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Neuronal Organization: Unsticking the Cadherin Code

Hindbrain cranial motor neurons are organized into discrete functional clusters. A new study demonstrates that coalescence of these nuclei is driven by the expression of distinct combinations of cadherin adhesion molecules by each motor neuron group.

Caroline A. Pearson[1,2], Samantha J. Butler[1,2,*], and Bennett G. Novitch[1,2,*]

Neurons prefer to keep the company of like-minded neurons, employing one of two general strategies to sort themselves into groups with shared function. First, many neurons — including the dorsal horn of the spinal cord, the inner plexiform layer of the retina and the cerebral cortex — are organized into stratified layers or laminae [1–3]. Second, neurons can cluster together into small groups called nuclei, in a process called nucleogenesis [4,5]. Nucleogenesis is an evolutionarily conserved feature of neuronal development observed throughout vertebrate nervous systems, including the motor pools in the ventral spinal cord, the numerous and varied nuclei of the hypothalamus and the cranial nuclei in the hindbrain [4–9]. Laminae and nuclei both contain neurons with common functionality and connectivity, presumably to facilitate neural circuit formation. However, while laminae often form by the radial migration of neurons born in immediately adjacent progenitor zones, many nuclei form from neurons born at considerable distance from each other. Thus, during nucleogenesis, newborn neurons must first migrate tangentially, sometimes for long distances, and then coalesce to form particular and distinct nuclei [2,8]. Remarkably little is known about how this process works.

The differential adhesion hypothesis suggests that intercellular adhesion directs clustering of different cell types [10]. The identification of large families of adhesive molecules, including the classical type I and II cadherins, beginning in the 1980s [11] supported this hypothesis, with Steinberg and colleagues then showing that a mere 50% difference in cadherin levels was sufficient to permit the sorting-out of intermixed cells *in vitro* [12,13]. More recently, the *in vivo* relevance of such mechanisms has been identified. In a series of elegant analyses [14,15], Price and co-workers demonstrated that type II cadherin function is required for spinal motor neurons to migrate and coalesce into motor pools. The mechanisms resolved how motor neurons are able to group with other motor neurons that perform the same function, i.e. innervate the same muscle. In a recent issue of *Current Biology*, Astick, Price and colleagues [16] now show that this mechanism is of general importance throughout nucleogenesis. They demonstrate that type II cadherins also organize the functionally distinct cranial motor neurons into discrete nuclei [16]. In essence, the ‘cadherin code’ is required to let like-minded cranial motor neurons stick together.

What are cranial motor nuclei? Cranial motor neurons, found mostly in the brainstem, control numerous processes fundamental to vertebrate life, including eye and facial movements, eating, speech and breathing. They are classified into three groups, somatomotor, visceromotor and branchiomotor neurons, which respectively innervate targets derived from the somites, the viscera and the pharyngeal arches [6,8]. Cranial motor neurons coalesce into nuclei, resulting in a segmented array of 9 pairs of cranial nerves, such as the glossopharyngeal (IXth) nerve, which innervates the tongue and pharynx. The organization of cranial motor neurons into functionally distinct nuclei is thus likely to be critical for the formation of functionally distinct cranial nerves. Astick *et al.* [16] focus on the formation of 8 distinct nuclei, containing both somatomotor and branchiomotor neurons, in rhombomeres r5 and r8, two of the transient segments in the embryonic chicken hindbrain. The authors followed the developmental progression of these nuclei, finding that, as the motor neurons arise from adjacent progenitor domains, they migrate in an intermingled stream before coalescing into distinct cranial motor nuclei. Many areas of the nervous system have developed temporal strategies to avoid such mixing, for example, the sequential generation of different classes of neurons in the cerebral cortex [1–3]. How do different classes of cranial motor neurons recognize each other in this confusion?

Building on their previous studies, Astick *et al.* [16] identify a ‘cadherin code’: a combination of six type II cadherins that uniquely identifies each of eight individual nuclei (Figure 1A) [16]. This code is predictive: the expression pattern of these key cadherins appears in subsets of motor neurons prior to nucleogenesis taking place. But are the cadherins needed? To address this question, the authors ubiquitously misexpressed a dominant negative form of cadherin to perturb all cadherin-mediated contacts in cranial motor neuron progenitors. Whilst this manipulation had no effect on the differentiation or migration of cranial motor neurons, it did result in all cranial motor neurons failing to coalesce and form discrete nuclei upon arrival at their final destinations in r5 or r8 (Figure 1B). Only the electroporated neurons were affected, demonstrating cadherins are cell-autonomously required for cranial motor nucleogenesis.

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levels, that determines their coalescence.

While these data demonstrate the importance of cadherin codes in hindbrain motor nucleogenesis, many questions still remain. First, what is the impact of cranial motor neuron mixing on their peripheral projections? A variety of manipulations in the spinal cord have suggested that motor neuron positions can be uncoupled from their axonal trajectories [17,18], and it will be important to determine whether this principle also holds true in the hindbrain. Second, what are the consequences of mispositioning on cranial motor neuron activities? Additional work in the spinal cord has suggested that one of the main purposes of motor neuron position is to ensure that appropriate sensory and central connectivity is achieved through spatial convergence of both presynaptic inputs and postsynaptic targets [19], and it seems likely that this organizational strategy should also apply to cranial motor neurons. Third, how is the cadherin code established? Presumably this code must be under transcriptional control, and it should thus be possible in future studies to begin parsing how the presence of particular transcription factors expressed by different cranial motor neuron groups contributes to cadherin selection. Finally, what are the underlying mechanisms that enable neurons to translate the cadherin code? Astick *et al.* [16] suggest that the different cadherins used by cranial motor neurons act through a common downstream partner, γ-catenin [16]. Does the identity or 'differential adhesivity' drive activation of γ-catenin to different levels? And how does this activity in turn influence the clustering and migratory behaviors of the neurons?

In summary, the study by Astick *et al.* [16] provides important mechanistic insights into an evolutionarily conserved mode of organizing functionally identical neurons. Type II cadherins are part of a large and diverse family, providing sufficient molecular diversity to direct the formation of many other neural nuclei, such as the varied nuclei of the limbic system, cerebral cortex and other structures [20]. The lessons learned from studying the role of cadherins in hindbrain motor neurons may thus have broad significance for understanding the basis of cellular

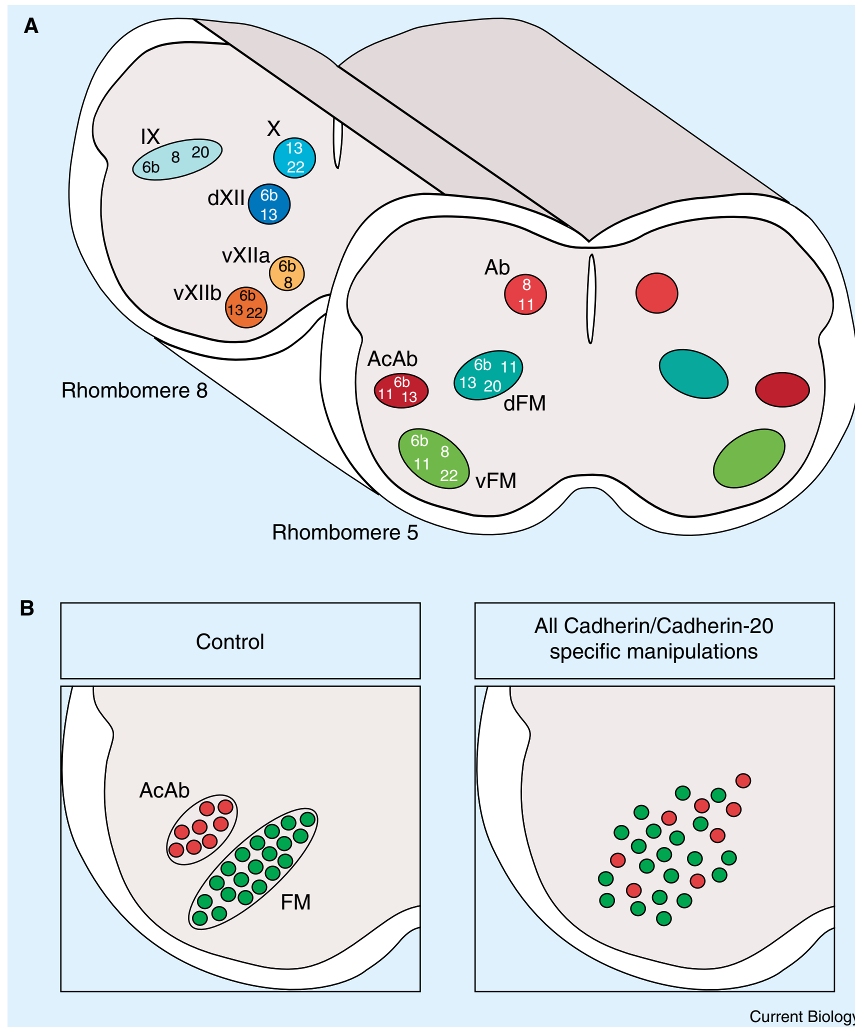

Figure 1. Expression of cadherins in cranial nuclei in the developing chick hindbrain at rhombomeres 5 and 8, and impact of cadherin manipulations.

(A) Somatic cranial motor neurons are labeled in red/orange, and branchiomotor/visceromotor neurons are labeled in blue/green. The dorsal facial nucleus (dFM) differs from the accessory abducens (AcAb) nucleus only in its expression of cadherin-20. Ab, abducens; AcAb, accessory abducens; dFM, dorsal facial motor; vFM, ventral facial motor; IX, glossopharyngeal; X, vagal; vXIIa, ventral hypoglossal a; vXIIb, ventral hypoglossal b. (B) At HH stage 29 in control embryos, the FM and AcAb nuclei coalesce to form two discrete populations. After functional manipulations of all cadherins or, more specifically, cadherin-20, FM and AcAb neurons fail to coalesce and remain intermingled.

Most importantly, is the cadherin code itself instructive? To answer this question, Astick *et al.* [16] selected two adjacent nuclei within r5, the dorsal facial (DF) and accessory abducens (AcAb) nuclei with a single difference in their cadherin code: the unique expression of cadherin-20 in the DF nucleus [16]. They then tested whether the addition or removal of cadherin-20 from motor neurons present in the DF and AcAb nuclei would disrupt their ability to resolve into separate groups. Importantly, neither manipulation affected cranial motor neuron generation, differentiation or positioning. However, both the DF and AcAb nuclei failed to coalesce, with their motor neurons remaining intermingled and dispersed (Figure 1B). This activity was specific to cadherin-20, as misexpression of either N-cadherin, which is not present in either AcAb or DF motor neurons, or cadherin-6b, which is common to both AcAb and DF, did not alter nuclei formation. These results together confirm that it is the unequal distribution of cadherins between nuclei, and not simply cadherin

and functional organization throughout the central nervous system.

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Evolution and Development: PINpointing the Origins of Auxin Transport Mechanisms

Specialists and non-specialists alike know that auxin regulates plant development, but the role of auxin transport mechanisms in the context of land plant evolution has been controversial. Two recent studies resolve the controversy by demonstrating that PIN-mediated auxin transport regulates morphogenesis in a moss.

Jane A. Langdale

Auxin (derived from the Greek word ‘auxein’ to grow) was the first plant growth regulator to be discovered. In now classic experiments, Fritz Went placed agar blocks under oat stems and then after a period of time transferred the blocks to the top of newly decapitated stems [1]. The decapitated stems resumed growth because auxin had moved downwards from the first stem into the agar and had then moved from the agar into the second stem. It was nearly 50 years before an active mechanism of polar auxin transport was first suggested [2] and another 24 years before the molecular basis of the transport process was revealed [3]. Key to the polar transport mechanism is the asymmetric location of PIN-FORMED (PIN) efflux carriers on the plasma membrane. PIN transmembrane proteins are mostly located on the basal side of cells and thus contribute to the general trend of moving auxin from the shoot down to the root. Phylogenetic analyses revealed the presence of a family of PIN genes in the flowering plant *Arabidopsis* and showed that representatives of the family can be found in all available land plant genomes [4,5]. Despite this observation, the origin of auxin transport mechanisms and the contribution of those mechanisms to the evolution of land plant form remained obscure, not least because of reports based on pharmacological studies which suggested that polar auxin transport does not occur in the leafy shoots of mosses [6]. Two papers in this issue of *Current Biology* resolve any uncertainty about the origins of PIN function by showing perturbed shoot development in *pin* mutants of the moss *Physcomitrella patens*. Polar auxin transport therefore regulates shoot development in one of the earliest divergent land plant lineages.

Land plants evolved from aquatic green algae ~470 million years ago, with phylogenetic analyses positioning charophytes as the land plant sister group [7]. Charophyte algae exhibit a range of vegetative body plans, ranging from single cells to highly branched multicellular structures, but these are all found in the haploid (gametophyte) generation of the lifecycle [8]. The diploid (sporophyte) generation of the lifecycle is invariant and unicellular. The alternation of haploid gametophyte and diploid sporophyte generations, both of which are multicellular, is a shared feature of all land plant lifecycles. However, the relative dominance of each generation changed as land plants evolved (Figure 1). In the earliest divergent bryophyte grade (liverworts, mosses and hornworts) the dominant generation is the gametophyte. In mosses, leafy shoots are characteristic of the gametophyte generation whereas the sporophyte develops just a single unbranched axis that subtends the spore-containing

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