AP2γ: A New Player on Adult Hippocampal Neurogenesis Regulation

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ABSTRACT: Since the recognition that the mammalian brain retains the ability to generate newborn neurons with functional relevance throughout life, the matrix of molecular regulators that govern adult neurogenesis has been the focus of much interest. In a recent study published in *Molecular Psychiatry*, we demonstrate Activating Protein 2γ (AP2γ), a transcription factor previously implicated in cell fate determination in the developing cortex, as a novel player in the regulation of glutamatergic neurogenesis in the adult hippocampus. Using distinct experimental approaches, we showed that AP2γ is specifically present in a subpopulation of transient amplifying progenitors, where it acts as a crucial promoter of proliferation and differentiation of adult-born glutamatergic granule neurons. Strikingly, deficiency of AP2γ in the adult brain compromises the generation of new glutamatergic neurons, with impact on the function of cortico-limbic circuits. Here, we share our view on how AP2γ integrates the transcriptional orchestration of glutamatergic neurogenesis in the adult hippocampus, and consequently, how it emerges as a novel molecular candidate to study the translation of environmental pressures into alterations of brain neuroplasticity in homeostatic, but also in neuropathological contexts.

KEYWORDS: Hippocampal neurogenesis, transcription factors, cortico-limbic circuits

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To understand how structural neuroplasticity is molecularly regulated in the adult central nervous system (CNS) is a demanding, yet crucial endeavor of modern neurosciences, ascribable to two major aims: first, it represents an open window to the mechanisms by which environmental pressures can reprogram the adult brain, leading to particular behavioral responses, both in health and in pathological contexts; second, deciphering this code may pave the way to innovative tools able to revert traumatic, neurodegenerative, and mental disorders driven by our endogenous regenerative potential. Looking back to the last decade, it is striking how many works contributed to shed light into the role of different transcriptional regulators of neurogenesis, as it was recently the case with the activating protein 2γ (AP2γ or AP2gamma).

Also known as Tcfap2c or Tfap2c, AP2γ encodes a transcription factor that, similarly to other members of the AP-2 family, binds to GC-rich consensus sequences and regulates, in different organs, the expression of multiple genes that modulate cell differentiation, proliferation, and apoptosis.[1] AP2γ plays broad and important biological functions during early mammalian development, organogenesis, and also during adulthood. Particularly in the CNS, AP2γ plays a critical role during cortical neuronal development, as part of the transcriptional network that governs glutamatergic neurogenesis, through a direct action on the genes of basal progenitor cells.[2] Interestingly, although the deletion of AP2γ in the developing cortex promotes a reduction of upper layer neurons in the occipital cerebral cortex, its overexpression fosters the generation of the cortical layers II/III.[2] In adulthood, and maybe not surprisingly in the light of its pro-proliferation profile, AP2γ was mostly known for its role in pathological conditions, particularly as a marker of tumor progression and poor prognosis in different malignant diseases, such as breast[3] and lung[4] cancer. Yet, and despite its importance in the developing brain, the presence or relevance of AP2γ in the adult brain was not clarified. Nevertheless, considering that the transcriptional network that controls glutamatergic neurogenesis during neurodevelopment is recapitulated, to some extent, in the adult CNS, the presence of AP2γ in the postnatal brain emerged as a plausible hypothesis.

As such, in a recent study published in *Molecular Psychiatry*,[5] we described for the first time the presence of AP2γ in the adult brain and explored its different roles on adult neurogenesis regulation and how it impacts on different behavioral modalities. After developmental stages, AP2γ persists in the adult CNS, although its presence is restricted to neurogenic niches. In

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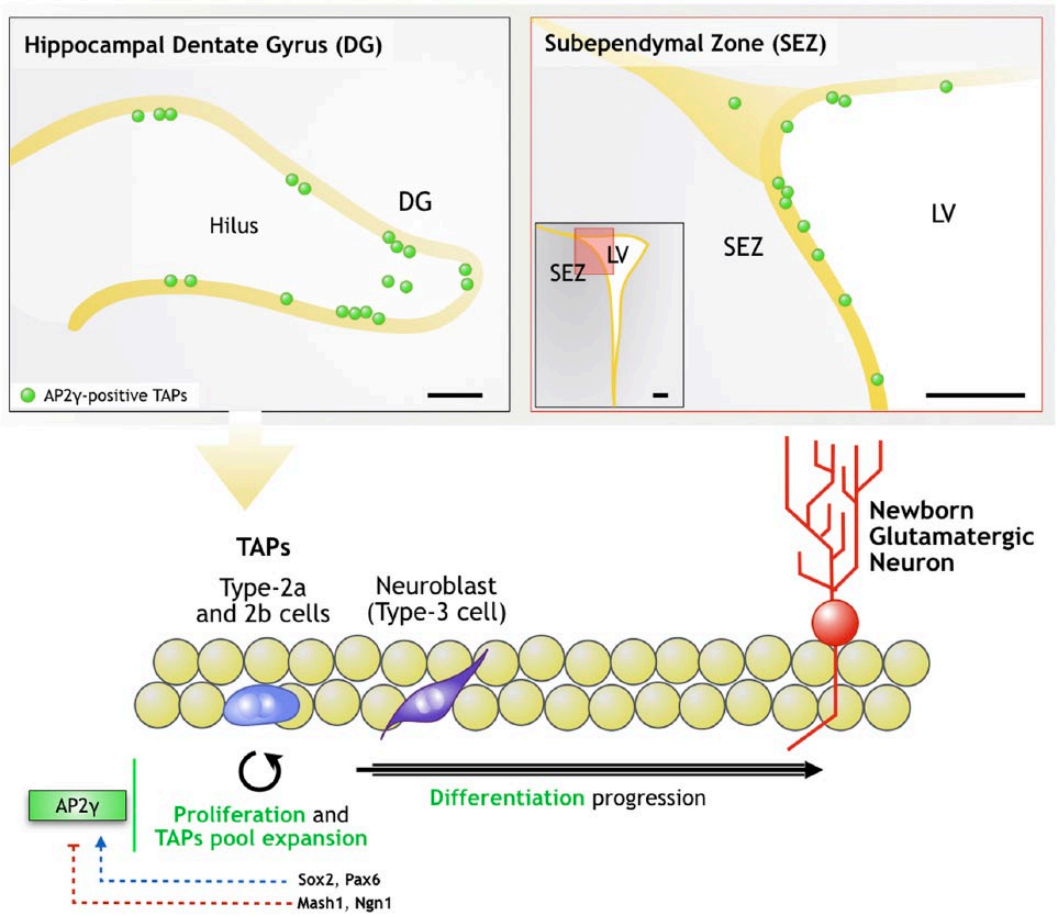

Figure 1. AP2γ in the adult neurogenic niches. AP2γ is present in a subpopulation of transient amplifying progenitors (TAPs) in the adult hippocampal dentate gyrus (DG) as well as in the subependymal zone (SEZ) lining the lateral ventricles (*top panel*). In dentate TAPs, AP2γ expression is promoted by Sox2 and Pax6, but negatively regulated by Mash1 and Ngn1. Through direct activation of glutamatergic neuronal fate determinants Tbr2 and NeuroD1, AP2γ is crucial in the expansion of TAPs pool and progression into subsequent differentiation steps (bottom panel).
DG, dentate gyrus; LV, lateral ventricles; SEZ, subependymal zone; TAP, transient amplifying progenitors.

particular, we identified AP2γ in sparsely defined cell subpopulations of the adult hippocampal dentate gyrus (DG), both in the dorsal and in the ventral hippocampal poles. This transcription factor was present in Tbr2-positive glutamatergic progenitor cells, as well as in doublecortin (DCX)-positive neuroblasts. Although in Mateus-Pinheiro et al, we focused on the histological and functional characterization of AP2γ within the hippocampus, additional results from our lab show that its expression, while restricted in the adult brain, is also extended to subependymal zone Tbr2-progenitors (Figure 1). In vitro assays suggest that AP2γ has the ability to specifically interact with different transcriptional regulators. Indeed, our work revealed that AP2γ levels are the result of the fine balance between positive regulators of its expression, Sox2 and Pax6, and the negative regulators, Ngn1 and Mash1 (known as transcriptional determinants of GABAergic neurogenesis).[6] Based on our results, AP2γ appears to be an important molecular promoter of adult hippocampal neurogenesis, through direct activation of the glutamatergic neuronal fate determinants Tbr2 and NeuroD1. Thus, it is likely that AP2γ displays an important role on the glutamatergic versus GABAergic fate determination *switch* on transient amplifying progenitors (TAPs). Due to this bivalent position in the transcriptional sequence regulating neurogenesis, it would be elucidative to study how different hippocampal-dependent tasks or even environmental factors known to have an impact on neurogenesis (eg, running or cognitive enrichment) affect AP2γ levels and neuronal fate determination in the adult brain. Accordingly, either viral-mediated overexpression or deletion of AP2γ was sufficient to rapidly expand or deplete the pool of hippocampal TAPs, respectively (Figure 1).

Although the functional correlates of adult cytogenesis have been the subject of many studies, it is still a matter of much debate, as many conflicting results coexist in literature.[7,8] By conditionally *knocking-out* (*cKO*) *Ap2γ* in adult mice, we further addressed this topic and explored the behavioral domains affected by loss of neural progenitors under AP2γ transcriptional control. These animals, which displayed a severe depletion of neuroblasts in both hippocampal poles, had marked cognitive deficits in a contextual fear conditioning task, as well as in behavioral flexibility on a water maze task.

More so, impaired ability to differentiate newborn glutamatergic neurons led to the preferential adoption of egocentric navigational strategies in the water maze task, as opposed to spatially oriented strategies normally evidenced by wild-type (wt) animals (Figure 1). The integrity of cognitive tasks altered in *Ap2γ-cKO* mice has been associated with adult hippocampal neurogenesis.[9,10] Although results in Mateus-Pinheiro et al did not allow us to claim that AP2γ-controlled newborn neurons are implicated in the regulation of anxiety-like behavior, they also raise the possibility that this may very well be the case (*please refer to Figure 3 in the work by Mateus-Pinheiro et al*). Indeed, both heterozygous and homozygous *Ap2γ-cKO* mice presented, on average, reduced exploratory behavior in the center of an open-field arena and in the open arms of an elevated plus maze task; however, maybe due to some variability on performance in these tasks, we found no significant group effects on anxiety-like behavior in this work. As AP2γ is only present in a subset of newly formed neuroblasts, it is also possible that the lack of this specific subpopulation is not sufficient to elicit an evident anxious-like phenotype in these tasks. Hence, it is likely that further studies, either in basal conditions or under an external challenge, such as stress exposure, may elucidate the participation of AP2γ in the control of anxiety in the adult brain.

Interestingly, AP2γ regulation of TAPs' population is essential not only for regional intra-hippocampal function but also for interregional communication between the hippocampus and other cortical areas. In particular, our electrophysiological studies showed how AP2γ deficiency leads to a decrease of coherence between the ventral hippocampus and the medial prefrontal cortex (PFC) as a measure of decreased functional interaction between these two brain regions. Importantly, the *hippocampus-to-PFC* link has been associated with complex cognitive behaviors,[11,12] but also implicated in the action of fast-acting antidepressant drugs, such as the much *in vogue* ketamine.[13] These findings further endorse the need to explore how AP2γ may serve as an important molecular mediator of environmental factors and pharmacologic agents into finely tuned alterations in hippocampal physiology and behavioral outputs.

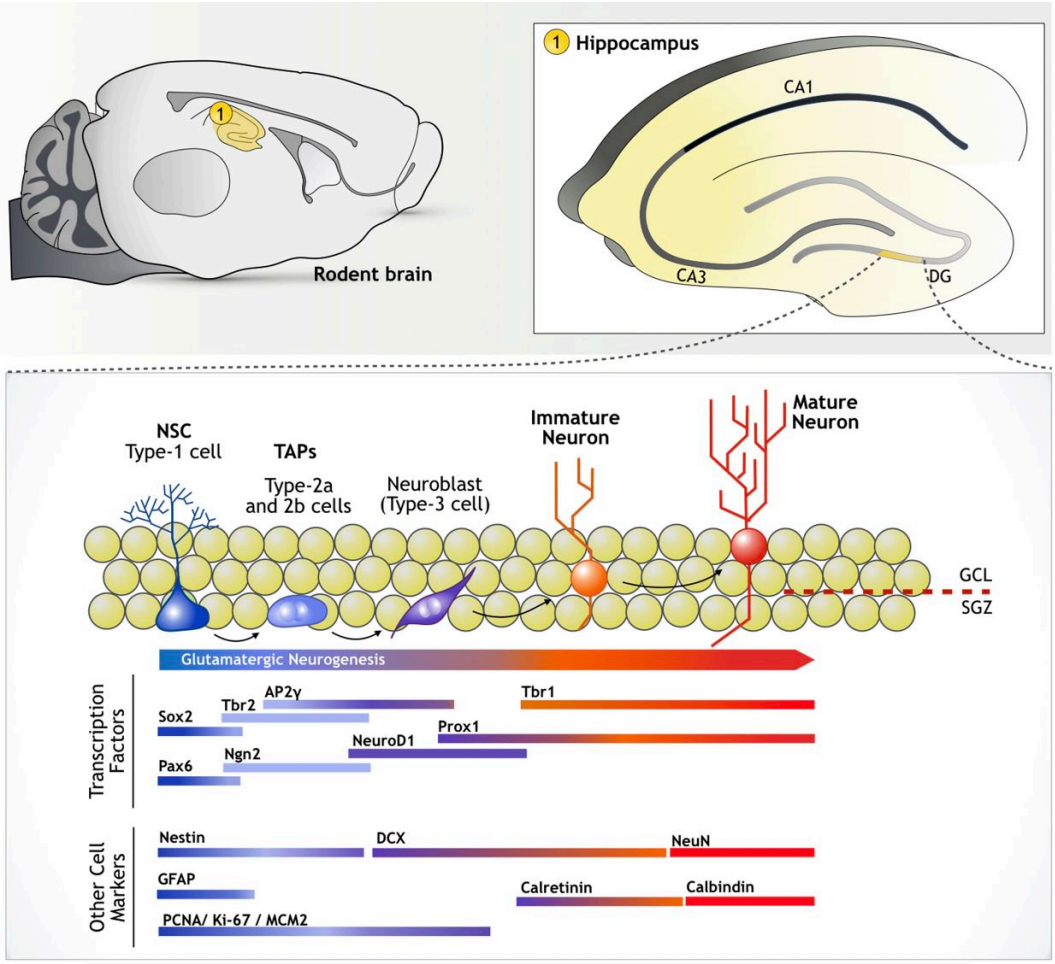

Figure 2. An integrative and updated view on the transcriptional program governing adult hippocampal neurogenesis. Adult hippocampal neurogenesis is a multistep process with an intricate transcriptional regulation. The time-window during which specific transcription factors are expressed is depicted, along with the expression of cell markers that identify newborn cells in different maturation phases (*for more details, please refer to the main text*). CA1 and CA3, cornu ammonis 1 and 3; DG, dentate gyrus; NSC, neural stem cell; TAP, transient amplifying progenitors.

Updating the Transcriptional Regulation of Neurogenesis in the Adult Hippocampus

This work brought to light a new molecular player crucial for the maintenance and differentiation of adult hippocampal TAPs into newly formed glutamatergic neurons. This preservation of neurodevelopmental mechanisms associated with AP2γ in the adult hippocampus was also recently confirmed by Hochgerner et al.[14] Although in that study, the authors found that quiescent and proliferating radial glial cells had differences in their molecular profile during early development, as compared with the postnatal period, intermediate progenitors and neuroblasts, where AP2γ is expressed, maintained very similar molecular identity at all ages.

Altogether, the bulk of evidence gathered in recent years allows to put forward the following renovated view on the sequence of transcriptional regulators of hippocampal glutamatergic neurogenesis in the adult brain (Figure 2; *for a full comprehensive view on this topic, please see the work by* Hodge and Hevner[15]). In radial neural stem cells (NSCs), transcription factors such as the *SRY-related HMG-box (Sox) family member*, Sox2, and the *Paired box protein* Pax6 are crucial for stem cell maintenance and regulation of the NSCs pool.[16,17] The transition from NSCs to neuronal lineage committed TAPs is then regulated and signaled by the upregulation of a particular sequence of transcription factors: Ngn2 (Neurogenin 2) is one of these transcription factors, whose expression is very limited and transient in TAPs, but overlapping with the expression of the *T-box transcription factor* Tbr2.[18,19] Tbr2 is the most well-documented transcriptional marker of TAPs in the adult DG and as its expression increases with TAPs maturation; markers of neuronal fate commitment, such as NeuroD1 or DCX, begin to be incrementally expressed.[18] To complement this picture, our data show how AP2γ is decisive to TAPs pool expansion and maturation progression, as it promotes the upregulation of Tbr2 and NeuroD1. Expression of NeuroD1 that persists into a subset of postmitotic neuroblasts[19] is then succeeded by expression of transcriptional regulators that are fundamental for survival and integration of postmitotic newborn neurons. The *prospero-related homeobox gene* Prox1 is one of these transcription factors, whose expression begins to be upregulated in the late-phase TAPs (type-3 cells), where it is required for maturation and survival of adult-born granule neurons.[20] Prox1 shares a very similar expression pattern with the *T-box transcription factor* Tbr1. Although the function of the latter remains to be fully elucidated, both transcription factors become constitutively expressed in mature granular neurons. The transcriptional code regulating adult hippocampal glutamatergic neurogenesis extends far beyond the summary made herein and many questions remain concerning the specific function of several of its players. New compelling findings may stem from expanding our comprehension on how this code is broken in pathological contexts known to challenge the hippocampal neurogenic niche, such as the case with stress-related disorders.

Author Contributions

AM-P, NDA, NS, and LP developed the structure and arguments for the paper and wrote the paper. AM-P designed and produced the figures. All authors approved the final version of the paper.

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REFERENCES

1. Eckert D, Buhl S, Weber S, Jäger R, Schorle H. The AP-2 family of transcription factors. *Genome Biol*. 2005;6:246.
2. Pinto L, Drechsel D, Schmid MT, et al. AP2gamma regulates basal progenitor fate in a region- and layer-specific manner in the developing cortex. *Nat Neurosci*. 2009;12:1229–1237.
3. Ailan H, Xiangwen X, Daolong R, et al. Identification of target genes of transcription factor activator protein 2 gamma in breast cancer cells. *BMC Cancer*. 2009;9:279.
4. Kang J, Kim W, Lee S, et al. TFAP2C promotes lung tumorigenesis and aggressiveness through miR-183- and miR-33a-mediated cell cycle regulation. *Oncogene*. 2017;36:1585–1596.
5. Mateus-Pinheiro A, Alves ND, Patrício P, et al. AP2γ controls adult hippocampal neurogenesis and modulates cognitive, but not anxiety or depressive-like behavior. *Mol Psychiatry*. 2017;22:1725–1734.
6. Parras CM, Galli R, Britz O, et al. Mash1 specifies neurons and oligodendrocytes in the postnatal brain. *EMBO J*. 2004;23:4495–4505.
7. Mateus-Pinheiro A, Patrício P, Bessa JM, Sousa N, Pinto L. Cell genesis and dendritic plasticity: a neuroplastic pas de deux in the onset and remission from depression. *Mol Psychiatry*. 2013;18:748–750.
8. Tanti Belzung AC. Hippocampal neurogenesis: a biomarker for depression or antidepressant effects? methodological considerations and perspectives for future research. *Cell Tissue Res*. 2013;354:203–219.
9. Garthe A, Behr J, Kempermann G. Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *PLoS ONE*. 2009;4:e5464.
10. Nakashiba T, Cushman JD, Pelkey KA, et al. Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell*. 2012;149:188–201.
11. Gordon JA. Oscillations and hippocampal-prefrontal synchrony. *Curr Opin Neurobiol*. 2011;21:486–491.
12. Oliveira JF, Dias NS, Correia M, et al. Chronic stress disrupts neural coherence between cortico-limbic structures. *Front Neural Circuits*. 2013;7:10.
13. Carreno FR, Donegan JJ, Boley AM, et al. Activation of a ventral hippocampus-medial prefrontal cortex pathway is both necessary and sufficient for an antidepressant response to ketamine. *Mol Psychiatry*. 2016;21:1298–1308.
14. Hochgerner H, Zeisel A, Lönnerberg P, Linnarsson S. Conserved properties of dentate gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing. *Nat Neurosci*. 2018;21:290–299.
15. Hodge RD, Hevner RF. Expression and actions of transcription factors in adult hippocampal neurogenesis. *Dev Neurobiol*. 2011;71:680–689.
16. Favaro R, Valotta M, Ferri AL, et al. Hippocampal development and neural stem cell maintenance require Sox2-dependent regulation of Shh. *Nat Neurosci*. 2009;12:1248–1256.
17. Maekawa M, Takashima N, Arai Y, et al. Pax6 is required for production and maintenance of progenitor cells in postnatal hippocampal neurogenesis. *Genes Cells*. 2005;10:1001–1014.
18. Hodge RD, Kowalczyk TD, Wolf SA, et al. Intermediate progenitors in adult hippocampal neurogenesis: Tbr2 expression and coordinate regulation of neuronal output. *J Neurosci*. 2008;28:3707–3717.
19. Roybon L, Hjalt T, Stott S, Guillemot F, Li JY, Brundin P. Neurogenin2 directs granule neuroblast production and amplification while NeuroD1 specifies neuronal fate during hippocampal neurogenesis. *PLoS ONE*. 2009;4:e4779.
20. Lavado A, Lagutin OV, Chow LM, Baker SJ, Oliver G. Prox1 is required for granule cell maturation and intermediate progenitor maintenance during brain neurogenesis. *PLoS Biol*. 2010;8:e1000460.