FUNDING PROGRAM FOR NEXT GENERATION WORLD-LEADING RESEARCHERS

Project Title: Genome-wide identification of non-coding RNA function for cell differentiation	
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1. Background of research

Although mammalian genomes contain less than 25,000 protein coding genes, the non-coding RNA (ncRNA) transcription constitute the most unexplored and prevalent part, Altogether there are hundreds of thousands transcription initiations of putative ncRNAs. Although functions of ncRNAs are known for specific ncRNAs a systematic elucidation of the function of ncRNA is needed to finally make sense of the largest output of the genome. There is growing evidence that ncRNAs are involved in the control of the epigenome. A large fraction of ncRNAs, particularly deriving from RE, are specifically localized in nuclear sub-compartments (unpublished). We have also found than treating mice with histone deacetylase causes dramatic epigenomic changes and reactivation of brain plasticity. Remarkably, this is associated with the expression of thousands of large ncRNAs and opening of chromatin on RE, suggesting a role of ncRNA in the neuron reprogramming. We set up to decipher the role of ncRNA in cell reprogramming.

2. Research objectives.

Aim 1: making a comprehensive map of the ncRNAs of iPS and MEF cells, using mainly sing CAGE, CAGE-scan RNA-seq.
Aim 2: We will preparation of reagents to experimentally alter ncRNAs expression including cloned ncRNAs and si/shRNAs.
Aim 3: Induction of de-differentiation by ncRNAs to produce iPS by using selected, highly expressed ncRNAs in MEF and in iPS for expression perturbation.
Aim 4: Differentiation mediated by ncRNAs from iPS to a differentiated state.
Aim 5: Identification of mechanisms of action and prediction.

3. Research characteristics (incl. originality and creativity)

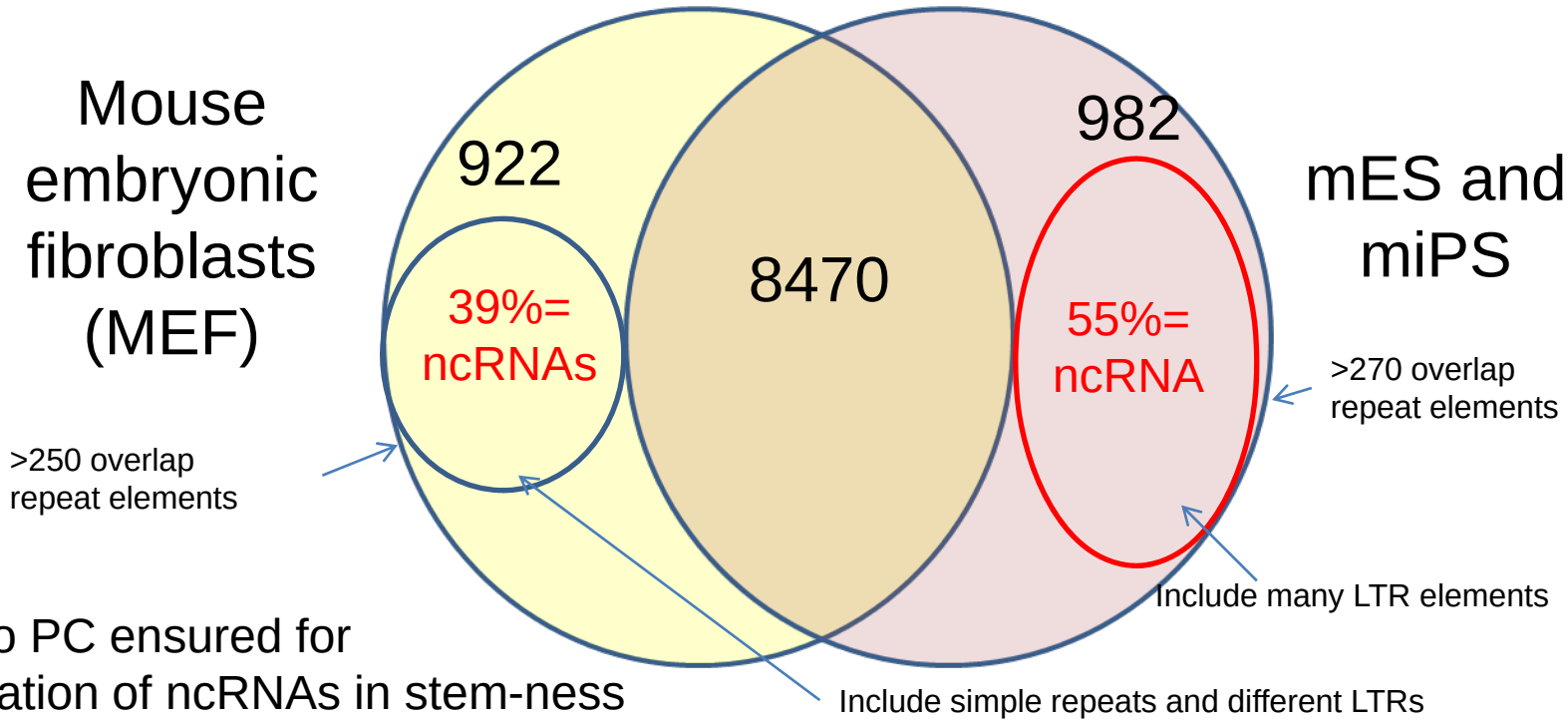
This is the first example of large scale screening of non-coding RNAs, including retrotransposon elements and other non-coding RNAs, to screen the role of these molecules in cell reprogramming. We believe that this is particularly significant because ncRNAs are likely to have a role in the epigenome and will help to reprogram the cells. In fact, although reprogramming iPS with the Yamanaka's factors is feasible, the efficiency is very far from being complete. We aim at filling the gap and providing new tools to regulate the cell programming.

4. Anticipated effects and future applications of research

This proposal aims at filling the existing gap between the discovery of ncRNAs, including those derived from RE elements, their role and function in the cell, as well as their utilization as tools for another emerging big field in modern science. We aim to use an "omics" approach to understand ncRNAs and RE function and connect them to their ability to promote or block cell differentiation, which may in turn affect the field of regenerative medicine. To complete the circle, this research will also provide in the early stage the most comprehensive transcriptome view so far of stem cells, creating an important resource that we will make available to the community.

Potential role of ncRNA in iPS and stem cells

- Differences in expression analyzed with CAGE



Grant to PC ensured for perturbation of ncRNAs in stem-ness

-Role of ncRNAs in the epigenome

15 April 2010| doi:10.1038/nature08975

Long non-coding RNA *HOTAIR* reprograms chromatin state to promote cancer metastasis

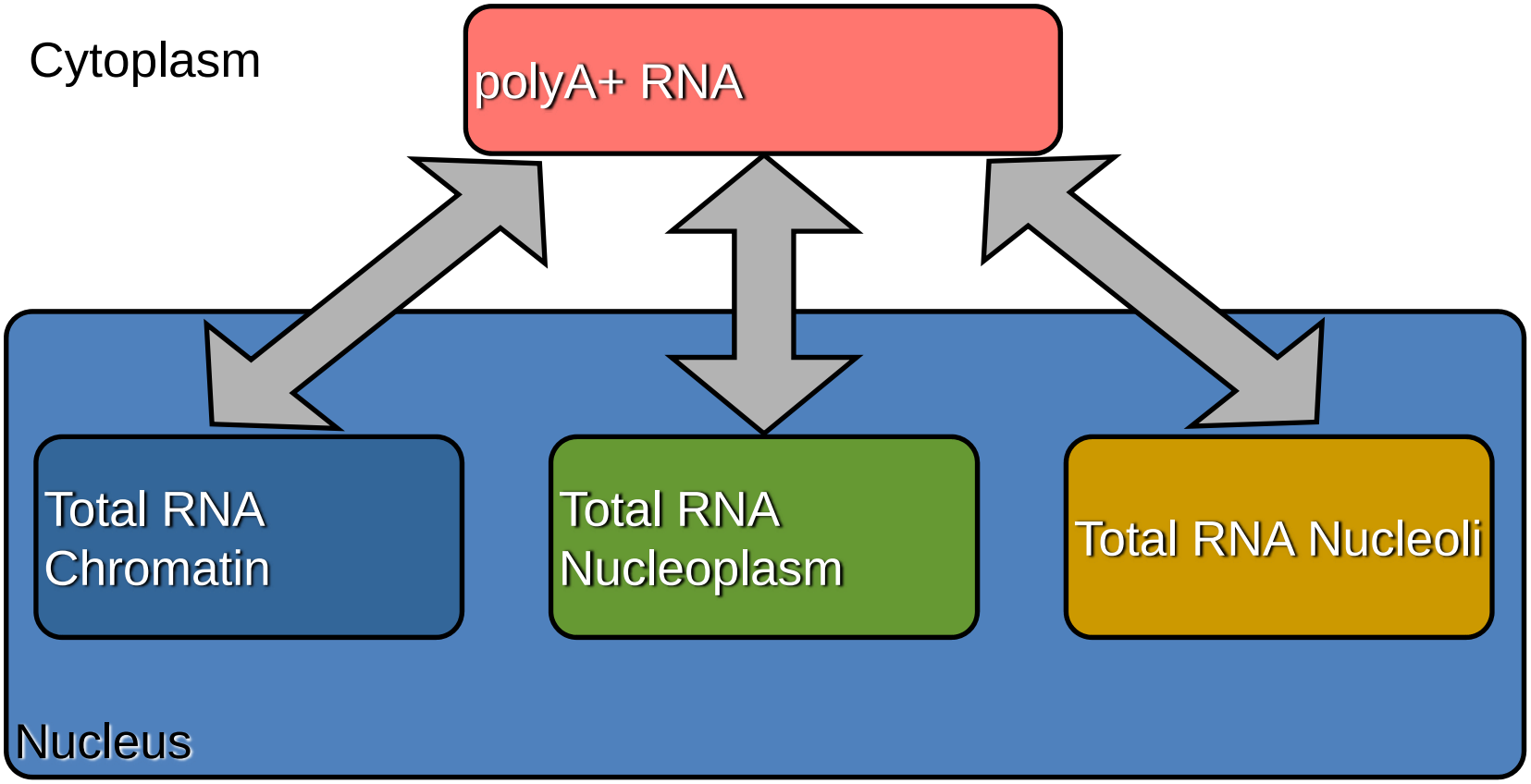
Rajnish A. Gupta[1], Nilay Shah[4], Kevin C. Wang[1], Jeewon Kim[2], Hugo M. Horlings[6], David J. Wong[1], Miao-Chih Tsai[1], Tiffany Hung[1], Pedram Argani[5], John L. Rinn[7], Yulei Wang[8], Pius Brzoska[8], Benjamin Kong[8], Rui Li[3], Robert B. West[3], Marc J. van de Vijver[6], Saraswati Sukumar[4] & Howard Y. Chang[1]

RE elements are indeed expressed
Nat Genet. 2009 May;41(5):563-71

The regulated retrotransposon transcriptome of mammalian cells

Geoffrey J Faulkner[1], Yasumasa Kimura[2], Carsten O Daub[2], Shivangi Wani[1], Charles Plessy[2], Katharine M Irvine[3], Kate Schroder[3], Nicole Cloonan[1], Anita L Steptoe[1], Timo Lassmann[2], Kazunori Waki[2], Nadine Hornig[4,5], Takahiro Arakawa[2], Hazuki Takahashi[2], Jun Kawai[2], Alistair R R Forrest[2,6], Harukazu Suzuki[2], Yoshihide Hayashizaki[2], David A Hume[7], Valerio Orlando[4,5], Sean M Grimmond[1] & Piero Carninci[2]

Non-coding RNAs will be investigated for their role in reprogramming .



We are investigating non-coding RNAs, including retrotransposon elements, because we see very clear segregation of ncRNAs into compartments like chromatin. There are, for instance, more than 10,000 LINE-derived ncRNAs (unpublished data) that are associated to the chromatin, as well as other ncRNAs.