All the Embryo's a Stage, and Olig2 in Its Time Plays Many Parts

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Olig2 is essential for the selection of motor neuron and oligodendrocyte fates and the choice of neural progenitors to either proliferate or differentiate. Three new studies demonstrate that these diverse actions of Olig2 are gated by phosphorylation at two distinct motifs and that Olig2's proliferative function acts in opposition to the p53 tumor suppressor pathway.

The exquisite cellular diversity and architecture of the central nervous system arises from the actions of a small number of regulatory molecules that are reused at different times to achieve radically disparate ends. A notable example of this phenomenon is the basic-helix-loop-helix (bHLH) transcription factor Olig2, which plays a central role in directing cell fate choices and controlling cell proliferation. In the early embryo, Olig2 first establishes a ventral domain of motor neuron progenitors and then promotes their cell cycle exit and neuronal differentiation (Mizuguchi et al., 2001; Novitch et al., 2001). Later in development, Olig2 directs the formation of oligodendrocyte precursors and mature oligodendrocytes (Lu et al., 2002; Park et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002; Zhou et al., 2001). Within the adult forebrain, Olig2 is expressed by a subset of Type C transit amplifying progenitors in the subventricular zone as well as cycling $NG2^+$ oligodendrocyte precursors (Jackson et al., 2006; Menn et al., 2006). Olig2 is further associated with pathological proliferation in all grades of glioma, and its presence strongly correlates with tumor forming potential (Ligon et al., 2007).

Olig2 thus acts as a multifaceted transcription factor: promoting both neuronal and glial fates and directing the differentiation or proliferation of progenitors at different places and times. The basis of these diverse activities, however, has remained mysterious. In this issue of *Neuron*, two studies by Li et al. (2011) and Sun et al. (2011) demonstrate that Olig2's activities in both cell fate specification and cell proliferation are guided by specific phosphorylation events. A companion study by Mehta et al. (2011) published in *Cancer Cell* further reveals that Olig2's ability to stimulate neural stem cell proliferation is mediated through its ability to oppose the p53 tumor suppressor pathway. Together, these studies cast a new spotlight on the way in which posttranslational modifications enable one protein to coordinate multiple functions in the developing nervous system.

Basic-helix-loop-helix (bHLH) transcription factors typically bind DNA as either homodimers or heterodimers with related proteins through their HLH dimerization domains. Li et al. (2011) demonstrate that the phosphorylation of Olig2 on Serine-147 (S147), a highly conserved residue within its HLH domain, strongly influences its dimerization preferences and thereby guides its activity in motor neuron and oligodendrocyte fate specification. Substitution of S147 with alanine (S147A) results in an Olig2 protein that is incapable of inducing motor neuron formation but still competent for oligodendrocyte precursor induction, both in vivo and in cultured cells. Interestingly, the Olig2 S147A mutant protein displays a reduced ability to form homodimers and instead shows a higher affinity for other bHLH proteins such as the proneural bHLH protein Ngn2. Thus, S147 phosphorylation appears to gate the composition of the Olig2 DNA binding complex, permitting Olig2 to form a motor neuron-producing homodimeric complex while removal of this phosphate favors the formation of heterodimeric complexes associated with oligodendrocyte formation (Figure 1).

These striking findings raise a number of questions. First, what is the endogenous composition of the Olig2 heterodimers associated with oligodendrocyte formation? Olig2 has been previously shown to be capable of associating with the bHLH proteins Ngn2 and E47 (Lee et al., 2005) and Li et al. (2011) indeed provide some evidence that Olig2 S147A has an increased affinity for Ngn2. However, since oligodendrocyte precursors do not normally express Ngn2, other dimerization partners may be more relevant in these cells. Second, how does Olig2's ability to form homodimers versus heterodimers explain its differential activities? It seems likely that the choice of binding partners could recruit Olig2 to distinct genomic sites important for either motor neuron or oligodendrocyte formation. Alternatively, Olig2 homodimer and heterodimer complexes could converge upon a common set of genes, but the nature of the complex could instead influence whether those genes are activated or repressed. These issues should be addressable through future investigations into the genomic targets of the Olig2 complexes in the context of both motor neuron and oligodendrocyte formation. Lastly, which kinases and phosphatases regulate S147 phosphorylation, and how are they controlled? Li et al. (2011) suggest that protein kinase A may be a

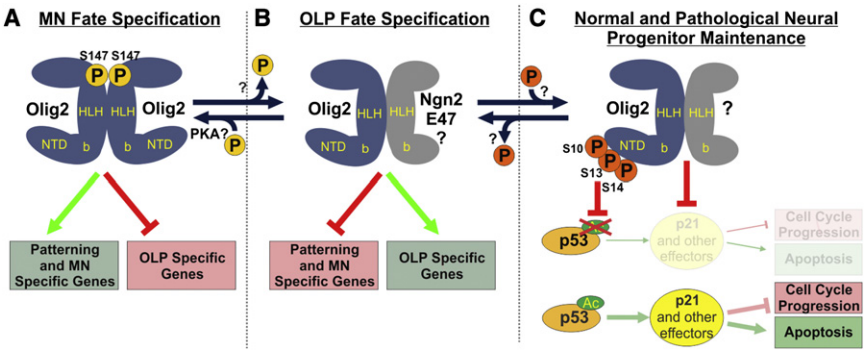

Figure 1. Olig2 Activity in Neural Development Is Regulated by Two Distinct Phosphorylation Events

(A) Early in development, Olig2 is phosphorylated at S147, and this modification directs the formation of Olig2 homodimers that promote the expression of motor neuron-specific genes and silence others.

(B) The transition to oligodendrocyte progenitor specification is facilitated by the dephosphorylation of S147, enabling Olig2 to form heterodimers with bHLH proteins such as Ngn2, E47, and others that induce the expression of oligodendrocyte progenitor-specific genes and suppress neuronal development.

(C) Within adult neural progenitors, the phosphorylation of a triple-serine motif (S10, S13, S14) near Olig2's N terminus (NTD) is necessary for Olig2 enhancement of proliferation. Triple-phosphorylated Olig2 reduces p53 acetylation and thereby limits p53's ability to activate its cell cycle arrest and apoptotic effectors.

relevant candidate, but direct testing of its function in this process is needed to confirm its role.

Later in development, Olig2 becomes essential for the proliferation of neural progenitors (Ligon et al., 2007). Sun et al. (2011) report here that this activity requires the phosphorylation of Olig2 at a distinct triple-serine motif (S10, S13, and S14) near its amino-terminus (Figure 1). The growth of *Olig2* mutant progenitors can accordingly be rescued and in some cases enhanced by the introduction of a triple phosphomimetic form of Olig2, but not by a triple phosphomutant form. Significantly, all forms of Olig2 investigated were able to restore oligodendrocyte formation, indicating that phosphorylation at the triple-serine motif selectively regulates the ability of Olig2 to promote neural progenitor proliferation and is dispensable for its fate-specifying functions. Given the distance of this motif from the HLH domain, it seems likely that this phosphorylation affects Olig2 activity independent of the dimerization preferences associated with S147 phosphorylation.

The molecular interactions that require the phosphorylation of Olig2's triple-serine motif are examined further in the companion study by Mehta et al. (2011). Olig2 has previously been shown to directly repress the p53 tumor-suppressor pathway effector $p21^{WAF1/CIP1}$ (Ligon et al., 2007). Mehta et al. (2011) now provide evidence that Olig2 has a much broader effect on the entire p53 pathway. In normal cells, DNA damage stimulates the activity of both p53 and p21 to reduce proliferation and induce apoptosis (Figure 1). Mehta et al. (2011) demonstrate that Olig2 mutant cells are more prone to cell cycle arrest following DNA damage and that this sensitivity can be overcome by removing p53 function. Thus, Olig2 and p53 appear to act in opposition to each other in modulating proliferation following genotoxic damage. Olig2 is further shown to suppress p53 acetylation, a posttranslational modification that is associated with its transcriptional activity, and impedes p53 binding to several known enhancer sites. The mechanism by which Olig2 carries out these functions remains unclear, though it strikingly requires the newly discovered triple-serine motif.

Both studies show that Olig2 expression is necessary for optimal proliferation of glioma cells and essential for their ability to form tumors when transplanted into SCID mice. Mehta et al. (2011) extend this observation by uncovering that Olig2 becomes dispensable for tumor formation in the absence of p53. Furthermore, Sun et al. (2011) have found that the triple-serine motif is highly phosphorylated in several glioma lines and that the phosphomimetic Olig2 protein is even more tumorigenic than the wild-type protein. These findings together strongly support the authors' contention that the ability of Olig2 to promote neural stem and progenitor cell proliferation is mediated through its opposition to the p53 pathway and that this mechanism contributes to the pathology of many human gliomas.

While the Sun and Mehta studies provide important new insights into the role of Olig2 in tumor formation, many questions remain unresolved. First, how does the phosphorylation of the triple-serine motif alter Olig2 interactions with regulators of p53 and other pathways? Second, how prevalent is the Olig2-mediated suppression of p53 within human gliomas? Although Sun et al. (2011) report that Olig2 was phosphorylated in several glioma samples, a more systematic survey is needed to determine the generality of this proposed mechanism for glioma pathogenesis and assess its implications for human disease. Third, what are the kinases and phosphatases that act upon the triple-serine motif, and how are they regulated? Finally, could the S147 and triple-serine phosphorylation events be combined to further expand the diversity of Olig2's function in the nervous system?

In summary, these papers provide an elegant example of how developmentally regulated phosphorylation events endow Olig2 with its unique biological functions. The findings further suggest a general strategy through which posttranslational modifications can enable single transcription factors to be co-opted for different purposes. Moreover, the correlation of Olig2 phosphorylation at the triple-serine motif with human gliomas make the removal of this modification a very promising avenue for the development of new therapies to combat glial tumor growth.

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A Cytokine-Dependent Switch for Glial-Neuron Interactions

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Though transmitters can be released from astrocytes, the conditions that permit their modulation of synaptic transmission are under debate. Santello et al. in this issue of *Neuron* now show that TNFα promotes a burst mode of glial transmitter release that escapes reuptake processes allowing access to neuronal NMDA receptors.

Seventeen years ago a quiet revolution in neuroscience began with the discovery that astrocytes, the major subtype of glia, could excite and activate neighboring neurons (Nedergaard, 1994; Parpura et al., 1994). One of these studies demonstrated the importance of the astrocytic release of the chemical transmitter glutamate (Parpura et al., 1994) in a process that has been termed gliotransmission. This observation, initially demonstrated in culture, moved to brain slice studies and more recently in vivo. In this issue of *Neuron*, Andrea Volterra and colleagues (Santello et al., 2011) now show that the presence of proinflammatory cytokine TNFα acts as a state-dependent switch to control the functional nature of gliotransmission.

Synapses are increasingly recognized to be tripartite structures, pre- and postsynaptic neurons and surrounding astrocytes (Figure 1), in which astrocytes actively modulate synaptic activity as well as support neuronal function (Araque et al., 1999). Astrocytes respond to synaptic activity with Ca^{2+} elevations, which leads to the release of gliotransmitters, such as glutamate. This astrocytic Ca^{2+} increase has the potential to contribute to synaptic activity by the activation of NMDA receptors or metabotropic glutamate receptors (Haydon and Carmignoto, 2006). With the identification of additional gliotransmitters and more evidence supporting this process in brain function have come recent papers with observations that challenge the relevance of gliotransmission, leading to what has been termed "the great glial debate" (Smith, 2010). Now, Santello et al. (2011) report a new observation concerning a state-dependence of gliotransmission that provides insights into the regulation of transmitter release from glia. They show that Ca^{2+}-dependent glutamate-mediated gliotransmission requires the presence of the proinflammatory cytokine TNFα. Interestingly, TNFα levels are modulated by sleep-wake cycles (Krueger, 2008), suggesting that astrocytes might modulate synapses in a diurnal manner.

In 2007, Volterra's group demonstrated that astrocytes modulate excitatory synapses of granule cells of the dentate gyrus through the Ca^{2+} dependent release of glial-derived glutamate (Jourdain et al., 2007). In that study, they demonstrated that the activation of P_2Y_1 receptors, which are enriched in astrocytes, caused an NMDA receptor dependent increase in the frequency of mEPSCs that was attenuated if Ca^{2+} elevations in astrocytes were inhibited. Amid the series of recent conflicting observations in the study of gliotransmission, it was shown that TNFα could potently augment the release of glutamate from astrocytes (Domercq et al., 2006), which prompted the authors to ask whether TNFα was required for the form of gliotransmission that they were studying.

Santello et al. (2011) first confirm that the P_2Y_1 receptor agonist 2MeSADP elevates mEPSC frequency and that astrocytic dialysis of the Ca^{2+} buffer BAPTA prevents this form of synaptic modulation. Having demonstrated that