Major processes in development of forelimb:

* Proliferation
* Migration
* Differentiation/specification
* Patterning
* Vascularization
* Immune system
* Nervous system

ReactomePA shows:

* E12/E11: Network of major pathways involved in ECM formation and remodeling
* E13/E12: ECM-related pathways are still predominant, but it also includes neuromuscular
* E14/E13: network of ECM has subsided. Networks of immune system and programmed cell death become dominant.

What are the potential roles of ECM in forelimb development?

* Differentiation/specification
* Migration
* Signaling/cross talk between satellite cells and nascent muscle fibers

What can the data potentially tell me?

* Timing of developmental events:
  + Transition from embryonic to fetal fibers
  + Fusion
  + A subpopulation already starts to commit to muscle cell line my expression of MyoD and Myog
  + A subpopulation is still in process of migration
  + Migration seems to cease at E14.
* Heterogeneity of the Pax3+ lineage (already discussed in Arun’s paper)
* Match the events listed above to the heatmap of transcription factor expression.

Pathway enrichment analysis with ReactomePA of the differentially expressed genes (E12/E11 and E13/E12) shows molecular pathways involved in ECM biosynthesis, assembly, and remodeling. It appears that the hallmark processes happening in this developmental states are heavily associated with ECM. ECM and its components have been shown to regulate myoblast fusion, proliferation, and differentiation independent of myogenic regulatory factors.

Collagen is required for myoblast differentiation

ECM-integrin interaction is required for successful terminal skeletal muscle differentiation1

When cultured with beta-D-xyloside and sodium chlorate, the expression of creatine kinase is suppressed. However, as exogenous ECM was added, it prevented the inhibitory actions of beta-D-xyloside and sodium chlorate on the expression. This observation suggests that ECM-muscle cells contact is required for differentiation1

Migration of myogenic progenitor cells are dependent on the expression of genes encoding for matrix metalloproteinases (MMP). Proteins of this family break down the extracellular matrix (ECM) allowing passage for cells to travel through. Thus, inhibition of MMP in vitro showed significant reduction in migration speed of muscle cells cultured with MMP-inhibitors2*. This observation established the critical role of MMP in ECM remodeling to allow migration of myogenic progenitor cells to migrate to their designated destinies*. Our results of the RNA-seq analysis of the Pax3+ cells isolated from the forelimbs of E11 to E14 mice strongly support the previously elucidated roles of MMP. During the developmental states of E11 to E13, the expression levels of MMP genes progressively increased suggesting on-going migratory activities of embryonic myogenic progenitor cells (EMPC). The expression of MMP genes, however, was down-regulated as Pax3+ cells transitioned from E13 to E14. These observations collectively not only corroborate the roles of MMP genes in skeletal myogenesis discovered by conventional knocked out gene studies, but also provide the temporal feature of EMPC’s migratory activities. Migration of Pax3+ cells was active from E11 to E13 then reduced at E14 signaling the end of fetal myogenesis in mouse forelimbs.

In order to gain understands about the roles of differentially expressed genes in the studied developmental states E11 to E14, Reactome pathway enrichment analysis reveals significantly enriched pathways involved in a dynamic ECM formation and breakdown.

ECM components are produced by fibroblasts; however, muscle fibers themselves are also capable of synthesizing

1. Nandan, D., Clarke, E. P., Ball, E. H. & Sanwal, B. D. Ethyl-3,4-dihydroxybenzoate inhibits myoblast differentiation: Evidence for an essential role of collagen. *J. Cell Biol.* (1990). doi:10.1083/jcb.110.5.1673

2. Nishimura, T. *et al.* Inhibition of matrix metalloproteinases suppresses the migration of skeletal muscle cells. *J. Muscle Res. Cell Motil.* (2008). doi:10.1007/s10974-008-9140-2