Marie Curie Intensive Training Course

Hadas Raveh-Amit

Oct 10, 2011

2007–2011 Ph.D.

***Thesis*: Translational Regulation of Protein Kinase C by Upstream Open Reading Frames**

Prof. Etta Livneh

2005–2007 M.Med.Sc.
Graduated *with excellence (SUMMA CUM LAUDE)*

***Thesis*: The role of the 5'-Untranslated Region of Protein Kinase C**

Department of Microbiology and Immunology, Ben-Gurion University of the Negev, Beer Sheva, Israel

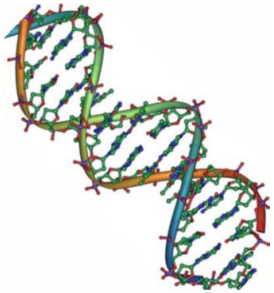
Main PhD research interests

The response of cells to stress

(e.g. nutrient deprivation, DNA damage):

- **Regulation of gene expression** at the translational level by regulatory elements in the mRNA
- **Signal transduction** pathways that lead to the adaptation to stress or **cell death**.

Research expertise

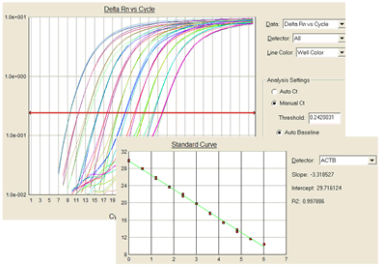


- ***Molecular biology***

DNA cloning and site-directed mutagenesis

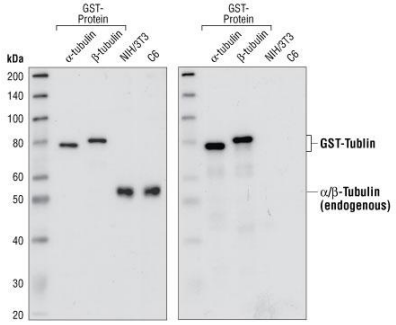
- ***Tissue culture***

Transfections, siRNA, reporter gene assays, and isolation of stably-transfected clones



- ***RNA profiling***

Real-Time PCR, Northern Blot Analysis, and RNA fractionation from sucrose gradients



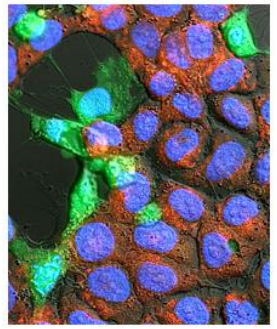
- ***Protein profiling***

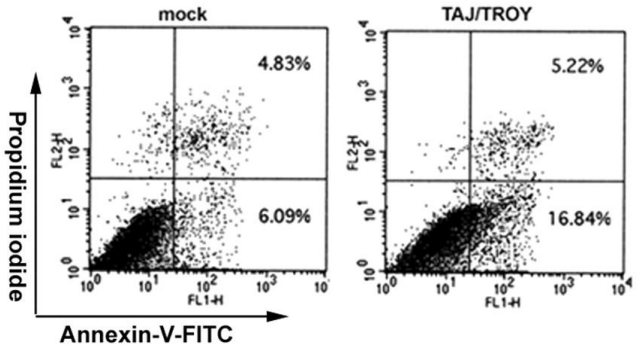
Sub-cellular fractionation of cultured cells, immunoprecipitation of proteins, western blot, immunofluorescence, and purification of proteins from bacteria

Research expertise

- ***Microscopy***

Visualization of fluorescent-tagged proteins using confocal microscopy



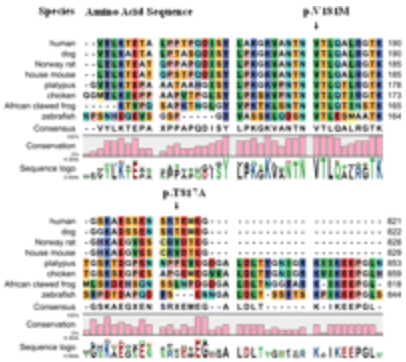


- ***Flow cytometry***

E.g. analysis of cell death

- ***Bioinformatics tools***

Amino acids and nucleic acids sequence analysis, blast, primer design, etc.



- Training on ***Fluorescence Resonance Energy Transfer (FRET)***, laboratory of Prof. Jonathan Chernoff, Fox Chase Cancer Center, Philadelphia, PA, USA (Jan. 2008)

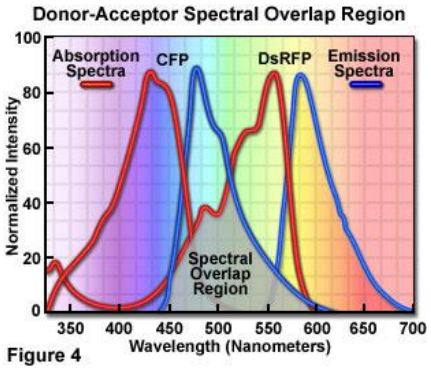


Figure 4

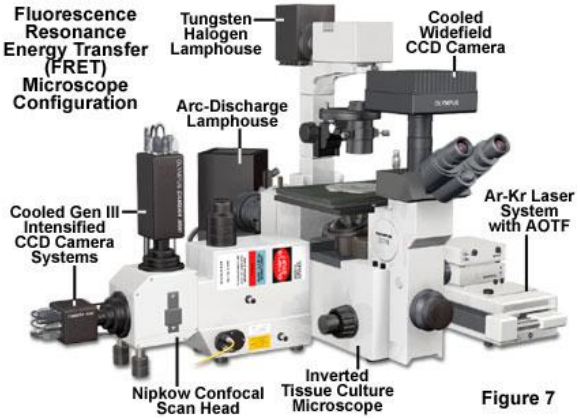


Figure 7

FOX CHASE CANCER CENTER

Publications

Raveh-Amit H, Hai N, Rotem-Dai N, Shahaf G, Gopas J, and Livneh E. **Protein Kinase C-eta activates NF-κB in Response to Camptothecin- induced DNA Damage** Biochem Biophys Res Commun. 2011 Aug 26;412(2):313-7.

Raveh-Amit H, Maissel A, Poller J, Marom L, Elroy-Stein O, Shapira M, and Livneh E. **Translational Regulation of PKCeta by Two upstream Open Reading Frames (uORFs)** Mol Cell Biol 2009 Nov;29(22):6140-8.

Shahaf G, Rotem-Dai N, Koifman G, Raveh-Amit H**,** and Livneh E. **PKCeta is a negative regulator of AKT inhibiting the IGF-1 induced proliferation** *(submitted)*

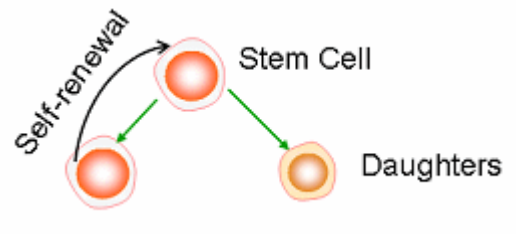
Part II: IDPbyNMR

Stem cells

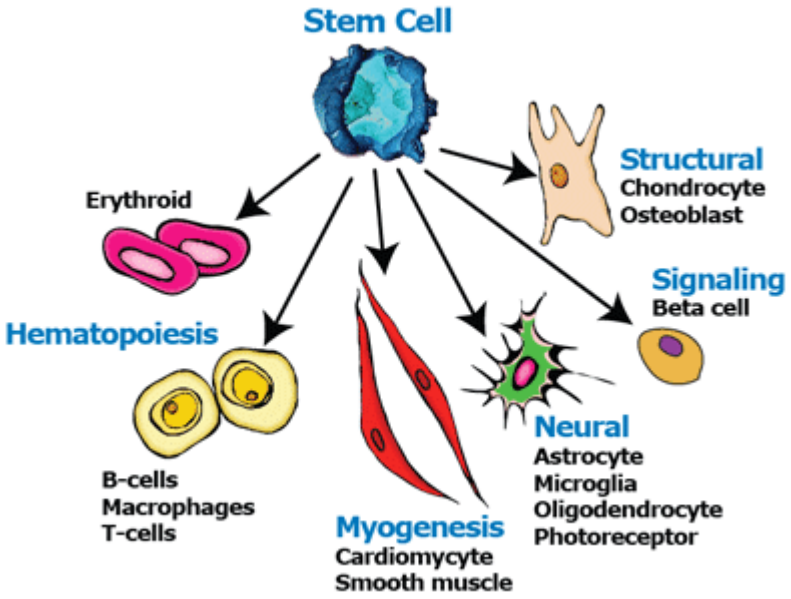
- Stem cells share two properties:

  > Self renewal

  > Potency



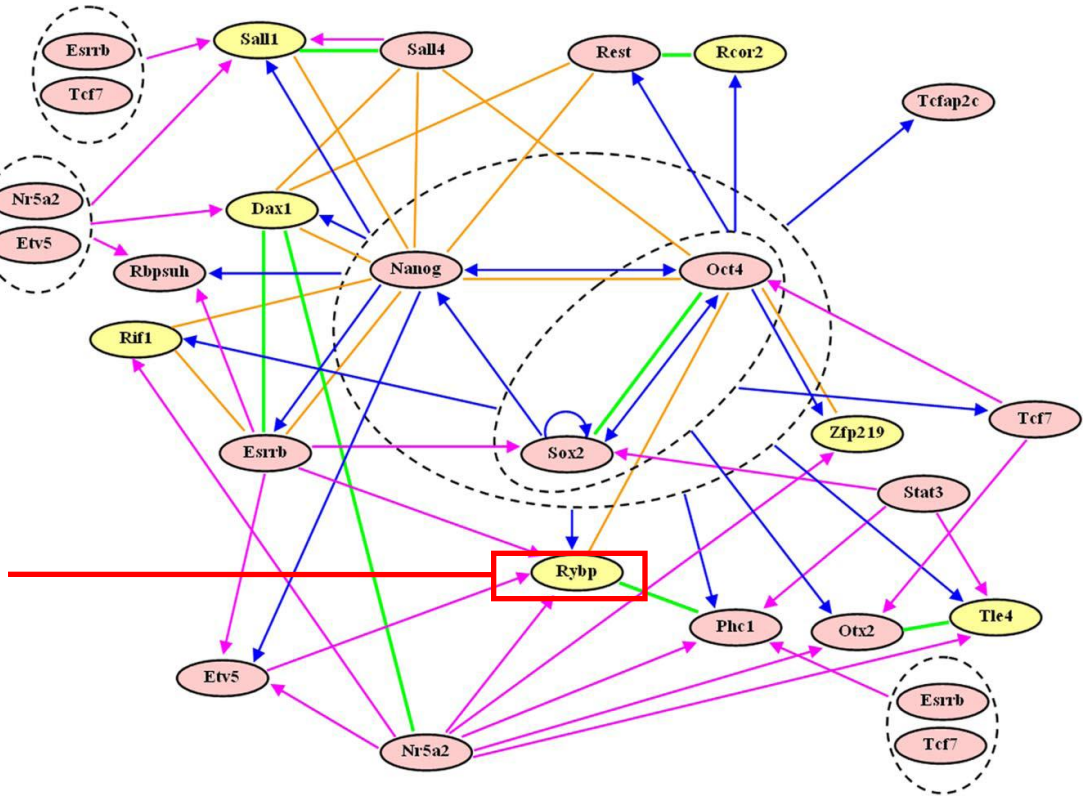
- Extensive research is dedicated to the understanding of the gene regulatory network that controls and maintains self renewal and potency of stem cells.



A gene regulatory network in mouse embryonic stem cells

RYBP

Is an IDP



PNAS

A gene regulatory network in mouse embryonic stem cells

Qing Zhou*, Hiram Chipperfield†, Douglas A. Melton†, and Wing Hung Wong‡§

*Department of Statistics, University of California, Los Angeles, 8125 Math Science Building, Los Angeles, CA 90095; †Department of Molecular and Cell Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138; and ‡Departments of Statistics, Health Research and Policy, and Biological Science Stanford University, 390 Serra Mall, Stanford, CA 94305

Edited by Philip P. Green, University of Washington School of Medicine, Seattle, WA, and approved August 21, 2007 (received for review February 5, 20

We analyze new and existing expression and transcription factor-binding data to characterize gene regulatory relations in mouse ES cells (ESC). In addition to confirming the key roles of Oct4, Sox2, and

ples produced 16 expression profiles, including 3 profiles of u ferentiated ESC (which is of course high in Oct4 expressio profiles from 2-, 4-, and 8-day EB with high Oct4 expression:

We also know that:

- **The regulatory network that controls ESC identity is enriched with proteins involved in the transcription and epigenetic control machineries which often bind multiple partners.**

- **Intrinsically disorder is believed to contribute to the ability of proteins to bind multiple partners.**

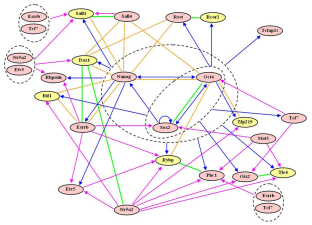
QUESTION 1:
Is the ESC regulatory network enriched with Intrinsically Disordered Proteins?

Search for predicted disordered proteins:
Experimental design

I

Extraction of proteins sequences

GenBank database



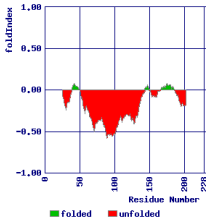
→ **FASTA**

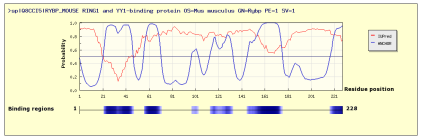
II

Prediction of intrinsically disordered proteins

FoldIndex server

IUPred server





ANSWER: Yes

70% of the proteins involved in stem cells identity were predicted to be disordered according to both servers (14/20)

- Moreover, many disordered proteins act by binding to a structured partner and undergo disorder-to-order transition.

QUESTION 2:
Can we find predicted disordered proteins that are likely to contain protein binding sites (in their disordered region)?

Identify potential interesting candidates for further structural and functional analysis in stem cells and induced pluripotent stem cells models

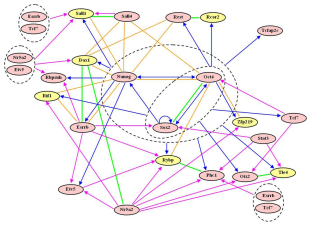
Search for predicted disordered regions that are likely to contain a protein binding site:

Experimental design

I

Extraction of proteins sequences

GenBank database



→ **FASTA**

II

Prediction of intrinsically disordered proteins

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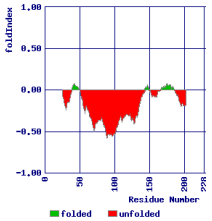
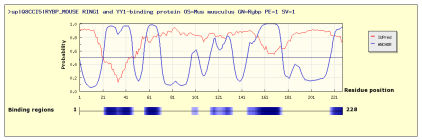
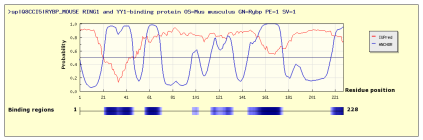




III

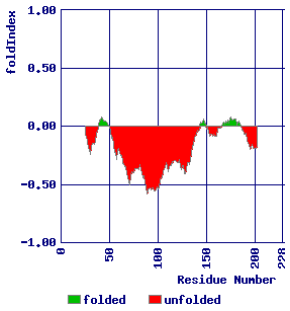
Search for predicted disordered regions that are likely to interact with a protein partner

ANCHOR server

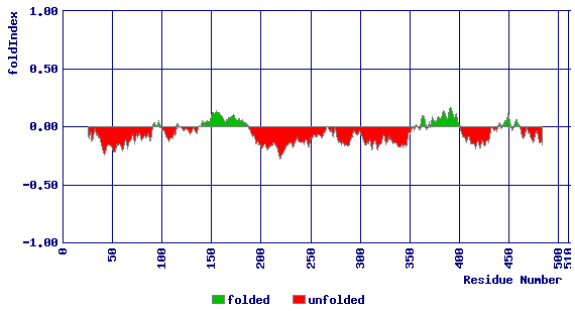


Four proteins turned out to be interesting…

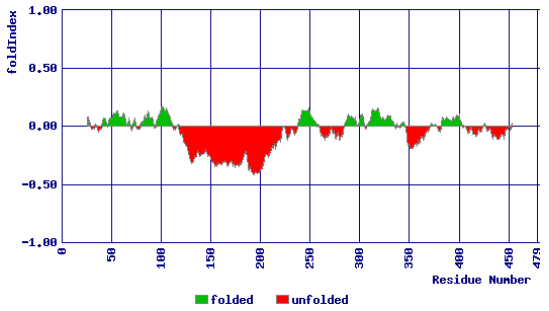
RYBP



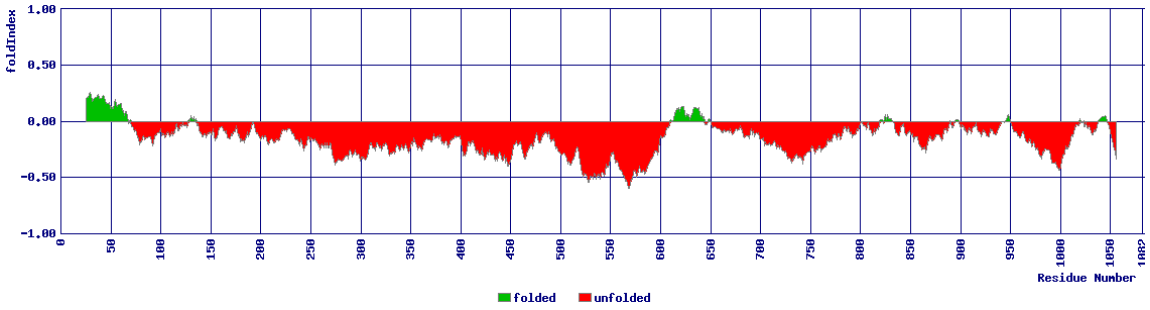
ETV5



RCOR2



REST

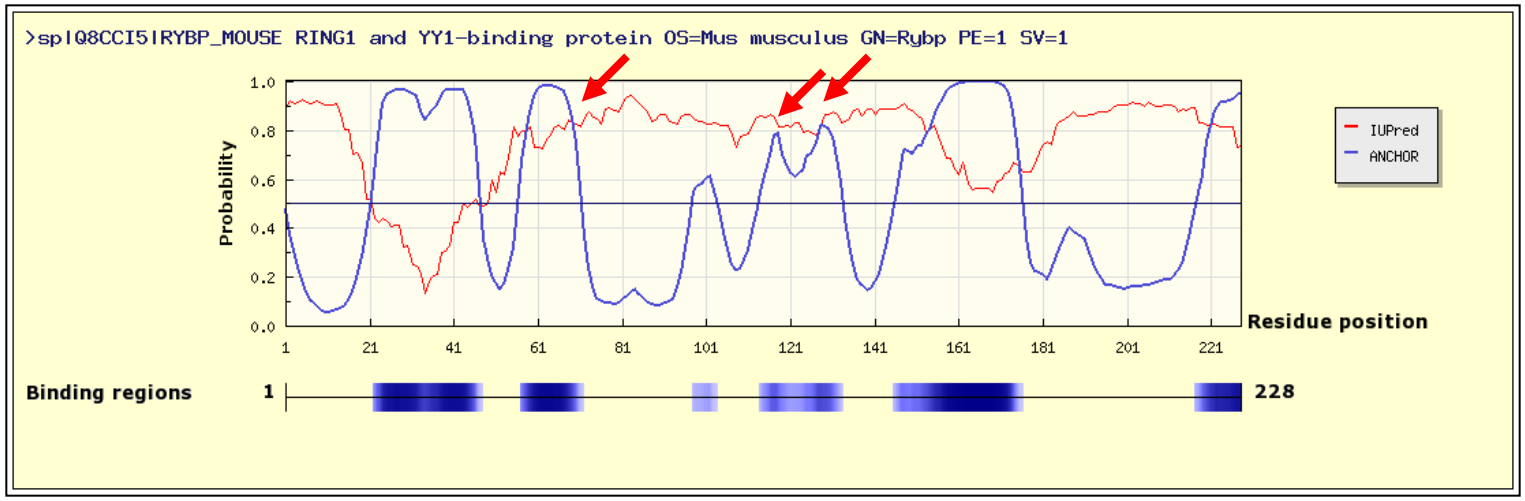


1. RYBP

Ring1- and YY1-binding protein

> Member of the polycomb group proteins that mediate transcriptional silencing and were shown to play a critical role in ESC identity

> RYBP was reported to be intrinsically disordered (2009)



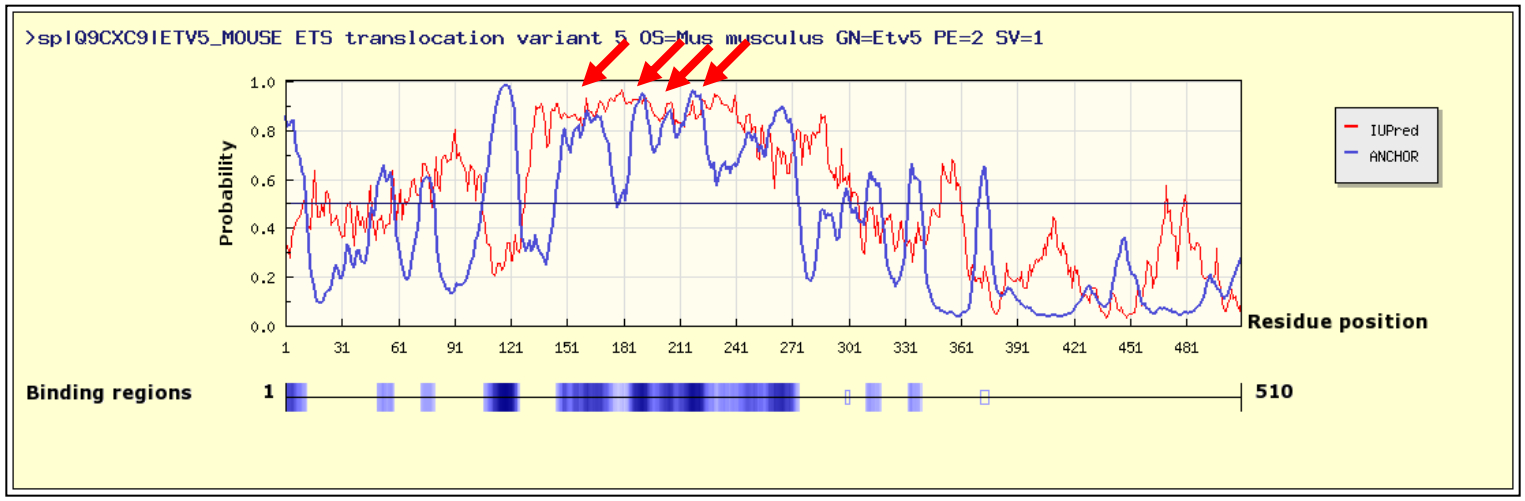
2. ETV5

ETS translocation variant 5 (ETV5)

> Member of the superfamily of transcription factors that share a conserved ETS domain

> Its role in ESC maintenance is not clear

> It wasn't reported to be intrinsically disordered (yet!)



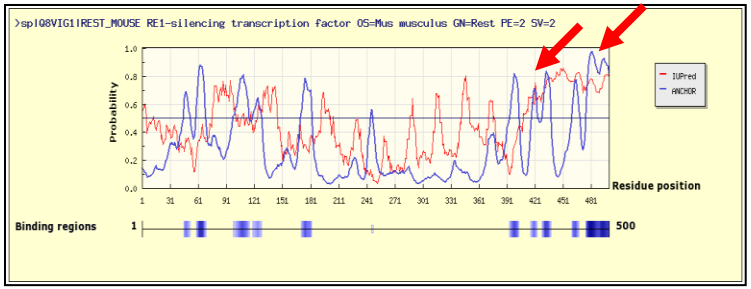
3. REST/NRSF

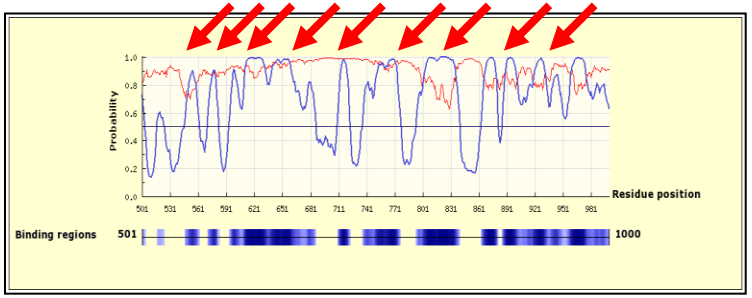
RE1-silencing transcription factor / Neural restrictive silencing factor

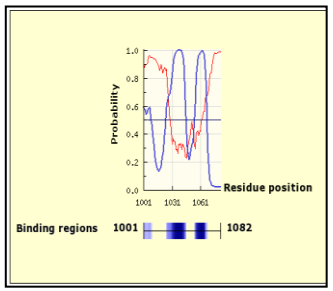
> Plays a critical role in stem cell fate determination

> Mediates cell type- and developmental stage-specific gene repression, gene activation, and long-term gene silencing

> It was reported to be intrinsically disordered (3 months ago)







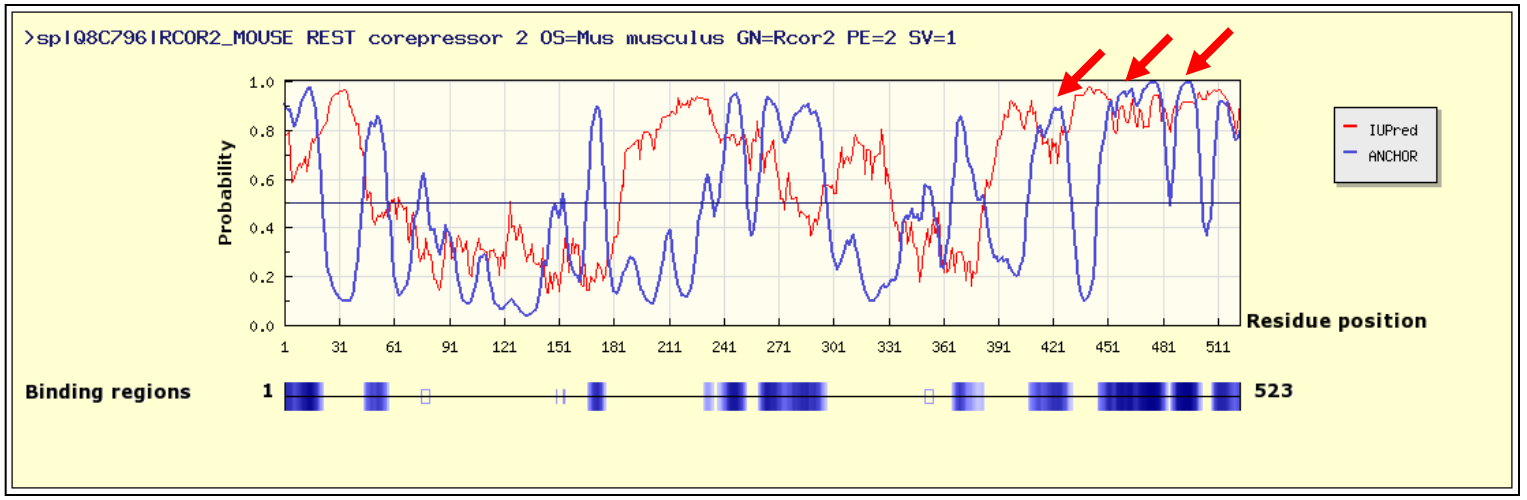
4. RCOR2

REST Corepressor2

> Plays a critical role in stem cell fate determination

> Recently, it was suggested that RCOR2 can substitute SOX2 in somatic cell reprogramming

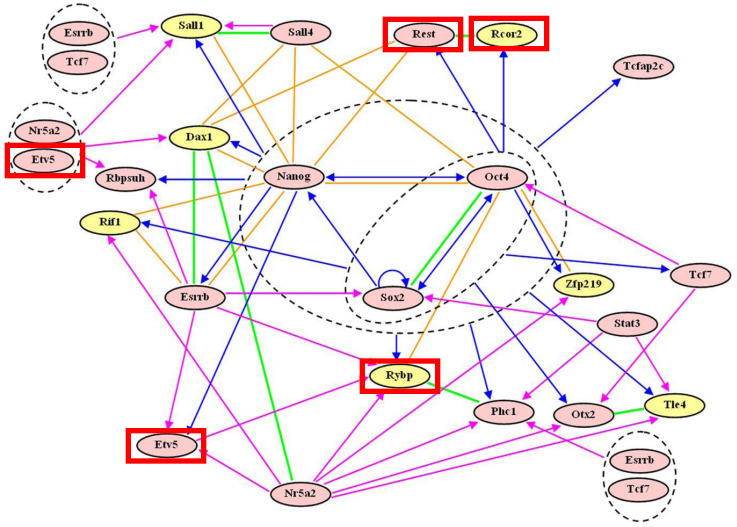
> It wasn't reported to be intrinsically disordered (yet!).



Summary

- We have identified two predicted and two already known IDPs that are likely to contain a protein binding domain within their intrinsically disordered regions.

- These proteins may serve as potential candidates for further research
  - Structural analysis by NMR
  - Test in our ES and iPS systems



BioTalentum

Prof. Andras Dinnyes

Dr. Anna Lovrics

Dr. Krisztian Kovacs

Thank you for your attention!

Questions?

IDPbyNMR

SEVENTH FRAMEWORK PROGRAMME