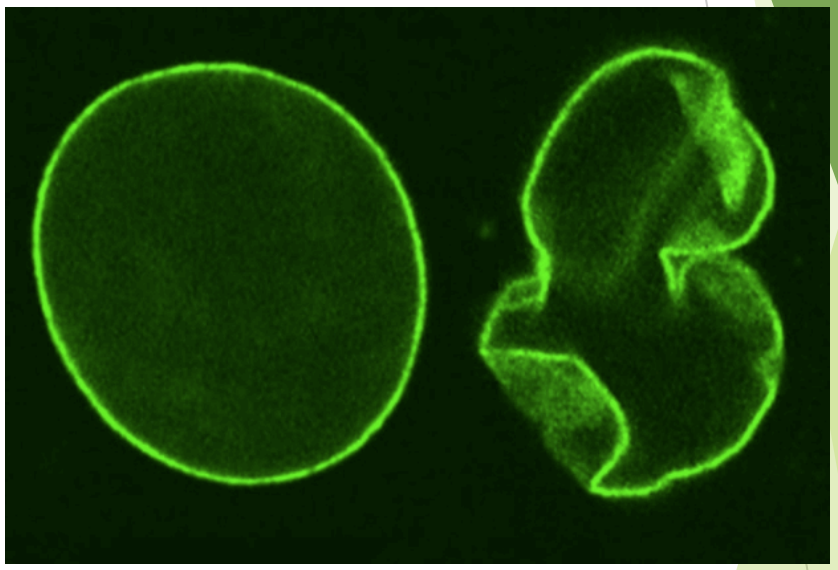
SAPIENZA
UNIVERSITÀ DI ROMA

LAMINOPATHIES AND LENTIVIRAL VECTORS: FOCUS ON RESTRICTIVE DERMOPATHY

Group 1

- Annapaola Angrisani
- Antonella Crispino
- Camilla Badiali
- Camilla Razionale
- Romain Bidanel

LAMINOPATHIES: group of rare genetic disorders caused by mutations in genes encoding proteins of the nuclear lamina or genes encoding lamin-binding proteins.



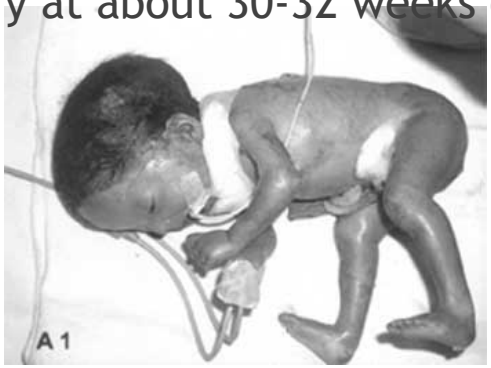
Most frequent mutation on:

- LMNA gene encoding lamin A/C
- ZMPSTE24 gene encoding zinc metallopeptidase STE24

RESTRICTIVE DERMOPATHY: sistemic disease presenting premature aging features, an autosomal recessive neonatal lethal genodermatosis

Phenotype

- Abnormal skin growth and differentiation
- Tight translucent and rigid skin
- Anomalous facial features
- Bone resorption of clavicles
- Arthrogryposis
- Pulmonary hypoplasia
- Intrauterine growth retardation
- Delivery at about 30-32 weeks of gestation



Histopathologic findings

- Smooth epidermis
- Thin dermis
- Abnormal dermal connective tissue structure
- Collagen fibers arranged more or less horizontally and parallel to the epidermis
- The number of elastin fibers shows a sharp decrease

Respiratory failure is the main cause of death becaus restriction of respiratory movements

RESTRICTIVE DERMOPATHY

Caused by dominant mutations on LMNA gene or by recessive mutations on ZMPSTE24 gene

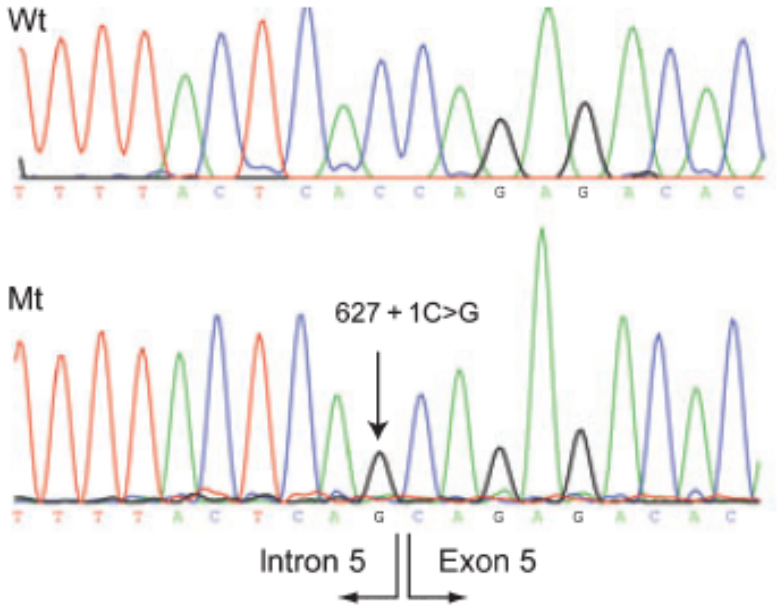
The majority of RD cases are caused by mutations in the ZMPSTE24 gene.

All known mutations in this gene are predicted to result in a complete loss of the Zmpste24 enzyme, which in turn results in the accumulation of farnesylated prelamin A.

One of most common mutations in Zmpste24 is the homozygous non-sense mutation **c. 627+1 G>C** on **1p34.2**

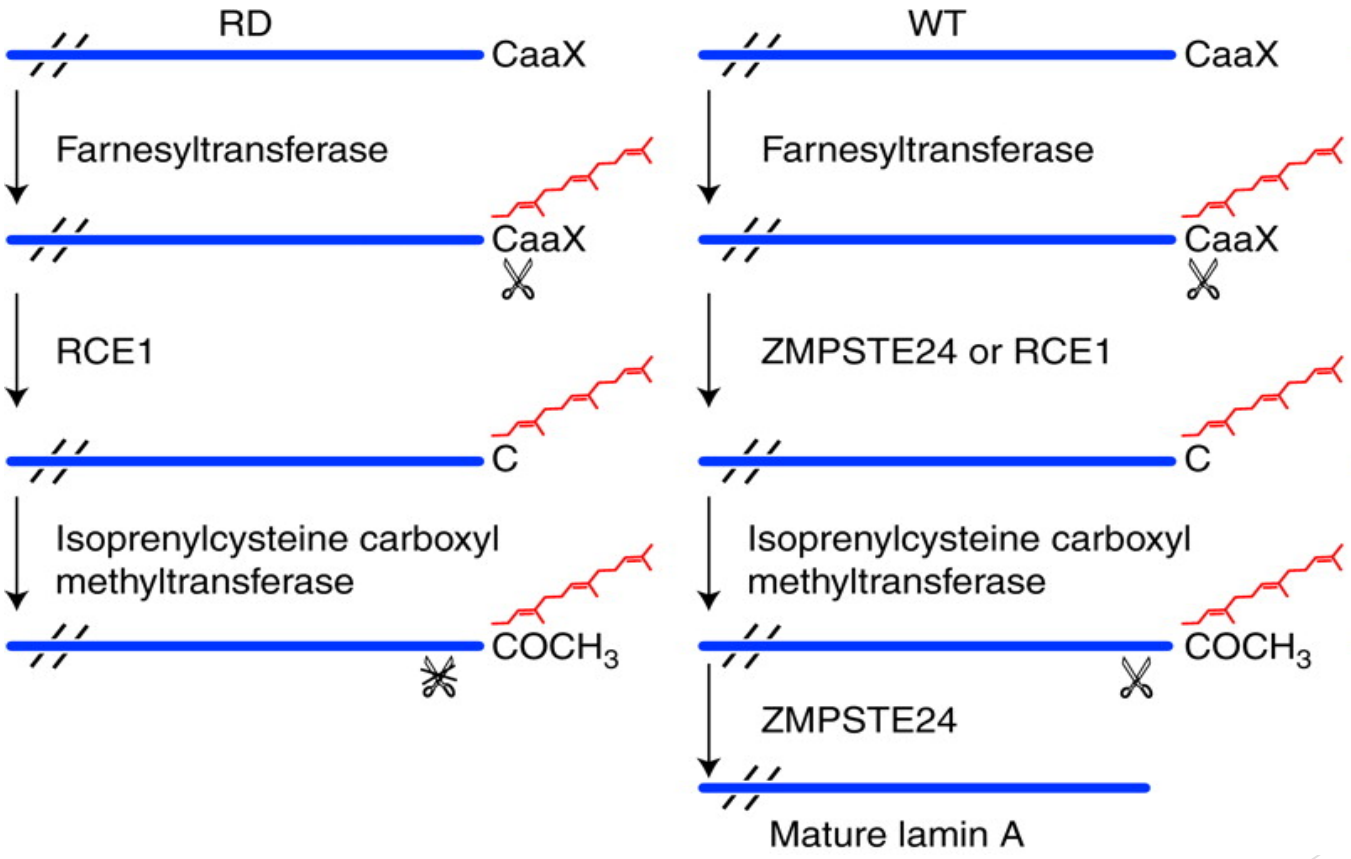
cDNA

cDNA



Sander et al., 2008

The role of Zmpste24 in the maturation of Prelamin A



RD: ACTUAL TREATMENTS

- Farnesyltrasferase inhibitor

BUT →

- Increase of misshapen nuclei

- Hypomorphic allele of isoprenylcysteine carboxyl methyltransferase (ICMT)

BUT →

- Is protein prenylation the only important factor in disease pathogenesis?

- Dermal filler

BUT →

- It's not a solution for the pathology

Davies et al., 2011 – Kwong et al., 2013

How improve skin phenotype in RD?

HYBRID TECHNOLOGY

LENTIVIRAL VECTORS+SLEEPING BEAUTY

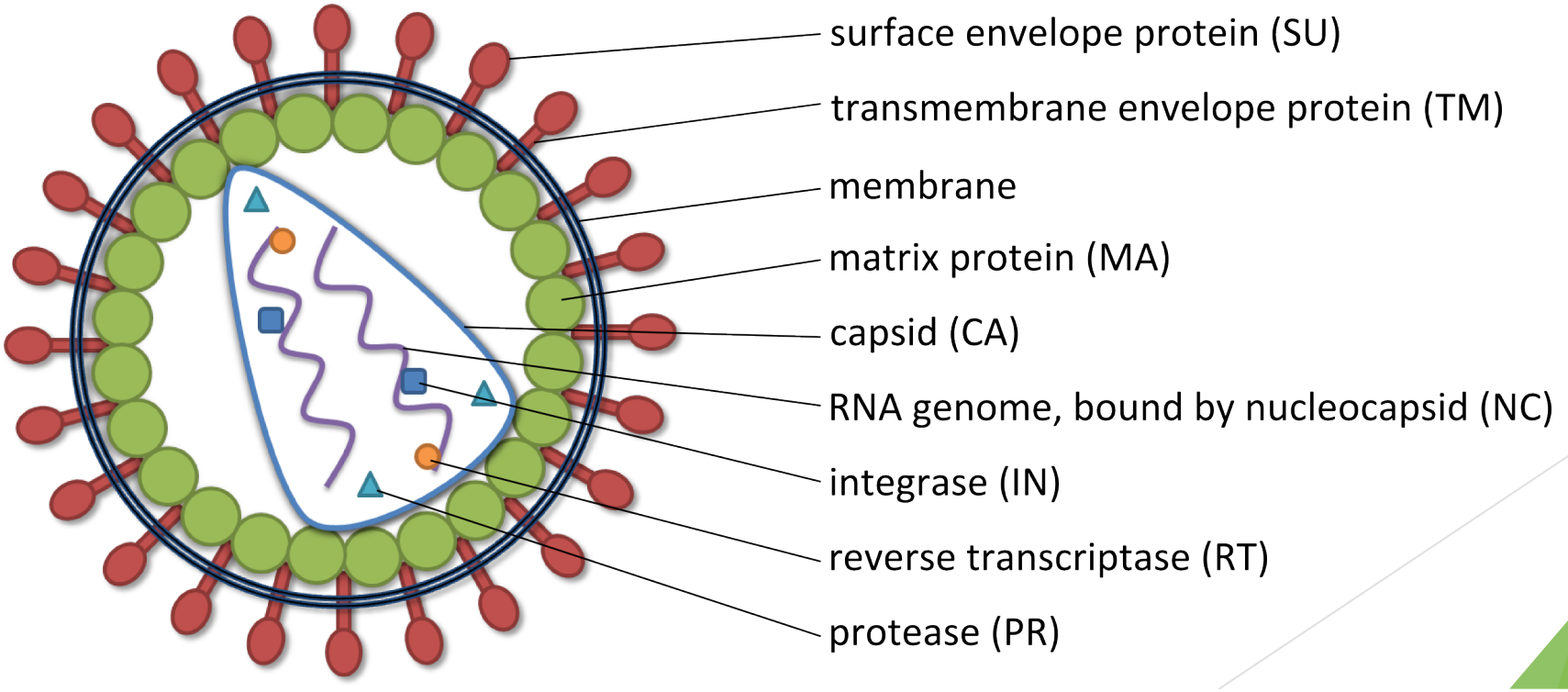
LENTIVIRAL VECTORS

Advantages

- Administration *in vivo*
- No inactivation by the complement
- Infect dividing and quiescent cells
- Large transgene (up to 10 kb).
- Stable integration and expression

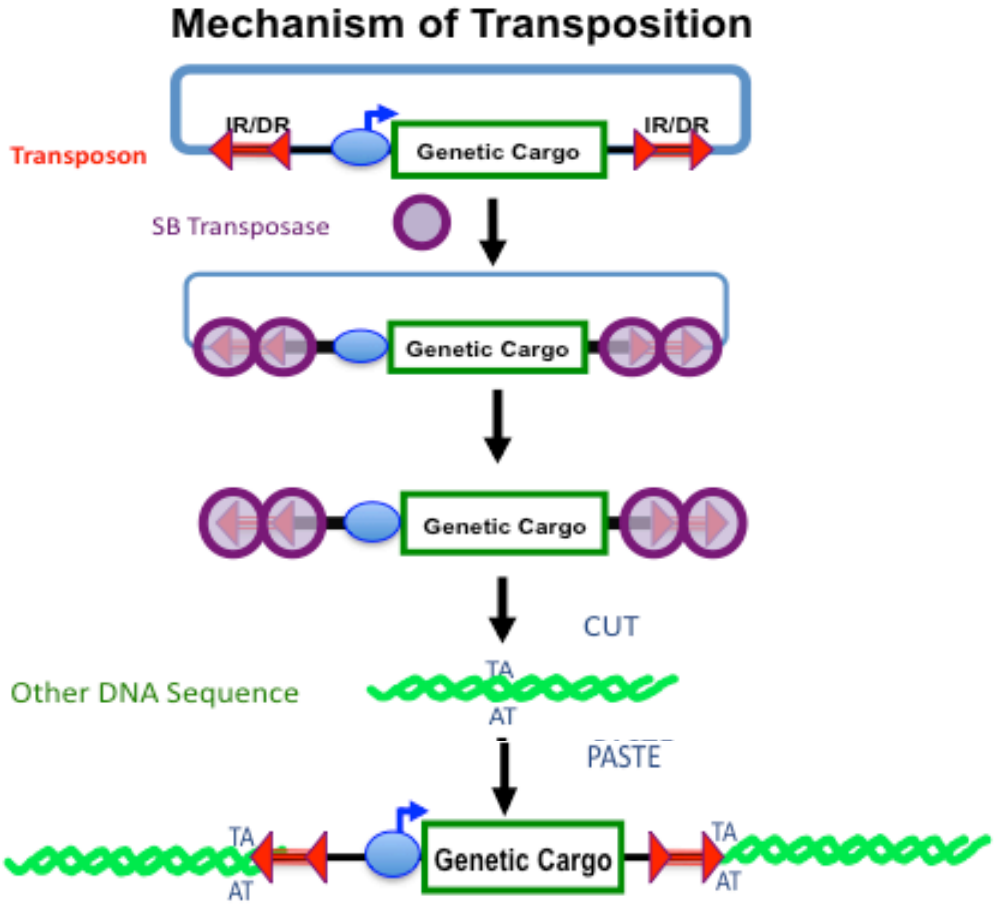
Disadvantages

- Random integration
- Oncogenic potential



SLEEPING BEAUTY

The Sleeping Beauty transposon system is an attractive gene delivery platform allowing stably integration of the gene of the interest through transposition into the target cell gene.



Advantages

- Random insertion in **TA sites** into intron or exon
- Low promotor/enhancer activity
- Conservation of sleeping beauty: it's not dangerous for the organism
- Insertion of a single copy of the gene in the genome
- Integrated gene is stable
- The transposase elevates the frequency of integration (100-fold or more)
- SB transposase is synthetic

Disadvantages

- Use of two vectors
- Inefficient delivery

Màtrai et al., 2009

LENTIVIRUS

- EFFICIENT DELIVERY
- HIGH INSERTIONAL MUTAGENESIS

SLEEPING BEAUTY

- INEFFICIENT DELIVERY
- LOW INSERTIONAL MUTAGENESIS

LV/Transposon Hybrid

The better of two worlds

Mobilization of DNA transposable elements from lentiviral vectors

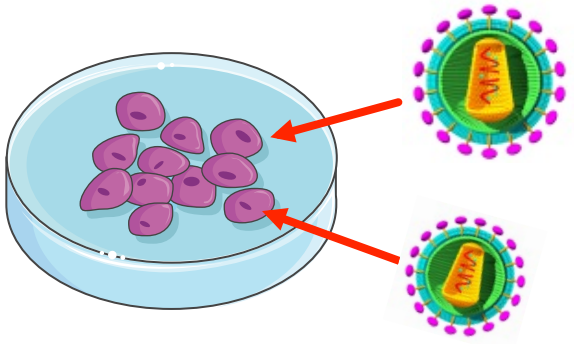
Rasmus O. Bak and Jacob Giehm Mikkelsen*
Department of Biomedicine, University of Aarhus, Aarhus C, Denmark

EXPERIMENTAL PLAN

pste24 -/- baby

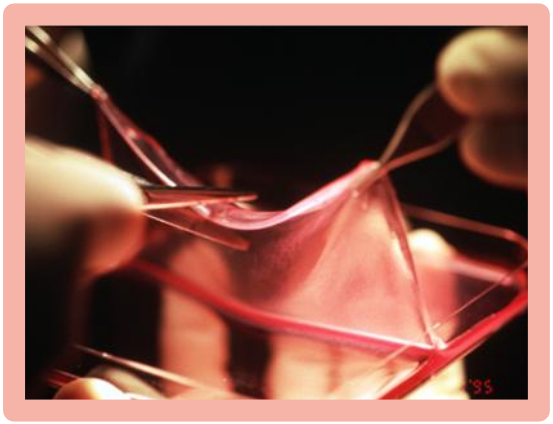
in vitro

Skin stem cells



Transfection

ex vivo



Skin tissue culture

Intravenous tail injection

BALB/c Nude Mouse

Stem Cells and Tissue-Engineered Skin

A. Charruyer and R. Ghadially
Department of Dermatology, University of California and Veterans Affairs Medical Center, San Francisco, Calif., USA

STRUCTURE OF VECTORS

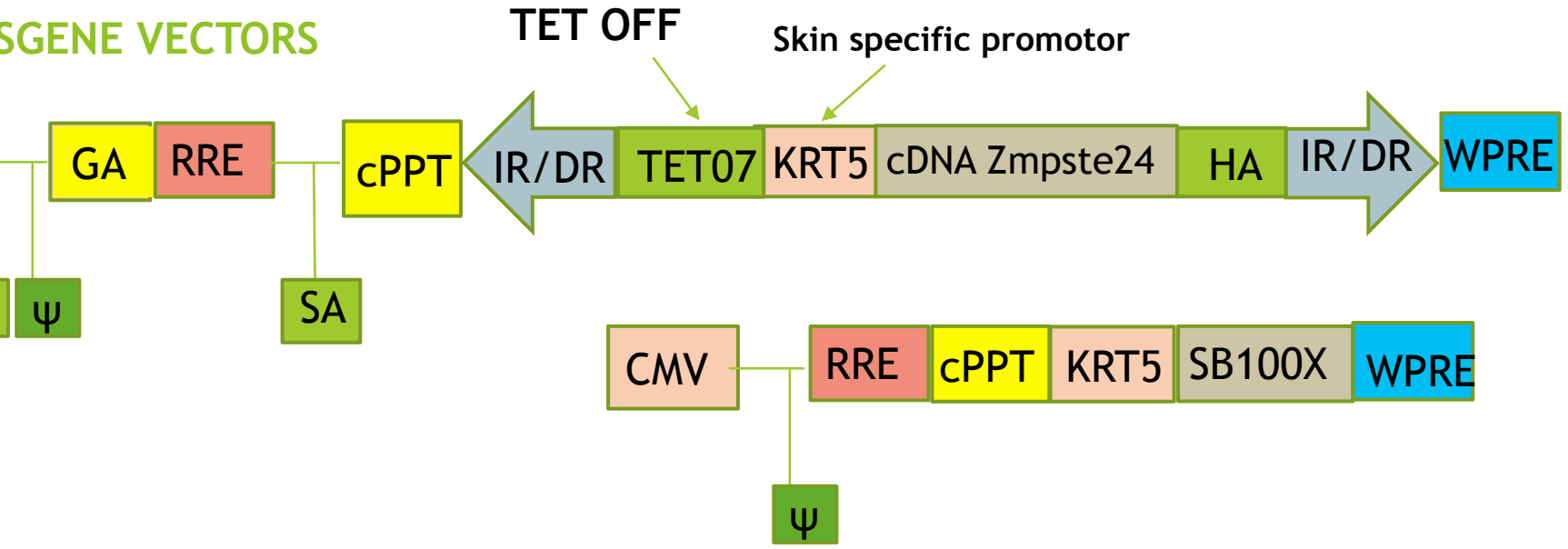
SD: Splice Donor
SA: Splice Acceptor

RRE: Exportation of mRNA from nucleus cytoplasm

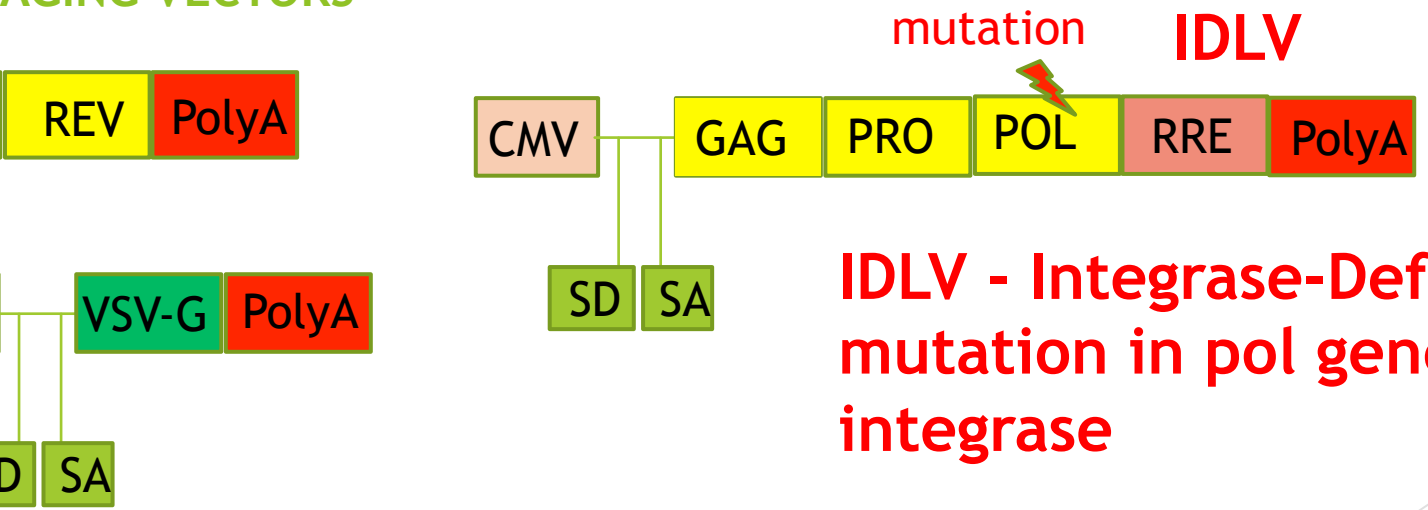
cPPT: central polypu tract sequence impo for transolacation in nucleus

WPRE: woodchuck posttranscriptional regulatory element

SGENE VECTORS

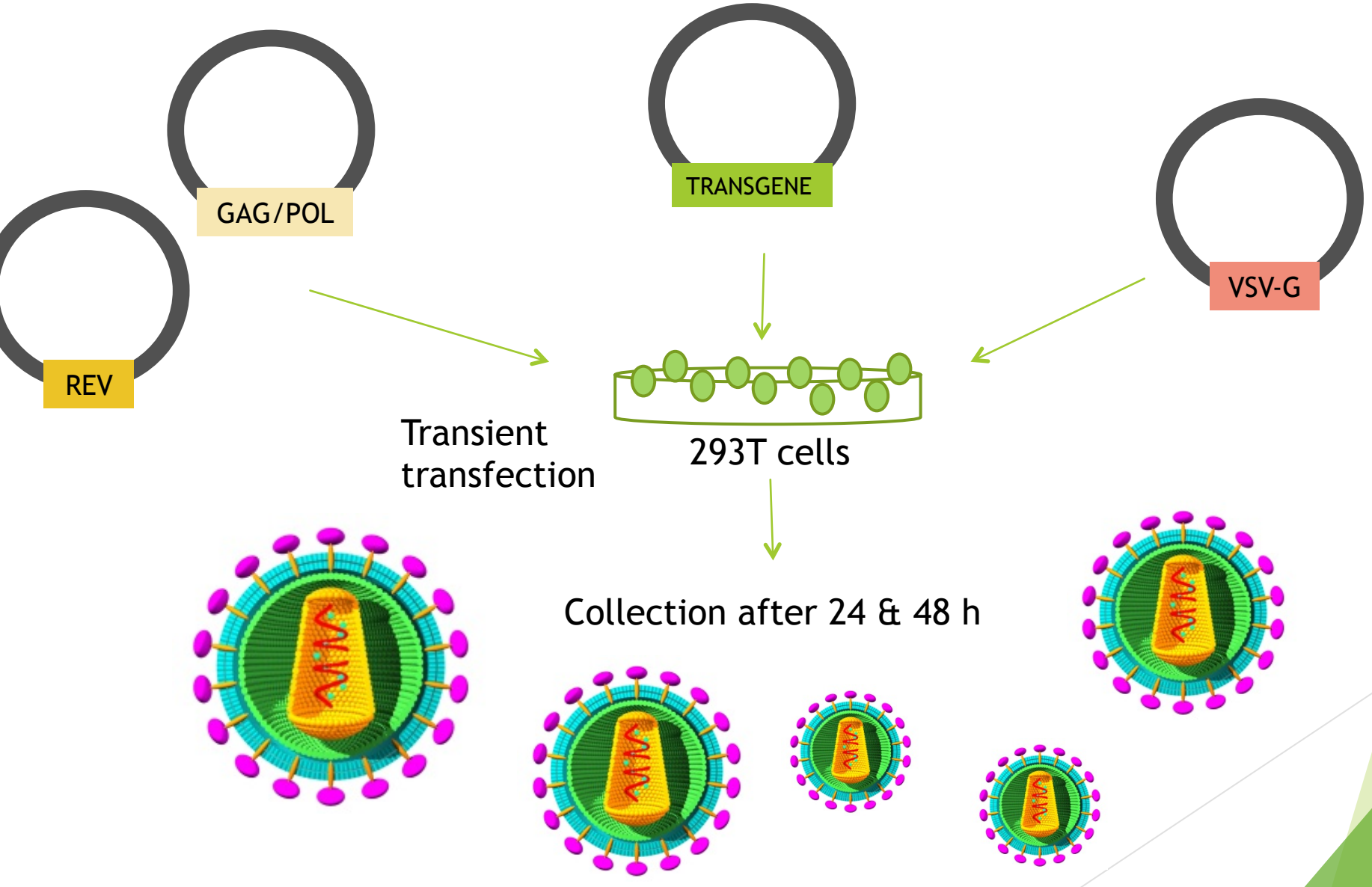


AGING VECTORS



IDLV - Integrase-Defective Lentiviral Vector: mutation in pol gene produces an inactive integrase

PRODUCTION OF ID-LENTIVECTORS



RD MODEL

vivo MODEL SYSTEM
ALB/c Nude Mouse

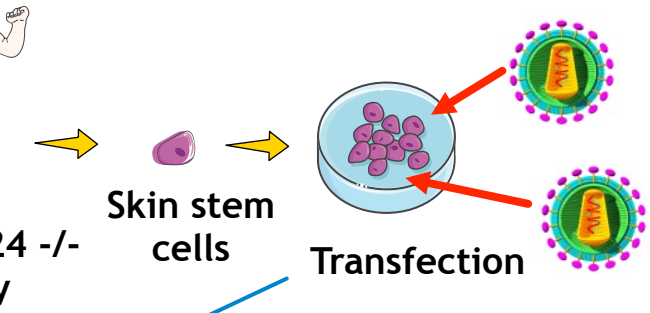
→ The animal lacks a thymus, is unable to produce T-cells, and is therefore immunodeficient

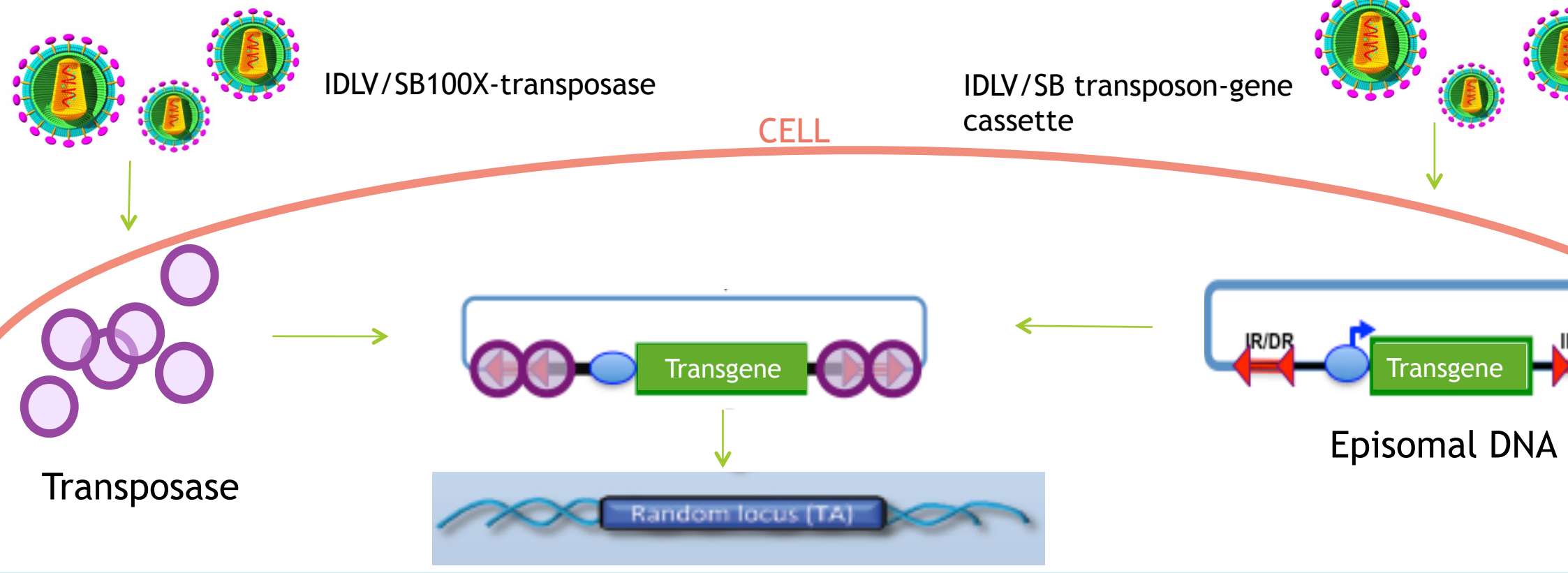
vitro MODEL SYSTEM
uman skin stem cells
mpste24 -/-

→ -Skin is the first tissue involved in RD
-Easy to extract
-Easy to culture *in vitro*
-Able to produce skin tissue

Cotransfection

How does LV/SB work?

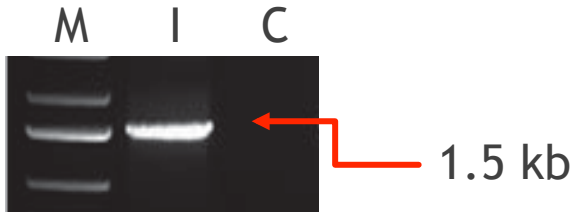




In vitro experiments

heck integration profile of Sleeping eauty :

. PCR → see if it is integrated
. LAM-PCR → site of integration

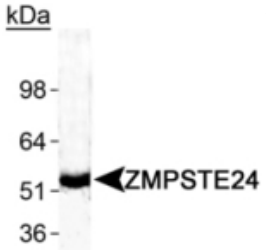


= Markers / I = Integration / C = Control

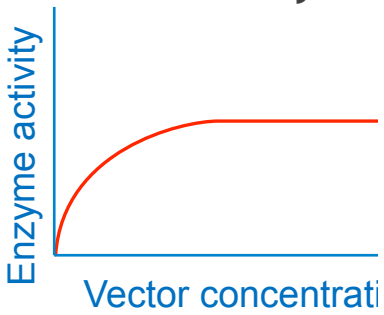
Presence and activity of the enzyme :

1. Western-blotting with ZMPSTE24 antibody
2. Titration of ZMPSTE24 activity
3. ELISA

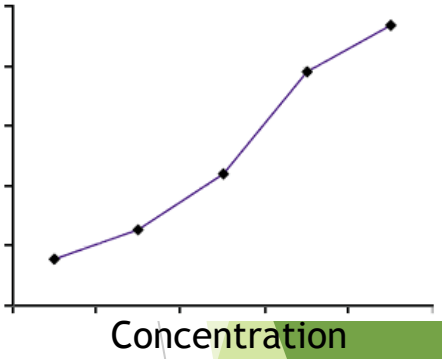
1.



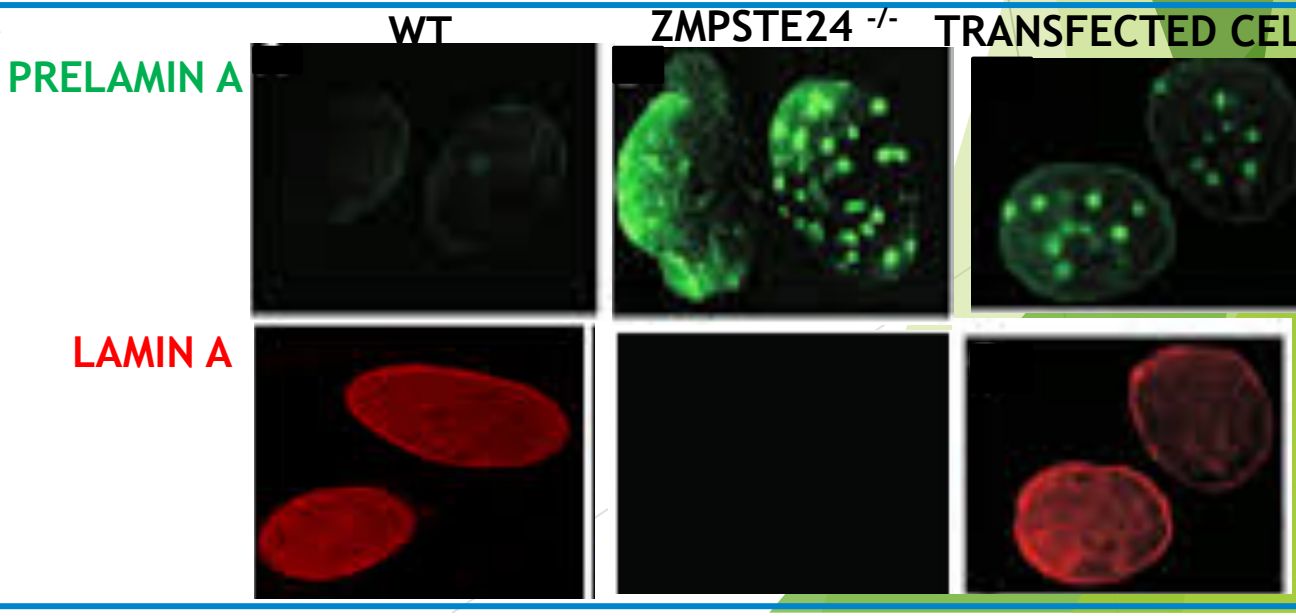
2.



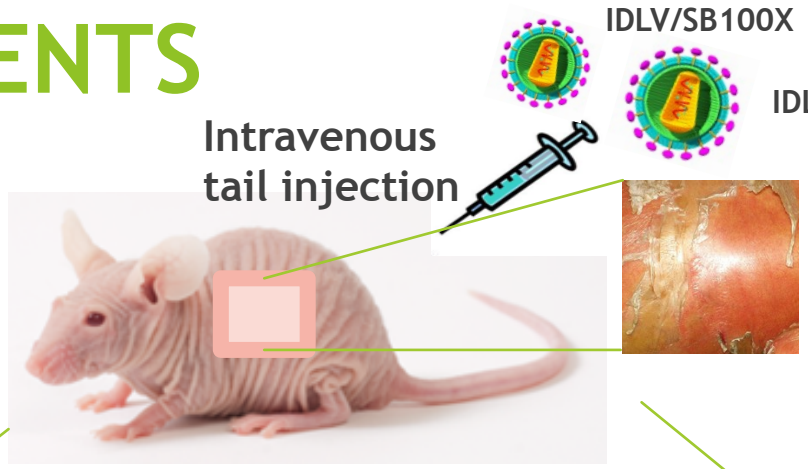
3.



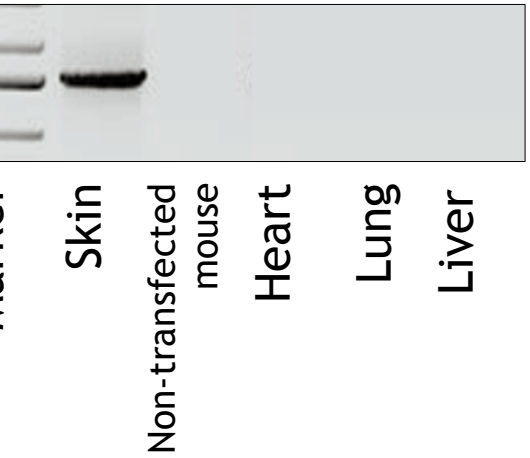
aturation of pre-lamin A is effective ith microscophy immunofluorence:



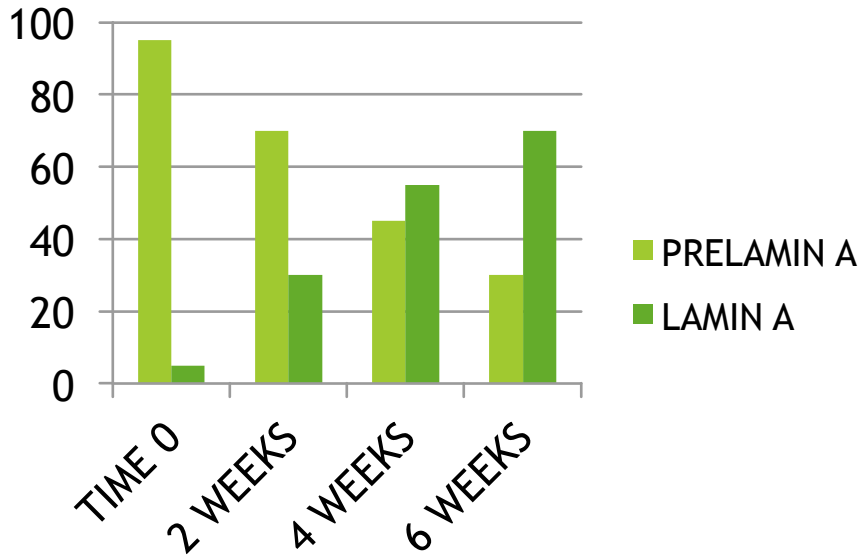
VIVO EXPERIMENTS



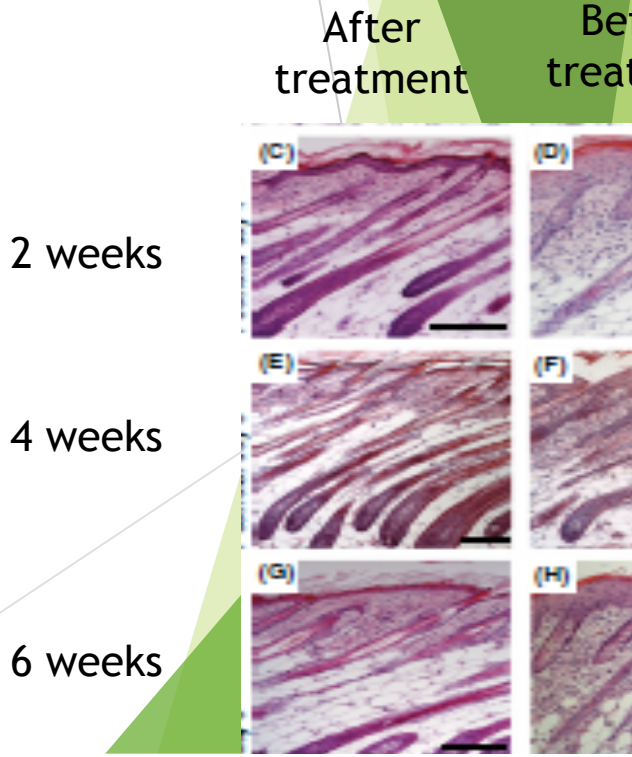
ck specificity and level of the Zmpste24
ession (Western-blot* & ELISA)
ck the decrease of Prelamin A (Western-blot)



Using Ab HA-tag



Microscopy on skin samples
to see the phenotype



Prospects:

After the validation of *in vivo* assay, clinical trials will start on infants, checking the efficiency of the vectors, its safety. Differents parameters will be followed as recovery of a functionnal pathway for maturation of prelamin A, recovery of a normal skin phenotype, survival of infants. Moreover this therapy could be applied to fix the other problems related to this disease in the other organs to improve the well-being of patients.

Pitfalls and solutions

- Proteins from cDNA could be non-functional because of a lack of post-transcriptional/translational modifications. This could be resolved using cDNA containing some important introns that are missing in our cDNA.
- Skin generation can be complicated and do not produce a skin layer that can be used. Indeed, skin tissue engineering is already existing and works but we don't know if the mutation will enable the production of a skin layer. In this case, we could change model:
  - Zmpste24 $^{-/-}$ mice which have accumulation of Prelamin A but they do not exhibit skin pathology;
  - LMNA $^{HG/+}$ mice in which Progerin is over-expressed and show a RD-like phenotype, but don't have mutation on Zmpste24.
- Zmpste24 is working but accumulation is still visible. Efficiency of the enzyme can be the cause, the activity is not enough to establish the wild type phenotype. Trial combining drug and the enzyme can be try to enhance the production of mature Lamin A. Moreover expression of enzyme could be enhanced with another stronger promoter.

MATERIALS AND COSTS:

-mouse BALB/c nude mouse: **250€**
- mouse WT (control): **50€**
- stabulation cost (each mice): **1000€**
- lamin A/C antibody: **302,15€**
- kit to extract skin stem cells: **1420€**
- struments used in lab (eppendorf, termomixer, vials, flasks, tubes, buffers, growth medium, chemical agents employed in our reserch, enzymes employed): **3000€**
- transfection and molecular analysis (antibodies, reagent PCR,, immunofluorescence, LAM PCR, western blot, GFP): **3000€**
- LENTIVIRUS: **440€**
-**SLEEPING BEAUTY** is cheaper than LV.

~ 25000 € / 3 years

ThermoFisher SCIENTIFIC charles river abcam® discover more

invitrogen™ INDUSTRIAL SCIENTIFIC CELL BIOLABS, INC. Creating Solutions for Life Science Research

BIBLIOGRAPHY:

1) R. O. Back, J. G. Mikkelsen. Mobilization of DNA transposable elements from lentiviral vectors. Mobile Genetics Element, 1:2, 139-144, 2011.
2) M. O. Bergo, B. Gavino, J. Ross, W. K. Schmidt, C. Hong, L. V. Kendall, A. Mohr, M. Meta, H. Genant, Y. Jiang, E. R. Wisner, M. van Bruggen, R. A. D. Carano, S. Michelis, S. M. Griffey, S. G. Young. Zmpste24 deficienty in mice caused spontaneous bone fractures, muscle weakness, and a prelamin A processing defect. Medical Sciences, 2002.
3) Y. Cai and J. G. Mikkelsen. Driving DNA transposition by lentiviral protein transduction. Mobile Genetic Elements, 4, 2014.
4) N. H. Staunstrup, B. Moldt, L. Mates, P. Villesen, M. Jakobsen, Z. Ivics, Z. Izsvak, J. G. Mikkelsen. Hybrid lentivirus-transposon vectors with a random integration profile in human cells. The American Society of Gene Therapy, 2009.
5) J. Màtrai, M. KL Chuah and T. VandenDriessche. Recent advances in lentiviral vector development and applications. The american society of gene and cell therapy, 2010.
6) A. Quigley, Y. Y. Dong, A. C. W. Pike, L. Dong, L. Shrestha, G. Berridge, P. J. Stensfeld, M. S. P. Sansom, A. M. Edwards, C. Bountra, F. von Delft, A. N. Bullock, N. A. Burgess-Brown, E. P. Carpenter. The structural basis of Zmpeste24 dependent laminopathies. Science, 2013.
7) J. Barrowman, C. Hanblet, M. S. Kane, S. Michaelis. Requirements for efficient proteolytic cleavage of prelamin A by Zmpste24. Plos one, 2012.
8) C. S. Sander, N. Salman, M. van Geel, J. L. V. Broers, A. Al-Rahmani, F. Chedid, I. Hausser, V. Ojii, K. Al Nuaimi, T. G. Berger, V. L. R. M. Verstraeten. Anewly identified splice site mutation in ZMPSTE24 causes restrictive dermopathy in the Middle East. British Journal ofDermatology, 2008.
9) B. SJ Davies, C. Coffinier, S. H Yang, R. H Barnes, HJ Jung, S. G. Young, L. G Fong. Investigating the purpose of prelamin A processing. Nucleus, 2011.
10) De Rosa L, Carulli S, Cocchiarella F, Quaglino D, Enzo E, Franchini E, Giannetti A, De Santis G, Recchia A, Pellegrini G, De Luca M. long-term stability and safety of transgenic cultured epidermal stem cells in gene therapy of junctional epidermolysis bullosa. Cell2014.
11) A.Coluccio et al.targeted gene addiction in human epithelial stem cell by Zinc-finger nuclease-mediated homologus recombination. Molecular Therapy, 2013.