Integration of Multilevel Genomic Data to Study Transcription Factor Mediated Epigenomic Regulation

The word epigenetics is a portmanteau coined by C. H. Waddington in 1942 CITATION. Its prefix means over or above, so epigenetics is the study of heritable changes in addition to the genetic (DNA) level of heritability. Some of the most well studied epigenetic changes across the genome are the insertion of varient histones in nucleosomes, modifications of the histone tails of canonical histones, and the methylation and hydroxymethylation of cytosines in DNA (what am I missing here?- eggs). These changes influence the access of transcription factors and the transcription machinery to the DNA, resulting in reversible positive or negative regulation of downstream products.

The transcription factor (transcriptional repressor) REST (also known as NRSF and XBR) was first identified as binding to repressor element 1 (RE1 or NRSE) (a 21bp cis-element) through 8 C2H2 zinc fingers and mediating the repression of some neuronal genes in non-neuronal tissues ([Chong, Tapia-Ramirez et al.](#_ENREF_8) ; [Schoenherr and Anderson 1995](#_ENREF_22)). [genes SCG10 and the type II sodium channel] These 21bp RE1 sites became known as canonical RE1s (cRE1s) which have two central basees with no sequence specificity ([Barski, Cuddapah et al. 2007](#_ENREF_4)). REST has also been found to bind to non-canonical RE1 sites (ncRE1s) which have between five and nine central positions with no sequence specificity, but the same flanking 19 bases, and half sites, which have one or the other flanking sequences. Gene repression mediated by REST is achieved through REST’s recruitment of a number of corepressors that changed the epigenetic state of the genes they interact with. (formation of REST complex) Some of these corepressors are a histone methylase, a histone demethylase, histone deacetylases, and a chromatin remodeling protein ([Ooi and Wood 2007](#_ENREF_19)). [probably should name these here] These histone modifying enzymes act to remove modifications that have been associated with gene expression CITATION and add modifications that have been associated with gene repression CITATION. REST also acts to repress genes through interaction with TATA-binding protein ([Murai, Naruse et al. 2004](#_ENREF_17)) which inhibits the formation of the preinitiation complex and through the recruitment of small CTD phosphatases, which downregulate RNApolII activity ([Yeo, Lee et al. 2005](#_ENREF_24)). Some recent studies have implicated REST (or various isoforms of REST) in the activation of some genes MORE CITATION([Bessis, Champtiaux et al. 1997](#_ENREF_5)). MECHANISM?

REST has a prevalent role in many diseases. REST was identified as a tumor suppressor in breast cancer ([Westbrook, Martin et al. 2005](#_ENREF_23); [Reddy, Greco et al. 2009](#_ENREF_21)), colorectal cancer ([Westbrook, Martin et al. 2005](#_ENREF_23)), and small cell lung cancer ([Coulson, Edgson et al. 2000](#_ENREF_9)). REST has also been identified as an oncogene in neuroblastoma ([Nishimura, Sasaki et al. 1996](#_ENREF_18)) and medulloblastoma tumors ([Fuller, Su et al. 2005](#_ENREF_10)) in pheochromocytomas that are associated with von Hippel-Lindau syndrome ([Huynh, Pacak et al. 2006](#_ENREF_12)). In ischemia and epilepsy, REST has been shown to be upregulated in the brain ([Palm, Belluardo et al. 1998](#_ENREF_20); [Calderone, Jover et al. 2003](#_ENREF_6)). The downregulation of REST disturbs development in the ES cell model for down syndrome ([Canzonetta, Mulligan et al. 2008](#_ENREF_7)) and REST’s inability to interact with the Huntington protein due to its mutation in Huntington’s disease increases the amount of REST found in the nucleus ([Zuccato, Tartari et al. 2003](#_ENREF_26)). REST may also play roles in opioid addiction, as it represses the mu-opioid receptor ([Kim, Hwang et al. 2004](#_ENREF_13)), in depression and anxiety as it represses the seratonin 1A receptor ([Lemonde, Rogaeva et al. 2004](#_ENREF_15)), and in cardiac dysfunction and arrhythmogenesis, as it is abnormally inhibited in these states ([Kuwahara, Saito et al. 2003](#_ENREF_14)).

REST's multitude of interactions with the epigenetic landscape, and its association with a wide variety of genes makes it a very interesting transcription factor to study context-specific patterns of epigenetic modifications.

Next generation sequencing is an expensive procedure, but the open source nature of published datasets means that this data is available to other researchers to analyze. In CD4+ T cells, there exists a plethora of previously sequenced data, such as 10 histone modifying enzymes (p300, CBP, HDAC1, HDAC2, HDAC3, HDAC6, Tip60, PCAF, MOF, and HMGN1), 2 transcription factors (CTCF and STAT6), a histone variant (H2A.Z), 18 histone acetylations (H2AK5ac, H2AK9ac, H2BK5ac, H2BK12ac, H2BK20ac, H2BK120ac, H3K4ac, H3K9ac, H3K14ac, H3K18ac, H3K23ac, H3K27ac, H3K36ac, H4K5ac, H4K8ac, H4K12ac, H4K16ac, and H4K91ac), 20 histone methylations (H2BK5me1, H3K4me1, H3K4me2, H3K4me3, H3K9me1, H3K9me2, H3K9me3, H3K27me1, H3K27me2, H3K27me3, H3K36me1, H3K36me3, H3K79me1, H3K79me2, H3K79me3, H3R2me1, H3R2me2, H4K20me1, H4K20me3, H2A + H4R3me2), RNA polymerases II and III, nucleosome positioning, DNAse sequencing data, three kinds of methyl sequencing (Me-seq, MRE-seq, MeDIP-seq), transcriptome (RNA-seq) sequencing [what kind of RNAseq how enriched?], and a miRNA array.

Hypothesis: REST functionally distinct groups are regulated by context specific patterns of within the epigenetic landscape and are a result of different modifying enzymes being recruited.

The above hypothesis has been explored before in Greenway, Zheng, Abrajano but either not genomically, or not as comprehensively as they will be addressed in this study. Secondary analysis / reanalyzed

Global picture / landscape

Locus-specific

Relate to transcriptome data -> “transcr outcome of REST modulated changes”

Exploit resources

How REST induces context-specific pat of chromatin mods on fnally distinct groups of targs, cluster based on pats of hist/etc mods, anal indiv groups for fnal sig. + seq features (near REST binding sites) that may serve as signals for recruiting other hist mod enz. Clust to show dep on REST for change (ala Zheng 2009)

This proposed integrated study will take a wide array of epigenetic data, and study the epigenomic changes that are regulated by REST binding. It will look not only at the narrow promoter regions surrounding the transcription start site, but will take a more comprehensive look at REST binding throughout the genome. Unlike: ([Greenway, Street et al. 2007](#_ENREF_11); [Abrajano, Qureshi et al. 2009](#_ENREF_2); [Abrajano, Qureshi et al. 2009](#_ENREF_1); [Zheng, Zhao et al. 2009](#_ENREF_25)) It will integrate methylation and rnaseq? And miRNA microarray data, none of which has been done before, to form a truly comprehensive look at the epigenetic context of REST binding. It will also take a stab at differences in REST epigenetic landscape in different functional groups, genomic locations, and other sub-sections of genes in the genome. The stratification is distinctly different then that used by others, esp Zheng 2009, in that the separation of anticipated bound sites, etc allows for a lot more teasing out of function than that study which just looked at REST bound and not bound RE1versus expression matched controls? (Zheng used RE1+REST, expr matched genes, and expr matched cRE1s-REST, change due to those two groups show change contingent of gene expr, but not due to binding). Look at diff complexes recruited by REST in ncRE1s and cRE1s both bound and unbound, esp important in forebrain, where we have coREST data. Prior genome wide studies have used data from jurkat and not the right cell line. No large scale studies of REST in cancer genomicly. Previous studies also used microarray expression data which is far less sensitive etc than rnaseq. Will also include HDACs and HATs unlike prior study

Zheng et al show nucleosome positioning at RE1 sites at promoter and at non-promoter, strat into more fine genomic regions, look at different functional groups nucleosome positioning.

DOES SUV39H1 (SUV39) BIND TO SOME KIND OF DNMT AS POSSIBLY INDICATED BY MUJUMDER? ALSO DOES IT BIND TO COREST? AS INDICATED BY SOMEONE? MUJUMDER? OR NOT? SEE NATURE REVIEW PAPER AGAIN?

REST’s context dependance is due to differential cofactor recruitment and/or endogenous chromatin structure([Greenway, Street et al. 2007](#_ENREF_11))

* “Differential corepressor recruitment occurs even in cell lines of similar embryological origin and … distinct chromatin environments are associated with individual target genes” Greenway
* in the mouse NSCs studied, REST is present at actively transcribed and silenced genes and acts as both a silencer and as a repressor
* others I forget who, have shown REST can mediate both long term and short term repression
* “REST recruitment results in modification of the local chromatin structure around the RE1 sites”
* There are HDAC inhibitor sensitive and insensitive REST repression sites (16,17,32,33)—Greenway
* Genes with the same corepressors bound act differently in Greenway paper (although they only looked at 7 genes and REST, coREST, SinA/SinB, and HDACs1+2), perhaps the other corepressor shit acts on these genes to explain all their behaviour?
* “the exact role of REST varies according to the source and regional identity of the cells and/or their developmental stage…differences exist in the corepressor platforms recruited…REST may precipitate changes in the epigenetic signiture without necessarily effecting gene expression”
* looked at 7 genes in 1 tissue type NSCs.

([Abrajano, Qureshi et al. 2009](#_ENREF_2))

* “During adult hippocampal neurogenesis, REST is converted from a transcriptional repressor into an activator by a small modulatory double stranded RNA (dsRNA)”
* “a truncated isoform of REST, REST4, exerts a dominant-negative effect on REST and possibly derepresses or activates expression of RE-1 containing genes in neurons”
* cRE1s and ncRE1s (Genome-wide analysis of repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) target genes + A new binding motif for the transcriptional repressor REST uncovers large gene networks devoted to neuronal functions)
* “REST and CoREST complexes can act with high degrees of context-specificity depending on developmental stage, cell type, and gene locus”
* “ REST and CoREST function as FSGs that act upstream of TSGs and have a broad range of effects on neuronal gene networks in order to promote the acquisition and maintenance of neuronal subtype identity while repressing alternative cellular fates”

([Abrajano, Qureshi et al. 2009](#_ENREF_1))

* REST complex is modular and macromolecular
* “ REST is now believed to have an increasing spectrum of developmental stage- and cell type-specific functions, including gene activation, repression, and long-term gene silencing, that are modulated by factors such as the levels of REST protein expression, the affinity of the REST complex for specific genomic loci, and the presence of regulatory cofactors (e.g., modulatory double-stranded ncRNAs and distinct isoforms of REST)”

([Zheng, Zhao et al. 2009](#_ENREF_25))

* refs 16 and 20 sequence of cRE1s and ncRE1s
* half-sites refs 19 and 21
* “local chromatin environment might affect the interaction between RE1 and REST”
* “the majority of RE1s are not located in promoters but rather in regions distant (>50 kb) from promoters”
* “*the inductive role of REST4 is mediated, in part, by the nucleosome remodeling factor BRG1”*
* “*the existence of a ncRE1 in the REST gene suggests a possible autoregulation of REST via a negative feedback loop” [19]*
* “ *involvement in regulating many non-coding RNAs**[*[*17*](http://genomebiology.com/2009/10/1/R9/#B17)*-*[*20*](http://genomebiology.com/2009/10/1/R9/#B20)*,*[*28*](http://genomebiology.com/2009/10/1/R9/#B28)*] . For example, REST has been shown to regulate the expression of several mouse microRNAs (mir-9, mir-124 and mir-132), all of which promote neuronal differentiation**[*[*28*](http://genomebiology.com/2009/10/1/R9/#B28)*]”*
* “*These modifications in turn create a platform for readers (or effectors) of histone code**[*[*42*](http://genomebiology.com/2009/10/1/R9/#B42)*] to orchestrate key biological processes for the establishment and maintenance of short- and long-term silencing of genes harboring RE1 motifs.”*
* “*several genes with REST-bound RE1 exhibited expression higher than the median expression level of all genes…* *REST can sometimes activate gene expression”*
* “*REST binding to a promoter does not always result in gene repression. However, our analyses have revealed that even the upregulated cRE1 genes exhibited REST-dependent deacetylations for most of the lysine residues interrogated”*
* “*REST likely interacts with additional histone methytransferase(s), such as polycomb repressive complexes (PRCs)”*
* “the average of our ncRE1 PSFM scores was higher than that of cRE1s (data not shown), suggesting that the degree of REST-mediated histone modifications may be affected by the affinity of a RE1 motif for REST. Such a correlation would also explain the significant correlation of PSFM score with the strength of gene repression regulated by REST [[17](http://genomebiology.com/2009/10/1/R9/#B17)] .”
* “*no correlation was found between REST occupancy and many histone modifications that exhibited a strong correlation (r < -0.2) with RE1, such as H3K9ac, H2BK20ac, H2BK120ac, and H3K36me1”*
* “ *the relationship between RE1 motifs and REST occupancy is extremely complex and heterogeneous, and perhaps inextricably linked to the modular nature of REST complexes”*
* “*RE1/REST near enhancers may be associated with a distinct pattern of histone modifications”*
* ***“separately interrogate the differential profiles of chromatin remodeling coordinated by REST but nevertheless occurring within distinct genomic, molecular and cellular contexts.”***
* “H3K4ac was lower in RE1 genes with REST binding regardless of high or low levels of gene expression, but the degree of H3K4 methylations (especially H3K4me1) was noticeably higher only in the group of cRE1/REST genes with up-regulated expression” (**what is the function of H3K4ac, look in H3K4me1 binding regions, enhancers or coactivators binding there? Sequence similarity, causing upregulation of genes?**
* Check out methods!

In the modern era of next generation sequencing, REST binding has been profiled throughout the genome in the Jurkat T cell line, which is a cancer cell line derived from acute lymphocytic lymphoma T cells CITATION, and in mouse ES cells and E12.5 extract.

also check out 4, 8-11(for dnmt activity) ? [some seem to say it exists through interaction with SUV39 others do not mention it? Consensus?]

The REST complex can also be associated with DNA methyltransferase 1 ([Majumder 2006](#_ENREF_16))

Communicate crosstalk (ability of TFs to mod epigentics, ability of epigenetics to allow TFs to bind IMPORTANT!)

Can we identify class 1 and class 2 genes? Is this theory still reasonable ([Ballas, Grunseich et al. 2005](#_ENREF_3))?

REST’s role in cancer is particularly interesting, as it has been shown to have both oncogenic and tumor suppressant functions CITATION.

Abrajano, J. J., I. A. Qureshi, et al. (2009). "Differential Deployment of REST and CoREST Promotes Glial Subtype Specification and Oligodendrocyte Lineage Maturation." Plos One **4**(11): e7665.

Abrajano, J. J., I. A. Qureshi, et al. (2009). "REST and CoREST Modulate Neuronal Subtype Specification, Maturation and Maintenance." Plos One **4**(12): e7936.

Ballas, N., C. Grunseich, et al. (2005). "REST and Its Corepressors Mediate Plasticity of Neuronal Gene Chromatin throughout Neurogenesis." Cell **121**(4): 645-657

Barski, A., S. Cuddapah, et al. (2007). "High-Resolution Profiling of Histone Methylations in the Human Genome." Cell **129**(4): 823-837

Bessis, A., N. Champtiaux, et al. (1997). "The neuron-restrictive silencer element: A dual enhancer/silencer crucial for patterned expression of a nicotinic receptor gene in the‚Äâbrain." Proceedings of the National Academy of Sciences **94**(11): 5906-5911.

Calderone, A., T. Jover, et al. (2003). "Ischemic insults derepress the gene silencer REST in neurons destined to die." Journal of Neuroscience **23**(6): 2112.

Canzonetta, C., C. Mulligan, et al. (2008). "DYRK1A-Dosage Imbalance Perturbs NRSF/REST Levels, Deregulating Pluripotency and Embryonic Stem Cell Fate in Down Syndrome." The American Journal of Human Genetics **83**(3): 388-400

Chong, J. A., J. Tapia-Ramirez, et al.

Coulson, J. M., J. L. Edgson, et al. (2000). "A Splice Variant of the Neuron-restrictive Silencer Factor Repressor Is Expressed in Small Cell Lung Cancer: A Potential Role in Derepression of Neuroendocrine Genes and a Useful Clinical Marker." Cancer Research **60**(7): 1840-1844.

Fuller, G. N., X. Su, et al. (2005). "Many human medulloblastoma tumors overexpress repressor element-1 silencing transcription (REST)/neuron-restrictive silencer factor, which can be functionally countered by REST-VP16." Mol Cancer Ther **4**(3): 343-349.

Greenway, D. J., M. Street, et al. (2007). "RE1 Silencing Transcription Factor Maintains a Repressive Chromatin Environment in Embryonic Hippocampal Neural Stem Cells." STEM CELLS **25**(2): 354-363.

Huynh, T.-T., K. Pacak, et al. (2006). "Transcriptional Regulation of Phenylethanolamine N-Methyltransferase in Pheochromocytomas from Patients with von Hippel–Lindau Syndrome and Multiple Endocrine Neoplasia Type 2." Annals of the New York Academy of Sciences **1073**(1): 241-252.

Kim, C. S., C. K. Hwang, et al. (2004). "Neuron-restrictive silencer factor (NRSF) functions as a repressor in neuronal cells to regulate the mu opioid receptor gene." J Biol Chem **279**(45): 46464-46473.

Kuwahara, K., Y. Saito, et al. (2003). "NRSF regulates the fetal cardiac gene program and maintains normal cardiac structure and function." EMBO J **22**(23): 6310-6321.

Lemonde, S., A. Rogaeva, et al. (2004). "Cell type-dependent recruitment of trichostatin A-sensitive repression of the human 5-HT1A receptor gene." J Neurochem **88**(4): 857-868.

Majumder, S. (2006). "REST in good times and bad: roles in tumor suppressor and oncogenic activities." Cell Cycle **5**(17): 1929-1935.

Murai, K., Y. Naruse, et al. (2004). "Direct interaction of NRSF with TBP: chromatin reorganization and core promoter repression for neuron‚Äêspecific gene transcription." Nucleic Acids Research **32**(10): 3180-3189.

Nishimura, E., K. Sasaki, et al. (1996). "Decrease in neuron-restrictive silencer factor (NRSF) mRNA levels during differentiation of cultured neuroblastoma cells." Neurosci Lett **211**(2): 101-104.

Ooi, L. and I. C. Wood (2007). "Chromatin crosstalk in development and disease: lessons from REST." Nat Rev Genet **8**(7): 544-554.

Palm, K., N. Belluardo, et al. (1998). "Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene." The Journal of neuroscience **18**(4): 1280.

Reddy, B. Y., S. J. Greco, et al. (2009). "RE-1‚Äìsilencing transcription factor shows tumor-suppressor functions and negatively regulates the oncogenic TAC1 in breast cancer cells." Proceedings of the National Academy of Sciences **106**(11): 4408-4413.

Schoenherr, C. and D. Anderson (1995). "The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes." Science **267**(5202): 1360-1363.

Westbrook, T. F., E. S. Martin, et al. (2005). "A genetic screen for candidate tumor suppressors identifies REST." Cell **121**(6): 837-848.

Yeo, M., S.-K. Lee, et al. (2005). "Small CTD Phosphatases Function in Silencing Neuronal Gene Expression." Science **307**(5709): 596-600.

Zheng, D., K. Zhao, et al. (2009). "Profiling RE1/REST-mediated histone modifications in the human genome." Genome Biology **10**(1): R9.

Zuccato, C., M. Tartari, et al. (2003). "Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes." Nature genetics **35**(1): 76-83.