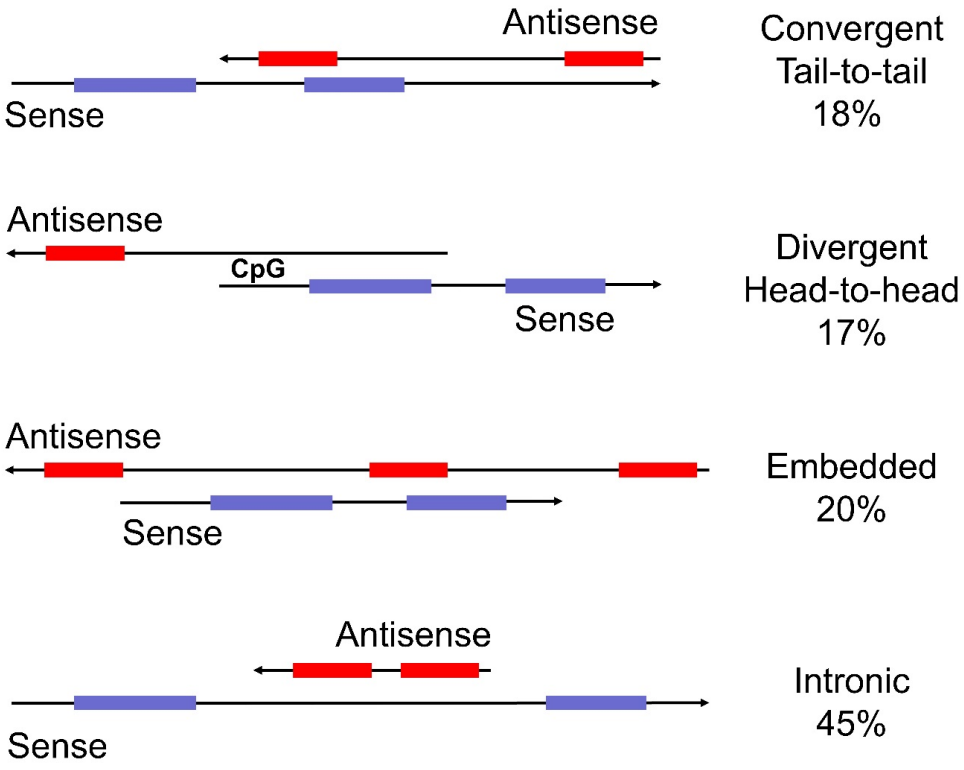
4 - 8 July 2018
Salzburg - Austria
EVOLUTION
Genetic Novelty/Genomic Variations by RNA Networks and Viruses

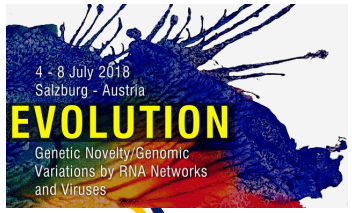
The two Faces of Natural Antisense Transcripts

Andreas Werner, Newcastle University, United Kingdom

Natural Antisense Transcripts (NATs)



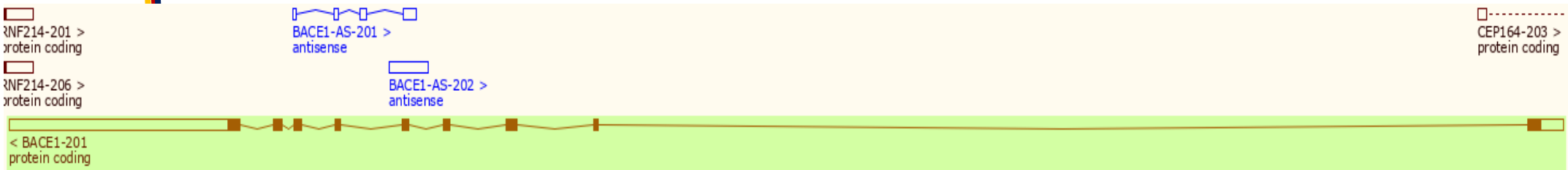
- Widespread (40-70% in human/mouse)
- Sense transcript coding
- Conserved in evolution
- Processed, mRNA-like
- Reminiscent of viral RNA

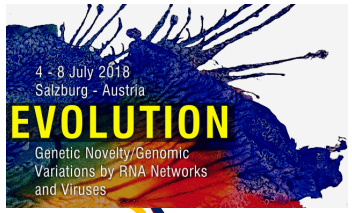


Biological Roles of NATs

1) Genomic role in evolution, transcriptome control
   - Transcriptome analysis
   - Epigenetics
   - Potential mechanism

2) Regulatory role in expression of a specific locus

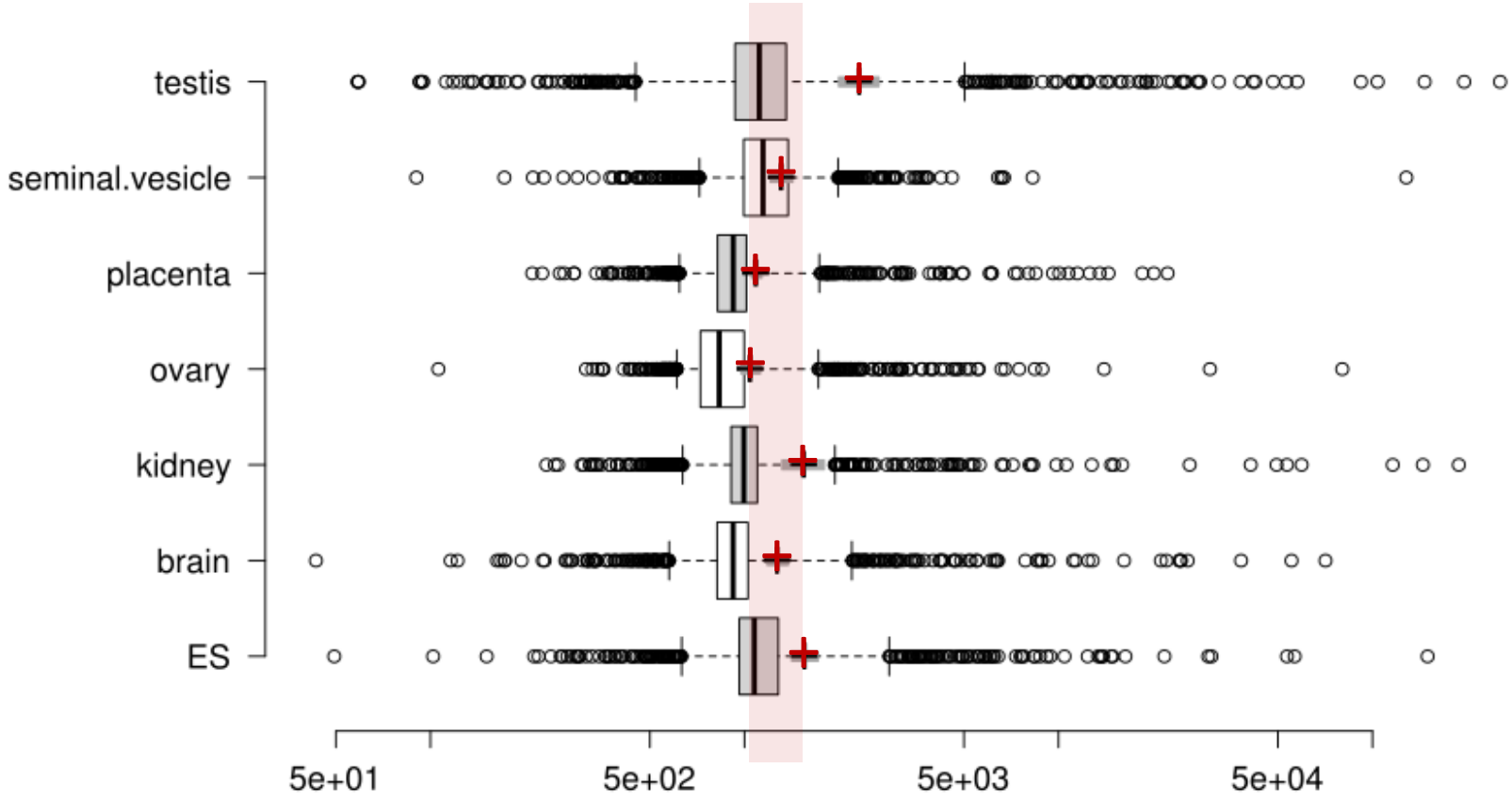




The role of NATs in evolution and transcriptome control

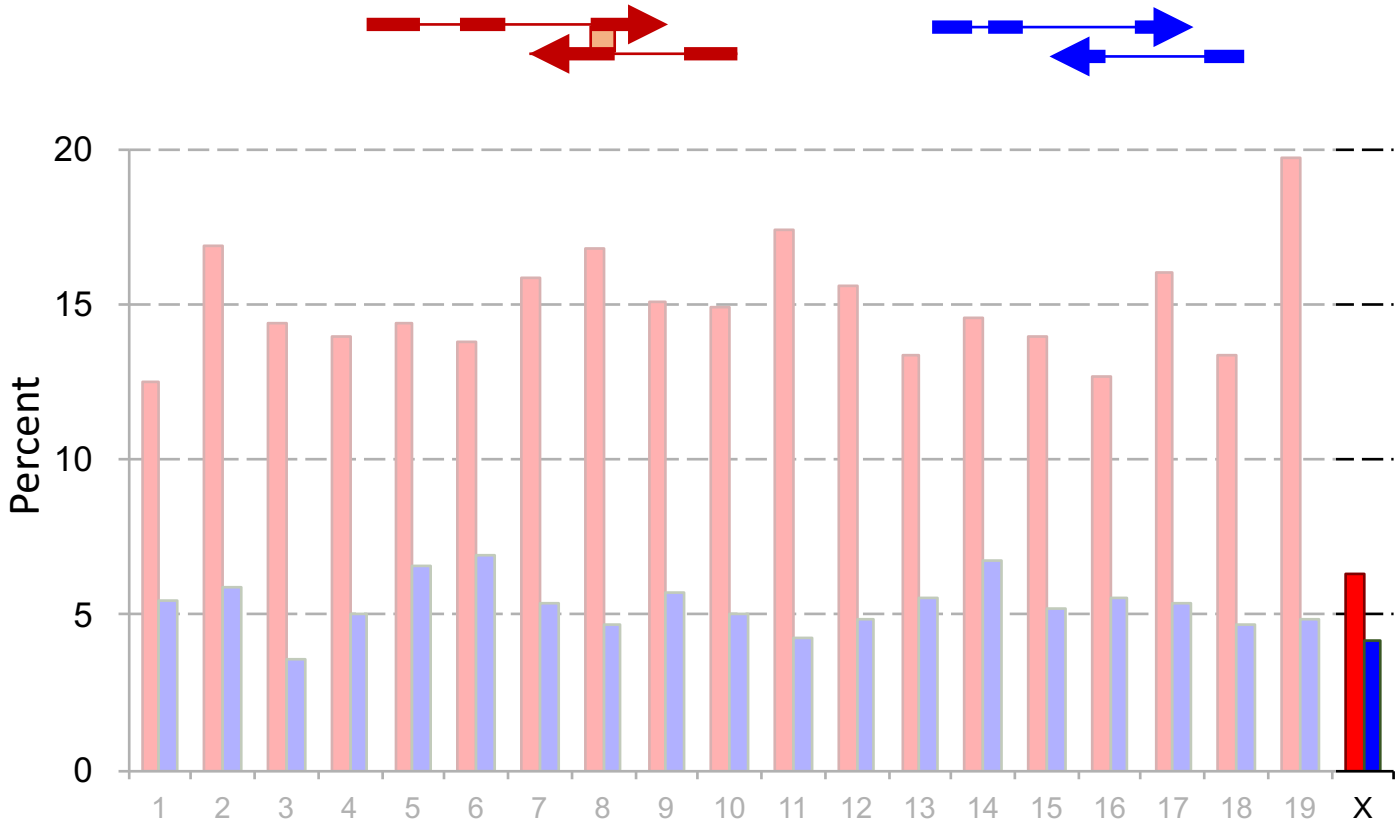
Transcriptome analysis
Potential mechanism

NATs are predominantly expressed in testis



Carlile et al. 2009, Nucleic Acids Res. 37: 2274

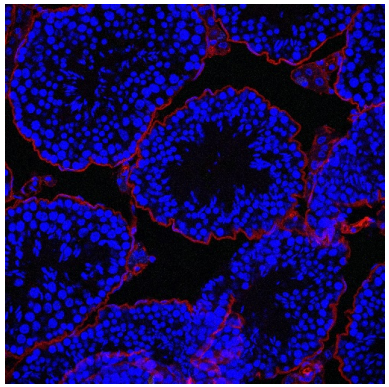
Chromosomal distribution of NATs



Kiyosawa et al. 2003, Genome Res 13: 1324

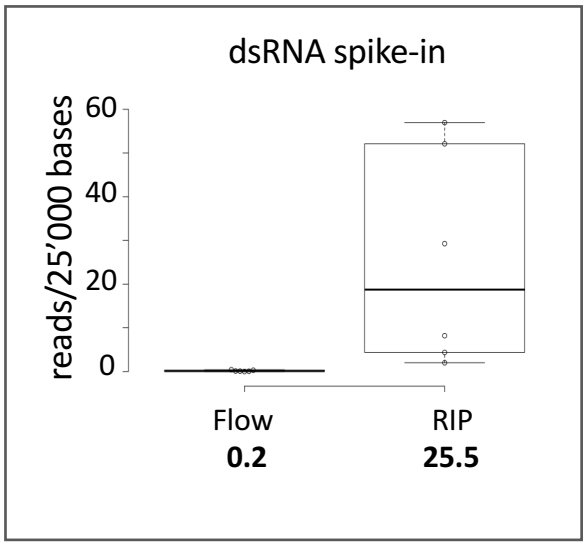
Double-stranded RNA in mouse testis

Mouse testis



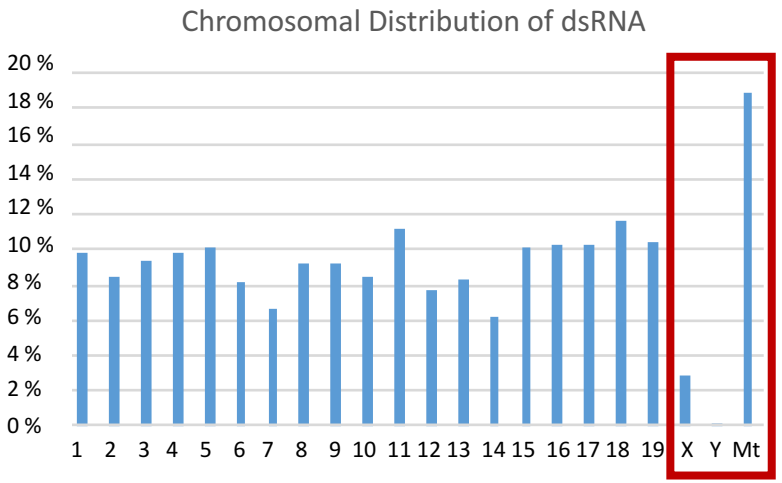
RIP using J2
Under non-denaturing conditions

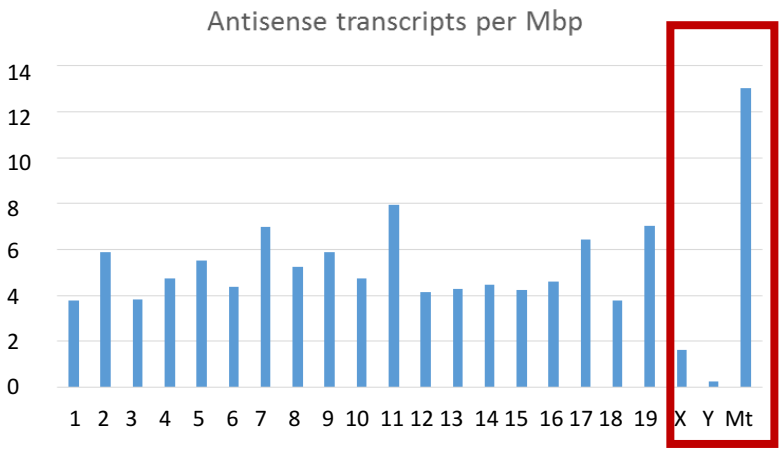
RNA-seq of dsRNA



Total of 3,277 genes are expressed (> 8x over background)

Sense/Antisense are co-expressed and form dsRNA





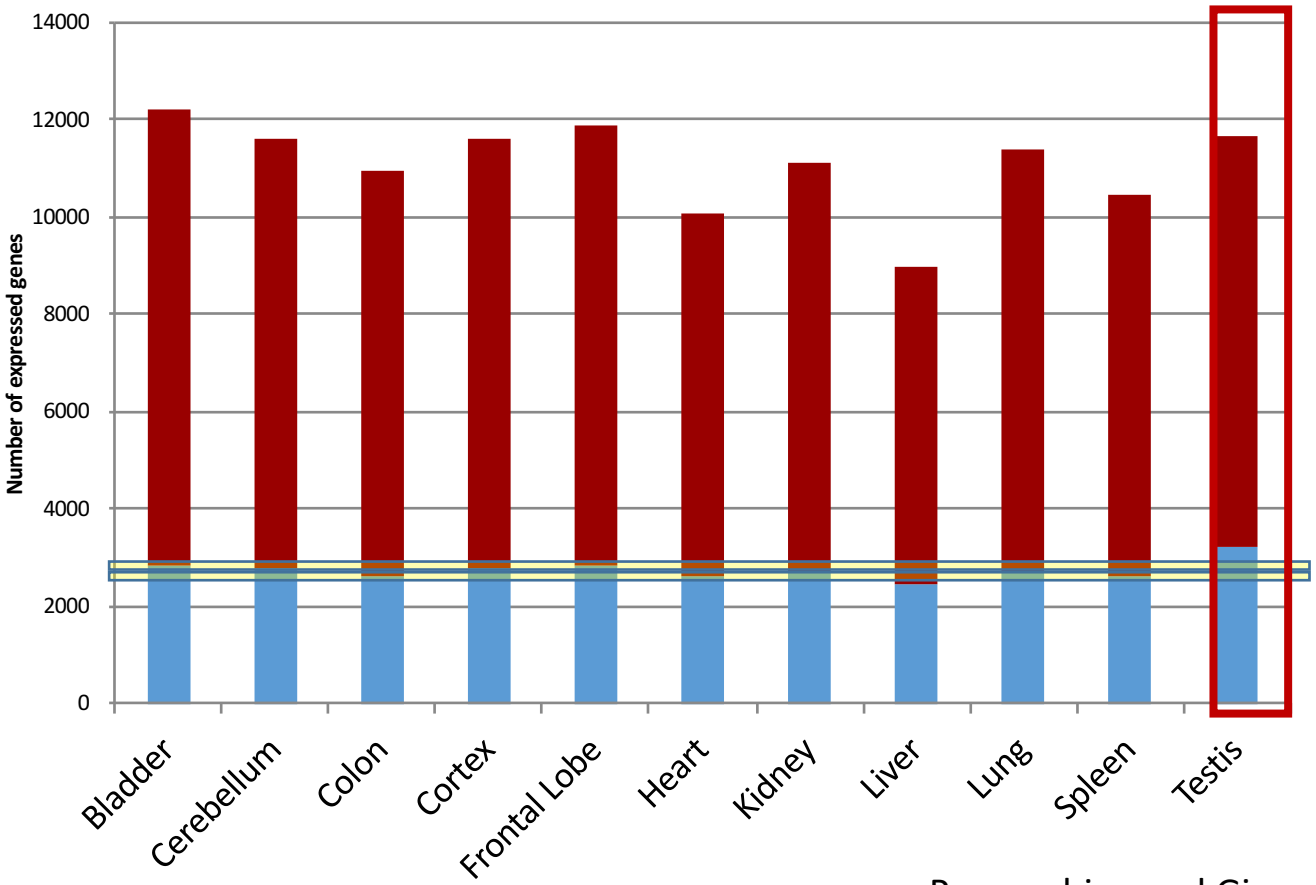
Antisense and dsRNA correlate

Highest on mitochondrial DNA

Lowest on X and Y chromosomes

Katayama et al. 2005, Science 309: 1364

Expression pattern of dsRNA



Average expressed genes

2693 ± 125

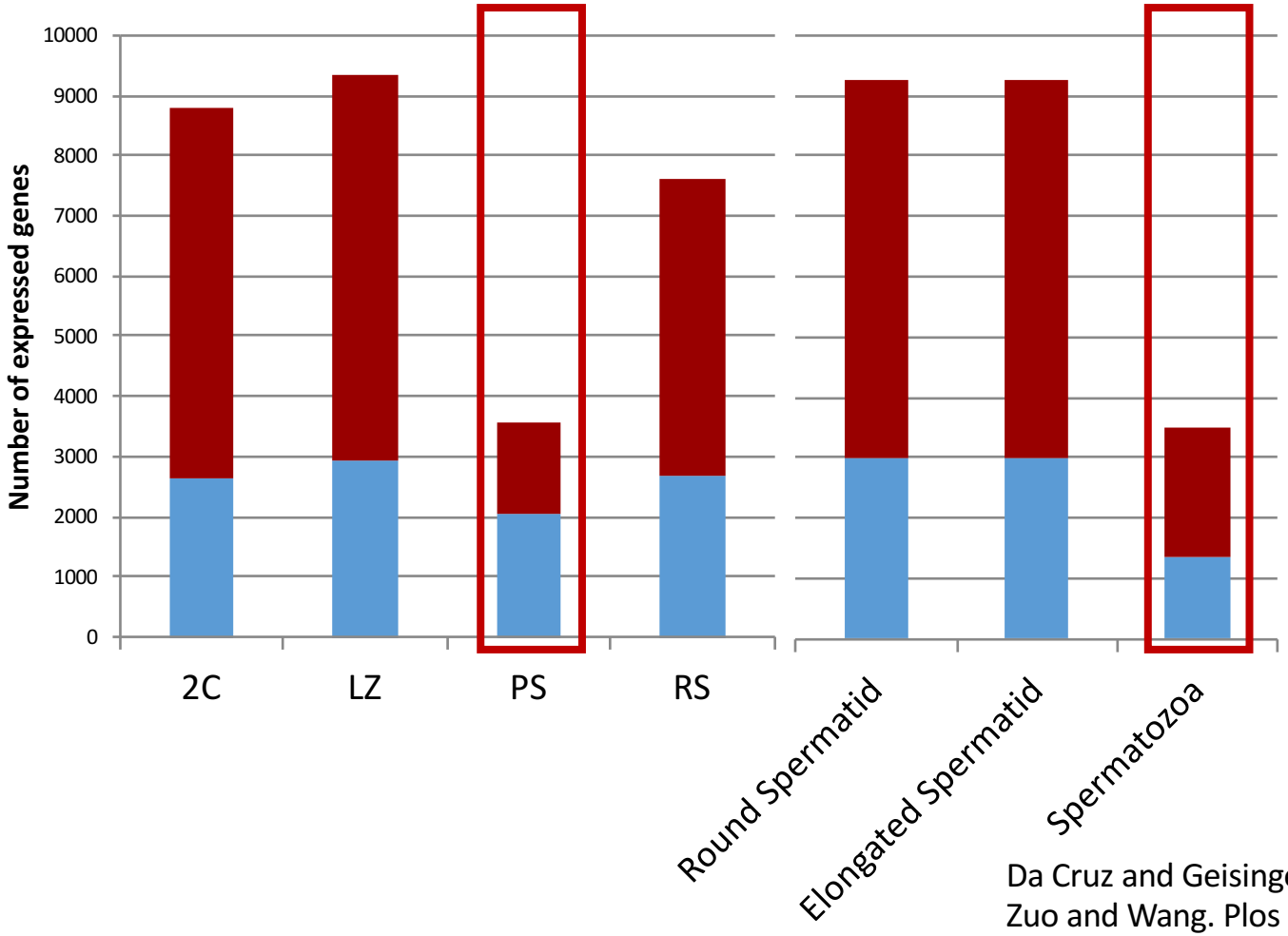
Testis: 3215

Does not form dsRNA

Forms dsRNA

Pervouchine and Gingeras, Nat Comm. 6, 5903, 2015

Expression pattern of dsRNA



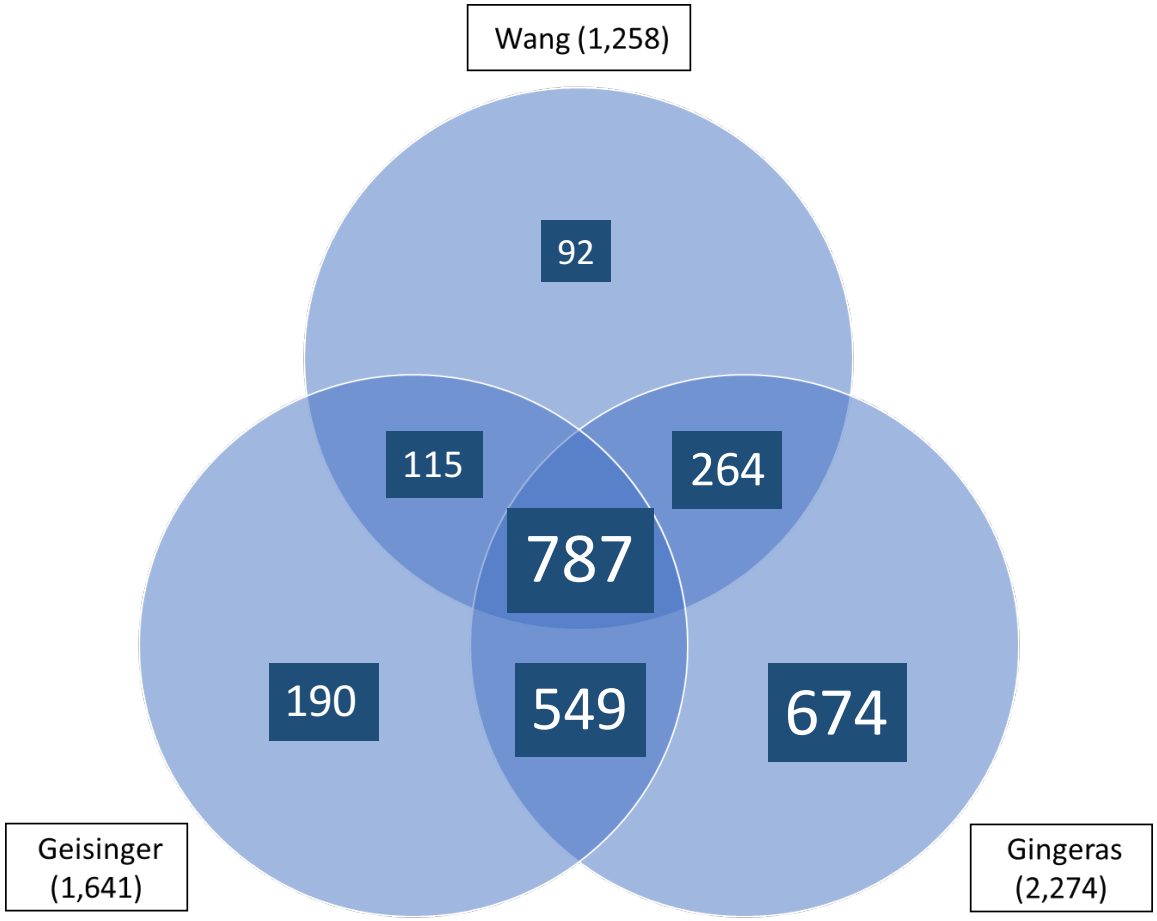
Average expressed genes

33.3 ± 3.1 %

Pachytene: 58.1 %

Da Cruz and Geisinger, BMC Genomics. 2016; 17: 294
Zuo and Wang. Plos ONE, 2017

Expression of dsRNA forming genes is not tissue specific

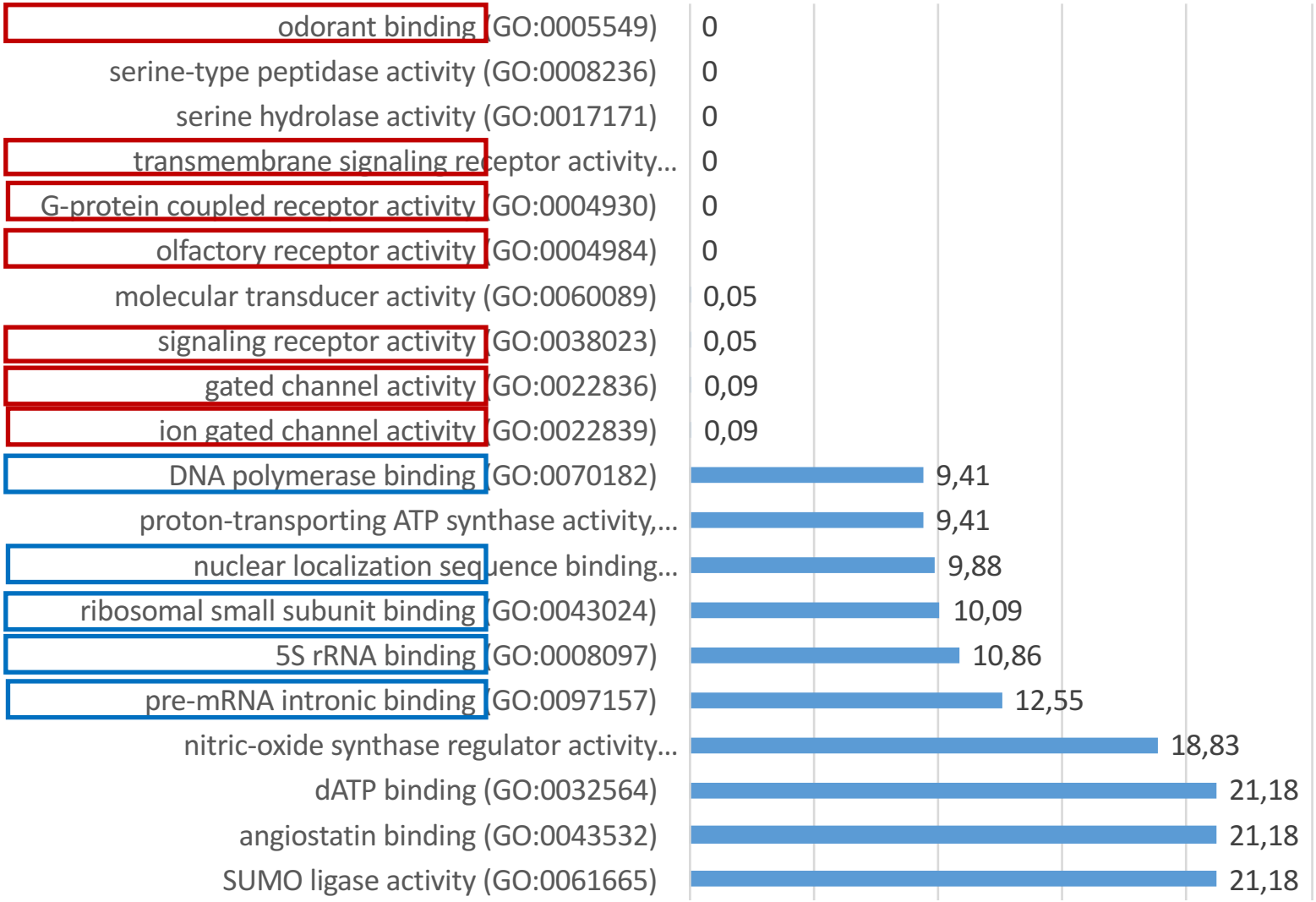


Gingeras:
Mouse tissue panel

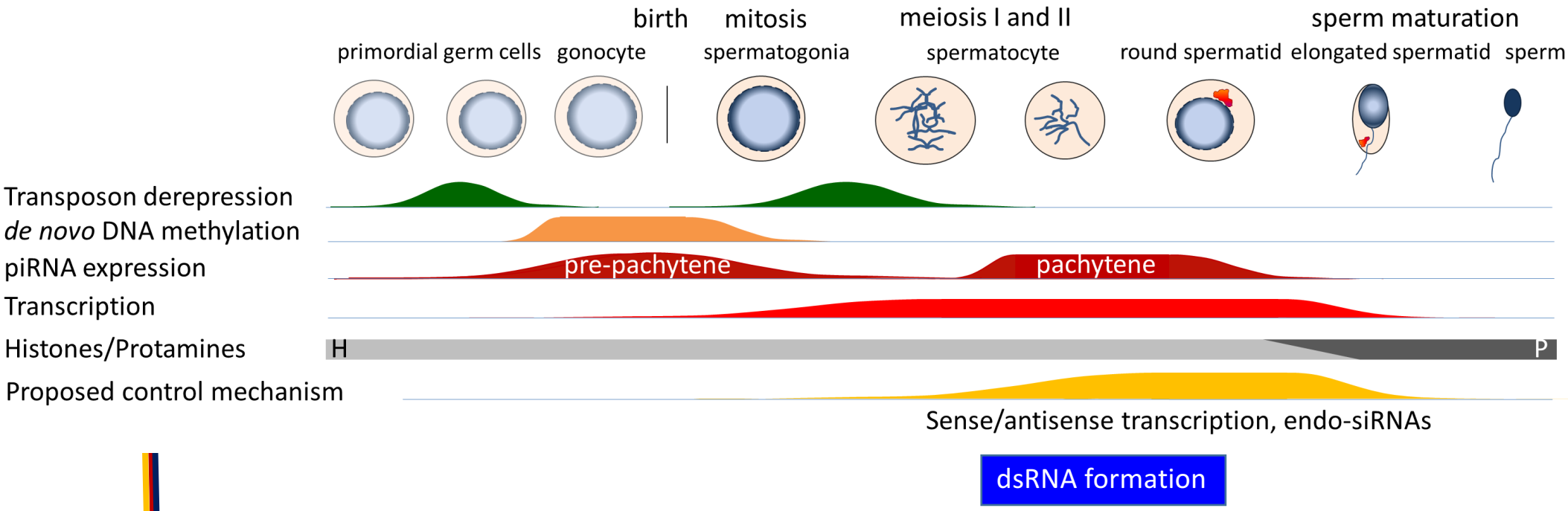
Wang:
Late spermatogenesis

Geisinger:
Early spermatogenesis

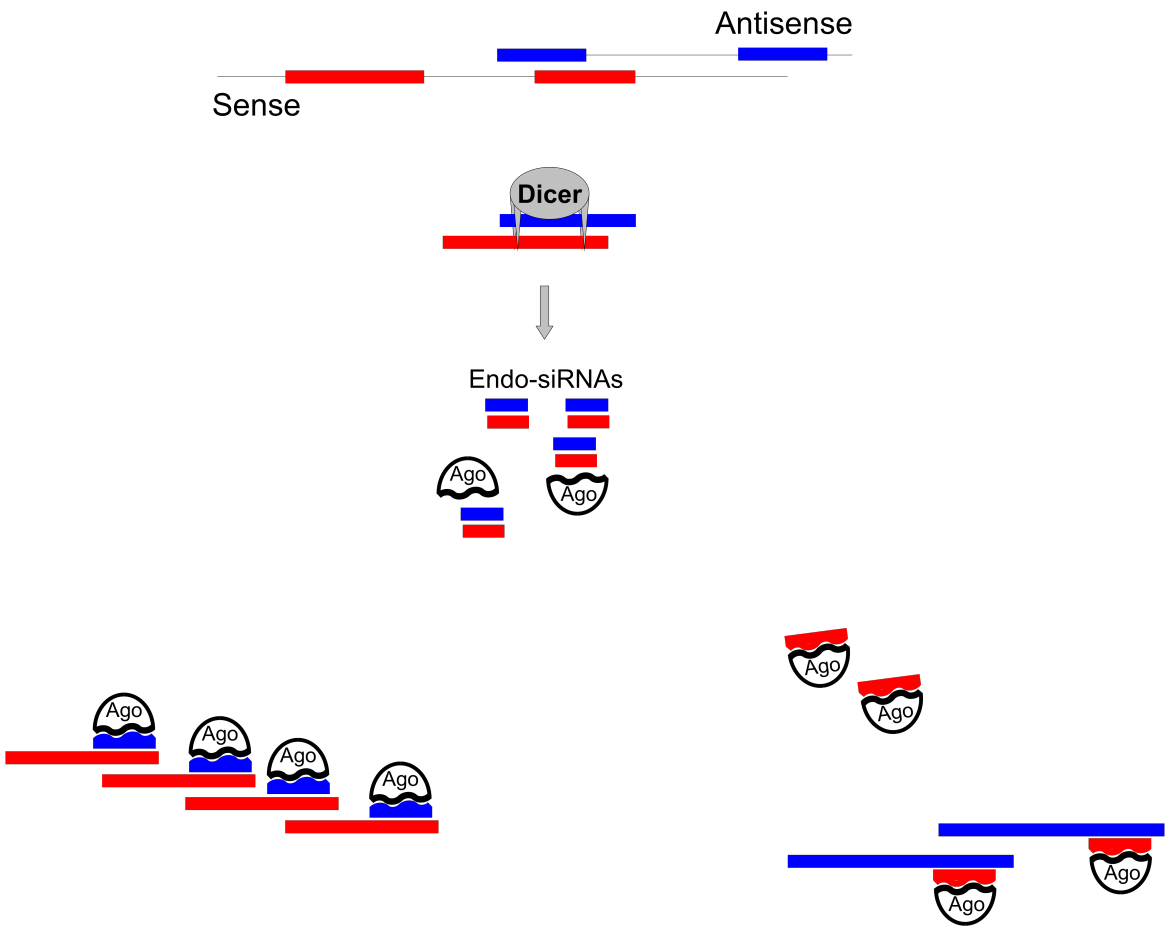
Ontology of dsRNA genes: Molecular function

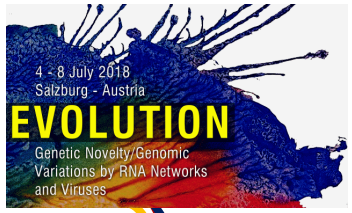


Expression pattern of dsRNA



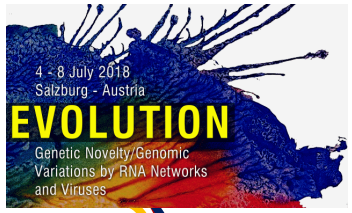
The mechanism, a hypothesis





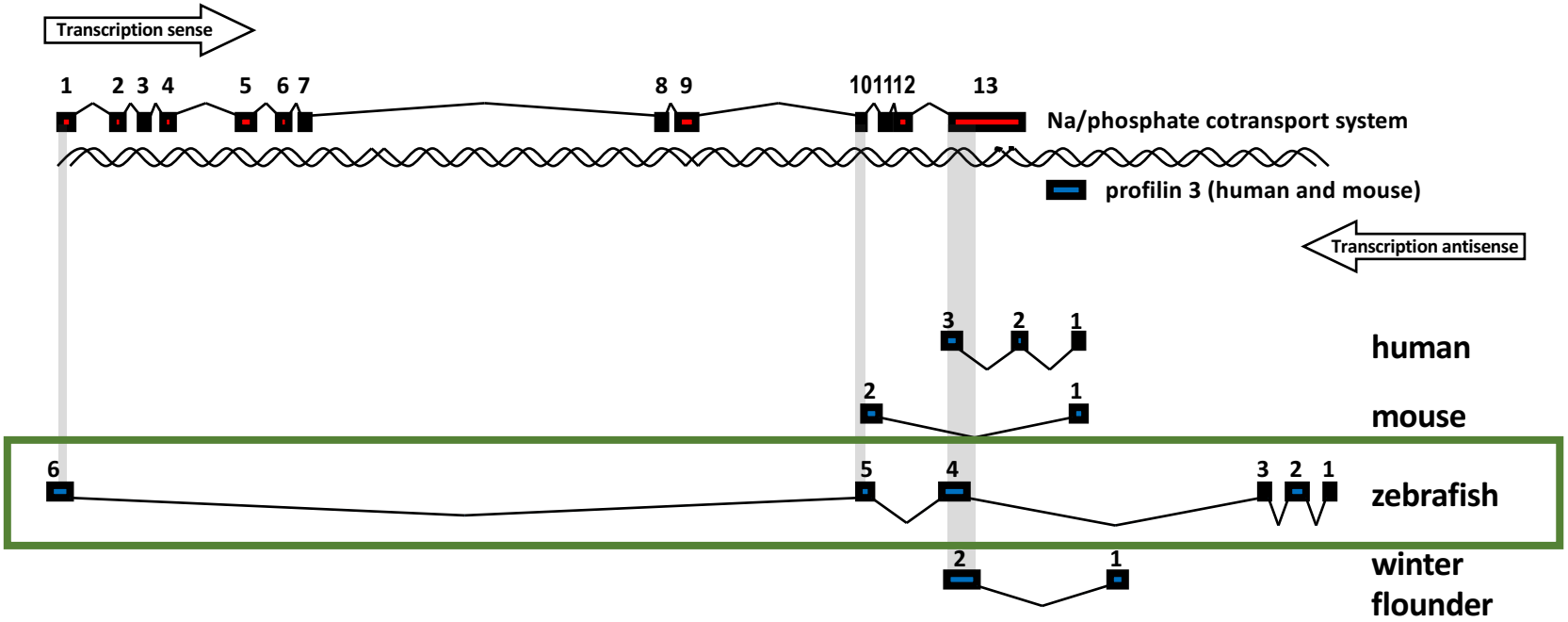
Conclusions part 1

- NATs are widely expressed and tend to form dsRNA
- dsRNA formation is most prominent in testis, in pachytene cells
- dsRNA transcriptome shows similarity in all tissues
- Indirect evidence suggests a NATs mediated gene quality control

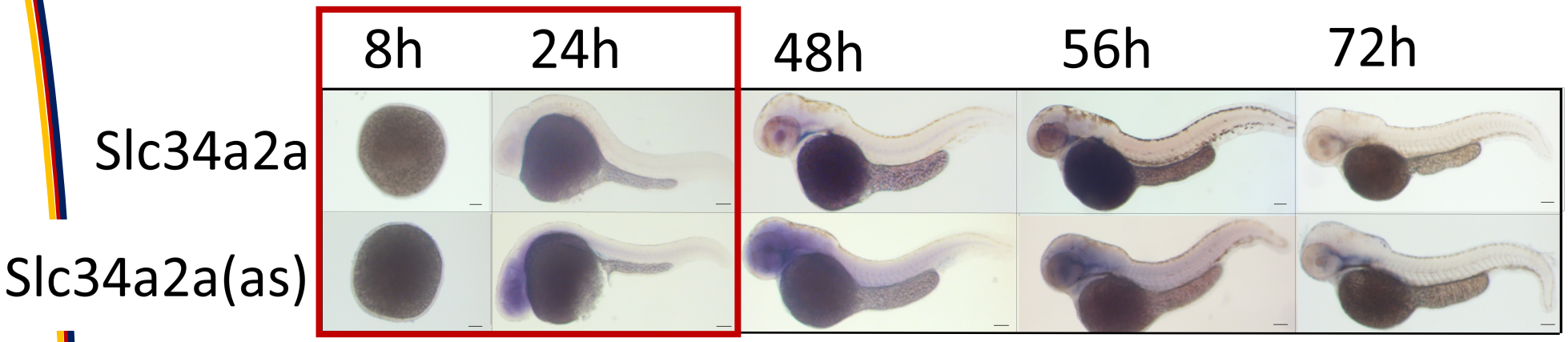


The regulatory role of NATs in expression of sense/antisense transcripts from a specific locus (*Slc34a1*)

Organization of *Slc34a1*, Na-phosphate transporter



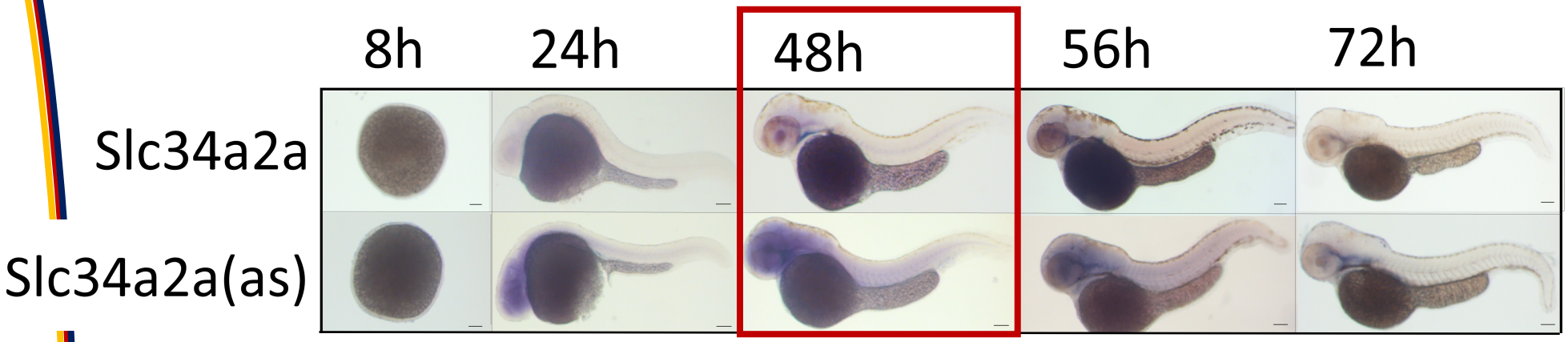
Expression of *Slc34a1 / as* in zebrafish embryos



- Early, no expression of the sense transcript, diffuse expression of antisense

Piatek et al. 2017, Plos ONE 12: e0178219

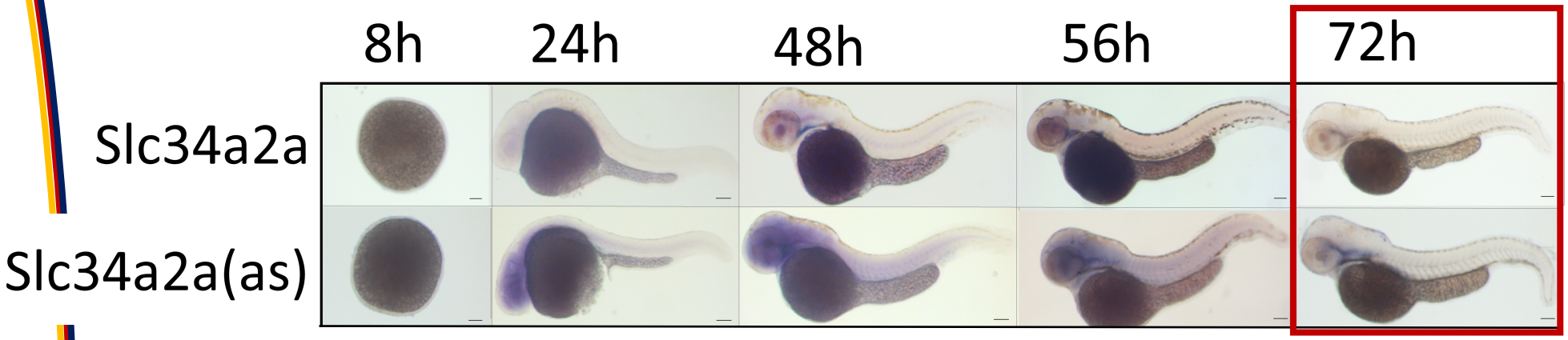
Expression of *Slc34a1 / as* in zebrafish embryos



- Early, no expression of the sense transcript, diffuse expression of antisense
- 48 hpf, distinct expression of sense in pronephric / intestinal structures, as diffuse

Piatek et al. 2017, Plos ONE 12: e0178219

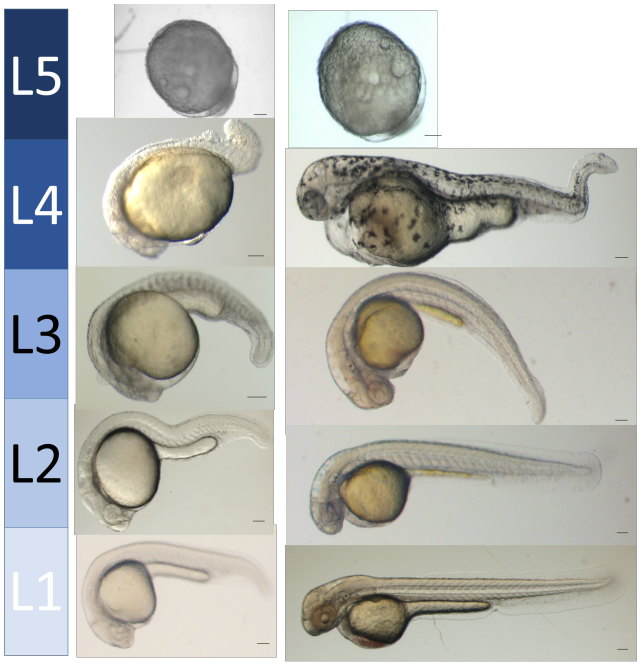
Expression of *Slc34a1 / as* in zebrafish embryos

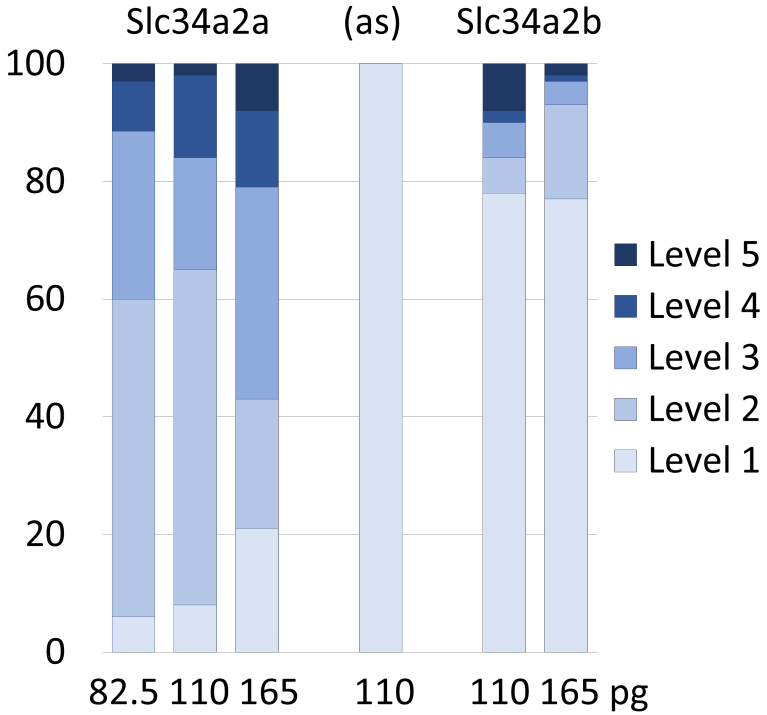


- Early, no expression of the sense transcript, diffuse expression of antisense
- 48 hpf, distinct expression of sense in pronephric / intestinal structures, as diffuse
- Late, distinct co-expression of sense and antisense

Piatek et al. 2017, Plos ONE 12: e0178219

Ectopic sense/antisense expression is toxic

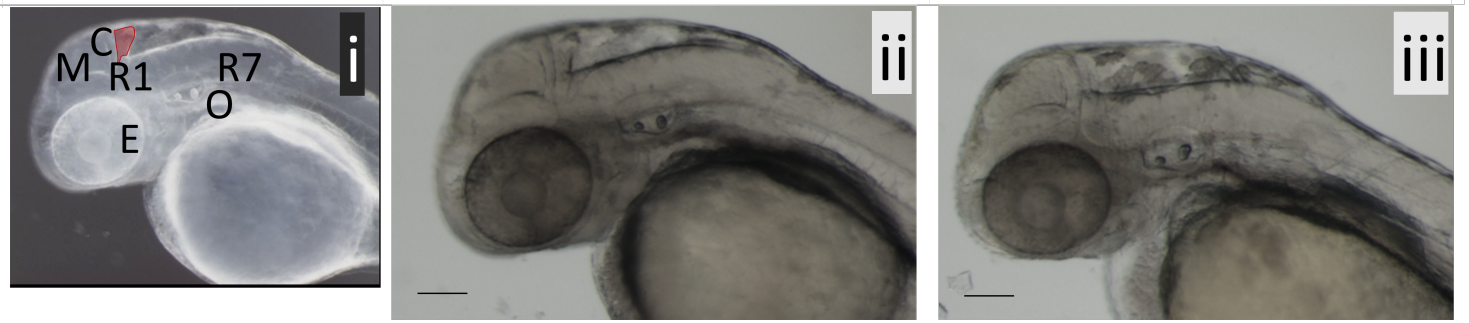




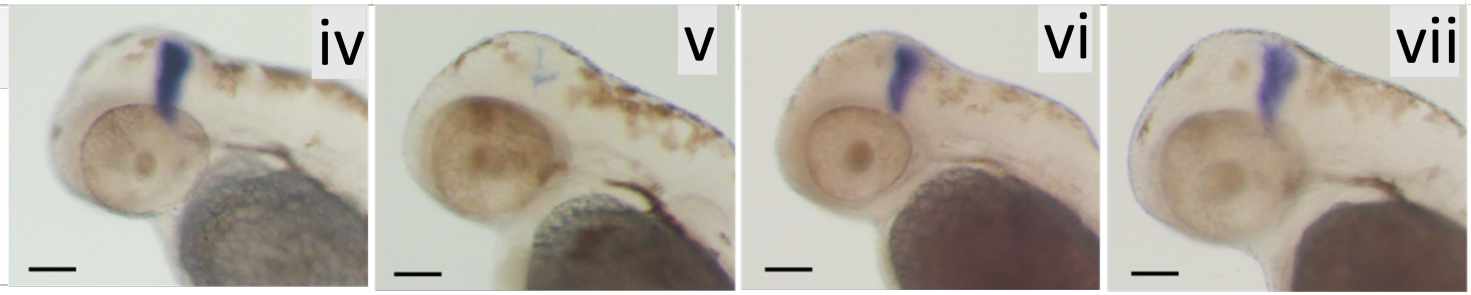
Piatek et al. 2017, Plos ONE 12: e0178219

Ectopic s/as impairs cerebellum development

RNA injected	none	none	Slc34a2a

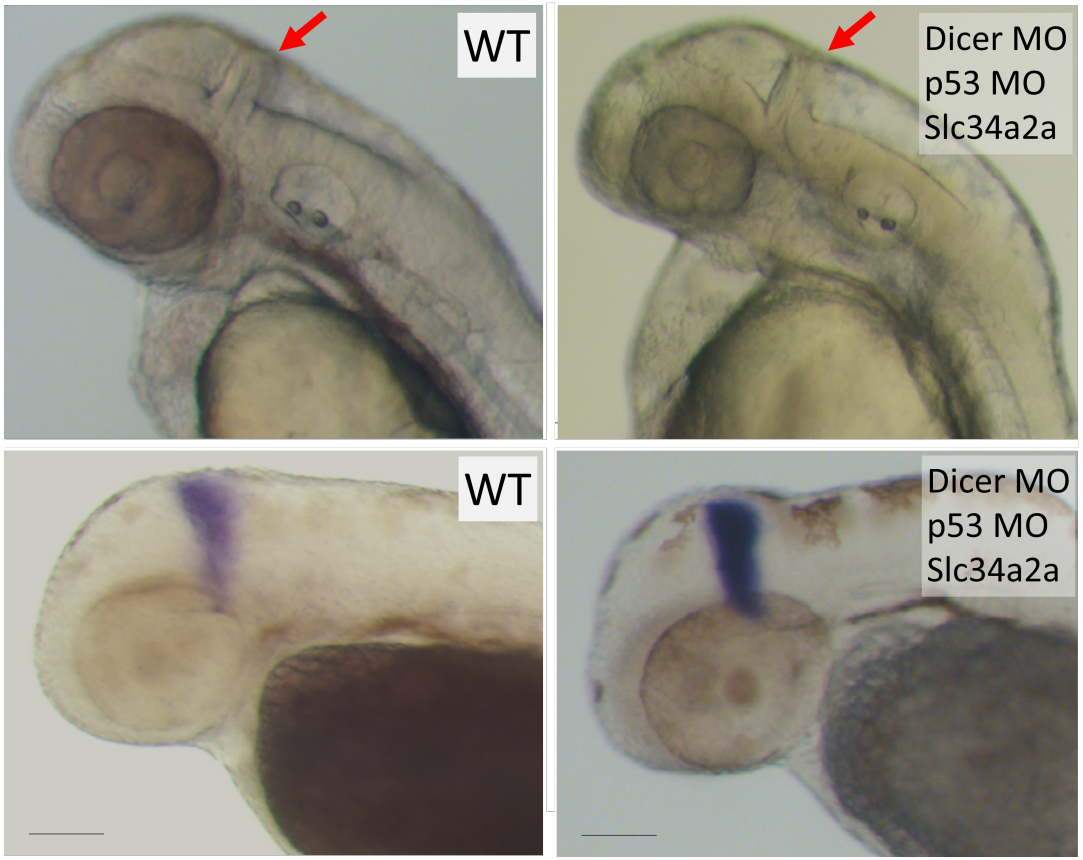


RNA injected	none	Slc34a2a	Slc34a2a(as)	Slc34a2b
ISH staining				
Eng2				



Piatek et al. 2017, Plos ONE 12: e0178219

Knock down of Dicer rescues cerebellum



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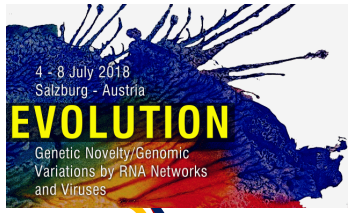


Mechanism of (Slc34a1) antisense regulation

1) Slc34a1 s/as form dsRNA

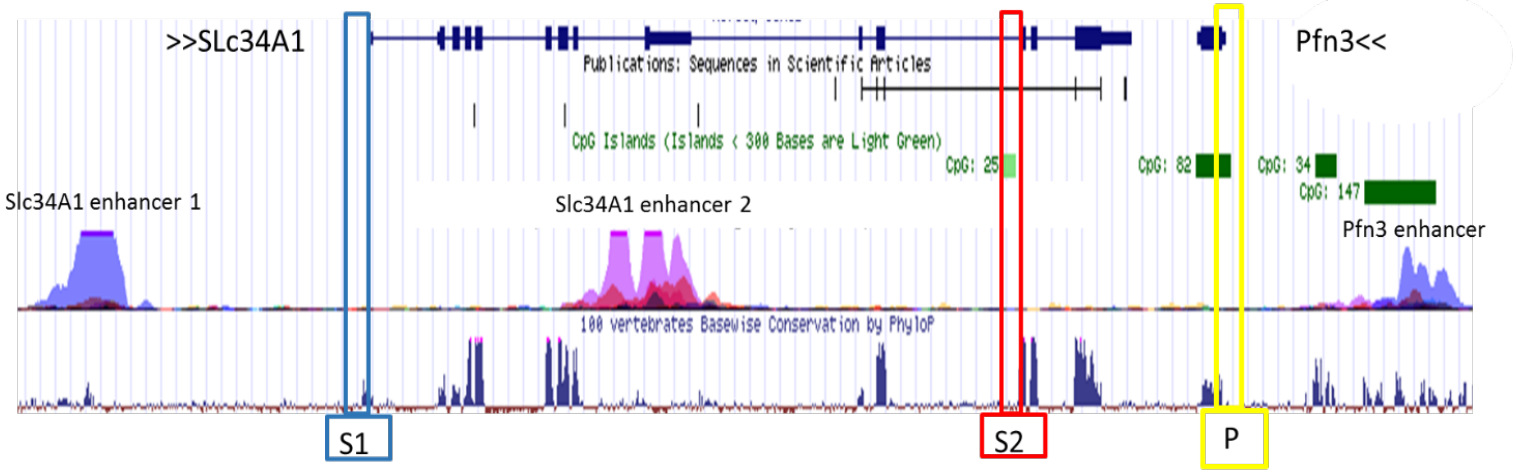
2) dsRNA is processed by Dicer

3) siRNAs feed into miRNA pathway and target endogenous mRNAs

4) THOUGH, hairpin RNAs against putative targets fail to fully reproduce the cerebellar phenotype

➔ **Better controlled system required**

Dexamethasone induces SLC34a1 s/as expression

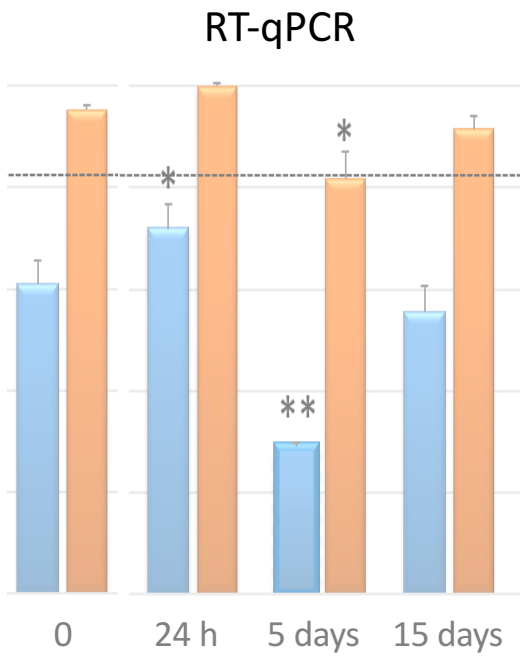


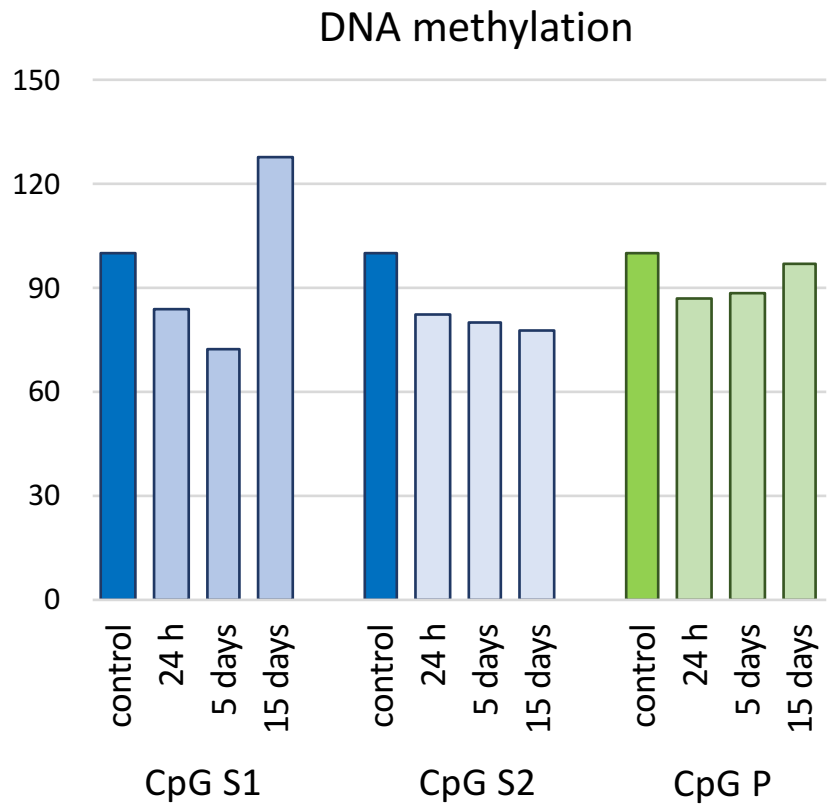
S1= CGTTTTTTTTAGTGTTTTCGCGGAGATTCGTTTTTTTAGTGTTTTCGCGGAGGTTCGTTTTTTTAG

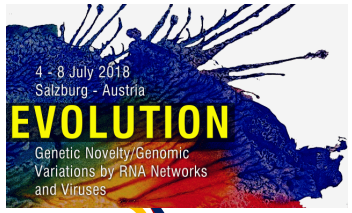
S2= CGGGGGATGTGTTTGGGTCGTGGTTG

P = CGGTTTCGTGAGTACGTTTATTTTTTGCGGCGAGATGGTCGTTAGTAGGTT

CpG de- methylation parallels expression changes



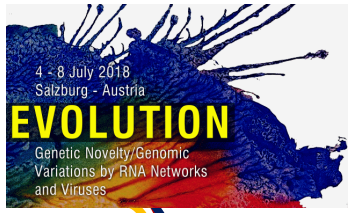




Conclusions 2

1) Experimental interference with s/as expression has an acute regulatory impact (that may be used for therapeutic approaches)

2) The gene specific regulatory functions depend on the experimental context and are not necessarily related to the role NATs play during evolution ?



Acknowledgments

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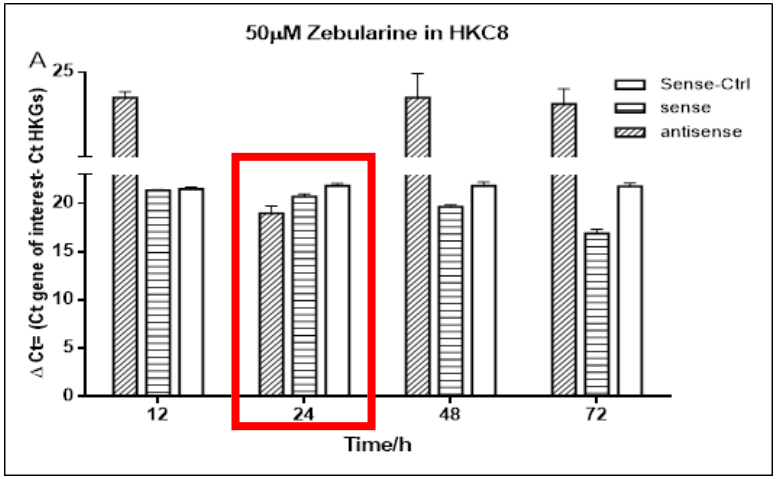
Martin Smith	
John Mattick	Sidney (Australia)

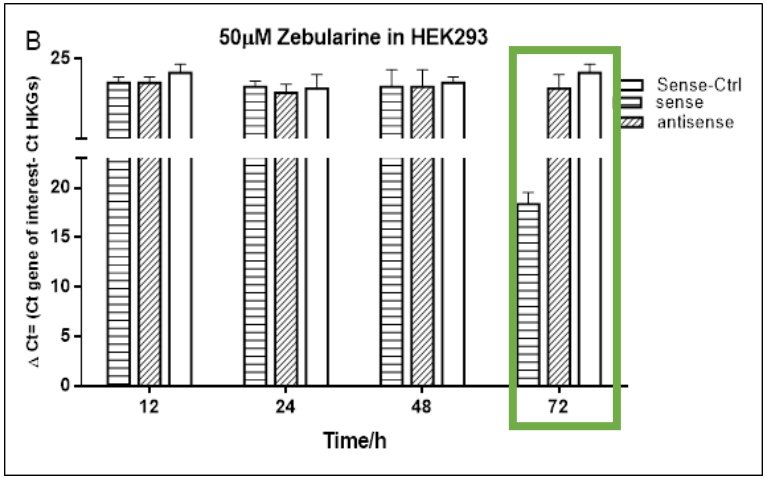
Baeza, Andalucia, Spain
29-31 October 2018
http://www.unia.es/biomedicine

Results

2. DNA methylation

- Temporal effect of zebularine on SLC34A1 sense and antisense transcripts





- Gradual increase in sense expression.
- No antisense transcripts were detected after 12, 48 and 72h.
- After 24h HKC8 showed expression of the antisense transcripts.

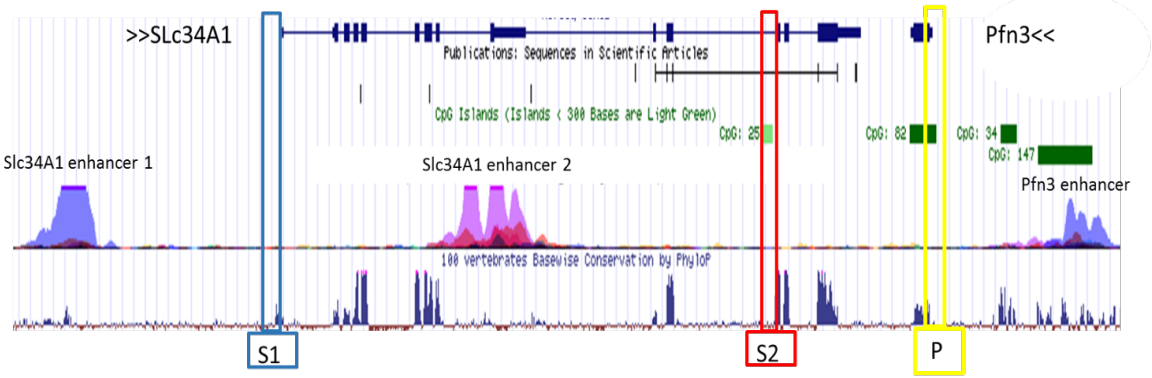
- Neither sense nor antisense expression was found at earlier time points.
- Only at 72h the sense transcripts was detected

Results

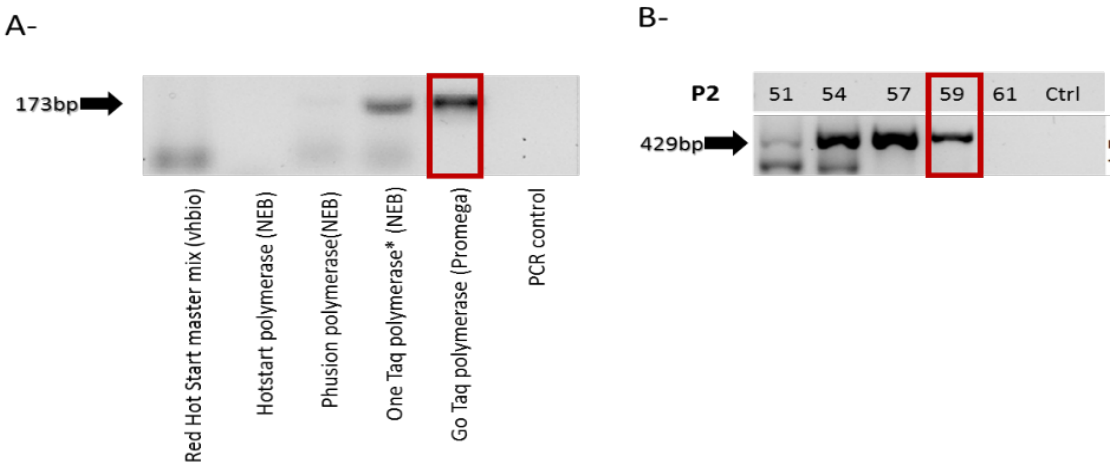
3. Pyrosequencing of SLC34A1/PFN3 CpG islands

3.1. Optimisation steps:

- For *SLC34A1*, two regions were identified with typical promoter features.
- For *PFN3* (antisense) one CpG island was investigated



S1= CGTTTTTTTTAGTGTTTTCGCGGAGATTCGTTTTTTTAGTGTTTTCGCGGAGGTTCGTTTTTTTAG
S2= CGGGGGATGTGTTTGGGTCGTGGTTG
P = CGGTTTCGTGAGTACGTTTATTTTTTGCGGCGAGATGGTCGTTAGTAGGTT



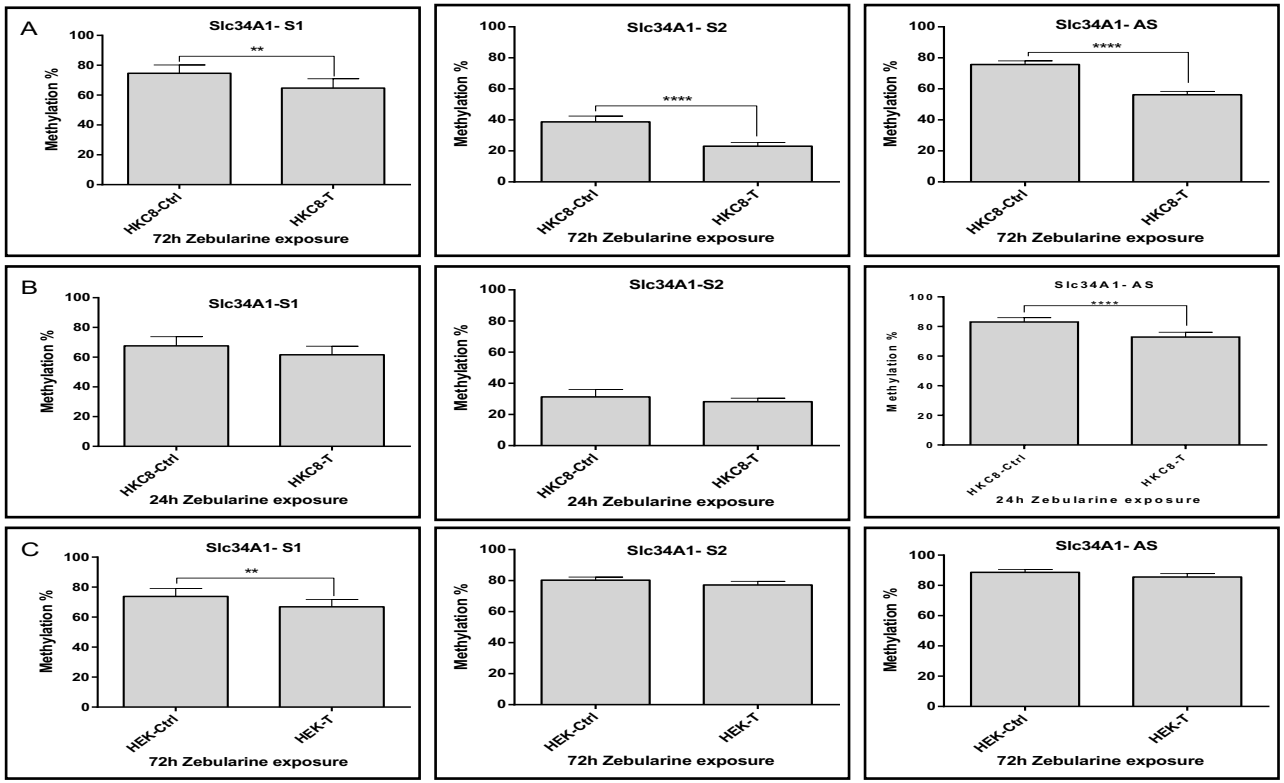
- PCR- optimisation for pyrosequencing experiments.

Results

3. Pyrosequencing of SLC34A1/PFN3 CpG islands

HKC 8

HEK293



Group	time/h	cells type	S1	S2	P1
A	72	HKC8	H.M	H.M	H.M
B	24	HKC8	M	M	H.M
C	72	HEK293	H.M	M	M

H.M=hypomethylated
M = Methylated