When we look around, we notice that humans are differ by size, proportions, colors of eyes, hair or skin. Yet, genetically, we are virtually identical. When we look further and compare genomes across species, we discover equally fascinating fact. At the protein level we share the same genes with fruit fly (60%), zebrafish (70%), almost 90% with mice and up to 98% with apes. Over the years of research it became apparent that not as much the proteins as the way they are regulated makes us so different. In fact, the main reason for this diversity is the differential regulation in space, time and intensity of the expression of protein coding genes. Many levels of that regulation have been and continuously are discovered. In 1965 Jacques Monod and Francois Jacob got Nobel Prize in Physiology and Medicine for the discovery of a basic gene regulatory circuit in bacteria- the Lac operon. This simple, repressor-based regulatory circuit is however insufficient to describe the complex regulatory landscape of eukaryotic genes. While it is at this moment not possible to study the regulatory networks as w whole, it is possible to analyze certain aspect of it. One of very important steps in the spatiotemporal regulation of gene expression takes place at the level of transcription. Here, the information encoded by DNA is converted to RNA which will serve as protein template later on. The spatiotemporal regulation of transcription as well as its intensity will determine the later phenotypic consequences of gene expression. One of examples of such a regulation is the expression of a gene, sonic hedgehog during limb development. It has been postulated that the duration and intensity of that expression participates in the determination of the shape and size of our limbs (work of labs of John Fallon, Cliff Tabin, Cheryl Tickle and many others). Consequently, studying of the transcriptional regulation is so important to understand the diversity within and among species.

This proposal focuses on investigation of genes expressed in joint interzone- the area between the developing bones in the limb that will later participate in the formation of tendon attachments to the bone, synovium and synovial membrane, ligaments and other joint associated structures. The two genes that we want to investigate, SMOC2 and DACT2, have a very specific expression in joint interzone. Importantly, although separated by 100,00bp they are transcribed at the same time and place. To behave like that, they most likely share the same regulatory sequences called enhancers. Additionally, they are very likely to be active within the same chromatin structure. Enhancers can be located anywhere on the same chromosome with respect to the gene they regulate. When the chromatin structure is modified, one of the consequences is bringing together enhancers and target genes resulting in tissue and/or time specific expression. Understanding these mechanisms is important for at least two reasons. From the basic science point of view it brings us closer to understand how the regulation of a gene expression can participate in big phenotypic differences among species. Medically, it is critical in unraveling disease associated mutations located in noncoding parts of DNA, especially since only about 2% of the genome encodes proteins while the rest is largely devoted to regulatory sequences. Our laboratory works for many years on joint induction and the possibility to study transcriptional regulation of a gene involved in the early stages of that process presents a unique opportunity to gain a better understanding of the dynamics of that process. Since SMOC2 has been associated with osteoarthritis, unraveling the mechanism of its expression in the joint is important in unraveling the contribution of that gene to this joint disease.

To accomplish our goal we will use most modern technologies available such as targeted chromatin capture followed by next generation sequencing. This technique permits the identification of chromatin domains that are transcriptionally active. We will also map active transcriptional domains in developing joints in vivo using chromatin immunoprecipitation to detect transcriptionally active domains near SMOC2/DACT2 genes. To accomplish that, we will identify these chromatin regions near SMOC2/DACT2 genes that bind proteins associated with active enhancers. Finally, we will use developing chick embryo to test *in vivo* identified enhancers and investigate consequences of these mutations that have been linked to joint disease on the spatiotemporal pattern of SMOC2/DACT2 expression. The results of our work may be used in the future to predict late onset joint disease, deliver potential targets for therapeutic intervention and used as a diagnostic tool to identify patients at risk.