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SOX2 en relación con NKX2.2, LHX2 y Calretinina en la Región Talámica de *Xenopus Laevis*

SOX2 in Relation with NKX2.2, LHX2 and Calretinin in the Thalamic Region of *Xenopus Laevis*

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Resumen

La región talámica emerge de la placa alar del prosómero p2. La región prospectiva del tálamo ha sido reconocida, y subdividida en los dominios histogenéticos rostral y caudal, en base a varios marcadores genéticos de desarrollo como Gbx2, Nkx2.2, Ngn2 and Dbx2. Sin embargo, la mayoría de estos genes se apagan después de los primeros estadios de desarrollo y los eventos moleculares que guían la compartimentalización nuclear en el cerebro adulto, aún son desconocidos. En este estudio, proporcionamos una descripción detallada del patrón de expresión de Sox2, que se expresa en células mitóticas y áreas específicas post-mitóticas, tales como el tálamo. Para este fin, se ha llevado a cabo una tinción de inmunofluorescencia para Sox2 y se ha combinado con otros genes de desarrollo como Lhx2 and Nkx2.2, así como con marcadores neuronales tales como la calretinina (CR) para distinguir diferentes poblaciones talámicas positivas para Sox2. Nuestros resultados han sido interpretados dentro del modelo prosomérico actual y con respecto a otros estudios recientes y clásicos de la región talámica en *Xenopus laevis*. Así, nuestros datos corroboran patrones altamente conservados y potenciales de nuevas subdivisiones de los núcleos talámicos en anfibios.

Palabras clave: diencéfalo, regionalización, anfibios anuros, tálamo.

Abstract

The thalamic region emerges from the alar plate of p2 prosomere. The prospective thalamus has been recognized and subdivided into rostral and caudal proliferative domains based on a number of developmental gene markers such as Gbx2, Nkx2.2, Ngn2 and Dbx2. However, most of these genes are down-regulated after early developmental stages and the molecular events that guide the late adult nuclear compartmentalization remains obscure. In this study, we provide a detailed description of the pattern of Sox2, expressed in mitotic cells and specific postmitotic areas, like the thalamus. To this end Sox2 immunohistofluorescence staining was combined with other developmental genes such as Lhx2 and Nkx2.2 and neuronal markers including calretinin (CR) to distinguish different thalamic Sox2 positive populations. We interpret our results within the current synthetic prosomeric model and with regard to other recent and classical studies of the thalamic region in *Xenopus laevis*. Thus, our data support mostly conserved patterns and potentially novel additional subdivisions within the amphibian thalamic nuclei. Grant by: BFU2015-66041 P.

Keywords: diencephalon, regionalization, anuran amphibians, thalamus.

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Introduction

Modern studies analyse the patterns of diverse genes to elucidate a possible common forebrain organization across tetrapods (Bandín, Morona & González, 2015; Dávila, Guirado, & Puelles; Morona & González, 2008). Thus, the transcription factors that regulate of the acquisition of neuronal identity are directly involved in the regionalization of a given brain area and the achievement of the adult nuclear functionality. It also regulates the transcription of downstream genes, as sonic hedgehog (Shh) (Zhang & Wiest, 2016) for thalamic regionalization. Sox2 is largely expressed in the immature neural epithelium and in a small proportion of differentiated neurons in the thalamus, striatum and septum (Ferri et al., 2004). To this end we have compared the expression pattern of Sox2 with other essential thalamic markers, such as Lhx2 and Nkx2.2.

Material and methods

According to the experimental procedures, in the present study adult *X. laevis* were used following laws of the European Union (2010/63/UE) and Spain (Royal Decree 53/2013) and the regulations of the Committee at the University Complutense. Adult *Xenopus* were obtained commercially from licensed suppliers (XenopusOne, Dexter MI). The animals were anesthetized by immersion in a solution of tricaine methanesulfonate (0.3 mg/ml, MS222, Sigma) and perfused with 0.9% NaCl, followed by the fixative MEMFA (0.1M MOPS [4-morpholinepropanesulphonic acid], 2mM EGTA [ethylene glycol tetraacetic acid], 1Mm MgSO4, 3.7% formaldehyde). The brains were dissected out and postfixed approximately 3-4 hours in the same fixative solution at 4ºC. Then, they were cryoprotected in a in a solution of 30% sucrose in PBS for 4-10h. After that, it was blocked in a solution of 20% gelatin with 30% sucrose in PBS, postfixed overnight in 3,7% formaldehyde in a solution of 30% sucrose in PBS and sectioned on a freezing microtome (Thermo Scientific Microm HM 450) at 35 µm in the transverse plane.. In accordance with double immunohistochemistry: 1) First, incubation was conducted for 72 hours at 4ºC in the dilution of each primary serum mouse anti-NKX2.2, mouse anti-CR (1:1,000, Swant), rabbit anti-CR (1:1,000, Swant 7699/3H), mouse antiLhx2 (1:100, DSHB, PCRP-Lhx2-1C11), rabbit anti-Sox2 (1:1000, AbCam ab97959). 2) All secondary antibodies were diluted 1:500 in PB containing 0.5 -1% Triton X-100and incubated for 90 minutes at room temperature: Alexa 594-conjugated goat anti-rabbit (Molecular Probes, Eugene, OR; catalog reference A11037) and Alexa 594-conjugated chicken anti-mouse (OR; catalog reference A-21201). 3) Finally, the sections were coverslipped with Vectashield mounting medium (Vector Laboratories, Burlingame).

Results

The distribution of Sox2 as a specific marker has been analysed in the thalamic region of *X.laevis* through an adult individual. The second diencephalic segment p2, contains the thalamus and was particularly rich in Sox2 positive cells, expressing in all over this prosomere. Overall, Sox 2 expression is very intense along the ventricular cells as well as in migrated cells in the ventral habenular nucleus, anterior rostral (Ar) and anterior caudal thalamic nucleus (Ac). It is also very intense in the dorsal and intermediate portions of the central thalamic nucleus (C). Calretinin immunoreactive (CR) cells showed also a widespread distribution in all thalamic nuclei, except the ventral portion of C and LPv, and was less intense in Ar. In the ventral habenula, Ar, Ac y C both markers (Sox2/CR) are widely coexpressed, whereas in the LPv most caudodorsal cells where single CR positive and a group of rostroventral cells where single Sox2 positive. Interestingly, in the central nucleus both markers revealed a partially segregated pattern, with a central part with double labeled cells (Fig.1 L, M), a dorsal part of single sox2 (Fig.1L) cells and a rostroventral band of single Lhx2 cells (Fig.1M). Lhx2 was absent in the Ac and expression in the intergeniculate leaflet (IGL), whereas was conspicuous in the LPv, where many Sox2/Lhx2 double labeled cells were found. To further characterize the Lhx2 population, double immunohistochemistry for Lhx2 and CR was carried out. The particular distribution of both markers also revealed within the central nucleus a single CR positive dorsal part (Fig.1 H,I), a double labelled central portion (Fig.1 H,I) and a single Lhx2 rostral band (Fig.1 H) .

Discussion

The expression pattern of Sox2 has been studied in mouse and chicken with a detailed description in mouse embryonic period, but studies in anamniote vertebrates are completely lacking. Thus, the main aim of this study is an exhaustive analysis of this transcription factor in the thalamus of the adult *Xenopus laevis*. However, double labelled cells were consistently observed in the La and LPv nuclei. We suggest that those double labelled cells could be immature Nkx2.2 positive cells that conserved Sox2, which is also a proliferative marker. However, cell lineage studies should be carried out to asses this question. Additional single Nkx2.2 positive cells were also observed in the *zona limitants intrathalamic*a (Zli). As Nkx2.2 was detected along the rostroventral band of the thalamus and the rostral shell of the of Zli but not in the Shh positive territory of the Zli itself (Bandín et al., 2015), these cells could correspond to migrated cells, either from the prethalamic region or the shell of the Zli. The coexpression with Sox2 corroborated a likely origin of the LPv, and C nucleus in the c-Th and revealed an heterogeneous population within these nuclei. Finally,

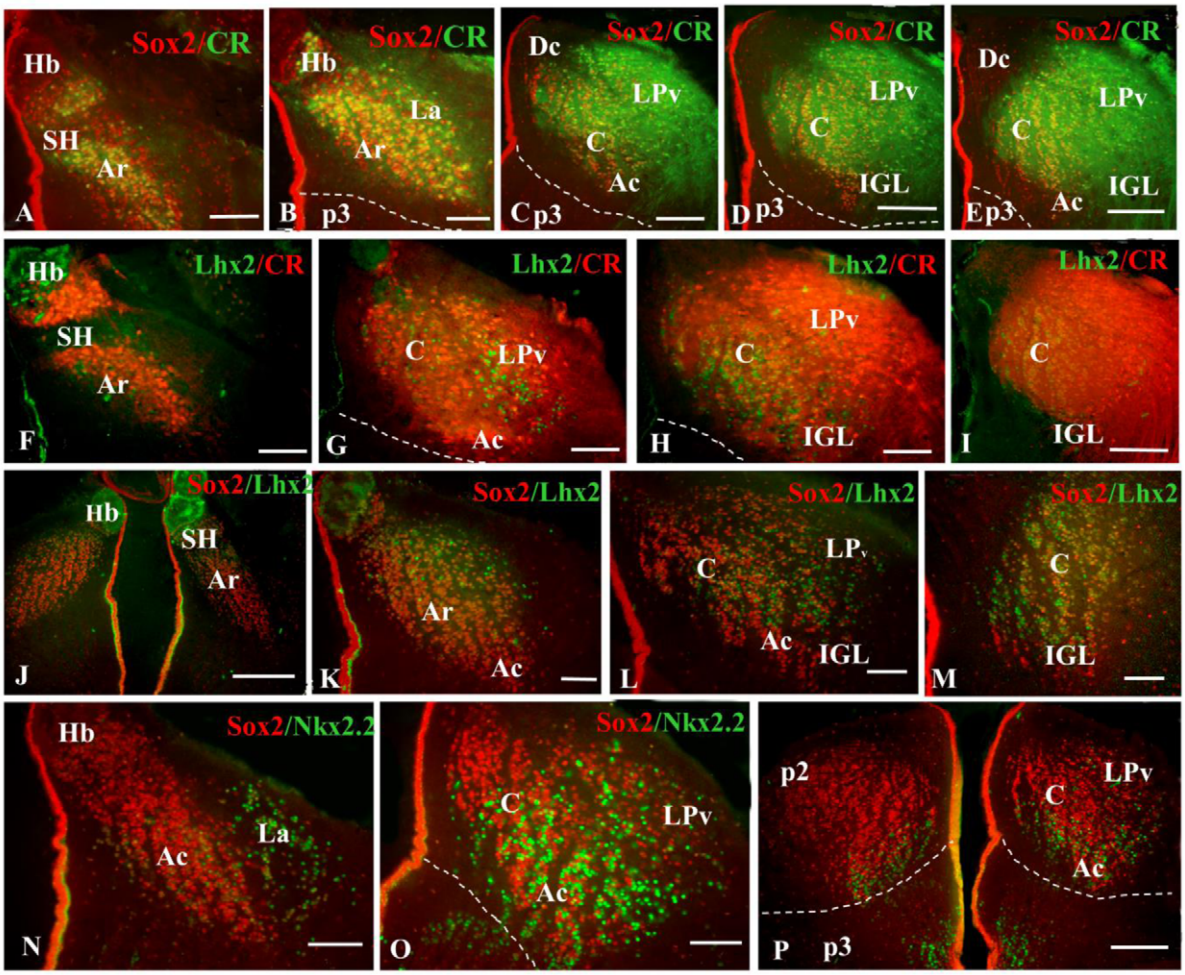

Figure 1. Photomicrographs of serial rostro caudal transverse sections through the thalamus of adult *Xenopus laevis*. The markers are indicated in the corresponding color code in the upper right corner. Scale bars=100 µm in A, B, F-I, L-N; 200µm in C-F,J,O,P.

the thalamic marker CR (Milán & Puelles, 2000; Morona & González, 2008) with Sox2 and Lhx2 corroborated, that Sox2 cells were post mitotically differentiated cells. The comparative pattern of the three markers also showed possible subdivisions within the central nucleus.

Conclusion

Sox2 is a marker of the c-Th derivatives together with CR and Lhx2 in the thalamic region of adult *X. laevis* and seems to be consistent across tetrapods. Positive cells for Nkx2.2 is a good marker for rostral thalamic derivatives as the Ar, Ac and La nucleus. It is also found far from the original histogenetic domain suggesting possible migratory routes across rostral territories. Heterogeneous populations of both histogenetic territories were found in several nuclei, suggesting a non- homogeneous and open concept of the anuran thalamic nuclei.

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