Cell adhesion molecules in olfactory connection formation

This dissertation study investigated expression, function and the regulatory mechanism of cell adhesion molecules in the formation of olfactory connections. Identification and characterization of a novel protocadherin, Pcdh20, provided additional evidence that multiple cell adhesion molecules are involved in the development of the olfactory system. In combination with several established studies by Sakano and Yoshihara's group, my study further supports the neural identity model and provides a regulatory mechanism involving MeCP2 in the establishment and maintenance of this combinatory cell adhesion molecule expression in the olfactory sensory neurons.

OR identity in correlation with cell adhesion molecule expression

Though many cell adhesion molecule expression patterns were described, few of them were correlated with specific ORs. On the other side of the coin, it is also unknown whether a specific OR is correlated with a specific set of cell adhesion molecules throughout development. In adult mice, Pcdh20 expression is in a subpopulation of OSNs and their axons terminate in a small number of discrete Pcdh20-positive glomeruli in the OB. Interestingly, the distribution and numbers of Pcdh20-positive glomeruli are markedly different across gender. More Pcdh20-positive glomeruli with a wider distribution pattern are observed in the male OB, whereas fewer glomeruli with more restricted clustering of Pcdh20-positive glomeruli are found in female OB. The sexually dimorphic expression of Pcdh20 suggests that there may be different ORs associated with Pcdh20 in different sexes. 103

If Pcdh20 expression is correlated with specific OR expression, identification of Pcdh20 associated ORs could reveal possible sex-specific OR expression and aid in further investigation of OR-specific ligand function. In previous studies, cDNA libraries from a single OSN were obtained. OR expression in a single OSN can be identified by PCR using degenerate primers (Dulac and Axel, 1995). In collaboration with Dr. T. Cutforth from Stanford University, I have initiated this study by isolating single OSNs by dissociation of OE and attempting to identify Pcdh20 expressing cells by PCR. Several attempts were made to confirm OR expression using degenerate primers designed by L. Buck (Buck and Axel, 1989). Though I will not be able to complete this study during my dissertation research, identification of Pcdh20 associated ORs will provide important insight into not only OR and CAM association but also understanding of differential OR gene expression in different sexes and whether or not the main OB is related to pheromone recognition.

Neuronal activity and regulation of cell adhesion molecule expression

Neuronal activity results in long term changes in neurons by regulating gene expression. OSNs constantly respond to external stimuli throughout the life of the animal. Using genetic models and surgical manipulations, it is shown that blocking odorant evoked activity alters the expression of selected cell adhesion molecules. Even though regulation of cell adhesion molecule expression is important for the formation of olfactory axonal converge into glomeruli, the regulatory mechanism of gene expression is undetermined.

I reported here that olfactory axon convergence is disrupted in MeCP2 null mice. Furthermore, I also obtained evidence that MeCP2 directly regulated

Kirrel 2/3 expression. In MeCP2 KO mice, significant increases in Kirrel2/3 gene transcripts were observed in OE, suggesting that MeCP2 is a transcription suppressor for Kirrel 2/3 gene expression. In addition, my data provide evidence that MeCP2 function is regulated by neuronal activity. With the presence of odorant evoked 104

activity, MeCP2 is phosphorylated at Serine80 and also possesses enhanced binding affinity to promoters of Kirrel2 and Kirrel3 genes. Though MeCP2 increased its binding to promoters of both Kirrel2 and Kirrel3, transcript level changes are markedly different between Kirrel2 and Kirrel3 under odorant stimulation. Other transcription factors were shown to be regulated by neuronal activity. It is likely that both Kirrel2 and Kirrel3 are regulated under multiple neural activity dependent transcription factors.

We propose a model in which a balanced transcriptional regulation from both repressors (like MeCP2) and enhancers (like CREB, MEF) determines the expression levels of Kirrel2 and Kirrel3. When both repressor and enhancer are under neural activity regulation, how the balance tilts will determine whether Kirrel2/3 expression will be up- or down-regulated in OSNs.

In this study, the olfactory system serves as an excellent model system to study gene regulation of MeCP2 by neuronal activity at physiological levels. Previously, the mechanism of MeCP2 on gene expression regulation by neuronal activity was only studied in vitro. The brain is composed of heterogeneous cells and their neuronal circuits are extremely complex. In contrast, the OE is composed of a single type of neuron. This property provides an opportunity to study neuronal subtype specific MeCP2 function.

In addition, the olfactory system provides an excellent system to study the effect of neuronal activity due to it accessibility. Odorant stimulation can be given to the OSNs in the nasal cavity to allow investigation of gene regulation under physiological level of stimulation. To further investigate the model we proposed, it is important to elucidate the full spectrum of MeCP2 target gene regulation. Future study should be done to screen MeCP2 target binding through ChIP-Chip analysis. MeCP2 binding sequence will provide information in the target genes they regulate in the OSNs. To further provide or block odorant evoked activity, changes in MeCP2 binding will allow us to identify target genes that are activity dependent. Furthermore, identification of odorant evoked activity dependent transcription enhancers in OSNs will allow further validation of the regulatory model we proposed here.

Understanding neural activity dependent MeCP2 function is critical in elucidating the mechanisms of Rett Syndrome. Though rapid progress has been made in the identification of MeCP2 targeting genes, we still do not understand how changes in gene expression result in neuronal structural and functional changes. Rett Syndrome is exacerbated during the early postnatal period. Neural activity plays a critical role in this process. Understanding the relationship between physiological levels of neuronal activity and MeCP2 regulation is the obvious next challenge. The olfactory system provides an excellent model for the easy manipulation of activity stimulation and examination of subtle axonal targeting defects. This study established that cell adhesion molecules are regulated by MeCP2 in an activity dependent manner. Further genomic analysis will provide a comprehensive

understanding of MeCP2 regulation of gene expression and could help in the development of treatment strategies for Rett Syndrome.