ChIP-seq Mapping of Distant-Acting Enhancers and Their *In Vivo* Activities

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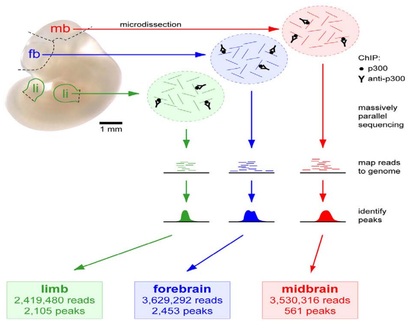
1. SUMMARY

- The genomic location and function of most distant-acting transcriptional enhancers in the human genome remains unknown
- We performed ChIP-seq for various transcriptional coactivator proteins (such as p300) directly from different embryonic mouse tissues, identifying thousands of binding sites
- Transgenic mouse experiments show that p300 and other co-activator peaks are highly predictive of genomic location AND tissue-specific activity patterns of distant-acting enhancers
- Most enhancers are active only in one or very few tissues
- Genomic location of tissue-specific p300 peaks correlates with tissue-specific expression of nearby genes
- Most binding sites are conserved, but the global degree of conservation varies between tissues

2. GENOME-WIDE MAPPING OF P300 IN EMBRYONIC TISSUES

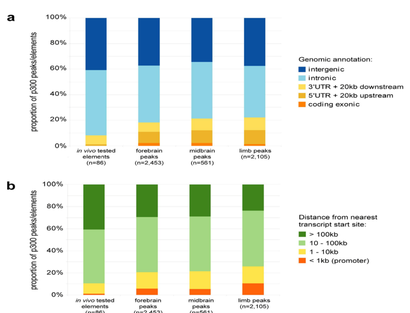


Overview of approach

- different tissues are collected from wild-type mouse embryos at embryonic day e11.5 – shown here: forebrain, midbrain, limb
- chromatin immunoprecipitation (ChIP) is performed directly from tissues, using an antibody directed against the p300 protein
- millions of sequence reads are obtained by massively-parallel sequencing and mapped to the mouse genome
- thousands of significantly p300-enriched genome regions ("peaks") are identified

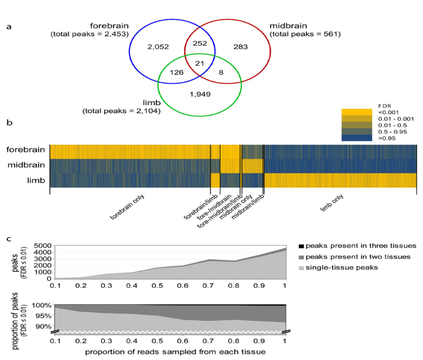
Genomic distribution of p300 peaks

- most peaks are intergenic or intronic, about 20% are close to transcript start or end sites, only a marginal fraction overlaps coding exons
- most peaks are located >10kb away from the nearest transcript start site



Most peaks are tissue-specific

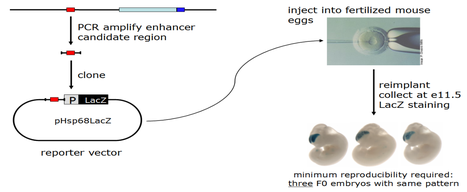
- among three tissues analyzed initially (forebrain, midbrain, limb), only 21 of 4,691 peaks (0.4%) were present in all three tissues, whereas 4,284 (91%) were significantly p300-enriched only in one of the three tissues
- re-sampling of subsets of reads suggests that deeper sequencing will identify additional peaks, most of them again only significantly enriched in a single tissue



3. P300 BINDING PREDICTS *IN VIVO* ENHANCERS

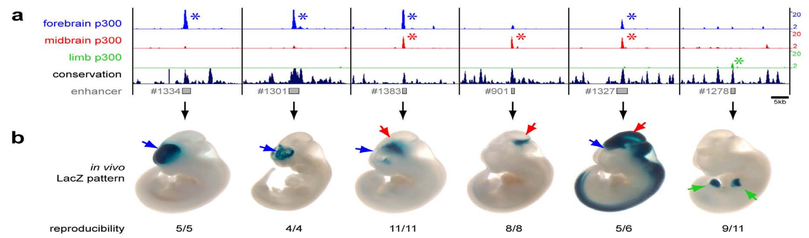
Transgenic enhancer assay

- p300 peaks are considered as enhancer candidate sequences
- human non-coding DNA fragments orthologous to mouse p300 peaks are coupled to an Hsp68 minimal promoter and a LacZ reporter gene
- transgenic mice (F_0 founder embryos) are generated by pronuclear injection and stained for reporter activity at e11.5

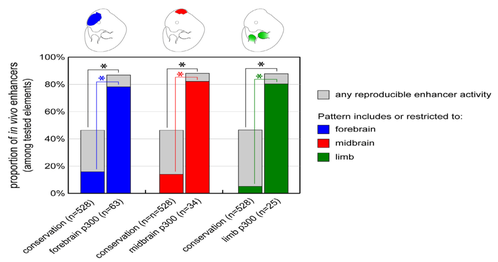


Prediction of *in vivo* enhancer activities

- presence or absence of p300 peaks in one of the three tissues correctly predicts *in vivo* reporter patterns observed in transgenic embryos (selected examples shown)



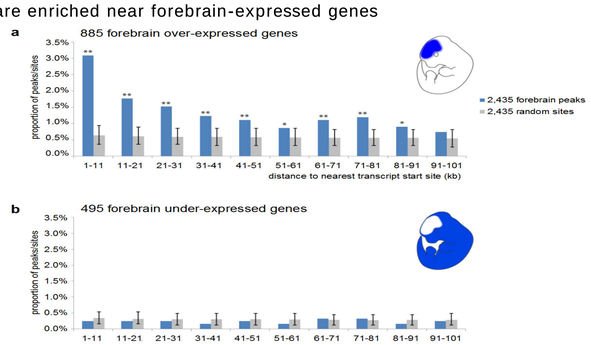
- in an initial large-scale assessment (n=86), we found that p300 peaks correctly predicted *in vivo* activity of enhancers in ~80% of cases, representing a dramatic improvement compared to conservation-based enhancer prediction methods



4. P300 PEAKS CORRELATE WITH TISSUE-SPECIFIC GENE EXPRESSION

Peaks are enriched near genes expressed in the same tissue

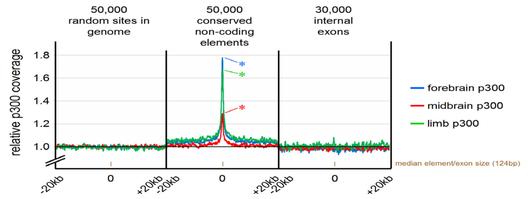
- genome-wide distribution of forebrain-p300 peaks was compared to microarray gene expression data from the same tissue
- forebrain p300 peaks are enriched near forebrain-expressed genes
- forebrain p300 peaks are NOT enriched near forebrain-underexpressed genes (compared to rest of body)
- a similar correlation was observed for limb peaks near limb-expressed genes (not shown)

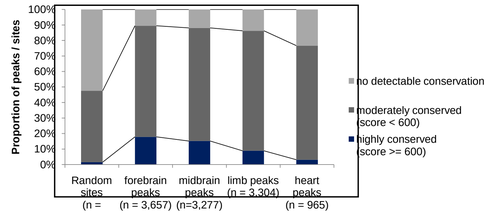


5. MOST P300 PEAKS ARE CONSERVED

p300 is enriched at conserved noncoding sequences

- extremely conserved noncoding sequences are enriched in p300 binding, consistent with their known enrichment in developmental enhancers (see Pennacchio et al. 2006; Visel et al. 2008)

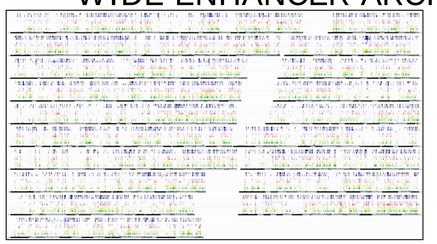




Most p300 peaks are constrained

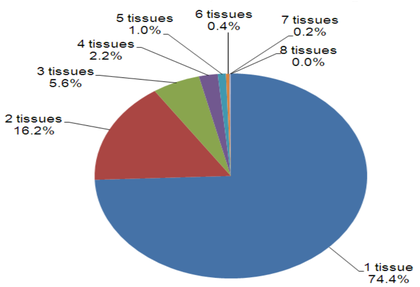
- the majority of p300-bound regions identified in forebrain, midbrain and limb overlap sequences that are under detectable evolutionary constraint in vertebrates
- proportion of extremely conserved peaks differs between tissues

6. A COMPREHENSIVE VIEW OF GENOME-WIDE ENHANCER ARCHITECTURE



Multiple Tissues

- based on the initial proof of principle, data for a total of 8 embryonic tissues was obtained (forebrain, midbrain, hindbrain, neural tube, heart, liver, limb, face; all at e11.5)
- 35 million non-redundant mapped reads identify 20,000 enhancer candidate sequences with predicted tissue-specific activities genome-
- most of the identified enhancer candidate sequences are bound by p300 only in one or two tissues
- very few regions are bound by p300 in most or all tissues, consistent with the hypothesis that these regions represent tissue-specific transcriptional enhancers
- large-scale transgenic validation of p300 peaks from all 8 tissues is in progress



LITERATURE AND RESOURCES

ChIP-seq identification of enhancers and activity patterns:

Visel et al. (2009), *Nature* 457:854-858

Visel et al. (2009) *Nature* 461:199-205.

Pennacchio and Visel (2010) *Nature Genetics* 42:557-8.

Blow et al. (2010) *Nature Genetics* 42:806-10.

Conservation-guided identification of developmental enhancers:

Visel et al. (2008) *Nature Genetics* 40:158-160

Pennacchio et al. (2006) *Nature* 444:499-502

Access to *In Vivo* Data:

http://enhancer.lbl.gov (Vista Enhancer Browser)

also see: Visel et al. (2007) *Nucleic Acids Research* 35:D88-92

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