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The Complex States of Astrocyte Reactivity: How Are They Controlled by the JAK–STAT3 Pathway?

Kelly Ceyzeriat, Laurene Abjean, Maria-Angeles Carrillo-de Sauvage, Lucile Ben Haim, and Carole Escartin

Abstract—Astrocytes play multiple important roles in brain physiology. In pathological conditions, they become reactive, which is characterized by morphological changes and upregulation of intermediate filament proteins. Besides these descriptive hallmarks, astrocyte reactivity involves significant transcriptional and functional changes that are far from being fully understood. Most importantly, astrocyte reactivity seems to encompass multiple states, each having a specific influence on surrounding cells and disease progression. These diverse functional states of reactivity must be regulated by subtle signaling networks. Many signaling cascades have been associated with astrocyte reactivity, but among them, the JAK–STAT3 pathway is emerging as a central regulator. In this review, we aim (i) to show that the JAK–STAT3 pathway plays a key role in the control of astrocyte reactivity, (ii) to illustrate that STAT3 is a pleiotropic molecule operating multiple functions in reactive astrocytes, and (iii) to suggest that each specific functional state of reactivity is governed by complex molecular interactions within astrocytes, which converge on STAT3. More research is needed to precisely identify the signaling networks controlling the diverse states of astrocyte reactivity. Only then, we will be able to precisely delineate the therapeutic potential of reactive astrocytes in each neurological disease context. © 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

Keywords: reactive astrocytes, STAT3, JAK–STAT pathway, neurological diseases, signaling cascades.

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INTRODUCTION

In response to the multiple pathological conditions that affect the central nervous system (CNS), astrocytes become reactive. This response develops after acute injuries such as ischemia, traumatic brain injury (TBI), spinal cord injury (SCI) or infection, as well as under progressive conditions like neurodegenerative diseases (ND) or multiple sclerosis (MS). Astrocyte reactivity was initially characterized by morphological changes (hypertrophy of soma and processes) and by the upregulation of intermediate filament proteins such as glial fibrillary acidic protein (GFAP) or vimentin. Besides these two hallmarks, astrocyte reactivity involves multiple transcriptional and functional changes that are still being elucidated (Burda and Sofroniew, 2014; Pekny and Pekna, 2014; Ben Haim et al., 2015a). Importantly, astrocyte reactivity is now recognized as a heterogeneous response resulting in various functional states depending on the disease context. In fact, it is important to note that reactivity is not the only change observed in astrocytes during diseases. For example, astrocytes may be dystrophic in the brain of patients with schizophrenia or even degenerate following encephalopathies. They may become reactive without stimulation (Ben Haim et al., 2015a). Among them, the janus kinase-signal transducer and activator of transcription 3 (JAK–STAT3) pathway seems to play a central role that we will cover in this review.

We will focus on the roles of STAT3 in astrocytes during various brain diseases, but we will also describe data from other cell types in the CNS or peripheral organs, when they give insight into the functions of the JAK–STAT3 pathway. Indeed, seminal discoveries on this cascade were made in cell cultures in the fields of Immunology and Oncology. Although very potent, in vitro studies have serious limitations when applied to the brain, because of the significant phenotypic changes occurring when brain cells are isolated in a dish. This is especially true for primary astrocytes that tend to become reactive without stimulation (Ben Haim et al., 2015a). In this review, we will thus present in vivo studies whenever possible, and landmark in vitro studies to illustrate the complex roles played by STAT3 in the control of astrocyte reactivity. We will (i) show that the JAK–STAT3 pathway plays a key role in the control of astrocyte reactivity, (ii) illustrate that STAT3 is a pleiotropic molecule operating multiple functions in reactive astrocytes and (iii) propose that each specific functional state of reactivity is governed by complex molecular interactions within astrocytes, which converge on STAT3.

THE JAK–STAT3 PATHWAY

A linear, canonical JAK–STAT3 pathway from the membrane to the nucleus

The JAK–STAT pathway is a ubiquitous, evolutionarily conserved signaling cascade, present in various species from Dictyostelium to mammals (Decker and Kovarik, 2000). It was discovered more than twenty years ago, as the cascade mediating interferon effects (Darnell et al., 1994; Stark and Darnell, 2012). There are four JAKs (JAK1-3, and TYK2) and seven STATs (STAT1-4, 5A, 5B, and 6) in mammals (Darnell, 1997). STAT3 was sequenced and cloned in 1994 (Akira et al., 1994; Zhong et al., 1994b). STAT3 is well expressed in the brain (Zhong et al., 1994a) and has been the most extensively STAT studied in the context of astrocyte reactivity.

The canonical JAK–STAT3 pathway is activated by the binding of polypeptides such as cytokines, hormones or growth factors to their multimeric receptor (Mertens and Darnell, 2007, Fig. 1). Conformational changes on the intracellular tail of the receptor bring the kinase domains of two JAKs in apposition (Brooks et al., 2014). JAKs are receptor-associated tyrosine (Tyr) kinases that phosphorylate each other and the receptor on several residues. The latent transcription factor STAT3 is then recruited to the phosphorylated receptor through its Src homology 2 (SH2) domain and is transphosphorylated by JAK on Tyr705 (Lim and Cao, 2006). Phospho-STAT3 proteins dimerize and accumulate in the nucleus. There, dimers of phospho-STAT3 bind specific sequences called STAT3-responsive elements (SRE) in the promoter of target genes and induce their transcription (Shuai et al., 1993; Darnell, 1997). These transcriptional changes impact cell growth, proliferation, differentiation and survival. This pathway is particularly important during development and immune responses, and its dysregulation is involved in cancer and immune diseases (see, Yu et al., 2009; O’Shea et al., 2013, for a complete review, as this will not be developed here). Activation of the JAK–STAT3 pathway increases the expression of several elements of the pathway, including stat3 itself, which promotes a positive feedback loop (Hutchins et al., 2013).

Additional branching points on the pathway increase the complexity of STAT3 signaling cascades

Besides the linear “canonical” pathway, STAT3 is connected to alternative signaling cascades within the cell (Fig. 1). First, some G-protein-coupled receptors (GPCRs), which are seven-transmembrane domain receptors for growth factors or purines, may be coupled to JAKs and phosphorylate STAT3 (Mertens and Darnell, 2007). Alternately, STAT3 can be phosphorylated on Tyr705 by other upstream kinases than JAKs (Fig. 1). They include receptors with an intrinsic Tyr kinase activity, like the receptor for epidermal growth factor (EGF), and non-receptor Tyr kinases, which are usually cytoplasmic and of viral origin, such as v-src (Mertens and Darnell, 2007).

In addition, STAT3 can be phosphorylated on Serine 727 (Ser727) by various Ser kinases, especially by mitogen-activated protein kinases (MAPK) (Wen et al., 1995; Decker and Kovarik, 2000). Depending on the
Experimental conditions (stimulus, promoter studied, cell type), phosphorylation at Ser727 has positive or negative effects on Tyr705-dependent transcription (Decker and Kovarik, 2000). Activation of STAT3 via P2Y receptors was reported in astrocyte cultures, resulting in the phosphorylation of both Tyr705 and Ser727 (Washburn and Neary, 2006). Other toxic stimuli were reported to trigger Ser727 phosphorylation like TBI (Oliva et al., 2012, see Fig. 2), or lipopolysaccharide (LPS, Moravcova et al., 2016).

Lastly, besides phosphorylations, STAT3 is subjected to multiple other post-translational modifications (PTM, see Fig. 1) that modulate its transcriptional activity (Lim and Cao, 2006). STAT3 is acetylated on several Lysine (Lys) residues. Acetylation levels are regulated by the balance of activity between histone acetyl-transferases like p300/CREB binding protein and deacetylases like histone deacetylases (HDAC, Zhuang, 2013). Acetylation of Lys685 by p300 promotes STAT3 dimerization, nuclear...
localization and transcriptional activation (Wang et al., 2005; Yuan et al., 2005). STAT3 acetylation was recently observed in hypothalamic neurons (Chen et al., 2015) and cultured microglia (Eufemi et al., 2015) but its physiological role in astrocytes is largely unexplored. STAT3 may also be methylated on several Lys residues, which influences the pattern of genes regulated by STAT3 (Yang et al., 2010; Kim et al., 2013; Dasgupta et al., 2015). In vitro studies suggest that even non-phosphorylated STAT3 is able to increase the transcription of specific genes (Yang and Stark, 2008).

Overall, STAT3 represents a hub for multiple signaling cascades that converge towards the nucleus to modulate transcriptional activity.

STAT3 has non-canonical functions independent of transcription

Another layer of complexity in STAT3 signaling was discovered more recently, when STAT3 was found to be more than a simple transcription factor (see, Gao and Bromberg, 2006, and Fig. 1). In the nucleus, STAT3 also induces global chromatin remodeling (Li, 2008). STAT3 is able to interact with microtubules to regulate their stability (Ng et al., 2006, and section "STAT3 modulates astrocyte morphology and migration"). In the cytoplasm, STAT3 may also participate in the control of the autophagic flow by binding to protein kinase R (PKR, Shen et al., 2012, and section "Other important functions for STAT3 in astrocytes") or contribute to long term depression in hippocampal neurons, by yet unknown mechanisms independent of transcription (Nicolas et al., 2012). Last, STAT3 is found in mitochondria where it modulates their functions (Yang and Rincon, 2016, and section "STAT3 regulates mitochondrial functions").

It is important to note that most of these alternative, non-transcriptional STAT3 functions have been characterized in cancer and immune cells in vitro (Mohr et al., 2012). Whether they also occur in astrocytes in situ, is mostly unknown.

**Box 1: An extensive toolbox to study the JAK–STAT3 pathway**

There are many molecular tools and techniques to study the JAK–STAT3 pathway in the brain.

A. To evidence the activation of the pathway by biochemical & histological techniques:

- Detection of phosphorylated active forms (e.g. gp130, JAK, STAT3). Phospho-specific antibodies may be used for western blotting, ELISA or immunostaining on slices, cultured cells or cell suspensions for cytomtery analysis.
- Detection of STAT3 nuclear translocation by immunostaining or subcellular fractionation.
- Detection of transcriptional induction of target genes by RT-qPCR (e.g. socs3, stat3).
- Detection of STAT3 binding to specific DNA sequences by electromobility shift assay or chromatin immunoprecipitation.

B. To monitor the activity of the pathway with reporter systems:

- To monitor transcriptional induction: Consensus SRE (TTrG(N)3CAA), coupled with a minimal promoter control the expression of a fluorescent protein or an enzyme whose activity is easily measured (e.g. luciferase, alkaline phosphatase, β-galactosidase). Such reporters are generally used in vitro, but they could be used to produce viral vectors (Gabel et al., 2010) to infect brain cells or to generate transgenic reporter mice.
- To monitor STAT3 intracellular trafficking: STAT3 is tagged with a fluorescent protein, and its subcellular location and trafficking is monitored by time-lapse fluorescent microscopy or fluorescent recovery after photobleaching (FRAP, Selvaraj et al., 2012).
- To monitor the formation of protein complexes: Förster resonance energy transfer (FRET) imaging can be used to reveal protein-protein interactions, for example, when STAT3 dimerizes (Kretzschmar et al., 2004) or when JAK2 is activated at the growth hormone receptor (Brooks et al., 2014).

C. To modulate the JAK–STAT3 pathway:

- Pharmacological inhibitors: They are numerous inhibitors of JAKs and STAT3. They were initially developed for the treatment of cancer and immune diseases (Yu et al., 2009; O’Shea et al., 2013). AG490 and Static are two commonly used inhibitors of JAK2 and STAT3 respectively (Meydan et al., 1996; Schust et al., 2006). Their use may be limited by poor blood brain barrier penetration and their potential side-effects on multiple cell types in the brain and periphery.
- Conditional KO: Given the important developmental and peripheral roles of the pathway, conditional recombination is required to restrict expression of the Cre recombinase to astrocytes by specific promoters such as the glap promoter (Herrmann et al., 2008). It is also advisable to control the time window of recombination with tet-operated promoters or by using tamoxifen inducible Cre (Pfrieger and Slezak, 2012).
- Expression of inhibitors or dominant negative mutants: Several mutant forms of STAT3 have been developed. They act as dominant negatives [e.g. STAT3(3215), STAT3D with a mutation in the DNA binding domain (Nakajima et al., 1996; He et al., 2005; Kohno et al., 2013)]. Alternatively, expression of endogenous inhibitors like SOCS3 in astrocytes prevents STAT3 activation (Ben Haim et al., 2015).
- Expression of constitutively active mutants: Introduction of two cysteine residues by point mutation in STAT3 was used to create STAT3-C, a mutant that should be capable of auto-dimerization by disulfide bonds (Bromberg et al., 1999). Recent studies suggest that STAT3-C still needs to be phosphorylated to bind DNA, but it does so with greater efficacy (Li and Shaw, 2006).
THE JAK–STAT3 PATHWAY IS A UNIVERSAL INDUCER OF ASTROCYTE Reactivity

Astrocytes become reactive in response to various pathological conditions affecting the CNS. While several signaling cascades are found activated in reactive astrocytes over the course of diseases or following injuries (Kang and Hebert, 2011; Ben Haim et al., 2015a), STAT3 appears as a key regulator of astrocyte reactivity.

Activation of the JAK–STAT3 pathway in acute diseases

STAT3 activation is detected by its phosphorylation, nuclear translocation and/or nuclear accumulation (Box 1). Activated STAT3 is observed in reactive astrocytes in various acute injury models, including TBI (Li and Shaw, 2006; Oliva et al., 2012 and see Fig. 2), excitotoxicity (Acarin et al., 2000), neonatal white matter injury (Nobuta et al., 2012), neuropathic pain (Tsuda et al., 2011), axotomy (Xia et al., 2002; Schubert et al., 2005; Tyzack et al., 2014), infection with scrapie (Na et al., 2007), glaucoma (Zhang et al., 2013), epilepsy (Choi et al., 2003b; Xu et al., 2011), ischemia (Justicia et al., 2000; Choi et al., 2003a) and after exposure to neurotoxins (Sriram et al., 2004; O’Callaghan et al., 2014) or LPS (Gautron et al., 2002). The activation of upstream cascades was explored in some studies: JAK2 and the cytokine receptor gp130 are also phosphorylated in a rat TBI model (Oliva et al., 2012, and see Fig. 2); JAK2 (but not JAK1 or Tyk2) is also activated by the injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, Sriram et al., 2004).

Overall, there is ample evidence that activation of the JAK–STAT3 pathway is associated with astrocyte reactivity. However, to demonstrate that STAT3 is really required for astrocyte reactivity, it is necessary to interfere with it (see Box 1). This was nicely demonstrated in the case of the glial scar, a dense structure of cells that aggregate at the site of parenchyma disruption (Sofroniew, 2009; Pekny and Pekna, 2014; Ben Haim et al., 2015a). Conditional knock-out (KO) of stat3 in reactive astrocytes (Nestin-Cre × stat3fl/fl) reduces glial scar formation while socs3 deletion (Nestin-Cre × socs3fl/fl) has opposite effects (Okada et al., 2006). Similarly, stat3 KO in astrocytes (Gfap-Cre × stat3fl/fl) attenuates GFAP upregulation and disrupts glial scar formation after SCI (Herrmann et al., 2008; Wanner et al., 2013).

STAT3 is also responsible for astrocyte reactivity in acute disease models without glial scarring. For example, pharmacological inhibition of JAK2 decreases STAT3 activation and astrocyte reactivity after hypoxic-ischemic damage in the neonatal mouse brain (Hristova et al., 2016), in the epileptic rat hippocampus (Xu et al., 2011), after MPTP injection (Sriram et al., 2004) and in a model of peripheral nerve injury (Tsuda et al., 2011). Khor et al. later used viral gene transfer of a dominant negative (DN) form of STAT3 to inhibit this cascade more specifically in spinal astrocytes after peripheral nerve injury in mice. They demonstrate that this pathway contributes to astrocyte reactivity, as the mRNA levels of gfap, vimentin and socs3 were reduced by STAT3-DN (Kohro et al., 2015). Genetic studies based on Gfap-Cre × stat3fl/fl transgenic mice further established the importance of STAT3 for astrocyte reactivity. By contrast to controls, transgenic mice display reduced astrocyte hypertrophy and GFAP upregulation following neonatal white matter injury in the brain (Nobuta et al., 2012) and spinal cord (Monteiro de Castro et al., 2015), axotomy (Tyzack et al., 2014), in a model of chronic itch (Shiratori-Hayashi et al., 2015) or after exposure to a range of neurotoxins causing neuronal death in different brain regions and species (O’Callaghan et al., 2014). Interestingly, STAT3 involvement in astrocyte reactivity is conserved in Drosophila. Stat92E, the Drosophila homolog of STAT3, controls glial reactivity after axonal injury (Doherty et al., 2014).

These results show that STAT3 is a central regulator of glial reactivity, conserved across evolution, as well as between disease conditions.

Activation of the JAK–STAT3 pathway in ND

In progressive pathological conditions such as ND, where astrocyte reactivity, neuroinflammation and neuronal death are gradually established over years, STAT3 appears to play a central role as well. STAT3 activation is reported in reactive astrocytes of patients with MS (Lu et al., 2013) or amyotrophic lateral sclerosis (ALS, Shibata et al., 2009). Similarly, STAT3 activation is found in mouse models of ALS (Shibata et al., 2010) and Alzheimer’s disease (AD, Ben Haim et al., 2015b and see Fig. 2), in pharmacological models of Parkinson’s disease (PD, Sriram et al., 2004; O’Callaghan et al., 2014) and in mouse and primate models of Huntington’s disease (HD, Ben Haim et al., 2015b).

While STAT3 activation is consistently detected in reactive astrocytes in ND models, few studies have investigated its role in the establishment of astrocyte reactivity (see Ben Haim et al., 2015a for review). Pharmacological inhibition of JAK2 and astrocyte-specific KO of stat3 in the MPTP model of PD attenuates astrocyte reactivity (Sriram et al., 2004; O’Callaghan et al., 2014). A similar reduction in reactivity is observed with JAK2 inhibition in a pharmacological model of HD based on striatal injection of an excitotoxin (Ignarro et al., 2013). To improve cell-type specificity, our group used lentiviral vectors to overexpress SOCS3 selectively in astrocytes, in mouse models of AD and HD (Ben Haim et al., 2015b). SOCS3 overexpression prevented STAT3 activation and GFAP upregulation in astrocytes of AD and HD mice. Furthermore, SOCS3-expressing astrocytes displayed resting-like morphology, demonstrating that the JAK–STAT3 pathway is a key pathway mediating astrocyte reactivity in ND models.

The JAK–STAT3 pathway is a central mediator of astrocyte reactivity in a variety of pathological conditions in the CNS. What are the functional outcomes of this cascade in reactive astrocytes?
WHAT DOES STAT3 DO IN ASTROCYTES?

**STAT3 induces the expression of intermediate filament proteins**

One of the best-known target genes of STAT3 is *gfap*, whose induction in astrocytes is the primary hallmark of their reactive state (Hol and Pekny, 2015). As described in section “The JAK–STAT3 pathway is a universal inducer of astrocyte”, pharmacological inhibition or genetic invalidation of *stat3*, consistently prevents or reduces the increase in *gfap* mRNA and/or protein levels in astrocytes following induction of reactivity. In fact, levels of GFAP are also reduced by STAT3 inhibition or invalidation in non-lesioned groups (Herrmann et al., 2008; Wanner et al., 2013; Levine et al., 2015), suggesting that STAT3 controls the basal expression of GFAP. The human *gfap* promoter is well characterized; it displays at least one SRE that contributes to high GFAP expression in multiple brain regions (Yeo et al., 2013). These SREs are conserved in the rodent *gfap* promoter (Nakashima et al., 1999). Vimentin, another intermediate filament characteristic of reactive as well as immature astrocytes, is similarly regulated by STAT3 (Herrmann et al., 2008). Interestingly, STAT3-mediated induction of intermediate filaments in reactive astrocytes recapitulates an important signaling cascade occurring at the time of astrogliogenesis (Kanski et al., 2014). The JAK–STAT3 pathway is inhibited during neurogenesis and activation of STAT3 coincides with the expression of glial markers GFAP and S100b at E18.5 in mice (He et al., 2005). STAT3 binds to the *gfap* promoter and increases GFAP expression in cortical progenitors (Bonnì et al., 1997). STAT3-dependent induction of GFAP is modulated by the pattern of histone and DNA methyla-
tion of the *gfap* promoter (Takizawa et al., 2001; Song and Ghosh, 2004).

**STAT3 controls proliferation of a subset of reactive astrocytes**

The JAK–STAT3 pathway is involved in normal proliferation of neuronal precursor cells during development (Kim et al., 2010) and abnormal proliferation of cancer cells (de la Iglesia et al., 2009; Yu et al., 2009). Does STAT3 also promote the proliferation of reactive astrocytes?

Recent studies show that contrary to the common belief, proliferation of reactive astrocytes is quite limited, being transient or involving only a small percentage of astrocytes (5–10%), especially in chronic diseases or injuries that do not involve rupture of the blood brain barrier (Dimou and Gotz, 2014). These subsets of proliferative astrocytes are located in specific niches, in contact with the vasculature (Bardehle et al., 2013) or the lesion core (Wanner et al., 2013; LeComte et al., 2015) and display stem cell properties (Sirkö et al., 2013). There is some indirect evidence that STAT3 controls the proliferation of reactive astrocytes. JAK inhibitors reduce the number of proliferating reactive astrocytes following spinal injury (Tsuda et al., 2011). The formation of the glial scar, which is composed of newly proliferated astrocytes, is also altered in *Gfap-Cre × stat3*Δ/Δ transgenic mice (Wanner et al., 2013; Anderson et al., 2016).

By which mechanisms does STAT3 control astrocyte proliferation? In cancer cells, it is well known that STAT3 promotes the expression of cell cycle genes, like cyclin D1 (Yu et al., 2009), and this regulation also occurs in cultured astrocytes (Sarafian et al., 2010). In addition, STAT3 activates the expression of anti-apoptotic genes (Sarafian et al., 2010, see section “STAT3 regulates mitochondrial functions”), which may promote the survival of proliferating reactive astrocytes. More recently, Lecomte et al. showed that following middle cerebral artery occlusion (MCAO), STAT3 is activated within a subset of reactive astrocytes sensitive to Notch signaling. STAT3 promotes the expression of the endothelin B receptor, which acts in an autocrine manner to stimulate the proliferation of this subset of cells (LeComte et al., 2015).

Of note, it was shown recently that the morphogen Sonic Hedgehog (Shh), which is abundant in the cerebrospinal fluid, also controls the proliferation of reactive astrocytes and their stem cell potential after invasive injuries (Sirkö et al., 2013). Whether and how the Shh cascade interacts with STAT3 in astrocytes is unknown.

**STAT3 regulates the secretome of reactive astrocytes**

In immune cells, STAT3 is an established transcriptional activator of cytokines (Yu et al., 2009). In astrocytes as well, STAT3 regulates the production of cytokines and chemokines during reactivity. Pharmacological inhibition of the JAK–STAT3 pathway reduces mRNA levels of interleukin 6 (*IL-*6), *IL-1β*, *IL-4* and vascular endothelial growth factor by astrocytes made reactive by high glucose concentration in culture (Wang et al., 2012). Likewise, expression of a siRNA against STAT3 reduces LPS-mediated induction of the chemokines Ccl20, Cx3cl1, Cxcl5 and Cxcl10 in primary astrocytes. Similar effects are observed in vivo, after intrathecal injection of the STAT3 inhibitor Statitty, in a LPS model of inflammation (Liu et al., 2013).

Lipocalin-2 (Lcn2) is one of the proteins released by reactive astrocytes (Zamanian et al., 2012). Its specific function in the brain is still unclear (Jha et al., 2015), but it may serve as an inflammatory mediator, as shown under chronic itch conditions (Shiratori-Hayashi et al., 2015). Lcn2 production by reactive astrocytes is dependent upon STAT3, as demonstrated by pharmacological inhibition in cultured astrocytes and astrocyte-specific KO of *stat3* in mice (Shiratori-Hayashi et al., 2015).

Molecules released by reactive astrocytes may further activate microglial cells or recruit immune cells from the periphery, contributing to a feed forward effect on neuroinflammation. Indeed, specific inhibition of the JAK–STAT3 pathway in astrocytes by SOCS3 reduces the mRNA levels of the microglial markers Iba1 and *CD11b* in a mouse model of HD (Ben Haim et al., 2015b). Likewise, the astrocyte-specific KO of *stat3* reduces microglial reactivity induced by hypoxia-ischemia (Hristova et al., 2016). The factors released by astrocytes in a STAT3-dependant manner do not only activate microglial cells but also modulate their activity. For example, reactive astrocytes release yet unidentified factors that reduce the production of transforming growth factor β by microglia in culture (Nobuta et al., 2012).

The STAT3-dependent release of proteins by reactive astrocytes not only impacts microglial cells but also neurons. For example, facial nucleus axotomy increases mRNA levels of *thrombospondin-1* (*tsp1*, a secreted protein promoting synapse formation and stability during development) in reactive astrocytes. This induction is reduced in *Gfap-Cre × stat3*Δ/Δ mice, which is associated with decreased synapse number and activity in neighboring neurons. Therefore, reactive astrocytes produce TSP1 in a STAT3-dependent manner, as further demonstrated by the direct binding of STAT3 to the *tsp1* promoter in astrocyte cultures (Tyzack et al., 2014). Recently, Anderson et al. showed that reactive astrocytes forming the glial scar after SCI, express a range of molecules in a STAT3-dependent manner (like chondroitin surface proteoglycans), which overall have axon-growth promoting effects (Anderson et al., 2016).

**STAT3 modulates astrocyte morphology and migration**

STAT3 plays a role in the migration of multiple cell types including glioblastoma cells (Zhang et al., 2015) and reactive astrocytes (Okada et al., 2006). The deletion of *Stat3* in reactive astrocytes (*Nestin-Cre × stat3*Δ/Δ) reduces the migration of reactive astrocytes after *in vitro* scratch injury. Impaired migration after SCI could be responsible for the altered wound closure and enhanced infiltration of inflammatory cells observed in these mice. The opposite is observed in *Nestin-Cre × socs3*Δ/Δ mice whose astrocytes display a quicker migration *in vitro* and an efficient compaction of the lesion in the spinal cord (Okada
et al., 2006). In different cell types, STAT3 regulates the transcription of genes implicated in matrix remodeling such as matrix metallo-proteinases MMP-1, MMP-2 and MMP-10 and the zinc transporter LIV-1, which regulates the expression of the adhesion molecule E-cadherin (Gao and Bromberg, 2006). In the spinal cord as well, Nestin-Cre × stat3 KO mice exposed to SCI display lower mRNA levels of LIV-1 than wild-type littermates and a concomitant increase in E-cadherin levels (Okada et al., 2006). The impairment of cell migration may thus be explained by the altered expression of cell adhesion proteins in absence of STAT3. But STAT3 may also regulate migration by non-canonical mechanisms. In neuronal cell lines and in fibroblasts, STAT3 was shown to interact with stathmin, a cytoplasmic protein that binds to tubulin and prevents its assembly into microtubules (Ng et al., 2006). The interaction of STAT3 with stathmin is also observed in cultured motoneurons, it promotes microtubule stability and axonal elongation. Intriguingly, this interaction requires Tyr705 phosphorylation but no transcriptional regulation (Selvaraj et al., 2012). It is currently unknown whether such non-canonical effects of STAT3 are involved in the migration of reactive astrocytes.

In fact, reactive astrocytes display limited capacity to migrate towards an injury site (Bardehle et al., 2013; Wanner et al., 2013); instead, astrocyte reactivity is characterized by striking morphological changes (hypertrophy, reorientation of processes). Such morphological plasticity appears to be regulated by STAT3 as well. During glial scar formation following SCI, the reorientation of astrocyte processes parallel to the lesion and the formation of bundles between apposed astrocytes are important to “corral” immune cells and fibroblasts. These morphological changes are disturbed in Gfap-Cre × stat3 KO mice (Wanner et al., 2013). Following axotomy of the facial nucleus, reactive astrocytes increase their coverage of axotomized neuronal cell bodies, a response found altered in stat3 KO astrocytes (Tyzack et al., 2014). Similarly, STAT3 induces the expression of growth associated protein 43, which contributes to increased process arborization in primary rat astrocytes exposed to LPS (Hung et al., 2016), confirming STAT3 importance for different types of morphological rearrangements. It is noteworthy that in neurons, STAT3 is also an important regulator of morphology and it promotes axonal regrowth (Moore and Goldberg, 2011).

STAT3 regulates mitochondrial functions

Interestingly, a pool of STAT3 proteins is present at the mitochondria (mSTAT3) and interacts with the complexes I and II of the electron transport chain (ETC) in the murine heart and liver (Wegrzyn et al., 2009). The KO of stat3 in cultured B cells reduces complex I and II activities, which is restored by viral gene transfer of stat3 (Wegrzyn et al., 2009). Strikingly, the effects of mSTAT3 on the ETC are mediated by its mitochondrial localization and its phosphorylation on Ser727 and not by its transcriptional activity. In other experimental conditions however, STAT3 does modulate metabolism by transcriptional regulation of genes involved in glycolysis and mitochondrial respiration. In particular, STAT3 induces the expression of hypoxia inducible factor 1α, which promotes glycolysis over mitochondrial respiration (Demaria et al., 2010).

Does STAT3 play similar roles in astrocytes? Sarafian et al. found that cultured astrocytes from Gfap-Cre × stat3 KO mice display lower mitochondrial mass and membrane potential and reduced ATP production (Sarafian et al., 2010). Inhibition of JAK2 by AG490 in wildtype astrocytes reproduces the decrease in mitochondrial membrane potential, suggesting that STAT3 operates through a canonical cascade involving JAK2 phosphorylation. The expression of several ETC enzymes is decreased in stat3 KO astrocytes, further confirming that STAT3 regulation of astrocyte metabolism is controlled at the transcriptional level.

STAT3 may also lower the production of reactive oxygen species (ROS) by the mitochondria, although the mechanisms are still unclear (Szczepanek et al., 2012; Yang and Rincon, 2016). Indeed, stat3 KO astrocytes display increased generation of ROS and reduced levels of the antioxidant glutathione (Sarafian et al., 2010). STAT3 also promotes ROS detoxification by activating the expression of several antioxidant genes. For example in the mouse brain, STAT3 directly binds to the promoter of the manganese superoxide dismutase (MnSOD) gene, a mitochondrial enzyme that metabolizes superoxide anions (Jung et al., 2009). Inhibition of the JAK2–STAT3 pathway by AG490 or STAT3 decoy DNA promoter of the manganese superoxide dismutase (MnSOD) gene, a mitochondrial enzyme that metabolizes superoxide anions (Jung et al., 2009). Inhibition of the JAK2–STAT3 pathway by AG490 or STAT3 decoy DNA

Other important functions for STAT3 in astrocytes

There are a few additional functions regulated by STAT3 that are important for brain homeostasis or response to injury.

Ozog et al. found that activation of the JAK2–STAT3 pathway by ciliary neurotrophic factor (CNTF) increases mRNA and protein levels of connexin 43 (Cx43),
resulting in an enhanced gap junction-coupling between astrocytes (Ozog et al., 2004). They identified three putative SRE on the cx43 promoter. Interestingly, CNTF-mediated increase in Cx43 levels is also observed in reactive astrocytes of the rat brain (Escartin et al., 2006). Gap junction coupling plays key roles in the normal and diseased brain, modulating synaptic transmission and metabolic supply to neurons (Giaume et al., 2010).

Recently, STAT3 was found to regulate autophagy, a key homeostatic mechanism whose alteration is linked to many brain diseases (Choi et al., 2013). Cytoplasmic STAT3, through a transcription-independent mechanism involving the binding to PKR, tonically suppresses autophagy in cell lines (Shen et al., 2012, see Fig. 1). STAT3 also regulates the expression of genes involved in the control of the autophagic flux (You et al., 2015).

Finally, Doherty et al. reported that Stat92E regulates the ability of glial cells to engulf debris of dead neurons by enhancing the expression of the Draper receptor in Drosophila (Doherty et al., 2014). Interestingly, they report that the upstream activator of Stat92E is the G-protein Rac1, and not the JAK homologue, suggesting that in Drosophila glia, non-canonical pathways operate as well.

Overall, the range of astrocyte functions regulated by STAT3 is extremely large, and most of them appear to be of great importance for neuronal survival in disease conditions.

**HOW DOES STAT3 GENERATE SO MANY FUNCTIONAL OUTCOMES?**

Previous paragraphs illustrate a puzzling fact: a unique pathway is able to control diverse functions ranging from cell proliferation to morphological remodeling in many cell types and organs. Even within reactive astrocytes, STAT3 mediates various effects depending on the disease, brain region or model studied. How does a single protein trigger so many functional outcomes?

An easy explanation is that STAT3 does not act alone. Besides the JAK–STAT3 pathway, other signaling cascades may be activated in reactive astrocytes (Buffo et al., 2010; Kang and Hebert, 2011; Ben Haim et al., 2015a) and STAT3 may interact with other transcription factors in the nucleus (Hutchins et al., 2013).

**Interactions between the JAK–STAT3 pathway and other cascades**

For example, the MAPK pathways and the nuclear factor-kappa-light-chain-enhancer of activated B cells (NF-kB) pathway are sometimes found activated in reactive astrocytes (Ben Haim et al., 2015a). For example, IL-6 activates the MAPK and STAT3 pathways, and the equilibrium between the two is controlled by a Tyr motif on the IL-6 receptor (Eulenfeld et al., 2012). Many interactions may take place between the JAK–STAT3 and these other pathways (Yu et al., 2009): they may act in synergy on the same target genes, activate one another in cascade (e.g. STAT3 target genes are activators of the NF-kB pathway, and reciprocally) or regulate one other (e.g. MAPKs phosphorylate STAT3 on Ser727, modulating its transcriptional activity). Conversely, these pathways can inhibit one another or compete for binding sequences on gene promoters (Ockinghaus et al., 2011).

The interaction between the JAK–STAT3 pathway and other signaling cascades is particularly well studied in the context of astrogliogenesis. The JAK–STAT3 pathway interacts with the fibroblast growth factor 2 pathway (Song and Ghosh, 2004), the Notch/Hes pathway (Kamakura et al., 2004), and the bone morpho-genetic protein-2/Smad1 pathway (Nakashima et al., 1999), to activate the gfap promoter during development (see, Kanski et al., 2014 for a recent review).

The pattern of activation of these cascades in a disease-specific manner may underlie the diverse transcriptional and functional outcomes observed in reactive astrocytes.

**A signaling code**

Even without resorting to interactions with other signaling cascades, the canonical JAK–STAT3 pathway has already a large potential for complexity and signaling subtleties. There are many ligands, acting on different receptors, activating several JAKs; STAT3 can form heterodimers with other STATs, and the pathway is retro-controlled by several inhibitors (see details in section “The JAK–STAT3 pathway”). New modulators or interactors of the pathway are still being discovered (Icardi et al., 2012; Matsuda et al., 2015). Overall, there is an immense potential for signaling complexity within the JAK–STAT3 pathway (Ernst and Jenkins, 2004).

The upstream ligands activating STAT3 influence the resulting transcriptional and functional effects. For example, IL-6 and IL-10, two cytokines relying on STAT3 as their effector, have opposite effects on inflammatory processes. Computational modeling and experiments on dendritic cells revealed that this could be explained by different temporal profiles of STAT3 activation, resulting in different transcriptional outcomes (Braun et al., 2013). An emerging theme in cell signaling is that the intensity, duration, frequency and pattern of receptor stimulation encode information that translates into different transcriptional profiles (Lemmon et al., 2016).

Another level of complexity resides in the numerous PTMs on STAT3 (see Fig. 1). The pattern of PTMs constitutes a molecular code that significantly impacts the profile of genes regulated at a given time by STAT3 (see section “Additional branching points on the pathway increase the complexity of STAT3 signaling cascades”).

Finally, the non-canonical actions that STAT3 performs outside of the nucleus may further diversify STAT3 functional outputs in reactive astrocytes. How these non-canonical functions are regulated in the context of astrocyte reactivity is an open question.

Overall, the JAK–STAT3 pathway is composed of multiple elements that can generate significant signaling
complexity and contribute to the observed diversity in astrocyte reactivity.

Differential abilities to engage in STAT3 signaling

The functional outcomes mediated by STAT3 may be influenced by the cell’s ability to mediate STAT3 signaling. Indeed, depending on the specific status of the cell, its response to the same stimulus on the JAK–STAT3 pathway will be different. Many factors can influence the capacity of a cell to efficiently operate the JAK–STAT3 pathway, including the abundance of pathway inhibitors, its epigenetic status, the activity of molecular machinery involved in signal transduction (e.g. nuclear import, ATP-dependent phosphorylation) and termination (e.g. nuclear export, phosphatase activity, degradation by the proteasome).

For example, the JAK–STAT3 pathway will be more or less active, depending on how much of SOCS and PIAS inhibitors are present and located at the right place to inhibit this pathway. SOCS3 is strongly induced by the JAK–STAT3 pathway, depending on the “signaling history” of the cell, the abundance of SOCS3 will vary (Linossi and Nicholson, 2015).

STAT3 binding to promoters is influenced by DNA and histone methylation, a regulation well described in the context of astrogliogenesis (Kanski et al., 2014, and section “STAT3 induces the expression of intermediate filament proteins”). Therefore, it is expected that the epigenetic status of the cell will impact the transcriptional outcomes of the JAK–STAT3 pathway.

The disease context itself may influence how a cell is able to respond to a stimulation of the JAK–STAT3 pathway. For example in Drosophila, the formation of tau aggregates in glia reduces STAT3-dependent promoter activity (Colodner and Feany, 2010). Likewise, the amyloid β protein reduces the activity of the JAK–STAT3 pathway in neurons (Chiba et al., 2009).

Global impairment in the cell’s ability to engage in a STAT3 response may occur in brain diseases. Several elements of the JAK–STAT3 pathway are sensitive to ROS, which are produced in many brain diseases (Duhe, 2013). Likewise, there is a consistent alteration in energy production in ND (Lin and Beal, 2006), which could affect the multiple energy-dependent steps of this cascade (e.g. phosphorylation, nuclear translocation, transcriptional induction). The activity of the JAK–STAT3 pathway is reduced in white matter astrocytes exposed to hypoxia (Raymond et al., 2011) and in the mouse brain after MCAO (Jung et al., 2009), confirming the importance of energy supply for proper STAT3 signaling. Finally, the nucleocytoplasmic transport is altered in some ND, due to the scavenging of key components of this system in toxic protein aggregates (Da Cruz and Cleveland, 2016), and this could directly prevent STAT3 signaling to the nucleus.

Therefore, depending on the disease and its stage of evolution, astrocytes will have variable intrinsic capabilities to trigger a STAT3-dependent response. This probably also contributes to the significant heterogeneity of the functional responses of reactive astrocytes in each disease context.

CONCLUSIONS AND PERSPECTIVES

Over the last decade, it has become clear that the JAK–STAT3 pathway, a signaling cascade initially described in the immune system, is very important for astrocyte development and response to injury. A thorough molecular dissection of the multiple interactors and regulators of this cascade has been performed in cell lines. It is now time to integrate this knowledge in the study of astrocyte response in vivo, and this is not a simple task (see Box 2). STAT3 appears to orchestrate numerous molecular and functional changes in reactive astrocytes. Much remains to be done to understand how a central signaling cascade mediates so many functional outcomes in astrocytes.

Deciphering the molecular code of astrocyte reactivity holds promising prospects for basic and medical science. It would make it possible to understand how the brain responds to each disease situation and to develop novel, efficient and, specific therapeutic strategies.

Box 2: Future questions and challenges

- What are the endogenous activators of this pathway during brain diseases (e.g. cytokines, growth factors, danger associated molecular patterns)?
- What is the time course of activation of the JAK–STAT3 pathway in reactive astrocytes, in relation to microglial activation or neuronal death?
- What are the effects of PTMs on STAT3 in reactive astrocytes?
- What are the non-canonical functions of STAT3 in reactive astrocytes, unrelated to transcriptional regulation?
- Can we define the networks of signaling cascades that control each specific functional state in reactive astrocytes?
- Can this pathway be targeted for therapeutic purposes?

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