Epigenetic regulation is an unexpected problem of transgenesis in animals

Andrew Kuznetsov, Freiburg i.Br.

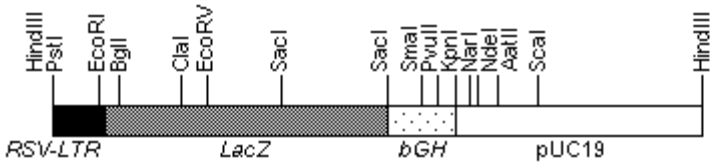
Content

- Genetic engineering improving the disease resistance (*Ad5*-asRNA, *MX1*-gene)
- Pilot experiments on transgenesis (MAR, MT1, RSV-LTR, CMVe *cis*-controlling elements; *bHG*, *lacZ*, *gfp* expressions)
  - DNA injection in the male pronucleus
  - Sperm Mediated Gene Transfer (SMGT)
  - DNA injection in somatic cells (testes, muscle)
- Signs of epigenetic regulation and possible controlling mechanisms affecting SMGT

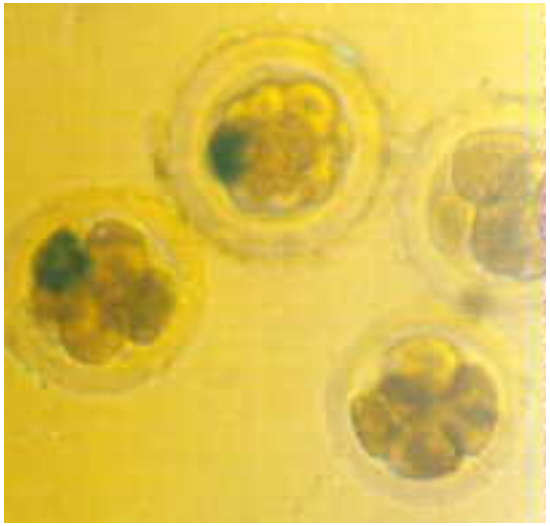
An idea to use transgenesis preventing diseases

- asRNAs, ribozymes by Thomas Tikhonenko and Boris Naroditsky (MT1-*antiAd5*, Ernst et al, 1990)
- *MX1* transgenesis by Gotfridt Brem (SV40-*Mx*, Muller et al, 1992)
- Unknown factors might make it hard
- Research started from ‘simple’ models with an extension to various species

Mosaic transient expression in rabbit embryos after microinjection of RSV-*lacZ*



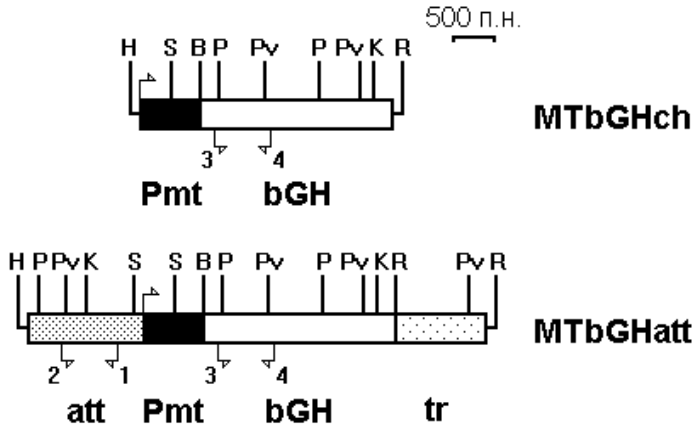
pRK3*lacZ*
7.1 kb

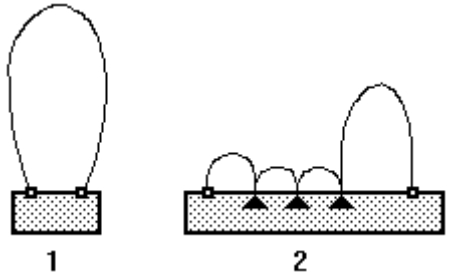


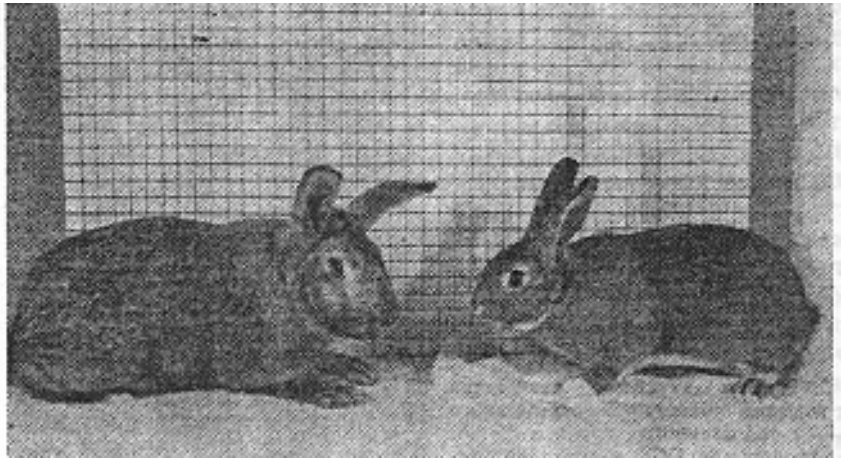
Preimplantation rabbit embryos injected with RSV-*lacZ* DNA and stained by X-gal

Gogolevsky et al, 1991

Unexpected anomalies in the transgenic rabbit with MAR-MT1-*bGH*





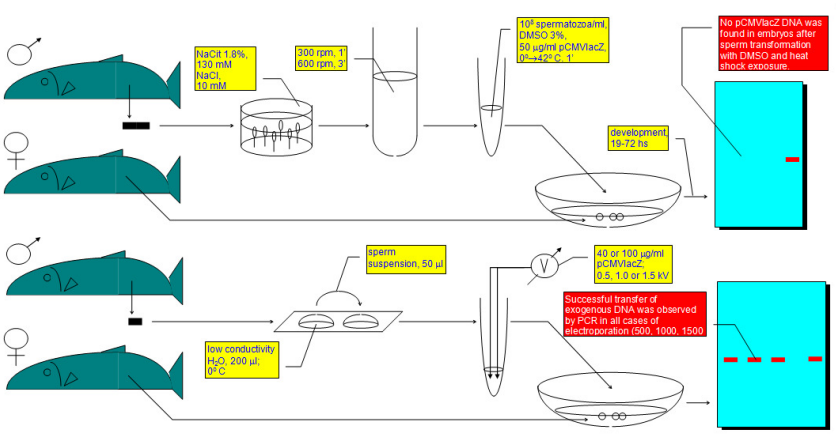


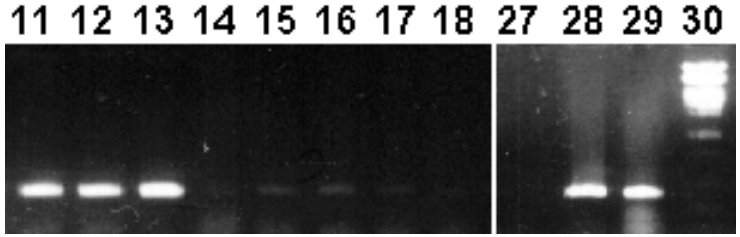
Problems:

- increased hepatocytes with pathological nucleus
- parenchyma cells elimination
- myocardial dystrophy
- the high level of cholesterol
- the 45th additional aberrant submetacentric chromosome in two clones of lymphocytes
- the infertility

Goldman et al, 1993

Mosaic transient expression in fish *M.fossilis* after SMGT with CMVe-*lacZ*





The negative and β-gal-positive fry of loach *M.fossilis* at 72 h after fertilization with pcDNA3-*lacZ* electrotransfected sperm cells

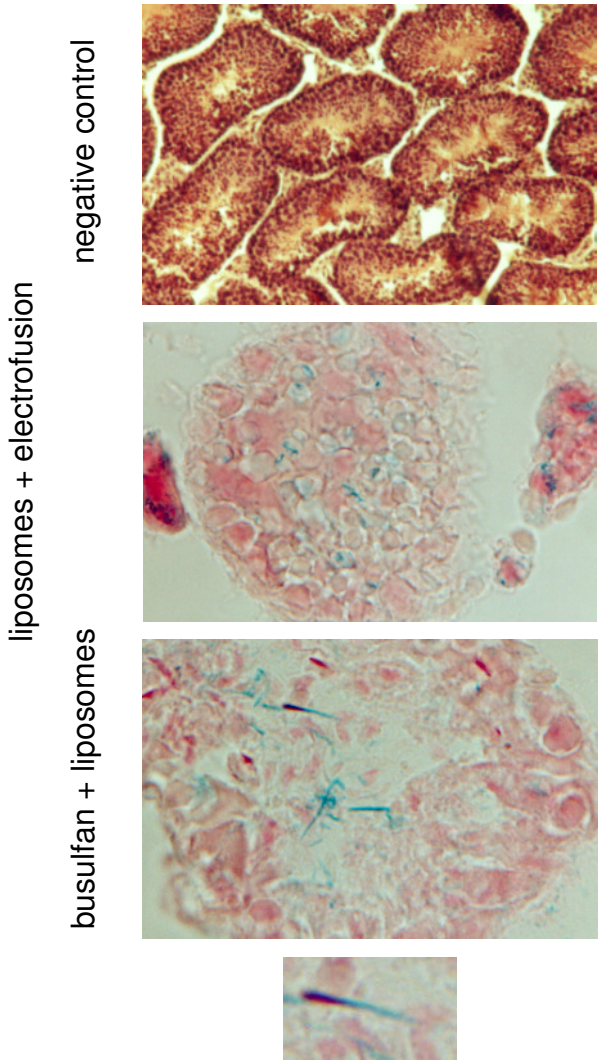
Andreeva et al, 2003

pCMV*gfp*(bind) transfer by sperm into mussel *M.galloprovincialis*, estimated by GFP fluorescence in swimming larvas

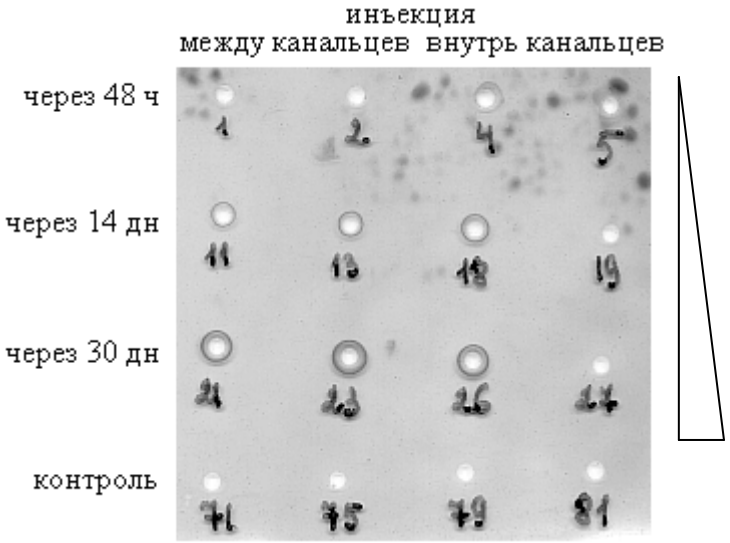
№	Variants of transfection	GFP total fluorescence intencity		
		A.U	%	Δ (%)
1	control (without DNA)	45.8	100	0
2	naked DNA	66.2	144.5	44.5
3	DNA+ V-GCN4	66.2	144.5	44.5
4	DNA+ V-GAL4 + lipids	93.0	216.2	**116.2**
5	DNA+ V-GCN4 + lipids	127.7	278.8	**178.8**
6*	DNA+ lipids	49.9	109.0	9.0
Note: * - DNA rearrangements detected by PCR-analysis				

Anisimova et al, 2000

pCMV-*lacZ* injection into mouse testes



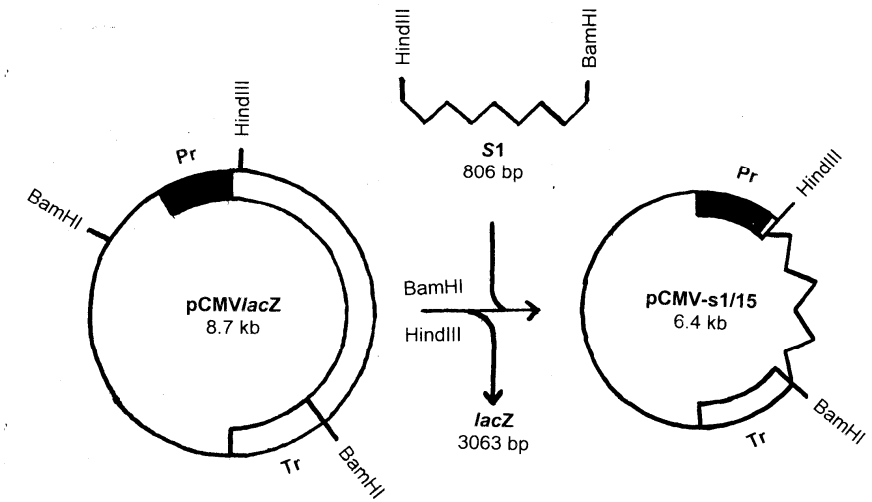
Paraffin sections of X-gal stained testes at day 14 after pCMV-*lacZ* injection

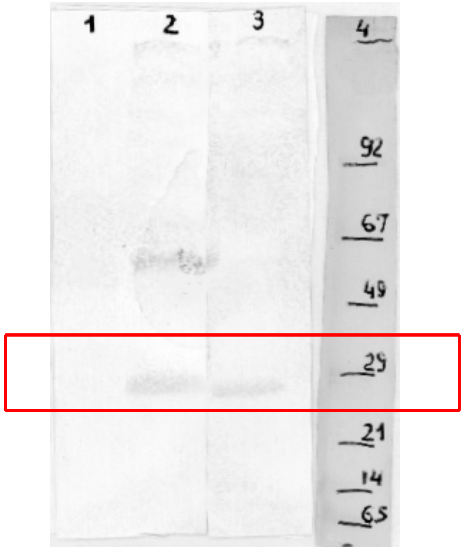


Mancini test revealed antibodies to bacterial β-galactosidase in the blood serum of pCMV-*lacZ*-electroporated mice

Shchit et al, 1996

Somatic DNA transient expression as a basis of genetic immunization





Gene *lac*Z in the plasmid pCMV-*lac*Z was substituted by the DNA fragment coding S1 subunit of *B.pertussis* toxin. Plasmid pCMV-*lac*Z and the resulting plasmid pCMV-*s1* were used for i/m injection. Serum was obtained at 11 day.

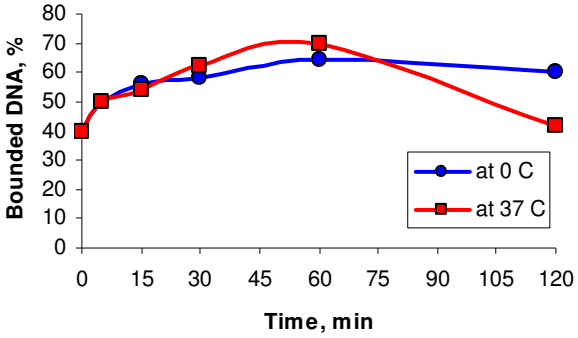
lacZ: interaction of serum with bacterial β-galactosidase was established by immunoprecipitation and Western-blot (the titre of serum 1:200).

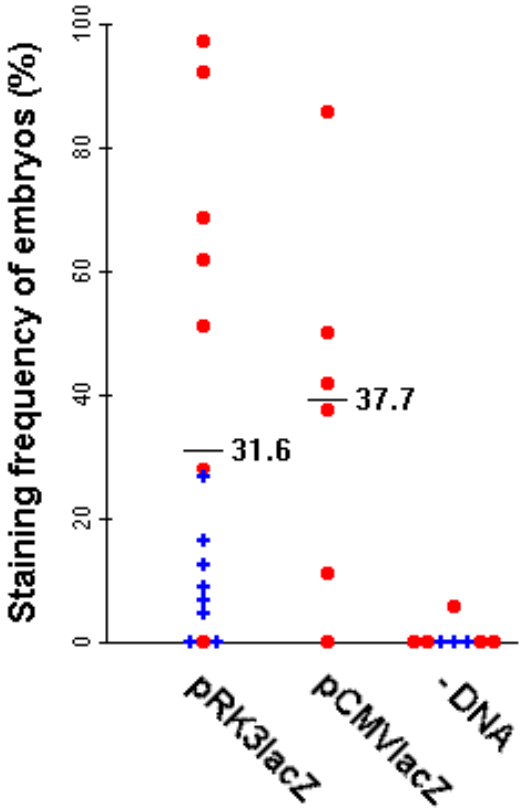
s1: specificity of S1-antiserum was analysed by Western-blot. Lysate of *B.pertussis* (slot 2) and purified pertussis toxin (slot 3) were used. The protein 26 kD, which is the same as S1 subunit of pertussis toxin, was revealed (slots 2, 3).

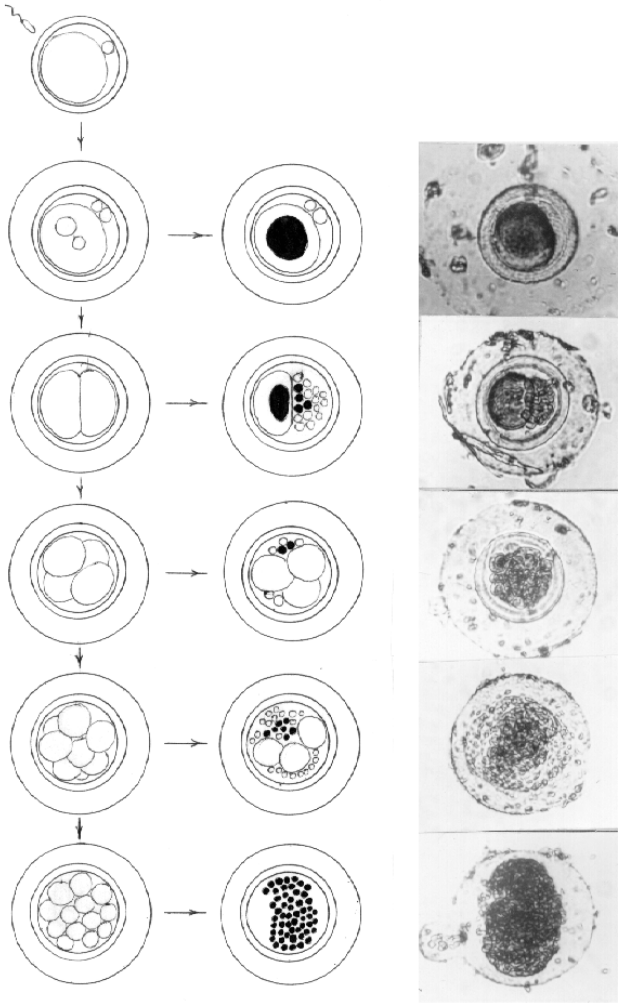
Panfertsev et al, 1996

Transient expression...

DNA uptake vs. variations in the transient expression and vs. degradation of embryos



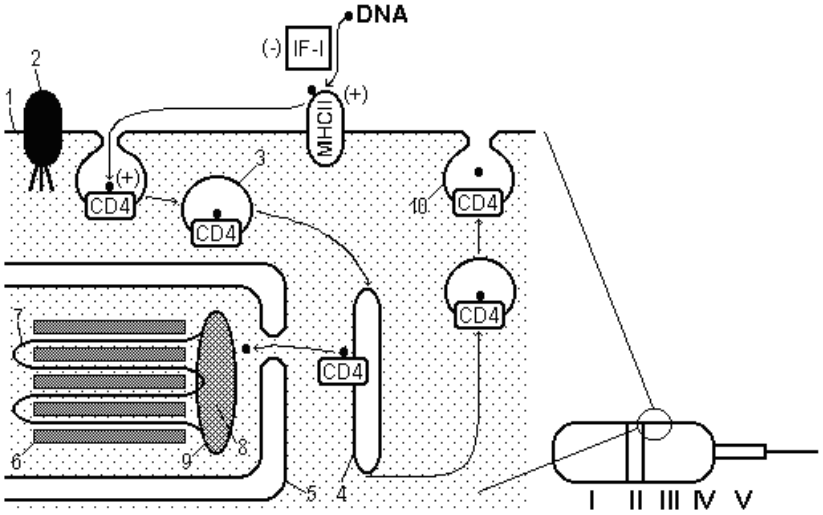




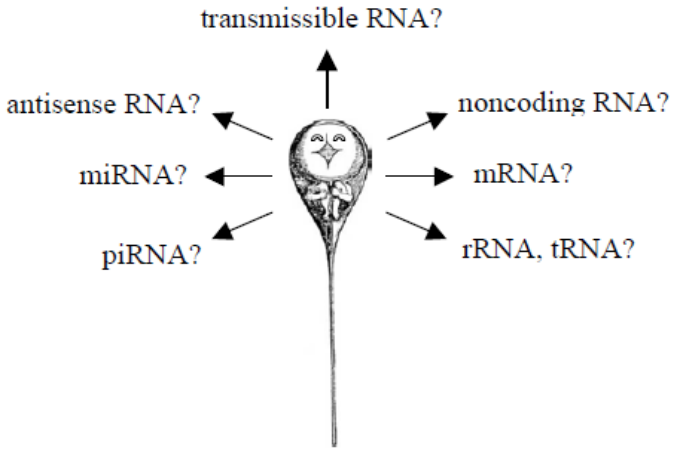
Kuznetsov et al, 2000

Shchit et al, 1998

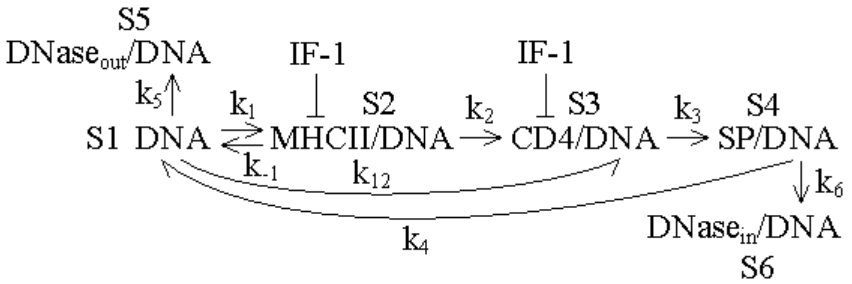
SMGT: unknown factors, unpredictable functions

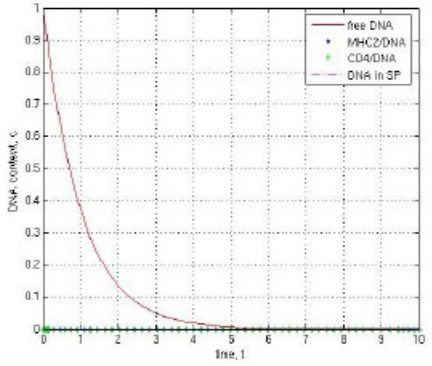


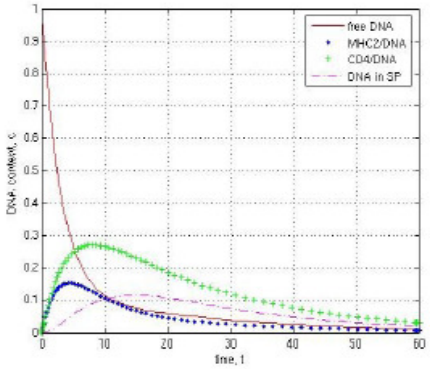
Kuznetsov et al, 1998



Kawano et al, 2007



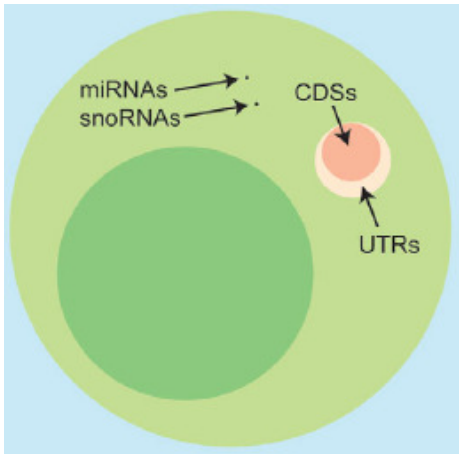




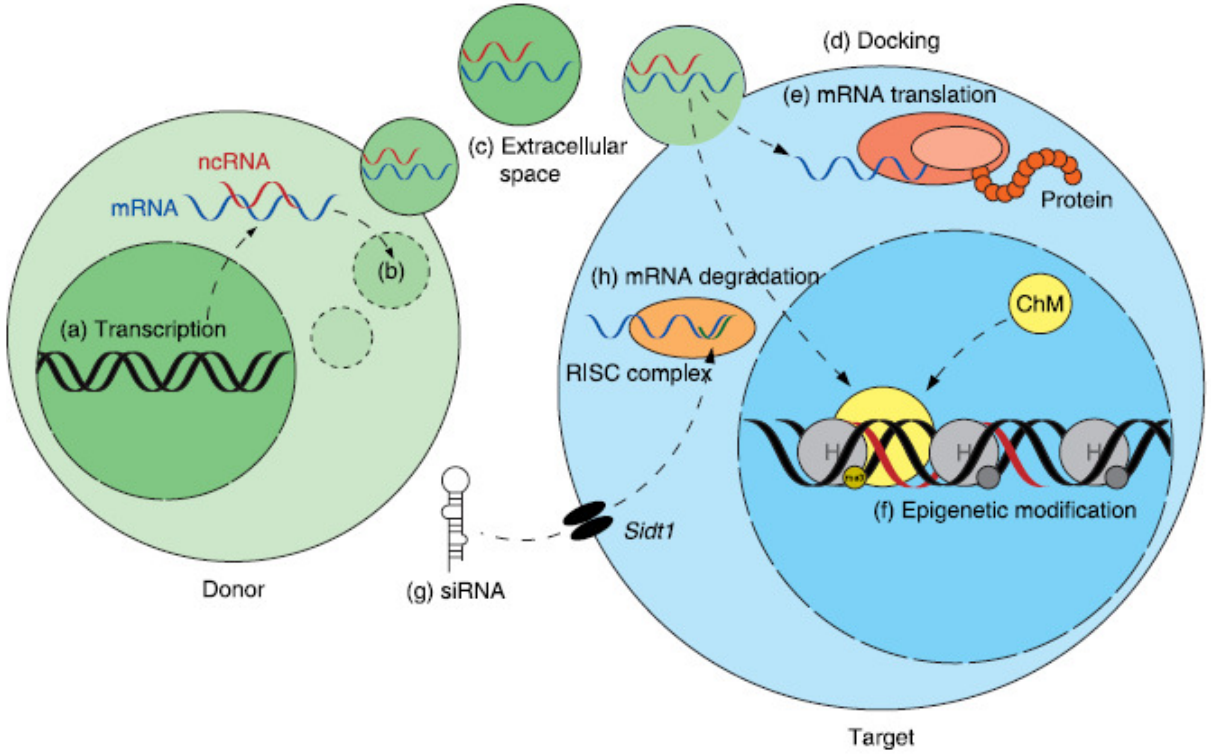
Kuznetsov, 2007

Incredible pool of ncRNAs

Transcription in mammals



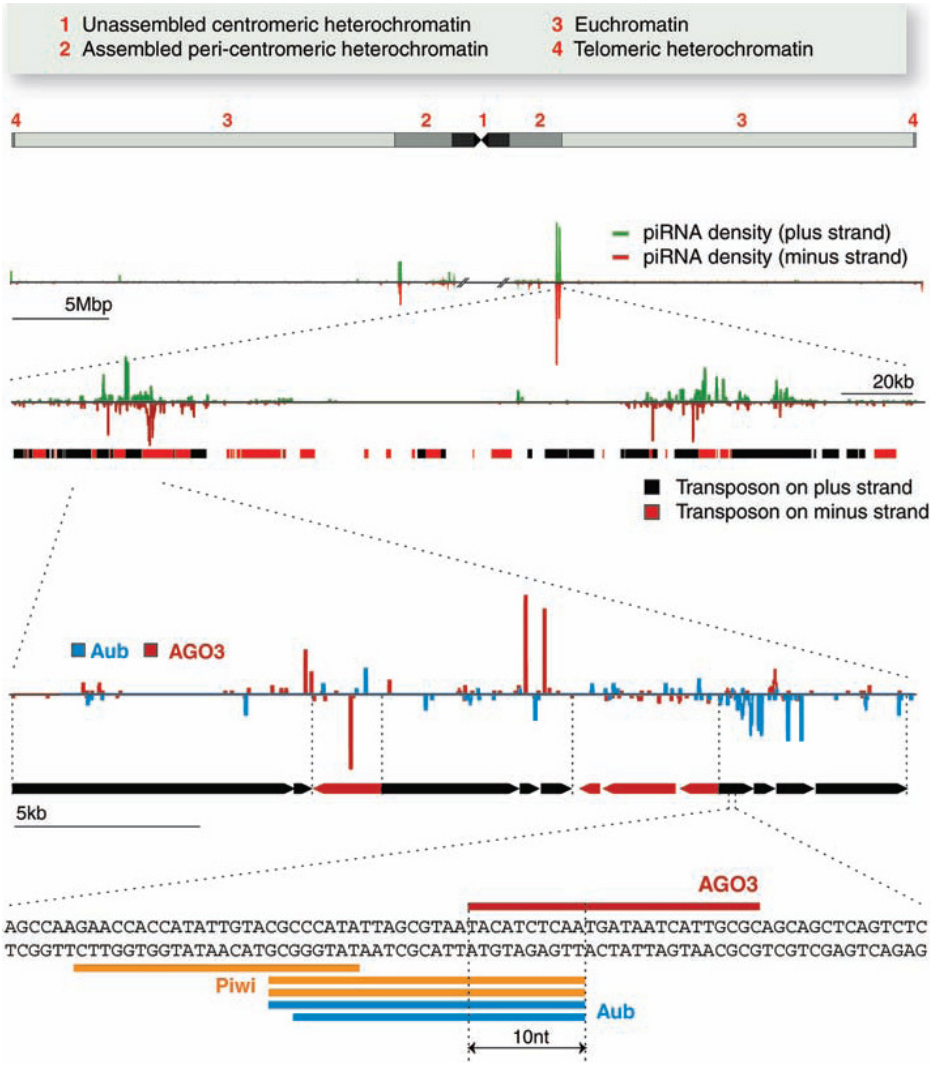
ncRNA pathways

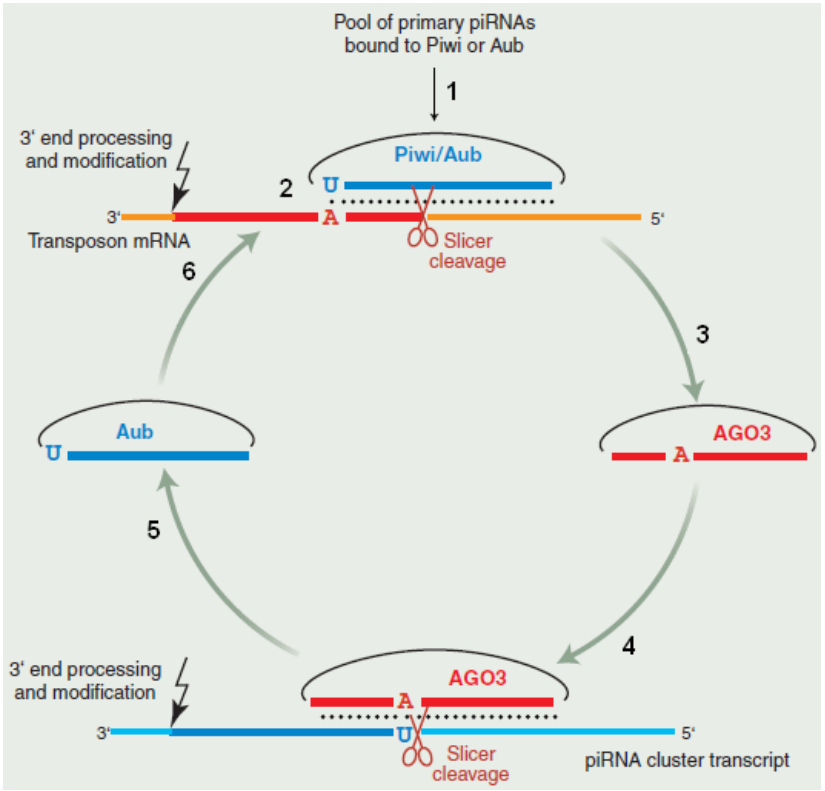


Mattick, Makunin, 2006

Dinger et al, 2008

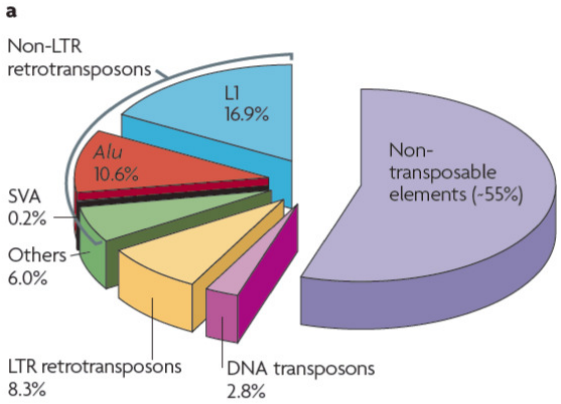
Relict innate immunity against foreign DNA/RNA, ping-pong mechanism



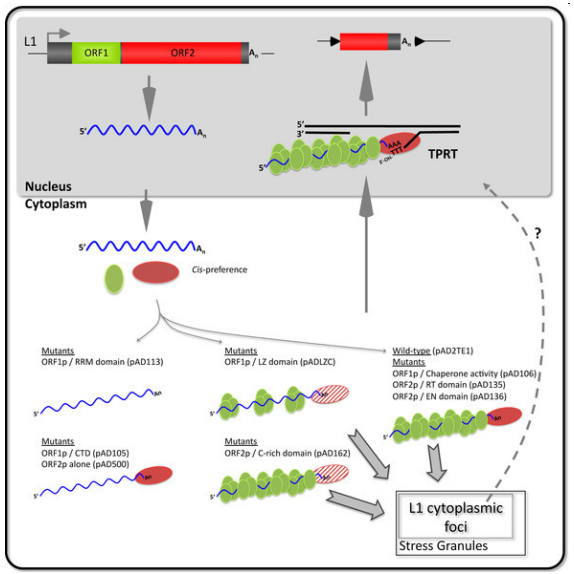


Aravin et al, 2007

LINE1 genetic parasites in humans



Cordaux, Batzer, 2009



Doucet et al, 2010

>500,000 L1 copies that comprise ~17% of the human genome

They arrived 150 Myr ago and expanded the genome of our ancestor

They play a significant role in the genome instability, early development, brain plasticity and cancerogenesis, because they can provoke the homologous recombination and co-transcription of the noncoding and coding RNAs

They are central “controlling elements”

They in turn are controlled by the MIWI-interacting piRNAs (!)

Components of L1-9092 are widely distributed in nature [Kuznetsov, 2011]

Conclusion

- Transgenesis may be unpredictable (position effects, DNA rearrangements, alternative splicing, apoptosis, unusual physiological effects, etc.)
- PCR, Southern/Northern/Western-blots are not enough to understand mechanisms of transgenesis
- It seems the pool of ncRNAs plays an important role in the regulation at different levels
- The introduced DNAs could interfere with ncRNA pathways
- ‘Omics’ research might give additional information about the mechanisms

Acknowledgements

- LAB: Ira Belikova, Dr. Ira Shchit, Dr. Irena Kuznetsova, Janna Kalmikova, Lena Martinova, Luba Sobennikova, Dr. Sveta Kaurova, Svetlana Diveeva, Valeria Sigaeva, and Dr. Evgeniy Dianov

- Many thanks to Dr. Lyudmila Andreeva, Dr. Anastasia Grigorenko, Dr. Gennady Dvoryanchikov, Dr. Alexander Gavrjushkin, and Dr. Vladislav Erokhin

- Special acknowledgements to Prof. Gottfried Brem and Prof. Lev K. Ernst

References

Andreeva L.E., Sleptsova L.A., Grigorenko A.P., et al. Loach spermatozoa transfer foreign DNA, which expression is discovered in the early development stages // Russian Journal of Genetics. 2003. V. 39(6). P. 758-761.

Anisimova S.A., Kuznetsov A.V., Erokhin V.E., et al. Sperm-mediated transfer of complex DNA:protein:lipids into mussel ova // II-d International Researh Conference Biotechnology in the plant and livestock industry and veterinary, Moscow, Russia, 18-19 October 2000, P. 132-134.

Gogolevsky P.A., Goldman I.L., Gusev V.V., et al. Study of expression of β-galactosidase gene in transgenic early embryos of rabbits // RAACS reports. 1991. V.10. P. 37-40.

Goldman I.L., Ernst L.K., Gogolevsky P.A., et al. Study of expression of recombinant cattle growth hormone gene mMT1/bGHatt with MAR element in rabbits // RAACS reports. 1993. V. 1. P. 58-71.

Kuznetsov A. DNA interaction with sperm cells: ODE model // BMC Systems Biology. 2007. V. 1(Suppl 1). P. 42.

Kuznetsov A. Identification of L19092-like retrotransposons and their components in the whole genome sequence datasets // Second Annual Conference, Models of Human Diseases, Toronto, Canada, 28 June 2011.

Kuznetsov A.V., Kaurova S.A., Kuznetsova I.V. Transfection of endometrium after rabbit insemination by transformed sperm cells. Model of DNA internalization into spermatozoon // Russian Journal of Human Reproduction. 1998. V. 4(6). P. 29-33.

Kuznetsov A.V., Kuznetsova I.V., Shchit I.Yu. DNA interaction with rabbit sperm cells and its transfer into ova in vitro and in vivo // Molecular Reproduction & Development. 2000. V. 56(2). P. 292-297.

Panfertsev E.A., Kuznetsova I.V., Kuznetsov A.V. Specific antibodies in rabbit serum as result of CMV-lacZ and CMV-s1 genes transient expression after intramuscular injection // 5-th International Expert Forum on Immunotherapy and Gene Therapy, Jerusalem, Israel, 4-7 June 1996, P. 173.

Shchit I.Yu., Kuznetsov A.V., Kaurova S.A., et al. Negative effects of exogenous DNA on fertilization and early development in experiments with gene transfer by sperm in rabbits // Russian Journal of Human Reproduction. 1998. V. 4(4). P. 5-10

Shchit I.Yu., Kuznetsova I.V., Denisova T.S., et al. Spermatozoon is a mobile vector for recombinant DNA // Bayev Memorial Conference, Moscow, Russia, 20-22 May 1996, P. 245.