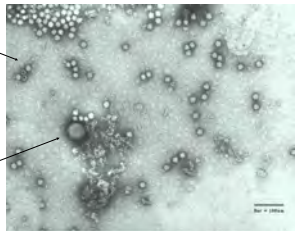
Adeno-associated virus (AAV)

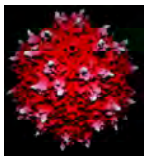


Taxonomy

Family: Parvovirus
Subfamily: Parvovirinae
Genus: Dependovirus
Type: AAV 1-12

Morphology

Particles are icosahedral, non-enveloped,
18-26 nm diameter, 50% protein (VP1-3) 50% DNA.
Resistant to inactivation by solvents, pH and heat.



Xie et al. 2002

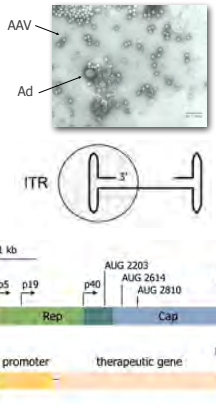
Genome

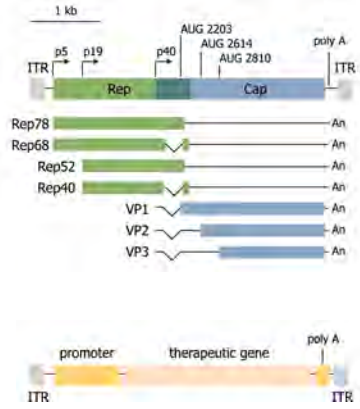
Linear, non-segmented, ssDNA ~5 kb.
AAVs package equal amounts of (+) and (-) strands.

Xie et al. 2002

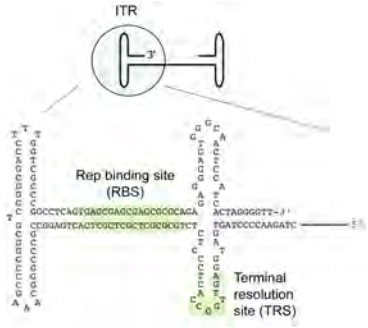
Gene transfer using Adeno-Associated Virus (AAV) vectors

1. Based on a broadly diffuse, non pathogenic virus
3. Vectors do not express viral proteins (not inflammatory and not immunogenic); long term persistence in vivo
4. Expression of the therapeutic gene can be driven by any desirable promoter
5. High titer vector preparations can be obtained by virion purification
6. Cells are transduced at high multiplicity of infection; mixing of different rAAV preparations results the simultaneous expression of gene combinations in vivo





AAV Inverted Terminal Repeat (ITR)

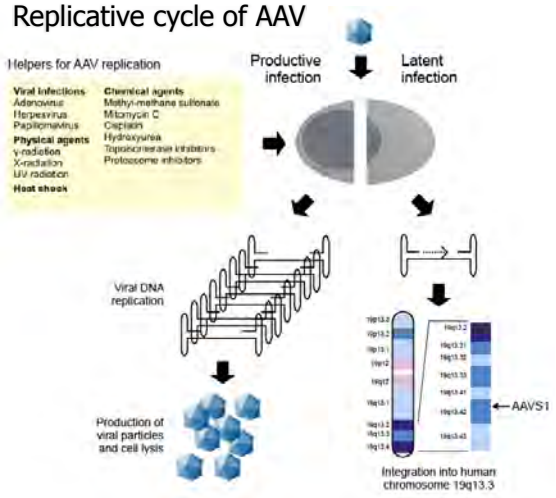


The AAV coding region is flanked by two ~145-nt-long ITRs, having an internal complementarity stretch in their first 125 nt and thus forming a T-shaped hairpin structure, identical at the two viral ends.

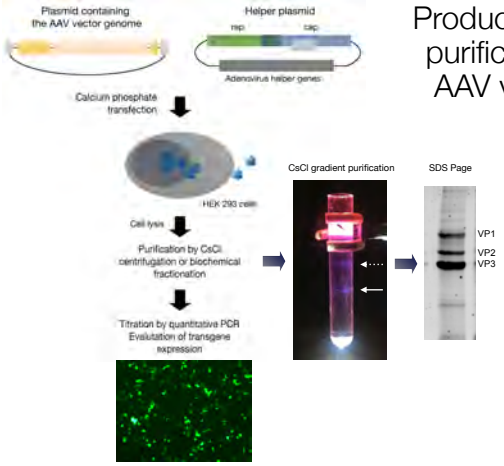
This palindromic sequence is the only cis-acting genetic element necessary for all AAV functions, including:
- viral DNA replication
- site-specific integration into the host cell DNA
- packaging of virions

The first two activities (replication and integration) require the presence of Rep68 or Rep78 proteins, which specifically bind a sequence within the ITR, the Rep binding site (RBS), and cleave in a site- and strand-specific manner at the terminal resolution site (TRS) located 13 nucleotides (nt) upstream of the RBS. An almost identical sequence in human chromosome 19q13.4 represents the minimal sequence necessary and sufficient for AAV site-specific integration

Replicative cycle of AAV

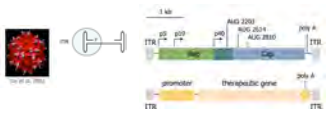


Production and purification of AAV vectors



AAV Vector Unit (AVU) facility at ICGEB Trieste

Yield: 1x10^{3}-1x10^{5} viral particles/cell.
Physical titer: 1x10^{12} to 1x10^{14} viral genome particles per ml



Operational since 2005

Over 2000 AAV vectors available

Over 150 vector preparations per year

During 2017:

160 AAV vector batches purified

40 AAV vector preparations for international collaborations

20 AAV vector preparations for Italian collaborations

Current International Collaborations

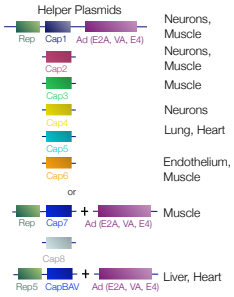
Eric Olson, University of Texas Southwestern Medical Center, Dallas, USA
Stefan Heymans, University Maastricht, Netherlands
David Klatzman, Hôpital Pitié-Salpêtrière, Paris, France
Jeffery D Molkentin, Cincinnati, USA
Georg Halder, Center for Human Genetics, Leuven, Belgium
Eva Van Rooij, Utrecht, The Netherlands
Leon J De Windt, University Maastricht, The Netherlands
Fatima Bosh, Universitat Autònoma de Barcelona, Spain
Charles E Murry, Seattle, USA
Thomas Thum, Hannover Medical School, Germany
Zimmermann Wolfram, University of Gottingen, Germany

AAV Vector Unit (AVU) facility at ICGEB Trieste, Italy: Production of AAV vectors with different serotypes

http://www.icgeb.org/avu-core-facility.html

The AVU produces, purifies, titers and characterizes research-grade recombinant AAV vectors

Both rAAV2 or hybrid vectors pseudotyped with capsid of serotypes from 1 to 9 are reproducibility obtained at a sterility and purity grade suitable for in vitro and animal experimentation



Mouse secretome collection
~1500 arrayed clones

Human microRNA collection
~800 arrayed clones

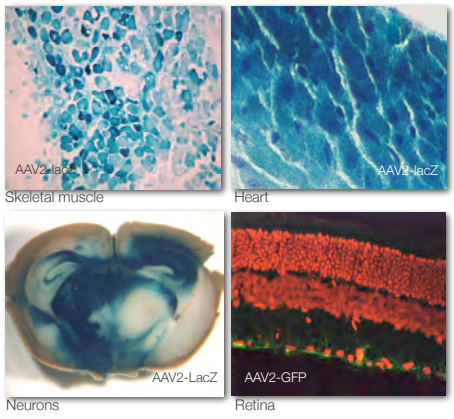
Lorena Zentilin

The origin of common AAV isolates, their receptors and tissue tropism

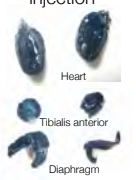
Serotype	Origin	Receptor and co-receptors[a]	Tissue tropism[b]
AAV1	Human or NHP	N-linked sialic acid	SM[murine, NHP, canine], CNS[murine, NHP], airway[murine, NHP], retina[murine], pancreas[murine], cardiac[murine, pig], liver[murine]
AAV2	Human	HSPG, FGFR1, HGFR, LamR, CD9, integrin $\alpha_V\beta_5$, $\alpha_5\beta_1$	SM[murine, canine], CNS[murine, monkey], liver[murine], kidney[murine], retina[murine, canine, human, NHP]
AAV3	Human	HSPG, FGFR, HGFR, LamR	HCC[human], SM[murine]
AAV4	NHP	O-linked sialic acid	CNS[murine], retina[murine, canine], lung[murine, NHP], kidney
AAV5	Human	N-linked sialic acid, PDGFR	SM[murine], CNS[murine, canine], airway[murine], retina[murine]
AAV6[c]	Human	N-linked sialic acid, EGFR	SM[murine, dog], heart[murine, dog, sheep, pig], airway[murine, dog, NHP]
AAV7	Rhesus macaque	?	SM[murine], retina[murine], CNS[murine], liver[murine]
AAV8	Rhesus macaque	LamR	Liver[murine, NHP, canine], SM[murine], CNS[murine], retina[murine, canine], pancreas[murine], heart[murine], kidney[murine]
AAV9	Human	LamR, N-linked glycans	Liver[murine], heart[murine, NHP, porcine], SM[murine, canine], lung[murine], pancreas[murine], CNS[murine, NHP], retina[murine, NHP], testes[murine], kidney[murine]
AAVrh10[d]	Rhesus macaque	LamR	Liver[murine], heart[murine], SM[murine, canine], lung[murine], pancreas[murine], CNS[murine, rat], retina[murine], kidney[murine]

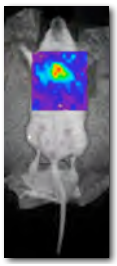
[a] Data compiled from Akache *et al.* [54], Shen *et al.* [55], Michelfelder and Trepel [56].
[b] Data compiled from Asokan *et al.* [18] and White *et al.* [57].
[c] Possibly AAV1/AAV2 hybrid.
[d] rh10 was superior to the other serotypes in transduction of neonates [54]. Abbreviations: AAV, adeno-associated virus; NHP, non-human primate

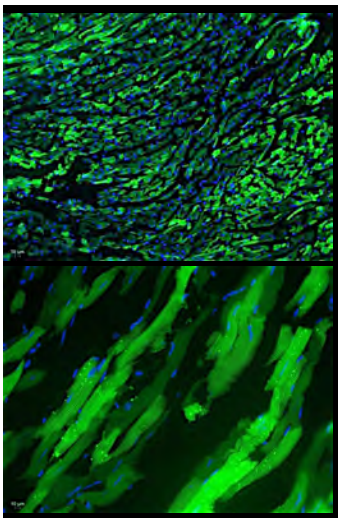
AAV vectors are outstanding tools for gene transfer into post-mitotic tissues in vivo



AAV-9
i.p. or i.v. injection







Cardiac transduction after systemic injection of AAV9 vectors

i.p. or i.v. injection

AAV9-ZsgGreen
(Zoanthus green fluorescent protein)

IP injection @day 7
5x10^{11} vg/mouse

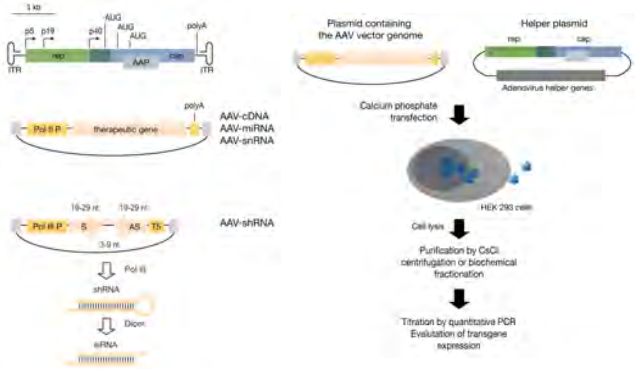
3 months post-injection
1x10^{8} - 1x10^{9} vg/heart

Zacchigna & Giacca 2014. Circ. Res.

AAV9-LacZ

AAV9-Luc

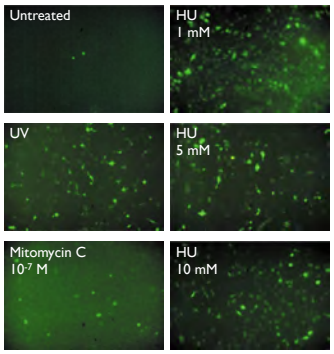
Production of AAV vectors for the expression of cDNAs and shRNAs in vivo



Tropism of AAV for post-mitotic cells in vivo.

Why?

Induction of rAAV-GFP transduction by genotoxic agents in wt CHO cells



Helpers for AAV replication

Viruses
- Adenovirus (E4-ORF 6)
- HSV-1

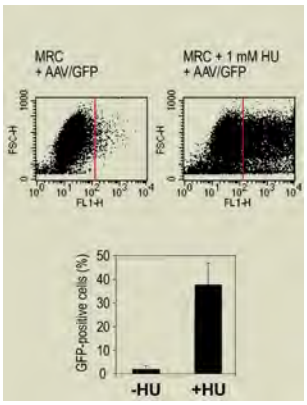
Physical agents
- γ-radiation
- X-ray
- UV
- Heat shock

Chemical agents
- Methyl methan sulfonate
- Mitomycin C
- Cisplatinum
- Hydroxyurea
- Topoisomerase inhibitors (novobiocin, etoposide, campthotecin)
- Protease inhibitors

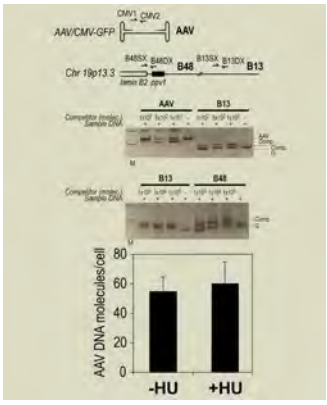
Zentilin et al. J. Virol. 2001

Hydroxyurea induces permissivity to rAAV transduction through a post-entry mechanism

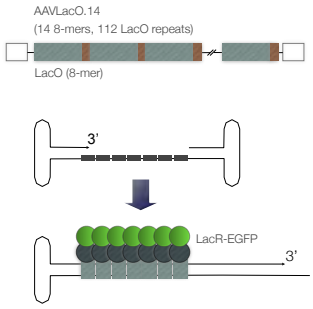
Efficiency of transduction



Quantification of intracellular DNA

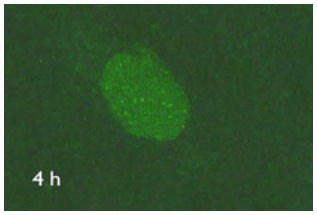


An intracellular reporter to visualize nuclear dsAAV DNA



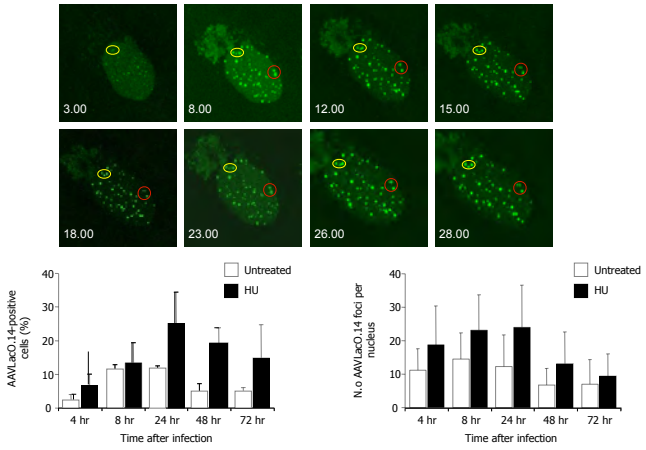
Formation of nuclear dsDNA AAV foci after transduction of poorly permissive cells

MRC/GFP-LacR cells

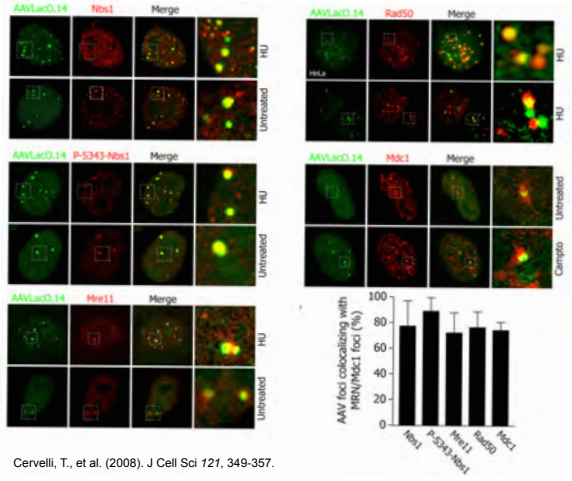


24 hour observation period

Kinetics of formation of AAVLacO.14 foci



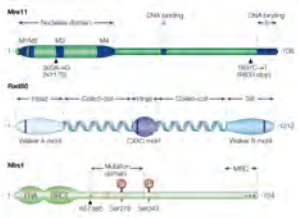
AAVlacO.14 foci co-localize with DDR foci

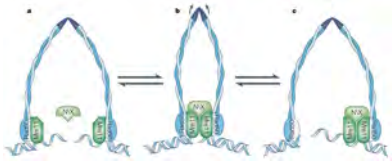


Cervelli, T., et al. (2008). J Cell Sci *121*, 349-357.

MRN complex

- Multisubunit nuclease composed of Mre11, Rad50 and Nbs-1
- Binds both ss and ds DNA and has pivotal role in sensing damaged or hairpin structured DNA

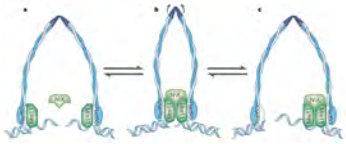




D'Amours and Jackson, Nat Rev Mol Cell Biol, 3, 317-327, 2002

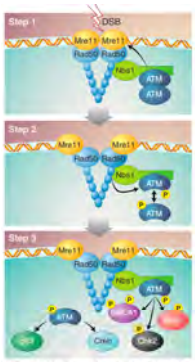
MRN complex

- Multisubunit nuclease composed of Mre11, Rad50 and Nbs-1
- Binds both ss and ds DNA and has a pivotal role in sensing damaged or hairpin structured DNA and process it



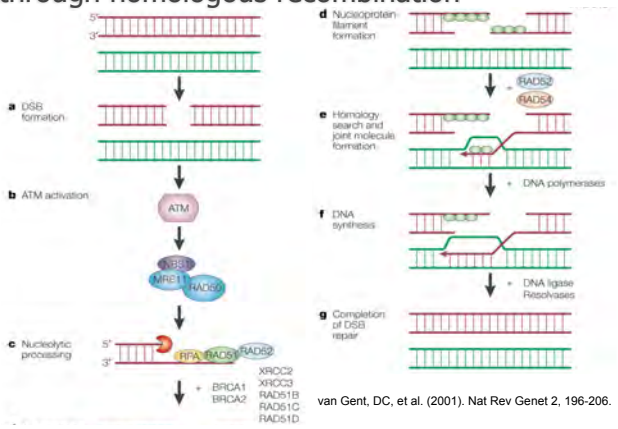
D'Amours and Jackson, Nat Rev Mol Cell Biol, 3, 317, 2002

MRN complex activates ATM and checkpoint signalling



Lee JH, Paull TT, Science 308, 551, 2005

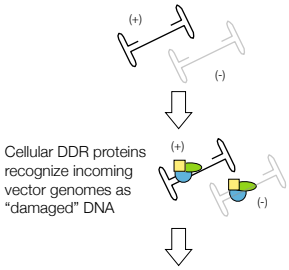
Double-stranded DNA break repair through homologous recombination



van Gent, DC, et al. (2001). Nat Rev Genet 2, 196-206.

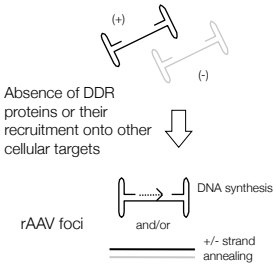
Cellular DNA Damage Response (DDR) proteins restrict AAV transduction

Non permissive cell



"The DDR inhibition model"

Permissive cell

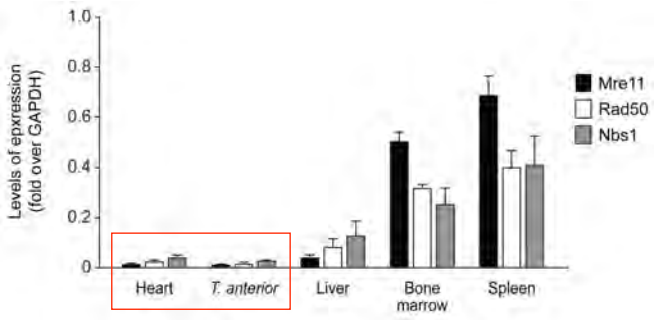


Zentilin L et al. 2001. J Virol 75, 12279
Cervelli T et al. 2008. J Cell Sci 121, 349
Schwartz RA et al. 2007. J Virol 81, 12936
Lovric J et al. 2012. Mol Ther 20, 2087

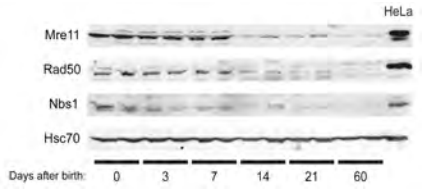
Why are post-mitotic cells permissive in vivo?

Post-mitotic tissues express low levels of MRN proteins in vivo

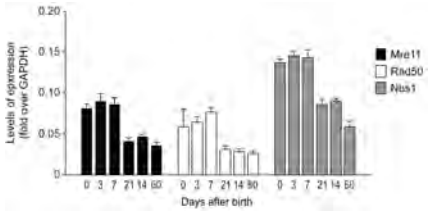
Jasmina Lovric



Cardiac MRN complex expression is strongly reduced after 2 weeks from birth

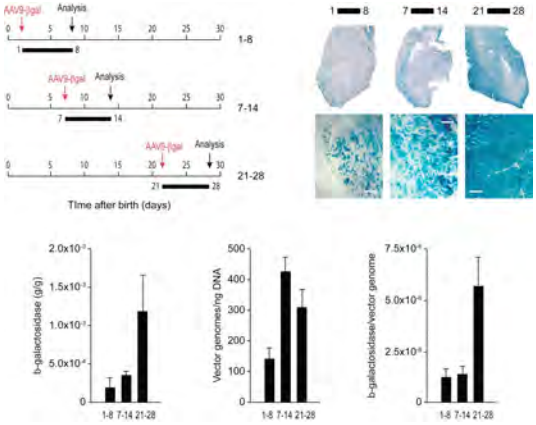


Protein

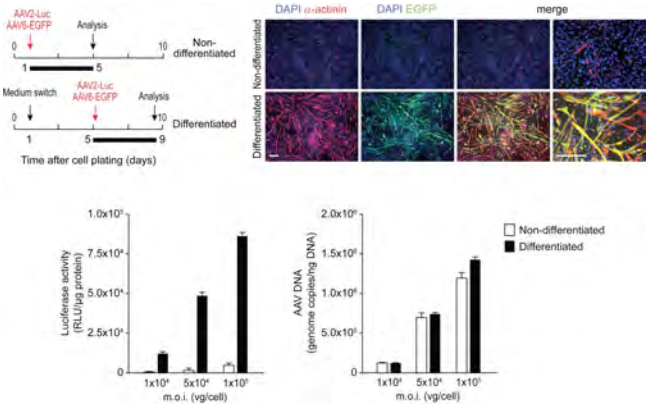


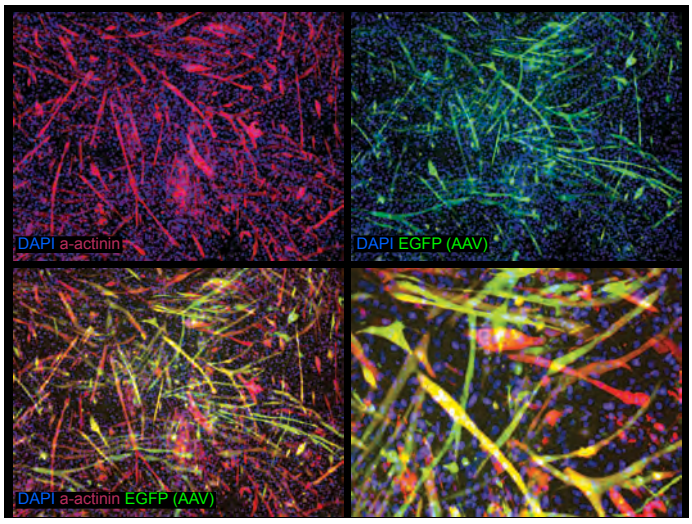
mRNA

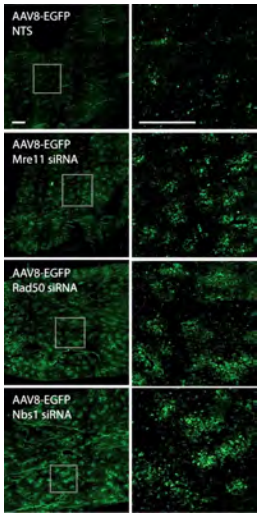
Cardiomyocyte permissivity to AAV transduction increases after birth



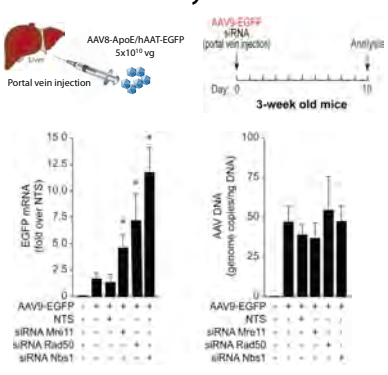
C2C12 differentiation increases permissivity to AAV transduction





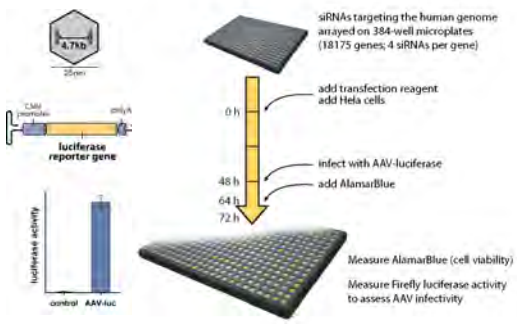


Transient knockdown of MRN improves in vivo liver transduction by AAV

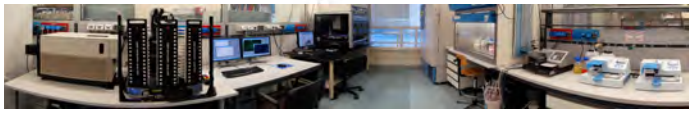


J Lovric et al. 2012. Mol Ther

High-throughput screening of a whole genome siRNA library for AAV transduction

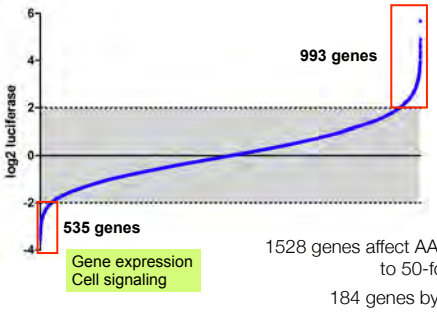


Miguel Mano



Molecular determinants of AAV transduction

GENOME-WIDE SCREENING – 18175 gene targets



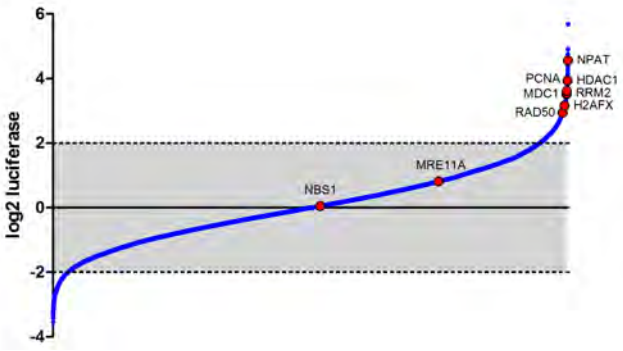
Cell cycle
Cell growth and proliferation
DNA replication,
recombination and repair

Gene expression
Cell signaling

1528 genes affect AAV by more than 4-fold (up to 50-fold change)

184 genes by more than 8-fold

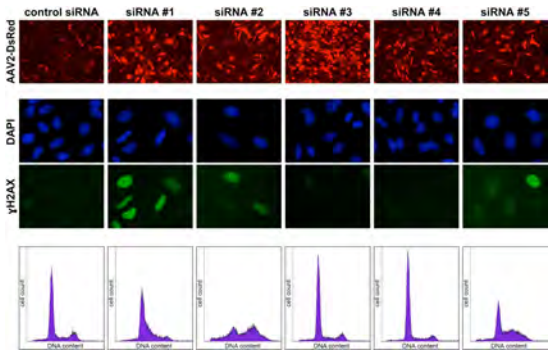
Molecular determinants of AAV transduction: enrichment for genes involved in the DNA Damage Response



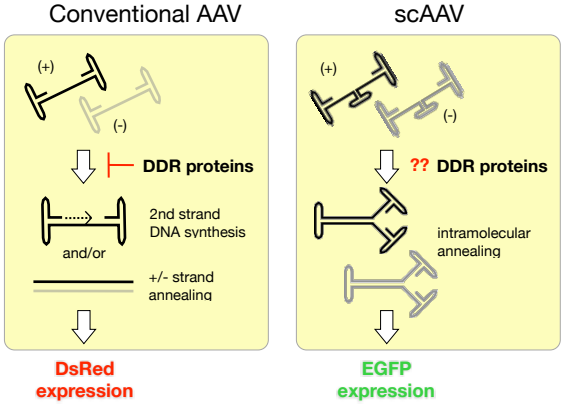
Screening for the induction of γH2AX foci by siRNAs modulating AAV permissivity

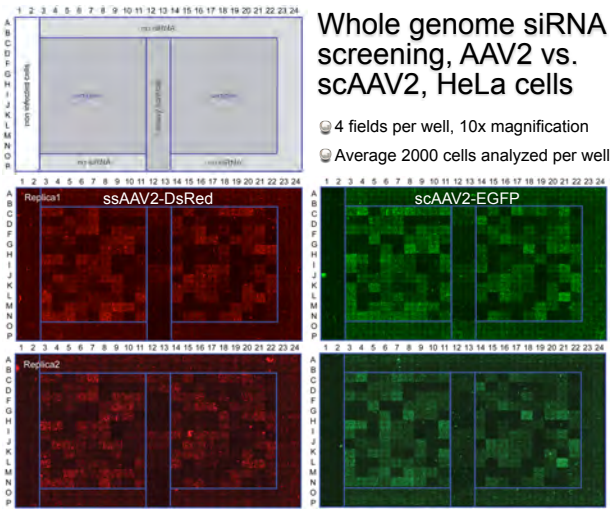
5 of the top-10 siRNAs that increase AAV transduction also induce γ-H2AX foci

13 of the top-50 siRNAs that increase AAV transduction also induce γ-H2AX foci



Self-complementary AAV





Whole genome siRNA screening, AAV2 vs. scAAV2, HeLa cells

- 4 fields per well, 10x magnification
- Average 2000 cells analyzed per well

Summary

- Cellular DNA Damage Reponse (DDR) proteins restrict AAV transduction
- Lack of MRN correlates with AAV permissitivity of post-mitotic tissues in vivo
- Results of whole genome, siRNA screening for AAV permissivity: 900+ genes improving AAV transduction; several genes involved in DNA repair or required for maintenance of DNA integrity
- No significant difference between ssAAV and scAAV to siRNAs inducing AAV permissivity