As a system for studying organogenesis, bone development has many advantages. First, it builds on a vast amount of knowledge of bone histology and pathology. Second, it includes a wealth of information on the genetic basis for human skeletal diseases and takes full advantage of powerful molecular genetic tools in mice. These advantages make the field of bone research extremely attractive for studies of important new as well as old questions about the formation and function of complex biological systems. In the last four years I have been studying bone development; more specifically, we have been interested in both genetic and epigenetic mechanisms that regulate bone formation.

The vertebrate skeleton is formed by cells that originate from cranial neural crest, somites and lateral plate mesoderm. Bones can be formed either by intramembranous ossification in which condensed mesenchymal cells differentiate into bone-forming osteoblasts or by endochondral bone formation in which condensing mesenchymal cells differentiate into cartilage-forming chondrocytes. As development proceeds, chondrocytes in the centers of the cartilage templates differentiate to hypertrophy; this is followed by invasion of blood vessels, osteoclasts and other mesenchymal cells from the perichondral tissue (perichondrium) into the cartilage, which is progressively eroded and replaced by bone marrow and trabecular bone.

The role of VEGF in mesenchymal condensations.

In vertebrates, the limb skeleton is formed by endochondral bone formation. During the initial stages of limb development, mesenchymal cells are homogeneously distributed throughout the limb and are vascularized by a primary vasculature. Interestingly, as development proceeds the vasculature rearranges, resulting in avascular areas that appear at specific sites where the mesenchymal cells form condensations. The mesenchymal cell condensations then differentiate into chondrocytes, forming a cartilage model that is further replaced by bone.

Although vascular rearrangement has been previously described, a comprehensive understanding of the coupling of cartilage development and angiogenesis and the molecular mechanisms that mediate them is still needed.
Vascular endothelial growth factor (VEGF) is a known angiogenic factor that has a role in bone development. We detected a dynamic expression of VEGF in the mesenchymal cells during early limb development. Interestingly, inactivation of VEGF in the mesenchymal cells caused a severe retardation in limb development, suggesting that VEGF is involved in early limb development.

These results show that cartilage development is necessary for normal vessel rearrangement and that VEGF affects both cartilage development and vessel rearrangement. These results suggest that VEGF is a key coordinator between cartilage development and angiogenesis. Cartilage development and vessel rearrangement are coordinated.

The role of VEGF in osteoblast differentiation.

Craniofacial bone develops via two pathways - endochondral ossification or intramembranous ossification. While most of what is presently understood of bone formation relates to endochondral ossification, very little is known about the regulation of intramembranous ossification. In our study, we demonstrate that osteoblasts forming intramembranous bones co-express both osteogenic and chondrogenic genes. Moreover, we provide direct genetic evidence for a role of VEGF in the continued differentiation of this cell type to a mature osteoblast, by using mice that only express the VEGF120 isoform, and mice where VEGF was conditionally deleted in neural crest cells or collagen type II-expressing cells. Alteration of VEGF signaling led to significantly reduced ossification at sites of intramembranous bone formation, due to a reduction in osteoblastic differentiation.

These findings identify VEGF as a key regulator of osteoblasts that co-express osteogenic and chondrogenic genes during craniofacial intramembranous bone development.

Our analyses of these mice have uncovered phenotypic abnormalities in several aspects of skeletogenesis. In particular, we observed fusion of hindlimb and forelimb joints, and focused on the humerus, radius and ulna in the forelimb. Abnormalities were revealed already during early joint formation where we observed histological differences in regions of future joints between wildtype (wt) and Sp2H/Sp2H embryos. Furthermore, detailed analysis of early joint marker expression revealed a decrease in expression of markers such as GDF-5 in mutant embryos, compared to its robust expression in wt. This was accompanied by a concomitant expression of the chondrocyte differentiation marker Col2 in this region, as well as an increase in cells staining positive for alcian blue. In addition, we observed an increase in cell proliferation in the area of joint fusion.

Recently the Wnt/b-catenin pathway was identified as a key regulator of joint formation. We examined the activation of the Wnt/b-catenin pathway in the absence of musculature by crossing mice that are heterozygous Sp2H with heterozygous TOPGAL mice. The TOPGAL mouse carries a LacZ reporter regulated by the LEF/TCF binding sites, which can be activated by the b-catenin. We found reduced b-catenin activity in E13.5 embryos that are heterozygous TOPGAL and homozygous Sp2H.

Acknowledgements
Our data therefore suggests that the musculature is required for early joint specification, which may be regulated by the Wnt pathway.

The role of mechanical load on embryonic bone development.

During the last 150 years data from several animal models support the conclusion that mechanical forces on bones created by muscles attached to them have a role in bone development. We studying this phenomenon in mice using all the benefits of genetic manipulation in mice. As was mention above It has been well established that mechanical forces on bones created by muscles attached to them play a role in bone homeostasis and remodeling in postnatal life. However, during embryonic development the contribution of mechanical load is not well understood. We aimed to study the regulatory roles of the musculature during skeletal development utilizing a “muscleless” mouse model carrying a mutation splotch in the Pax3 gene(Sp2H/Sp2H). In these mice primordial myoblasts fail to migrate into the growing limbs resulting in a lack of limb musculature.