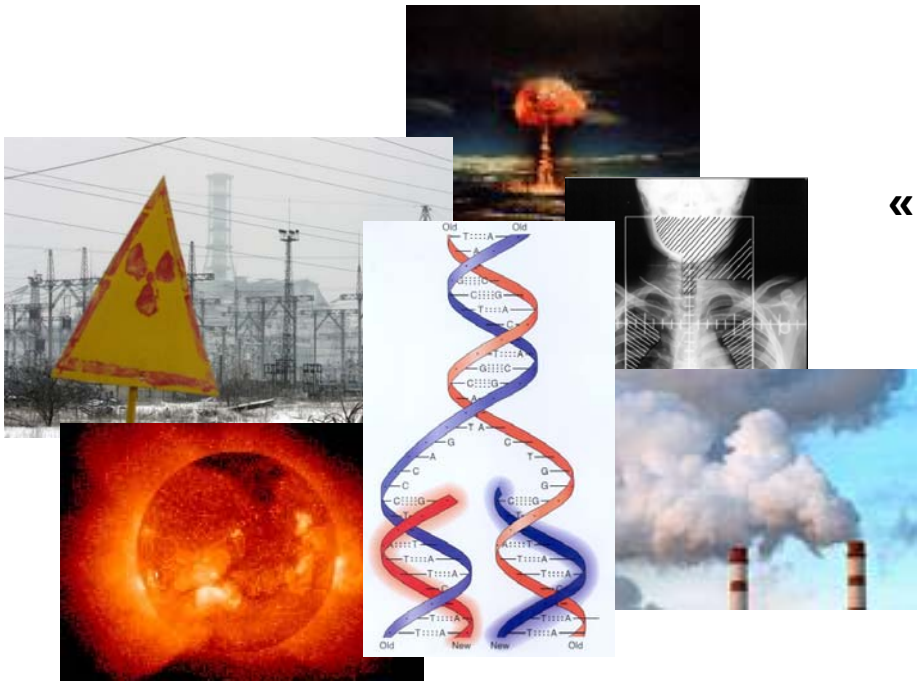


« Stress » génotoxiques

Lésions de l’ADN

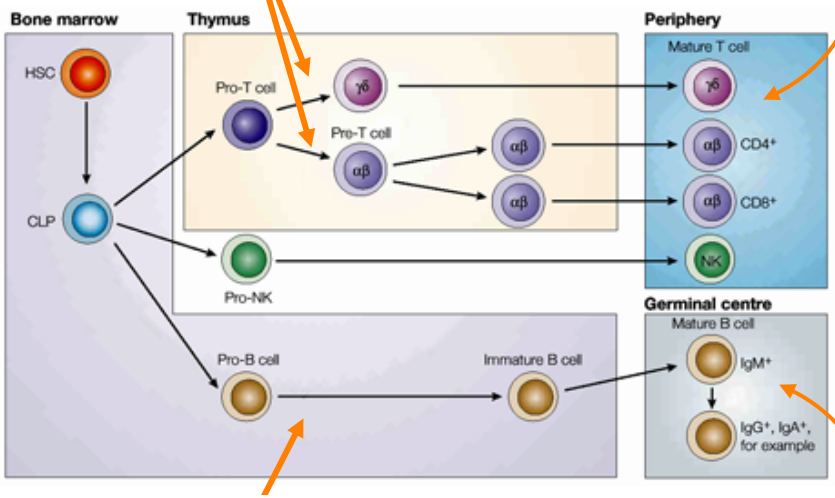
- Sensibilité individuelle
- Mécanismes de réparations
- Pathologies asociées

Intérêt du Système Immunitaire dans l’étude des lésions de l’ADN et des mécanismes assurant leurs réparations

Genetic plasticity in the immune system

- V(D)J recombination
- Cell proliferation

- Cell proliferation
- Cell activation

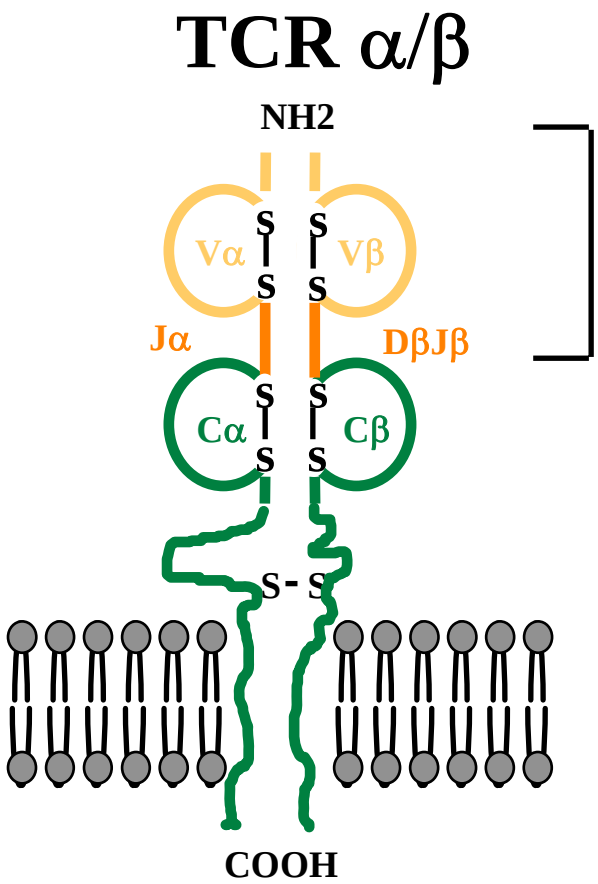


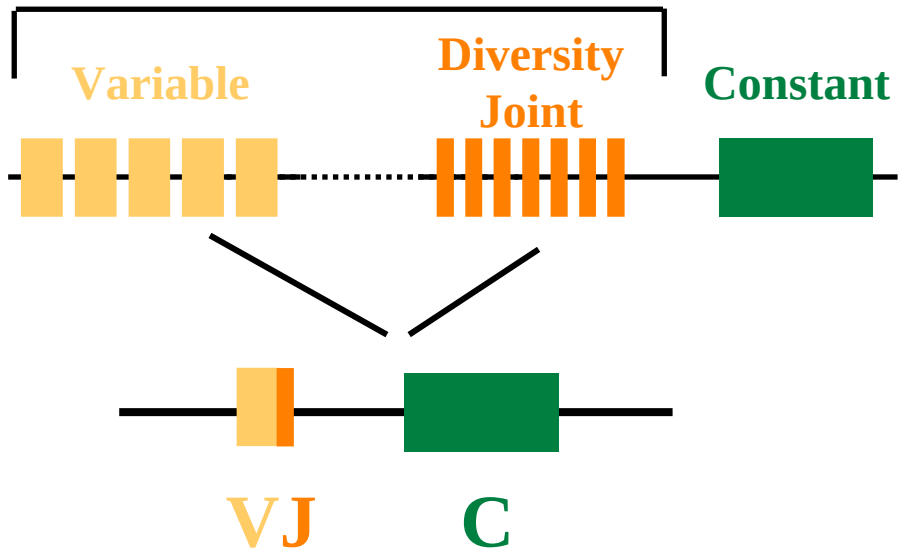
- V(D)J recombination
- Cell proliferation

- CSR, SHM
- Cell proliferation
- Cell activation

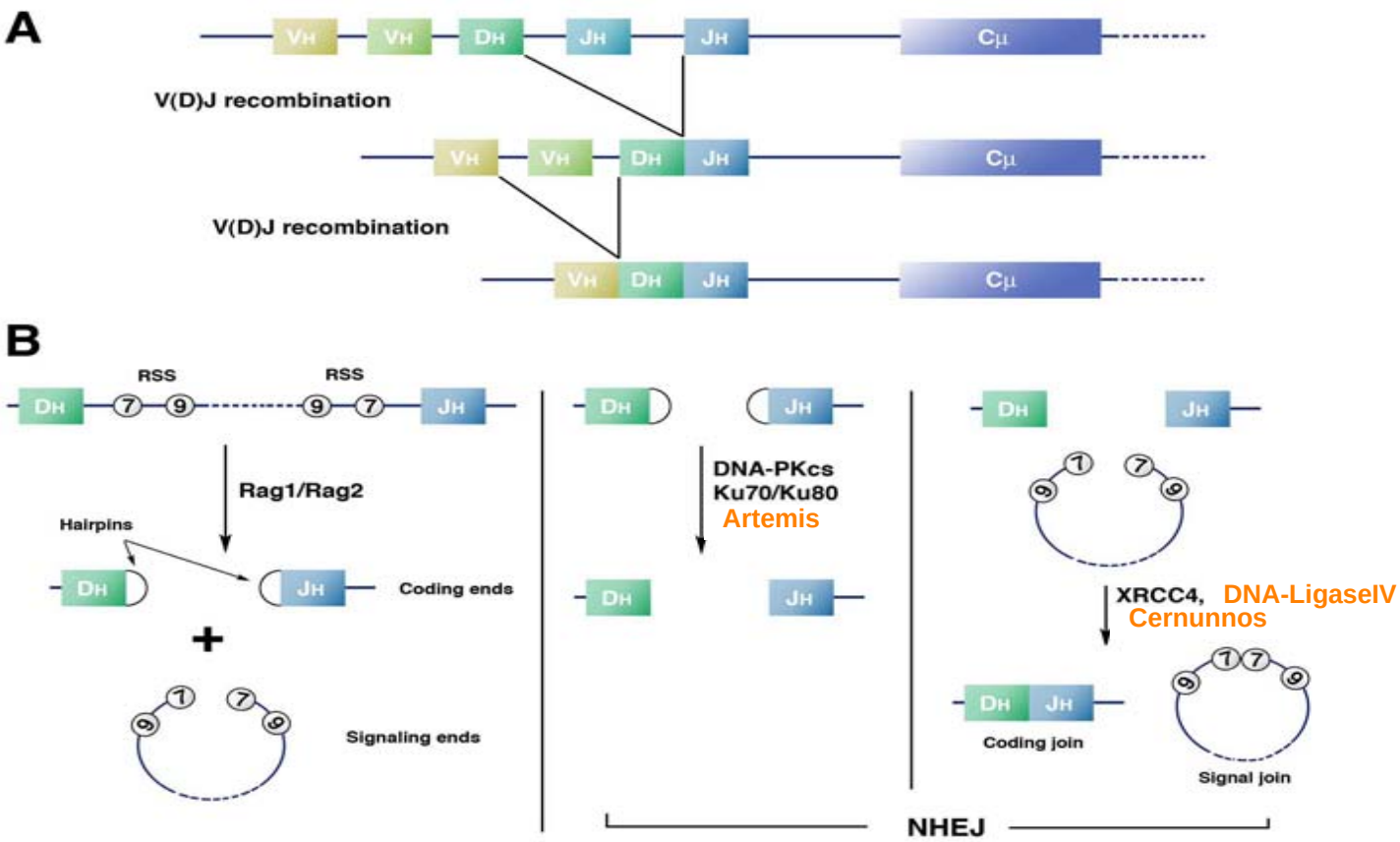
DNA “damages” / modifications

- **Programmed/natural**
  - **V(D)J recombination**
  - **CSR, SHM**
- **Random/accidental/environmental**
  - **Cell proliferation**
    - Stalling of replication forks
    - Telomere maintenance
  - **Cell activation**
    - Reactive oxygen species (ROS)


TCR α/β
NH2
Vα
Vβ
Jα
DβJβ
Cα
Cβ
S-S
COOH


Variable Domain
Variable
Diversity
Joint
Constant
VJ
C

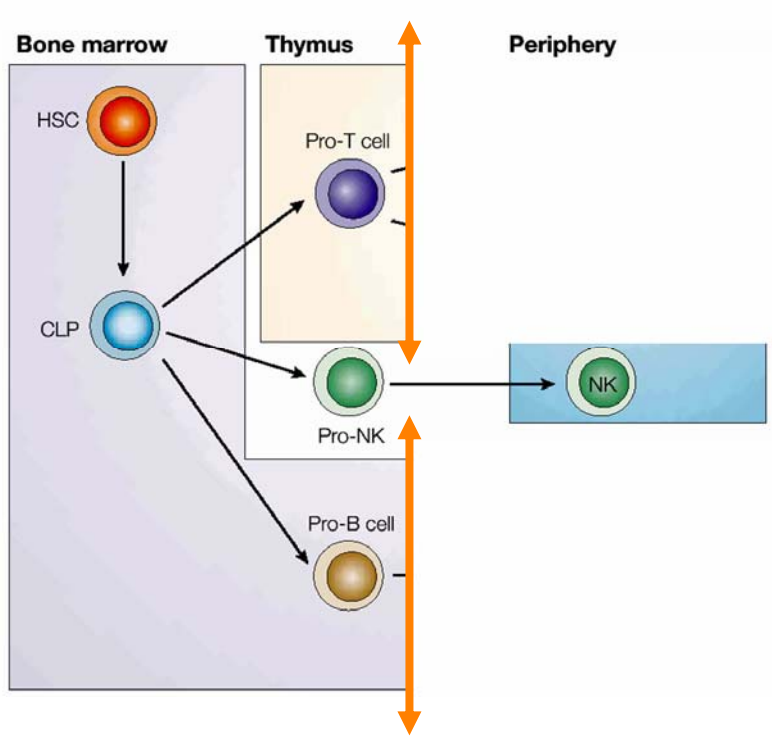
V(D)J recombination



- **Diversity of the immune system**
- **Developmental checkpoint of the adaptive immune system**

T-B-NK+ SCIDs

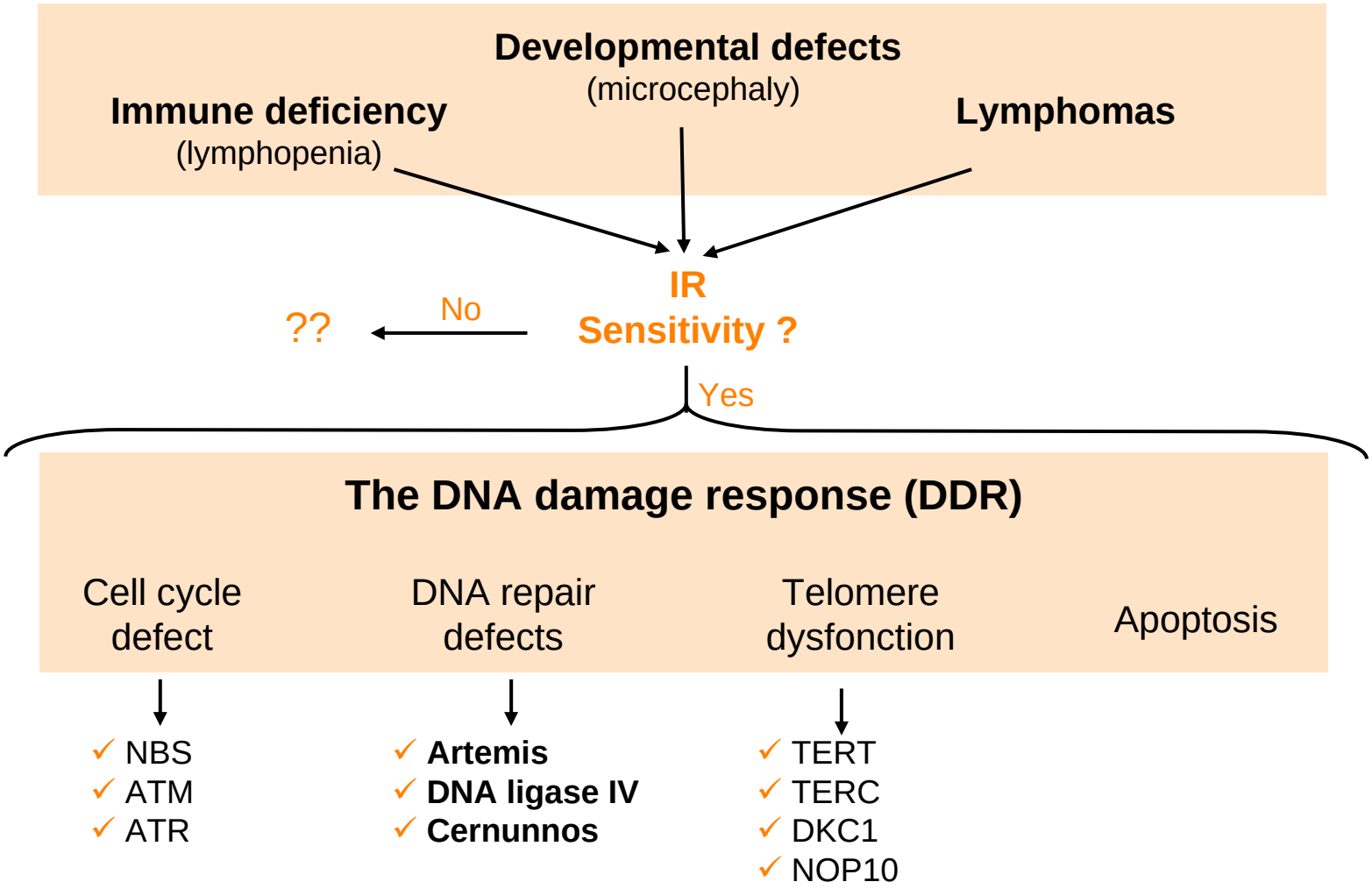
V(D)J recombination deficiency



V(D)J recombination defect

- T-B-NK+ SCID
  - 20% of SCIDs
  - Autosomal recessive inheritance
  - HSC transplantation
- Rag1/2 SCIDs
- RS-SCID
  - T-B-NK+
  - V(D)J recombination defect
  - No Rag1/2 mutations
  - Increased sensitivity to IR
  - General DNA-dsb repair defect

Survey of human immune deficiencies

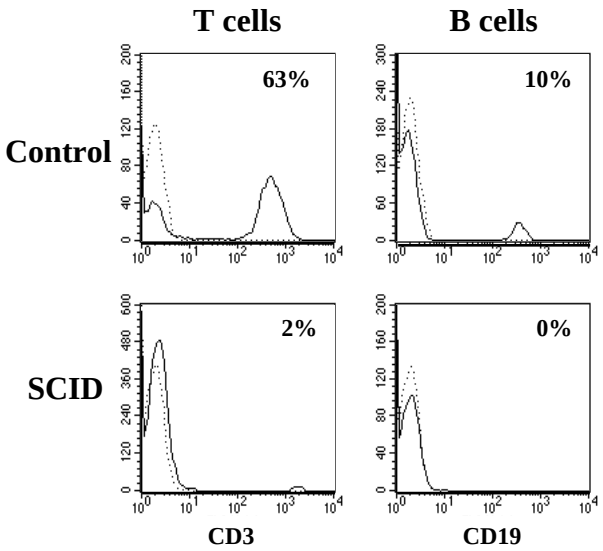


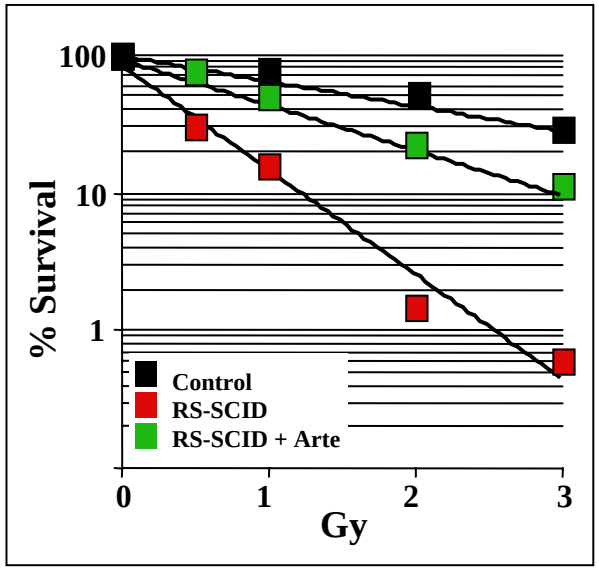
Cell

Cell, Vol. 105, 177–186, April 20, 2001, Copyright ©2001 by Cell Press

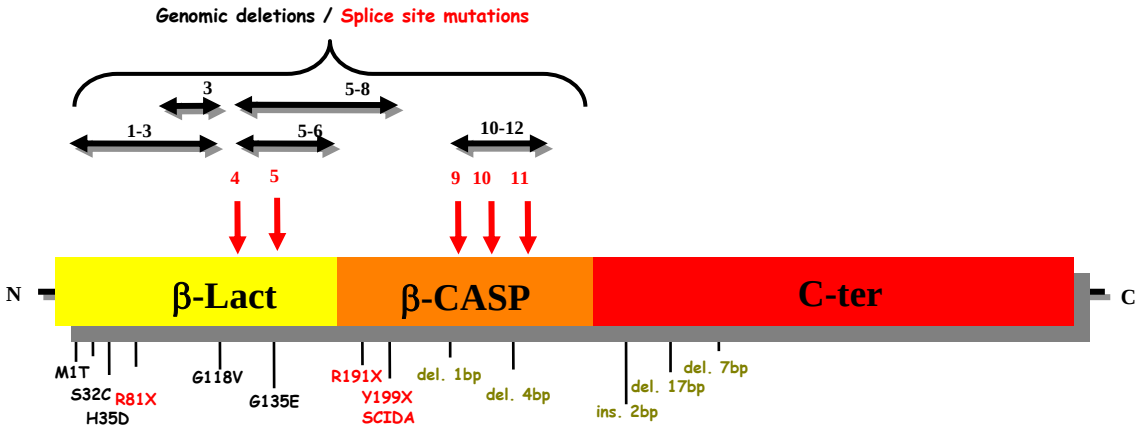
Artemis, a Novel DNA Double-Strand Break Repair/V(D)J Recombination Protein, Is Mutated in Human Severe Combined Immune Deficiency

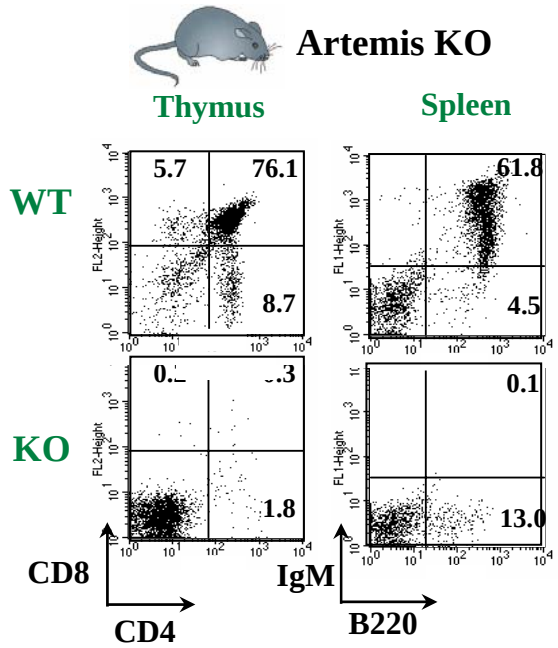
Despina Moshous,* Isabelle Callebaut,†
Régina de Chasseval,* Barbara Corneo,*
Marina Cavazzana-Calvo,* Françoise Le Deist,*
Ilhan Tezcan,‡ Ozden Sanal,‡ Yves Bertrand,§
Noel Philippe,§ Alain Fischer,*
and Jean-Pierre de Villartay*

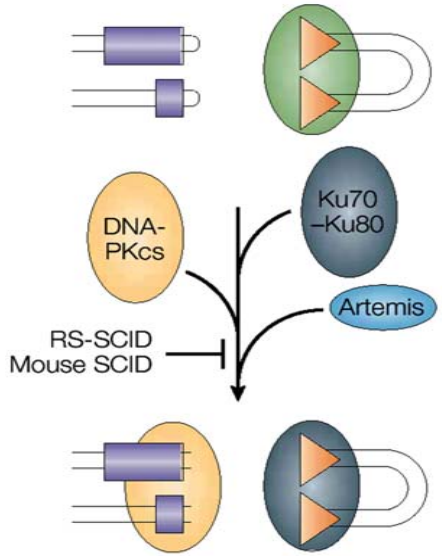




Artemis


Genomic deletions / Splice site mutations
3
5-8
1-3
5-6
10-12
4 5
9 10 11
N
β-Lact
β-CASP
C-ter
C
M1T
S32C
R81X
H35D
G118V
G135E
R191X
Y199X
SCIDA
del. 1bp
del. 4bp
ins. 2bp
del. 17bp
del. 7bp


Artemis KO
Thymus
Spleen
WT
KO
5.7
76.1
8.7
61.8
4.5
1.8
0.1
13.0
CD8
CD4
IgM
B220


DNA-PKcs
Ku70
-Ku80
Artemis
RS-SCID
Mouse SCID

European Journal of Immunology

Severe combined immunodeficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase IV

Dietke Buck[1], Despina Moshous[1], Régina de Chasseval[1], Yunmei Ma[2], Françoise le Deist[1], Marina Cavazzana-Calvo[1,3], Alain Fischer[1,4], Jean-Laurent Casanova[4,5], Michael R. Lieber[2] and Jean-Pierre de Villartay[1,4]

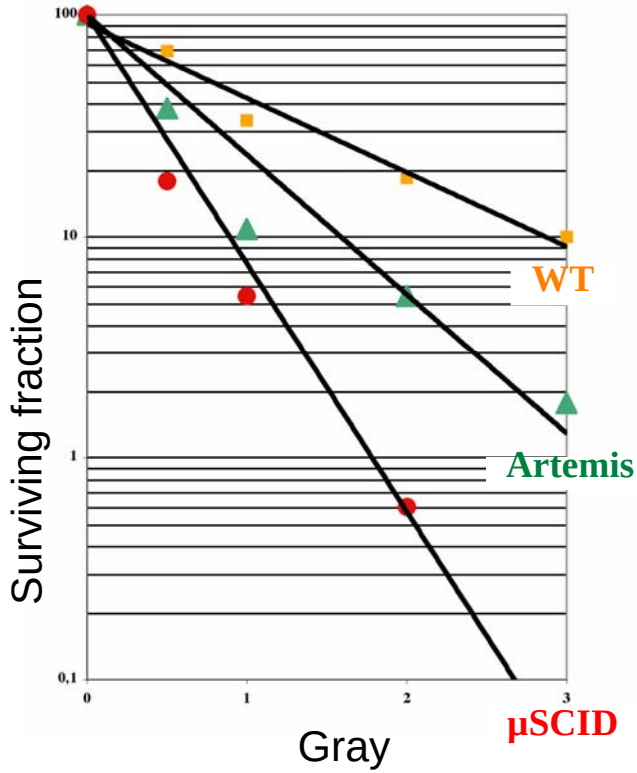
- 2 sisters from non-consanguineous family
- Microcephaly (-3SD), repeated infection
- Profound lymphocytopenia
  - Virtual absence of B ly.
  - Diversified and functional T ly.

Presentation similar to NBS patients although ID more severe

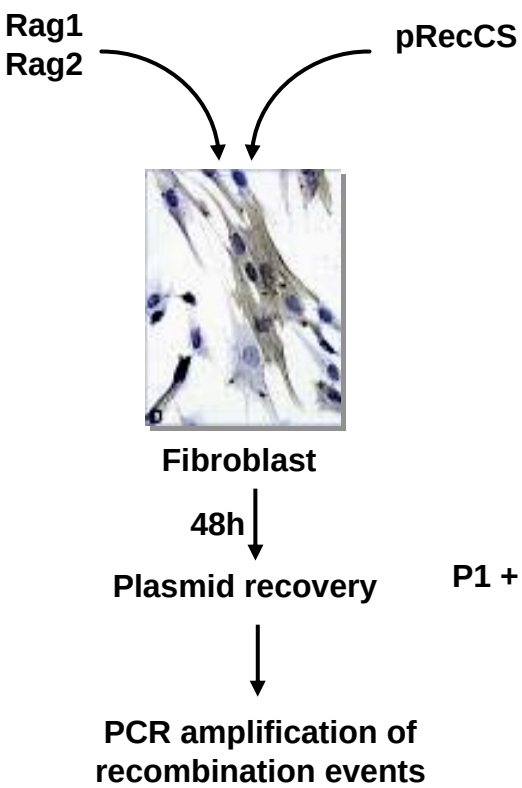
- Increased cellular radiosensitivity
- Normal G1/S cell cycle checkpoint following IR

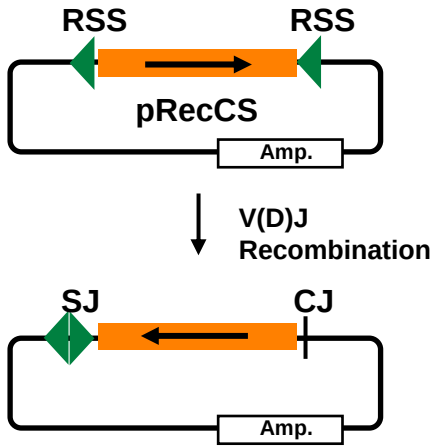
Hypothesis of a defect in a DNA repair factor

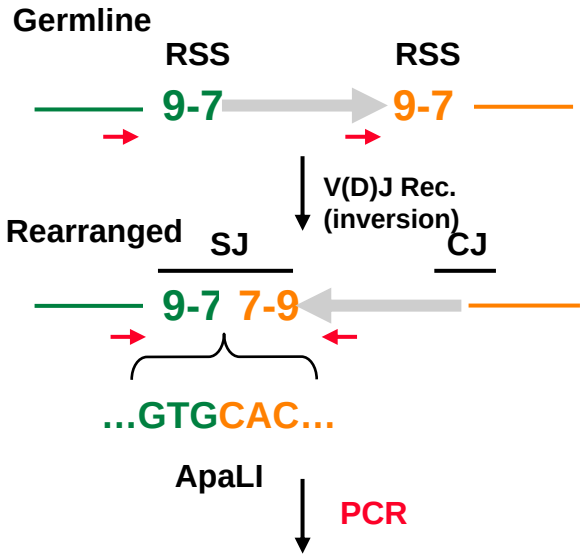
Radiosensitivity

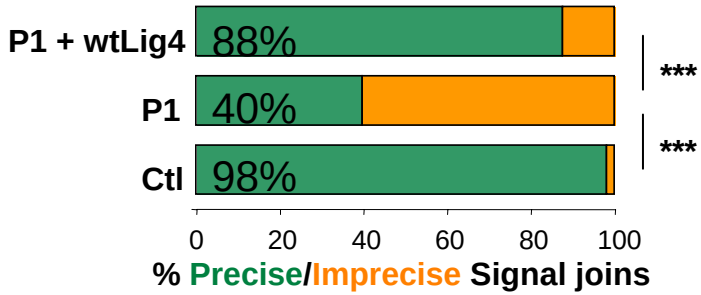


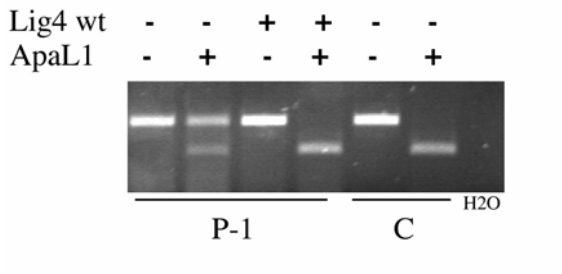
Extrachromosomal V(D)J Recombination assay



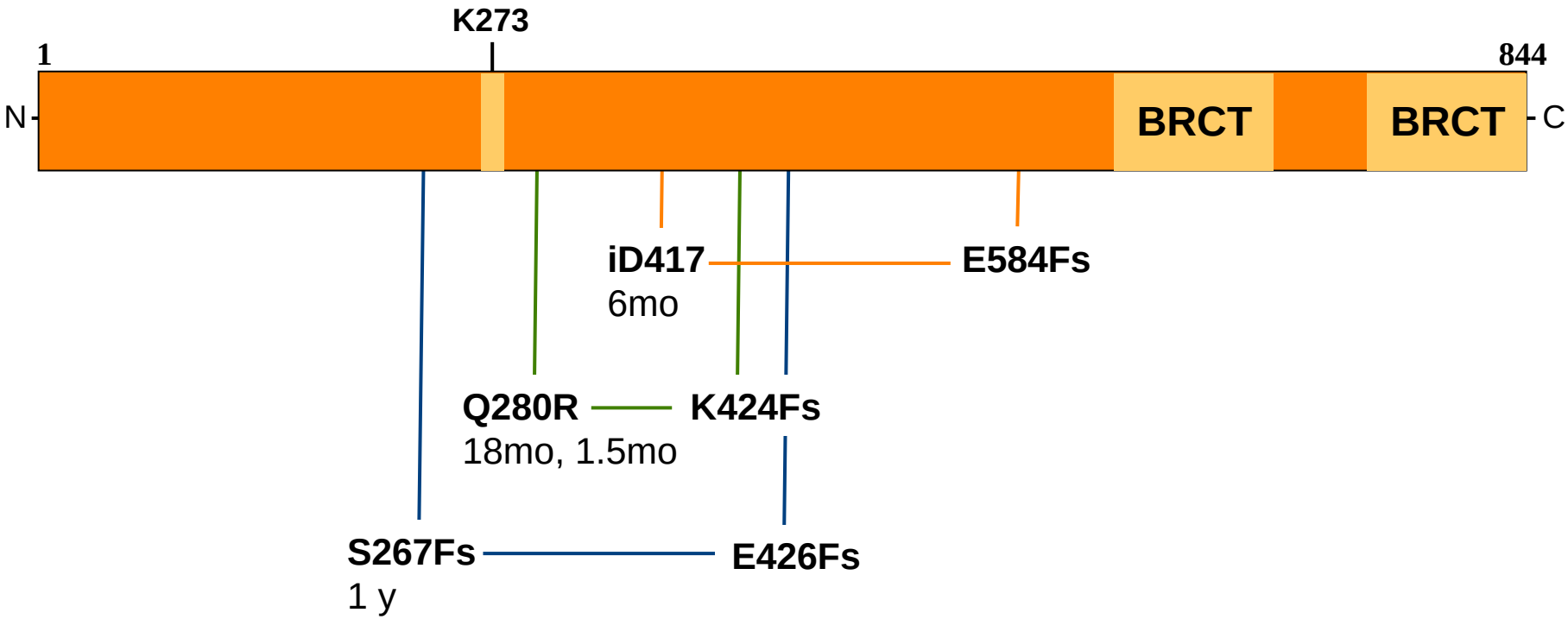




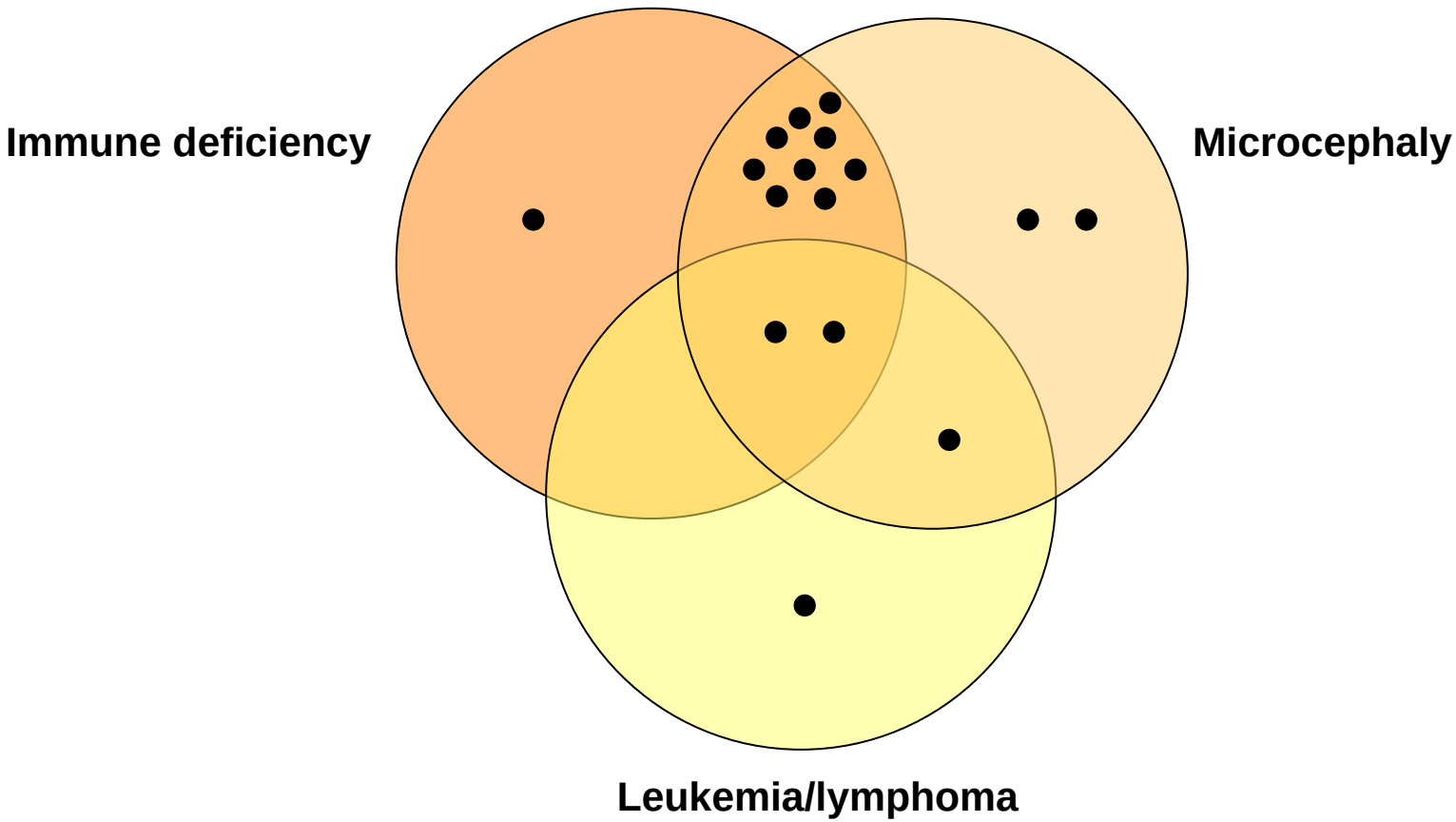




DNA-Lig4 mutations



DNA-Lig4 deficiency: 16 patients (09/2007)

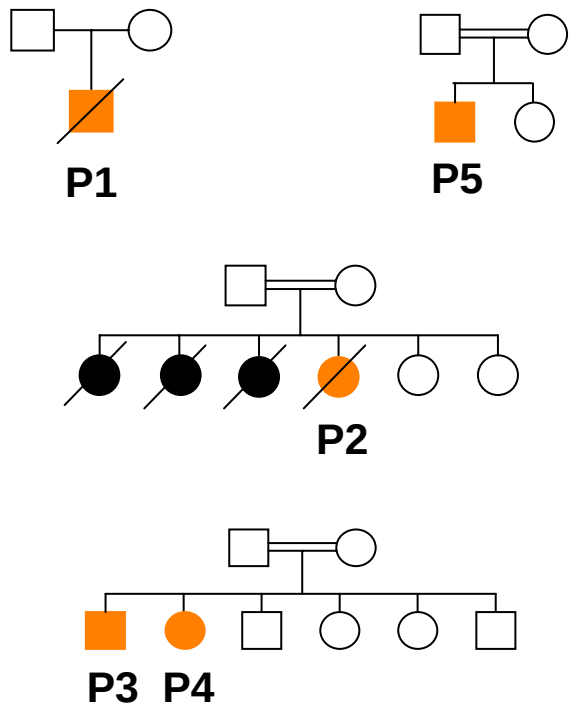


Cell

Cernunnos, a Novel Nonhomologous End-Joining Factor, Is Mutated in Human Immunodeficiency with Microcephaly

Dietke Buck,[1] Laurent Malivert,[1] Régina de Chasseval,[1] Anne Barraud,[1] Marie-Claude Fondanèche,[1] Ozden Sanal,[2] Alessandro Plebani,[3] Jean-Louis Stéphan,[4] Markus Hufnagel,[5] Françoise le Deist,[1,6] Alain Fischer,[1,6] Anne Durandy,[1,6] Jean-Pierre de Villartay,[1,6,*] and Patrick Revy[1]

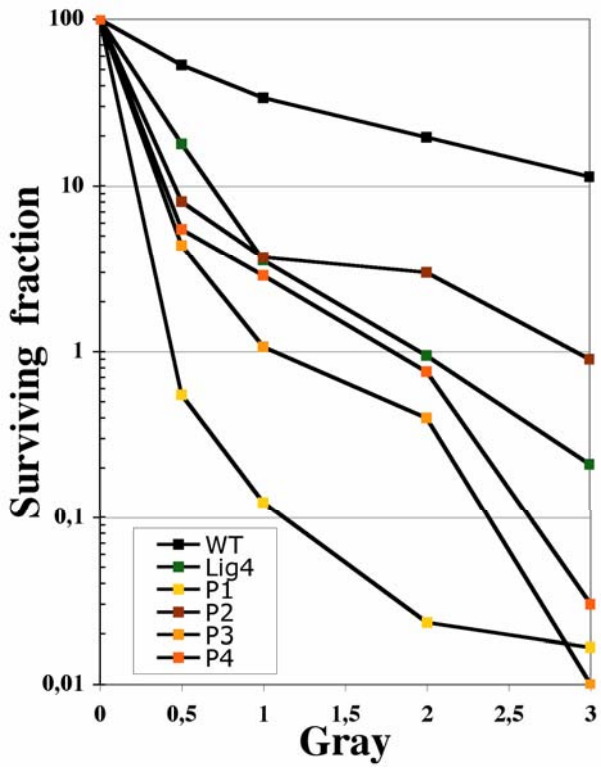
- 5 patients with microcephaly and various degree of immune deficiency
- Progressive B and T lymphocytopenia
- Hyper-IgM syndrome
- “Memory” only T lymphocytes



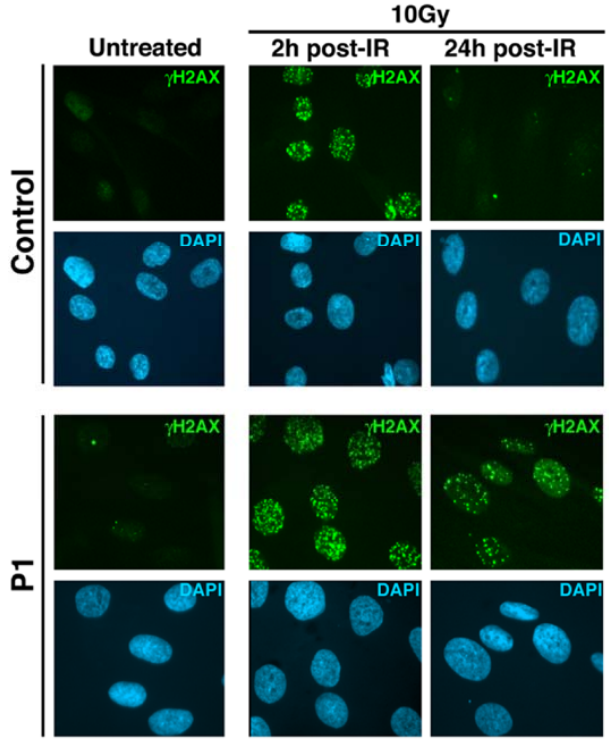
Presentation similar to NBS and Lig4 conditions

DNA repair defect in µSCID-II

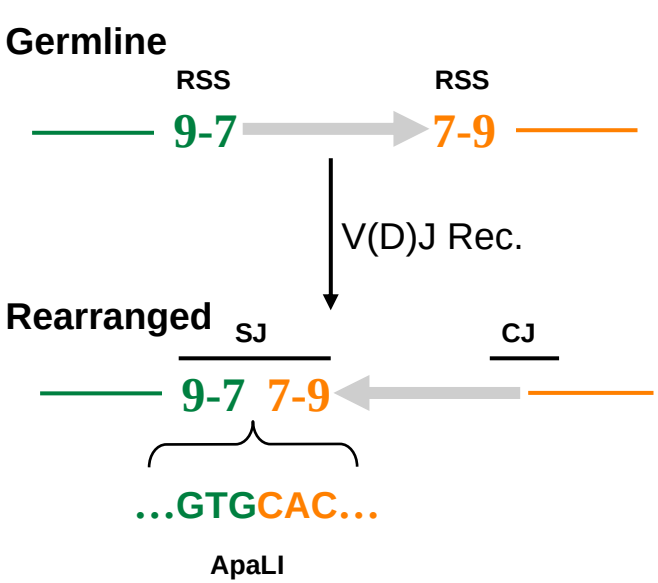
Radiosensitivity

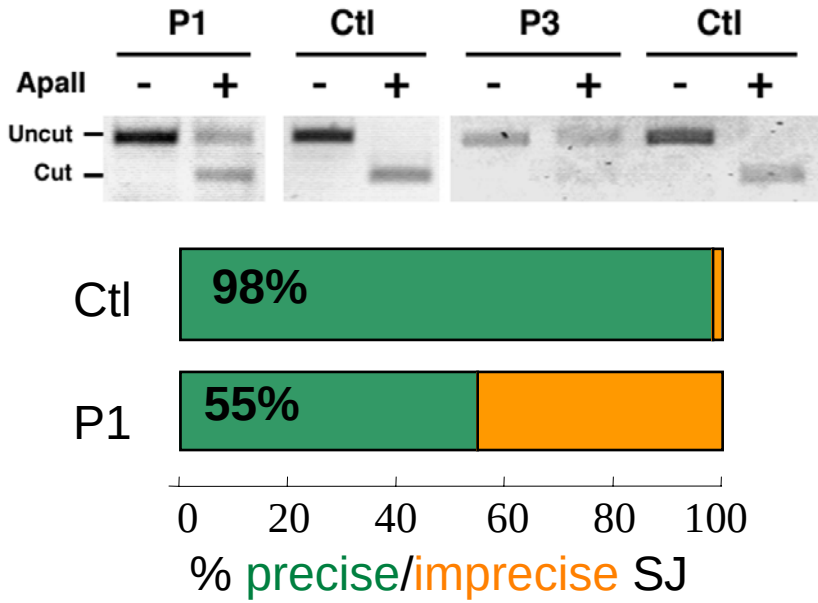


Persistence of γH2AX foci following IR



V(D)J recombination defect in µSCID-II

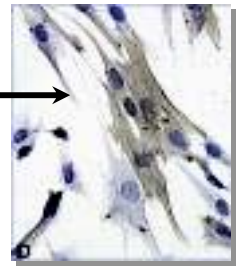




- **Signal joins infidelity is a hallmark of a NHEJ defect (seen in Lig4 patients)**

cDNA functional complementation cloning

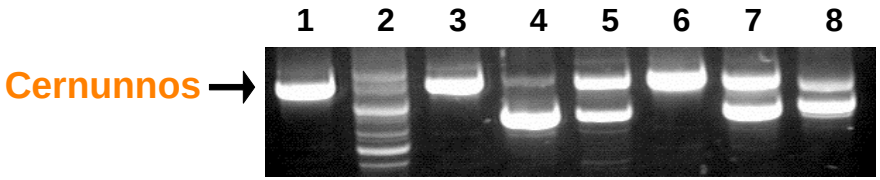
Retroviral thymic cDNA library



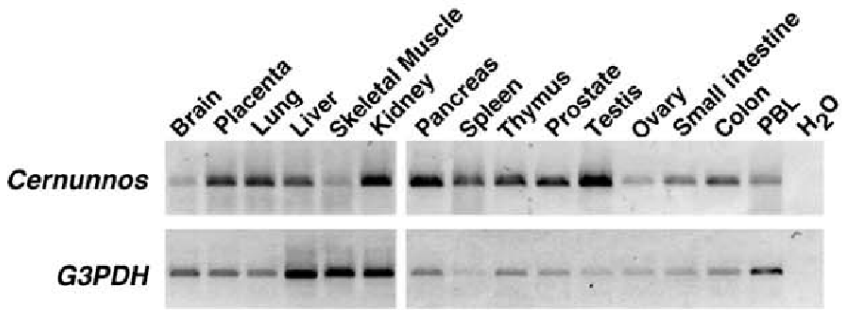
SV40, hTert fibroblasts (8 pools)

9x Bleo. (0.5µg/ml)
90 days

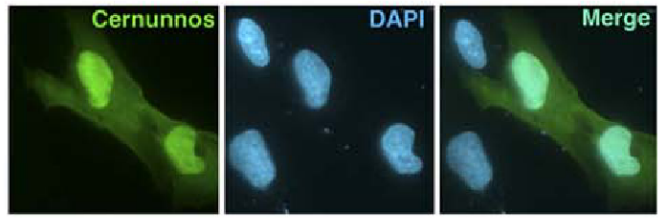
PCR amplification of retroviral inserts



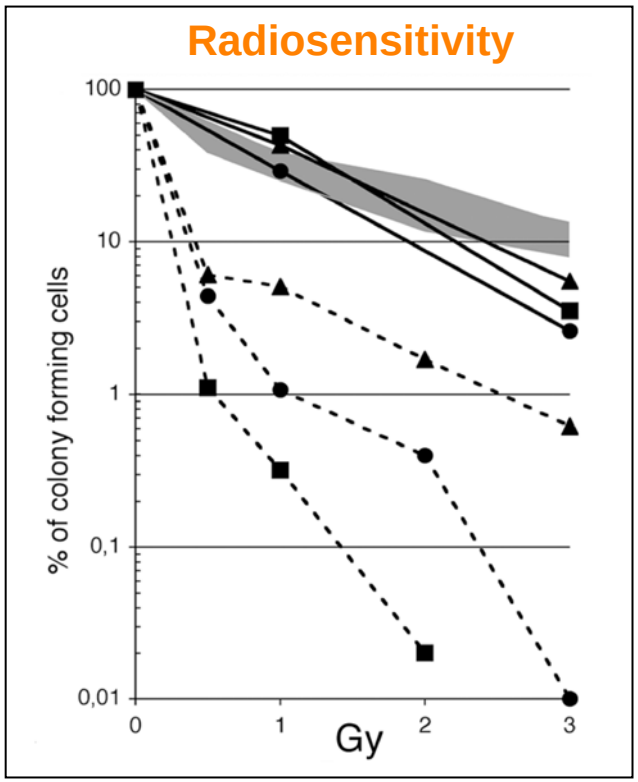
- **2,063 bp; 299aa**
- **Hu Chr 2q35; 8 exons**
- **Ubiquitously expressed**

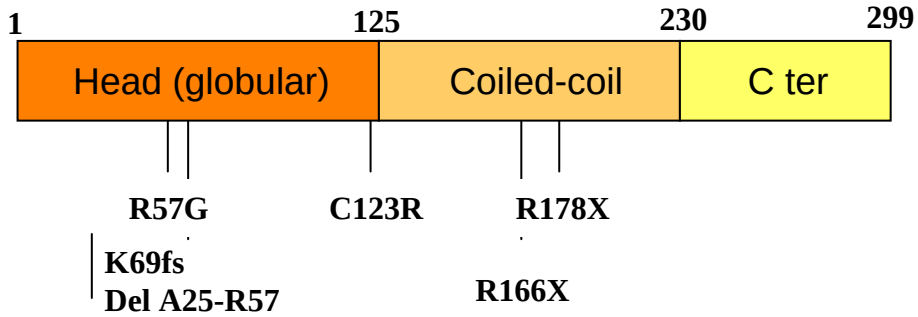


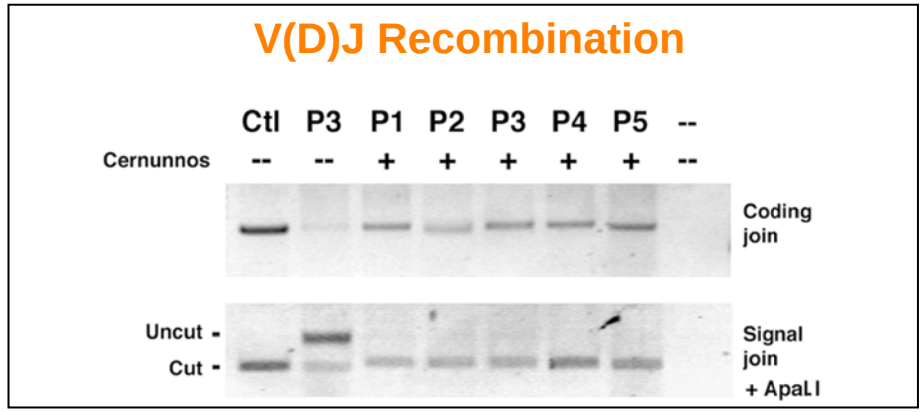
- **Nuclear**



Cernunnos mutations







NHEJ DNA repair factors are essential for the development of the immune system

- ✓ *Artemis*
  - Immune deficiency (RS-SCID)
- ✓ *DNA Ligase 4*
  - µSCID (I)
- ✓ *Cernunnos*
  - µSCID (II)

- ✓ *Ku70, Ku80, DNA-PKcs, Artemis*
  - Immune deficiency
- ✓ *XRCC4, DNA-Ligase IV*
  - Immune deficiency
  - Embryonic lethality

Radiosensibilité:
variabilité individuelle et tests prédictifs

- Des mutations/polymorphismes dans les gènes du NHEJ sont ils associés à un risque particulier de sur-réponse aux thérapeutiques anti-cancéreuses?
- Une haplo-insuffisance des facteurs du NHEJ est-elle également associée à un risque particulier?
- Quels tests prédictifs développer pour identifier les individus à risque?
  - Test de radiosensibilité sur fibroblastes
  - Test de radiosensibilité sur lymphocytes
  - Analyse des produits de recombinaison V(D)J et Ig switch à la recherche d'anomalies

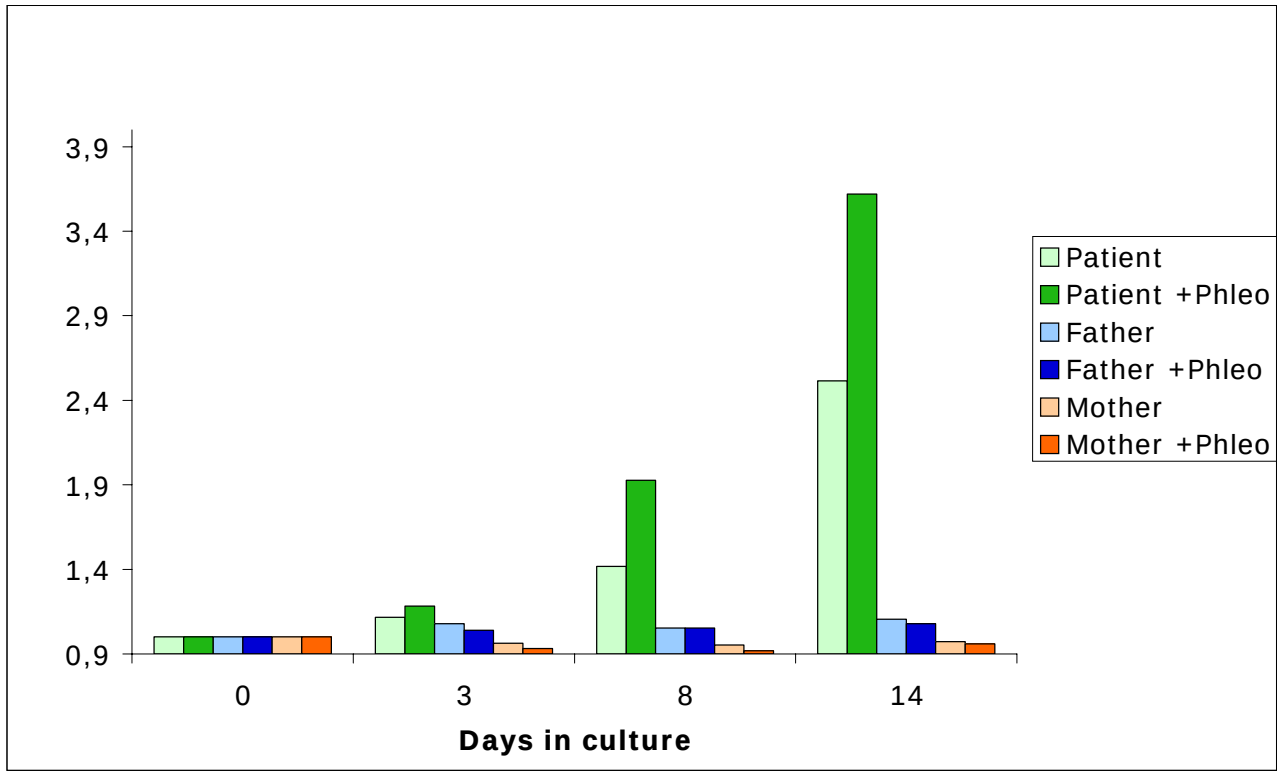
An instance of clinical radiation morbidity and cellular radiosensitivity, not associated with ataxia-telangiectasia

PN Plowman, BA Bridges, CF Arlett, A Hinney and JE Kingston
Department of Radiotherapy, St Bartholomew's Hospital, London.

The British Journal of Radiology, Vol 63, Issue 752 624-628, Copyright © 1990 by British Institute of Radiology

- 14 years old Turkish-Cypriot boy with no previous medical history
- Diagnosis of T cell acute lymphoblastic leukemia treated by Chemotherapy (vincristine, daunorubicin, 1-asparaginase, prednisone)
- Cranial radiation prophylaxis (1800cGy 10 fractions/12days)
  - Marked scalp erythema and desquamative reaction behind both ears (d5)
  - Tiredness and lethargy during 3wks (d33)
  - Severe pain in the right ear -> necrotic ulcer (m4)
  - EEG -> radiation induced encephalopathy (m7)
  - Died 8 months following radiotherapy
- Skin fibroblast cell line “180BR”
  - Hypersensitivity to ionizing radiation
- R278H homozygous mutation in the DNA-Ligase IV gene (Riballo E et al. 1999)

DNA-Lig4 functional complementation



- DNA Lig4 haplo-insufficiency does not lead to increased radiosensitivity

Conclusions

- Le système immunitaire présente une grande plasticité génétique
- Le développement harmonieux du système immunitaire nécessite une machinerie efficace de réparation des lésions de l'ADN
- Un défaut de réparation des lésions de l'ADN entraîne des déficits immunitaires sévères et/ou le développement d'hémopathies malignes
- Risque de sur-réponse à des traitements génotoxiques dans les thérapeutiques anti-cancéreuses par l'existence de « mutations » hypomorphes de facteurs de réparation de l'ADN
- Nécessité de développement d'outils prédictifs de la sensibilité individuelle aux agents génotoxiques utilisés en thérapeutique
- Le système immunitaire représente un laboratoire naturel d'étude des voies de réparation de l'ADN