Comparative analysis in bat and mouse identifies wing-specific genes and regulatory regions

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Abstract
The bat is the only mammal capable of powered flight. To identify the molecular mechanisms underlying the development of bat forelimbs into wings, we studied comparative gene expression and chromatin data from mouse and bat.

We have collected gene expression (mRNA-seq) and genome-wide regulatory chromatin data (ChIP-seq for the active H3K27ac and the repressive H3K27me3 marks) from developing forelimbs and hindlimbs, during three early developmental stages of the Natal long-fingered bat (Miniopterus natalensis) and the mouse (Mus musculus).

We then developed a computational model to compare expression or ChIP data across organism, limbs and time. We identified a set of ~2000 genes and ~1500 genomic loci that present significant forelimb-specific patterns in the bat.

As we show, our analysis reveals with great confidence genes and DNA regions that are well known for their roles during limb development, as well as many less studied ones. This study offers interpretation of how local changes in regulatory elements are translated into dramatic functional and phenotypic differences. Furthermore, the signalling pathways involved in limb development are well conserved among species, suggesting that identification of novel genes and distal regulatory regions could shed light on limb malformations in humans.

Analysis of variance
We use a statistical design to predict the read-counts or peak heights that were observed in any specific expression or chromatin activation level. Our null hypothesis is that as first order approximation, bat and mouse are indistinguishable.

H0, the zero-model (reduced model)
Main effects: Species (Bat vs. Mouse), Stage (E12.0, E13.0, E13.5) and Tissue (forelimb vs. hindlimb).

Expect: regions where forelimb is differentiated in activation levels from hindlimb in bat relative to the activation levels in mouse design: Tissue + Species + Stage

H1, the full model
Add interactions: Stage x Species, Tissue x Species.

Expect: greater confidence in the predicted fold change.

design: Tissue + Species + Stage + Species x Tissue

To resolve a differential model we test the likelihood ratio between H0 and H1, which would reveal differential patterns due to the interaction terms.

The developing bat wing

![Figure 1. Limb development in bat and mouse. (A) Forelimb development in bat (Miniopterus natalensis), from development stage CS15 (top) to CS17 (bottom). Shown are mesenchymal condensations (white), cartilage (blue) and tissue interdigital expression (red), which are combined to form the wing. In parallel, we present hindlimb development in bat and mouse (developmental stages E12, E13 and E13.5). Adapted from Eckelbair et al., manuscript under review. (B) Forelimbs and hindlimbs anatomy. Shown is ventral view of adult bat (M. natalensis) and its wing, compared to hindlimb (red), with thumbs (I) and digits (II-IV) marked (Adapted from Hochman et al. [1]).](image)

Change in expression pattern

![Figure 2. Change in expression pattern between forelimb and hindlimb in mouse and bat.](image)

Confidence in the predictions

![Figure 3. Confidence in the predictions between forelimb and hindlimb in mouse and bat.](image)

Discussion

Takeaway: This methodology breaks down in great genomic detail the epigenetics that give rise to wing development.

- Tissue, and time-specific analysis uncovers the set of genes and chromatin regions that operate differentially during limb development causing the bat to grow wings, when other animals don’t.

Following this computational study, our group will enter an experimental phase to check the effect of subsets of these genes and regions.

References

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