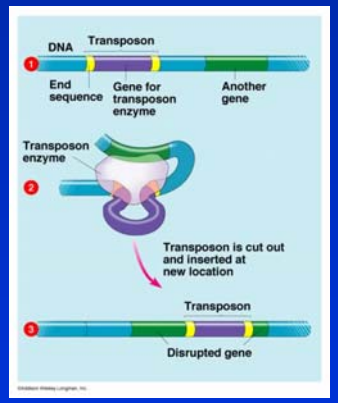
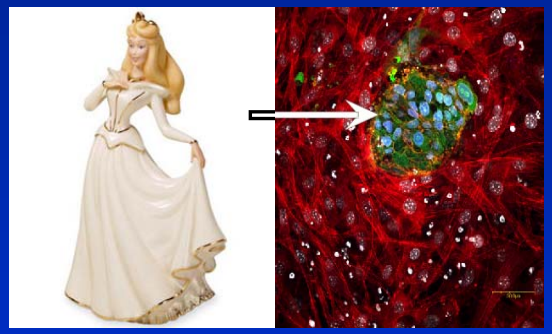
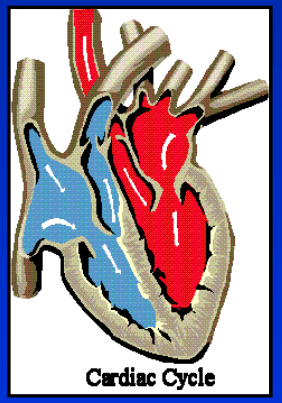
Eukaryotic DNA transposons and their use in modern molecular genetics







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Hungarian Research Network

What is really a transposon? # 1

- "jumping genes" – mobile genetic element is more correct

- Barbara McClintock discovered them in maize experiments: 1940's and 1950's
  Nobel Prize: 1983

Ac and Ds elements

A bit of nomenclature

- old nomenclature in prokaryotes:

- the more correct and up-to-date definition:

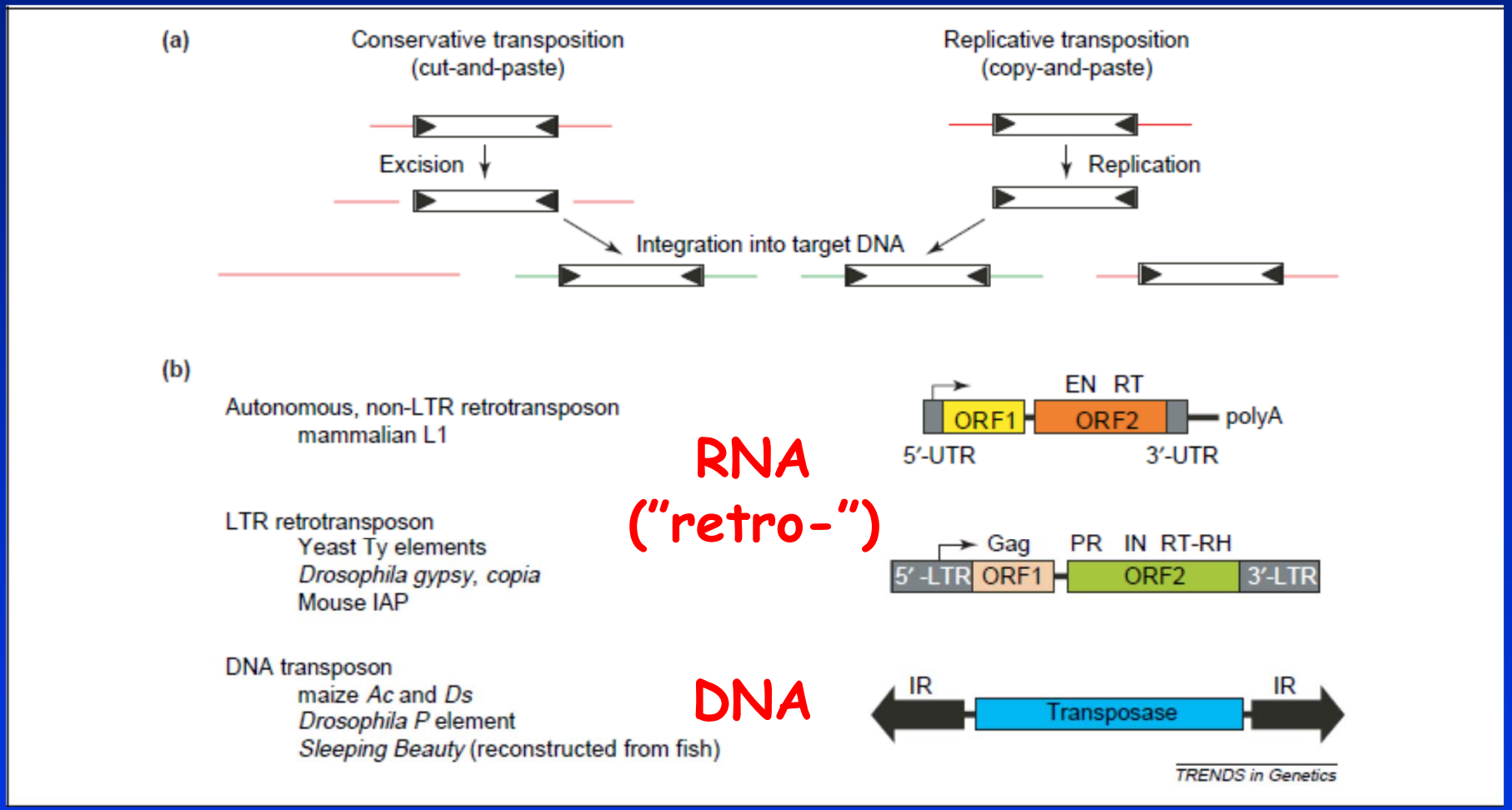
transposon = mobile genetic element

What is really a transposon? # 2

- molecular parasites? "selfish genes"?
  - partially true…

- but they are important players in genome evolution:
  - spreading antiobiotic resistance genes (especially in bacteria)
  - promoting genetic recombinations; sources of new genes: "domestications", e.g. RAG recombinases, Drosophila telomerase…
  - they represent at least 45% of the human genome !!!

Transposon/transposition types



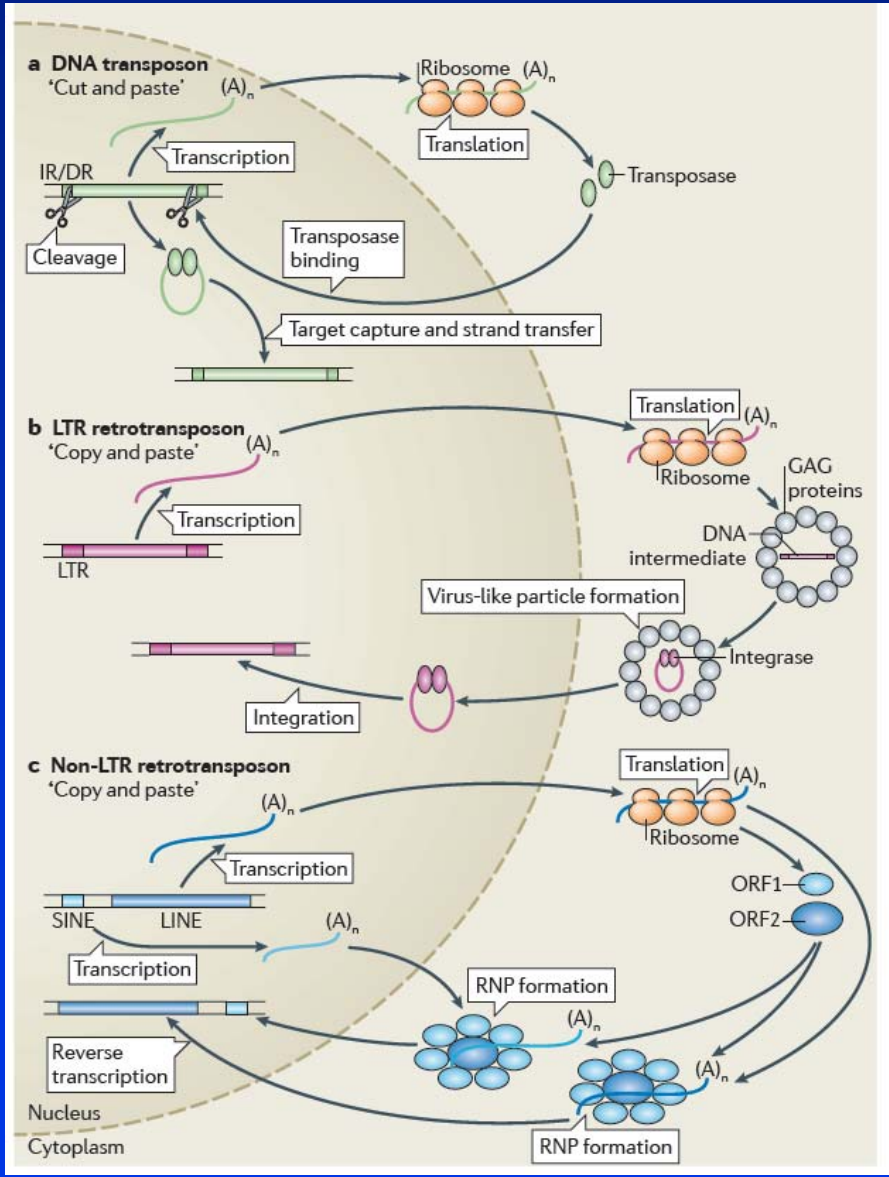
Trend Genet, 2005; 21(1):8

Classification of eukaryotic mobile elements

Classification		Structure	TSD	Code	Occurrence
Order	Superfamily				
Class I (retrotransposons)					
LTR	*Copia*	GAG AP INT RT RH	4–6	RLC	P, M, F, O
	Gypsy	GAG AP RT RH INT	4–6	RLG	P, M, F, O
	Bel–Pao	GAG AP RT RH INT	4–6	RLB	M
	Retrovirus	GAG AP RT RH INT ENV	4–6	RLR	M
	ERV	GAG AP RT RH INT ENV	4–6	RLE	M
DIRS	*DIRS*	GAG AP RT RH YR	0	RYD	P, M, F, O
	Ngaro	GAG AP RT RH YR	0	RYN	M, F
	VIPER	GAG AP RT RH YR	0	RYV	O
PLE	*Penelope*	RT EN	Variable	RPP	P, M, F, O
LINE	*R2*	RT EN	Variable	RIR	M
	RTE	APE RT	Variable	RIT	M
	Jockey	ORF1 APE RT	Variable	RIJ	M
	L1	ORF1 APE RT	Variable	RIL	P, M, F, O
	I	ORF1 APE RT RH	Variable	RII	P, M, F
SINE	*tRNA*		Variable	RST	P, M, F
	7SL		Variable	RSL	P, M, F
	5S		Variable	RSS	M, O
Class II (DNA transposons) - Subclass 1					
TIR	*Tc1–Mariner*	Tase*	TA	DTT	P, M, F, O
	hAT	Tase*	8	DTA	P, M, F, O
	Mutator	Tase*	9–11	DTM	P, M, F, O
	Merlin	Tase*	8–9	DTE	M, O
	Transib	Tase*	5	DTR	M, F
	P	Tase	8	DTP	P, M
	PiggyBac	Tase	TTAA	DTB	M, O
	PIF–Harbinger	Tase* ORF2	3	DTH	P, M, F, O
	CACTA	Tase ORF2	2–3	DTC	P, M, F
Crypton	*Crypton*	YR	0	DYC	F
Class II (DNA transposons) - Subclass 2					
Helitron	*Helitron*	RPA Y2 HEL	0	DHH	P, M, F
Maverick	*Maverick*	C-INT ATP CYP POL B	6	DMM	M, F, O

(Nat Rev Genet, 2007; 8:973)

Transposition mechanisms in brief



Nat Rev Mol Cell Biol, 2011; 12(4):246

But the euk. genome is protected by:

- promoter methylation, chromatin modifications
- RNA interference:
  - endogenous siRNAs
  - piRNAs
  (- also certain miRNAs)

Which is more prevalent in genomes?

DNA transposons

or

retrotransposons?

Transposons are everywhere…

Human Genome ~3200 Mb		# of Copies (×1000)	Total Length (Mb)	% of Genome	Active
LINEs		**868**	**558.8**	**20.42**	
LINE1[1]		516	462	16.89	Active
LINE2		315	88.2	3.22	
LINE3		37	8.4	0.31	
SINEs		**1558**	**359.6**	**13.29**	
Alu[1]		1090	290.1	10.6	Active using L1 RT
MIR		393	60.1	2.2	
MIR3		75	9.3	0.34	
SVA[1]		2.76	4.2	0.15	Active using L1 RT
LTR retro-transposons		**443**	**227**	**8.29**	
ERV class I		112	79.2	2.89	
ERV (K) class II		8	8.5	0.31	
ERV (L) class III		83	39.5	1.44	
MaLR		240	99.8	3.65	
DNA transposons		**294**	**77.6**	**2.84**	
hAT	Charlie	182	38.1	1.39	
	Zaphod	13	4.3	0.16	
Tc-1	Tigger	57	28	1.02	
	Tc2	4	0.9	0.03	
	Mariner	14	2.6	0.1	
PiggyBac-like		2	0.5	0.02	
Unclassified		22	3.2	0.12	

Cell, 2008; 135:poszter

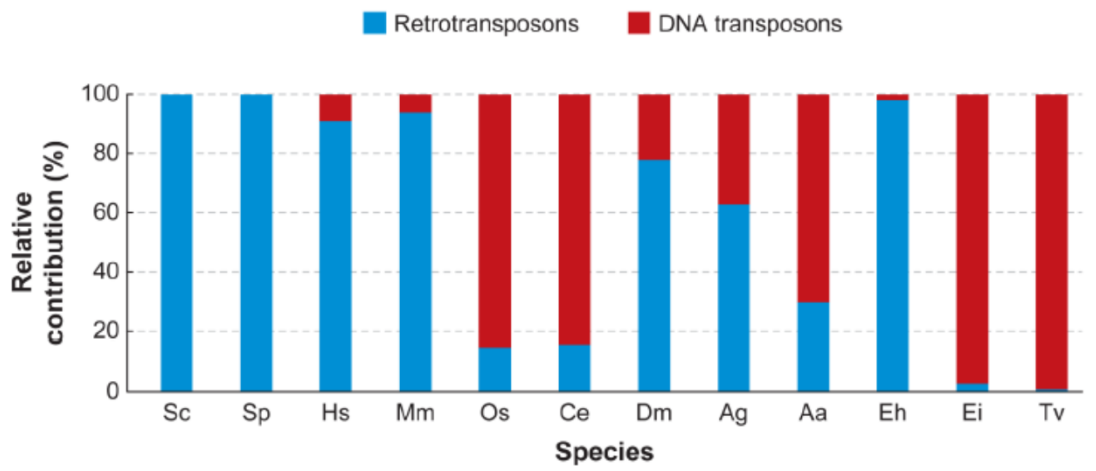
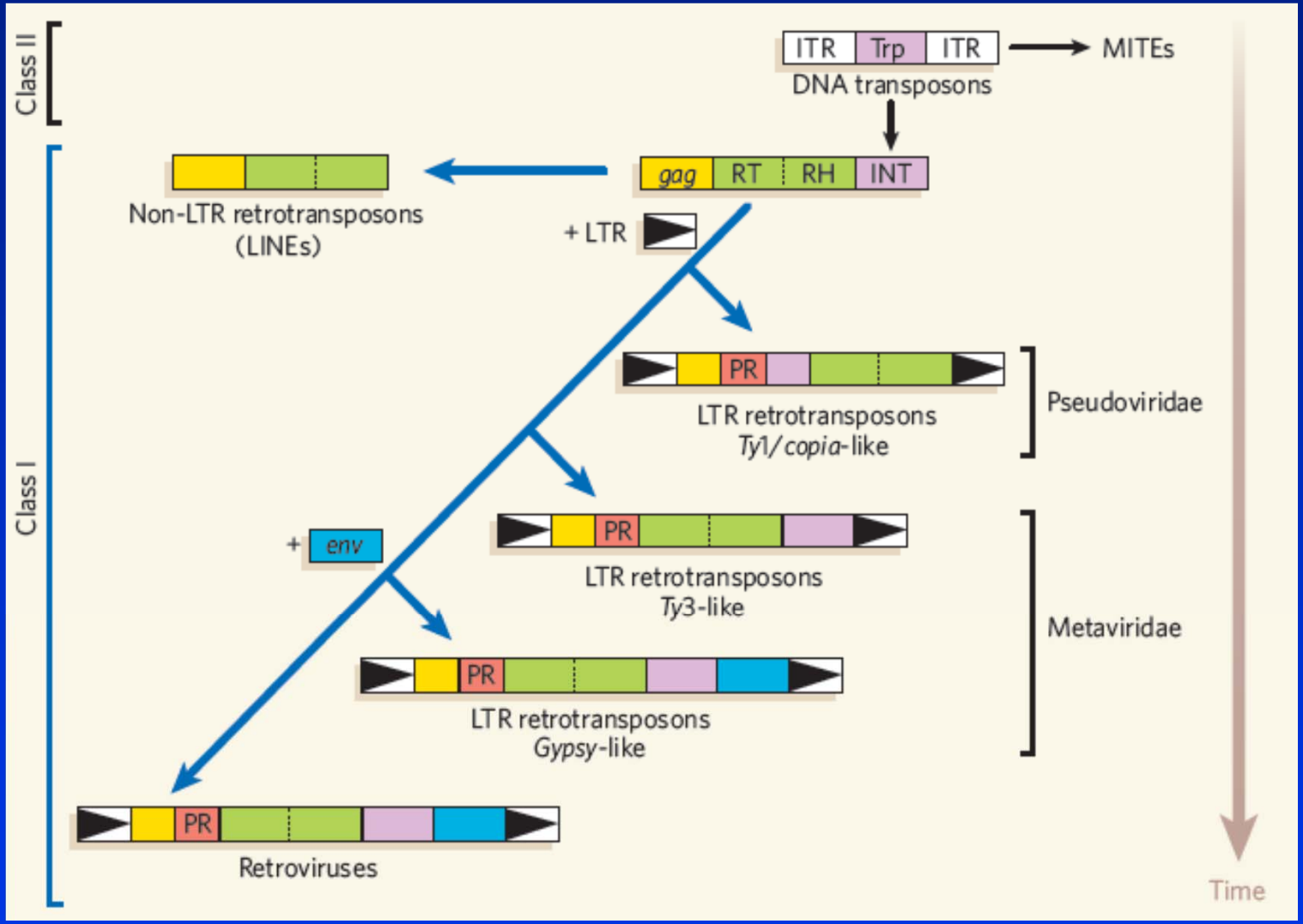


Figure 2.
The relative amount of retrotransposons and DNA transposons in diverse eukaryotic genomes. The graph shows the contribution of DNA transposons and retrotransposons in percentage relative to the total number of transposable elements in each species. The data were compiled from papers reporting draft genome sequences (references available upon request) and from the Repeatmasker output tables available at the UCSC Genome Browser (http://genome.ucsc.edu) or from the following sources: *E. histolytica* and *E. invadens*: (159); *T. vaginalis*: E. Pritham, unpublished data. Species abbreviations: Sc: *Saccharomyces cerevisiae*; Sp: *Schizosaccharomyces pombe*; Hs: *Homo sapiens*; Mm: *Mus musculus*; Os: *Oryza sativa*; Ce: *Caenorhabditis elegans*; Dm: *Drosophila melanogaster*; Ag: *Anopheles gambiae*, malaria mosquito; Aa: *Aedes aegypti*, yellow fever mosquito; Eh: *Entamoeba histolytica*; Ei: *Entamoeba invadens*; Tv: *Trichomonas vaginalis*.

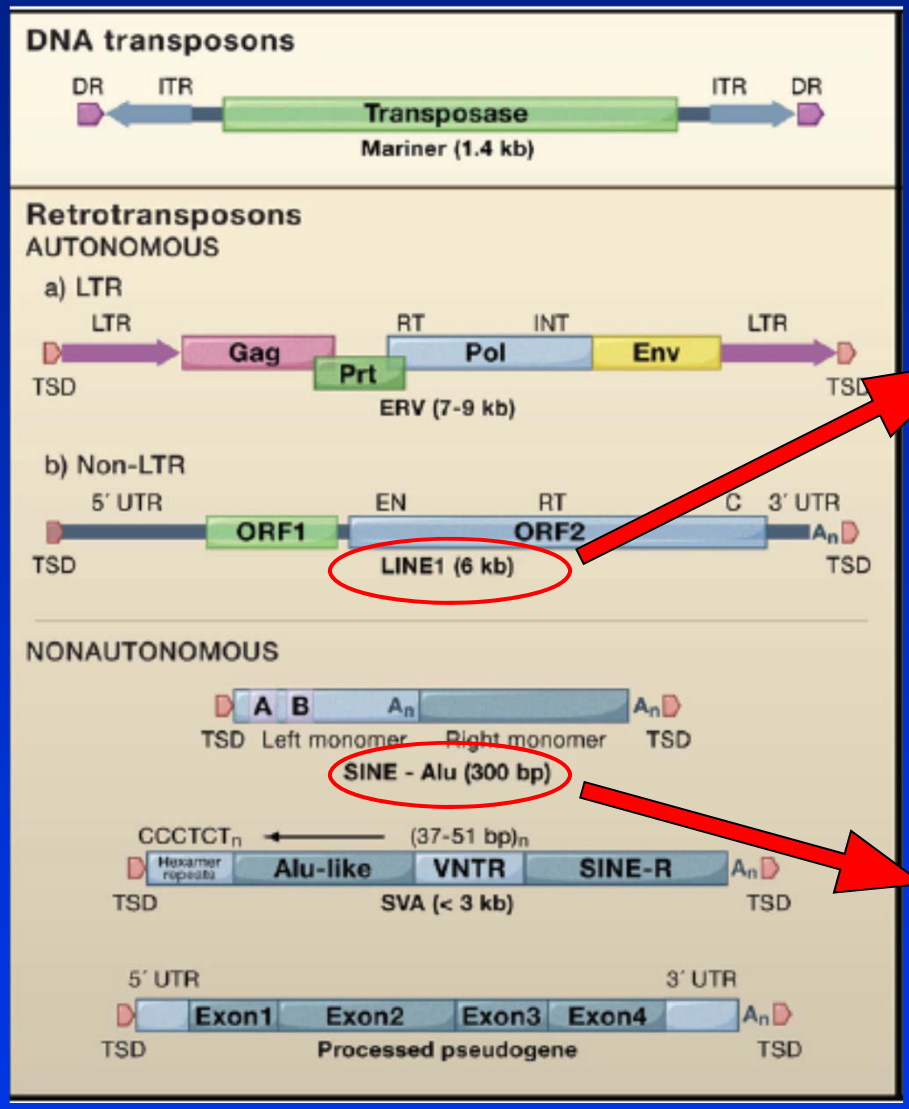
Annu Rev Genet, 2007; 41:331

A bit of (transposon) evolution # 1



Nature, 2006; 443(7111):521

A bit of (transposon) evolution # 2



Cell, 2008; 135(1):23

LINE1 elements become active in neuronal progenitors →
somatic mosaicism in neurons!
(Trend Neurosci 2010; 33(8):345)

'Alu' elements are extremely numerous in primates; a great progress in genome evolution →
a reason or a consequence?
(PNAS, 2011; 108(7):2837; NSMB, 2016; 23(11):1011)

Questions...

Transposons as genetic tools

1. **Applications:**
   - insertional mutagenesis
   - cloning, gene traps
   - gene delivery → transgenic animals

2. **Invertebrate model organisms:**
   - D. melanogaster → P-element (a new acquisition!)
   - C. elegans → Tc1/Mariner superfamily

3. **Vertebrates: DNA tnp tools were missing for long time**
   → retrotransposons have some drawbacks:
   - ☹ higher mutation rate (reverse transcription)
   - ☹ can be re-mobilized (genetic instability)
   - ☹ unfavorable integration profile

Used vertebrate transposon systems

Eukaryotic transposon:

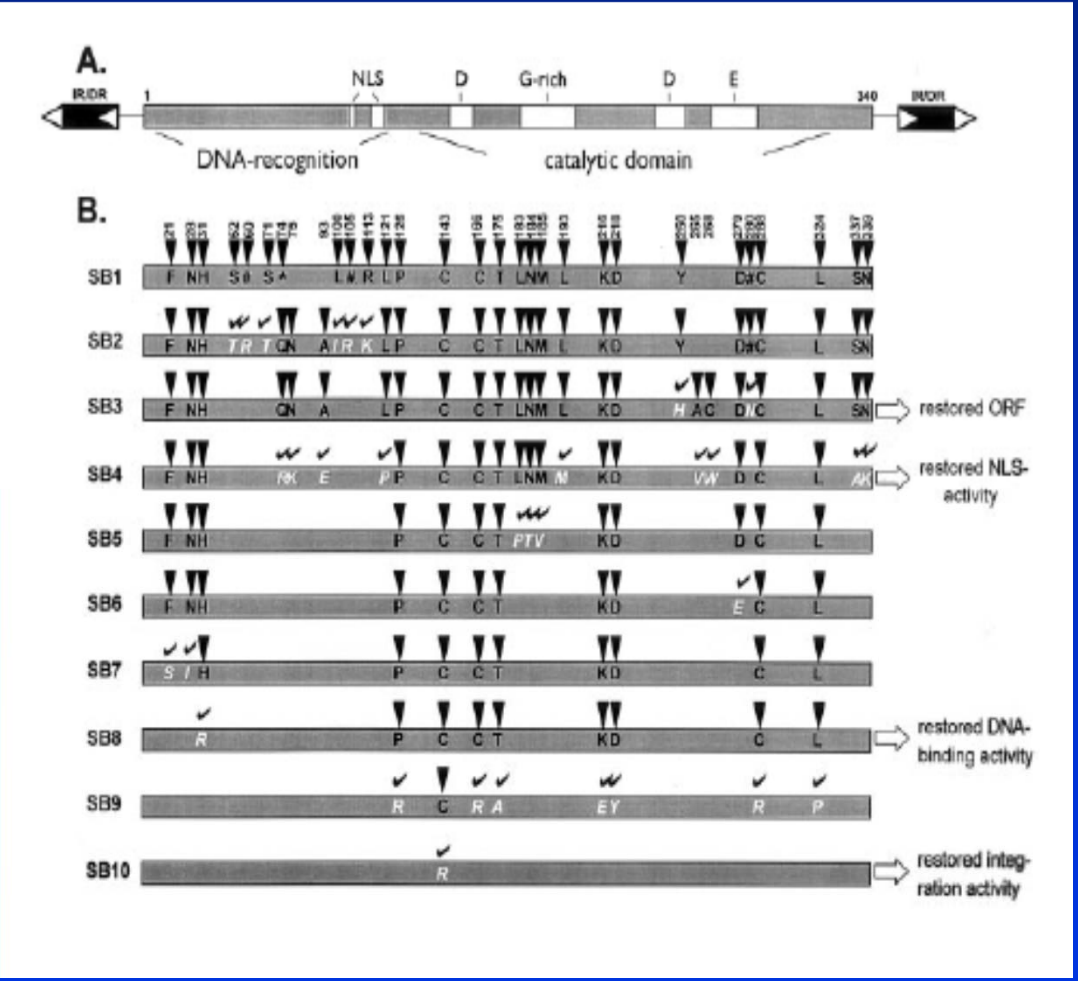
- Class II (DNA transposons)
  - Tc1/mariner superfamily

"cut & paste" transposition mechanism

„Sleeping Beauty"

„Frog Prince"

Sleeping Beauty / Frog Prince origin

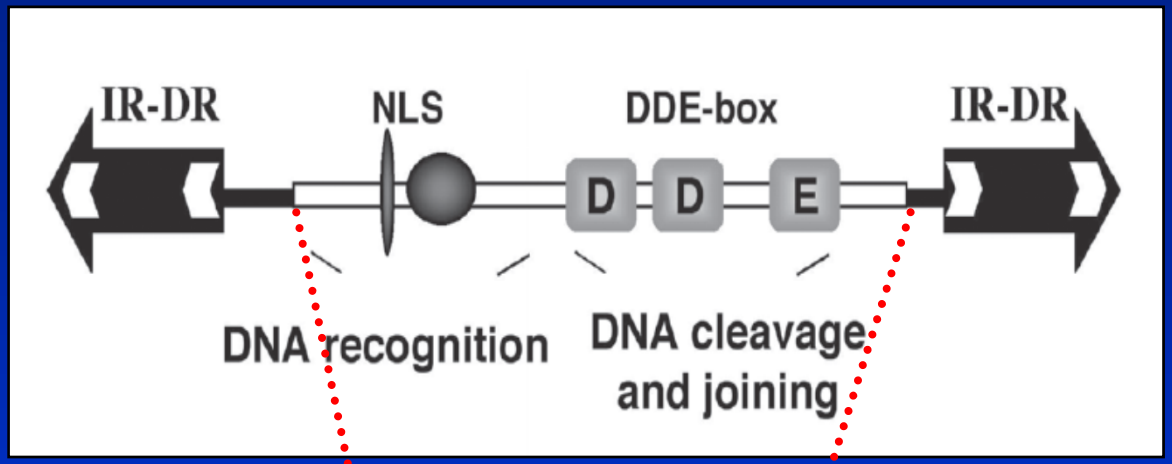


Fish & frogs:
start from inactive (dead) elements

transposase activity resurrected by directed *in vitro* mutagenesis

Zoltán Ivics, Perry B. Hackett, Ronald H. Plasterk and Zsuzsanna Izsvák, Cell, 1997; 91:501
Csaba Miskey, Zsuzsanna Izsvák, Ronald H. Plasterk and Zoltán Ivics, NAR, 2003; 31:6873

Structure of the active transposon



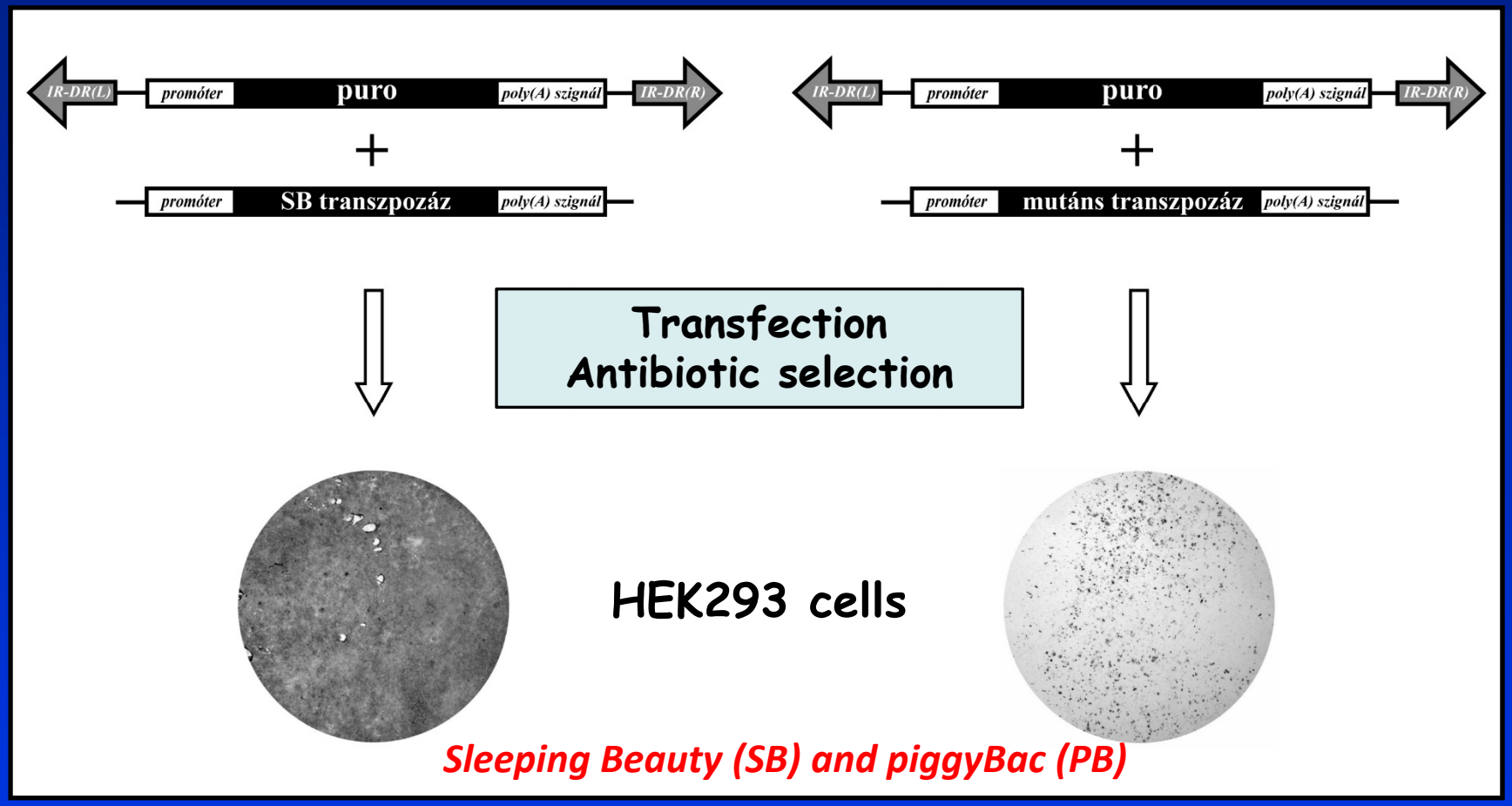
Transposase

IR | your favorite gene | IR

CMV | transposase

Ivics and Izsvak, Methods in Molecular Biology, 2004; 260: 255-276.

DNA transposons: gene delivery tools



Kolacsek et al. (2011) Mobile DNA

Kolacsek et al. (2014) Human Gene Therapy Methods

Advantages of DNA transposons versus viral vectors

- Cheaper, easier to make
- Less safety concerns
- Random integration profile,
  no preference towards active genes (→ true for SB)
  (⇔ *piggyBac or Tol2 !*)
- Activity in non-dividing cells
- But transfection efficiency is a limiting factor…

One more argument… SB100x

Molecular evolution of a novel hyperactive *Sleeping Beauty* transposase enables robust stable gene transfer in vertebrates

Lajos Mátés[1,6], Marinee K L Chuah[2,6], Eyayu Belay[2], Boris Jerchow[1], Namitha Manoj[1], Abel Acosta-Sanchez[2], Dawid P Grzela[1], Andrea Schmitt[1], Katja Becker[1], Janka Matrai[2], Ling Ma[2], Ermira Samara-Kuko[2], Conny Gysemans[3], Diana Pryputniewicz[1], Csaba Miskey[1], Bradley Fletcher[4], Thierry VandenDriessche[2], Zoltán Ivics[1] & Zsuzsanna Izsvák[1,5]

Nat Genet, 2009; 441(6):753

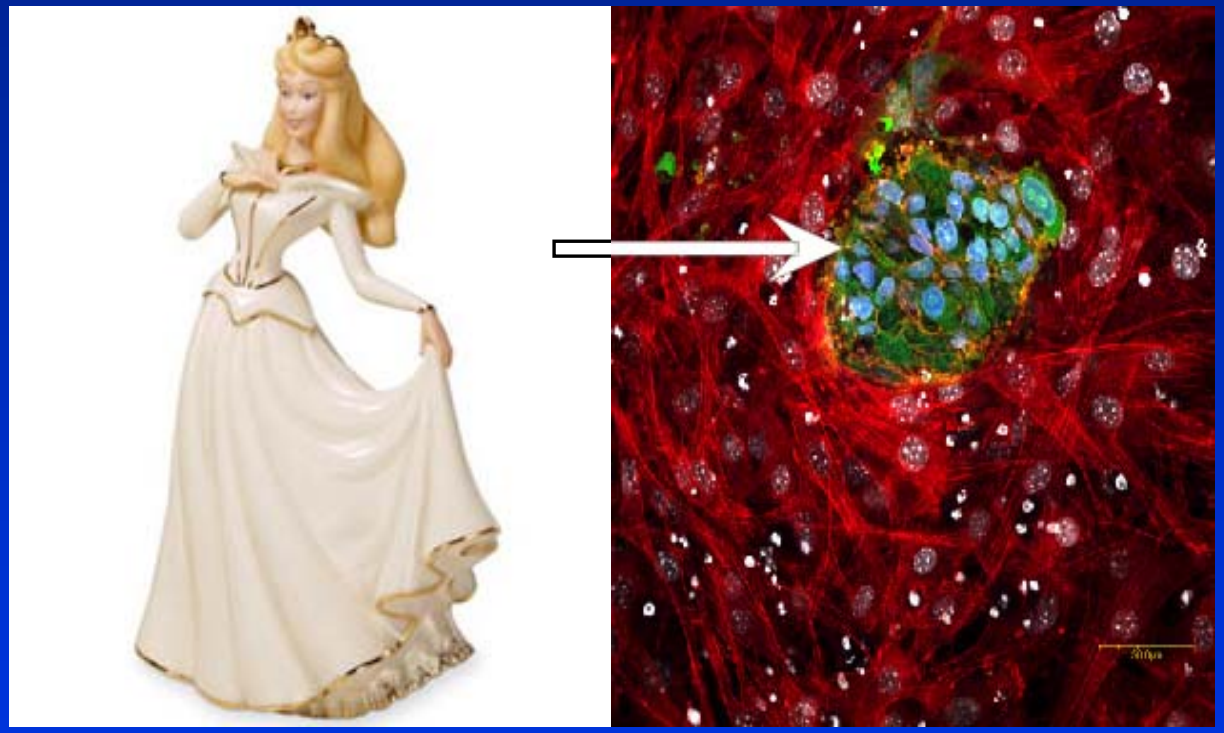
The activity of the new hiperactive SB100x transposase is comparable to that of the most efficient viral-based gene delivery tools.

SB100x transposase was the 'molecule of the year' in 2009 – selected by the Science journal:

http://www.biotechniques.com/news/Sleeping-Beauty-named-Molecule-of-the-Year/biotechniques-187068.html?autnID¼191663

Questions...

Gene delivery into human embryonic stem cells the *Sleeping Beauty* transposon system

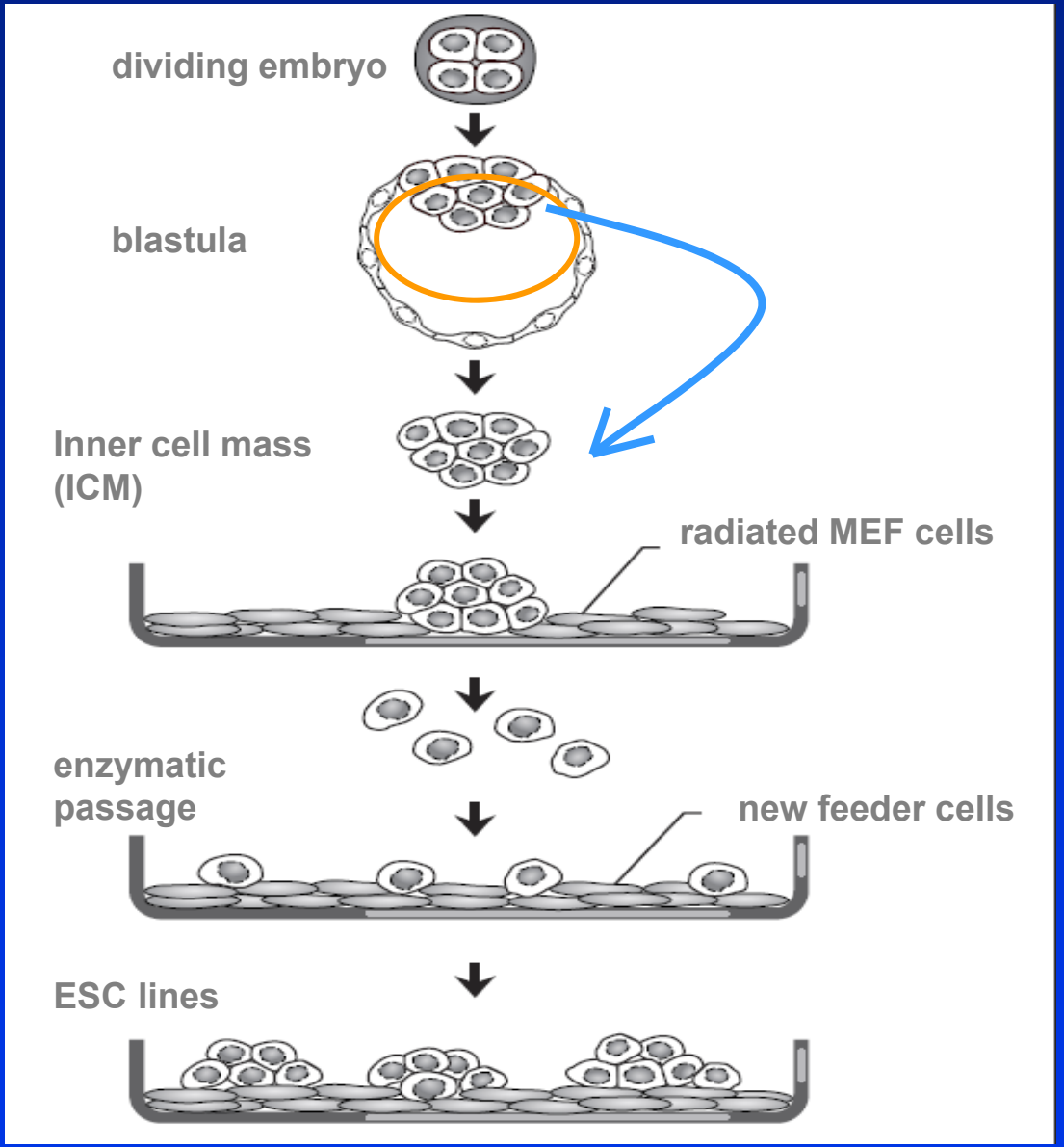


Origin of human embryonic stem cell lines

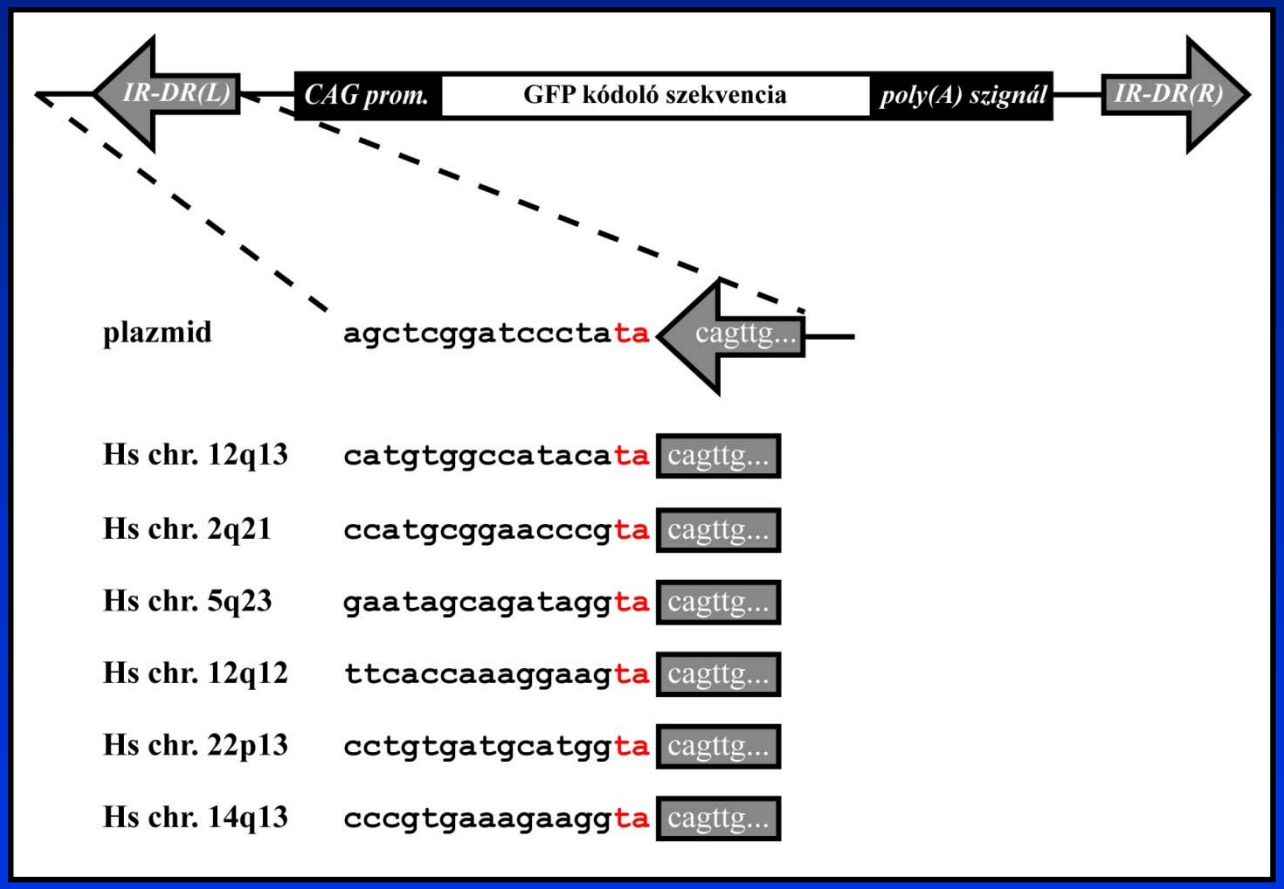
there are two distinct cell populations with distinct differentiation potentials

- inner cell mass
- trophectoderm cells

cells of ICM are cultured

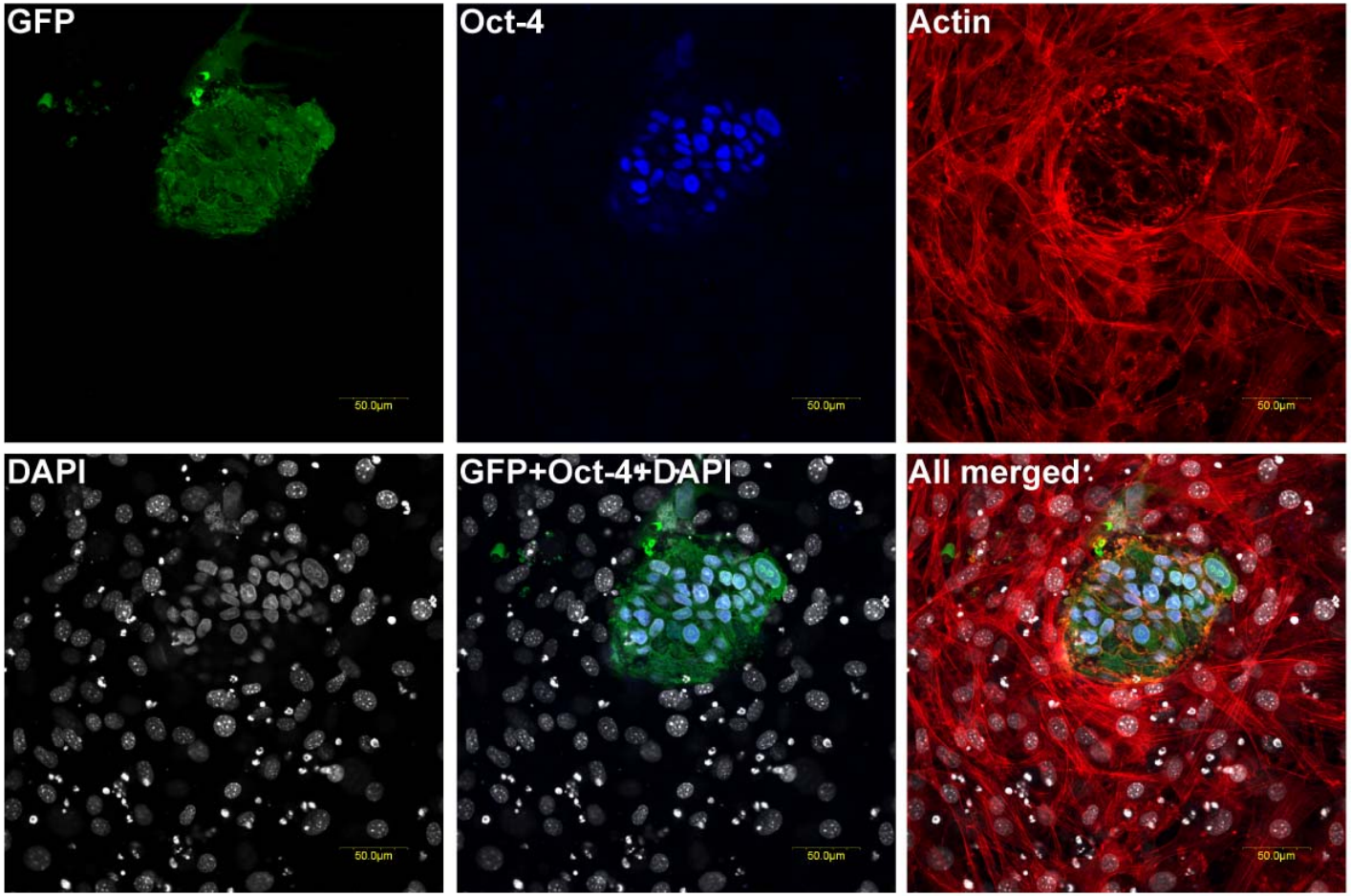


Proof of transposition: integration sites



Orbán et al. (2009) Stem Cells

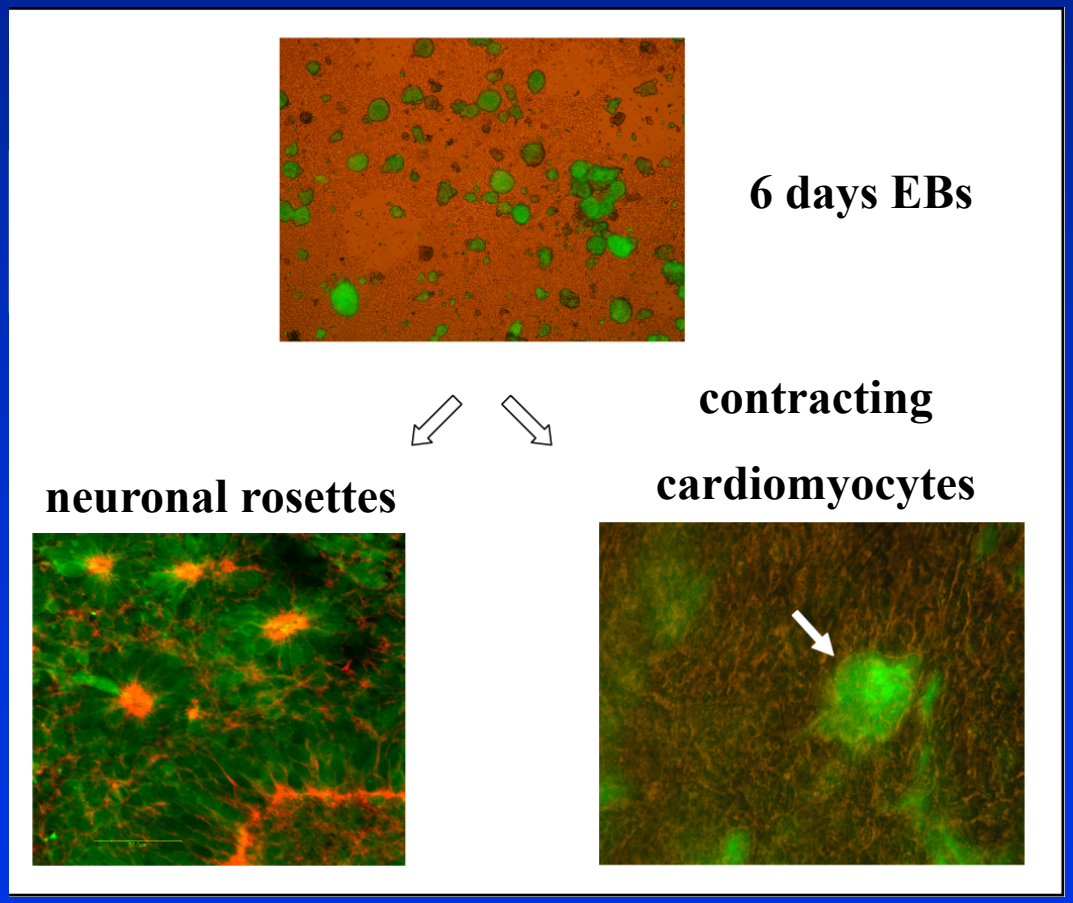
Pluripotency of GFP-expressing clones: the Oct4 protein as an example



Orbán et al. (2009) Stem Cells

Differentiation of stem cell clones

- spontaneous differenctiation via embryoid bodies (EB): teratoma-like structures

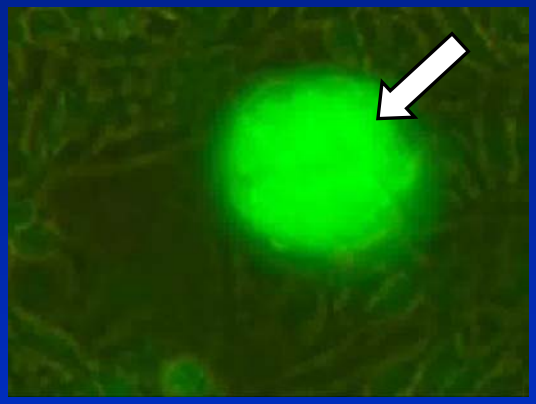


CAG promoter:
strong expression
in cardiomyocytes ?!

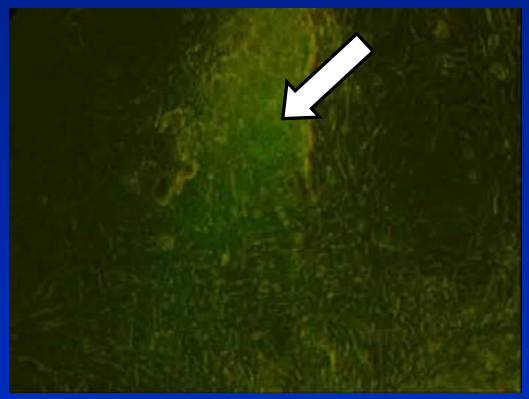
Orbán et al. (2009) Stem Cells

Differentiation toward cardiomyocytes

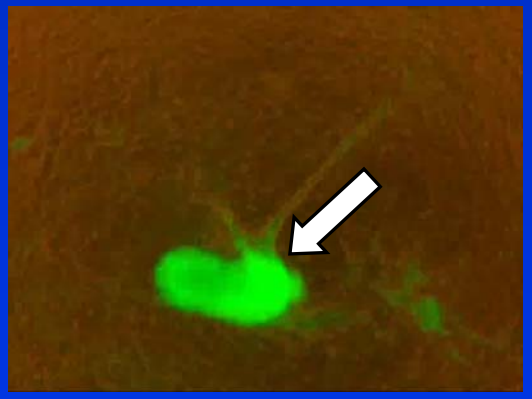
SB-CAG-GFP



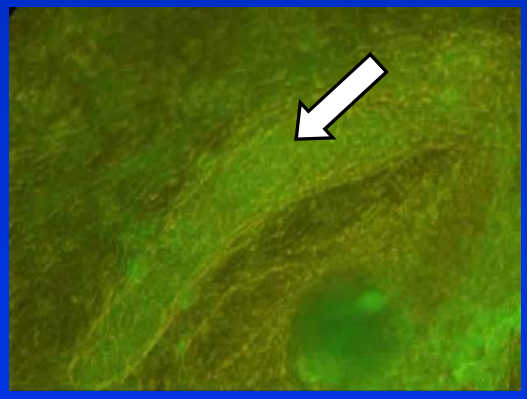
SB-EF1α-GFP



LV-CAG-GFP

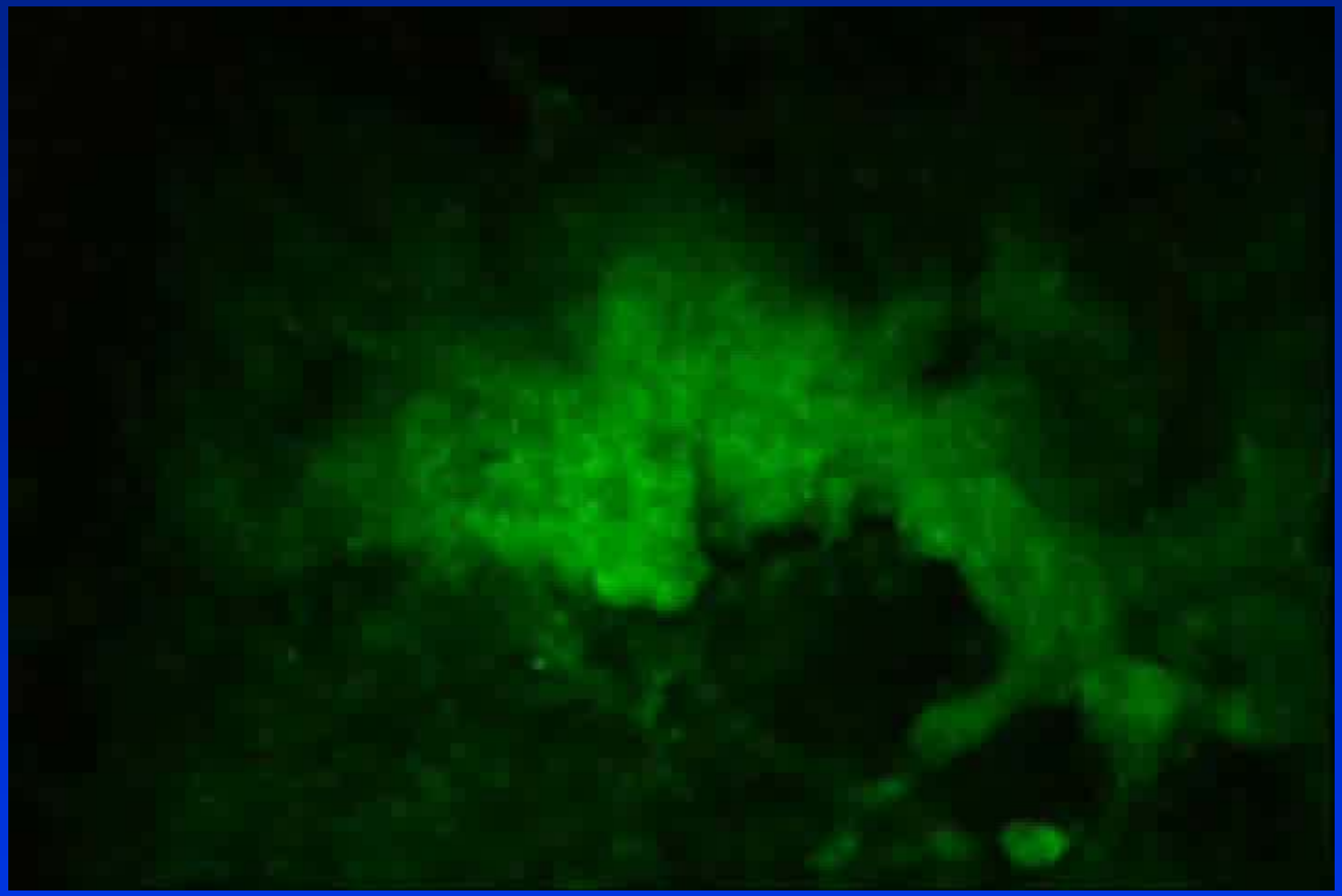


LV-EF1α-GFP



Orbán et al. (2009) Stem Cells

Pharmacological testing

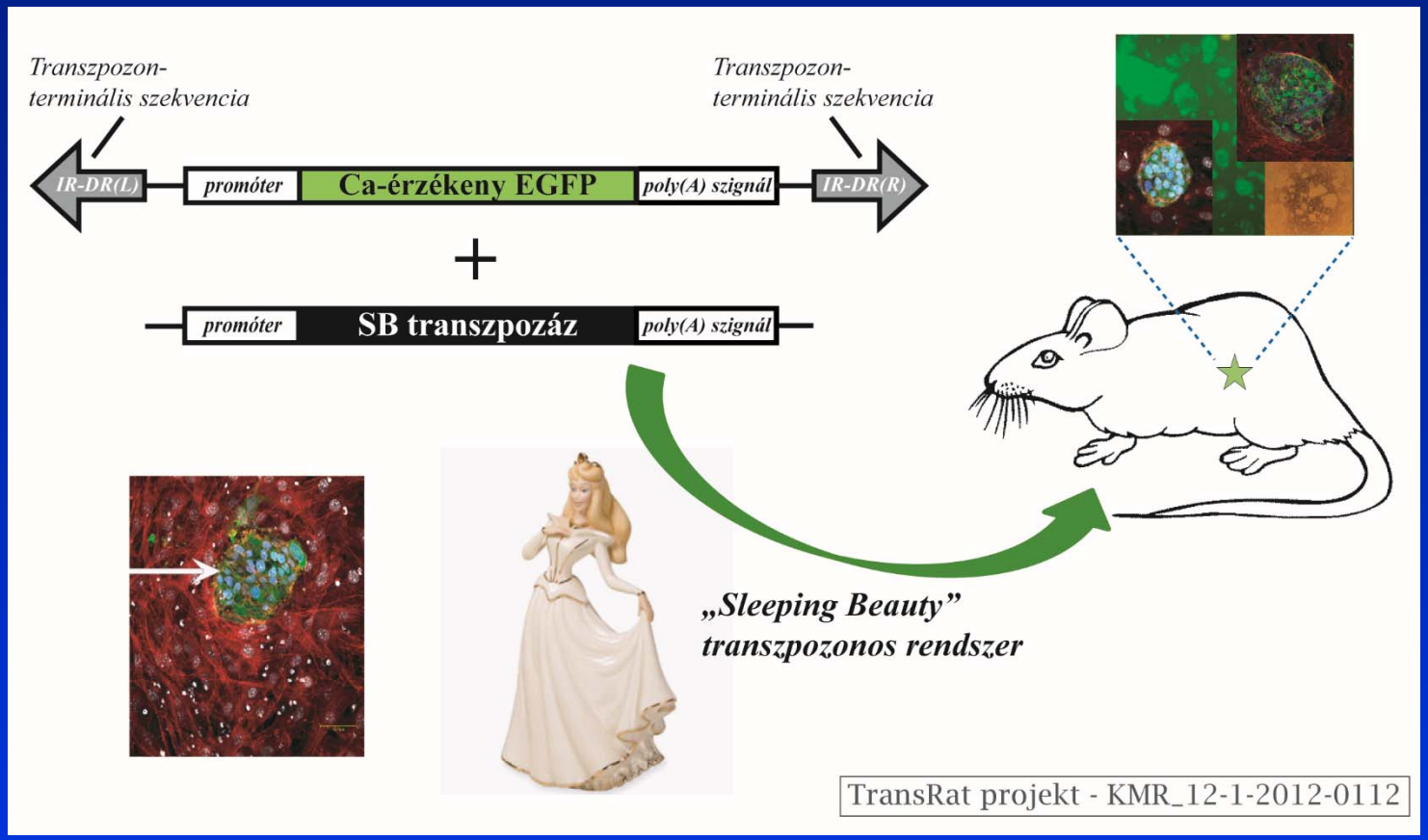


+adrenalin / +verapamil

Orbán et al. (2009) Stem Cells

Questions...

Transgenic rats established using transposons

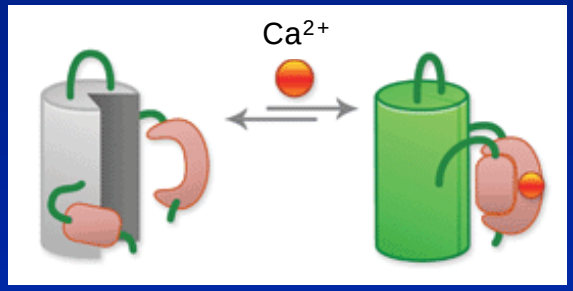


CAG-GCaMP2 / rGFA-RGECO

Szebényi et al. (2015) J Am Soc Nephrol

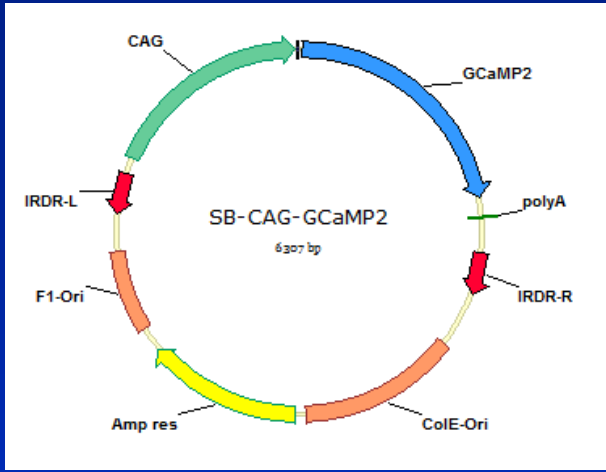
Szebényi et al. (2015) Scientific Reports

Calcium signals with GCAMP2

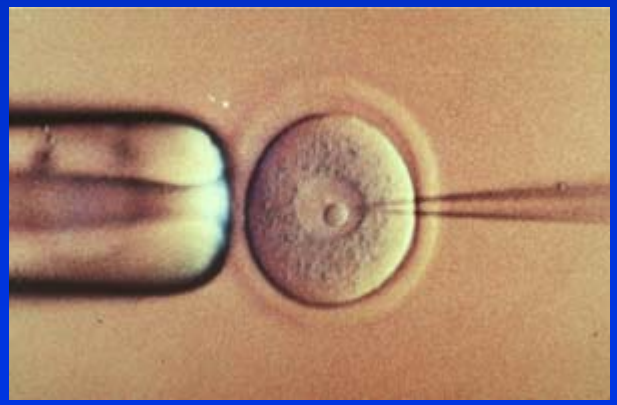


GCaMP2: - calcium-sensitive GFP
- a calmodulin domain is used

Transgene microinjection in zygotes



+ *SB100x* as mRNA source



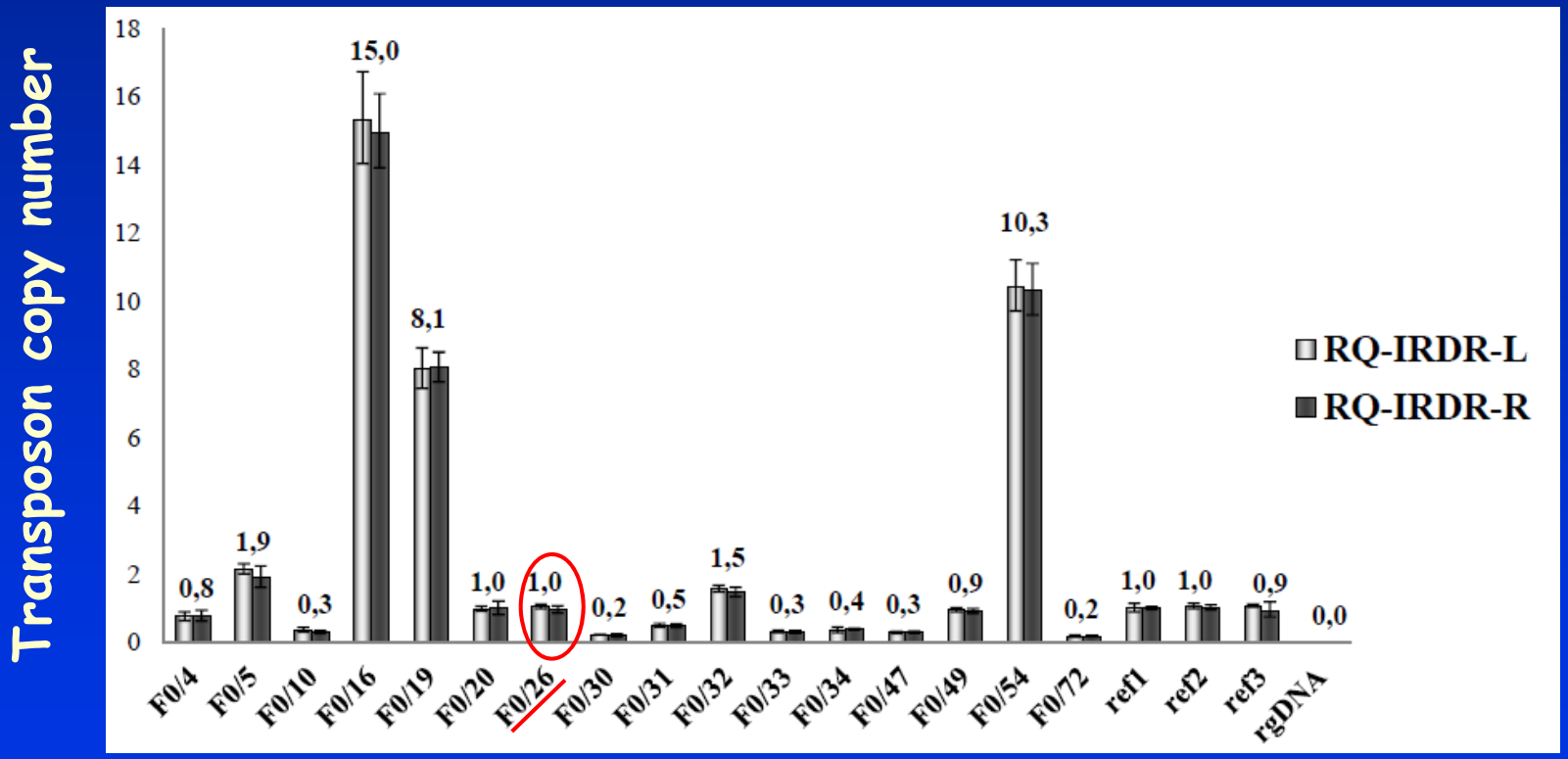
1. Microinjection into the male pro-nucleus
2. Implant into pseudopregnant females
3. Founder (F0) generation is born

(Oocytes from Sprague-Dawley strain into Wistar female recipients)

Genetic screen of the F0 generation

PCR and real-time PCR → selection based on transgene copy number

16 carriers among 75 newborns → ~21% efficiency!

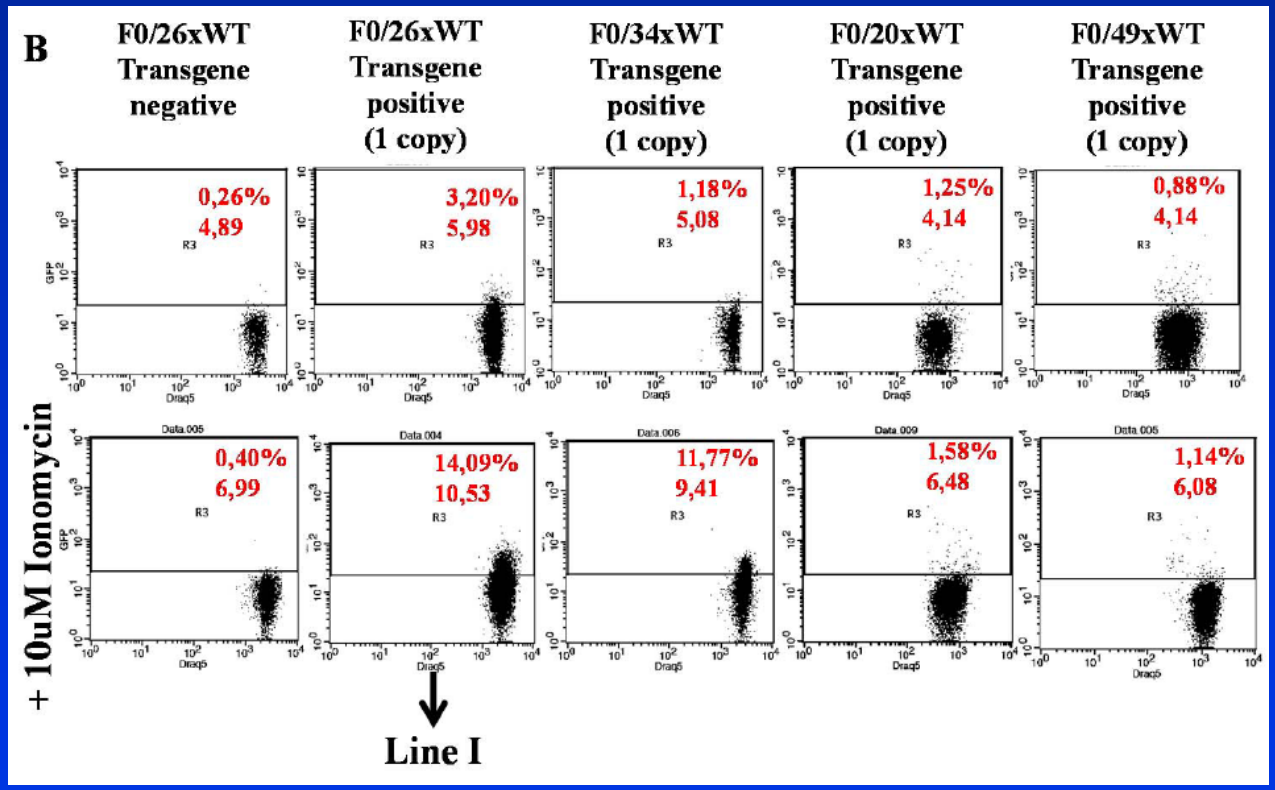


Szebényi et al. (2015) Scientific Reports

Establishing and screening the F1 generation

Start: crossing low copy number F0 rats with WT individuals

Phenotype screen of F1 generation:
GCaMP2 expression in leukocytes, FACS-based measurement



+ Ionomycin

↓

Ca^{2+} level ↑

↓

GCaMP2 exp.↑

Szebényi et al. (2015) Scientific Reports

Establishing a stable line (2 transgene copies)
(1 copy / haploid genome)

- Several crosses with WT individuals
- Crossing heterozygotes, inbreeding
- Verification: genetic stability, phenotype monitoring

stable copy number (2)
and integration site
over >25 generations

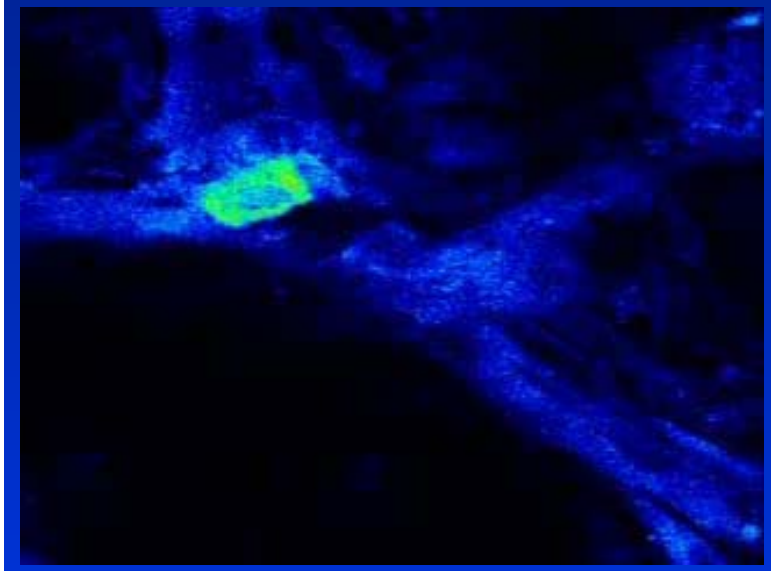
GGGACTAGGTTGGGCTAAGAGTGAAGACTCTTTAGC
TGTCGTTCTATGGCAATCCTGACAGGATTCCACTCCC
CTTGTAAAGCAGGTA**CAGTTGAAGTCGGAAGTTTA**
CATACACCTTAGCCAATCACTAGTGAATTCGCGGC
CGCCT

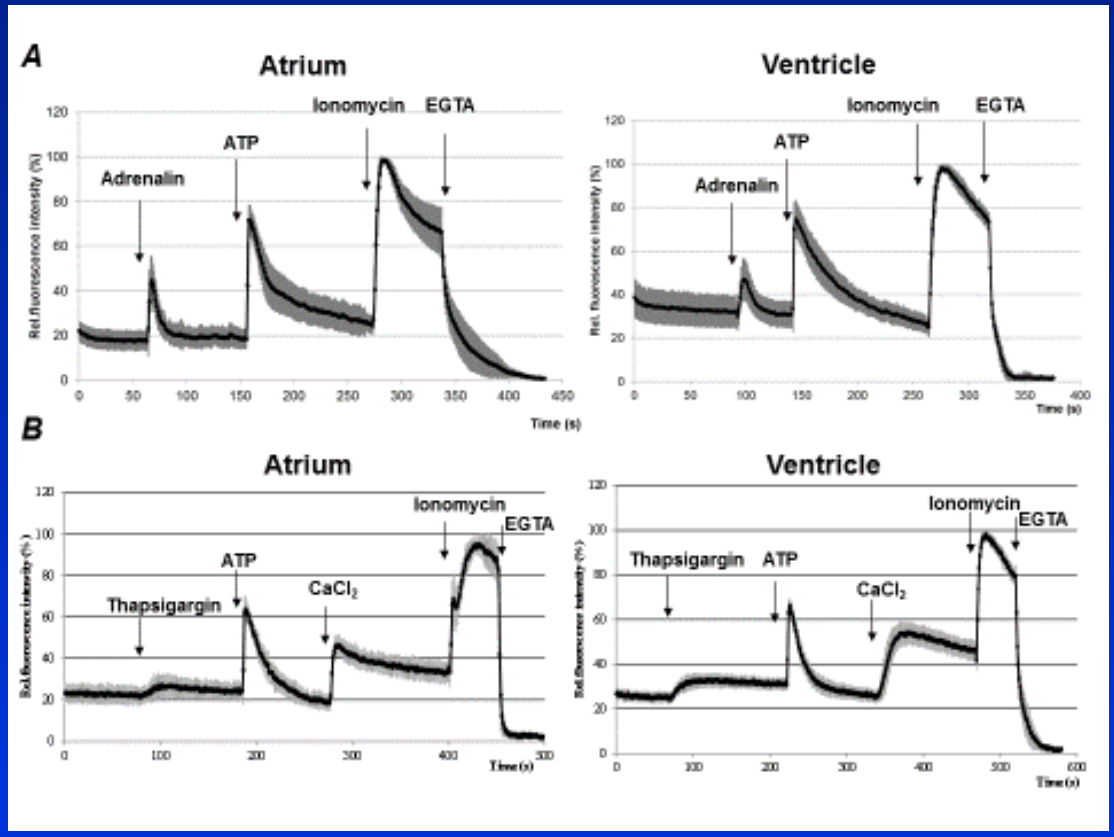
Line I. – *Sleeping Beauty* transposon integration locus
in the rat genome/ Chromosome 9, intergenic region
Ref. seq: NC_005108.3, nucleotide position:78819834
(**bold**: IRDR-R transposon sequence)

normal and stable
karyotype
(21 pairs of chromosomes)

Szebényi et al. (2015) Scientific Reports

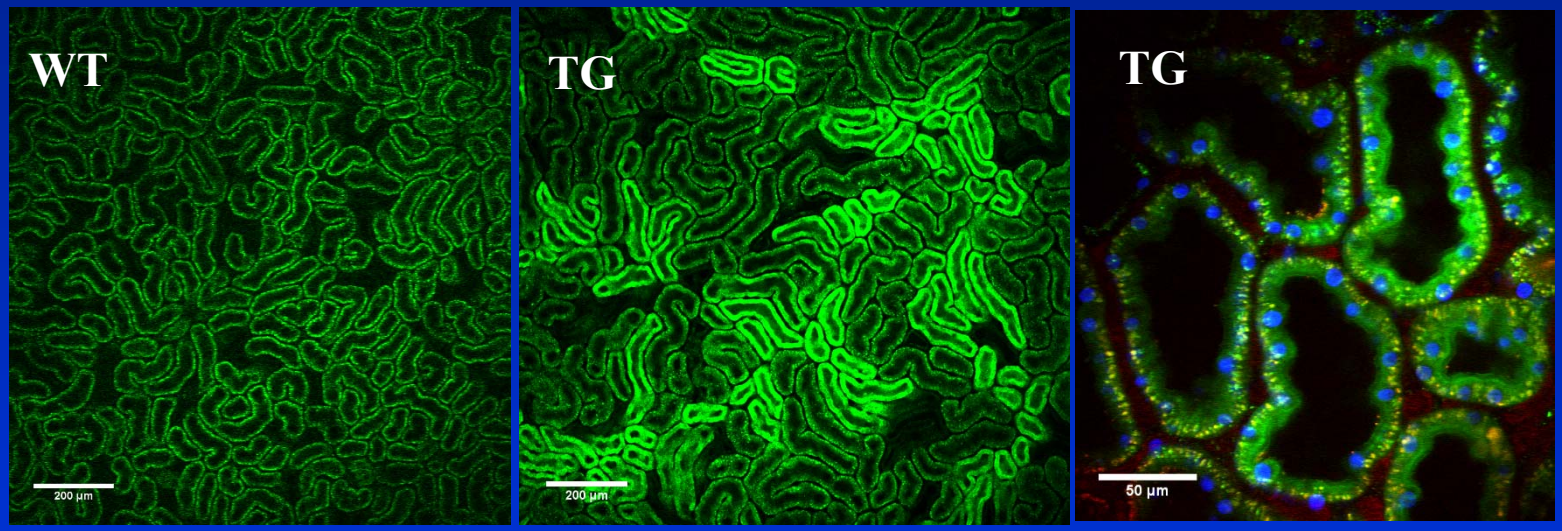
In vitro cardiomyocyte cultures





Szebényi et al. (2015) Scientific Reports

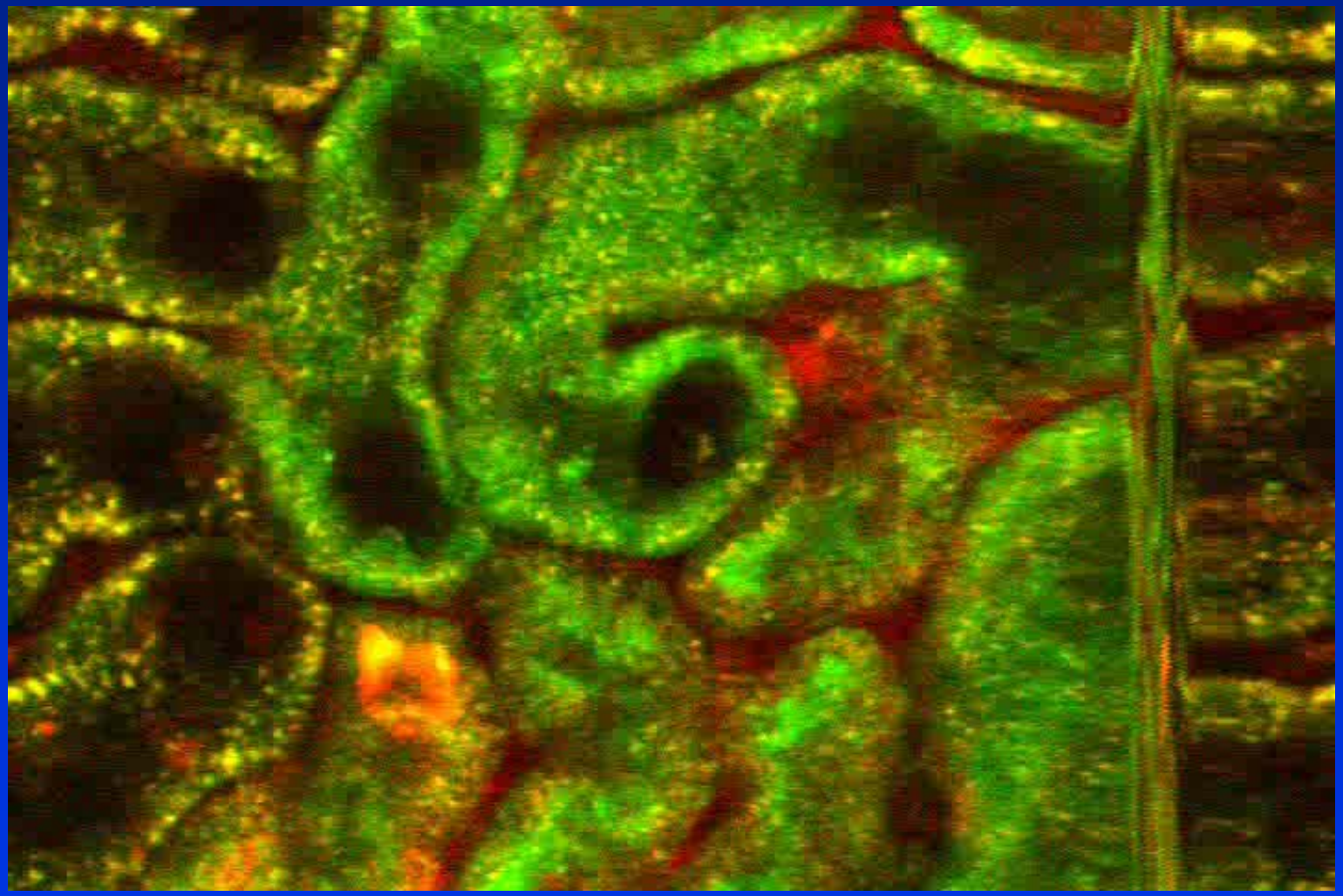
Expression of the CAG-GCaMP2 Ca^{2+} indicator protein in rat kidneys *in vivo*



Why is it so heterogeneous?

Szebényi et al. (2015) J Am Soc Nephrol

Expression of the GCaMP2 indicator in rat kidneys *in vivo*



Szebényi et al. (2015) J Am Soc Nephrol

rhodamine-dextrane conjugate

Thank you
for your attention!