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BRAIN RESEARCH REVIEWS

Review

What determines neurogenic competence in glia?

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ARTICLE INFO

Article history:
Accepted 14 January 2010
Available online 21 January 2010

Keywords:
Stem cells
Neurogenesis
Proneural gene
Brain repair
Reactive gliosis
Müller glia

ABSTRACT

One of the most intriguing discoveries during the last decade of developmental neurobiology is the fact that both in the developing and adult nervous system neural stem cells often turn out to have a glial identity: Radial glia generates neurons in the developing telencephalon of fish, birds and mammals and astro/radial glial stem cells in specialized neurogenic zones give rise to new neurons throughout life. What are the extrinsic signals acting on and the intrinsic signals acting within these glial populations endowing these with a neurogenic potential, whilst most other glia seemingly lack it? Studies on postnatal astroglia shed interesting light on this question as they are the intermediate between neurogenic radial glia and mature parenchymal astrocytes. At least in vitro their decision to acquire a glial fate is not yet irrevocable as forced expression of a single neurogenic transcription factor enables them to transgress their lineage and to give rise to fully functional neurons acquiring specific subtype characteristics. But even bona fide non-neurogenic glia in the adult nervous system can regain some of their radial glial heritage following injury as exemplified by reactive astroglia in the cerebral cortex and Müller glia in the retina. In this review first we will follow the direction of the physiological times' arrow, along which radial glia become transformed on one side into mature astrocytes gradually losing their neurogenic potential, while some of them seem to escape this dire destiny to settle in the few neurogenic oases of the adult brain where they generate neurons and glia throughout life. But we will also see how pathophysiological conditions partially can reverse the arrow of time reactivating the parenchymal astroglia to re-acquire some of the hallmarks of neural stem cells or progenitors. We will close this review with some thoughts on the surprising compatibility of the co-existence of a neural stem cell and glial identity within the very same cell from the perspective of the concept of transcriptional core networks.

Contents

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doi:10.1016/j.brainresrev.2010.01.002

1. Introduction

The last ten years have seen the rise of specialized glial cells to the rank of neuronal precursors both in the developing and adult nervous system. In the developing telencephalon neuroepithelial cells differentiate into radial glia which in turn generate either directly or indirectly via intermediate progenitors most forebrain neurons (Anthony et al., 2004; Kriegstein and Alvarez-Buylla, 2009; Malatesta et al., 2003; Malatesta et al., 2000; Pinto and Gotz, 2007). While most of the radial glia (RG) eventually transform into parenchymal astrocytes at the end of neurogenesis (Alves et al., 2002; Voigt, 1989), some of them give rise to astroglial stem cells in the adult subependymal zone (SEZ) or ependymal cells (Merkle et al., 2007; Spassky et al., 2005). Both, radial glia and astroglial stem cells display hallmark features of classical astrocytes [for a detailed review on the glial nature of radial glia see (Pinto and Gotz, 2007)]. Thus the question arises as to what endows these glial cells with a neurogenic competence so noticeably lacking in astroglia in most other regions of the brain, such as e.g. the cerebral cortex. What signals are required for the neurogenic endowment of glia and what processes take place in radial glia while they transform into non-neurogenic astrocytes? Does the loss of neurogenic potential occur abruptly or gradually? Is it in fact irreversible? Our studies during the last few years have led to the recognition that astroglial cells at an early stage of postnatal development are not irrevocably fixed in their lineage, but forced expression of single neurogenic transcription factors can render these cells capable of transgressing their own lineage and generating diverse types of neurons, at least in vitro (Berninger et al., 2007a; Heins et al., 2002). However, similar processes might be evoked in the early postnatal brain by damage such as caused by hypoxia (Fagel et al., 2009; Fagel et al., 2006). Finally, partial neurogenic competence can be regained by glial cells in the adult nervous system following injury as they de-differentiate and resume proliferation, as shown in the retina and the cerebral cortex (Buffo et al., 2008; Fischer and Reh, 2001; Karl et al., 2008). Thus it appears that not all the radial glial heritage is spent during early life, but some of it is latently preserved stimulating the hope that it might be possible to unearth this potential for the development of novel strategies for brain repair.

2. Radial glia: the founding fathers of the telencephalon and the adult neurogenic zones

Radial glial cells (RGCs) comprise a specialized cellular population in most regions of the vertebrate brain during restricted developmental periods, the functions of which have been highly disputed since their first description at late 19th century [reviewed in (Rakic, 2003)]. Nowadays, there is a general consensus that RGCs differentiate from neuroepithelial cells acquiring typical astroglial features, as for instance the presence of glycogen storage granules and the expression of astroglial markers, such as e.g. the astrocyte-specific glutamate and aspartate transporter (GLAST), brain lipid-binding protein (BLBP) and tenascin-C [for review see (Pinto and Gotz, 2007)]. In addition, it is now well established that RGCs function both as neuronal and glial progenitors at least in the developing telencephalon (Anthony and Heintz, 2008; Anthony et al., 2004; Malatesta et al., 2003; Malatesta et al., 2000; Miyata et al., 2001; Noctor et al., 2001; Tamamaki et al., 2001). Finally, with completion of most neuro- and gliogenesis they sign responsible for generating ependymal cells as well as particular astro/radial glial cells that function as neural stem cells (NSCs) in the adult brain (Chojnacki et al., 2009; Kriegstein and Alvarez-Buylla, 2009; Merkle et al., 2004). The fact that at least some RGCs are multipotent, i.e. can generate both neuronal and glial progeny, and are capable of self-renewing cell divisions generating either two new RGCs (symmetric division) or one RGC and a fate-restricted progenitor (asymmetric division) (Miyata et al., 2001; Noctor et al., 2004) indicate that RGCs exhibit defining stem cell hallmarks and are thus often considered as embryonic NSCs (Kriegstein and Alvarez-Buylla, 2009). The generation of adult NSC from RGCs may be considered as a particular case of RGC self-renewal raising interesting points about the precise lineage relationships between RGCs and adult NSCs. For example, given the evidence that there are virtually no quiescent RGCs during embryonic development (Hartfuss et al., 2001), consequently all adult NSCs must be derived from RGCs that have been previously contributing to neuro- or gliogenesis. Moreover, given the notion that NSCs in the adult SEZ are quite heterogeneous with respect to the distinct progenies they give rise to (Brill et al., 2009; Brill et al., 2008; Hack et al., 2005;

Merkle et al., 2007), it can be argued that each class of adult generated neuron and glia may likely descend from a different type of RGC. Indeed there is compelling evidence that the adult SEZ exhibits a similar dorsoventral organization as the developing telencephalon [for review see (Kriegstein and Alvarez-Buylla, 2009)]: for instance, transcription factors involved in interneuron specification in the developing ventral telencephalon such as Dlx2, (Petryniak et al., 2007) are highly expressed in the ventral SEZ (Brill et al., 2008), while transcription factors expressed dorsally such as Pax6 and Neurogenin2 (Neurog2) (Brill et al., 2009; Brill et al., 2008) are well known for their important role in the developing cerebral cortex (Bertrand et al., 2002). This regionalisation of the SEZ has been taken as sign for the construction of the adult SEZ from "building blocks" of different origins (Kohwi et al., 2007), not unlike a mosaic (Merkle et al., 2007; Young et al., 2007). Finally, while the majority of adult NSCs generate neurons, some of them give rise to oligodendrocytes and possibly also non-stem cell astroglia (Hack et al., 2005) leading to two interrelated questions: (i) Do all adult NSCs in vivo generate neuronal and glial progeny, possibly even in a sequential manner, or are we dealing here with distinct sets of neuro- and gliogenic progenitors (which would no longer justify the use of the term "stem cell")? (ii) In the latter case are different adult progenitors derived from different types of RGCs, i.e. adult neurogenic progenitors from neurogenic RGCs and adult gliogenic progenitors from gliogenic RGCs?

3. Lineage relationships between adult NSC astroglia and embryonic radial glia

Astro/radial glial cells functioning as NSCs in the adult brain have been long suggested to derive from embryonic radial glia (Alvarez-Buylla et al., 2001; Chojnacki et al., 2009). However, embryonic RGCs are heterogeneous (Malatesta et al., 2000) and it is not clear whether adult NSCs would belong to the lineage of multipotent (Fig. 1, scenario I), lineage-restricted (Fig. 1, scenarios II and III) or even of a very small and hence previously missed set of quiescent RGCs (Fig. 1, scenario IV). Recently, we have provided additional evidence indicating that the early developing cerebral cortex is devoid of glia-restricted progenitors (Costa et al., 2009). By using retrovirally-based transduction and video time-lapse microscopy to follow the progeny of single cortical progenitors in vitro and in vivo, we showed that the majority of early cortical progenitors generate exclusively neurons, a pool which becomes depleted with time, whereas a subset of cortical progenitors generate neurons before giving rise to glia-restricted progenitors observed after mid-neurogenesis. Therefore, every actively dividing cortical progenitor seems to generate neurons at early developmental stages and only a subset of progenitors undergoes the alleged sequence of transitions from neurogenesis to gliogenesis during cortical development (Qian et al., 2000). As RGCs have been shown to constitute the majority of progenitor cells in the developing cerebral cortex at the developmental stages when most of these lineage studies were performed (Hartfuss et al., 2001; Malatesta et al., 2000; Noctor et al., 2002, 2004), most of the cortical progenitors studied in previous work [see (Costa et al., 2009) and references herein] are RGCs and, therefore, cell lineage data would indicate that early RGCs comprise two major populations, namely a neuron-restricted and a multipotent population. Since the former seem to become depleted over time as they generate neurons, this may suggest that adult NSCs are encompassed in the lineage of multipotent RGCs contributing both neurons and macroglial cells during development (Alvarez-Buylla et al., 2001) (Fig. 1, scenario I). Although this may be considered to be the most likely scenario, there is no direct experimental support to dismiss the possibility that adult NSCs are derived from symmetrically dividing RGCs generating only daughter RGCs (Fig. 1, scenario III) or from a small, and therefore difficult to be detected, subpopulation of RGCs that did not divide at all during development (Fig. 1, scenario I). None of these alternatives has been directly tested, yet the fact that several signalling pathways involved in the transition from neurogenesis to gliogenesis in the developing cerebral cortex also play a role in the specification of adult NSCs (see below) may lend some support to the view that adult NSCs are indeed encompassed in the lineage of multipotent radial glia (Fig. 1, scenario I).

4. The role of intercellular signalling pathways for the endowment of glia with neurogenic potential

Irrespective of the exact lineage relationship between RGCs and adult NSCs, there is good experimental support for the notion that embryonic RGCs transform into adult SEZ astroglia at postnatal stages (Merkle et al., 2007, 2004). These two cell populations share an intriguing property: they exhibit astroglial features and at the same time have the potential to generate neurons. In contrast, other astroglial population directly derived from RGCs, such as parenchymal astroglia in the cerebral cortex (Alves et al., 2002; Voigt, 1989) and ependymal cells (Spassky et al., 2005) do not generate neurons in the adult brain under physiological conditions. What are the factors endowing RGCs and SEZ astro/radial glia with neurogenic potential and what restricts parenchymal astroglia and ependymal cells to their lineage and prevents them from generating neurons notwithstanding their close ties to RGCs and SEZ astro/radial glia? To gain some more insights into the differences between stem cell and parenchymal glia we need to have a closer look at the signalling pathways activated in these cells. We will focus here on the discussion of Notch and sonic hedgehog (Shh) signalling as several lines of evidence suggest that these pathways play a major role in stem cell fate determination and may hence shed light on the question how astroglial cells are endowed with stem cell properties.

4.1. *Notch signalling*

On first sight, the canonical Notch pathway seems to be gliogenic: Forced expression of Hes1, a downstream target and effector of Notch, impairs neurogenesis in the embryonic cortex (Ishibashi et al., 1994), whereas expression of an active form of Notch1 (NICD1) or Notch 3 (NICD3) promotes increased generation of parenchymal astrocytes and ependymal cells (Dang et al., 2006; Gaiano et al., 2000). These effects of Notch

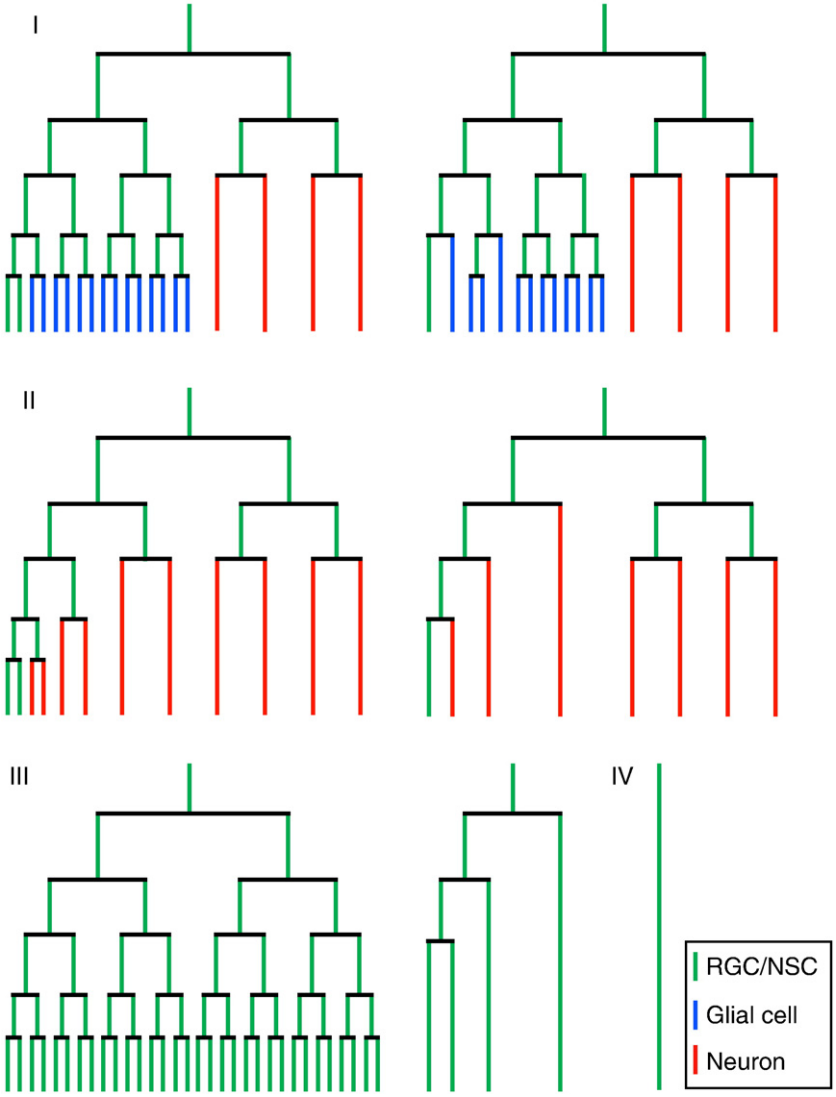

Fig. 1 – Possible lineage relationships between RGCs and adult NSCs. In the first scenario (I) RGCs generate neurons and macroglial cells during development before becoming adult NSCs. In the second scenario (II), RGCs that give rise to adult NSCs generate only neurons during development. The third scenario (III) suggests that adult NSCs are derived from a separate population of RGCs that do not contribute neurons or macroglial cells during development. The two lineage trees depicted in each of these 3 scenarios are fictious and have the purpose to illustrate that RGCs may progress to adult NSCs via symmetric and asymmetric cell divisions. The last scenario (IV) suggests the existence of quiescent RGCs that do not divide during development and directly become adult NSCs.

signalling in RGCs have been thought to depend on the repression of proneural genes such as Neurogenin1 (Neurog1), Neurog2 and the mouse homologue of achaete–scute 1(Mash1) by the Notch effectors Hes1 and Hes5 (Hatakeyama et al., 2004). However, it has also been shown that most RGCs express Notch target genes at early embryogenesis (Mizutani et al., 2007), indicating that Notch signalling occurs at stages when RGCs are actively engaged in the generation of neuronal progeny. Moreover, expression of NICD1 or NICD3 significantly increases the frequency of embryonic telencephalic progenitors that form neurospheres (Dang et al., 2006; Yoon et al., 2004), suggesting that Notch signalling might be involved in the maintenance of a stem cell state in RGCs. Accordingly, active Notch 1 and 3 promote the generation of subependymal astrocytes (Dang et al., 2006; Yoon et al., 2004). Although these data seem at first glance contradictory with previous findings pointing to a gliogenic role for Notch, it might only highlight a limitation inherent to studies based on forced expression or deletion of genes, namely their "all or none" effect that is blind to the temporal fine tuning of physiological expression levels. In fact, it has been recently demonstrated that the levels of Hes1 and Neurog2 oscillate out of phase to each other within RGCs (Shimojo et al., 2008), indicating that the susceptibility of RGCs for fate decisions is dynamically regulated and may thus depend on temporally coincident signalling events impinging on them rather than on the static activation of isolated signalling pathways, such as mimicked by forced expression of Notch effectors or their counter-players. Indeed, promotion

of gliogenesis by Notch depends on the concomitant activation of the janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway (Ge et al., 2002). When these two pathways are not simultaneously activated, the Notch signalling mediator CBF1 binds to a repressive cofactor protein, NCoR, which functions to repress gliogenic genes (Hermanson et al., 2002). Therefore, Notch signalling in embryonic RGCs may prevent neurogenesis by two different means: i) by maintaining RGCs in an undifferentiated, proliferative state and ii) by promoting gliogenesis. In the adult SEZ, Notch signalling functions to maintain ependymal cells in a quiescent state and inhibition of this signalling allows these cells to produce olfactory bulb neurons (Carlen et al., 2009). In contrast, activation of Notch signalling has also been shown to be important for survival and proliferation of adult NSCs (Alexson et al., 2006; Androutsellis-Theotokis et al., 2006), suggesting that similarly to the embryonic brain, the role of Notch signalling in the adult neurogenic niches may also depend on dynamic interactions with other signalling pathways. Interestingly, Notch signalling becomes activated in the cerebral cortex after stroke (Arumugam et al., 2006), a condition in which parenchymal astroglia de-differentiate and resume proliferation and can generate multipotent neurospheres (Buffo et al., 2008, 2005) (see discussion below).

4.2. *Sonic hedgehog (Shh) signalling*

The Shh member of the Hedgehog protein family plays critical roles in the patterning of the developing neural tube and in the induction of ventral forebrain structures [for a recent review see: (Dessaud et al., 2008)]. Recently, it has been shown that conditional knockout of the *Shh* and *Smo* genes in the early developing dorsal telencephalon resulted in a smaller dorsal telencephalon by prolonging the cell cycle of RGCs (Komada et al., 2008). Moreover, Shh signalling has been shown to be crucial for the generation of NSCs in the postnatal brain (Palma et al., 2005). Consistent with a role in neural stem cell maintenance, Shh signalling in the adult neurogenic regions is important to maintain normal levels of NSC proliferation and neurogenesis (Balordi and Fishell, 2007; Han et al., 2008; Lai et al., 2003). Furthermore, in an elegant study, Ahn and Joyner provided unequivocal evidence that a small population of cells (probably RGCs) in the developing brain respond to Shh and establish the two major neurogenic compartments in the adult brain (Ahn and Joyner, 2005). Using a genetic fate-mapping strategy based on the expression of the Shh target Gli1 in vivo, they showed that Shh responding cells are established at late embryogenesis and comprise NSCs capable of self-renewal and generation of multiple cell types in the adult brain. More recently, it has also been shown that NSCs fail to develop in animals with defective Shh signalling caused by embryonic ablation of ciliary genes in RGCs (Han et al., 2008). Collectively, these data suggest a pivotal role of Shh for the establishment of NSCs within the neurogenic germinative zones of the adult brain. Surprisingly, yet consistent with such role, Shh can stimulate differentiated parenchymal astroglia from the adult cerebral cortex to initiate the formation of multipotent neurospheres in vitro (Jiao and Chen, 2008). The latter data suggest that this factor may not only be important for the establishment and maintenance of astroglial NSCs within the stem cell niche, but rather may exert a stem cell identity inducing activity over other astroglial cell populations.

Other molecular determinants may also contribute to the neurogenic potential of stem cell astroglia. For example, members of the bone morphogenetic protein family (BMPs) promote neuronal differentiation at early embryogenesis (Li et al., 1998) and BMP signalling is also active in GFAP-positive cells in the adult SEZ where it is required for neurogenesis (Colak et al., 2008). Likewise, Wnt signalling has also been shown to be involved in the control of cortical progenitors/RGCs proliferation (Chenn and Walsh, 2002; Woodhead et al., 2006) and adult neurogenesis (Kuwabara et al., 2009; Lie et al., 2005). The precise role of these factors in maintaining the pool of NSCs in developing and in the adult neurogenic germinative zones is currently under active investigation and lessons learned from these studies may help to reactivate neurogenic programs in classically non-neurogenic areas of the brain, as illustrated by the case of Shh.

5. Postnatal astroglia: a transition state between neurogenic radial glia and mature parenchymal astrocytes?

As previously discussed, while some RGCs give rise to astroglial stem cells of the adult neurogenic zones, ependymal cells or late oligodendroglial progenitor cells (Kessaris et al., 2006), the majority is thought to transform directly or indirectly into parenchymal astroglia (Kriegstein and Alvarez-Buylla, 2009). Eventually many of these astroglial cells will take up functions quite different from generating neurons such as regulation of blood flow, ion homeostasis, energy metabolism and regulation of synaptic function (for review see articles within this issue of Brain Research Reviews), i.e. intricate functions which require a high degree of specialization (Wang and Bordey, 2008). Very little is known about the molecular mechanisms underlying the specialization of astroglia, but evidence from the spinal cord white matter points to the possibility that transcription factors involved in neuronal specification also contribute to astrocyte specification, with Pax6 being a prime example for a transcription factor not only regulating neuronal, but also astroglial specification (Hochstim et al., 2008). For instance, astroglia in the ventral and dorsal spinal cord white matter can be distinguished by their mutually exclusive expression of reelin and Slit1, respectively. Intriguingly, reelin requires expression of Pax6, while Nkx 6.1 promotes expression of Slit1 (Hochstim et al., 2008). This data suggest a transcriptional code for astrocyte positional identity in the white matter of the spinal cord. Does expression of neurogenic fate determinants play a similar role in the specification of cortical astroglia? Indeed Pax6 has been detected in postnatal astrocytes and their progenitors (Sakurai and Osumi, 2008) (S. Gascón and M. Götz, unpublished observation) suggesting that continued expression of neurogenic fate determinants – most likely at drastically reduced levels – by cortical astrocytes at early postnatal stages ensures a gradual change from neurogenic RGCs to gliogenic RGCs and finally astrocytes. Indeed several lines of evidence point to such a gradual change: for instance, postnatal astroglia can be induced to initiate the formation of

clonal aggregates called neurospheres when cultured in the presence of epidermal growth factor (EGF) and fibroblast growth factor 2(FGF2) (Laywell et al., 2000) (Heinrich et al., unpublished observation). These neurospheres are characterized by their ability to self-renew (i.e. can give rise to secondary, tertiary etc. neurospheres) and are multipotent, i.e. can generate all three major neural lineages, thus exhibiting the hallmarks of neurospheres generated by NSCs. The neurosphere-forming capacity of astroglia rapidly declines during the second postnatal week (Laywell et al., 2000). Thus, under the influence of EGF/FGF2 early postnatal astroglia can sufficiently de-differentiate to revert to a multipotent lineage. But by postnatal days 14–18 apparently changes take place within astroglia that disable the neurosphere-forming capacity of these cells. Conceptually, one may assume that these changes correspond with the in parallel increasing involvement of astroglia in their above mentioned regulatory functions. For instance, by about 2 weeks astroglia acquire a mature expression of inwardly rectifying potassium channels (K_{ir}), thought to be involved in buffering of potassium (Bordey and Sontheimer, 1997; Seifert et al., 2009). Interestingly, up-regulation of $K_{ir}4.1$ has been associated with cell cycle exit, while loss of functional K_{ir} channels is associated with re-entry into cell cycle and gliosis (Olsen and Sontheimer, 2008). Indeed, functional specialization of astroglia is likely to require these cells to cease proliferating and to acquire a postmitotic status thereby rendering these cells refractory to the mitogenic action of EGF and FGF2. However, as we will see below, astroglia can de-differentiate and resume proliferation following injury, an effect which is accompanied by the regaining of neurosphere-forming capacity (Buffo et al., 2008, 2005).

5.1. *Stable reprogramming of postnatal astroglia*

Another striking example for the plasticity of early postnatal astroglia can be seen in their high susceptibility for neuronal reprogramming following forced expression of neurogenic fate determinants. Based on the fact that neurogenic RGCs in the developing cortex express high levels of Pax6 (Gotz et al., 1998), our laboratory has demonstrated that forced re-expression of Pax6 in culture via retroviral vectors can drive some mouse astroglia towards the neuronal lineage (Heins et al., 2002). Subsequently, we found that retrovirally-mediated expression of the Pax6 target gene Neurog2 or the related proneural gene Mash1 are even more efficient in driving early cultured postnatal astroglia towards neurogenesis (Berninger et al., 2007a). Intriguingly, forced expression of these transcription factors does not only induce expression of neuronal markers, but also results in the gradual acquisition of neuronal conductances and the ability to generate repetitive action potentials discharges suggesting that forced expression of these transcription factors had caused early postnatal astroglia to undergo a stable lineage transgression.

Single cell tracking of astroglia derived from transgenic mice expressing GFP under the human GFAP promoter revealed interesting details about the reprogramming process. First of all, consistent with the viral integration, cells undergoing reprogramming typically divided before undergoing neuronal metamorphosis with many cells apparently dying from apoptosis (Berninger et al., 2007a). It is currently not clear whether this apoptosis is a specific mechanism for aborting failed reprogramming, but it is conceivable that forced expression of neurogenic fate determinants may induce a transcriptional program that enters in catastrophic conflict with the intrinsic astroglial differentiation. Another lesson learned from these single cell tracking experiments is the fact that cells undergoing successful reprogramming exhibit morphological changes surprisingly similar to early cortical progenitors. For instance, like early cortical precursors (LoTurco and Bai, 2006), astroglial cells expressing Neurog2 seem to pass through distinct morphological stages, such as a multipolar followed by a bipolar phase (Fig. 2), and eventually enter a migratory stage (Berninger et al., 2007a). These data are consistent with the recent finding that one of Neurog2's direct transcriptional targets is the small GTP binding protein Rnd2, the activity of which is constitutive and plays a fundamental role in regulating the morphology and migration of early cortical precursors (Heng et al., 2008).

5.2. *Synapse formation by reprogrammed postnatal astroglia*

Notably, astroglia-derived neurons were capable of receiving functional synapses from co-cultured embryonic cortical neurons suggesting a remarkable ability for functional integration. Somewhat surprisingly, however, astroglia-derived neurons failed to form functional presynaptic outputs. Yet, consistent with Neurog2 playing a fundamental role in specifying a glutamatergic identity in telencephalic precursors (Bertrand et al., 2002), a substantial proportion of astroglia expressing Neurog2, but not Mash1 or Pax6, up-regulated the T-box transcription factor Tbr1 (Berninger et al., 2007a), a hallmark in glutamatergic neurogenesis (Hevner et al., 2006). However, the failure of forming functional presynaptic specializations argued that neuronal reprogramming remained only partial. In an effort to overcome these limitations by using viral vectors providing higher levels of expression and less susceptible to silencing we have been recently able to show that early postnatal astroglia can be directed towards fully functional synapse forming neurons, at least in vitro (Heinrich et al., unpublished observation). Importantly, this study showed that forced expression of distinct fate determinants such as Neurog2 and Dlx2, known for their critical roles in specifying glutamatergic and GABAergic neurons, respectively, within the developing telencephalon (Bertrand et al., 2002; Petryniak et al., 2007) directs astroglial cells towards distinct neuronal transmitter subtypes (Heinrich et al., unpublished observation), consistent with their developmental role. Thus, forced expression of neurogenic fate determinants can result in the stable and complete lineage reprogramming of early postnatal astroglia. Notably, in parallel to the decline in neurosphere-forming capacity, the susceptibility of undergoing reprogramming gradually decreases with the age of the cultured astroglia (Berninger, unpublished observation) again arguing for a gradual change in the internal milieu of astroglia undergoing maturation. Future studies will have to show whether these changes are accounted for by epigenetic modifications and whether and under what conditions they are reversible.

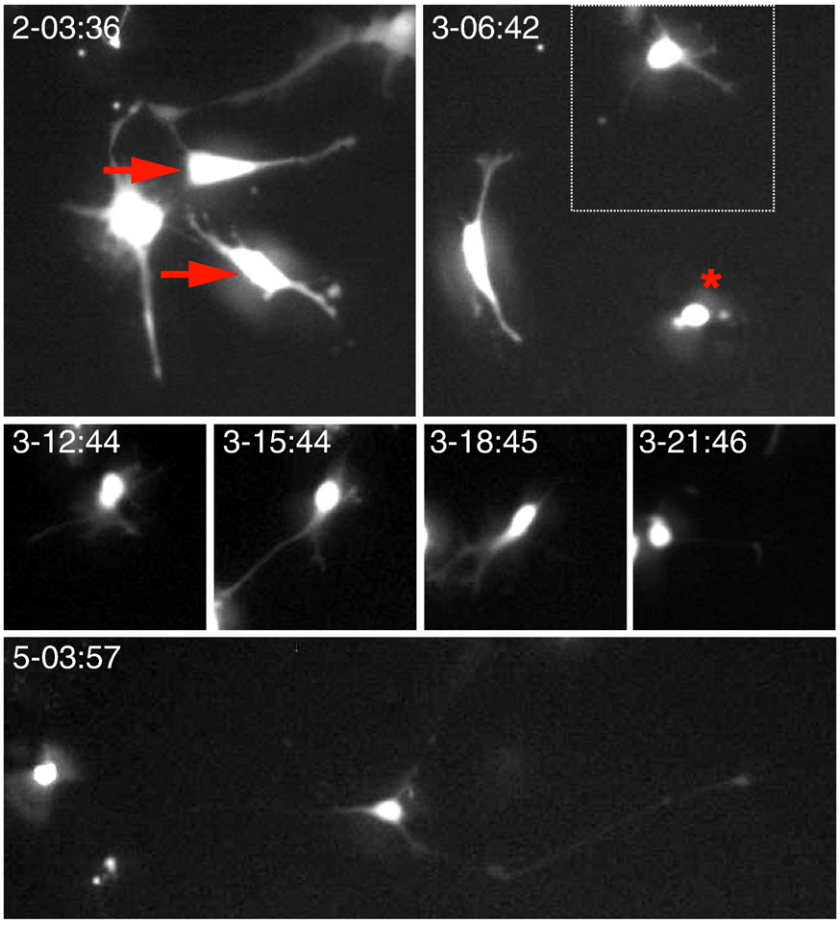

Fig. 2 – Metamorphosis of an astrocyte from the postnatal cerebral cortex into neuron upon forced expression of Neurog2. Panels depict different stages of cellular siblings (red arrows) derived from a hGFAP-GFP positive postnatal astroglia transduced with Neurog2. Time is shown as "days-hours:minutes". One of the daughter cells dies at time point 3-06:42 (red asterisk), whereas the second cell (dashed box) undergoes progressive morphological changes finally acquiring a neuronal morphology and TuJ1 expression (not shown) after 5 days.

5.3. *Recruitment of local or SEZ astroglia following injury of the postnatal cortex?*

While the present data argue for a high degree of plasticity of early postnatal astroglia in culture, astroglia fate-mapping studies from the Vaccarino lab suggest that to some low degree (<1%) astroglia can spontaneously generate neurons even in the postnatal cortex in vivo (Ganat et al., 2006). Moreover, following hypoxic insult, which causes a marked reduction in cortical thickness and neuron number at early postnatal stages, a much more substantial incorporation of new neurons can be observed (Fagel et al., 2009, 2006) which may eventually result in total recovery of cortical size and cell number. However, it is currently not known whether these newly incorporated neurons are derived from astroglia endogenous to the injured cortex or are rather from astroglial cells residing within the emerging adult neurogenic SEZ, which responds to hypoxic injury with a marked increase in proliferation (Fagel et al., 2009, 2006; Sundholm-Peters et al., 2005; Szele and Chesselet, 1996). The fact that many of the newly generated neurons were found to express Tbr1 (Fagel et al., 2006), as mentioned above a hallmark of glutamatergic neurogenesis, may on first sight argue for local generation, as postnatally generated neurons in the SEZ comprise largely GABAergic neurons. However, a recent study has demonstrated that the adult SEZ also contains a pool of glutamatergic progenitors which can be recruited from the SEZ upon injury (Brill et al., 2009).

6. Re-acquisition of a stem or progenitor-like state by mature glia following injury

6.1. *Reactivation of astroglia following injury of the cerebral cortex*

Thus, several lines of evidence suggest that the abysm between RGCs and early postnatal astroglia is not yet unbridgeable, but becomes wider and wider with development. Upon reaching adulthood at the latest, however, astrocytes resident to the cortical parenchyma seem to have lost all neurogenic potential and accordingly the adult cortex is devoid of newly generated neurons. Recently, Buffo and et al. re-assessed the question whether astroglia proliferates in

the mature cerebral cortex by genetic fate-mapping, using a mouse line expressing a tamoxifen-inducible Cre recombinase driven by the astrocyte-specific GLAST promoter crossed to a reporter line (Buffo et al., 2008). Notably, at that stage astroglia was found to become largely quiescent and hardly any genetically fate-mapped astroglia incorporated the thymidine analogue BrdU. Similar data were also obtained by double-labelling immunohistochemical makers for astrocytes with BrdU (Buffo et al., 2008) (C. Simon and M. Götz, unpublished observation). This situation changes dramatically following injury. When the cerebral cortex was locally injured by a stab wound, genetically fate-mapped astroglia was found to resume proliferation along side with the up-regulation of GFAP and other classical markers of reactive glia. Despite the observation that some injury paradigms appear to be inductive for low degree neurogenesis in the cerebral cortex (Magavi et al., 2000), no new neurons could be found in the stab wound model (Buffo et al., 2008, 2005). In fact, virtually all fate-mapped astroglia were found to give rise to new astrocytes (Buffo et al., 2008). Does this mean that these proliferating astroglia are intrinsically restricted to the astroglial lineage? In fact, in vivo the answer to this question seems to be yes. Surprisingly, however, when these astroglial cells were placed in vitro, these, but not their counterparts of the intact cortical hemisphere, could give rise to neurospheres characterized by the ability to generate secondary and tertiary neurospheres, suggestive of self-renewal capacity, and by multipotency, i.e. the ability to generate not only new astroglia, but also oligodendrocytes and neurons (Buffo et al., 2008). Intriguingly, ectopic grafting of committed neuronal precursors derived from the SEZ into a non-neurogenic milieu such as the adult striatum causes their glial conversion (Seidenfaden et al., 2006) suggesting that the lack of neurogenesis from reactivated astroglia in vivo may not be due to cell-intrinsic shortcomings of these cells compared to astro/radial glial stem cells of the SEZ, but their exposure to a highly non-neurogenic microenvironment.

Notably, data from our laboratory show that these neurosphere cells can be efficiently reprogrammed in vitro to generate glutamatergic and GABAergic neurons by forced expression of Neurog2 and Dlx2, respectively (Heinrich et al., unpublished observation). Can these cells also be reprogrammed in vivo? The answer to this question is a partial yes. Retrovirally-mediated forced expression of Pax6 was found to induce doublecortin (DCX) in some of the transduced progeny suggesting early stages of neurogenesis, but this population of DCX positive cells eventually disappeared (Buffo et al., 2005). This abortive neurogenesis may have many reasons, such as only partial transdifferentiation leading to failure of functional integration and ultimately apoptosis, therein resembling the high incidence of cell death following recruitment of DCX positive cells from the SEZ into the ischemic striatum (Arvidsson et al., 2002). This data suggests that successful reprogramming of mature astroglia represents only one of the hurdles to be taken if aiming at functional neurological reconstitution of damaged brain circuits. At the same time the microenvironment must be made favourable or at least permissive. Suffice it to say that neurons generated from reprogrammed astroglia must be able to form functional synaptic connections between themselves and the adjacent intact tissue, a process which will require the help of astroglia, well known to play a fundamental role in regulating synapse formation (Christopherson et al., 2005; Slezak and Pfrieger, 2003; Song et al., 2002) and plasticity (Perea and Araque, 2007).

Studies performed in our laboratory clearly indicate that while some early stages of neurogenesis can be indeed attained by forced expression of single neurogenic transcription factors, the response falls short in comparison to the effect observed in early postnatal astroglia. Yet, hope for improvement originates from work in adult pancreas. The Melton laboratory could show that while forced expression of single transcription factors failed to induce an effective reprogramming of exocrine cells into insulin secreting beta cells, a quite substantial degree of transdifferentiation was observed in the adult pancreas in vivo following expression of three defined factors involved in distinct stages of beta cell specification and differentiation (Zhou et al., 2008). It is thus conceivable that joint expression of several neurogenic transcription factors may be more effective in achieving stable lineage transgression towards neuronal identity and thereby also increase the chances for functional integration which in case of incomplete neuronal reprogramming will remain highly compromised.

Although the neurosphere-forming capacity seems to originate from reactive astroglia, we do not know whether all types of parenchymal astroglia de-differentiate to the same degree as to re-acquire NSC like properties. Intriguingly, however there are some hints to which molecular pathways may account for this remarkable response. As mentioned above, the morphogen Shh can induce GFAP-positive cells from the adult cerebral cortex to generate neurospheres (Jiao and Chen, 2008). A recent study has now revealed that Shh expression is indeed induced within cortical astroglia following a local injury {Amankulor, 2009 #267}. Surprisingly, the induction of Shh expression was found to depend on astroglia–macrophage interactions. There is an obvious need to gain more insights into the molecular processes underlying reactive gliosis. Indeed, recent progress unravelled a key role of astroglia–basement membrane contact mediated by integrins in maintaining astroglia in a non-reactive state (Robel et al., 2009). Of greatest interest in regard to the theme of astroglial de-differentiation into stem cells, loss of β1-integrin-mediated signalling leads to the activation of all hallmarks of reactive astrocytes except proliferation and stem cell de-differentiation, thereby highlighting that specific aspects of reactive gliosis are elicited by distinct signalling pathways.

6.2. *Reactivation of Müller glia following retinal injury*

Intriguingly, a similar response to injury can be observed in the retina. In teleost fish, substantial regeneration of the neural retina can be spontaneously achieved from Müller glia which may be a pendant to astroglia within other central nervous tissues (Lamba et al., 2008a). Indeed, Müller glia in the teleost retina have a complex response to local injury that includes some features of reactive gliosis (up-regulation of glial fibrillary acidic protein, GFAP, and re-entry into the cell cycle) along with characteristics associated with radial glia (expression of BLBP) and re-acquisition of molecular

characteristics of multipotent retinal progenitors, such as activation of Notch–Delta signalling and expression of Pax6 (Raymond et al., 2006). The regenerative capacity of the neural retina appears to be progressively lost during evolution. In birds, following acute damage to the neural retina Müller glia still re-enter the cell cycle with many expressing transcription factors characteristic of embryonic retinal progenitors such as Pax6 (Fischer and Reh, 2001). Notably, some of these progenitors then give rise to new neurons, while others produce new Müller glia or remain progenitors. Thus, despite some regenerative capacity, the response remains limited. In mammals such as mice, the regenerative response to neurotoxic injury resulting in the selective death of amacrine and retinal ganglion cells is even more restricted as Müller glia does not spontaneously enter the cell cycle (Karl et al., 2008). However, following intraocular injection of growth factors such as EGF, FGF2 or FGF2 in combination with IGF, it is possible to stimulate Müller glia proliferation and de-differentiation, which now do express Pax6 (Karl et al., 2008). Of note, this up-regulation of Pax6 is in strong contrast to what can be observed in reactive astroglia in the cerebral cortex where instead the basic helix loop helix transcription factor Olig2 is up-regulated, persistent expression of which is anti-neurogenic in the telencephalon (Buffo et al., 2005; Colak et al., 2008; Hack et al., 2005). Turning back to the retina, consistent with the induction of Pax6, when stimulated with growth factors Müller glia even generate some amacrine neurons. Again, many of these newly generated neurons die (Karl et al., 2008). One may speculate that this could be in part due to the fact that no new retinal ganglion cells are regenerated and thus functional integration of newly generated amacrine neurons remains incomplete eventually leading to their apoptosis.

Using the retinal injury model the Reh laboratory has provided some intriguing evidence to what actually happens when reactive glia de-differentiate. They found that Müller glia start to re-express a component of the SWI/SNF core complex called BAF60c (Lamba et al., 2008b). The SWI/SNF complex is an ATP dependent chromatin remodelling complex that undergoes changes in its composition in parallel to the differentiation from neural stem cell to neuron or glia. Notably, BAF60c is normally expressed in retinal progenitors and re-expression of this factor by Müller glia following injury combined with growth factor treatment suggests that the target specificity and hence remodelling activity SWI/SNF complex becomes progenitor-alike. One particular interesting aspect of BAF60c is its role in stabilizing the interaction between activated Notch and its DNA-binding partner RBP-J (Takeuchi et al., 2007). Thus, re-expression of BAF60c may render Müller glial cells more susceptible to Notch signalling. Intriguingly, in birds Notch signalling seems to play a dual role, namely it contributes to the de-differentiation process, but when maintained at high levels counteracts neurogenesis (Hayes et al., 2007) not unlike to what has been stated above about the role of Notch signalling in RGCs of the developing cerebral cortex.

Taken together, reactive gliosis in the cerebral cortex and the retina results in the re-acquisition of stem cell or progenitor-like properties, but in order to promote a regeneration eventually leading to the reconstitution of neurological function, the details of the reactive response need to be much better understood.

7. The concept of a transcriptional network underlying neural stemcellness

The fact that neural stem and progenitor cells often come in the disguise of glia leads us to one particularly perplexing question: what are the common denominators of the transcriptional core network regulating neural stem cell and (astro)-glial identity? Under transcriptional core network we understand a set of transcription factors which mutually regulate each other, thereby stabilizing the differentiation status of a cell. The concept of a transcriptional core network has been extremely fruitful in the case of embryonic stem cells which eventually led to the discovery of a defined set of transcription factors that allow for the reprogramming of somatic cells (Jaenisch and Young, 2008; Takahashi and Yamanaka, 2006). Forced expression of Oct4, Sox2, Klf4 and c-myc eventually superimposes onto fibroblasts a transcriptional circuitry such that these stably acquire the status of pluripotency. Is there a similar transcriptional circuitry underlying the differentiation state of a "neural stem cell"? The observation that neural stem cells are fate-restricted in a region-specific manner, at least with respect to neuronal subtype specification (Merkle et al., 2007), may suggest that there is indeed not a single transcriptional core network conveying the status of a neural stem cell, but potentially more than one. Yet, SEZ stem cells can be easily forced to acquire other neuronal subtype identity following forced expression of appropriate neurogenic fate determinants (Berninger et al., 2007b; Brill et al., 2008; Hack et al., 2005) indicating that they possess the competence to correctly interpret these transcriptional cues. These may argue in favour of a neural stem cell core network that is not region-specific, but universal. Furthermore, some transcription factors such as Sox2 (Cavallaro et al., 2008; Favaro et al., 2009; Graham et al., 2003; Kessaris et al., 2006; Suh et al., 2007; Taranova et al., 2006), Tlx (Liu et al., 2008; Roy et al., 2004; Shi et al., 2004) and Bmi-1 (Fasano et al., 2009; Molofsky et al., 2005, 2003; Moon et al., 2008; Zencak et al., 2005) appear to play a critical role in the maintenance and self-renewal of NSCs of different regions and developmental stages, suggesting that these might be either constituents of a NSC transcriptional network or at least closely related to it. A functional proof for the existence of a NSC transcriptional core network would be provided if forced expression of transcription factors defining this network could superimpose a NSC fate on parenchymal astroglia or even other types of somatic cells that are ontogenetically more distant than astrocytes from NSCs.

Two aspects merit further consideration: first the surprising closeness of neural to pluripotent stem cells. While fibroblast require several transcription factors for reprogramming into induced pluripotent stem cells, sole expression of Oct4 is sufficient to reprogram mouse adult as well as human foetal neural stem cells into pluripotent cells (Kim et al., in press, 2009). These data suggest that the stable transition between a neural and a pluripotent stem cell core networks may be rather simple, and reflect the other side of the coin that neural determination often appears a default pathway of embryonic stem cell differentiation (Tropepe et al., 2001). Such

facile transition may reflect a high degree of commonalities between these core networks, as exemplified by the common expression of Sox2.

The second point deals with the question posed at the beginning of the paragraph: How can a NSC core network co-exist with a hypothetical transcriptional core network conveying "glianess" within a single cell? Obviously, a NSC transcriptional network is not active in all astroglia, as classical parenchyma astroglia are not functioning as NSCs. However, some candidate constituents like Sox2 are expressed not only in NSCs, but also in parenchymal astroglia (Komitova and Eriksson, 2004). Other candidate genes such as Tlx seem to be restricted to stem cell astroglia (Liu et al., 2008), and loss of Bmi-1 even increases the genesis of non-stem cell astroglia at the expense of NSCs (Zencak et al., 2005), while forced expression of Bmi-1 induces stem cell characteristics in cultured astroglia (Moon et al., 2008). Thus, there seems to be a clear partitioning between stem cell and parenchymal astroglia. Yet, as the studies in the injured cerebral cortex or retina have shown, parenchymal astroglia have the capacity to regain NSCs characteristics (Buffo et al., 2008; Karl et al., 2008). Possibly, this capacity is due to a partial overlap of the two otherwise distinct transcriptional circuits, as represented by the common expression of Sox2, which may enable astroglia in some circumstances to switch from one to the other mode of being, i.e. being stem or parenchymal glia.

Acknowledgment

The authors would like to thank Dr. Christophe Heinrich for discussion and comments on the manuscript. Work by the authors was supported by the DFG, the BMBF and the Bavarian State Ministry of Sciences, Research and the Arts (for NeuroCell).

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