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ARCHITECT GENES OF THE BRAIN

A LOOK AT BRAIN EVOLUTION THROUGH GENOARCHITECTURE

José Luis Ferran

The brain of modern humans is the result of the evolution of a building plan (*Bauplan*) that began its design 500 hundred millions years ago. The process began in basal chordates (sea animals that were living immersed in the sand) and gave rise to the first building plan of the central nervous system; this was progressively modified and shared by all vertebrates. Behind the story are gene networks, key actors in the process to give identity to the different brain regions. This evolutionary scenario provides the basis for studies that seek to understand what is «conserved» and what is «new» between different vertebrates, as well as the underlying mechanisms involved in this process. This article explores the role of genoarchitectonic studies in this human scientific endeavor.

Keywords: brain evolution, gene expression patterns, brain regionalization, gene networks.

IS THE HUMAN BRAIN DIFFERENT TO THAT OF A SHARK, CHICKEN OR CHIMPANZEE?

All vertebrates have a common evolutionary origin, which means that they have a common ancestor. One of the main steps on the long road to produce the brain of extant vertebrates was to obtain a basic building plan to be used in the construction of the brain. One way to understand the meaning of this construction plan is to compare it with the plans for a house. Since the beginning of the cultural evolution of mankind, the house model has changed from a single multi-purpose compartment to the current model that has different «parts» such as the kitchen, bedroom, bathroom and living room. These «parts» were added to serve the needs of each era; for instance, a parking in later stages. However, all houses have the same basic spaces. Something similar applies to the evolution of the vertebrate brain. The basal chordates 500 million years ago gave rise to a relatively simple central nervous system with few compartments; this simple model was modified when vertebrates emerged, leading to the development of a greater number of regions.

Several experimental studies have shown that the brain of all vertebrates is based on a general plan that is established early during development. The first thing that occurs during the formation of the central nervous system is the generation of a neural tube from a sheet of cells known as neural plate (this process occurs in humans during the third and fourth week of embryonic development). From this time, the plate and the tube begin to be «regionalized» both in its anteroposterior dimension and around the circumference of the tube (known as dorsoventral dimension) (Nieuwenhuys, Voogd, & Van Huijzen, 2008). Thus, from rostral to caudal, the different regions of the central nervous system will be generated. The most rostral part of the tube produces the forebrain and includes regions such as the hypothalamus and the telencephalon (the telencephalon produces the cerebral hemispheres, which, among other derivatives, give rise to the mammalian cerebral cortex), followed caudally by the diencephalon (which produces prethalamus, thalamus and pretectum); caudal to the forebrain, we find the midbrain, the hindbrain (giving rise to the cerebellum, among other structures), and the spinal cord (Figures 1 and 2).

> «HOW DO WE KNOW THAT AN ANATOMICAL STRUCTURE IS DIFFERENT FROM ANOTHER? THIS SEEMS SIMPLE, BUT IS NOT EASY TO ANSWER»

Today we know that these regions contain smaller anteroposterior compartments that serve as building blocks, known as neuromeres (e.g., the forebrain

includes five neuromeres: two rostral ones, producing different parts of the hypothalamus and telencephalon, and three caudal ones containing different parts of the diencephalon; Figure 1). These neuromeres are developing units that are part of the construction plan shared by all vertebrates. Each neuromere is further subdivided into smaller units along the dorsoventral axis. The details about how the vertebrate brain regionalization is achieved are accurately addressed in the prosomeric model proposed by Puelles and Rubenstein (2003, 2015). According to this model, these main anteroposterior and dorsoventral compartments are present in all vertebrates and are established during early embryonic development. This means that we will find the same general compartments (such as the neuromeres and their dorsoventral subdivisions) in a crocodile, a duck, an elephant or a human brain.

However, if the compartments are the same, what are the differences between these brains? The main difference that we can find (and this is still undergoing experimental study) concerns to the derivatives that each compartment can give rise to. One of the most interesting examples corresponds to the cerebral cortex of humans or rodents, which shows a considerable expansion (Figure 2). This cortex, which is part of the telencephalon, is a region greatly expanded in many mammals, yet it appears very different in non-mammals and it is currently being discussed what the comparable (homologue) region is in birds and reptiles. As a consequence, a particular brain compartment of birds may produce a structure that differs in size and morphology from that derived from the same compartment in mammals. Moreover, comparing the cerebral cortex between rodents, chimpanzees and humans stresses that the latter has undergone the biggest expansion.

Thus, while all vertebrates have the same plan and share the same general compartments, each species is able to change their size and may even produce «new» derivatives. Returning to the example of the basic parts of a house that allows to convey the idea abstractly: all homes have a kitchen but it is not the same in all households, because not all families have the same needs; the same applies to the brain when compared among different vertebrates. The same

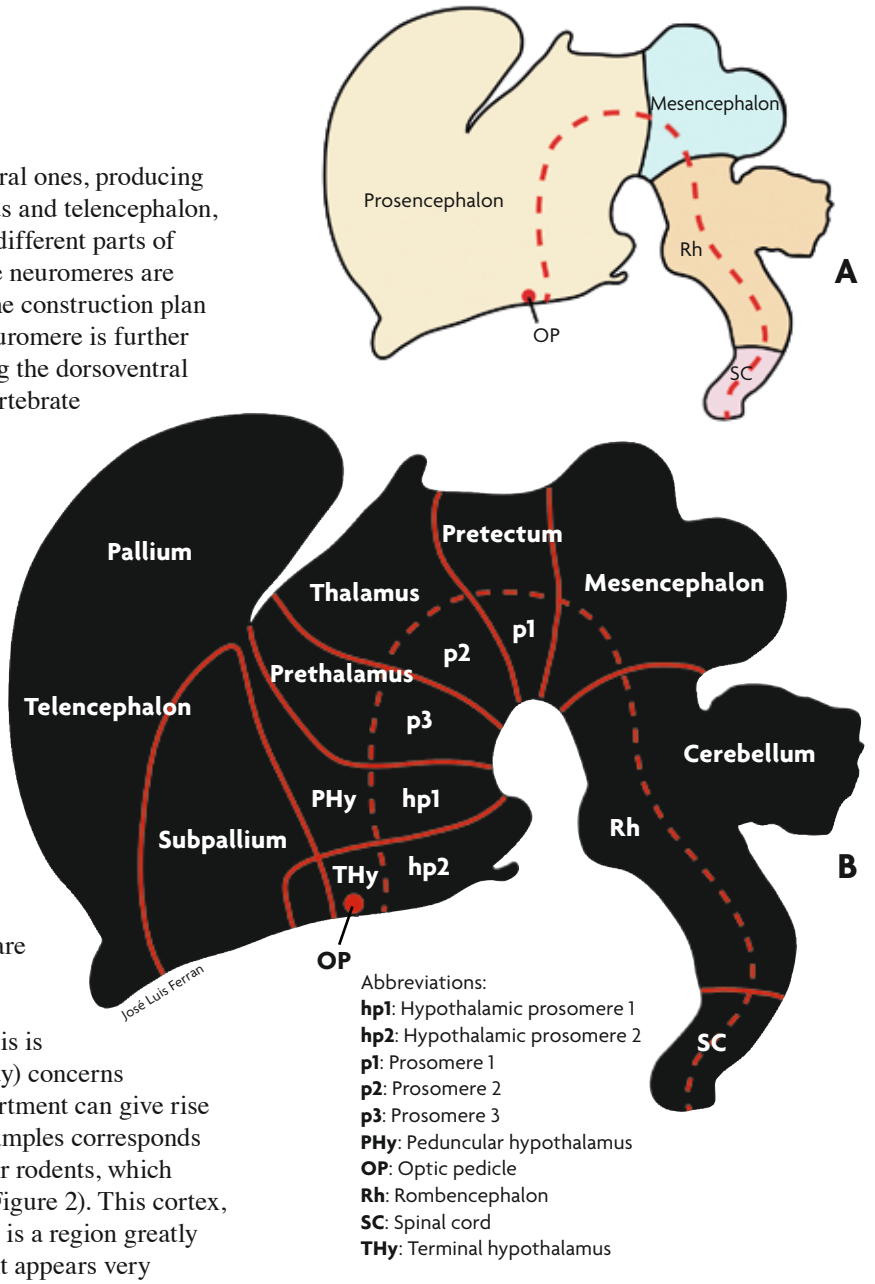

Figure 1. Diagrams of a side view of the central nervous system in which some of the major regions shared by all vertebrates (common *Bauplan*) are indicated. **A)** During regionalization of the neural tube, the prosencephalic (or forebrain – secondary prosencephalon plus diencephalon), mesencephalic (or midbrain), rombencephalic (or hindbrain) and spinal cord regions are generated from rostral to caudal levels, respectively. **B)** In the most rostral region of the central nervous the secondary prosencephalon is located, formed by two prosomeres (hp1 and hp2) which give rise to the hypothalamus; at dorsal levels these prosomeres produce the telencephalon. Caudally, the secondary prosencephalon continues with the diencephalon that contains the prosomeres 1 (pretectum), 2 (thalamus) and 3 (prethalamus). This is followed by the mesencephalic region (with two neuromeres not shown here), the rombencephalic region (eleven neuromeres not shown) and the spinal cord. Due to differential growth in different regions, the axis of the neural tube is curved (the dotted red line separates dorsal and ventral parts of the brain and serves as a guide to see the anteroposterior axis orientation). Note that most of the telencephalic derivatives (pallial and subpallial regions) correspond to the dorsal extension of the first or peduncular hypothalamic prosomere (hp1).

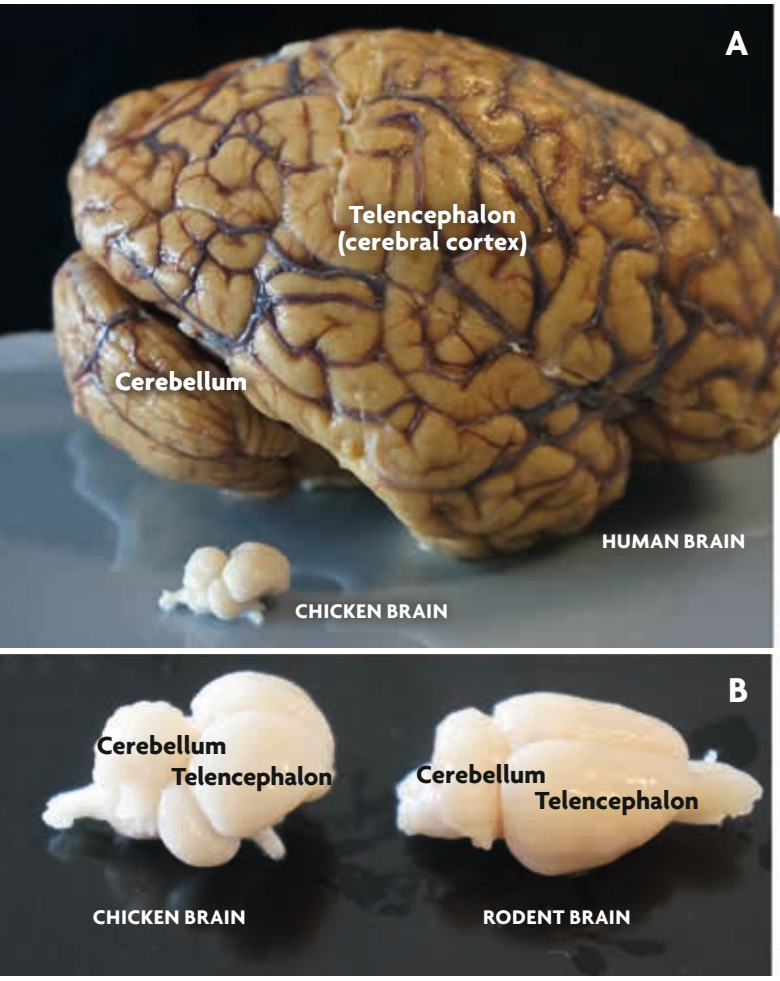

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Figure 2. Central nervous system of mammals and birds. **A)** Side view of a human brain compared to a chicken brain, highlighting the size of the cerebral cortex of the human one. **B)** View of a chicken brain after one month of postnatal life, and from a rodent (rat) after two and a half months of postnatal life (young adult). In the rodent, the cortex is also expanded, but unlike the human cortex, it is devoid of folds. However, in the chicken the telencephalon corresponds largely to structures different from the cerebral cortex (currently, what is the homologous region to the cerebral cortex in birds is still debated). In all cases the cerebellum (a part of the hindbrain) is observed.

«NEURAL GENOARCHITECTURE REFERS TO THE DESCRIPTION OF THE NEURAL STRUCTURE IN TERMS OF GENE EXPRESSION PATTERNS, AND INVOLVES THE USE OF MRNA PROBES AS MORPHOLOGICAL MARKERS»

partitions are present in all brains, but they can change in size, number or type of derivatives, and even in the location of these derivatives (if there are changes in the migration of neurons produced in each compartment during development). The question we should be asking at this point is: how do scientists reach these findings? Or what are some of the strategies to achieve this conclusion? This is the point where genes start to take a leading role, and by studying them scientists act as detectives trying to understand what might have happened during the evolution of the brain.

WHAT IS THE ROLE OF GENES IN BRAIN DEVELOPMENT?

When genes become activated, they start to synthesize RNA molecules (a process known as transcription), which are then used to produce a protein (this mechanism is known as translation). Proteins are molecules that have different functions, such as those related to the maintenance of the cell (housekeeping genes), specification of the cell identity during development, increase in cell number (proliferation), changes in adhesiveness, etc. Gene activity is controlled by DNA regions whose function is to regulate gene expression. These regulatory regions will determine when and where a gene is expressed, by activating or repressing promoter regions that initiate transcription. The primary function of some genes is to regulate the expression of other genes; they encode protein products known as transcription factors, which transport into the nucleus and interact with regulatory regions that control the expression of other genes. For a gene to be activated, the action of a group of protein products from other genes is usually required; in some cases, its own activation can also lead to activating or repressing the expression of other genes. This mode of operation, presented here in a simplified way, wherein several gene products may interact determining the expression of other genes, underlies how a gene network works (Davidson, 2006; Puelles & Ferran, 2012).

To summarize, we could say that, during brain development, the neural tube is formed initially by dividing into compartments, which acquire their own identity due to the active participation of the gene networks that are active in differential patterns in space (giving rise to different regions) and time (at each stage of development). The anatomical derivatives that progressively arise from each compartment are closely related with the effects of these gene networks. However, the number of genes that humans possess is not infinite, but rather around 20,000 genes. Most of them are used in the brain, either in its construction

or functioning. Having in mind the great diversity of processes of regionalization and specification from the beginning of the development of the organism, and the wide variety of cell types that each organ possesses, this number of genes is too low to associate a gene to a function. A gene product is often used both in development and in postnatal life in multiple events, and the context in which they are expressed (i.e., other genes that interact with this gene and the previous molecular history of the region) is what will determine its ultimate effect on the organ. The key elements in this process are the networks of gene expression.

One of the key events in the evolution of vertebrates (and specifically their central nervous system) was a double round of whole genome duplication that occurred in the ancestors of vertebrates. Susumu Ohno (1970) proposed the so-called theory of double genomic duplication (2R hypothesis) as a key event in the origin of vertebrates. This proposal has been reinforced and is now virtually assumed as valid, after confirmation derived from the analysis of complete genomic sequences from different vertebrates and the *Amphioxus*, an extant basal chordate (Putman et al., 2008). As a result of these duplications in the initial ancestor who had one copy of each of the genes, vertebrates could potentially have four copies. This redundancy of information allowed not only that initial functions were preserved, but also gave the possibility to add new or separated functions to the «new» genes. While many genes that resulted from these duplications of entire genomes have been lost as a result of the evolutionary process, the current genomes of birds and mammals have more than one copy for many of its genes. Some teleost fish and amphibian species of the genus *Xenopus* have additional rounds of genomic duplications. The double genomic duplication was not the only way to increase the number of genes during these 500 million years; but in many cases the genes were also individually duplicated and aligned one after another. The increased number of genes is paralleled to the increase in complexity of organisms, and in the case of the central nervous system we can find a more complex building plan in vertebrates than that observed in basal chordates.

Genes have functions; work in networks and in specific anatomical locations. If the building plan of the central nervous system is preserved or shared between vertebrates, very similar gene networks operating in the same region of the brain could be found in different vertebrates; but we must also assume that, in some cases and at a particular location, these networks could have changed significantly. As

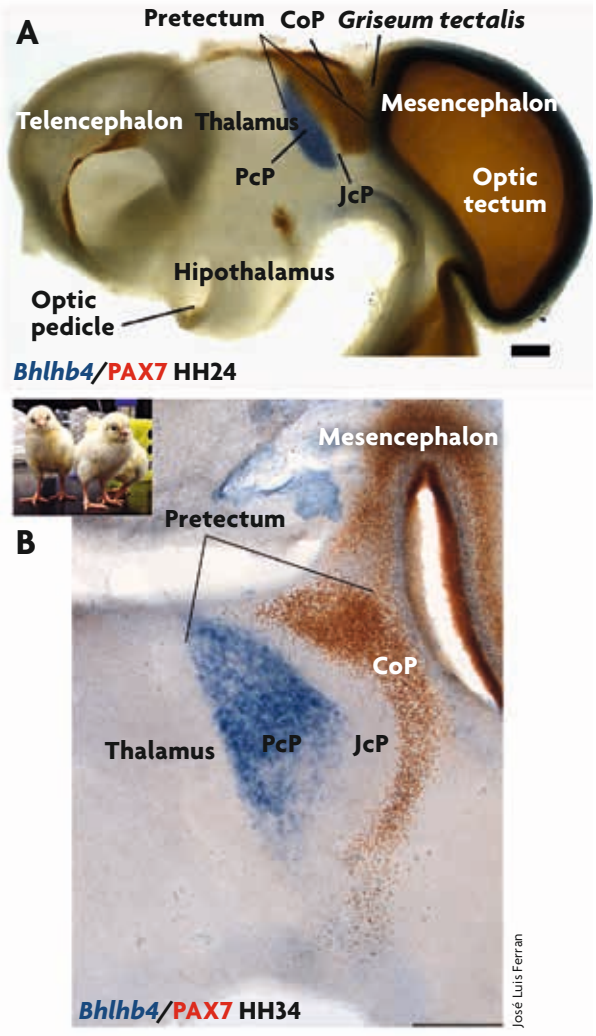

Figure 3. Brain genoarchitecture in birds. **A)** Side view of a chicken brain of four days of development (HH24). In the example, the analyzed region (pretectum) is indicated, showing two different expression patterns that can help to define distinct domains (*Bhlhb4* mRNA, detected by *in situ* hybridization; and PAX7 protein, detected by immunohistochemistry). **B)** Parasagittal section from a chicken embryo after eight days of development (stage HH34). The analysis of these expression patterns (messenger and protein) throughout development emphasizes that both genes are expressed in the same, non-overlapping regions of the brain since previous stages. Three anteroposterior subdivisions or expression domains are visualized in the pretectal region (precommissural or PcP; juxtacommissural or JcP; and commissural or CoP) through genoarchitectonic exploration, and they are evident at all stages analyzed.

«GENOARCHITECTURE IS A POWERFUL TOOL THAT HELPS TO CHARACTERIZE REGIONS OF THE NERVOUS SYSTEM FROM VERY EARLY STAGES»

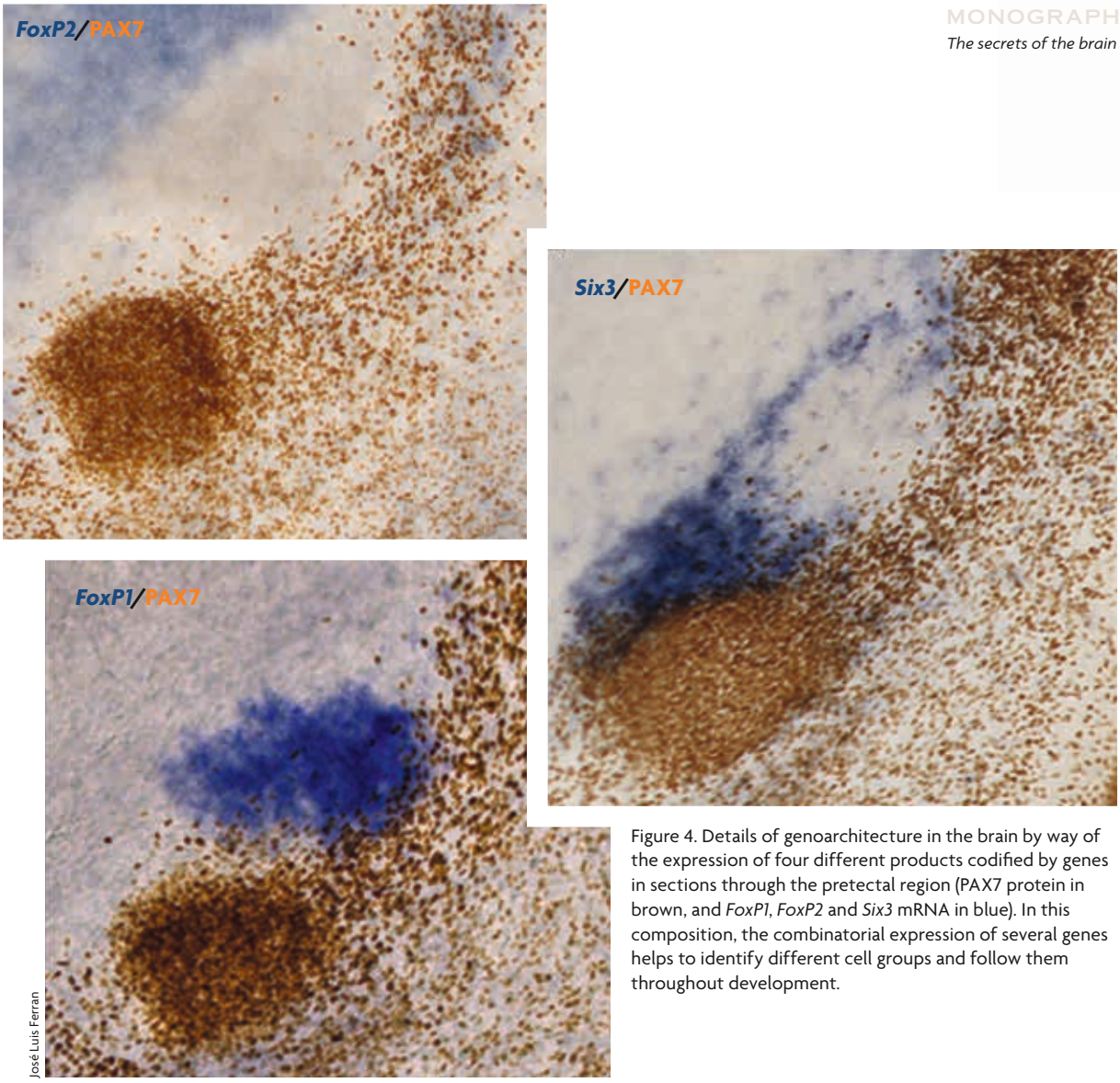

Figure 4. Details of genoarchitecture in the brain by way of the expression of four different products codified by genes in sections through the pretectal region (PAX7 protein in brown, and *FoxP1*, *FoxP2* and *Six3* mRNA in blue). In this composition, the combinatorial expression of several genes helps to identify different cell groups and follow them throughout development.

discussed in the next section, the gene products that are active during early development and are involved in the specification of the identity of a region or in the specific identity of its derivatives are useful tools for comparative studies since they can help to recognize components that were originated from the same region in different species.

GENOARCHITECTONIC STUDIES ALLOW TO UNDERSTAND THE EVOLUTION OF THE BRAIN

How do we know that an anatomical structure is different from another? This seems simple, but is not easy to answer. If we want to know how to recognize an anatomical structure in relation to another, we need powerful tools to indicate the extent of a territory clearly (its boundaries or borders), or the common identity that a group of cells may have. As previously explained, genes are behind the establishment of a building plan of the brain and the generation of its specific derivatives. Therefore, if we can determine which genes are active in each territory, we could characterize the boundaries between them and also define very precisely how many different components were arisen from each region. Genoarchitectonic or neural genoarchitecture studies refer to descriptions of a neural structure in terms of discrete gene expression patterns. We know that the identity of a territory is given by gene networks that are expressed at specific both position and time during development. Therefore, the strategy is to find the messenger RNA (mRNA), which is the transcription product of an active gene, in the area of interest by using *in situ* hybridization (Figure 3). Thus, neural genoarchitecture refers to the description of the neural structure in terms of gene expression patterns, and involves the use of mRNA probes as morphological markers. These genoarchitectonic studies allow a morphological discrimination that is changing modern neuroanatomy (Ferran et al., 2015; Puelles & Ferran, 2012).

We know that genes are expressed during many stages of development and during the postnatal life of an individual, but many times they are also expressed in different anatomical regions. Therefore, the mere presence of a gene product is not enough to determine whether or not the identified territory is comparable between two stages of development from the same species (or between two species of vertebrates). You must also know the position occupied by these gene products within an overall building plan of the central nervous system. Thus, the gene expression domain detected needs to be related to a specific location within the plan of the brain. Genoarchitecture is a powerful tool that helps to characterize regions of the nervous system from very early stages, as well as what emerges from them during later stages and in postnatal life. For example, Figure 3 shows a picture of the central nervous system from chicken during development, in which we can recognize a brain region known as «pretectal region», which is located in the caudal part of the diencephalon. At early stages, when the territory still has few cells, these can be identified by its genoarchitecture, highlighting different subdivisions within this region. If we move forward in development, the cells that derived from these subdivisions seem to maintain the expression of these genes (Figure 3). That is, this tool allows grouping cells with common identities, thus allowing us to have an anatomical characterization of the brain much more elaborated than the one we previously had (Figures 3 and 4). That is, using the genoarchitecture we can observe with fine detail how the anatomical characterization of this area is throughout all embryonic development and during the postnatal life of a species (ontogeny) (Ferran et al., 2009; Ferran, Sánchez-Arrones, Sandoval, & Puelles, 2007).

Moreover, the comparative study of the expression of these genes in different vertebrate species allows the identification of equivalent derivatives of the brain. But at this point it is important to note that it is only possible to compare regions from different species that are placed in the same location within the general plan (same topological position); or cells that were originated from the same site in both species (this is known as «field homology»; the anatomical structures characterized are recognized as field homologues; Puelles & Medina, 2002). In our example, the pretectal region seen in Figure 5 shows that the same active gene is expressed in the same subdivision of this region in both chicken and mouse. This, on the one hand, allows us to recognize the same area in two different species that in many cases are evolutionary very distant from each other; but, on the other hand,

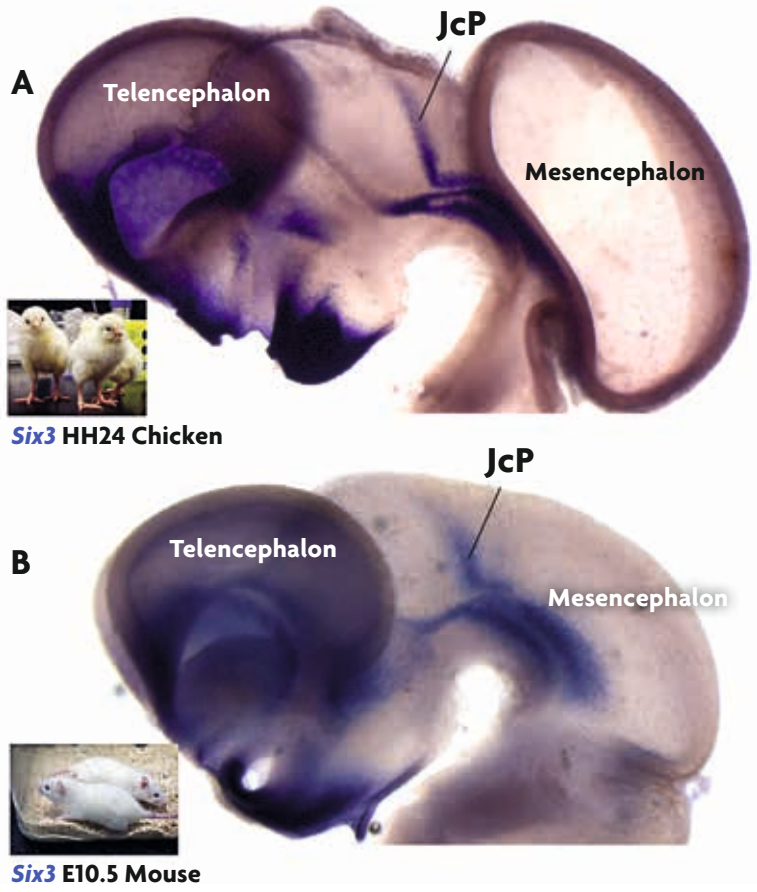

Figure 5. Brain genoarchitecture comparing two vertebrate species. **A)** Side view of a whole mount chicken embryo (4 days of development) in which *Six3* gene expression is observed in different brain regions. A well-defined expression is observed in a particular domain of pretectal region (called juxtacommissural pretectal subdivision or JcP). **B)** Side view of a whole mount mouse embryo (10.5 days of development) showing the mRNA expression of the gene *Six3* at the level of the pretectal region in the same domain as in chicken. Since both expression domains occupy the same position within the overall brain organization or building plan (*Bauplan*), we can say that they are homologous.

this example also reveals that birds and mammals are using the same genes in the construction of that region at early developmental stages (Ferran et al., 2008; Merchán, Bardet, Puelles, & Ferran, 2011; Morona, Ferran, Puelles, & González, 2011).

The most difficult task is to recognize what is homologous when we compare brain regions at advanced developmental stages or from the postnatal life; in this case, the study must rely on other tools to indicate where the origin of the anatomical structure or cell group of interest is. The reason is that to know whether two derivatives are homologous they must have originated in the same compartment and, in some cases, the regions derived from them become very different in distinct species, with different active genes as well; moreover, sometimes cells

migrate a long distance throughout different domains, making their site of origin very difficult to know. Genoarchitecture is currently being used to study all brain regions in different species of vertebrates and provides details on the development and evolution of different neuron groups at levels that were unthinkable decades ago.

CAN WE EXPECT MORE ANATOMICAL LEVELS OF COMPLEXITY?

We have explained that the expression of a gene product, i.e., the messenger RNA, is an extremely useful tool in the anatomical characterization of brain regions and derivatives. However, today we know that the messengers produced by a single gene can be assembled differently, through a process known as «alternative splicing». According to this mechanism, a gene can give rise to mRNA products with differences in the final composition of the mRNA molecule; this would mean that not all products of the same gene would work in the same way when they are transformed into proteins. Regarding our study, this means that two derivatives that are expressing the same gene could be producing molecules that are not exactly identical. In some cases, the characterization of alternative splicing of mRNA has shown an additional level of complexity in the recognition of different anatomical structures.

«AT THE BEGINNING OF DEVELOPMENT THE SIMILARITIES BETWEEN BRAINS ARE SIGNIFICANT, BECAUSE WE START FROM A COMMON PLAN; BUT WHEN DEVELOPMENT PROGRESSES, THERE ARE VARIATIONS IN THE SIZES OF EACH REGION, OR IN THEIR DERIVATIVES»

ARCHITECT GENES OF THE HUMAN BRAIN

The gene products have their fundamental role in brain development and function; but they can also help us to recognize the level of detail in which the human brain resembles that of other vertebrates. At the beginning of development the similarities are significant, because we start from a common plan; but when development progresses, there are variations in the sizes of each region, or in their derivatives, and in many cases evolutionary «novelties» appear. The detailed knowledge of the brain-building plan from other vertebrates allows us to advance both in understanding how the human brain is constructed as well as in understanding the possible changes that our species could face on this planet or in any we might colonize in the near future.

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José Luis Ferran. Associate Professor at the Department of Human Anatomy and Psychobiology of the University of Murcia (Spain). He is a researcher in Murcia Institute for Biosanitary Research (IMIB), where he is the head of the functional genoarchitecture unit of the Research Group in Brain Regionalization and Development Genes. He is the author of many papers in this topic.