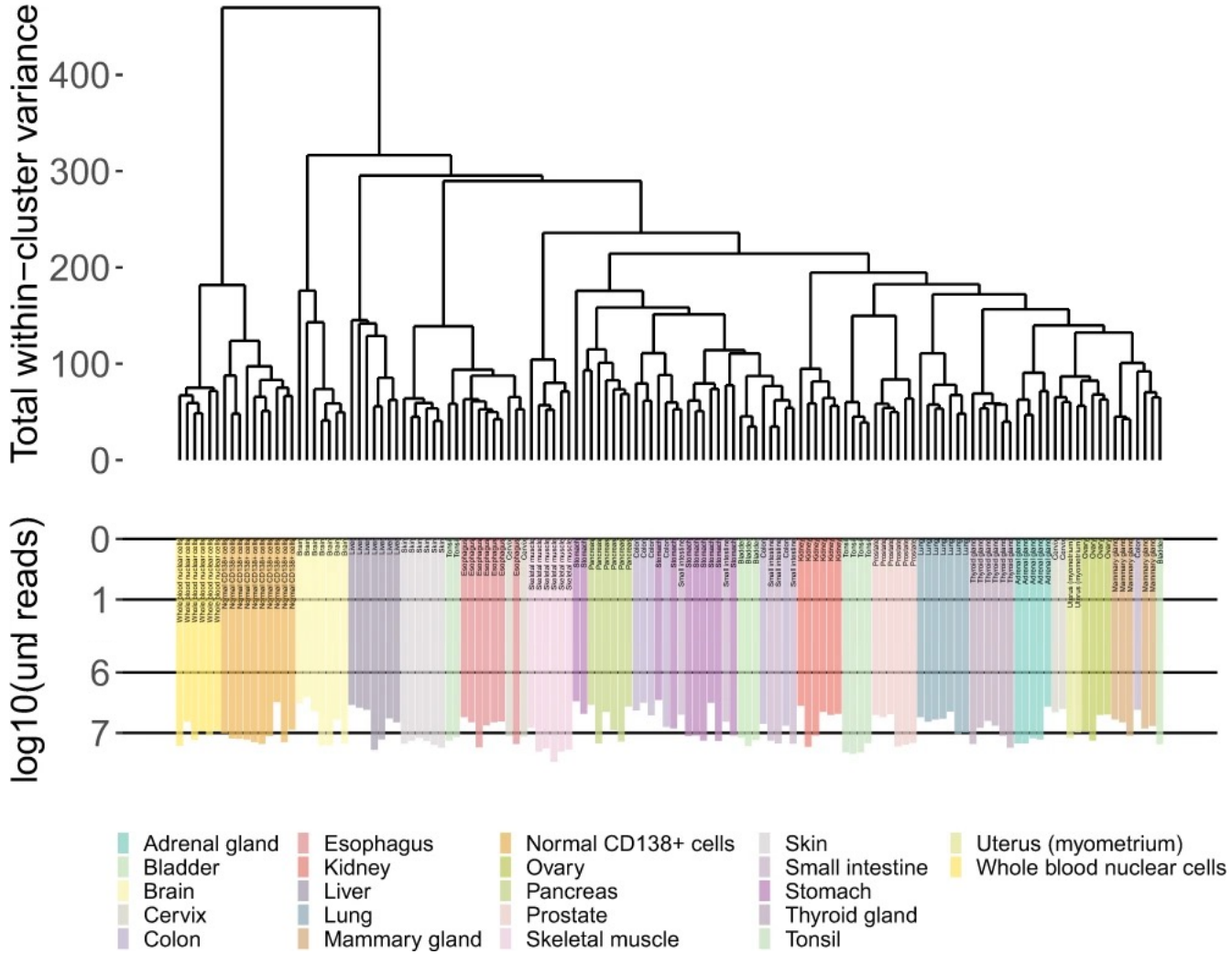
RNA introduction

RNA-seq data analysis

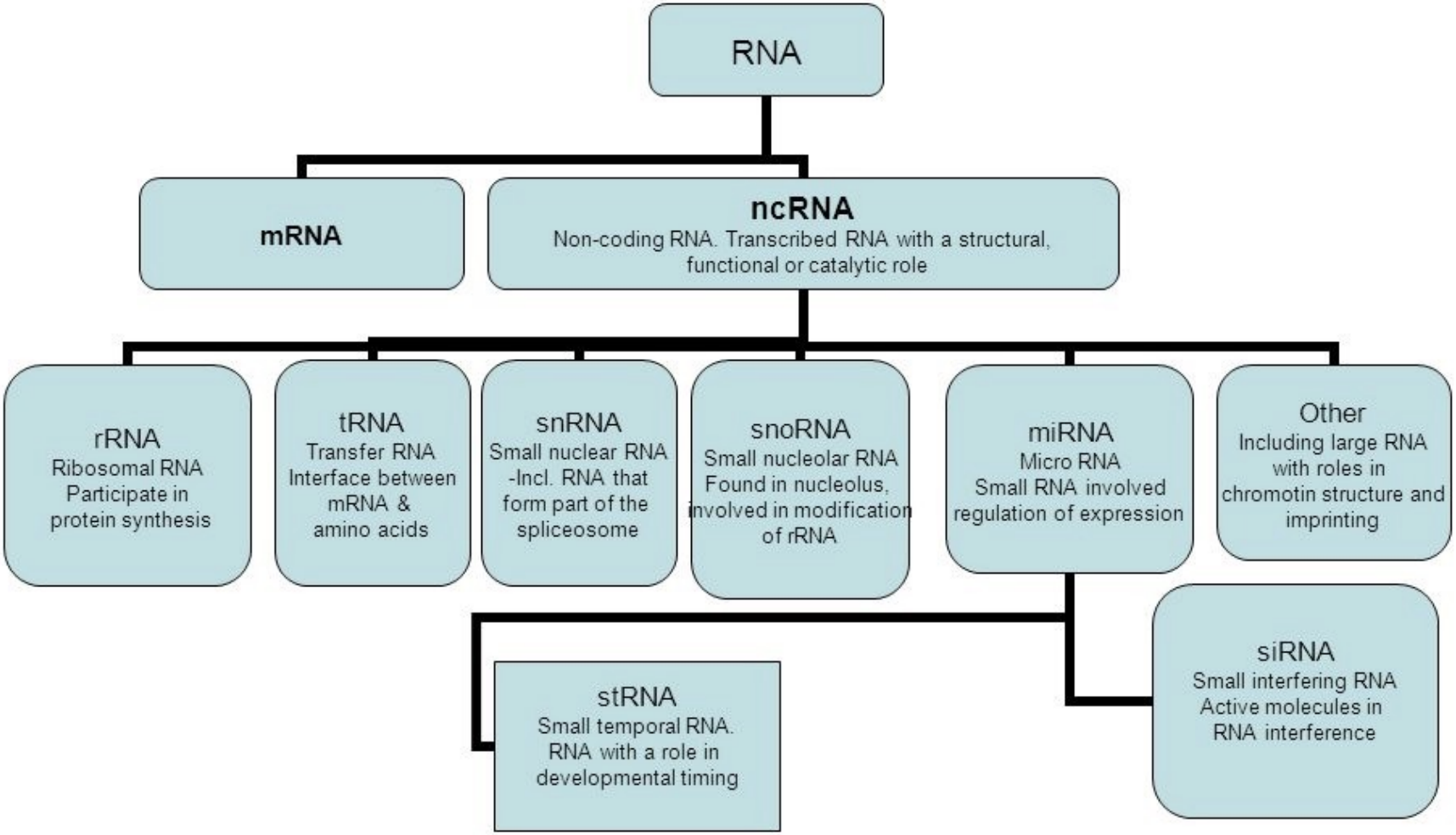
Johan Reimegård |

DNA is the same in all cells
RNAs are different in all cells



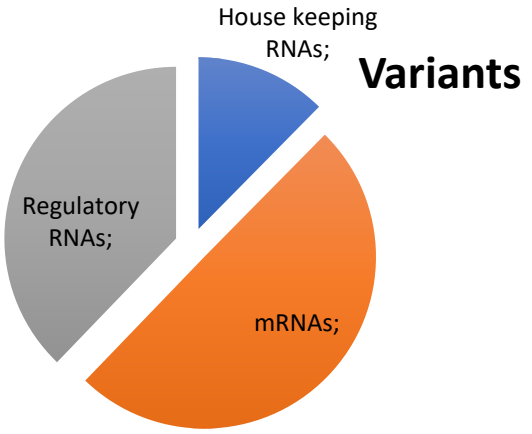
Atlas of RNA sequencing profiles for normal human tissues (Scientific data 2019)

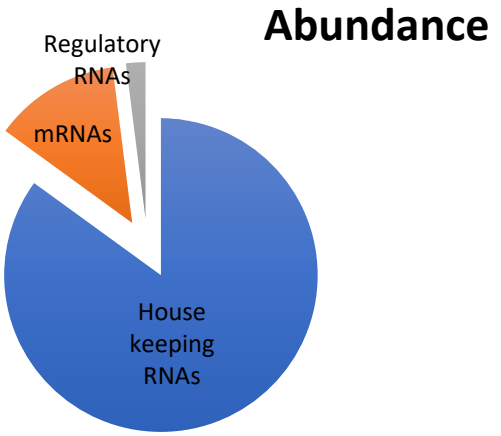
There is a wide variety of different functional RNAs



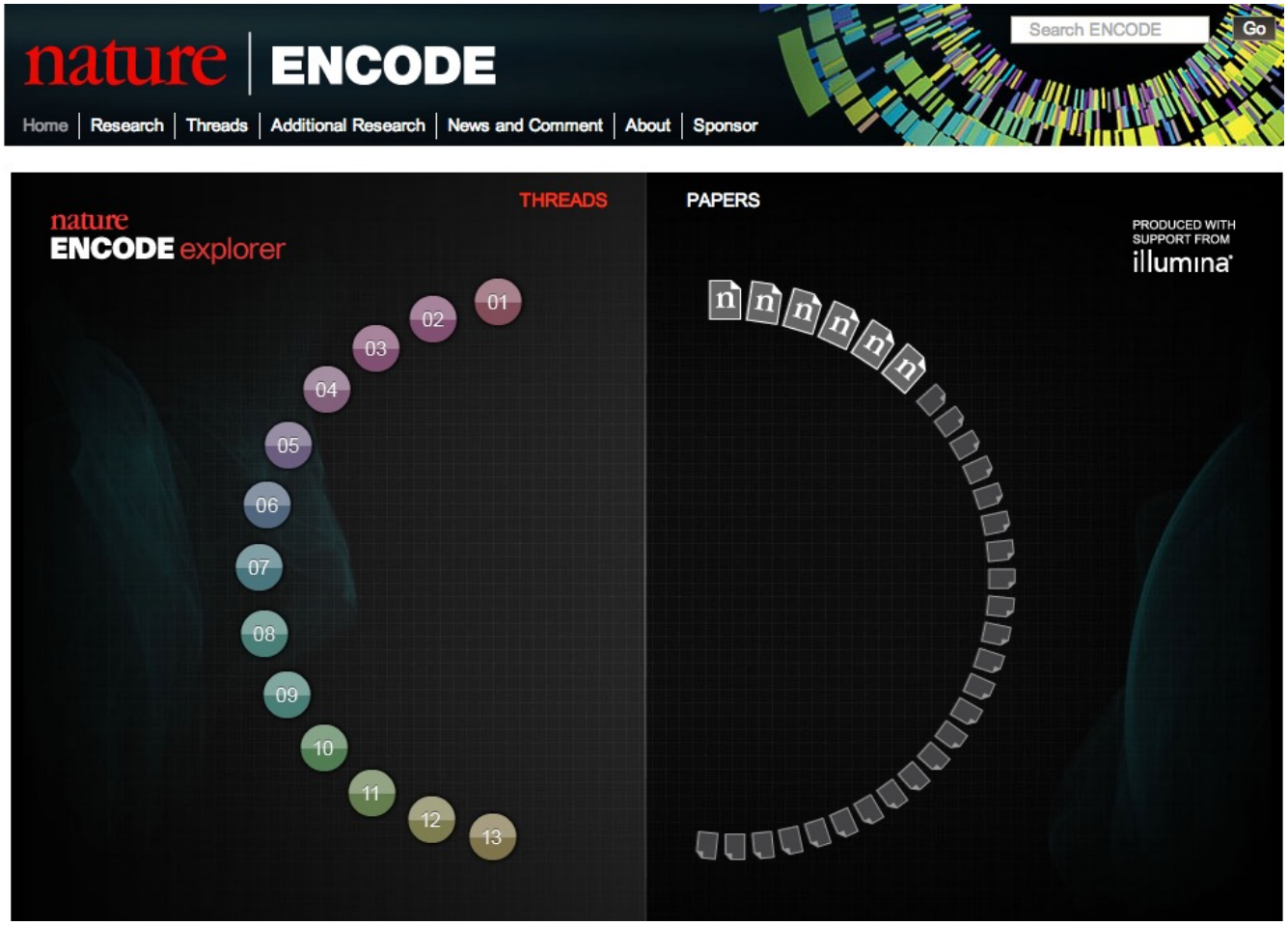
Depending on how you count there are more or less of the different RNAs

- House keeping RNAs
  - rRNAs, tRNAs, snoRNAs, snRNAs…
- Protein coding RNAs
  - 1 coding gene – many mRNAs)
- Regulatory RNAs
  - sRNAs, CRIPSR, miRNAs, piRNAs, lincRNAs….



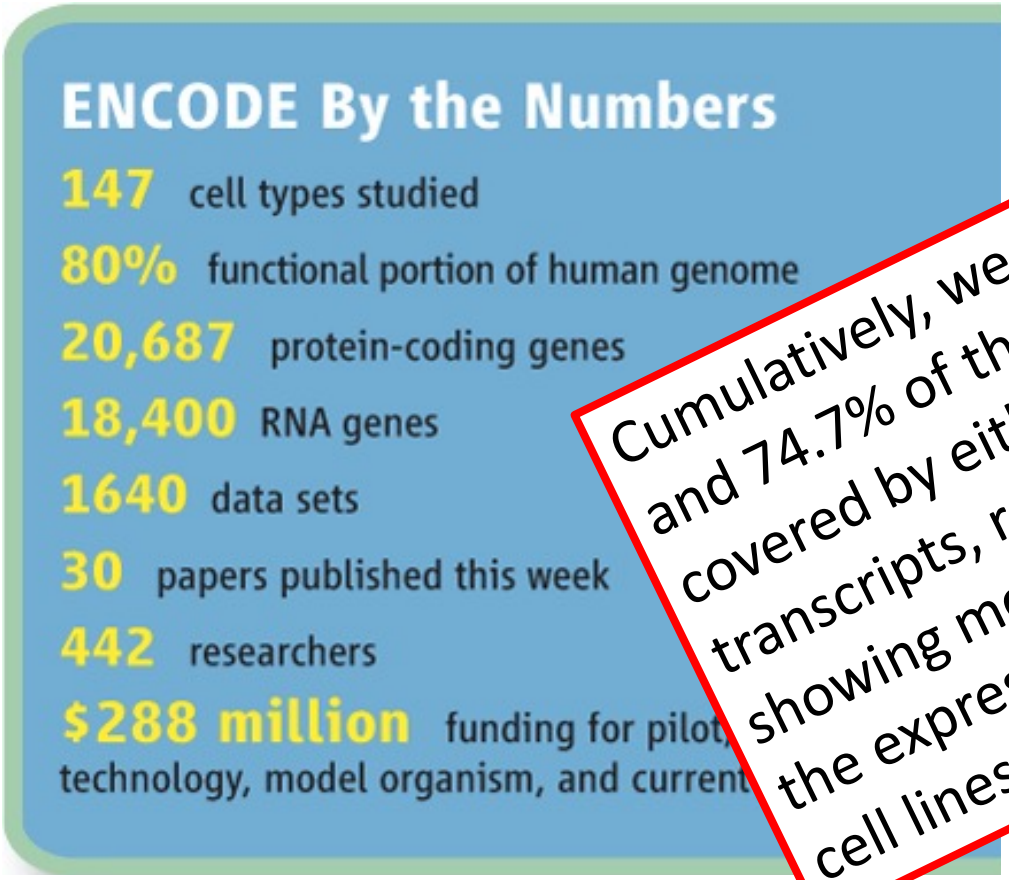


Landscape of transcription in human cells, S Djebali *et al.* ***Nature 2012***



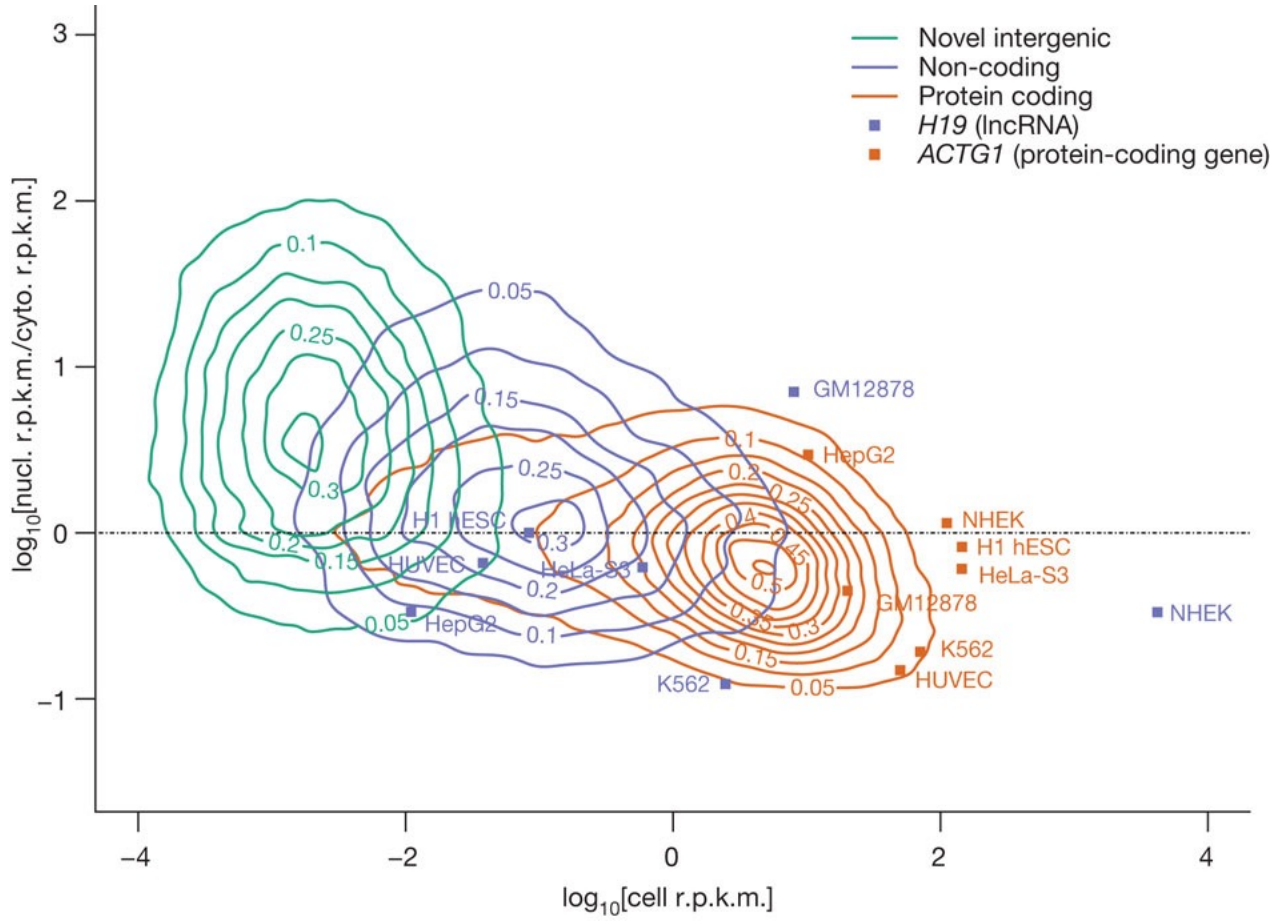
ENCODE, the Encyclopedia of DNA Elements, is a project funded by the National Human Genome Research Institute to identify all regions of transcription, transcription factor association, chromatin structure and histone modification in the human genome sequence.

ENCyclopedia Of Dna Elements



Cumulatively, we observed a total of 62.1% and 74.7% of the human genome to be covered by either processed or primary transcripts, respectively, with no cell line showing more than 56.7% of the union of the expressed transcriptomes across all cell lines.

Coding genes are more highly expressed than non-coding



RNA flavors

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PLoS BIOLOGY

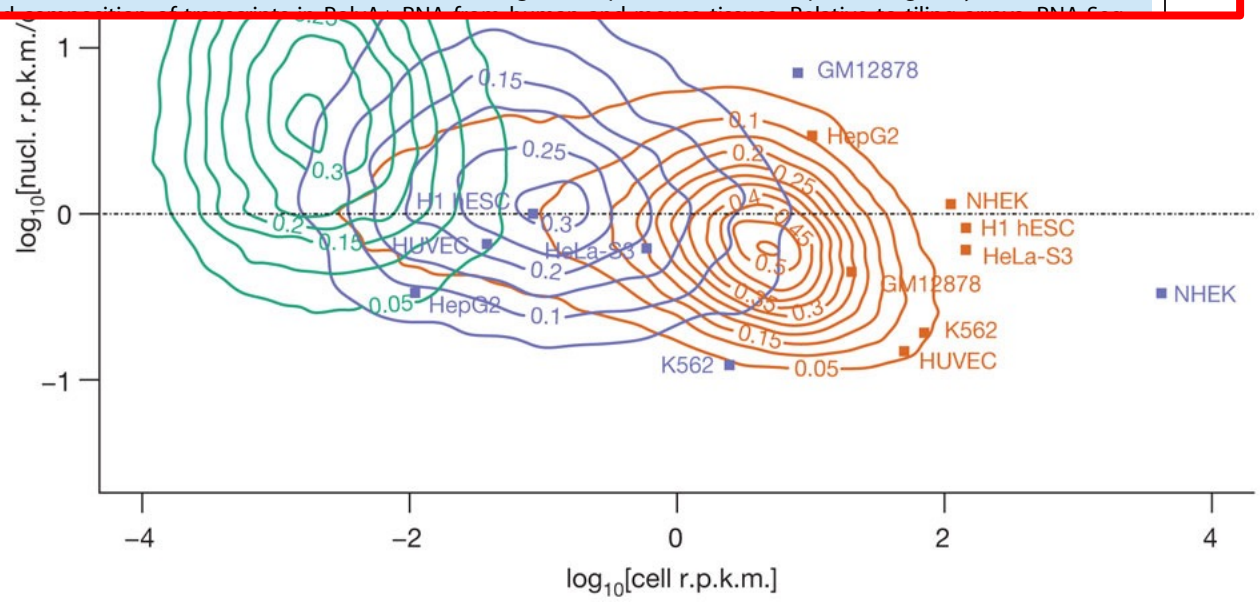
Most "Dark Matter" Transcripts Are Associated With Known Genes

Harm van Bakel[1], Corey Nislow[1,2], Benjamin J. Blencowe[1,2], Timothy R. Hughes[1,2]*

1 Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada, **2** Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

Abstract

A series of reports over the last few years have indicated that a much larger portion of the mammalian genome is transcribed than can be accounted for by currently annotated genes, but the quantity and nature of these additional transcripts remains unclear. Here, we have used data from single- and paired-end RNA-Seq and tiling arrays to assess the



RNA flavors

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Most "Dark Matter" Transcripts Are Associated With K

Ha

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Ont

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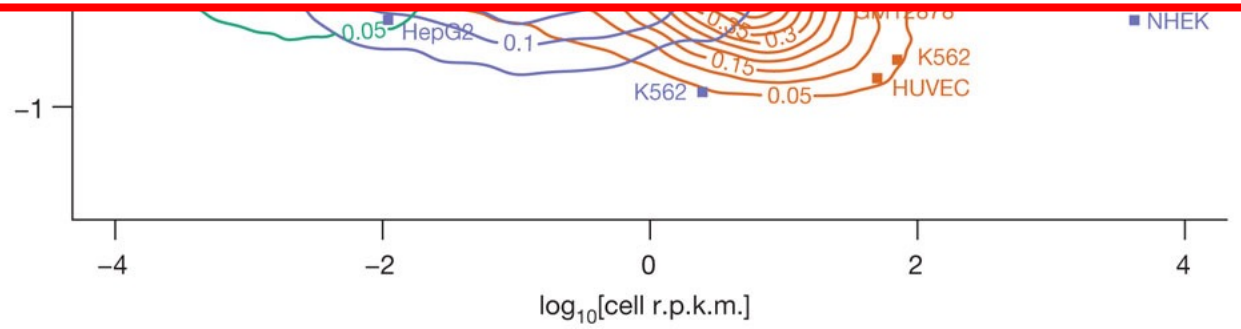
Perspective

The Reality of Pervasive Transcription

Michael B. Clark[1], Paulo P. Amaral[1,9], Felix J. Schlesinger[2,9], Marcel E. Dinger[1], Ryan J. Taft[1], John L. Rinn[3], Chris P. Ponting[4], Peter F. Stadler[5], Kevin V. Morris[6], Antonin Morillon[7], Joel S. Rozowsky[8], Mark B. Gerstein[8], Claes Wahlestedt[9], Yoshihide Hayashizaki[10], Piero Carninci[10], Thomas R. Gingeras[2]*, John S. Mattick[1]*

1 Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia, **2** Watson School of Biological Sciences, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, United States of America, **3** Broad Institute, Cambridge, Massachusetts, United States of America, **4** MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom, **5** Department of Computer Science, University of Leipzig, Leipzig, Germany, **6** Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, California, United States of America, **7** Institut Curie, UMR3244-Pavillon Trouillet Rossignol, Paris, France, **8** Computational Biology and Bioinformatics, Yale University, New Haven, Connecticut, United States of America, **9** University of Miami, Miami, Florida, United States of America, **10** Omics Science Center, RIKEN Yokohama Institute, Tsurumi-ku, Yokohama, Kanagawa, Japan



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The

Micha
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Miami, M

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Perspective

Response to "The Reality of Pervasive Transcription"

Harm van Bakel[1], Corey Nislow[1,2], Benjamin J. Blencowe[1,2], Timothy R. Hughes[1,2]*

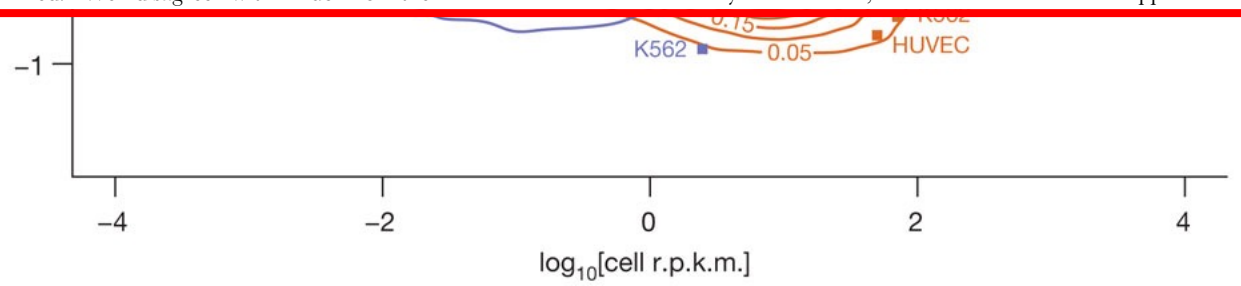
1 Banting and Best Department of Medical Research and Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario, Canada, **2** Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

Clark et al. criticize several aspects of our study [1], and specifically challenge our assertion that the degree of pervasive transcription has previously been overstated. We disagree with much of their

tic" transcripts greatly increases their abundance [7,8].

We acknowledge that the phrase quoted by Clark et al. in our Author Summary should have read "stably transcribed", or

emphasized the lack of abundant pervasive transcription in our study. Clark et al. cite papers that have previously documented pervasive transcription, and point out that several different approaches have been



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Comments on van Bakel et al. (2011) Response to "The Reality of Pervasive Transcription"

Comments by Mike Clark

Van Bakel et al. 2011 (vB 11) have published their reply to our critique of their paper van Bakel et al. 2010 (vB 10).

Firstly lets briefly review some of our main criticisms of vB 10:

1. vB 10 didn't properly consider previous evidence for pervasive transcription (especially that from cDNA analysis in the mouse) when claiming the genome was not as tra previous evidence was unreliable due to false positives.

2. vB 10 incorrectly conflated pervasive transcription with the relative abundance of transcripts when the correct (and known) definition was the amount of the genome that

3. The tiling arrays vB 10 performed and then used to claim that previous array studies suffered from high false positives were atypical and lacked any validation of the fal

4. The RNA sequencing carried out by vB 10 was severely limited in its ability to address the question of pervasive transcription. The depth of sequencing was too shallow complex samples and then the assembly of what was found into transcripts was poor. Since it couldn't detect and/or characterize rare transcripts this meant it couldn't eva

5. vB 10 claimed that low level intergenic transcription may be due to "random initiation events" and/or transcriptional "byproducts" (ie: transcription noise), when the limitat differentiate properly between this and genuine transcripts under their detection threshold.

mattick

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nks
- QuARC
- NRED
- lncRNAdb

Novel intergenic
ding
coding
RNA)
(protein-coding gene)

Defining functional DNA elements in the human genome

A priori, we should not expect the transcriptome to consist exclusively of functional RNAs.

Zero tolerance for errant transcripts would come at high cost in the proofreading machinery needed to perfectly gate RNA polymerase and splicing activities, or to instantly eliminate spurious transcripts.

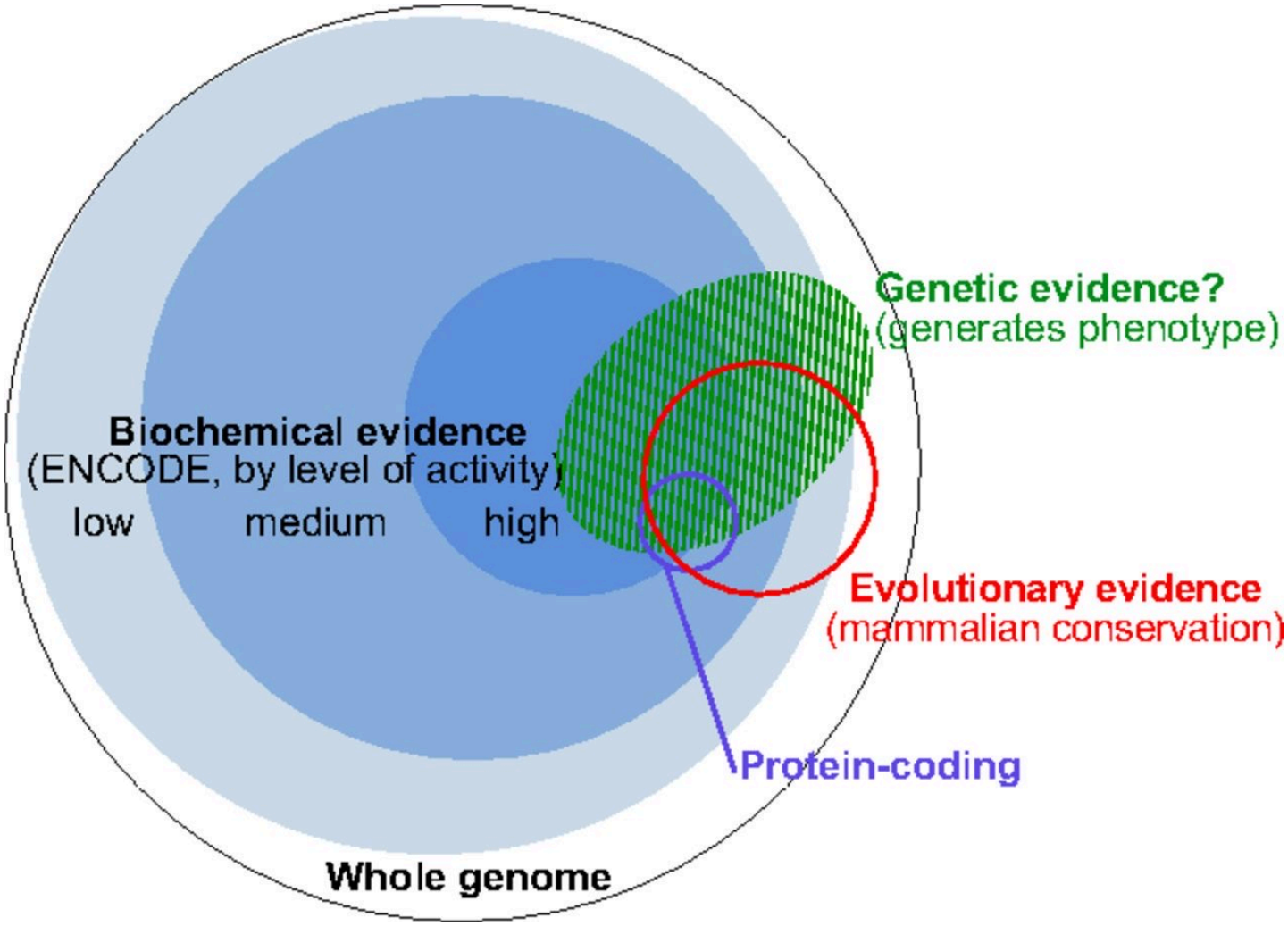
In general, sequences encoding RNAs transcribed by noisy transcriptional machinery are expected to be less constrained, which is consistent with data shown here for very low abundance RNA

Thus, one should have high confidence that the subset of the genome with large signals for RNA or chromatin signatures coupled with strong conservation is functional and will be supported by appropriate genetic tests.

In contrast, the larger proportion of genome with reproducible but low biochemical signal strength and less evolutionary conservation is challenging to parse between specific functions and biological noise.

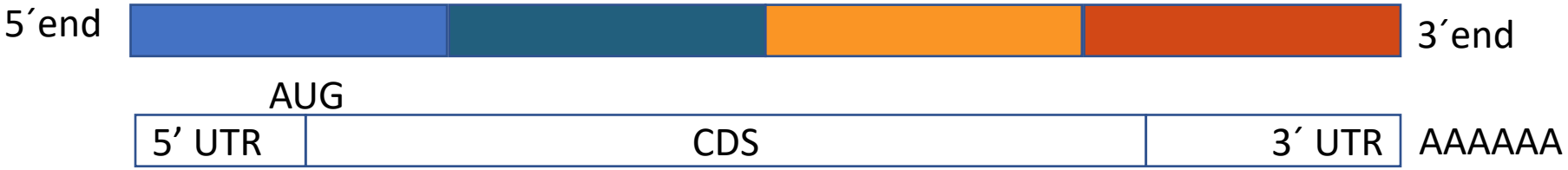
Defining functional DNA elements in the human genome
Kellis M et al. PNAS 2014;111:6131-6138

The complementary nature of evolutionary, biochemical, and genetic evidence.



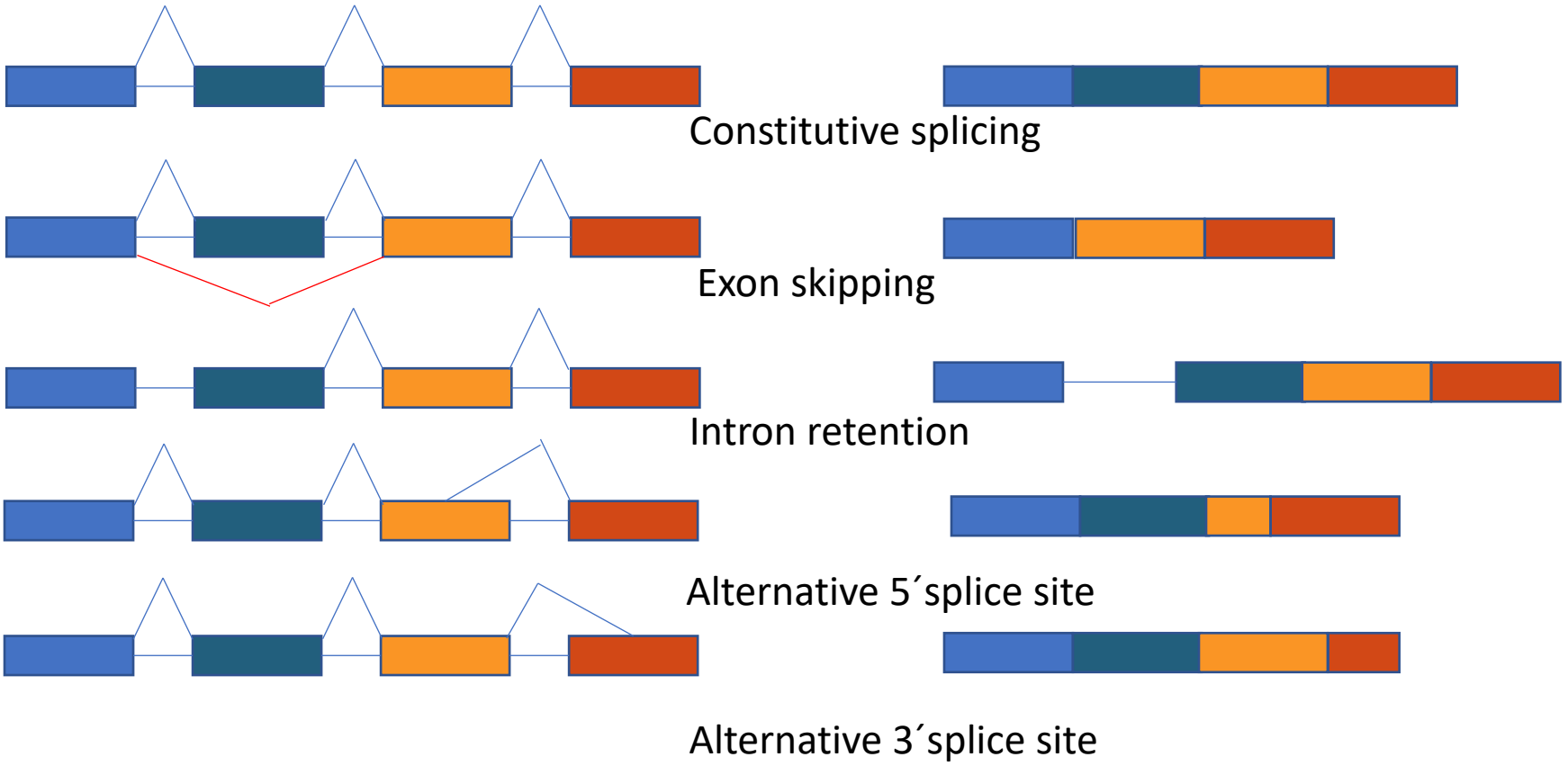
Defining functional DNA elements in the human genome
Kellis M et al. PNAS 2014;111:6131-6138

RNA structure

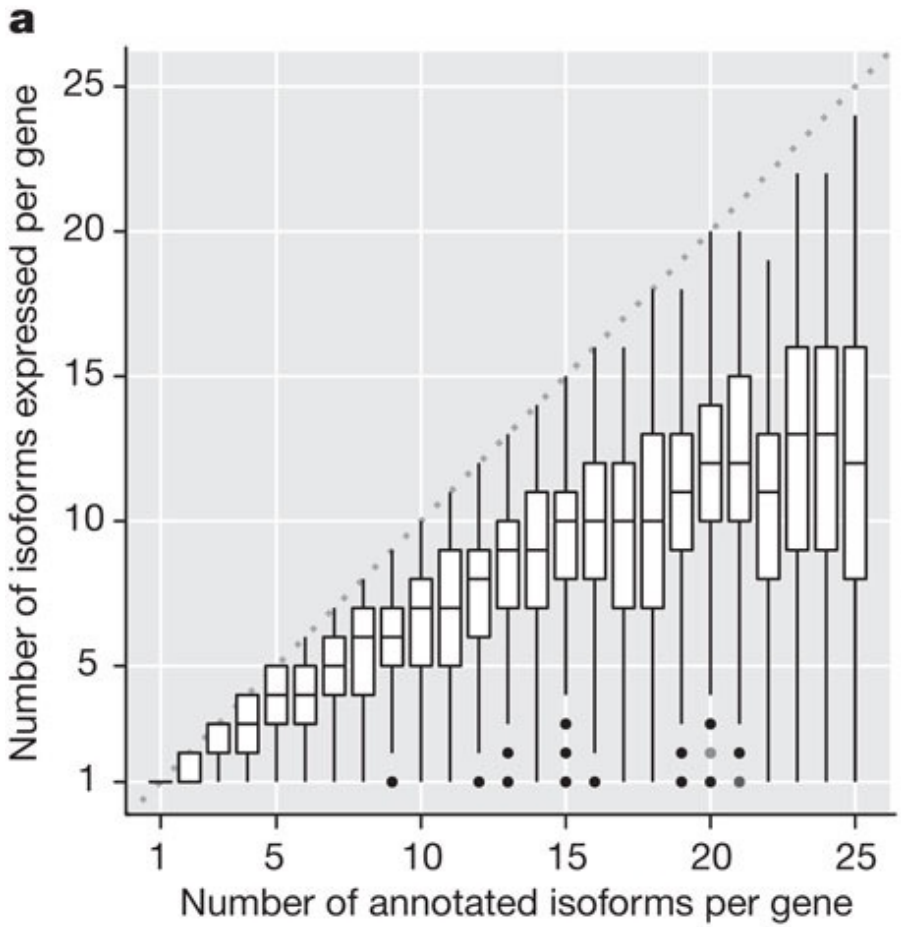


UTR = Untranslated region
CDS = Coding sequence

One gene can produce many different isoforms

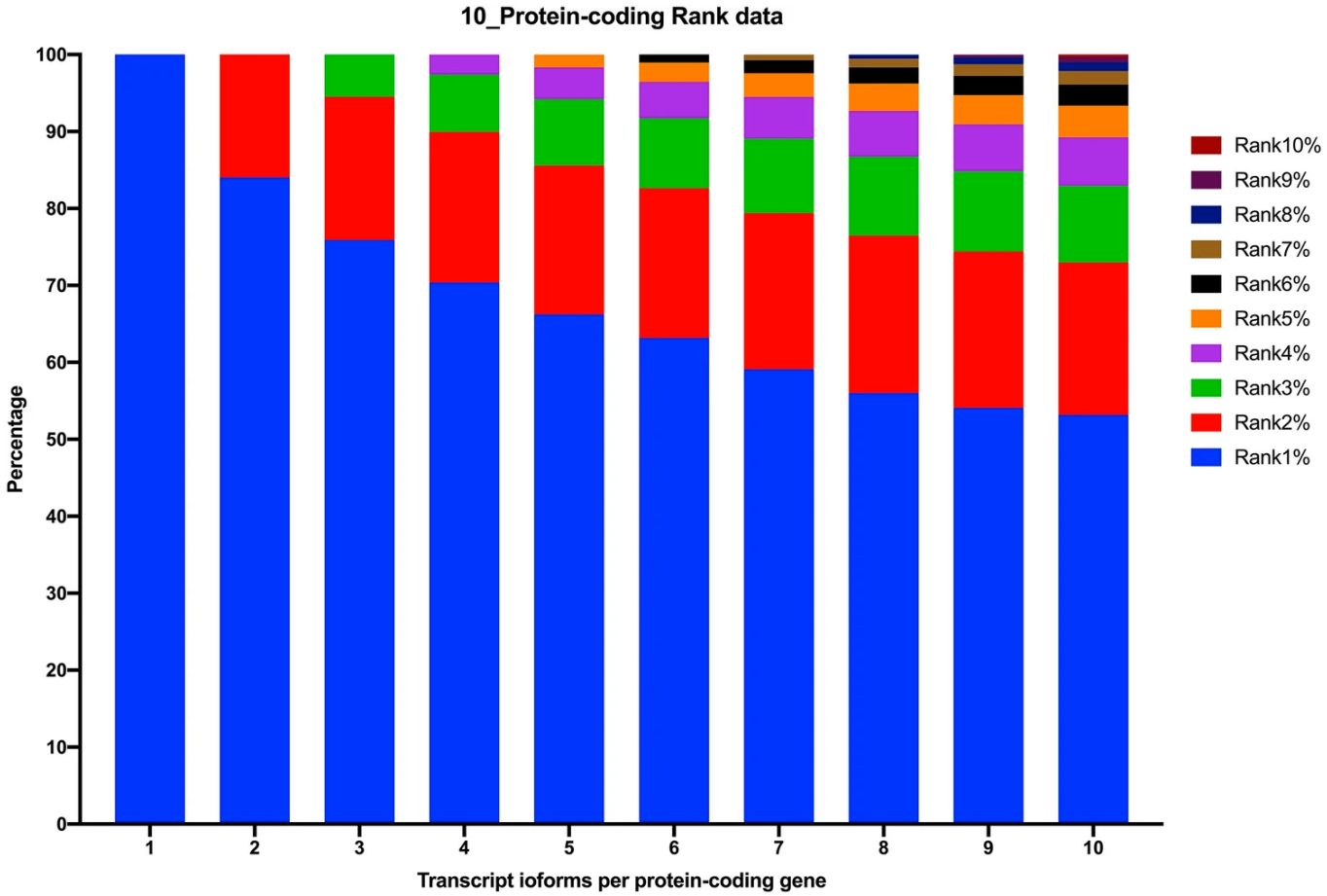


Encode: most isoforms being transcribed



Landscape of transcription in human cells, S Djebali *et al.* ***Nature 2012***

Now: Only a few isoforms being transcribed at a high concentration



Top-ranked expressed gene transcripts of human protein-coding genes investigated with GTEx dataset, Tung *et al*. Scientific reports. 2020

Summary

- Many nt of the genome is being transcribed
  - Most of them are NOT functional.
- Many RNAs are being transcribed in a cell
  - Most of them are functional.
- One gene can produce many isoforms (transcripts)
  - Only a few of those isoforms are likely to be functional
  - Conservation in other species, Functional analysis, coding ability and genetic information can help in identifying which that are important.
- Just because a RNA is differentially expressed between two setting does NOT mean that they are important for the phenotypic difference.

Thank you.

Johan Reimegård | 30-November-2020