To determine whether a binding site for the transcription factor, Pros, is found in the upstream promoter region of *notch*, two pieces of data were needed. Empirical data allowed for the determination of a consensus binding sequence for Pros, C-A/t-c/t-N-N-C-T/c (Hassan et al., 1997). In the context of this sequence, 'N' indicates that any nucleotide is equally likely for that position, two nucleotides separated by a slash indicate the occurrence of each nucleotide in that position across sequences. In the latter case, uppercase letters indicate a strong preference for that nucleotide. This binding sequence consensus was written in the Python code as a string, with dashes removed but dashes kept intact. The sequence was manually converted to one case (lowercase), except where indicated otherwise (in determining "strong preference" binding sites). Since the binding site and a portion of the promoter region could only be exact matches if they were in the same case, this manual edit made such comparisons easier. The promoter region of *notch* was estimated as the 1000 nucleotides upstream of the *notch* promoter, and was obtained through the University of California, Santa Cruz (UCSC) *Drosophila* Genome Browser.

The program first seeks to find a specific binding site sequence within the promoter region. If an exact match is found (i.e., a k-mer within the promoter region exactly matches the binding site of length k), then this k-mer and its start location is printed. While this function is useful if a binding site is highly conserved, it does not allow for any variation such as is seen in the consensus reported by Hassan et al. (1997). To handle this issue, the program enumerates all possible binding motifs from the consensus. First, a function is written to build a "tree" of possible binding sites. Branch points represent "choices" in the consensus, such as an "N" or "A/T" (Figure 1). The nucleotide on the branch is determined by an assigned alphabet, which is "ACTG" for "N" versus "AT" for "A/T." Motifs are built to include every possible choice at each position, and added to a list of possible binding sites. Then, the previous function, which looks for exact matches in the promoter region, was modified slightly to identify exact matches with elements in a list. Finally, a list of binding sites with strong preference (i.e., capital letter in a "A/t") was generated, and exact matches with the promoter region were identified.

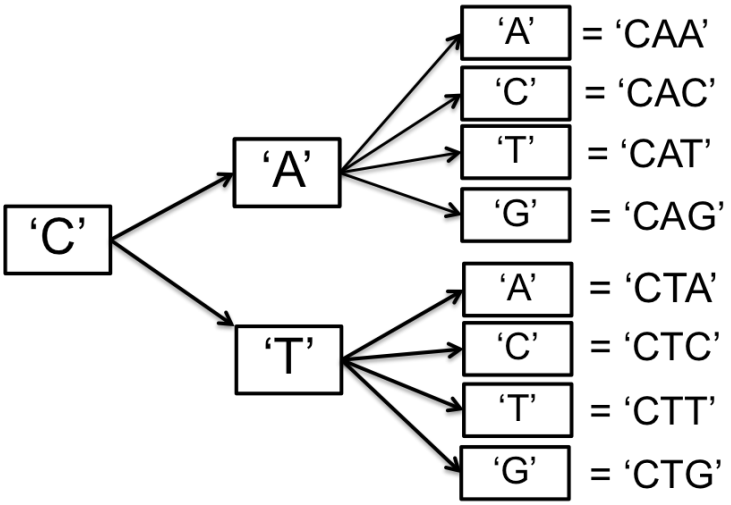

Figure 1. Schematic of the "tree" built to allow for all possible choices from a toy consensus string, 'CA/TN.'

Twelve total binding sites, and eleven unique binding sites, were identified within the *notch* promoter region, from the 128 possible binding sites. Eight binding sites were identified with at least one capital letter, corresponding to strong preference for that nucleotide over its partner (e.g., preference for "A" in "A/t").

These results suggest a possible novel mechanism of cell fate determinant regulation. Prospero is vital to stem cell fate determination, particularly in the neural system (Choksi et al., 2006; Spana and Doe, 1995; Doe et al., 1991). The Notch signaling pathway is a well-studied system that controls cell fate via several mechanisms. Lateral inhibition, or selective expression of Notch and related signaling elements to allow for opposite cell fate between the cells, is an important facet of cell fate control throughout the nervous system (Artavanis-Tsakonas et al., 1999; Haines and Irvine, 2003). Previous literature establishes a regulatory role of Notch on Prospero expression in cell fate determination of retinal and sensory cells (Charlton-Perkins et al., 2011; Reddy and Rodrigues, 1999), demonstrating that these systems interact in cell type specification. However, whether Pros acts in the *notch* promoter region has not been examined previously, and such investigation is highly relevant to the study of stem cell mechanism and cancer development.

A large portion of cancer research examines the overexpression of growth factors or deficits in tumor suppressors, which result in neoplasticity. However, many cancers arise from misregulation of cell fate determination (Arnold et al., 2015; Sancho et al., 2015). Typically, when a tissue is wounded, the system generates specific cell types to counter this damage. When cell fate determinants are disrupted, stem cells do not

proliferate and differentiate appropriately, allowing for development of a tumor, which is often compared to a wound that does not heal (Dvorak, 1986). Disrupted lateral inhibition due to altered Notch signaling may allow for rampant proliferation of one cell type rather than a controlled cell fate distribution (Arnold et al., 2015). In fact, Notch dysregulation has been implicated in several cancers (Nickoloff et al., 2003). Null mutation of *prospero* in neural cells was linked with tumorigenesis when these cells were transplanted to the abdomen (Caussinas and Gonzales, 2005). Furthermore, the disruption of Prospero-mediated expression of stem cell genes and differentiation genes induced excessive cell proliferation (Choksi et al., 2006). Interestingly, vertebrate homolog of Pros, Prospero homeobox 1 (PROX1) is considered a tumor suppressor in the context of some cancers, and a progressive factor in others (Lu et al., 2012). Together, these findings implicate a role for Prospero and Notch in cancer, and beg further elucidation of possible interactive roles.

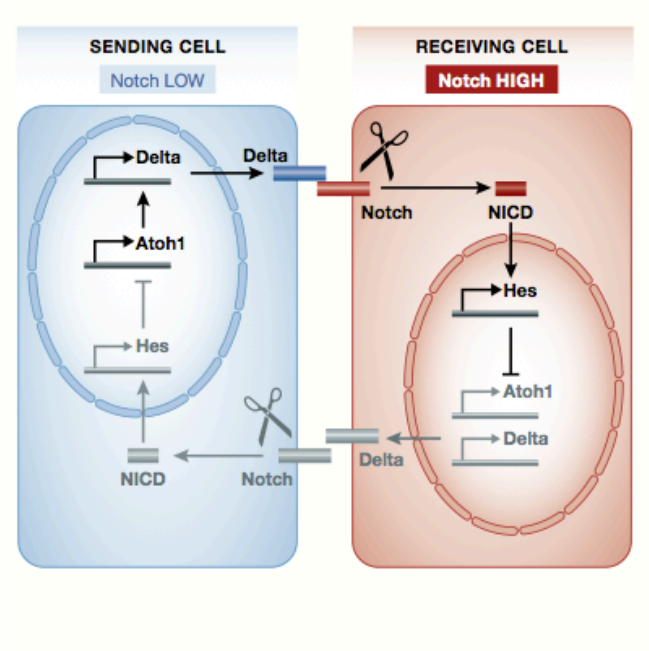

Figure 2. Schematic of lateral inhibition regulated by Notch in the intestine. Briefly, reduced Notch activity in one cell (blue) is associated with increased production of the ligand, Delta. Production of this ligand induces heightened Notch activity and reduced ligand production in neighboring cells (red). This forms a positive feedback loop, and initially small differences in Notch levels are amplified, resulting in opposite cell fate. From Sancho et al., 2015 (Figure 2).

Several studies have demonstrated that Prospero is a downstream target of Notch (Hayashi et al., 2008). However, the literature examining the effects of Prospero on

Notch signaling is limited. As a notable exception, Charlton-Perkins et al. (2011) report that in the *Drosophila* retinal system the transcription factors Prospero and Pax2 regulate cell fate via the Notch pathway in the retinal system. However, the authors propose that Pros acts indirectly on the Notch pathway by increasing pERK levels, thereby activating Delta and reducing Notch expression. In contrast to this model, the current results suggest a direct action of Pros on the *notch* promoter region.

Further computational, statistical, and empirical investigation is needed to determine the validity of this direct action by Prospero on Notch signaling. First, the statistical significance of this event (i.e., eleven unique 7-mers found in a 1000 nucleotide sequence from a list of 128 possible 7-mers) should be determined. Furthermore, the same code may be applied to examine individual components of the Notch pathway, including pERK and Delta, to determine whether binding sites are distributed across elements of the signaling pathway. If both these measures prove promising, lab work may confirm the existence of Pros binding sites in the *notch* promoter region. For instance, DNA footprinting may be used to examine the regions of DNA that are bound versus unbound. Thus, future experiments may determine whether DNA is bound at the identified binding site positions when Pros is present. Overall, this project suggests a direct effect of the Prospero transcription factor on Notch expression as a part of a complex feedback system, and begs further elucidation of such a mechanism.

References

Arnold, K. M., Opdenaker, L. M., Flynn, D., & Sims-mourtada, J. (2015). Cancer Growth and Metastasis of Treatment Resistance in Breast Cancer. *Cancer Growht and Metastasis*, 1–13. http://doi.org/10.4137/CGM.S11286.RECEIVED

Artavanis-tsakonas, S., Rand, M. D., & Lake, R. J. (1999). Notch Signaling : Cell Fate Control and Signal Integration in Development. *Signal Transduction*, *284*(April), 770–776. http://doi.org/10.1126/science.284.5415.770

Caussinus, E., and Gonzalez, C. (2005). Induction of tumor growth by altered stem-cell asymmetric division in Drosophila melanogaster. Nat. Genet. 37, 1125–1129.

Charlton-Perkins, M., Whitaker, S. L., Fei, Y., Xie, B., Li-Kroeger, D., Gebelein, B., & Cook, T. (2011). Prospero and Pax2 combinatorially control neural cell fate decisions by modulating Ras- and Notch-dependent signaling. *Neural Development*, *6*(1), 20. http://doi.org/10.1186/1749-8104-6-20

Choksi, S. P., Southall, T. D., Bossing, T., Edoff, K., de Wit, E., Fischer, B. E., … Brand, A. H. (2006). Prospero acts as a binary switch between self-renewal and differentiation in Drosophila neural stem cells. *Developmental Cell*, *11*(6), 775–89. http://doi.org/10.1016/j.devcel.2006.09.015

Doe, C., Chu-LaGraff, Q., Wright, D., & Scott, M. (1991). The prospero gene specifies cell fates in the Drosophila central nervous system. *Cell*. Retrieved from http://www.sciencedirect.com/science/article/pii/0092867491904639

Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med*. 1986;315(26):1650–1659.

Haines, N., & Irvine, K. D. (2003). Glycosylation regulates Notch signalling. *Nature Reviews. Molecular Cell Biology*, *4*(10), 786–797. http://doi.org/10.1038/nrm1228

Hassan, B., Li, L., Bremer, K. a., Chang, W., Pinsonneault, J., & Vaessin, H. (1997). Prospero is a panneural transcription factor that modulates homeodomain protein activity. *Proceedings of the National Academy of Sciences*, *94*(20), 10991–10996. http://doi.org/10.1073/pnas.94.20.10991

Hayashi, T., Xu, C., & Carthew, R. W. (2008). Cell-type-specific transcription of prospero is controlled by combinatorial signaling in the Drosophila eye. *Development (Cambridge, England)*, *135*(16), 2787–2796. http://doi.org/10.1242/dev.006189

Lu, M.-H., Huang, C.-C., Pan, M.-R., Chen, H.-H., & Hung, W.-C. (2012). Prospero Homeobox 1 Promotes Epithelial-Mesenchymal Transition in Colon Cancer Cells by Inhibiting E-cadherin via miR-9. *Clinical Cancer Research*, *18*(23), 6416–6425. http://doi.org/10.1158/1078-0432.CCR-12-0832

Nickoloff, B. J., Osborne, B. a, & Miele, L. (2003). Notch signaling as a therapeutic target in cancer: a new approach to the development of cell fate modifying agents. *Oncogene*, *22*(42), 6598–6608. http://doi.org/10.1038/sj.onc.1206758

Reddy, G. V, & Rodrigues, V. (1999). Sibling cell fate in the Drosophila adult external sense organ lineage is specified by prospero function, which is regulated by Numb and Notch. *Development*, *126*(10), 2083–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10207134

Sancho, R., Cremona, C. a, & Behrens, A. (2015). Stem cell and progenitor fate in the mammalian intestine: Notch and lateral inhibition in homeostasis and disease. *EMBO Reports*, *16*(5), 571–581. http://doi.org/10.15252/embr.201540188

Spana, E. P., & Doe, C. Q. (1995). The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in Drosophila. *Development (Cambridge, England)*, *121*, 3187–3195. http://doi.org/10.1016/0168-9525(96)81394-1