Lecture 2. MCB 141. Regulation of differential gene expression.
Reading: Essential Cell Biology, Chapter 8.

1. On Tuesday we discussed evidence for nuclear equivalence and the importance of differential gene expression in development. For example, it is possible to convert any tissue into iPS cells (induced pluripotent stem cells) by forced expression of three transcription factors (TFs): Nanos, Oct4, and Sox2. iPS cells, in turn, can form any cell type, including cardiomyocytes, neurons, muscles, blood cells, and skin. It is therefore possible to convert a given cell into any other cell type. This is compelling proof for nuclear equivalence: all of the genes in the genome are retained in every specialized cell type, even if they are silent and not used by that cell.

2. In general, TFs such as Nanog, Oct4 and Sox2 work by binding to specialized regulatory DNAs called enhancers. These are typically 100-1000 bp in length and can map quite far from the genes that they regulate. For example, the ZRS enhancer maps about one megabase from the sonic hedgehog promoter. It remains a mystery how remote enhancers such as the ZRS communicate with distant promoters. It is generally thought that this involves chromosomal looping of the enhancer to the promoter.

3. TFs work in a combinatorial fashion to activate gene expression in specific cell types. For example, the MesP gene is selectively activated in the progenitors of the cardiac mesoderm, the B7.5 blastomeres, by the combination of Lhx3 and Tbx6. These TFs are co-expressed only in B7.5 and not in any other cells of the Ciona embryo. What is the basis for Tbx6 + Lhx3 synergy?

4. Before delving into this question I would like to summarize a few more examples of combinatorial gene control in development. Let's consider the process of neurulation seen in vertebrate embryos. During this process the dorsal surface of the embryo undergoes involution to form the neural tube. This tube receives two signals, one from the "top", the dorsal ectoderm and the other from the "bottom", the notochord. BMP signals emanating from the dorsal ectoderm induce expression of Msx in dorsal regions of the neural tube. In contrast, SHH signals emanating from the notochord induce expression of Nkx6. This localized expression of Msx and Nkx6 helps pattern the neural tube.

5. How do BMP and SHH signaling molecules specifically activate their target genes in the neural tube when both are used repeatedly in a variety of developmental processes? For example, as I previously mentioned, SHH is expressed in the developing limb buds, not just the notochord. Why is Nkx6 selectively activated in the neural tube but not the limb buds?

6. The answer is combinatorial gene control. SHH is not sufficient to induce Nkx6 expression. Instead, it works in concert with another TF, SoxB1, which is expressed throughout the neural tube. It is the combination of SoxB1 and Gli

(SHH effector TF) that activates Nkx6. Since SoxB1 is restricted to the neural tube, SHH is unable to induce Nkx6 in the limbs. Similarly, BMP works in concert with SoxB1 to activate Msx in the neural tube, but not in any of the other tissues receiving BMP signals.

7. There are many other examples of signaling pathways working in concert with localized TFs to obtain tissue-specific patterns of gene expression. We previously discussed localized expression of the MesP TF in the B7.5 progenitors of the sea squirt heart. FGF signaling induces the anterior daughter cell of B7.5 to express genes essential for heart development, including FoxF and Nkx2.2. FGF signaling is pervasively used in a variety of developmental processes, but it selectively activates FoxF and Nkx2.2 in the B7.5 lineage by working in concert with MesP.

8. There are several cell signaling pathways that are used for a variety of developmental processes, including FGF, SHH, BMP, and WNT. You will hear a lot more about each of these pathways in future weeks. They primarily function by inducing the activities of specific TFs. FGF induces the activity of ETS, SHH induces GLI, BMP induces SMAD, while WNT induces TCF. In general, binding of the extracellular ligand to a membrane-tethered receptor leads to the activation of a TF effector protein. For example, FGF and BMP signaling lead to the phosphorylation and movement of the ETS and SMAD TFs from the cytoplasm to the nucleus.

9. OK, so we've seen a number of examples of TF combinations activating gene expression. Tbx6 + Lhx3 activate MesP, SoxB1 + Smad activate Msx, SoxB1 + Gli activate Nkx6, and MesP + Ets activate FoxF and Nkx2.2. How do these TFs work synergistically to activate transcription?   There are three key modes of transcriptional activation: (i) chromatin remodeling by Swi/Snf, (ii) histone modifications by CBP and Trr/Set, and (iii) Mediataor-TFIID/Pol II interactions.

10. Once TFs bind to target enhancers they recruit "cofactors" responsible for the binding and release of RNA polymerase II (Pol II) from the promoter.   CBP acetylates lysine residue 27 on the histone H3 tail (H3K27 acetylation), Trr/Set methylates H3K4. These histone marks, H3K27Ac and H3K4me1, are highly correlated with active enhancers and promoters.   It is possible that these modifications facilitate the activities of Swi/Snf, which displaces inhibitory nucleosomes, thereby permitting the binding of additional TFs.

11. So, one model for combinatorial activation is that one TF, say Tbx6, recruits CBP and Trr/Set, which in turn, lead to the binding of Swi/Snf. This "opens" the enhancer and exposes Lhx3 binding sites. Once bound, Lhx3 might recruit Mediator, which is critical for the binding of TFIID/Pol II at the core promoter. According to this model, neither TF alone could mediate efficient transcription. Tbx6 fails to recruit MED, while Lhx3 can't interact with enhancers unless Swi/Snf first displaces nucleosomes and exposes it binding sites.