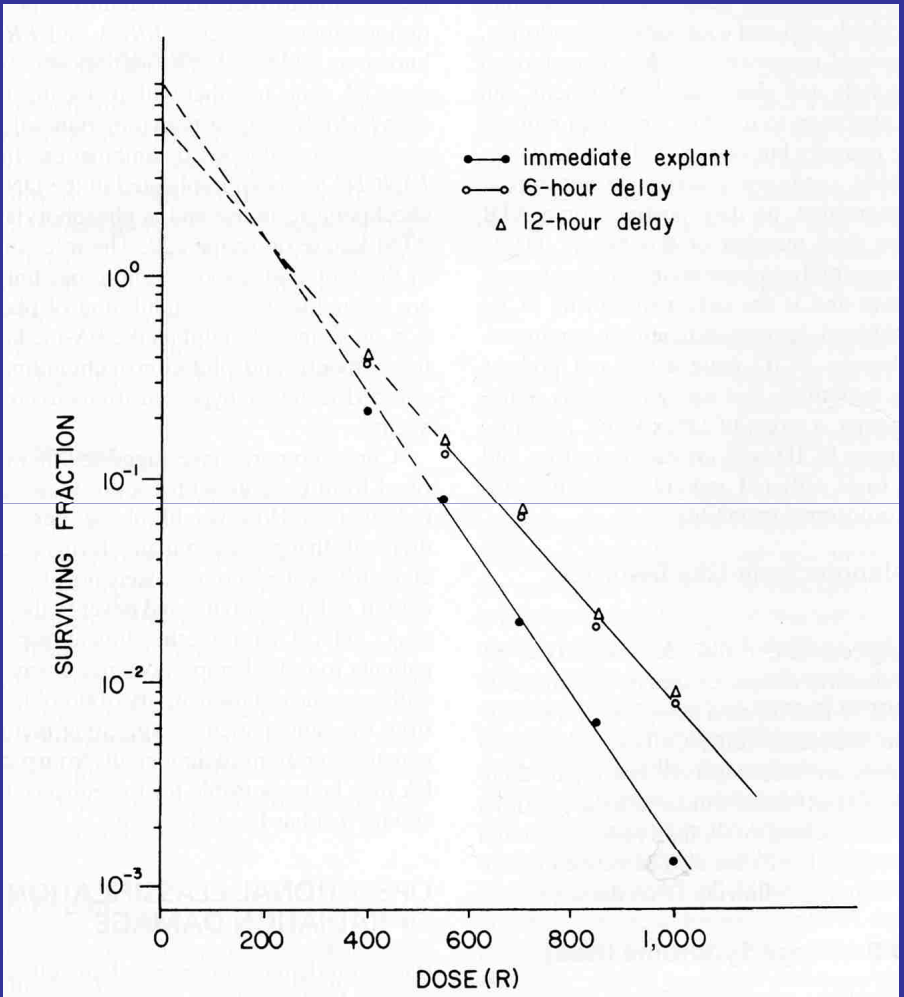


FIGURE 5.4 ● X-ray survival curves for density-inhibited stationary-phase cells, subcultured (trypsinized and plated) either immediately or 6 or 12 hours after irradiation. Cell survival is enhanced if cells are left in the stationary phase after irradiation, allowing time for the repair of potentially lethal damage. (From Little JB, Hahn GM, Frindel E, Tubiana M: Repair of potentially lethal radiation damage *in vitro* and *in vivo*. *Radiology* 106:689–694, 1973, with permission.)

Megosztott dózisok hatása (szubletális károsodások kijavítása)

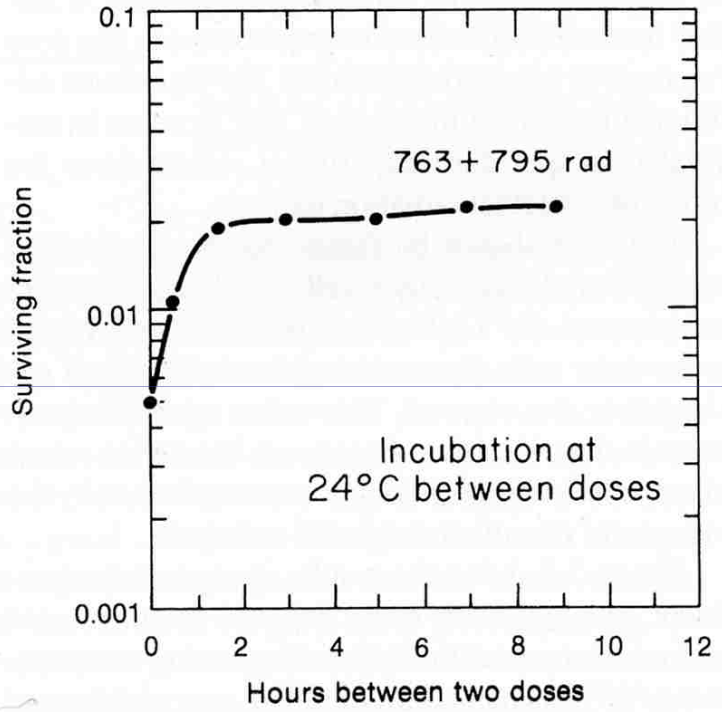


FIGURE 5.6 Survival of Chinese hamster cells exposed to two fractions of x-rays and incubated at room temperature for various time intervals between the two exposures. (From Elkind MM, Sutton-Gilbert H, Moses WB, Alescio T, Swain RB: Radiation response of mammalian cells in culture: V. Temperature dependence of the repair of x-ray damage in surviving cells [aerobic and hypoxic]. *Radiat Res* 25:359–376, 1965, with permission.)

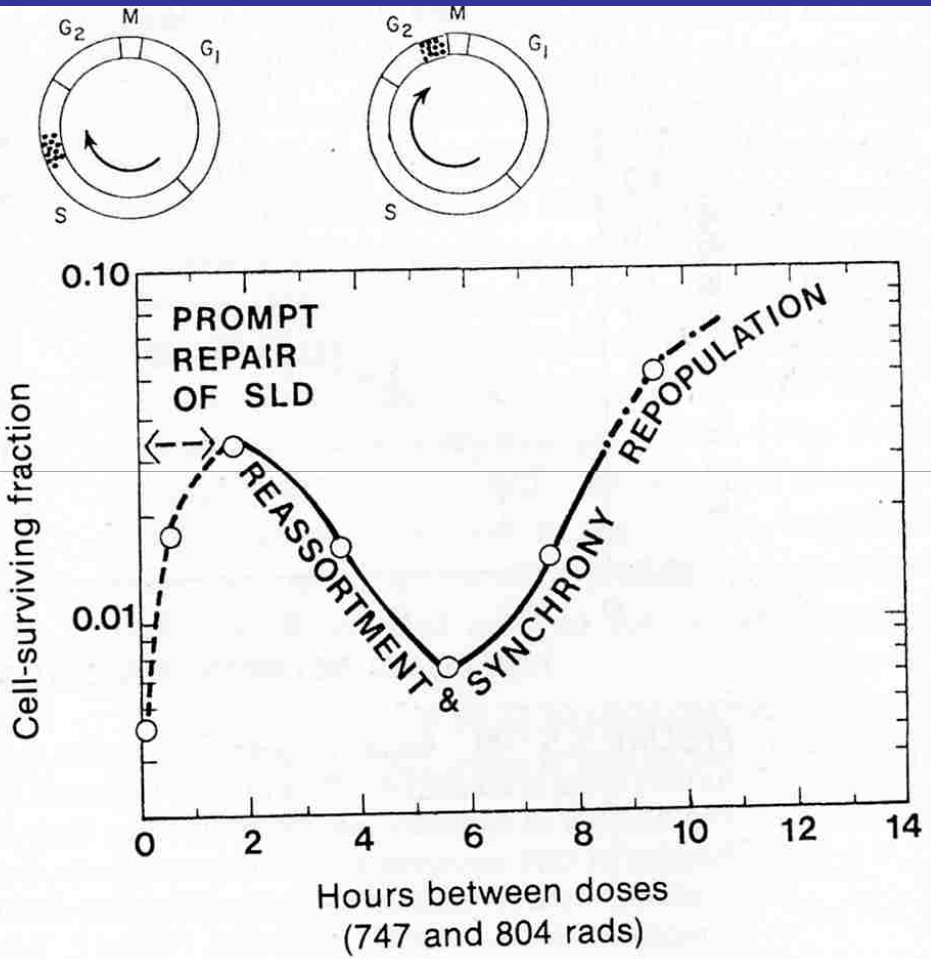
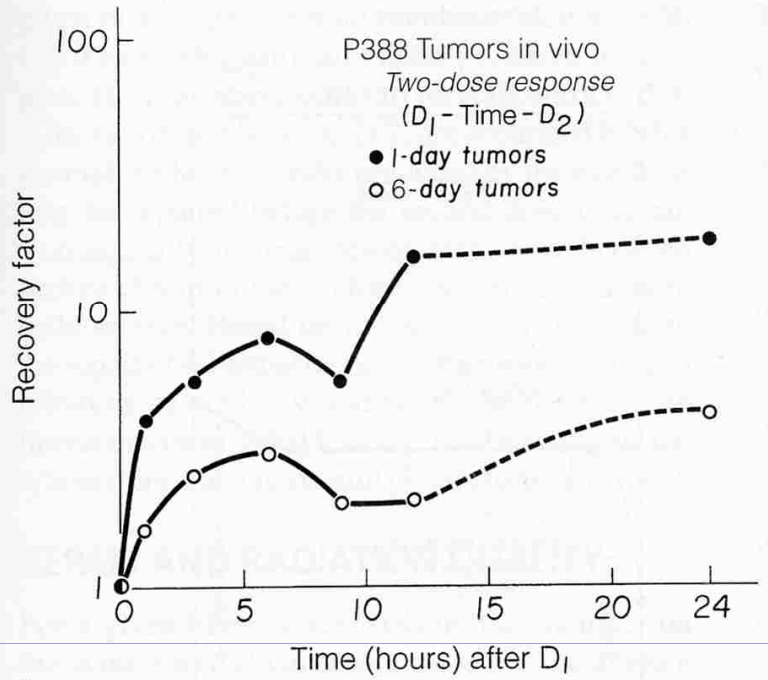


FIGURE 5.7 ● Survival of Chinese hamster cells exposed to two fractions of x-rays and incubated at 37°C for various time intervals between the two doses. The survivors of the first dose are predominantly in a resistant phase of the cycle (late S). If the interval between doses is about 6 hours, these resistant cells have moved to the G_2/M phase, which is sensitive. (Adapted from Elkind MM, Sutton-Gilbert H, Moses WB, Alescio T, Swain RB: Radiation response of mammalian cells in culture: V. Temperature dependence of the repair of x-ray damage in surviving cells [aerobic and hypoxic]. *Radiat Res* 25:359–376, 1965, with permission.)

Chapter 4. If an asynchronous population of cells is



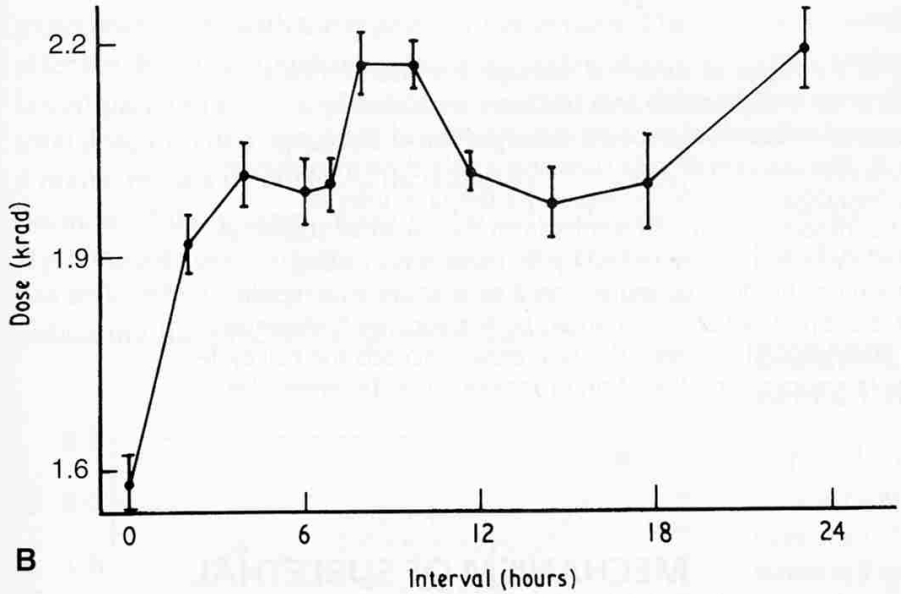


FIGURE 5.8 ● Repair of sublethal damage in two *in vivo* mammalian cell systems. **A:** Split-dose experiments with P388 lymphocytic leukemia cells in the mouse. The recovery factor is the ratio of the surviving fraction resulting from two-dose fractionation to the survival from a single equivalent dose. One-day-old tumors are composed predominantly of oxygenated cells; the cells in 6-day-old tumors are hypoxic. (From Belli JA, Dicus GJ, Bonte FJ: Radiation response of mammalian tumor cells: 1. Repair of sublethal damage *in vivo*. *J Natl Cancer Inst* 38:673–682, 1967, with permission.) **B:** Split-dose experiments with skin epithelial cells in the mouse. The total x-ray dose, given as two fractions, required to result in one surviving epithelial cell per square millimeter is plotted against the time interval between the two doses. (From Emery EW, Denekamp J, Ball MM: Survival of mouse skin epithelial cells following single and divided doses of x-rays. *Radiat Res* 41:450–466, 1970, with permission.)

Nagy LET értékű sugárzások

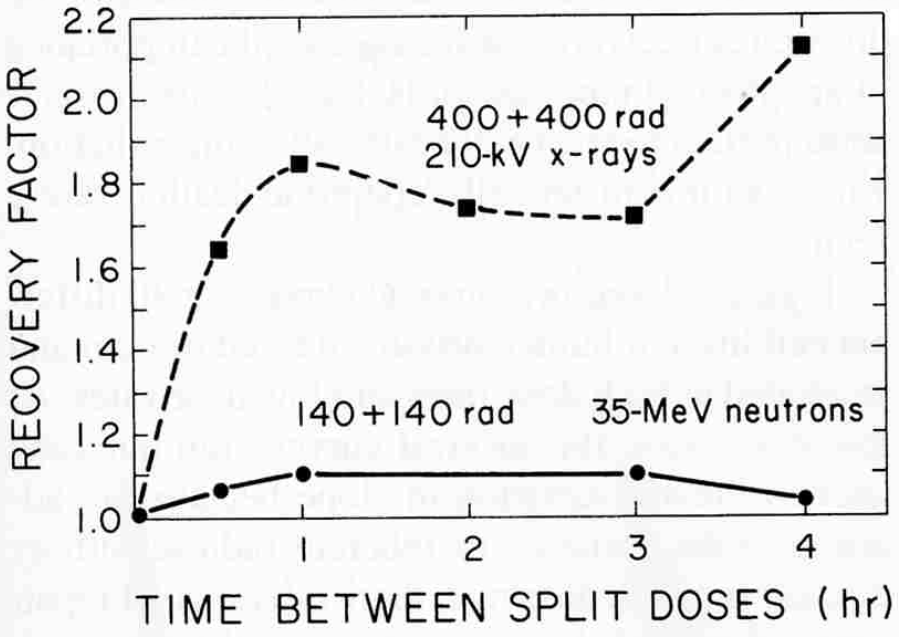
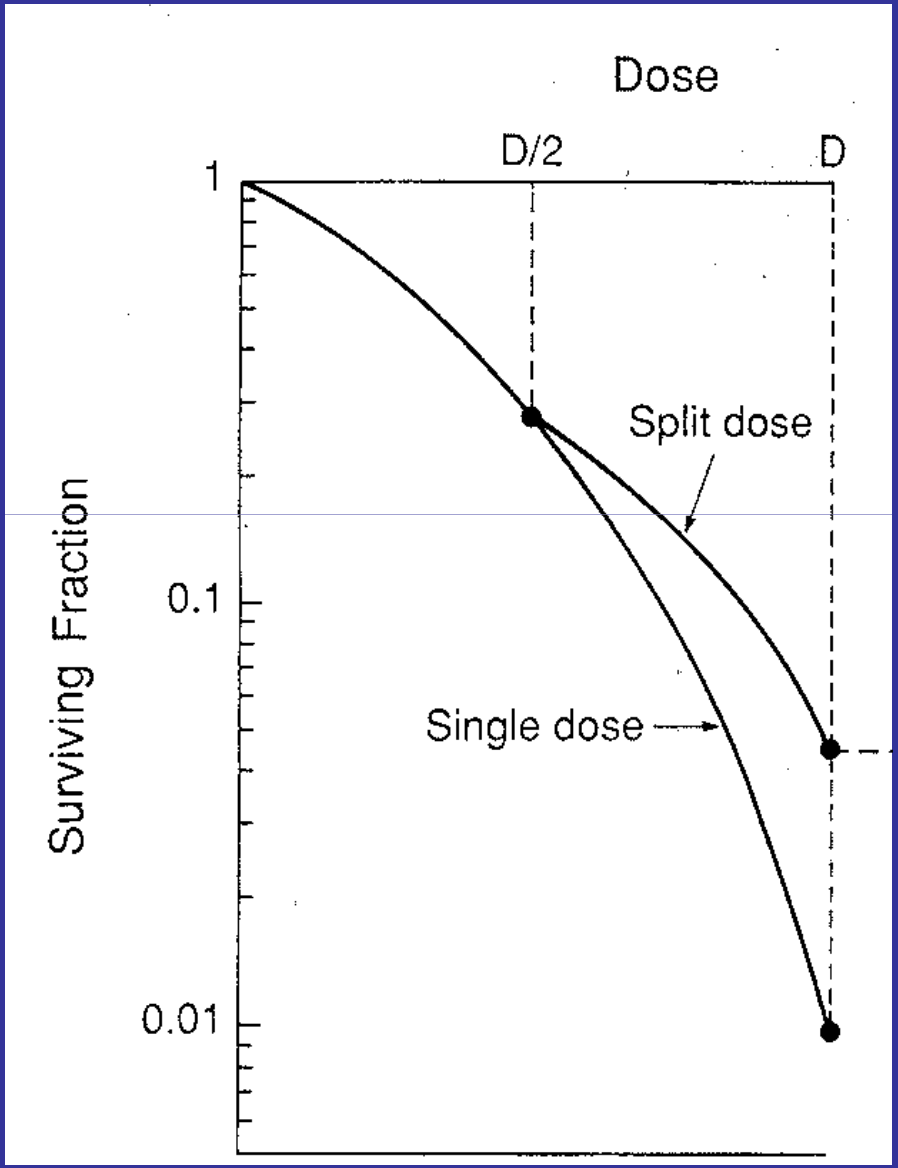


FIGURE 5.10 ● Split-dose experiments with Chinese hamster cells. For 210-kV x-rays, two 4-Gy (400-rad) doses, separated by a variable interval, were compared with a single dose of 8 Gy (800 rad). For neutrons (35-MeV $d^+ \rightarrow$ Be), two 1.4-Gy (140-rad) doses were compared with a single exposure of 2.8 Gy (280 rad). The data are plotted in terms of the recovery factor, defined as the ratio of surviving fractions for a given dose delivered as two fractions compared with a single exposure. It is evident that repair of sublethal damage during the interval between split doses is virtually nonexistent for neutrons but is a significant factor for x-rays. (From Hall EJ, Roizin-Towie L, Theus RB, August LS: Radiobiological properties of high energy cyclotron produced neutrons used for radiotherapy. *Radiology* 117:173–178, 1975, with permission.)

Frakcionált sugárterápia alapjai



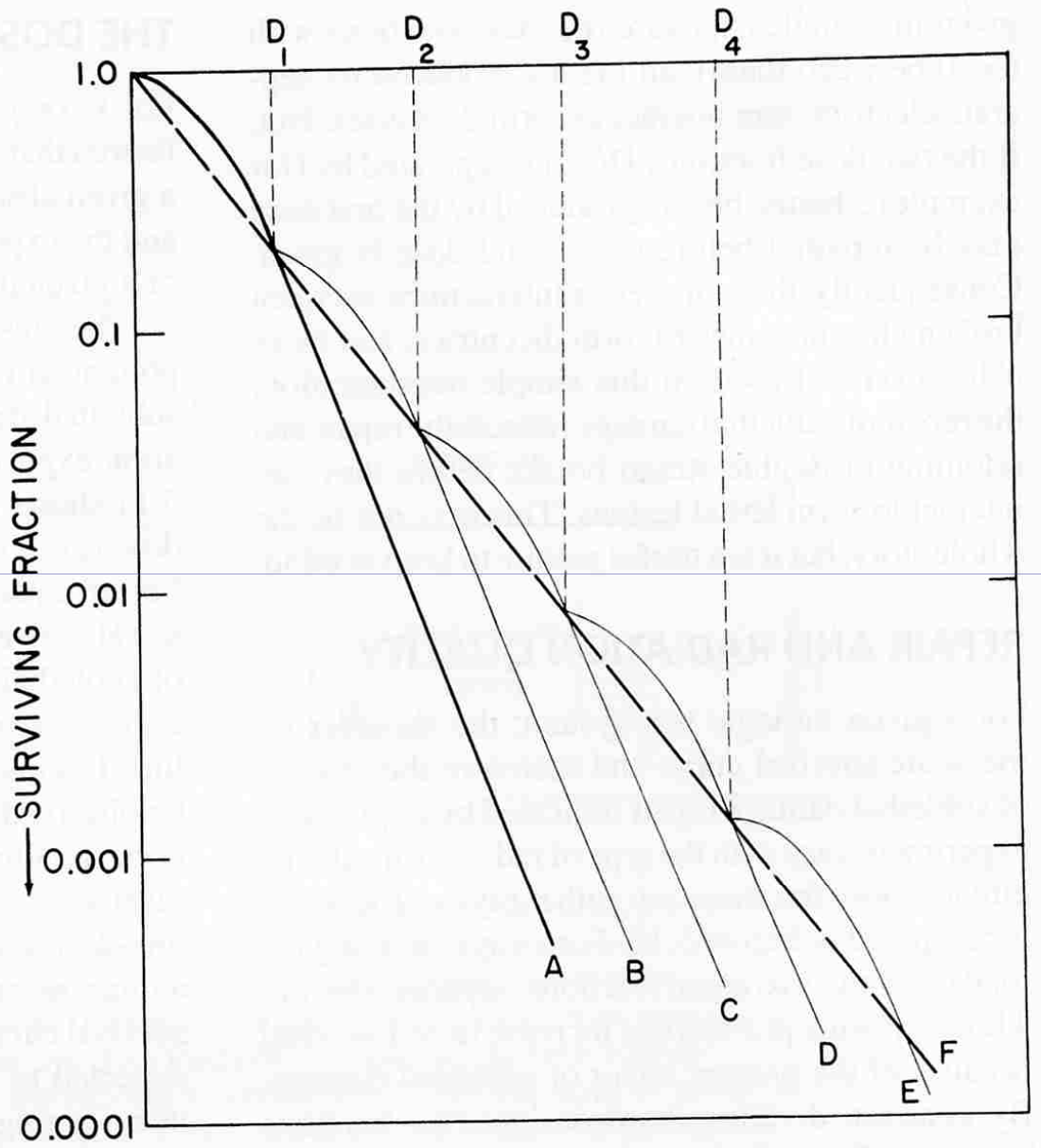


FIGURE 5.11 ● Idealized fractionation experiment. Curve A is the survival curve for single acute exposures of x-rays. Curve F is obtained if each dose is given as a series of small fractions of size D_1 with an interval between fractions sufficient for repair of sublethal damage. Multiple small fractions approximate to a continuous exposure to a low dose rate. (From Elkind MM, Whitmore GF: *Radiobiology of Cultured Mammalian Cells*. New York, Gordon and Breach, 1967, with permission.)

Dózis-teljesítmény hatása a sejtek túlélésére

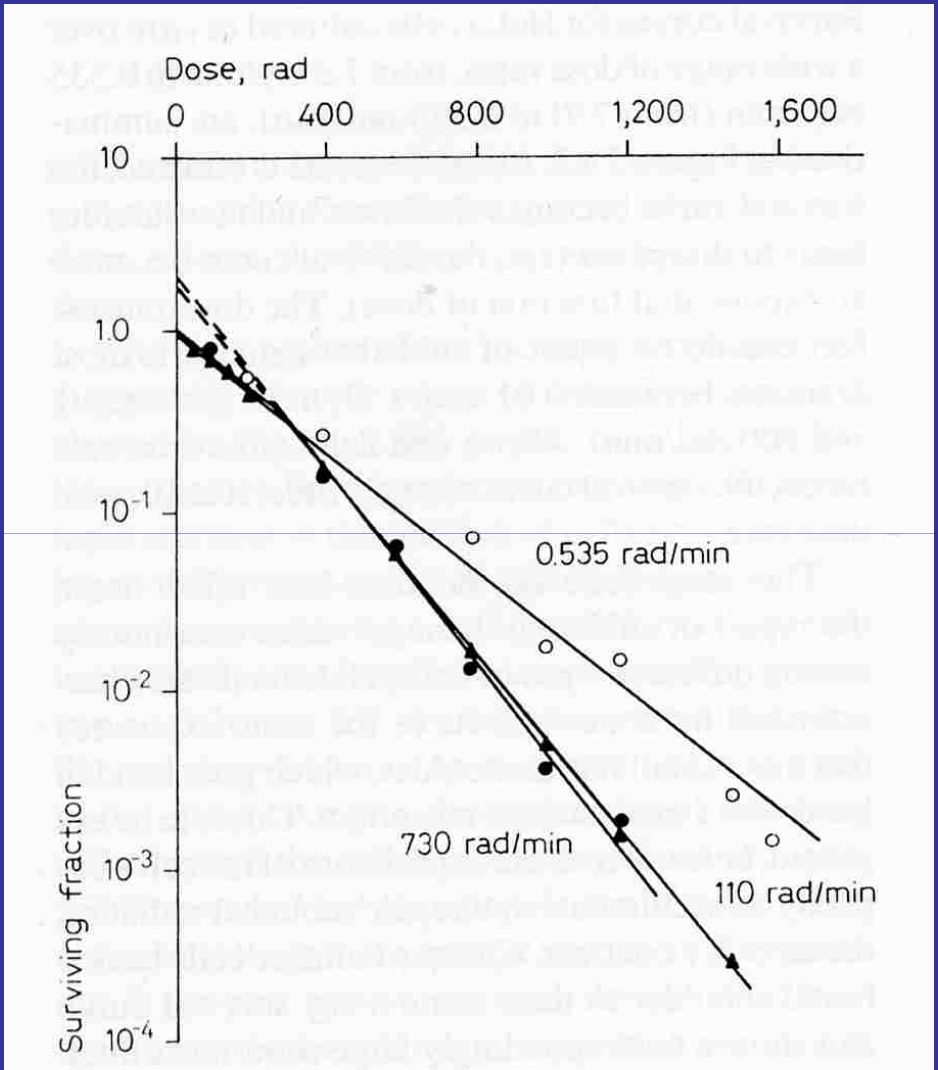


FIGURE 5.12 Survival curves for HeLa cells cultured *in vitro* and exposed to γ-rays at high and low dose rates.

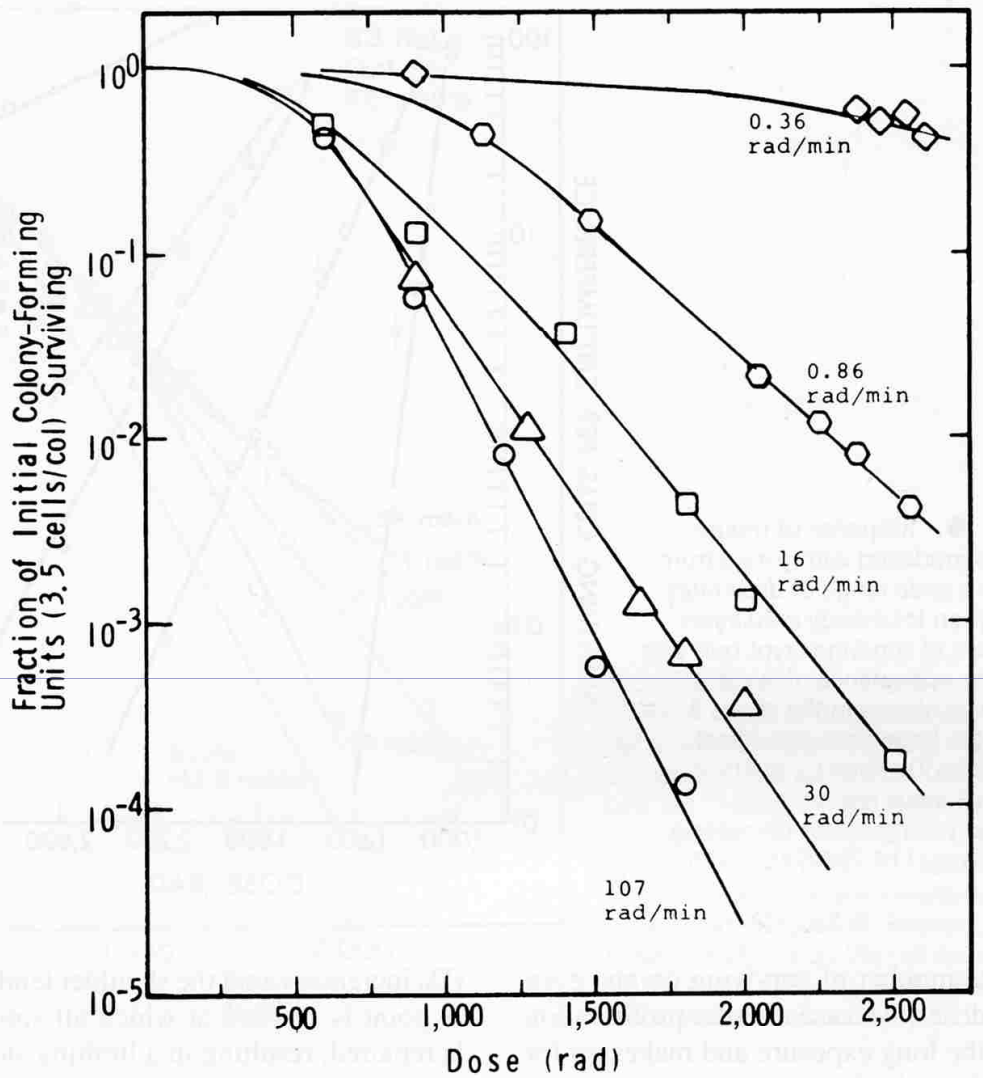


FIGURE 5.13 ● Dose–response curves for Chinese hamster cells (CHL-F line) grown *in vitro* and exposed to cobalt-60 γ-rays at various dose rates. At high doses, a substantial dose-rate effect is evident even when comparing dose rates of 1.07, 0.30, and 0.16 Gy/min (107, 30, and 16 rad/min). The decrease in cell killing becomes even more dramatic as the dose rate is reduced further. (From Bedford JS, Mitchell JB: Dose-rate effects in synchronous mammalian cells in culture. *Radiat Res* 54:316–327, 1973, with permission.)

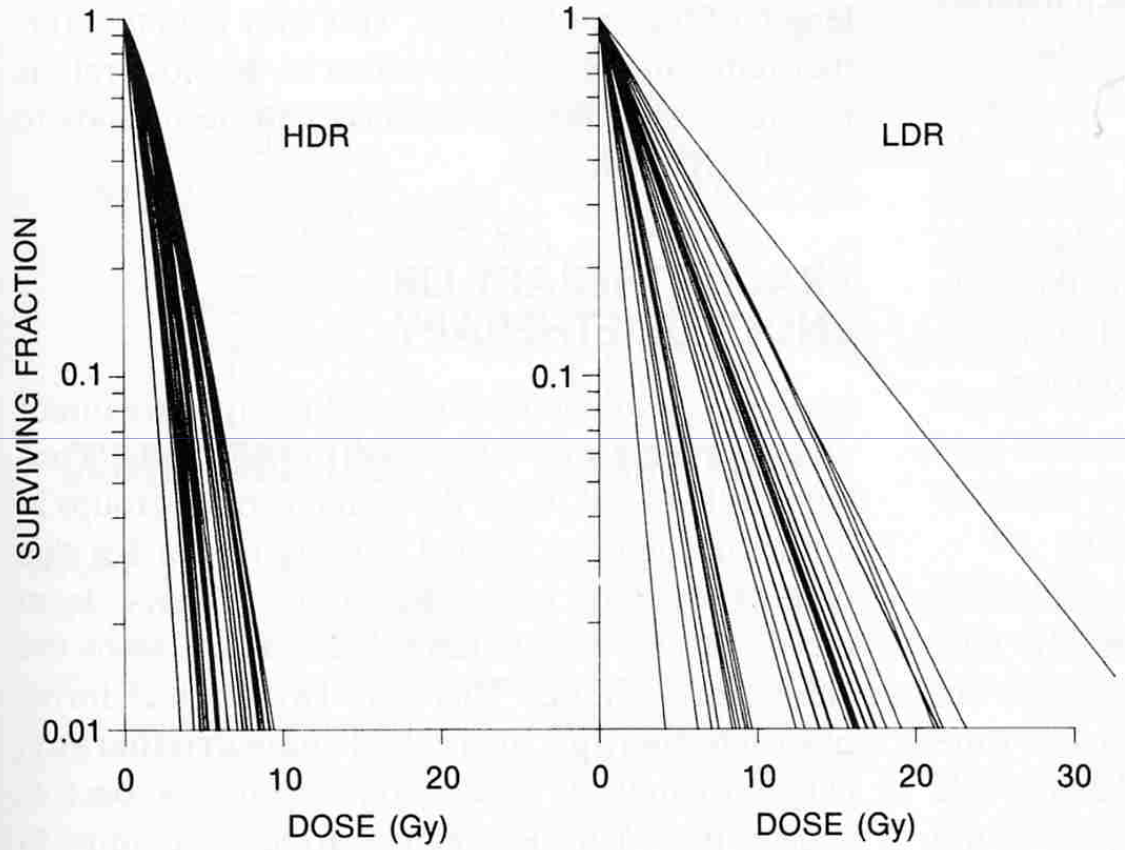


FIGURE 5.14 ● Dose–survival curves at high dose rates (HDR) and low dose rates (LDR) for a large number of cell lines of human origin cultured *in vitro*. Note that the survival curves fan out at low dose rates because in addition to a range of inherent radiosensitivities (evident at HDR), there is also a range of repair times of sublethal damage.

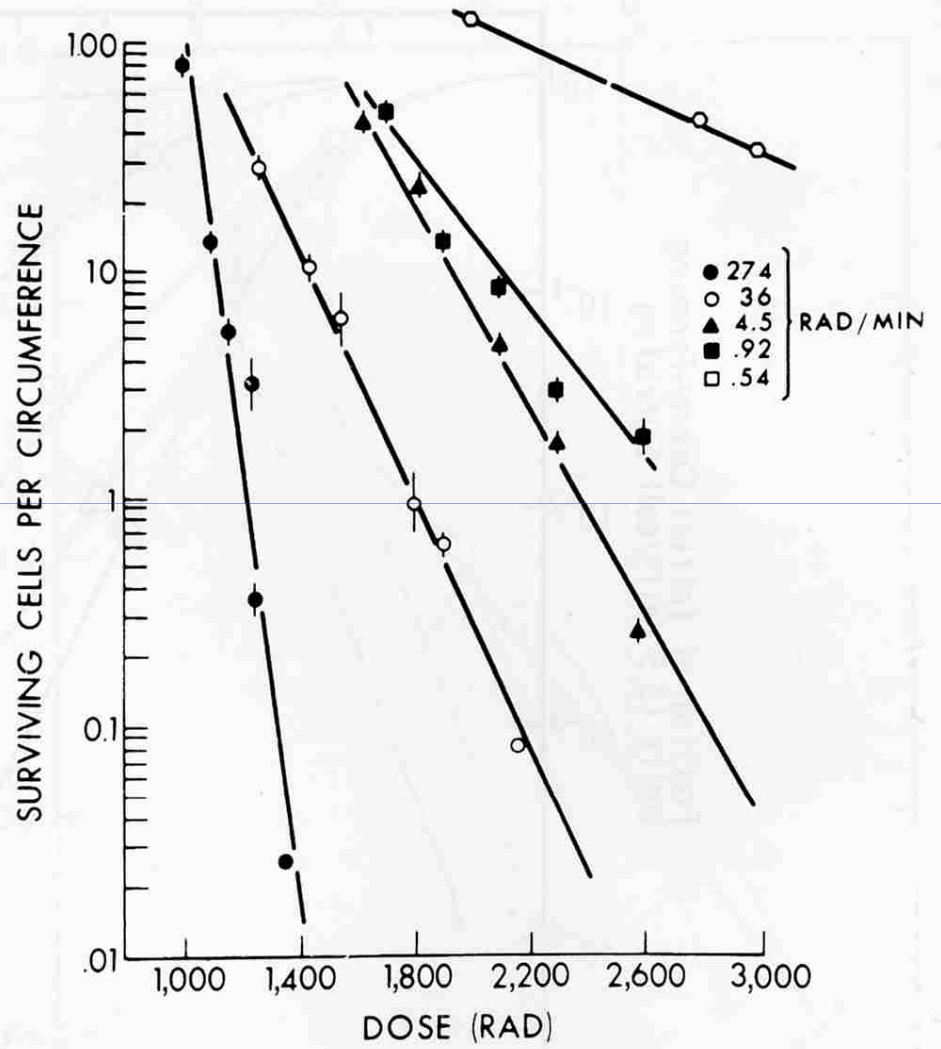
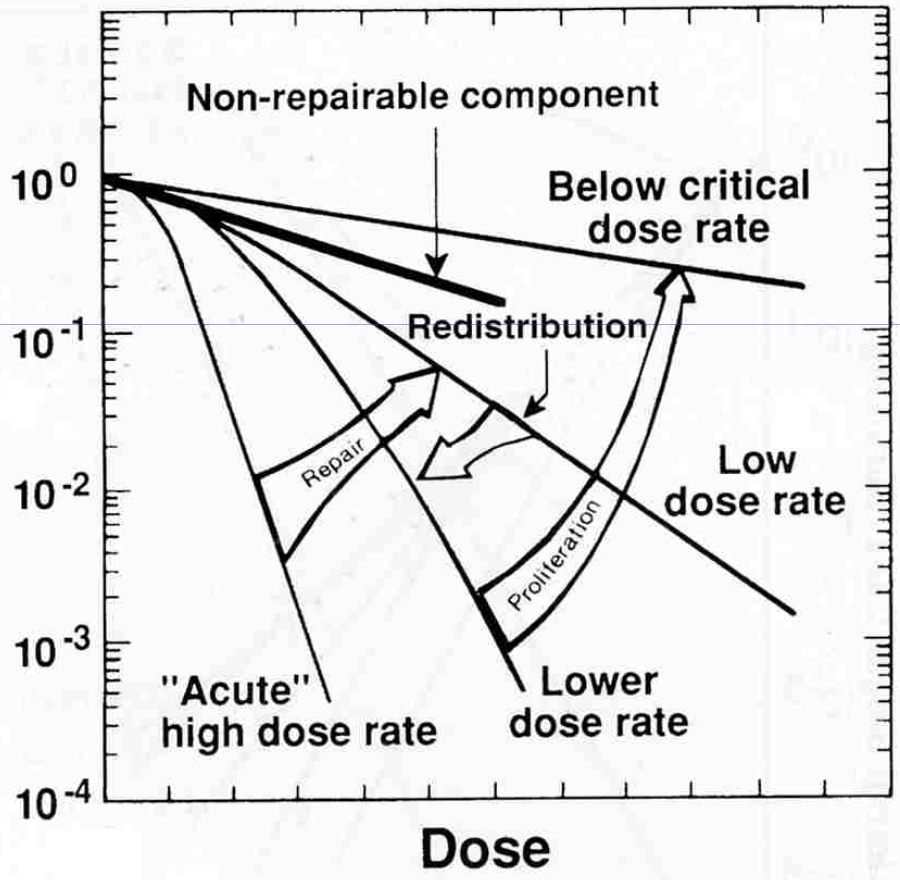


FIGURE 5.15 ● Response of mouse jejunal crypt cells irradiated with γ-rays from cesium-137 over a wide range of dose rates. The mice were given total-body irradiation, and the proportion of surviving crypt cells was determined by the appearance of regenerating microcolonies in the crypts 3 days later. Note the large dose-rate effect. (From Fu KK, Phillips TL, Kane LJ, Smith V: Tumor and normal tissue response to irradiation *in vivo*: variation with decreasing dose rates. *Radiology* 114:709–716, 1975, with permission.)

A sejtek túlélését befolyásoló tényezők

FIGURE 5.18 ● The dose-rate effect resulting from repair of sublethal damage, redistribution in the cycle, and cell proliferation. The dose–response curve for acute exposures is characterized by a broad initial shoulder. As the dose rate is reduced, the survival curve becomes progressively more shallow as more and more sublethal damage is repaired, but cells are "frozen" in their positions in the cycle and do not progress. As the dose rate is lowered further and for a limited range of dose rates, the survival curve steepens again because cells can progress through the cycle to pile up at a block in G_2, a radiosensitive phase, but still cannot divide. A further lowering of dose rate below this critical dose rate allows cells to escape the G_2 block and divide; cell proliferation then may occur during the protracted exposure, and survival curves become shallower as cell birth from mitosis offsets cell killing from the irradiation. (Based on the ideas of Dr. Joel Bedford.)



Összefoglalás

- A kétláncú DNS törések kijavításában az elsődleges a NHEJ, de a HR-nek is szerepe van
- Az NHEJ repair alapvető szerepet játszik az immunrendszer fejlődésében
- A frakcionált sugárterápia alapja a szubletális károsodások kijavítása az egyes frakciók között
- Az alacsony dózisteljesítményű besugárzás lehetővé teszi a károsodások besugárzás alatti kijavítását
- A sugárterápia eredményességét a repair, reasszociáció, repopuláció és a reoxigenáció befolyásolja