Math1: Waiting to Inhale

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The proneural gene Math1 is known to be involved in numerous functions within the nervous system, including unconscious proprioception, audition, and arousal. Two recent papers by the Zoghbi group in this issue of *Neuron* and a recent issue of *PNAS* now identify a critical role for this gene in the development of brainstem regions critical for conscious proprioception, interoception, and respiration.

Numerous actions of the autonomic nervous system are essential in all mammalian organisms. However, with the exception of a beating heart, control of respiration is perhaps the most fundamental to life. Work over the past decade from a number of laboratories has focused on the molecular means by which the autonomic nervous system is assembled. Among these, the *mouse atonal homolog 1* (*Math1*, *Atoh1*) has proven to be critical for the development of the unconscious proprioceptive, auditory, and arousal systems (Ben-Arie et al., 1997; Bermingham et al., 1999; Machold and Fishell, 2005; Wang et al., 2005). Two recent papers from the laboratory of Huda Zoghbi now provide novel insights into the requirement for *Math1* in the development of the complex neural circuits in the brainstem (Rose et al., 2009a [a recent issue of *PNAS*], and Rose et al., 2009b [this issue of *Neuron*]). The first paper (Rose et al., 2009a) identifies a number of previously unknown Math1-dependent neuronal lineages that contribute to the conscious proprioceptive and interoceptive systems, as well as to respiratory circuits within the brainstem. The second paper (Rose et al., 2009b) further characterizes the role of Math1 in brainstem respiratory circuit development, as well as the alterations in brainstem respiratory activity that occur in the absence of Math function, thereby providing a novel window into the potential causes of respiratory dysfunction in this mutant. Thus, it now appears that Math1 also joins the ranks of transcription factors such as *Phox2*, *Lbx1*, and *Egr2* that are necessary for the proper assembly and function of the brainstem neurocircuitry required for respiration.

Respiration involves complex interactions between the nervous system and the periphery. Residing within the mammalian brainstem are specialized groups of neurons that generate rhythmic respiratory activity and are modulated by nearby nuclei that adjust this rhythm in response to changes in the environment. In humans, mutations in a number of genes result in dysfunction of these respiratory circuits, and as a consequence, devastating respiratory disorders such as sudden infant death syndrome (SIDS). Collectively, the rhythmogenic respiratory neurons are located within the ventral respiratory column (VRC) of the medulla. The rhythmic activity that drives respiration originates within the pre-Bötzinger complex (preBötC), but this region receives input from other nuclei within the VRC, including the parafacial respiratory group and retrotrapezoid nucleus, which are referred to in the Rose et al. (2009b) manuscript as the pFRG/RTN. Although many neurotransmitter and neuromodulatory pathways are involved in the regulation of respiration, glutamatergic signaling is particularly important for preBötC activity. Previous work from the Zoghbi lab demonstrated that the proneural transcription factor *Math1* is required for the development of a wide variety of nuclei in the brainstem (Ben-Arie et al., 1997; Wang et al., 2005), particularly glutamatergic populations that contribute to the unconscious proprioceptive, auditory, and arousal systems. Interestingly, mice lacking functional Math1 protein die immediately after birth due to respiratory failure. This observation prompted the authors to examine the specific defects in brainstem development in Math1 null animals that could account for this phenotype (Rose et al., 2009b).

The authors first rule out the presence of any overt defects in the peripheral components of the Math1 null respiratory system, indicating that the brainstem circuits responsible for establishing respiration were likely to be absent or dysfunctional in Math1 null animals. To test this directly, the authors utilized electromyography (EMG) to examine respiratory rhythmic activity in brainstem preparations from mutant and control animals, in which the phrenic nerve and diaphragm remained connected. They found that in comparison to controls, EMG activity in brainstem preparations from Math1 null animals was greatly diminished, as indicated by a reduced frequency of firing and poor rhythmic coherence. The rhythmic defect was apparent in direct recordings of preparations lacking the diaphragm and even in slice preparations of the medulla that contained the preBötC region. This indicated that the origin of the respiratory failure in Math1 null animals likely resides within the medulla itself.

To determine which specific Math1 dependent lineages within the medulla were required for the establishment of respiratory rhythmic activity, the authors reexamined the Math1-expressing populations arising in this region. During embryogenesis, *Math1* expression is largely restricted to neural precursors originating within the rhombic lip neuroepithelium and is for the most part rapidly downregulated following their migration

away from the latter. The well-known exceptions to this are the granule cell precursors of the cerebellum, which maintain Math1 expression throughout their postnatal proliferation in the external granule layer (EGL). Interestingly, by in situ hybridization, the authors find that Math1 mRNA expression is also evident outside of the rhombic lip in cells surrounding the trigeminal (V) and facial (VII) motor nuclei from E11.5 until birth. Paramotor expression of the Math1 gene persisted in the Math1 null animals, although the "Math1-expressing" cells were somewhat displaced in the vicinity of the motor nuclei. Examining heterozygote Math1$^{LacZ/+}$ embryos at E16.5, the authors noted the presence of labeled cells in the intertrigeminal region and pFRG/RTN suggesting that this is the normal location of the displaced populations observed in mutant animals. Taken together with the Math1 mutant data, this suggests a specific role for Math1 in these areas that is independent of the requirement of this protein during neurogenesis and migration within the rhombic lip.

Recognizing that the stability of β-galactosidase expressed from the Math1 locus makes it impossible to assess when cells labeled in such a manner actually expressed the Math1 protein itself, the authors created a Math1-EGFP fusion protein knockin mouse line to characterize the Math1-expressing populations within the medulla in greater detail. Neurons of the pFRG/RTN express the transcription factors Phox2b and Lbx1, in addition to the substance P receptor NK1R. Combining anti-EGFP immunocytochemistry to detect the Math1-EGFP fusion protein with immunocytochemical labeling of Phox2b and NK1R, the authors reveal coexpression of Math1 with Phox2b and NK1R that was only observed within the pFRG/RTN and not in the Math1 lineages emerging from the rhombic lip. The authors examined the developmental trajectory of Math1 and Phox2b/Lbx1/NK1R expression within the pFRG/RTN and find that Phox2b/Lbx1 expressing neurons are readily detectable by E11.5, just prior to Math1 expression, which becomes evident in a subset of these neurons twelve hours later (E12.0). The population of triple labeled cells (Math1/Phox2b/Lbx1) was observed from E12.0 to E16.5 at the periphery of the motor nuclei, in a manner suggesting that Math1 plays a role in cell migration in this region.

To determine the importance of this perimotor Math1 expression, the authors reexamined the medullas of Math null and control animals for changes in marker expression that might help elucidate the Math1 null respiratory phenotype. They found that the expression of NK1R is lost in the pFRG/RTN, as well as in other nuclei relevant for respiration (e.g., parabrachial), but is preserved in the preBötC. Within the pFRG/RTN, the expression of Phox2b and Lbx1 was also found to be severely decreased in the Math1 mutant, again indicating a failure of these nuclei to develop properly in the absence of Math1. Utilizing the β-galactosidase expressed from the Math1 locus in the null animals (Math1$^{LacZ/LacZ}$), the authors find that those Math1^{+} cells abnormally distributed in the periphery of the pFRG/RTN showed a striking reduction in their numbers caudally. Thus, Math1 is required both for the proper specification, migration, and acquisition of substance P responsiveness of pFRG/RTN neurons.

Considering that the pFRG/RTN provides key glutamatergic input to the preBötC, the authors examined the Math1 null animals for changes in glutamatergic innervation within the medulla by assessing Vglut2^{+} fibers. Somewhat surprisingly, while decreased numbers of glutamatergic fibers were evident in the rostral medulla in Math1 nulls, the Vglut2^{+} innervation of the preBötC and rVRG appeared to be preserved. This could be at least partially explained due to the persistence of locally projecting glutamatergic preBötC neurons that are not affected by the loss of Math1. Thus, it is possible that any loss of preBötC innervation arising from abnormal development of the pFRG/RTN in the Math1 null would be obscured by other glutamatergic inputs from Math1-independent neuronal lineages.

To examine the projections arising specifically from Math1 lineages within the medulla, the authors employed a strategy similar to one that we previously used to demonstrate the transient nature of Math1 expression in early born rhombic lip lineages within rhombomere 1 (Machold and Fishell, 2005). By generating a mouse allele where a hormone-activated Cre recombinase (Cre*Pr) is expressed from the Math1 locus (Rose et al., 2009a), the authors were able to permanently mark Math1 expressing neurons originating at different stages of development. In this case, the authors combine the use of two Cre-dependent reporters to examine the cell bodies and projections of Math1 lineages arising between E9.5 and E14.5 in the medulla at P0. They find that Math1 lineages labeled specifically at E10.5 contribute to the VRC, with cell bodies and projections evident in the preBötC and rVRG, whereas Math1 lineages labeled at earlier or later stages did not exhibit projections within the VRC for the most part. The origin of projections within the VRC from the collective pool of Math1 lineages labeled at E10.5 is complex and could include fibers from neurons located in the parabrachial, dorsal column, spinal trigeminal, and medullary reticular nuclei (Rose et al., 2009a). However, closer analysis of the E10.5 labeled Math1 lineages in the preBötC revealed that while these neurons did not express NK1R or somatostatin, they did project in the vicinity of preBötC neurons expressing the latter markers. Thus, the authors identify a novel population of Math1-dependent preBötC neurons that could possibly contribute to respiratory control in this region. In the future, by evaluating the consequences to respiration in a conditional loss of function context, the authors will be able to define more specifically the requirement of *Math1* for proper respiratory activity.

To characterize in more detail the nature of the failure to establish rhythmic respiratory activity in the Math1 null animals, the authors revisited the brainstem preparation described in the beginning of the Results and tested a panel of neuromodulators for their ability to rescue the abnormal EMG output observed in Math1 null medullas. Application of substance P resulted in an increase in respiratory rhythm, consistent with the persistence of NK1R^{+} preBötC neurons in the Math1 null, but did not rescue the frequency or pattern of rhythmic activity. Noradrenaline application reduced the rhythmic activity in both control and Math1 null preparations similarly, indicating a regulatory role for this pathway that operates independently of brainstem lineages that require Math1. Inhibition

of GABAergic or glycinergic signaling slightly increased the burst frequency of the Math1 null, but the effect was relatively mild. Other neuromodulators known to be expressed in Math1-dependent brainstem lineages, such as acetylcholine, corticotropin-releasing hormone (CRH), and nitric oxide (Rose et al., 2009a) had no effect. Although overlap between Math1 lineages and serotonergic neurons has not been demonstrated, given serotonin's known role in respiration, its effects on this preparation may be interesting to examine in future studies. Notably however, in contrast to their negative findings with other neuromodulators, application of the glutamate reuptake inhibitor dihydrokainic acid (DHK) caused the Math1 null preparation to exhibit a striking increase in rhythmic frequency to wild-type levels. Furthermore, the rhythmic pattern was also rescued to a large extent in the Math1 null preparation upon application of DHK. Thus, the authors conclude that the respiratory defects observed in the Math1 null animals are due to decreased glutamatergic signaling.

The insights into brainstem development and function provided by these studies are tantalizing (Rose et al., 2009a, and Rose et al., 2009b). However, given the large numbers of cell types that express Math1 in the brainstem, the precise correspondence between the cells expressing this protein and their roles in conscious proprioception, interoception, and respiration remains to be clarified. For instance, with regards to respiration specifically, it is still not clear which Math1-dependent lineage (or lineages) is critical for maintaining proper activity within the preBötC. Although increasing glutamatergic activity in the Math1 null brainstem preparation rescued the rhythmic activity, the authors did not detect any obvious changes in glutamatergic innervation of the preBötC in Math1 null animals. Thus, it seems likely that other excitatory circuits that regulate the activity of the preBötC are themselves critically dependent on Math1 lineages. In this regard, the unique role of Math1 in the development of the pFRG/RTN will be particularly interesting to explore. Happily, given the availability of a conditionally null *Math1* allele, these authors have at hand precisely the right tool to address these questions. Hence, we can all eagerly await for Math1 to take its next breath.

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Complexins Living Up to Their Name—New Light on Their Role in Exocytosis

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Ca^{2+}-dependent exocytosis of synaptic vesicles is mediated by the SNARE proteins synaptobrevin/VAMP, SNAP-25, and syntaxin. SNARE function is controlled by conserved regulatory proteins, including the complexins. In a study by Xue et al. in this issue of *Neuron*, contradictory data from *Drosophila* and mouse complexin mutants have been resolved, revealing a complex pattern of facilitatory and inhibitory domains.

When an action potential arrives in a nerve terminal, voltage-gated calcium channels open and calcium enters, triggering exocytosis of synaptic vesicles. The protein machinery mediating fusion of the vesicle with the plasma membrane includes the SNARE proteins as core components. Upon membrane contact, the SNAREs interact and form membrane-bridging *trans*-complexes. These complexes progressively assemble toward the membrane anchors in the vesicle and plasma membrane, respectively, forming an extended bundle of four intertwined α helices. The energy released during assembly is thought to overcome the energy barrier for fusion (Rizo and Rosenmund, 2008). SNAREs form a superfamily of conserved proteins, and thus SNARE assembly between membranes destined to fuse appears to be a common mechanism for intracellular fusion reactions (Kloepper et al., 2007). Synaptic exocytosis, however, is one of the most specialized