

m8 expression, which in wild-type cells is normally downregulated in Naa and Nab.

It is however unclear why *E(spl)*m8 expression only reappears in Naa in wild-type cells following normal Ham downregulation in later stages of Naa and Nab development. Possibly, this is a result of Pon-mediated inhibition in Nab, that is, the asymmetric distribution of Notch signaling inhibitors occurring in parallel. In fact, even in Ham-deficient precursor cells, the late downregulation of *E(spl)*m8 expression is intact, whereas the initial downregulation early after Naa and Nab are born is blocked in Ham-deficient cells. It is crucial to note here that although *E(spl)* reporter transgenes are useful read-outs of Notch transcriptional activity¹⁵, there is no evidence yet that quantitative changes in *E(spl)* regulation are in fact linked to the phenotypic effects of Ham function. Nonetheless, this nicely illustrates the requirement for precise timing and selectivity of Ham function in ORN development.

How much of the Ham-mediated histone methylation affects the immediate response to Notch signaling and how much will result in longer lasting changes in promoter activity?

In other words, how short or long lasting (and how widespread) are the chromatin ‘Notches’ induced by Hamlet during cell fate choices? One usually thinks of chromatin modification as a mechanism for marking a gene locus to generate a rather persistent pattern of activation or silencing. Here, however, Ham loss-of-function changes the dynamics of *E(spl)* reporter expression in a relatively dynamic, subtle and quantitative fashion. As explained above, Ham and *E(spl)* expression are not mutually exclusive and different cells do different things. Thus, relatively small changes in the expression of multiple (possibly many) genes together appear to mediate the observed cell-transformation effects. Alternatively, Ham may regulate olfactory receptor expression and ORN axon targeting via additional unknown regulatory factors.

One can speculate that the special case of Ham-Notch interrelations in the ORN lineage is not the exception, but rather reveals a more general principle of how epigenetic modifications are used to diversify the outcome of a single reiteratively used signaling module. Moreover, even if one regards only the Notch

pathway, this study provides a useful tool (Ham-controlled target promoters) for identifying in a global analysis a much-needed larger complement of target genes selectively involved in Notch-dependent fate specification.

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MeCP2: only 100% will do

Hsiao-Tuan Chao & Huda Y Zoghbi

A knock-in mouse mimicking a MeCP2 mutation found in human Rett syndrome recapitulates disease features, including progressive motor and cognitive impairments—and correlations emerge between MeCP2 dosage in mice and phenotype.

Typical Rett syndrome (RTT) is characterized by apparently normal development for the first 6–18 months of life, followed by developmental regression, cognitive deficits, motor impairments, autism-like features and seizures. RTT can be caused by any of a host of mutations in virtually any region of the X-linked methyl CpG-binding protein 2 (*MECP2*)¹, and it has been thought that different mutations might impair different protein functions, giving rise to the phenotypic variability seen in the syndrome. Curiously, however, mouse models have shown that

RTT-like features can be caused not only by *Mecp2* mutations, but also by loss of expression, underexpression and even overexpression of the protein^{2–7}. These findings raise the question of whether the different mutations observed in individuals with RTT lead to different degrees of impaired MeCP2 function. In this issue of *Nature Neuroscience*, Goffin *et al.*⁸ shed light on this question by discovering that a mutation in a key residue affects both the function and levels of MeCP2.

Thr158, a residue located near the C-terminal end of the methyl CpG-binding domain (MBD), is the most common site for mutations in Rett syndrome. The most common alteration at this site is T158M, with T158A occurring in rare instances. To explore the importance of the Thr158 residue, the authors generated a T158A knock-in mouse model, as the larger methionine residue would most likely create steric interference that on its own might perturb MeCP2 function. These *Mecp2*^{T158A/y} mice recapitulated nearly all of the features of RTT seen in *Mecp2*^{-y} mice, including progressive motor impairments,

cognitive impairments and premature lethality, but in a somewhat less severe form. Protein instability leads to a reduction of MeCP2 levels in these mice by 40–50%, which is comparable to the 50% reduction observed in the partial loss-of-function *Mecp2*^{loxP/y} allele that was previously shown to cause RTT features (albeit without diminishing lifespan)⁴. Despite similar reductions in MeCP2 protein level, however, the phenotypes observed in *Mecp2*^{T158A/y} mice are more severe than those of the previous *Mecp2*^{loxP/y} mice. This suggests that the T158A allele leads to a loss of MeCP2 function beyond that conferred by the diminished protein levels.

Residue Thr158 is thought to be important not only for stabilizing the tertiary structure of the MBD, but also for allowing MeCP2 to bind methylated CpG sequences. Indeed, Goffin *et al.*⁸ found that T158A mutation severely impairs MeCP2 binding to methylated CpG DNA. Closer examination using chromatin immunoprecipitation assays against loci known to be bound by MeCP2 (*Xist*, *Snrpn* and *Crh*) revealed an overall 70–75% reduction in MeCP2 binding. This suggests that, in addition

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to the 50% reduction in MeCP2-T158A protein levels, the efficiency of MeCP2-T158A binding to methylated CpG DNA is further impaired by approximately 50%. It is worth noting that the *Mecp2*^{T158A/y} mice share many features, including premature lethality, with *Mecp2*^{R168X/y} mice, which have a *Mecp2* allele that is truncated at the MBD. That truncation leads to a 50% reduction in mRNA expression relative to wild type, concomitantly reduced protein expression and a predicted impairment in DNA binding⁷. Further comparison of the *Mecp2*^{T158A/y} mice with previous RTT models proves revealing in several respects.

First, it illuminates a correlation between MeCP2 levels and behavior: the lower the levels of MeCP2 protein, the less anxiety-like behavior the mice show. Goffin *et al.*⁸ quantified the amount of time *Mecp2*^{T158A/y} and *Mecp2*^{-/-} mice spent in the open and closed arms of an elevated zero maze and found that both mutants showed less anxiety than wild-type mice. This reduction in anxiety-like behavior was more pronounced in *Mecp2*^{-/-} than in *Mecp2*^{T158A/y} mice⁸ and is consistent with an earlier study, which found that a 50% reduction in MeCP2 suppresses anxiety-like behavior⁴. In contrast, mice of the *Mecp2*^{308/y} model, the C-terminal truncation of which preserves several of the main functional domains of the protein, show more anxiety-like behavior than wild-type mice^{6,9}. One explanation for the increased anxiety-like behavior in *Mecp2*^{308/y} mice is the increase in *Crh* (corticotropin-releasing hormone) mRNA and the resulting increase in stress-induced corticosterone levels⁹. Goffin *et al.*⁸ found that the T158A mutation hindered MeCP2 binding to the *Crh* promoter, with a consequent decrease in *Crh* mRNA. Together, the *Mecp2*^{308/y} and *Mecp2*^{T158A/y} mice suggest that anxiety-like behavior correlates with *Crh* expression and that not every *MECP2* mutation will alter *Crh* expression in the same manner. This is particularly relevant because anxiety is a salient symptom of many, but not all, individuals with RTT¹⁰. Understanding the functional disturbance and phenotypic consequences of various pathogenic *MECP2* alleles will be necessary so as to manage specific phenotypes.

Second, by examining electroencephalograms (EEGs) in the *Mecp2*^{T158A/y} mice at different time points, Goffin *et al.*⁸ found neurological deficits that are age dependent. Earlier slice and EEG studies in mice lacking MeCP2 revealed hyperexcitability in the hippocampal network^{11–13}. Such disruptions in network activity may underlie the behavioral and cognitive features observed in RTT. Goffin *et al.*⁸ recorded EEGs from the hippocampus to examine auditory evoked event-related potentials (ERPs) during

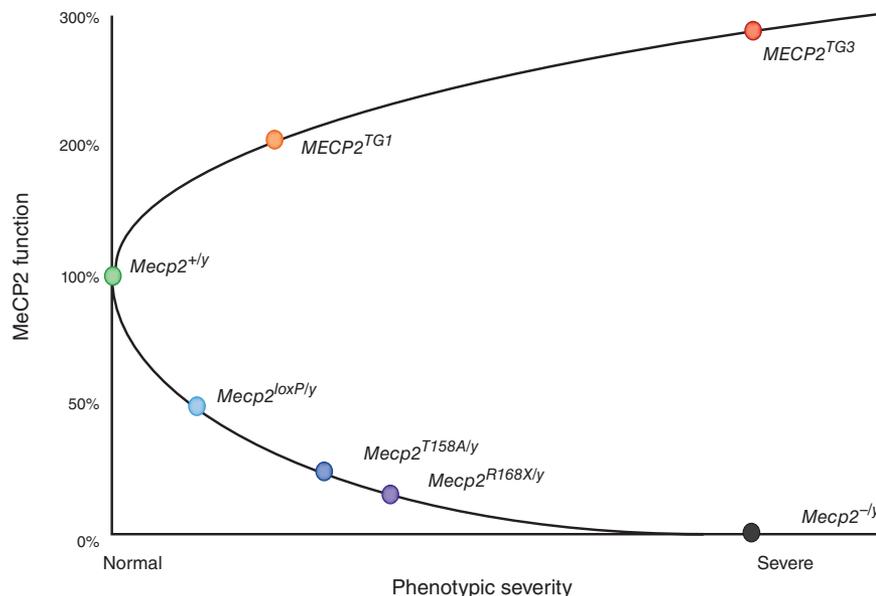


Figure 1 Phenotypic severity increases as MeCP2 function decreases, as shown by comparison of *Mecp2*^{+/y}, *Mecp2*^{loxP/y}, *Mecp2*^{T158A/y}, *Mecp2*^{R168X/y} and *Mecp2*^{-/-} mice. Severity also increases, however, as MeCP2 increases beyond normal levels, as seen in *MECP2*^{TG1} (twofold overexpression) and *MECP2*^{TG3} (threefold overexpression) mice.

passive network processes. ERP analysis from presymptomatic *Mecp2*^{T158A/y} mice at postnatal day 30 (P30) revealed responses similar to those of wild-type control mice. Symptomatic *Mecp2*^{T158A/y} mice at P90, however, showed alterations in the latency and amplitudes of their ERP peaks. This age-dependent effect could occur because of either an acquired impairment of network activity as a result of abnormal development as the mice mature or a failure to maintain appropriate neuronal function and/or development. Accumulating evidence favors the latter possibility. First, postnatal restoration of MeCP2 expression in symptomatic *Mecp2* null mice is sufficient to rescue several important phenotypic features, indicating that neurological development is largely intact despite the absence of MeCP2 function and that network impairments are reversible¹⁴. Second, deletion of *Mecp2* in adult mice recapitulates RTT-like features. This strongly suggests that the primary function of MeCP2 is to maintain neurological function and neural network activity¹⁵. The absence of gross alterations in ERP at P30 lends further force to the hypothesis that the initial development of the neural network remains largely intact, despite a 70–75% reduction in MeCP2 function. It would be interesting in future studies to examine the mechanisms underlying the age-dependent neural network dysfunction and to relate these mechanisms to specific alleles.

Last, but not least, the study by Goffin *et al.*⁸ nicely illustrates how the interaction between protein level and function determines the phenotypic severity in response to an age-

dependent requirement for MeCP2 activity. If we consider the *Mecp2*^{T158A/y} mice alongside previous models^{2–4,7}, we can array the phenotypes of *Mecp2*^{-/-}, *Mecp2*^{T158A/y}, *Mecp2*^{R168X/y} and *Mecp2*^{loxP/y} mice along a spectrum of severity (Fig. 1). The lower the MeCP2 function, the more severe the phenotype. These studies suggest that therapeutic interventions that increase the function of some alleles could mitigate symptom severity. These findings make it clear that comprehensive analysis of the effects of different mutations on MeCP2 expression level and function will be critical for creating and optimizing allele-specific therapies.

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