A Never-Ending Search for the Evolutionary Origin of the Neocortex: Rethinking the Homology Concept

Loreta Medina Antonio Abellán Ester Desfilis

Faculty of Medicine, Institute of Biomedical Research of Lleida (IRBLleida), University of Lleida, Lleida, Spain

Scientific literature on the evolutionary origin of the neocortex is quite abundant and continued interest can be explained by our need to understand the origin of human cognitive abilities. In a recently published article, Dugas-Ford et al. [2012] analyze the origin of the neocortex using gene expression data at the cellular level, which they compare in representative mammals, birds and reptiles. They find molecular support for similarity between neurons in specific neocortical layers of mammals and specific neurons in the pallium of birds and reptiles [including neurons of the dorsal ventricular ridge (DVR)], thus reinforcing the cell-type homology hypothesis initially proposed by Karten [1969; 1997]. Any new contribution to our understanding of the organization and evolution of the pallium is welcome. However, in our opinion, the data of Dugas-Ford et al. [2012] are inconclusive for homology purposes, and the search for the evolutionary origin of the neocortex remains open. We explain our reasoning below, and add some thoughts on the contribution of evo-devo (evolutionary developmental biology) to understanding the mechanisms of evolution and the homology concept.

Genes Chosen for Analyzing Homology

Dugas-Ford and colleagues studied a number of genes that encode proteins related to various functional features of the cells (such as different ion channels or proteins related to cell homeostasis) or late development and functional maturation. However, not all genes play an equal role in morphological evolution and, therefore, not all genes are equally useful for identifying homologies. Current views on evolution, which incorporate evo-devo ideas, point to the relevance of developmental regulatory genes for explaining morphological evolution [Carroll, 2008]. In contrast to structural and house-keeping genes, the genes that regulate early development often encode proteins (transcription factors or morphogens) that play a crucial role in the formation and patterning of specific body structures. The function of these proteins is so important that their coding DNA sequences are under considerable evolutionary constraint and are, therefore, highly conserved across phyla (key functions of these regulatory proteins are often also conserved; see Gehring [2005]). Since their effects are dosage-dependent, there is also a

constraint against duplication of these genes [Carroll, 2008]. On the other hand, the regulation of their expression is more complex than it is for structural/house-keeping genes. Whereas structural/house-keeping genes usually contain only one *cis*-regulatory element (CRE), developmental regulatory genes contain multiple CREs, each driving the expression of the gene in a particular temporal and spatial sequence [Carroll, 2008]. Evolutionary variations in the CREs of developmental regulatory genes and/or variations in the activators or repressors binding the CREs, or in their cofactors, appear to have been a driving force for morphological evolution [Carroll, 2008]. Therefore, focus on the expression of developmental regulatory genes and on the regulation of their expression should provide us with better clues to understanding cortical evolution (or evolution of any other part of the brain or the body) and for the search of homologies.

Cells and Areas Compared

Dugas-Ford and colleagues analyzed the expression of a battery of genes in cells of different layers in the neocortex of mam-

mals (mouse and ferret), and compared them to cells showing similar expression profiles in the dorsal cortex of a reptile (the red-eared turtle) as well as to the hyperpallium and DVR of two avian species (chicken and zebra finch). The genes *Eag2/Kcnh5* (a potassium ion channel) and *Rorb/Nr1f2* (retinoid-related orphan receptor-beta; a transcription factor gene related to circadian rhythms) were used as neocortical layer 4 markers. In the avian and reptilian pallium, they were found in thalamorecipient areas, including the entopallium and L field of avian DVR, the interstitial nucleus of the avian apical hyperpallium (HA) and the turtle dorsal cortex. Six genes were used as markers of neocortical layer 5: the transcription factor genes *Er81/Etv1* (which targets genes that modulate cell proliferation and differentiation in different tissues and is involved in maturation and synaptic function of cerebellar circuitry [Abe et al., 2011]) and *Fezf2/Znf312* (which controls the development of dendrites in layer 5 pyramidal neurons, and is possibly involved in fasciculation of their projections), the ion channel gene *Cacna1h*, the sulfatase gene *Sulf2*, the gene *Pcp4* (encoding a calmodulin-binding protein that promotes neuronal or neuroendocrine cell differentiation and neurotransmitter release [Harashima et al., 2011]) and one gene of uncertain function, *Tmem200a/Kiaa1913*. In the avian and reptilian pallium, most of these genes were expressed in neurons with long descending projections, including neurons in the avian arcopallium and HA, and the reptilian dorsal cortex.

Although the authors use these data to defend homology between layer-specific neocortical neurons and specific neuron groups in the avian and reptilian pallium, some of the genes employed in this study are not specific to particular neocortical layers, as they are also expressed in other parts of the pallium, including the hippocampal formation (*Eag2*, *Rorb*, *ER81*, *Fezf2*, *Cacna1h*, *Sulf2*, *Pcp4* and *Tmem200a*), the pallial amygdala (*Eag2*, *Rorb*, *ER81*, *Fezf2*, *Cacna1h* and *Pcp4*) and/or the olfactory bulb (*Eag2*, *Rorb*, *Er81*, *Cacna1h*, *Sulf2* and *Pcp4*) (see also [Saganich et al., 2001] and the Allen Brain Atlas). Therefore, these genes are only general markers of thalamorecipient pallial neurons (including both lemnothalamorecipient and collothalamorecipient neurons; [Butler, 1994a, b]), or descending projection pallial neurons (assuming that they can be shown to have these specific features in all pallial areas).

In addition, it is worth noting that some of the studied genes are also expressed in parts of the subpallium (and in other parts of the brain; see the Allen Brain Atlas) and can therefore not be used to distinguish pallial from subpallial neurons, either individually or in combination. This is likely due to the fact that the genes employed by Dugas-Ford et al. [2012] are not involved in early developmental events, but are rather related to the late maturation and/or functional activity of the neurons.

Homology of Genes, Cell Types or Brain Regions: Are They Complementary?

Karten's original proposal of homology between layer-specific neocortical neurons and specific neuron groups in the avian and reptilian pallium was based on the similarity of connections. In contrast, Dugas-Ford et al. [2012] use molecular data to draw conclusions about such cell-type homology. However, as noted above, the presented molecular data support only a more general homology between thalamorecipient pallial cells or between descending projection pallial neurons of different amniotes.

How useful are claims about cell-type homologies, and how do they relate to the homology of gene networks and brain regions? To deal with such questions, some comparative neurobiologists have invoked the hierarchical nature of biological organization, proposing that genes, developmental processes, morphological structures, physiological function and behaviors all constitute different levels of organization and that homology at any of the levels does not necessarily imply homology at the other levels [Striedter and Northcutt, 1991; Striedter, 1999]. In this sense, homology at the cellular level can be useful as a tool to identify comparable cell types in different species, such as thalamorecipient cells of the pallium. If proven that the genetic networks involved in the production of these cells are the same, it could be a case of *deep homology* as proposed by Shubin et al. [2009] for photoreceptors, which would not necessarily imply homology of the pallial subdivisions in which those cells are found.

However, the nervous system of vertebrates contains millions or billions of neurons (depending on the species) and each neuron is unique, as unique is its specific set of connections. In such a system, it is ex-

tremely difficult to define specific neuron types if we do not refer to regions or fields. For example, it is useful to distinguish the motor neurons born in the midbrain (oculomotor) from those in rhombomere 2 (trigeminal) or those in specific cervical spinal segments (as noted below, the specific embryonic origin within the neural tube will determine the distinct muscles each motor neuron group innervates). Similarly, in the pallium, it would be useful to distinguish between thalamorecipient cells in the neocortex and those found in other parts of the pallium (e.g. the amygdala). Moreover, it would make little sense to compare neocortical neurons to cerebellar cortical neurons or to thalamic neurons, even if they express similar sets of genes (e.g. *ER81* and *Fezf2* are expressed in neocortical layer 5 and in the cerebellar cortex; Allen Brain Atlas). Therefore, to get meaningful definitions of specific neuron types, such as pallial neurons versus subpallial neurons or subtypes within these major groups, we find it necessary to refer to the location of these neurons in the nervous system.

The most useful approach for defining the basic divisions of the nervous system and comparing them across species is to analyze the expression patterns of multiple genes involved in embryonic brain development within the context of a topological structural framework [Puelles et al., 2000; Puelles and Medina, 2002; Puelles and Ferran, 2012]. The combination of regulatory proteins expressed in each progenitor zone controls the features of its adult derivatives, including neurotransmitter(s), neuropeptide(s), membrane receptors, ion channels and adhesion molecules as well as connections. Therefore, a cell's embryonic origin determines much of its adult identity [Puelles and Medina, 2002; Medina, 2007].

In the case of the vertebrate pallium, at least four subdivisions have been observed in the embryonic brain of different amniotes: medial, dorsal, lateral and ventral pallia [Puelles et al., 2000; Abellán et al., 2009; Medina and Abellán, 2009; Medina et al., 2011]. These embryonic pallial subdivisions have similar topological positions and express similar combinations of developmental regulatory genes. Based on these features, these pallial subdivisions have been proposed to be field-homologous across amniotes. For example, the embryonic medial pallium is located dorsomedially and expresses the transcription factors *Emx1*, *Lhx2*, *Lhx9*, *Pax6* and *Tbr1* in mouse, chicken and some reptiles, such as

turtles or lizards [Fernandez et al., 1998; Puelles et al., 2000; Abellán et al., 2009; Desfilis et al., 2012]. The embryonic dorsal pallium in mouse and chicken and its putative corresponding area in reptiles is located in a dorsal position within the pallium, and is characterized by expression of the transcription factors *Emx1*, *Lhx2*, *Pax6* and *Tbr1*, but a lack of *Lhx9* [Fernandez et al., 1998; Puelles et al., 2000; Abellán et al., 2009; Desfilis et al., 2012]. In contrast, the lateroventral pallium (lateral plus ventral subdivisions) of mouse, chicken and reptiles is located more laterally, abuts the boundary with the striatum ventrally, contains the lateral olfactory tract at its surface [Striedter, 1997] and expresses *Lhx2*, *Pax6*, *Tbr1*, *Lmo3* and *Lhx9* [Puelles et al., 2000; Abellán et al., 2009; Desfilis et al., 2012] (data on *Lmo3* only known in mouse and chicken). In particular, in different amniotes, the embryonic ventral pallium lacks ventricular expression of *Emx1* [Fernandez et al., 1998; Puelles et al., 2000], but expresses *Lhx9* in the ventricular or subventricular zone and in the mantle (later in development, *Lhx9* expression becomes restricted to rostral, bulbar and caudal amygdalar levels of the ventral pallium) [Abellán et al., 2009; Desfilis et al., 2012]. In the mouse, the embryonic ventral pallium is additionally characterized by the expression of *Dbx1* [Yun et al., 2001; Medina et al., 2004], but data on *Dbx1* in nonmammals are incomplete and inconclusive [Bille et al., 2005].

In the mouse, mutations in developmental regulatory genes that are expressed strongly in the dorsomedial pallium (such as *Lhx2*) have dramatic effects on the development of this pallial territory (and its derivatives, the hippocampal formation and neocortex), but the lateroventral pallium (and the piriform lobe that derives from it) remains fairly unchanged based on gene expression patterns (e.g. spared expression of *Lmo3* [Vyas et al., 2003]). This type of evidence supports the notion that dorso-medial and lateroventral pallial divisions are truly different progenitor zones and produce different structures.

As the mammalian neocortex derives from the dorsal pallium, while the DVR of reptiles and birds derives from the lateroventral pallium, these data disfavor homology of the neocortex and DVR. On the contrary, such data would favor homology between the claustroramygdaloid region in mammals and the DVR in birds and reptiles, since both derive from the lateroventral

pallium [Striedter, 1997; Puelles, 2001; Medina et al., 2011].

However, as noted by Striedter and Northcutt [1991] (also [Striedter 1999]), embryonic homology does not necessarily imply homology of all adult derivatives. So, the dorsal pallium produces the neocortex in mammals and the Wulst (hyperpallium) in birds [Striedter, 1997; Puelles, 2001], but can we safely say that the neocortex is homologous to the Wulst? Similarly, the lateroventral pallium produces the claustroramygdaloid region in mammals and the DVR in reptiles and birds, but does this imply one-to-one homology between their adult derivatives? Homologous embryonic domains have clearly undergone divergent evolution in different lineages. Some of their adult derivatives are easily homologized across species, whereas others are much less clear. For example, the mammalian neocortex, the avian Wulst and the reptilian dorsal cortex (which apparently includes a sector derived from the dorsal pallium) seem to contain a homologous primary visual area receiving input from the thalamic dorsal geniculate nucleus, which was likely present in the amniotes' common ancestor [Butler, 1994a, b; Medina and Reiner, 2000]. However, some cell types in this area, such as those involved in corticocortical (or pallio-pallial) connections, may have evolved independently in mammals and birds [Reiner, 1991]. A pending issue is that, for now, we lack a set of developmental regulatory genes that clearly distinguishes the embryonic dorsal pallium from the adjacent pallia (many of the regulatory genes that characterize the dorsal pallium are also expressed in the adjacent medial pallium and/or the lateral pallium [Medina and Abellán, 2009]). Therefore, a clear definition of a true dorsal pallium in reptiles is still missing, which is essential for deciphering the evolutionary origin of the neocortex.

Furthermore, tangential migrations of cells between domains during development can complicate the analysis of homology between adult structures of different animals. For example, although the mammalian neocortex originates from the dorsal pallium, it includes neuron subpopulations derived from the subpallium (i.e. the GABAergic interneurons [Marín and Rubenstein, 2001]). This tangential migration of GABAergic interneurons from the subpallium to the pallium has been found in different vertebrates, even lampreys [Medina and Abellán, 2009]. In addition, the neocortex also contains a transient sub-

population of glutamatergic cells derived from the ventral pallium, which dies postnatally [Puelles, 2011]. These cells include transient Cajal-Retzius cells, but also other cells that show a dispersed location across layers (i.e. they do not occupy any specific cortical layer); this has only been described in mammals [Puelles, 2011].

Another complication in the effort for analyzing the homology of the adult structures and their cellular components is that derivatives of different, nonhomologous embryonic domains may have acquired similar features, to perform similar functions, by convergent evolution. This may have involved convergence at the level of function-related genes. For example, the independent evolution of infrared vision in rattlesnakes, pythons and boas involves the convergent evolution of similar function-related genes [Yokoyama et al., 2011]. Therefore, the neurons and neuronal circuits involved in complex behaviors may well have evolved independently in different vertebrates. This may be the case for some neural circuits of the songbird DVR and the mammalian neocortex, both subserving some similar cognitive functions, such as vocal learning and communication [Jarvis et al., 2005] or perception of human faces [Marzluff et al., 2012]. It is important to state that nonhomology does not diminish, but, on the contrary, increases the interest in their study for trying to understand, for example, which are the neural requirements for a certain function. Moreover, it is marvellous to see and to try to understand how evolution can find independent solutions for a similar functional achievement.

In conclusion, the analysis of homologies at different levels (genetic, cellular or regional) is useful and complementary for trying to decipher evolution of brain complexity. However, for acquiring deeper conclusions on specific homologies of adult structures, or their cellular components, it is extremely important to refer to their embryonic origin and the genetic regulatory networks involved in their early development, as these are usually highly conserved in evolution and help to reveal the morphotypic organization of different brains. Moreover, it is also very important to identify the developmental changes that are responsible for evolutionary variation and divergence, or the developmental mechanisms responsible for evolutionary convergence. For both of these, more comparative data are necessary.

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