



Daños inducidos sobre el DNA

Alteraciones cromosómicas

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BIOLOGICAL EFFECTS OF IONIZING RADIATION

Ionizing radiations have many beneficial applications, but they also have detrimental consequences for human health and for the environment.

Since X-rays were discovered in 1895, it was seen that they could produced detrimental health effects.

To protect people and environment from the detrimental effects of radiation, it is essential to know, as in detail as possible, all the radiation-induced effects.





The original radiograph of Frau Rontgen's hand.

22 December 1895.

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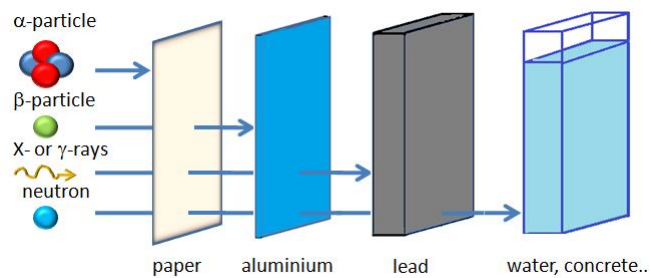
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What is ionizing radiation?

A radiation can be considered as ionizing if the deposited energy is higher enough to ionize the traversed material

- Types
- RI electromagnetic (X and γ - rays),
 - RI corpuscular (α - and β -particles and neutrons)

Each type interacts in its own way with the material



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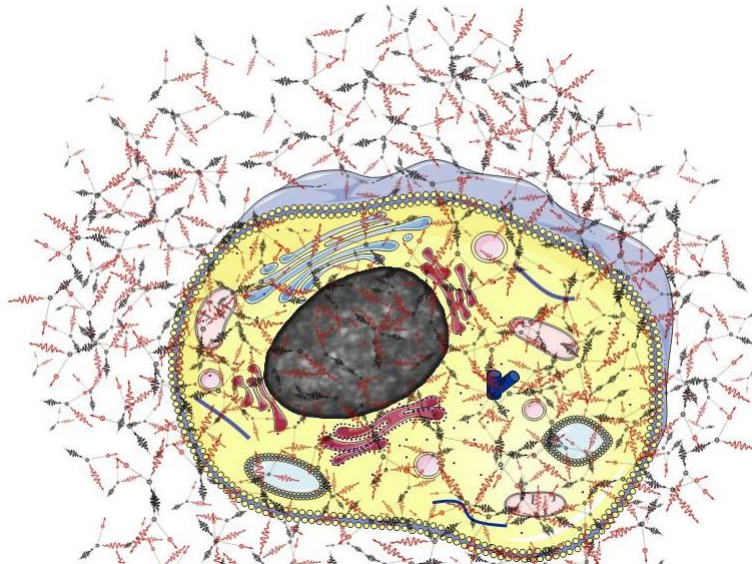
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Quantities used in radiation studies

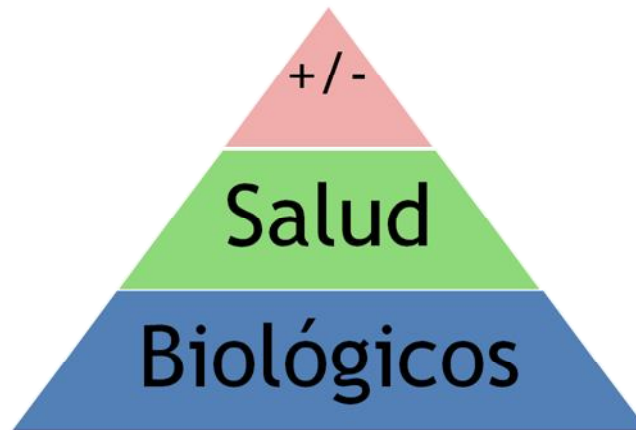
The amount of radiation producing an effect is specified as the energy deposited per unit mass in the irradiated system. This is termed as **absorbed dose (D)**

$$D = \frac{\Delta E}{\Delta m}$$

Where ΔE is the energy absorbed in the mass Δm . In SI is J/kg and is called the Gray. The obsolete rad is sometimes still used and $100 \text{ rad} = 1 \text{ Gy}$



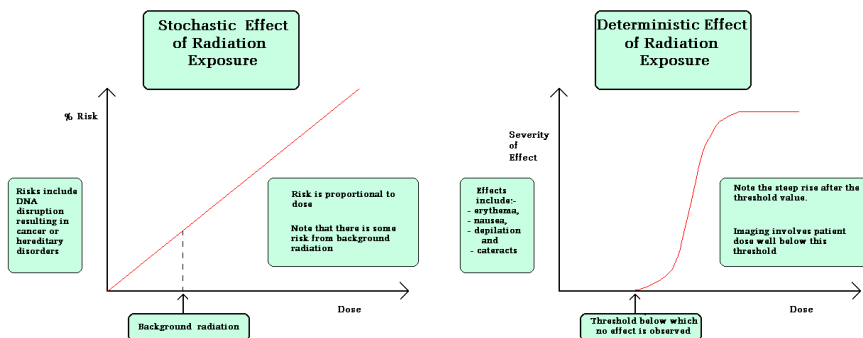
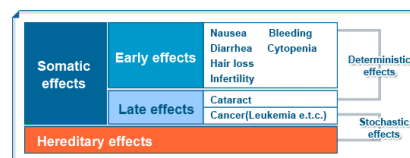
Efectos biológicos de las radiaciones ionizantes



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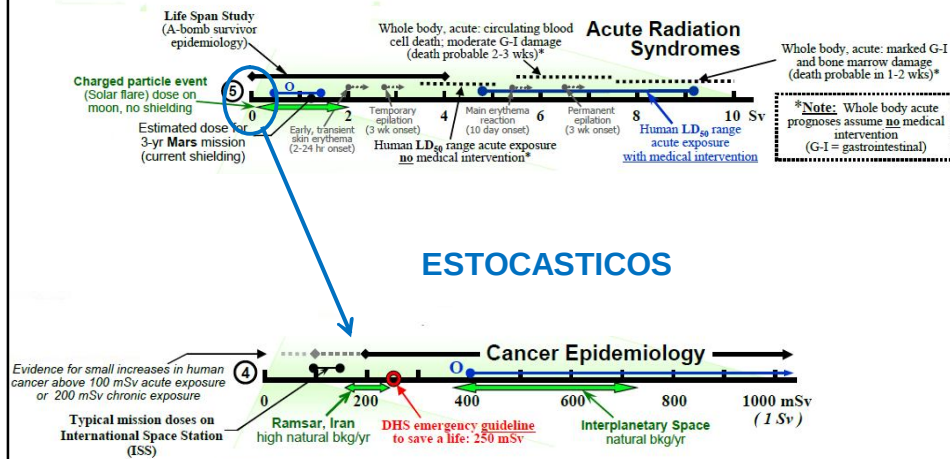
EFFECTOS SOBRE LA SALUD



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DETERMINISTICOS

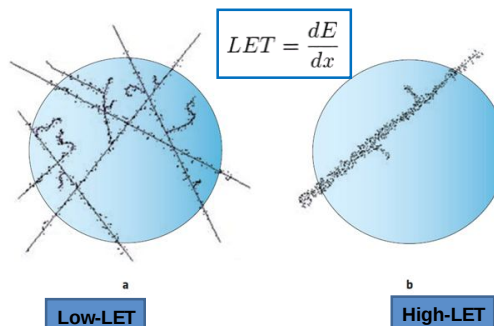


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However, each type deposits the energy in a different way

Linear energy transfer (LET) is a measure of the energy transferred by an ionizing particle to the traversed material. This measure is typically used to quantify the effects of ionizing radiation on biological specimens and is usually expressed in units of keV/μm.



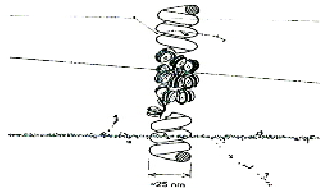
- X and γ-rays are sparsely ionizing radiations
- Energy is distributed homogeneously

- α-, β-particles and neutrons are densely ionizing radiations.
- The energy isn't distributed homogeneously

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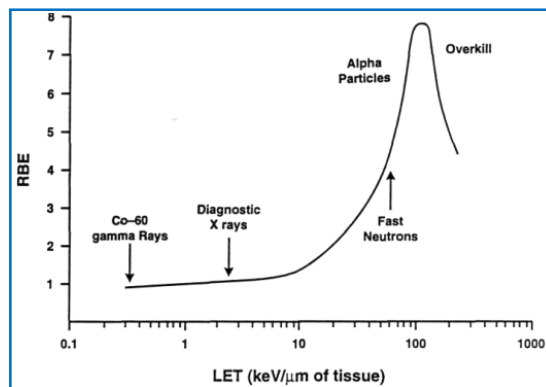
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High LET radiation types are more efficient in producing damage

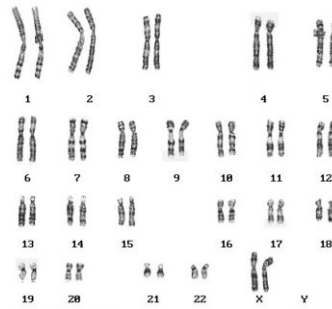
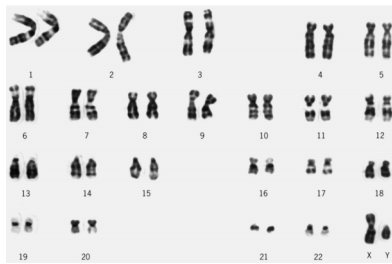


To normalize the Relative Biological effectiveness is used

$$RBE = \frac{\text{Dose of } 150 \text{ V X-rays required to cause effect } x}{\text{Dose of radiation required to cause effect } x}$$

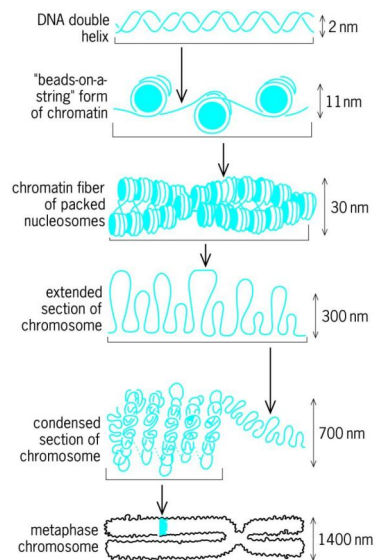
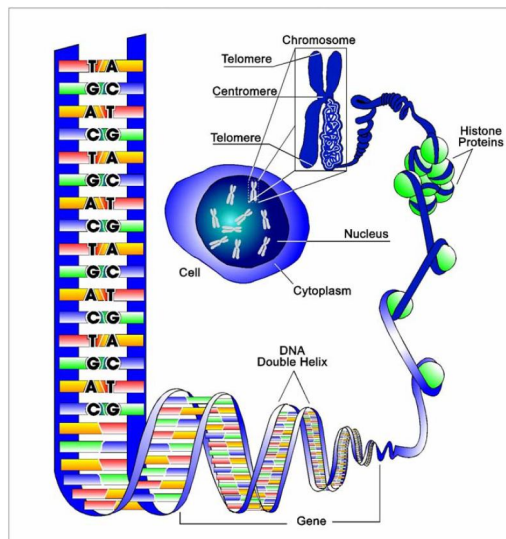


There is a clear relation between RBE and LET



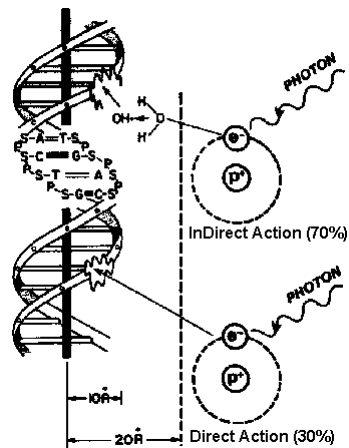
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Incident X-Ray Photon

Fast Electron (e⁻)

Ion Radical

Free Radical

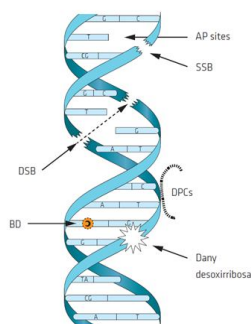
Chemical Changes

Breakage of Bonds

Biological Effects

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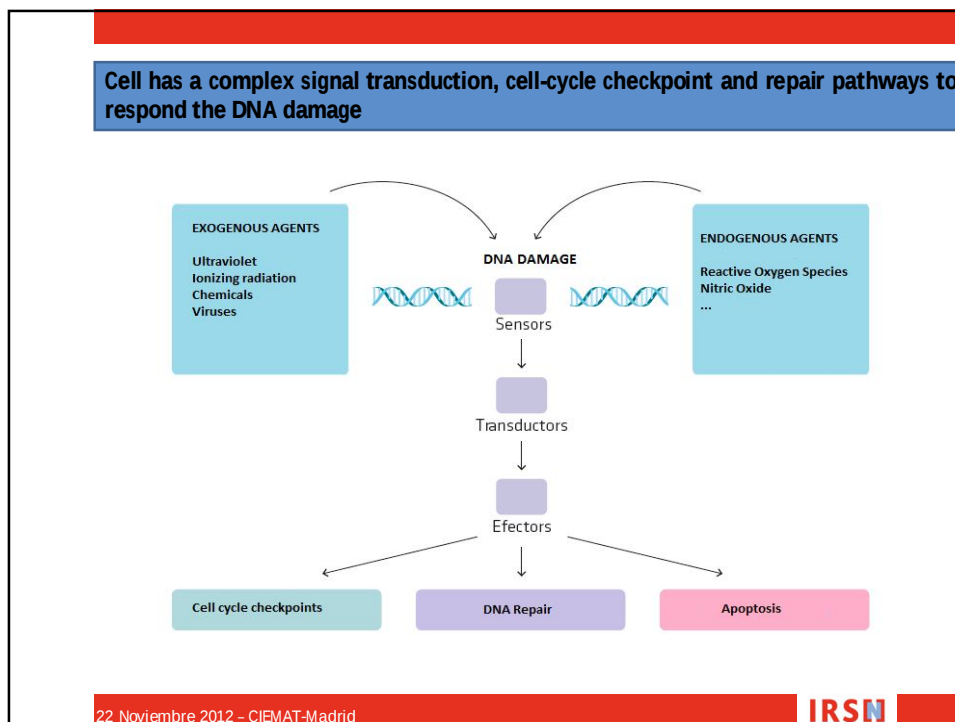
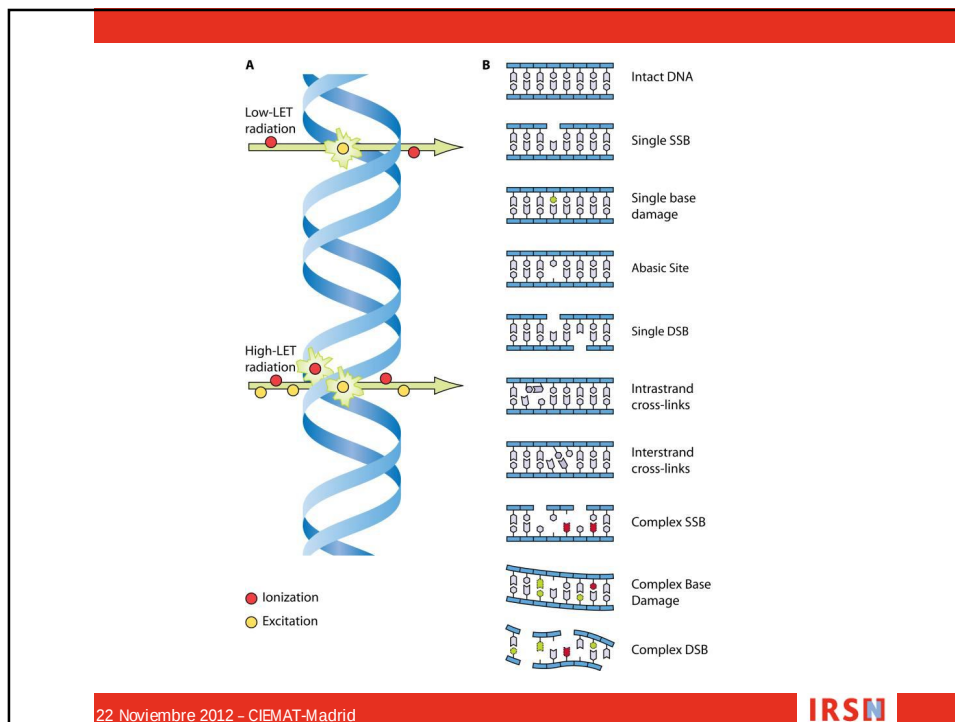
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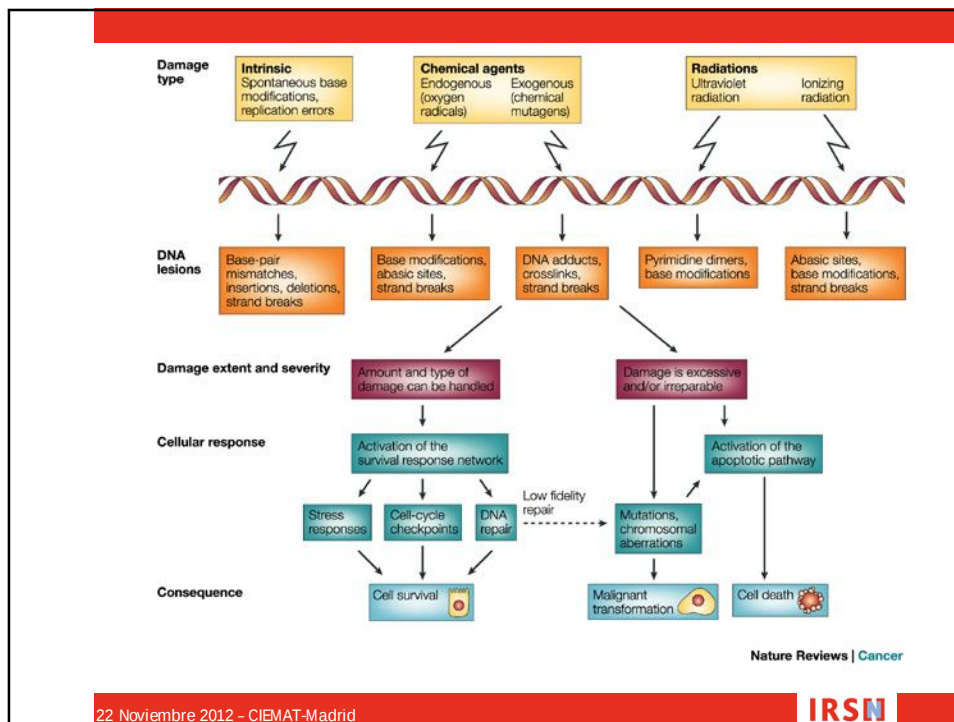


Domage ADN	Lésions spontanées/cellule/jours	Lésions radio-induites/Gy
Cassures simple brin	10 000 à 55 000	1000
Perte de base	12 600	Non évaluée
Domage de base	3 200	2 000
Cassure double brin	8	40
pont ADN/ADN	8	30
pont ADN-proteine	quelques	150
sites multilésés	Non évalué	quelques

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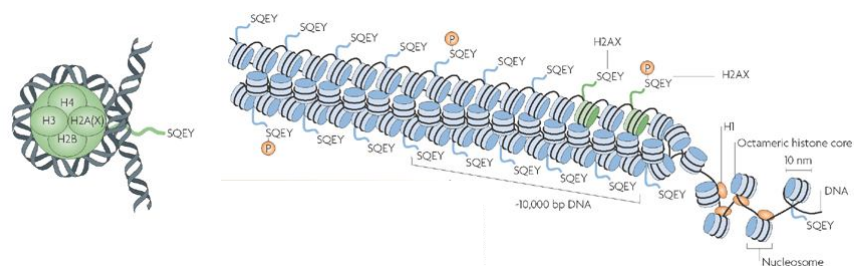




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Histone H2AX

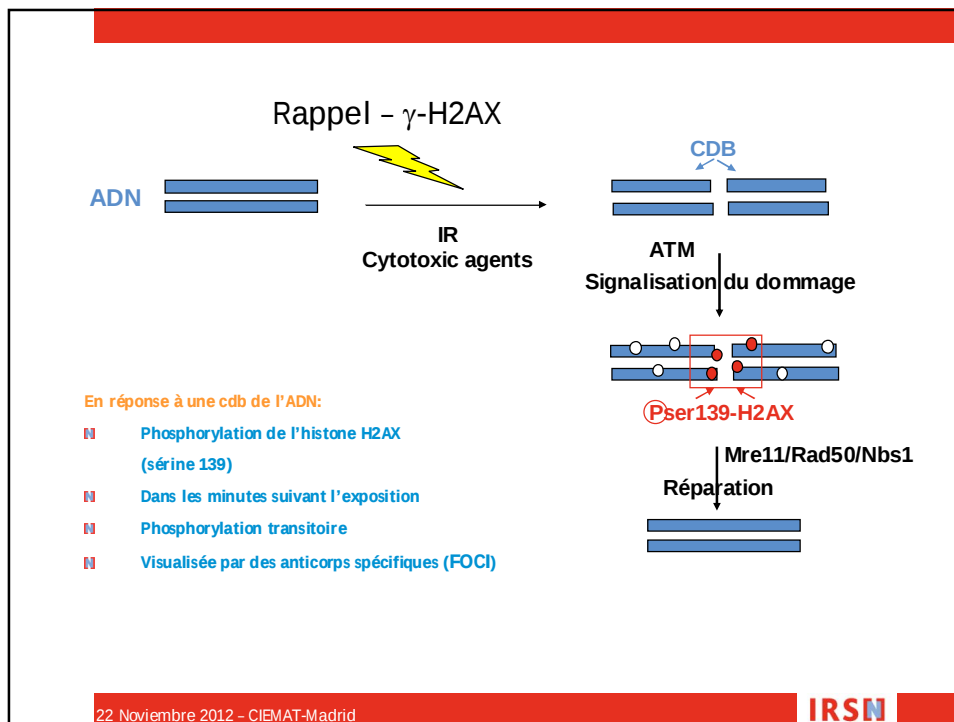


En fonction du type cellulaire et de l'espèce : H2AX représente de 2 à 25% des histone H2A d'un noyau.

Chez les mammifères cela représente en moyenne 1 nucléosome sur 5.

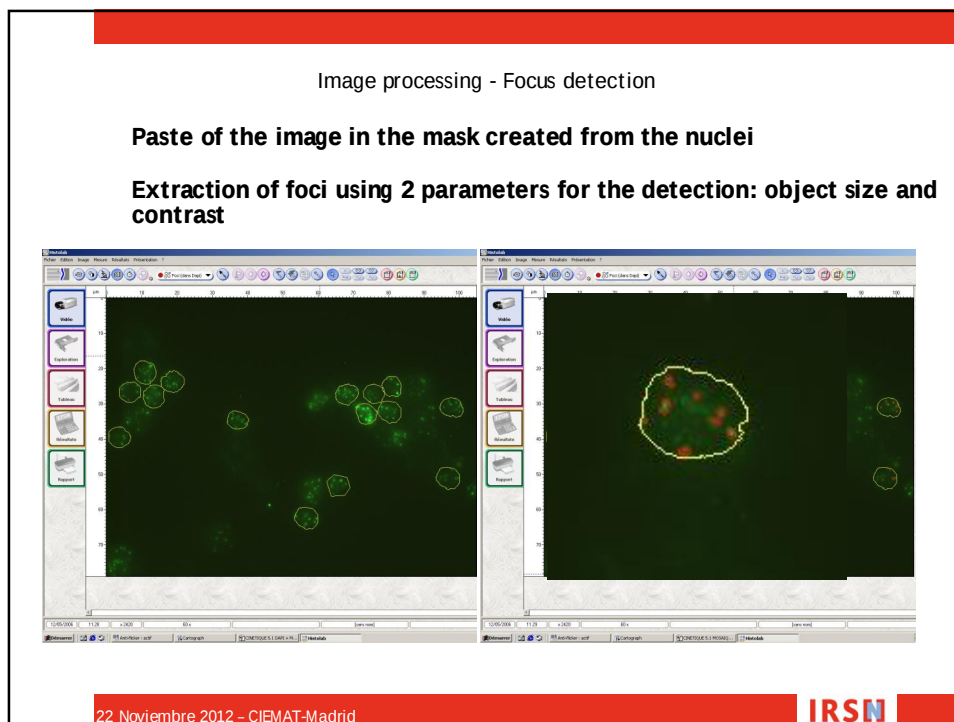
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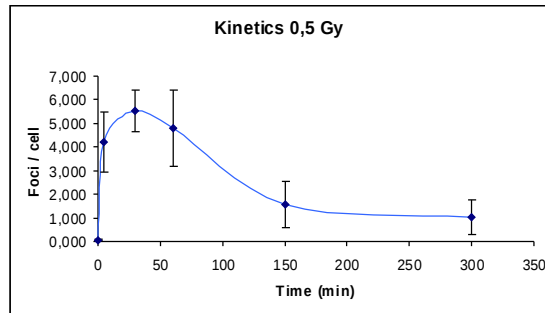
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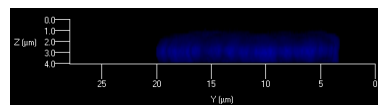
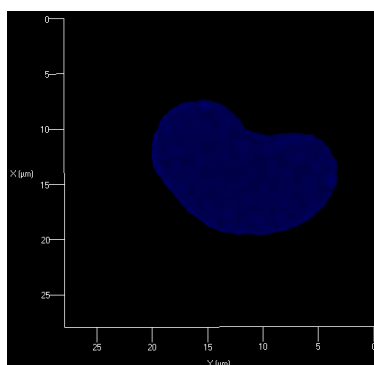
Cinética de γ -H2AX à 0.5 Gy



Hasta los 30 min post- irradiación, el número de foci aumenta,
5 h post-IR, 85 % desaparece,

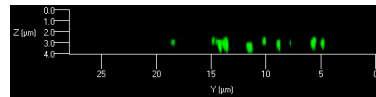
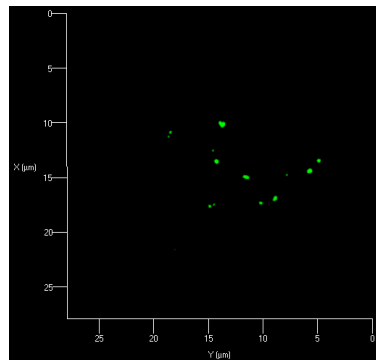
Caractérisation de la méthodologie

- Caractéristiques topologiques de la cible (noyaux)



Caractérisation de la méthodologie

- Caractéristiques topologiques de la cible (noyaux)



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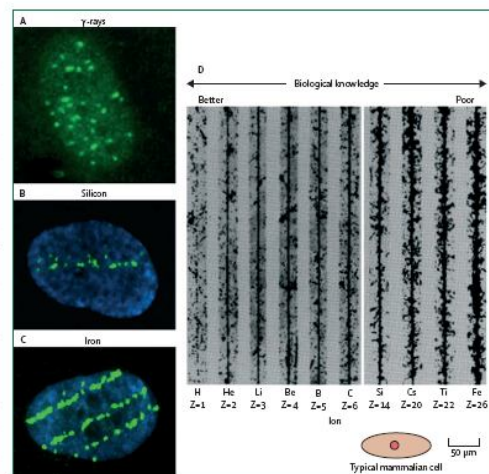


Figure 3: Comparison of particle tracks in nuclear emulsions and human cells
Three nuclei of human fibroblasts exposed to (A) γ -rays (B) silicon ions or (C) iron ions; and immunostained for detection of γ -H2AX. * Every green focus corresponds to a DNA double-strand break. In the cell exposed to sparsely ionising γ -rays (A), H2AX foci are uniformly distributed in the nucleus. Cells exposed to HZE particles show DNA damage along tracks—one silicon (B) and three iron (C) particles, respectively. Spacing between DNA double-strand breaks is reduced at very high-LET. (B) Tracks of different ions, from protons to iron, in nuclear emulsions, show increasing ionisation density ($LET \sim \Delta E/\Delta x$) as charge, Z, increases. Biological knowledge increases with increasing atomic number.

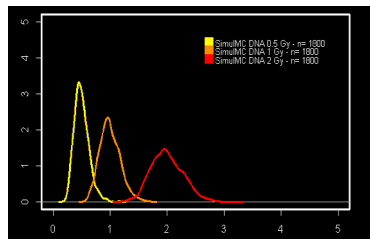
Punto de encuentro física-biología

Cancer risk from exposure to galactic cosmic rays: implications for space exploration by human beings Francis A Cucinotta, Marco Durante, 2006

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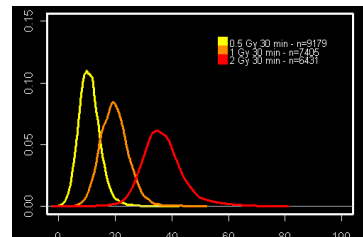
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- Distribution de la dose à l'échelle de la molécule d'ADN



Simulations Monte Carlo de la dose absorbée aux molécules d'ADN

- Variabilité observable au niveau des dommages à l'ADN



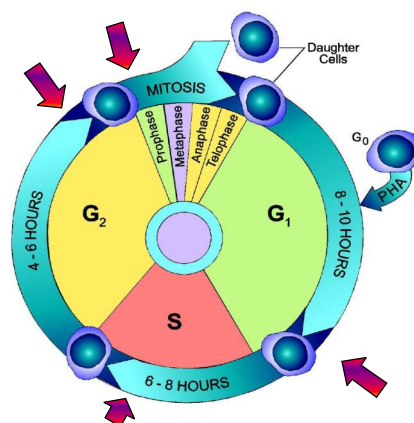
Observations de foci γ H2AX

Travaux Gruel, Villagrasa et al.

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BD, AS, and SSB are repaired by different processes like excision repair (BER), nucleotide excision repair (NER), and single strand break repair (SSBR). DPCs are repaired by NER and Homologous Recombination repair (HR)



The cell cycle and the checkpoints

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DSBs are critical DNA lesions. Their misrepair or unrepair is directly related to the origin of chromosome damage (i.e chromosomal aberrations like dicentrics).

DSBs are life-threatening lesions whose repair is promoted by an intricate network of multiple DNA repair pathways.

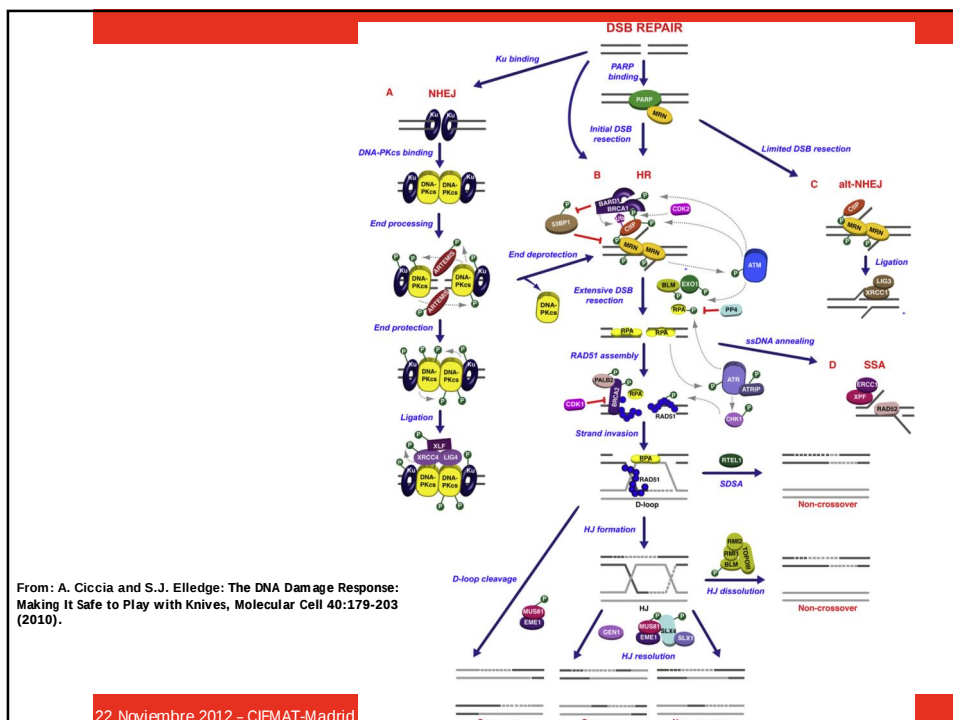
At least four independent pathways can repair DSBs:

Homologous recombination (HR)
Non-Homologous End Joining (NHEJ)
Alternative-NHEJ (alt-NHEJ)
single-strand annealing (SSA)

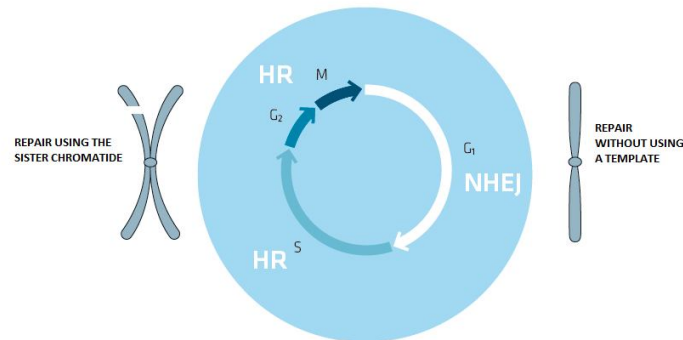
A main factor influencing the pathway choice is the extent of DNA end processing. Classical NHEJ does not require DNA end resection whereas alt-NHEJ (also known as microhomology-mediated end joining or MMEJ), HR, and SSA are dependent on DSB resection, which is limited for alt-NHEJ (5-25 nt) and more extensive for HR and SSA.

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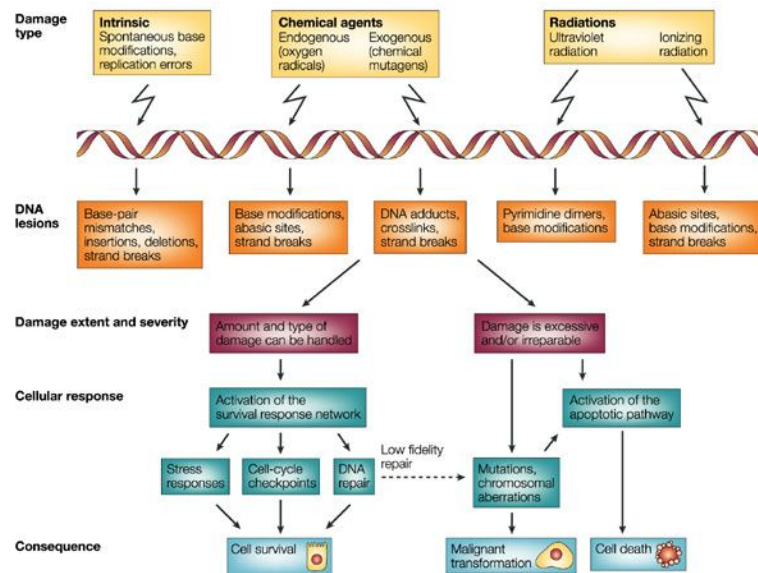
There are two main mechanisms to repair DSB: Homologous recombination repair (HRR), and the DNA non-homologous end-joining (NHEJ)



The two mechanisms operate in different phases of the cell cycle. The NHEJ occurs mainly in the quiescent G₀ phase and during the cell cycle in G₁, but can also give the remaining phases, the HR can occur only when the DNA is replicated, in S and G₂ phase, after the repair system crashes

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CHROMOSOME ABERRATIONS INDUCED BY X-RAYS

KARL SAX

Arnold Arboretum, Harvard University, Jamaica Plain, Massachusetts

Received June 3, 1938

INTRODUCTION

SINCE the discovery by MULLER and STADLER that X-rays induce mutations in animals and plants, a new field has been developed in experimental genetics. This work on radiation genetics has been reviewed by MULLER (1932), HANSON (1933), OLIVER (1934), STADLER, GOOD-SPEED, GOWEN, et al. (DUGGAR 1936), STUBBE (1937), and TIMOFEEFF-RESSOVSKY (1937). The genetic results show that (1) the mutation rate increases directly with dosage, (2) the X-ray effect is not delayed or indirect, (3) there is no temperature coefficient, (4) differential susceptibility is found in different stages of development, (5) the X-rays cause translocations, inversions, and deletion of chromosome segments, (6) the induced mutations are not distributed entirely at random in the chromosomes, (7) there is no differential effect of the various wave lengths in the X-ray range, and (8) the gene string is already partially split in *Drosophila* sperm and in *Zea* pollen grains.

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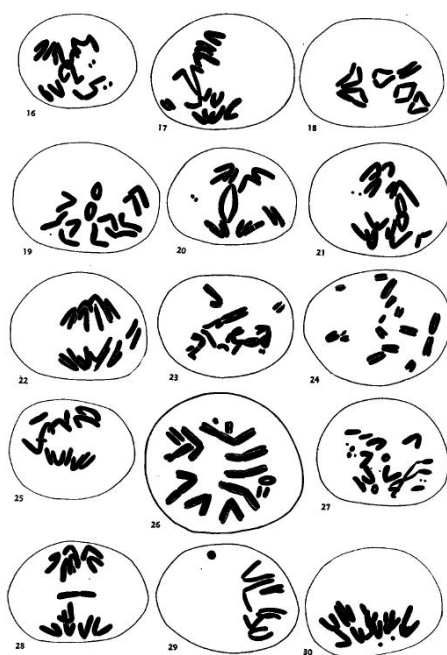


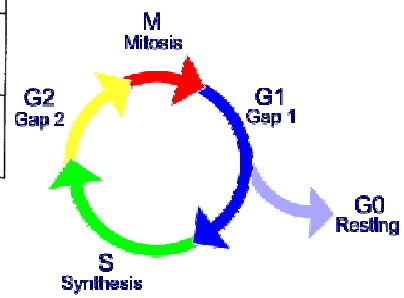
PLATE 2



Figure 2. Chromosome aberrations: a) aneuploid cell with chromosome fragments (arrowed); b) aneuploid cell showing dicentric chromosomes (arrowed); c) root tip cell with two nuclei.

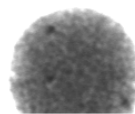
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		Chromosome aberrations			Chromatid aberrations		
		Resting	Pre-metaphase	Anaphase	Prophase	Pre-metaphase	Anaphase
One Hit	Hit 1						
	Hit 2						
	Hit 3						
Two Hit	Hit 1						
	Hit 2						
	Hit 3						



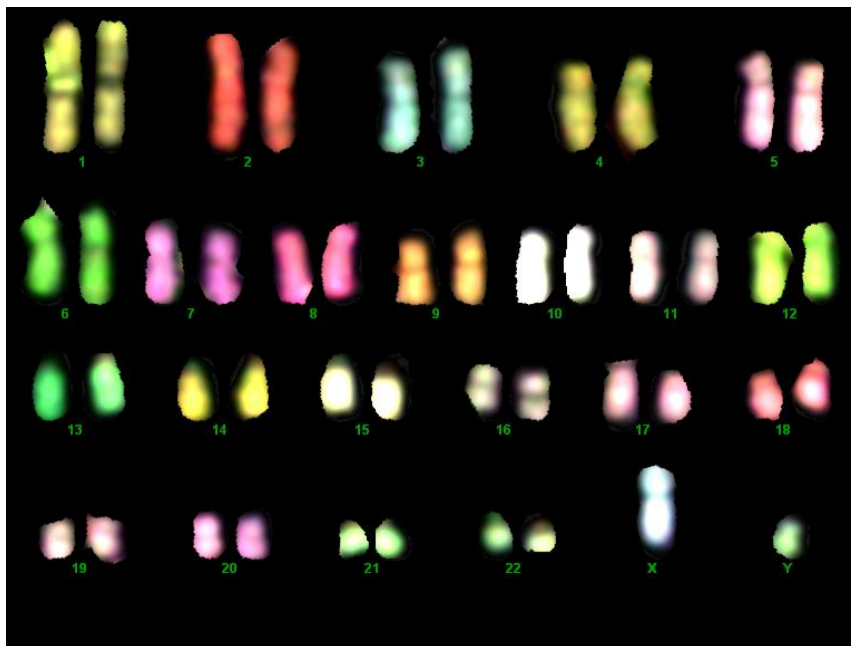
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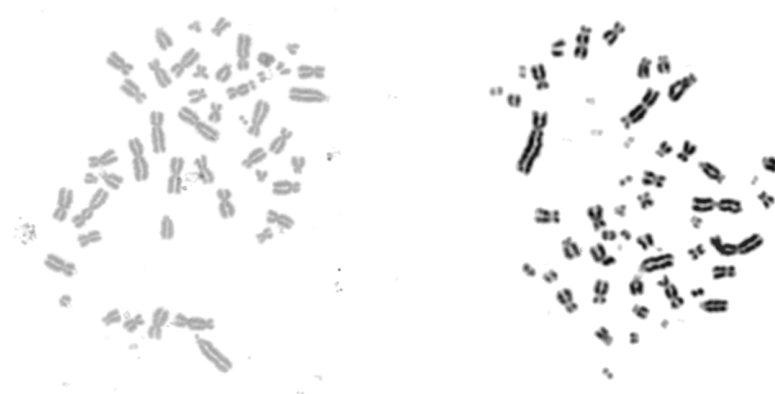


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Las radiaciones de baja *LET* inducen predominantemente daños de base y *SSB*. La proporción de *DSB* respecto *SSB* es de 1:10-20.

Las radiaciones de elevada *LET*, en cambio, inducen una proporción más alta de *DSB* y generalmente provocan un daño mayor que las de baja *LET*.



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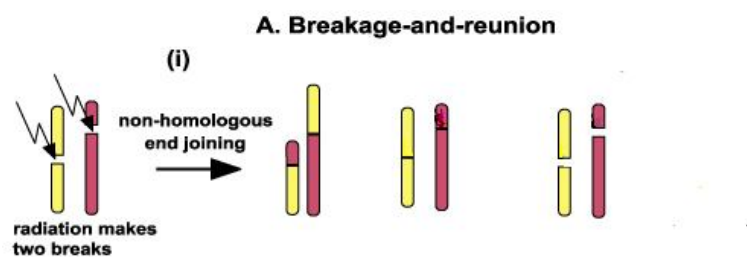
La reparación errónea de las lesiones se puede observar en metafase en forma de **alteraciones cromosómicas**.

Modelos principales de formación de alteraciones cromosómicas inducidas por las RI:

- **Modelo de la rotura y reunión (*breakage-and-reunion*)**, Sax, 1938; Lea y Catcheside, 1942
- **Teoría de intercambio** (Revell 1955)
- **Modelo *one-hit*** o de **recombinación de homólogos** (Chadwick i Leenhouts, 1978)

Modelo de la rotura y reunión

Las RI inducirían la rotura de dos cromosomas dejando los extremos abiertos

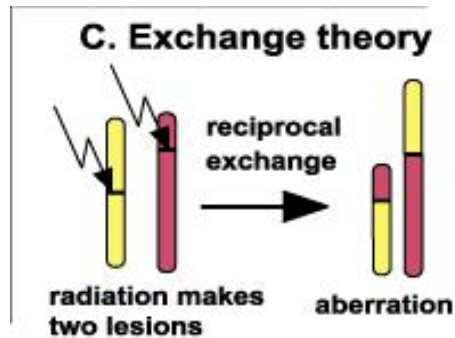


adaptado de: L. Hatky et al., BioEssays 24, 714-723, 2002.

- Reconstruir los cromosomas originales.
- Si las dos roturas son cercanas en el espacio y tiempo, los extremos rotos podrían interaccionar entre ellos, reorganizándose de manera ilegítima y formando alteraciones tipo intercambio.
- Una fracción de las rotura primarias no se reunirían y aparecerían en la metafase como a deleciones terminales.

Teoría de intercambio

El primer efecto de la radiación no es una rotura del cromosoma sino una lesión inestable, que si se repara no da lugar a alteraciones.

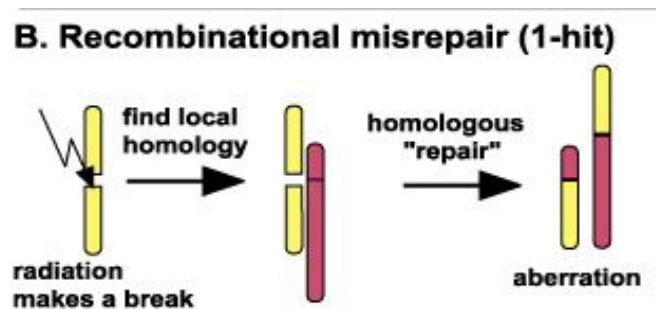


adaptado de: L. Hlatky et al., BioEssays 24, 714-723, 2002.

Si dos lesiones inestables coinciden en el tiempo y el espacio, podrían formar una alteración tipo intercambio. Es necesario un proceso de contacto entre las dos cromátides (bucles de cromátide o bucles de Revell).

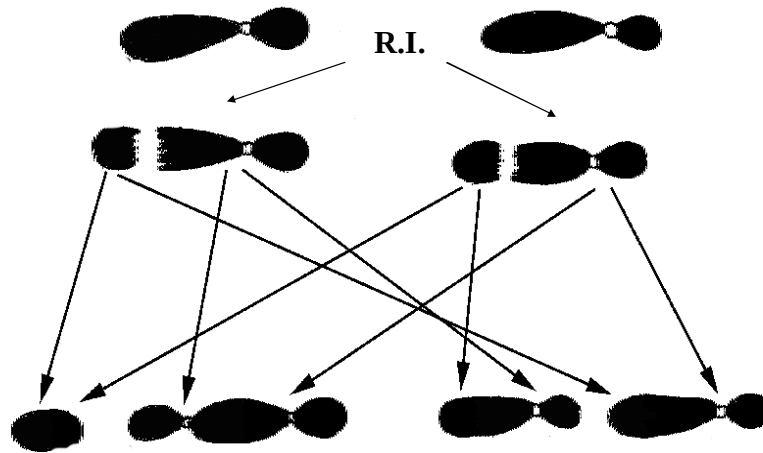
Modelo *one-hit* (recombinación de homólogos)

Las deleciones y los intercambios se originan a partir de una sola rotura de doble cadena y los mecanismos de reparación inducirían una segunda rotura en una cadena no dañada con regiones de homología con la primera.



adaptado de: L. Hlatky et al., BioEssays 24, 714-723, 2002.

Actualmente la teoría clásica de **rotura y reunión** es la más aceptada.



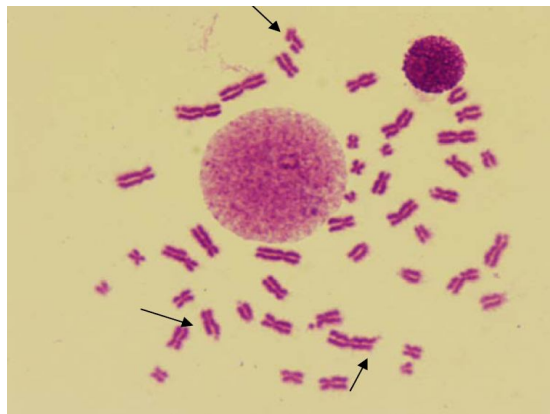
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TIPOS DE ALTERACIONES CROMOSÓMICAS INDUCIDAS

•**Tipo cromatídico** (afectan una sola cromátida del cromosoma)



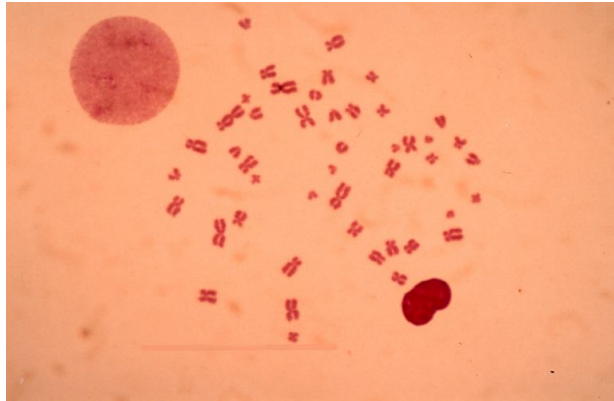
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TIPOS DE ALTERACIONES CROMOSÓMICAS INDUCIDAS

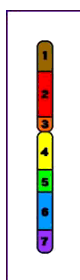
- **Tipo cromosoma** (afectan a las dos cromátidas).



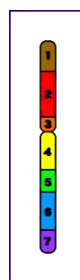
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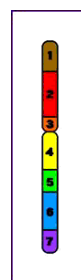
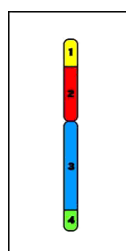
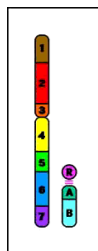
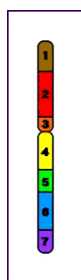
TIPOS DE ALTERACIONES CROMOSÓMICAS INDUCIDAS



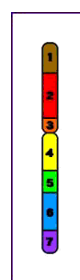
terminales



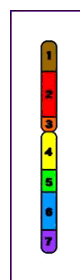
Intersticiales



Pericéntrica



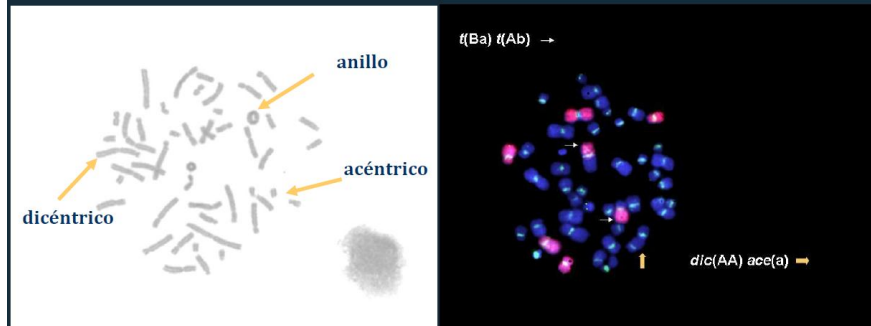
Paracéntrica



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- **Técnica tinción uniforme:** permite identificar **dicéntricos**, **anillos** y **acéntricos**
- **Técnicas de hibridación *in situ* fluorescente (FISH)**
 - sondas cromosomas enteros y sonda pancentromérica → detección inequívoca de **translocaciones** y de **dicéntricos**



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Dosimetría biológica: estimación de la dosis de una exposición a RI mediante parámetros biológicos

Recuento de **alteraciones cromosómicas** en linfocitos de sangre periférica

➤Elaboración curvas de calibración dosis – efecto

Radiaciones de baja LET

$$Y = C + \alpha D + \beta D^2$$

$\alpha D \rightarrow$ proporción de alteraciones inducidas por un único haz de RI

$\beta D^2 \rightarrow$ alteraciones inducidos por dos haces de RI diferentes

Radiaciones de alta LET

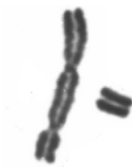
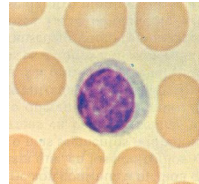
$$Y = C + \alpha D$$

Y = número de alteraciones cromosómicas por célula
C = frecuencia basal de la alteración
D = dosis
 α y β = coeficientes lineal y cuadrático obtenidos del análisis estadístico

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DOSIMETRÍA BIOLÓGICA

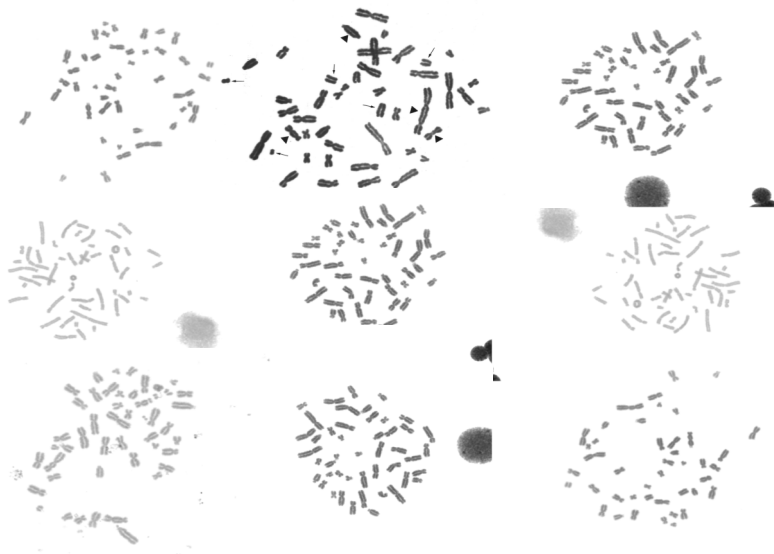
El método más ampliamente aceptado es el análisis de las alteraciones cromosómicas, particularmente los cromosomas dicéntricos, presentes en linfocitos de sangre periférica.



Buena relación dosis-respuesta

Baja frecuencia basal (1-2 por 1000)

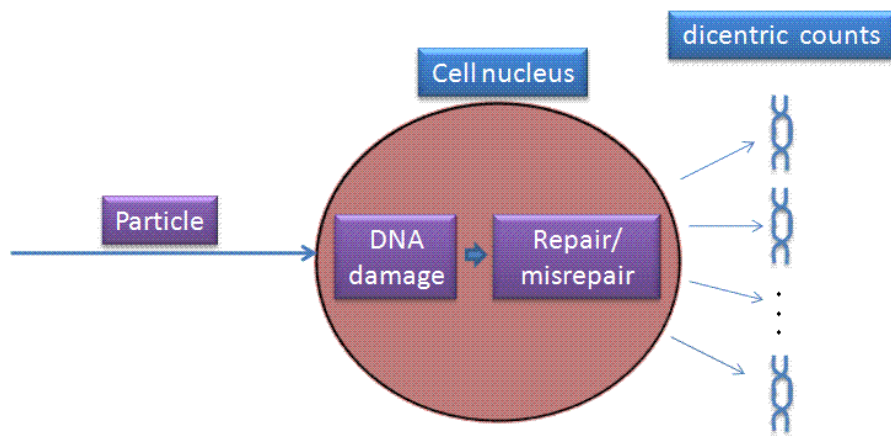
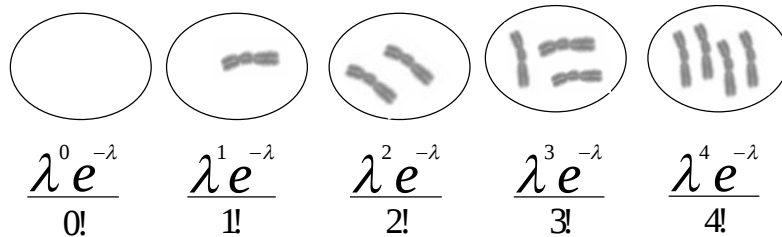
Alteración cromosómica bastante específica de RI



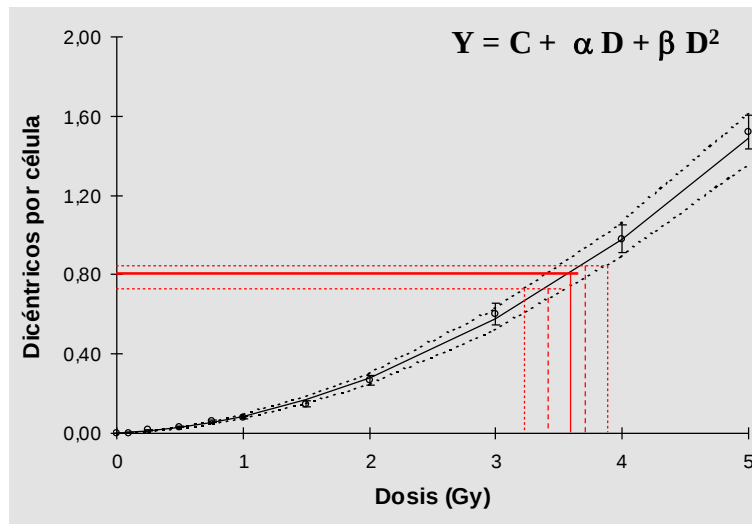
For dicentric, irradiation with X or gamma rays produces a distribution of damage which is very well represented by the **Poisson distribution**.

If the expected number of occurrences in this interval is λ , then the probability that there are exactly n occurrences (n being a non-negative integer, $n = 0, 1, 2, \dots$) is equal to:

$$f(n; \lambda) = \frac{\lambda^n e^{-\lambda}}{n!}$$



ANÁLISIS CUANTITATIVOS DEL EFECTO DE RI



53

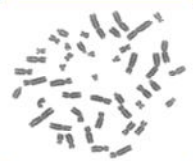
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A key element for the evaluation of the risks associated to an exposure to ionizing radiation is the precise determination of the received dose

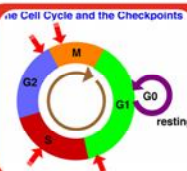
Biological dosimetry is of great interest in radioprotection

The frequency of dicentrics present in peripheral blood lymphocytes is considered as "gold standard" method



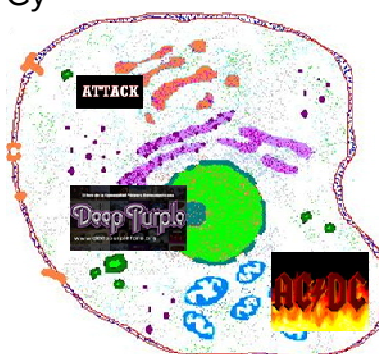
The analysis of radio-induced dicentrics is made in first division mitotic cells.

- blood lymphocytes remain in the non-proliferative phase G0, and it is necessary to stimulate their division using a mitogen like phytohaemagglutinin
- The standard method is to culture the stimulated cells during 48 hours and to arrest mitotic cells adding Colcemid to inhibit the formation of the spindle-apparatus



After cell irradiation, and before reaching metaphase, cells progress through the cell-cycle-checkpoints where the progression can be arrested in response to the damage level

Heavily damaged cells are not able to reach mitosis, and the use of dicentrics as a biological indicator is restricted to doses up to 4-5 Gy

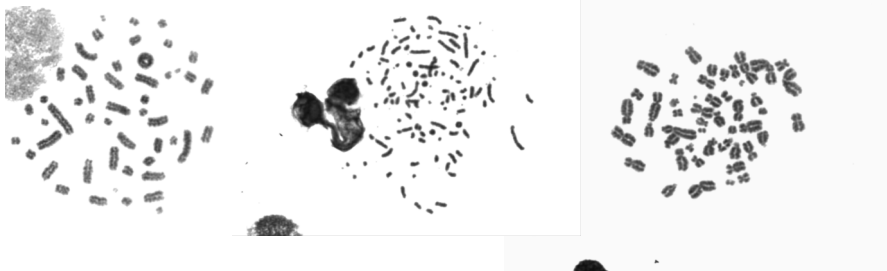


For higher doses cells can show a greater difficulty or impossibility to reach mitosis, and it is very difficult to obtain metaphase spreads from lymphocytes irradiated at 10Gy

Different techniques which condensates prematurely the chromosomes during interphase are being accepted to be useful for dose-assessment after irradiations to very high doses.

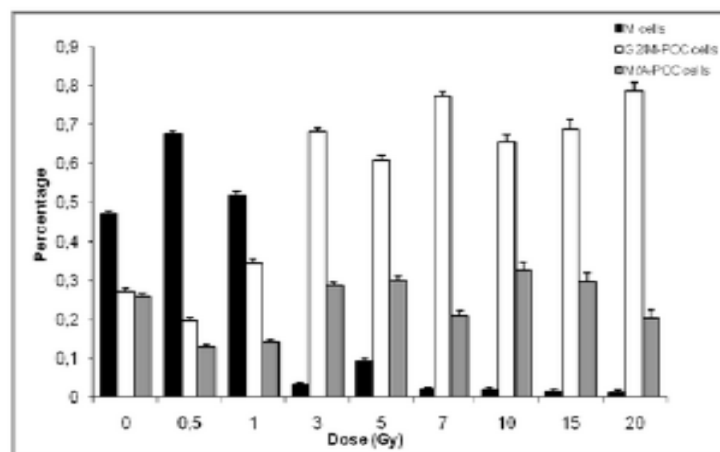
Lately the chemically induced premature condensation of chromosomes (PCC) by Calyculin-A or Okadaic Acid has been proposed as a method for dose assessment after expositions to high doses.

The advantage of the chemically induced PCC is that heavily damaged cells that will not pass through the G2/M checkpoint can be analyzed in G2 phase.



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Percentages of the different cell morphologies observed at each dose. Black bars represent M cells, white bars G2/M-PCC cells and grey bars M/A-PCC cells. The error bars indicate the standard error of the mean.

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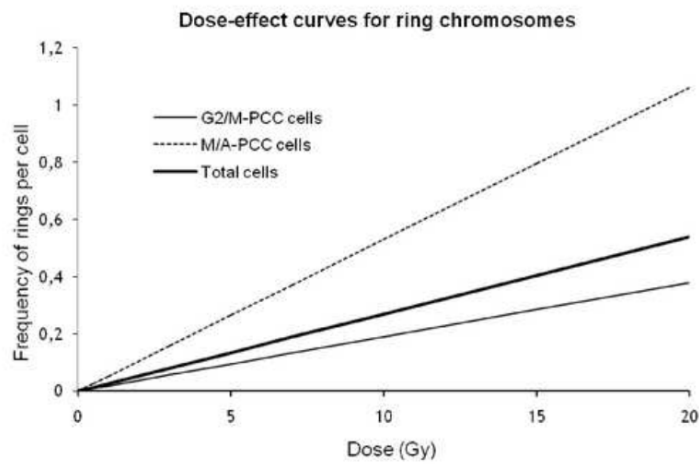


Fig. 3. Linear relationships for chromosome rings. The values ($\pm$ SE) of the α coefficients are: 0.019($\pm$ 0.002) for G2/M-PCC cells, 0.054($\pm$ 0.005) for M/A-PCC cells and 0.027($\pm$ 0.003) for total cells

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THE USE OF CAFFEINE TO ASSESS HIGH DOSE EXPOSURES TO IONISING RADIATION BY DICENTRIC ANALYSIS



Caffeine has important effects on cell cycle checkpoints inhibiting the catalytic activity of ATM and its related kinase (ATR)

While the presence of caffeine reverses S and G2/M checkpoint response to IR damage, it has a very little effect on G1 checkpoint, and only an ATM- and p53-independent G1 delay has been described

Treatments with caffeine during the late G2 phase has been lately used to visualize at metaphase the chromosome damage induced

Here it is presented some studies to evaluate the suitability of using caffeine for biological dosimetry purposes

1- G0 lymphocytes irradiated at doses from 1 to 15 Gy were cultured to obtain metaphase spreads in two different ways:

- harvesting after a conventional treatment with Colcemid
- harvesting after a co-treatment with Colcemid and caffeine.

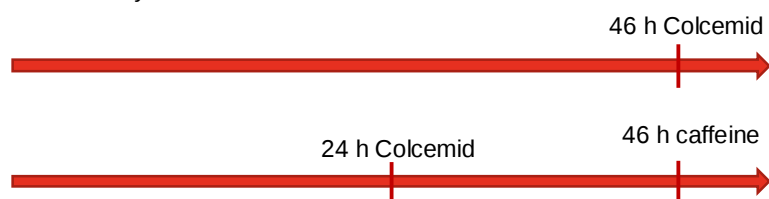
The main purpose is to evaluate if the mitotic index increases and to check if the co-treatment with caffeine can be a potential method for dose assessment after exposures to very high doses.

Peripheral blood samples were obtained from a 45 years old healthy male with no history of exposure to IR or clastogenic agents,

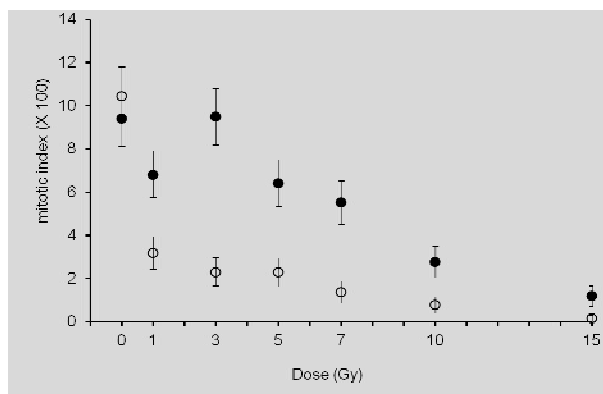
Samples were irradiated at 0, 1, 3, 7 10 and 15 Gy with gamma rays

Two different protocols were applied:

cultures only treated with Colcemid



Heavily damaged cell observed after 15 Gy irradiation. The cell has three dicentrics, two tracentrics, and two tetracentrics, that was considered as a cell carrying 13 dicentrics equivalent. The metaphase also contains one ring and several acentric fragments.



MI only considering stimulated cells.

At 0 Gy no differences were observed when the two treatments were compared

In the irradiated samples, the cultures treated only with Colcemid the MI (100) decreases clearly as dose increases.

Similar results are observed when the MI was calculated considering all cells, unstimulated and stimulated ones.

Table 1. Number of cells and dicentric cell distribution observed.

Dose (Gy)	Treatment	Cells	CA	Dicentric cell distribution																Dic	Y ± SE	DI	u
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
0	Col	1000	3	999	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0.00 ± 0.00	1.00	—
	Col+Caf	683	0	683	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00 ± 0.00	—	—
1	Col	509	102	455	49	4	1	0	0	0	0	0	0	0	0	0	0	0	0	60	0.12 ± 0.02	1.12	1.89
	Col+Caf	500	97	412	76	12	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0.20 ± 0.02	1.04	0.67
3	Col	100	80	31	38	22	9	0	0	0	0	0	0	0	0	0	0	0	0	109	1.09 ± 0.09	0.82	-1.29
	Col+Caf	200	169	37	40	79	29	11	2	2	0	0	0	0	0	0	0	0	0	351	1.76 ± 0.09	0.86	-1.44
5	Col	150	148	3	17	63	34	19	12	1	1	0	0	0	0	0	0	0	0	394	2.63 ± 0.10	0.59	-3.58
	Col+Caf	137	136	0	26	49	34	21	6	1	0	0	0	0	0	0	0	0	0	346	2.53 ± 0.10	0.51	-4.02
7	Col	50	50	0	6	10	15	10	5	2	2	0	0	0	0	0	0	0	0	162	3.24 ± 0.21	0.70	-1.49
	Col+Caf	100	100	2	7	23	34	23	10	1	0	0	0	0	0	0	0	0	0	303	3.03 ± 0.12	0.47	-3.74
10	Col	20	20	0	1	0	0	7	2	5	1	2	2	0	0	0	0	0	0	110	5.50 ± 0.45	0.74	-0.81
	Col+Caf	34	34	0	1	0	3	11	7	6	4	1	1	0	0	0	0	0	0	170	5.00 ± 0.28	0.52	-1.95
15	Col	5	5	0	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0	40	8.00 ± 1.14	0.81	-0.27
	Col+Caf	42	42	0	0	0	1	0	1	3	4	1	2	7	5	5	6	5	1	444	10.57 ± 0.46	0.85	-0.66

Treatment with Colcemid (Col), and with Colcemid and caffeine (Col+Caf); number of aberrant cells (CA); total number of dicentrics (dic) and frequency (y); dispersion index, variance/mean (DI); u-test values, values between ± indicated a Poisson distribution.

^aEnlarging the culture time up to 60 h.

^bThe 48 h culture were irradiated blood was mixed with non-irradiated simulating a 50 % partial irradiation.

When all doses were considered together there was no significant trend of one treatment in showing higher or lower frequencies of dicentrics respect to the other treatment (Wilcoxon matched-pairs test p=0.93).

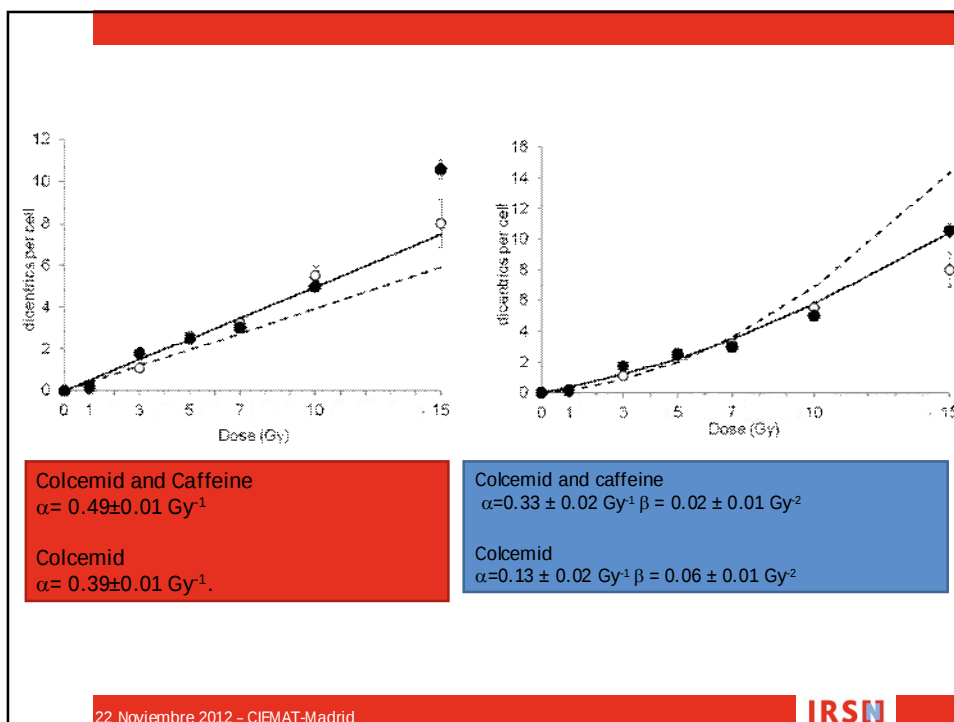


Table 1. Number of cells and dicentric cell distribution observed.

Dose (Gy)	Treatment	Cells	CA	Dicentric cell distribution																Dic	Y $\pm$ SE	DI	u		
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					16	
15 ^a	Col	8	8	0	0	0	0	0	0	1	1	2	1	1	1	0	0	1	0	0	0	65	8.13 $\pm$ 0.90	0.79	-0.40
	Col+Caf	100	99	1	0	1	4	12	16	17	19	9	7	7	3	3	0	1	0	0	665	6.65 $\pm$ 0.24	0.89	-0.77	
15 ^b	Col+Caf	200	8	193	0	0	0	3	1	1	2	0	0	0	0	0	0	0	0	0	37	0.19 $\pm$ 0.07	5.44	44.87	

Treatment with Colcemid (Col), and with Colcemid and caffeine (Col+Caf); number of aberrant cells (CA); total number of dicentrics (dic) and frequency (y); dispersion index: variance/mean (DI); χ^2 test values: values between $\pm$ indicated a Poisson distribution.

In the cultures from blood irradiated at 15 Gy and incubated during 60 hours, the MI ($\times 100$) observed after the treatment with Colcemid and caffeine was 4.52 ± 0.92 , significantly higher than the one treated only with Colcemid, 0.82 ± 0.36 , and than the one observed in the 48 hours culture treated with Colcemid and caffeine 1.20 ± 0.49 ($p < 0.01$ in both cases).

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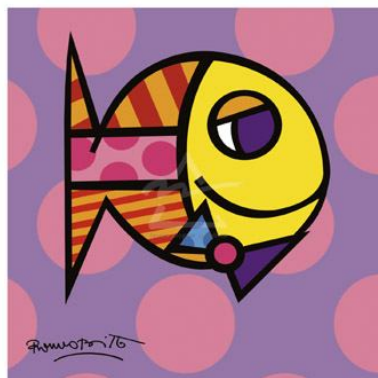
Simple o complejas

Simple: implican 2 roturas en 1 ó 2 cromosomas

Complejas: implican 3 ó más roturas en 2 ó más cromosomas

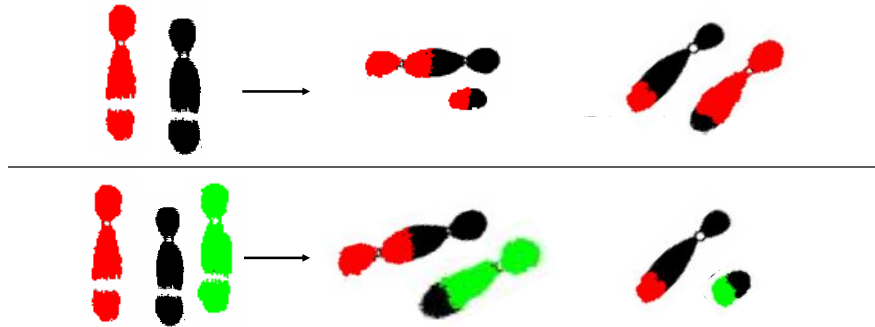
Completas: todos los extremos producidos se han reparado incorrectamente → presencia de telómeros

Incompletas: todos o alguno de los extremos producidos no se han reparado → ausencia de telómeros



ANÁLISIS CUALITATIVOS DEL EFECTO DE RI

- **Alteraciones simples**, aquellas que implican sólo dos roturas



- **Alteraciones complejas**, aquellas que implican un mínimo de tres roturas

Anomalías complejas

mFISH
Complex exchange
CAB_{actual} 4/4/4
Cycle structure c4



Counterstain only
(or solid staining)
Pseudosimple
Dicentric



Yellow probe
Pseudosimple
CAB minimal 2/2/2
S&S pattern 2B



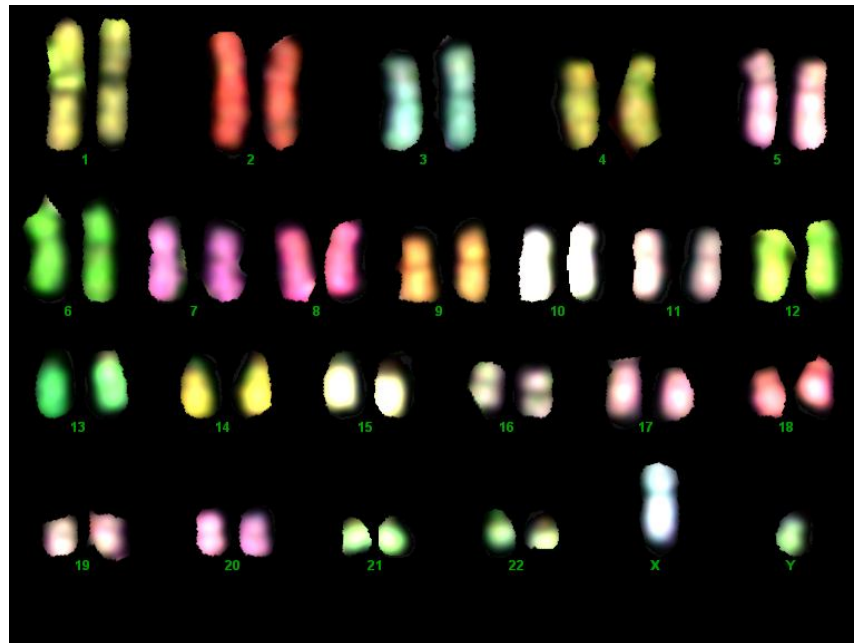
Orange probe
Visibly complex
CAB minimal 3/3/3
S&S pattern 2G



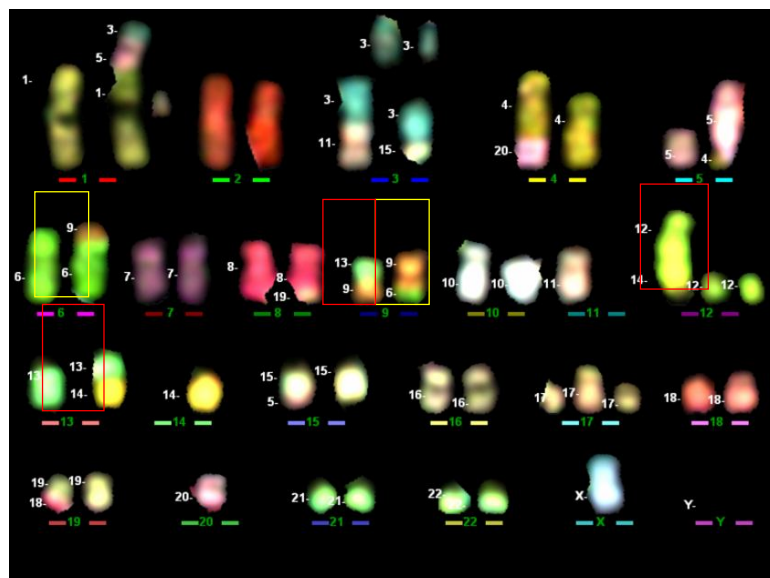
Green probe
Pseudosimple
CAB minimal 2/2/2
S&S pattern 2A



mFISH:



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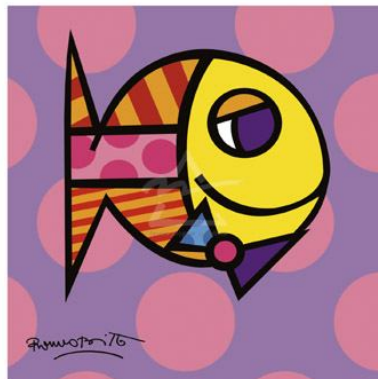
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ANÁLISIS CUALITATIVOS DEL EFECTO DE RI

Alteración cromosómica completa



Alteración cromosómica incompleta

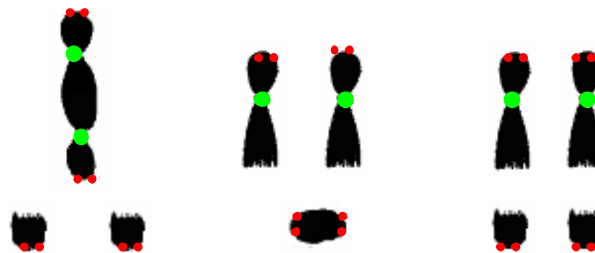


LA APLICACIÓN DE SONDAS PAN-TELOMÉRICAS Y PAN-CENTROMÉRICAS

Alteración cromosómica completa

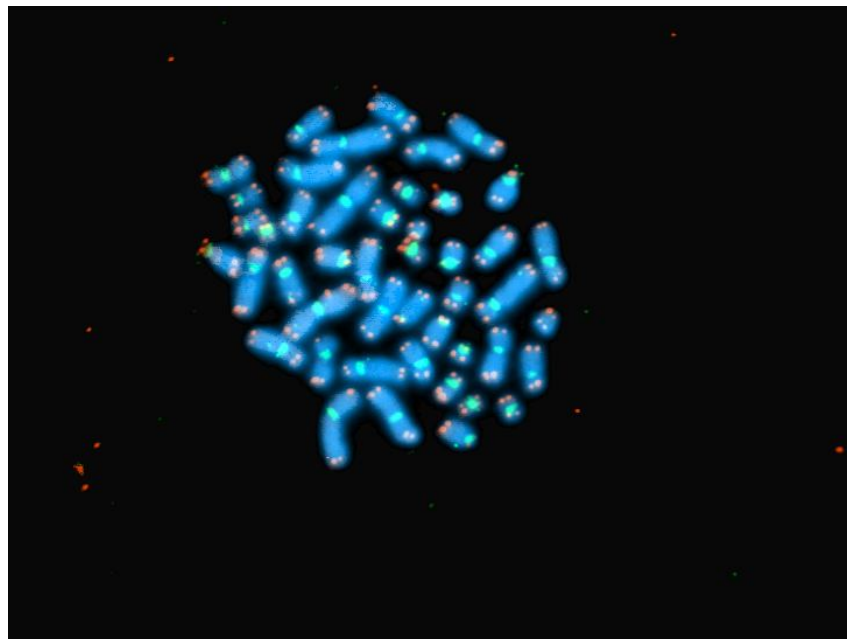


Alteración cromosómica incompleta



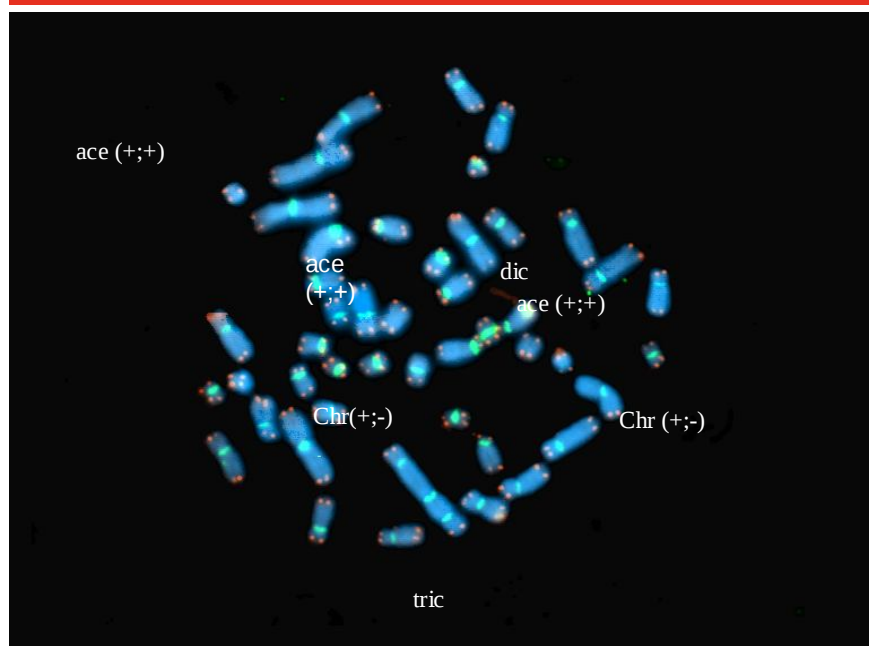
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A cytogenetic study on the biological effect of low energy X-rays

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Actualmente se asume $w = 1$ para todos los fotones (rayos X y γ) (ICRP)

- Existen evidencias de que los **rayos X de baja energía** podrían tener un efecto superior al asumido
 - Especial relevancia en rayos X de mamografías (29-30 kVp)
 - Cuestiona la **relación beneficio/riesgo** de su uso como diagnóstico precoz

Eficacia biológica relativa máxima a bajas dosis (RBE_M)

- Se irradia a diferentes dosis $\rightarrow$ curva dosis - efecto

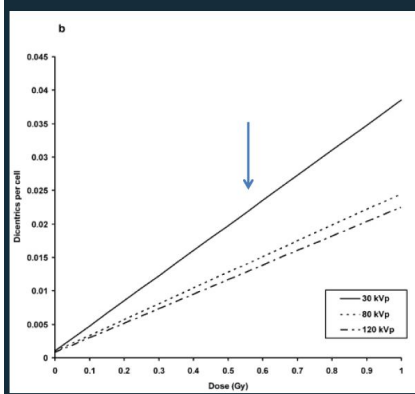
$$RBE_M = \frac{\text{coeficiente } \alpha \text{ radiación problema}}{\text{coeficiente } \alpha \text{ radiación referencia}}$$

For each radiation quality, and taken into account published empirical data, the *International Commission on Radiological Protection* (ICRP-60, ICRP-92) proposed values some *weighting factors* (W_R).

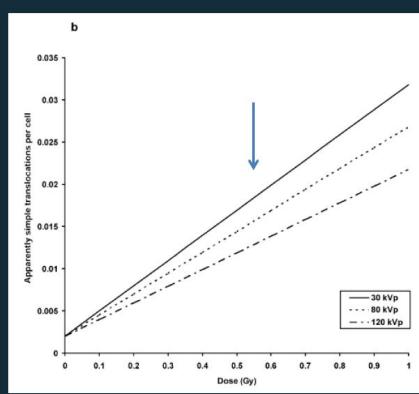
Tipos de radiación	Energía	w	
		ICRP-60	ICRP-92
Fotones	Todas	1	1
Electrones y muones	Todas	1	1
Neutrones	<10 keV	5	Función
Neutrones	10-100 keV	10	Función
Neutrones	de >100 a 2 MeV	20	Función
Neutrones	de 2 MeV a 20 MeV	10	Función
Neutrones	>20 MeV	5	Función
Protones	<2 MeV	5	2
Partículas alfa, fragmentos de fisión, núcleos pesados	Todas	20	20

➤ Relación dosis – efecto utilizando únicamente el factor lineal αD de las curvas

Dicéntricos



Translocaciones



RBE de las diferentes energías de rayos X

$$RBE_M = \frac{\text{coeficiente } \alpha \text{ rayos X 30 kVp}}{\text{coeficiente } \alpha \text{ radiación referencia}}$$

➤ Radiación de referencia: Rayos X de 120 kVp

$$RBE_M \text{ dicéntricos 30 kVp} = 1.73 \pm 0.59$$

$$RBE_M \text{ dicéntricos 80 kVp} = 1.08 \pm 0.43$$

$$RBE_M \text{ mnb 30 kVp} = 1.42 \pm 0.41$$

$$RBE_M \text{ mnb 80 kVp} = 1.38 \pm 0.39$$

$$RBE_M \text{ translocaciones 30 kVp} = 1.51 \pm 0.47$$

$$RBE_M \text{ translocaciones 80 kVp} = 1.26 \pm 0.40$$

➤ Radiación de referencia: Rayos γ 60-Co

$$RBE_M \text{ dicéntricos 30 kVp} = 1.79 \pm 0.56$$

$$RBE_M \text{ translocaciones 30 kVp} = 2.08 \pm 0.41$$

La RBE_M estimada para 30 kVp (dicéntricos, mnb y translocaciones) es superior a 1 y está de acuerdo con la de estudios de inducción de micronúcleos, dicéntricos y con los modelos teóricos

La similitud entre la RBE_M obtenida para dicéntricos y para translocaciones
→ rayos X de 30 kVp son igual de eficaces en producir alteraciones cromosómicas inestables y estables

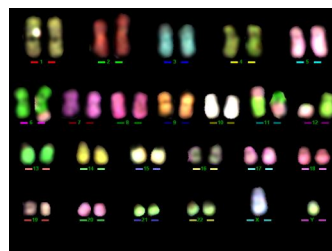
La **RBE** podría aumentar a medida que decrece la energía del fotón debido a la diferencia en el mecanismo de interacción con la materia

- fotones de elevada energía: efecto Compton
- fotones de baja energía: efecto Compton + fotoeléctrico

(ICRP-92, Kellerer 2002, Frankenberg et al. 2002, Hunter y Muirhead 2009, Chadwick y Leenhouts 2009)

To evaluate by mFISH technique the radiation induced complex chromosome abnormalities.

$$\text{ratio} = \frac{\text{complex aberrations}}{\text{total aberrations}}$$

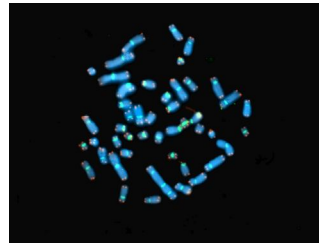


Energía rayos X	30 kVp	80 kVp	120 kVp
Alteraciones complejas	26	24	12
Frecuencia por célula ± SE	0.14 ± 0.03	0.9 ± 0.02	0.06 ± 0.02
Alteraciones intercambio totales	140	216	130
Frecuencia por célula ± SE	0.75 ± 0.06	0.82 ± 0.06	0.63 ± 0.06
Alteraciones totales	186	269	166
Frecuencia por célula ± SE	0.99 ± 0.07	1.02 ± 0.06	0.8 ± 0.06

➤ Esta frecuencia para rayos X de 30 kVp es significativamente superior a la de 120 kVp

To evaluate by FISH technique using pantelomeric and pancentromeric probes

$$\text{ratio} = \frac{\text{chr}(+,-) + \text{ace}(+,-)}{\text{dic}}$$



➤ Alteraciones incompletas

➤ Considerando todas las alteraciones

30 kVp = $0,6 \pm 0,18$

80 kVp = $0,5 \pm 0,18$

120 kVp = $0,53 \pm 0,21$

Alteraciones incompletas = no intercambio + intercambio incompletas

➤ Considerando sólo las alteraciones tipo intercambio

30 kVp = $0,16 \pm 0,04$

80 kVp = $0,1 \pm 0,03$

120 kVp = $0,13 \pm 0,04$

Rayos X de 30 kVp → siempre muestran ratios de incompletas superiores
(sin diferencias estadísticamente significativas)

Los rayos X de 29 kVp producen DSB que se reparan de forma errónea más frecuentemente que los inducidos por los rayos γ del Co-60

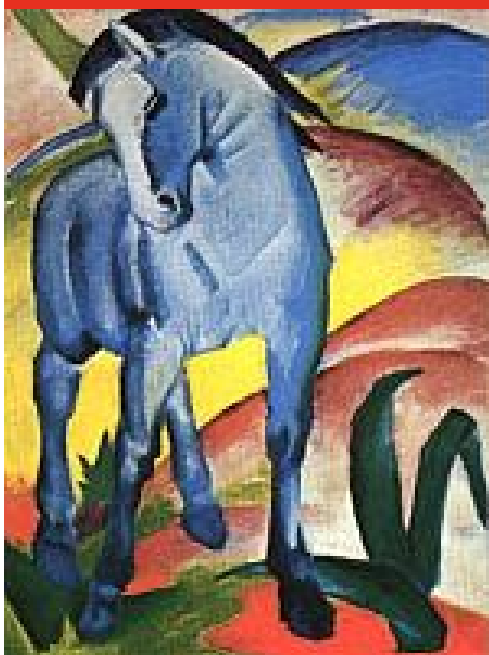
(Kuhne *et al.* 2005)

Esta reparación errónea → aumento anomalías complejas observado

En las radiaciones de **alta LET**, la cantidad de energía se deposita localmente y produce más daño en el DNA. Por tanto, es más difícil de reparar y se relaciona con la mayor eficiencia en producir alteraciones complejas e incompletas

(Goodhead 1989, 1993; Jenner 1993; Stenerlöw 1996; Anderson 2002, 2003; Barquinero 2004)

El daño producido por rayos X de 30 kVp se acerca al tipo de daño producido por radiaciones de alta LET



**MUCHAS
GRACIAS POR SU
ATENCION!**

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