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Regulation of Neuronal Proliferation and Differentiation by Nitric Oxide

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Abstract

Many studies have revealed the free radical nitric oxide (NO) to be an important modulator of vascular and neuronal physiology. It also plays a developmental role in regulating synapse formation and patterning. Recent studies suggest that NO may also mediate the switch from proliferation to differentiation during neurogenesis. Many mechanisms of this response are conserved between neuronal precursor cells and the cells of the vascular system, where NO can inhibit the proliferative response of endothelial and smooth-muscle cells to injury. In cultured neuroblastoma cells, NO synthase (NOS) expression is increased in the presence of various growth factors and mitogens. Subsequent production of NO leads to cessation of cell division and the acquisition of a differentiated phenotype. The inhibitory action of NO on neuroblast proliferation has also been demonstrated in vivo for vertebrate and invertebrate nervous systems, as well as in the adult brain. Potential downstream effectors of NO include the second messenger cyclic GMP, activation of the tumor-suppressor genes *p53* and Rb, and the cyclin-dependent kinase inhibitor *p21.* These studies highlight a new role for NO in the nervous system, as a coordinator of proliferation and patterning during neural development and adult neurogenesis.

Index Entries: Nitric oxide synthase; cyclic GMP, neurogenesis; PC12 cell; neuroblast; *p53; p21;* cell division; retinoblastoma.

Introduction

NO Synthesis and Molecular Targets

One cornerstone of development is the generation of appropriate cell types at the correct locations within the embryo. Cells in the nervous system face additional challenges, as developing neurons must also find and make connections to create functional circuits. The timing of these events must be tightly regulated within the embryo to ensure the appropriate matching of neurons and target cells. Although the early processes of neuronal induction and patterning have been extensively studied *(1–3)*, the mechanisms underlying the timing and coordination of these

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events presents another avenue of research that is just beginning to be explored. Recent studies have highlighted a potentially important role for the free radical nitric oxide (NO) in this process. This article focuses on increasing evidence from many sources suggesting that NO and its downstream effectors are involved in regulating the processes of cell proliferation and differentiation in the developing nervous system. These studies add a new dimension to the role of NO as an important modulator of primary inductive and patterning events in the embryo.

The gaseous molecule NO was first described over a decade ago as an important regulator of vasodilation *(4).* Subsequent research has also revealed a role for NO in the nervous system, generating numerous research articles and reviews. Much of this work has focused on the role of NO as a neuromodulator, which regulates circuit activity in both vertebrates *(5)* and invertebrates *(6,7).* NO may also regulate the induction and maintenance of mammalian long-term potentiation (LTP) *(8)* and long-term depression (LTD) *(9).* In the developing nervous system, NO has been the focus of many studies showing its involvement in the establishment and patterning of retinal inputs in the visual system of many species *(10–15).* In each of these systems, it appears that the NO produced by target cells interacts with newly arrived retinal inputs to regulate the location and numbers of synaptic connections. In some cases, the role of NO in the developing visual system has been shown to be modulated through the activity of soluble guanylate cyclase *(12,15,16).*

NO is produced by a family of enzymes known as nitric oxide synthases (NOS), through the conversion of L-arginine to citrulline. These are large, heme-containing enzymes that contain binding sites for flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and nicotinamide adenine dinucleotide phosphate (NADPH) (*17–19;* Fig. 1). The dimeric catalytic domain contains

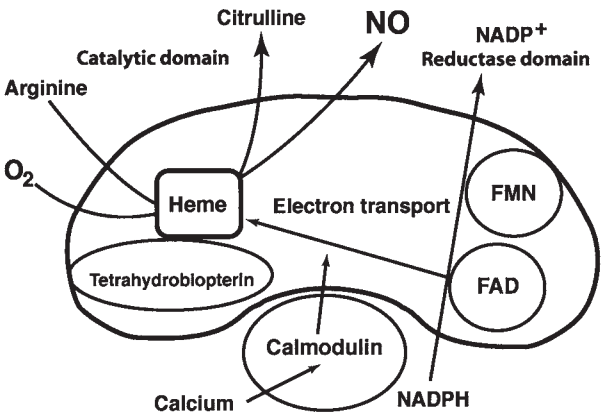

Fig. 1. Components of the nitric oxide synthesis pathway.

the heme group and tetrahydobiopterin, which binds the arginine substrate *(20).* A regulator site binds calcium/calmodulin, and facilitates electron transfer between the catalytic domains *(21).* The formation of NO occurs in the presence of NADPH, which results in oxidation of the guanadino nitrogen group of arginine. This "NADPH-diaphorase" activity of NOS remains active after tissue fixation, and has been used extensively as the basis for histochemical localization of NOS expression and activity in the nervous system *(22,23).*

There are several isoforms of NOS, which are encoded by a large multigene family. These isoforms were originally named for the tissues in which they were first identified: endothelial NOS (eNOS), neuronal NOS (nNOS), and the macrophage inducible form (iNOS). In mammals, these different types correspond to three genes: *NOS1* (nNOS), *NOS2* (iNOS), and *NOS3* (eNOS) *(24,25).* Of the lower vertebrate and invertebrate NOS genes to have been cloned, most seem to show homology with *NOS1* (nNOS), as is the case for *Drosophila (26), Xenopus (27),* zebrafish *(28),* and others *(29).* Although the expression of eNOS and nNOS were shown to be constitutively expressed and dependent on calcium/calmodulin, iNOS was character-

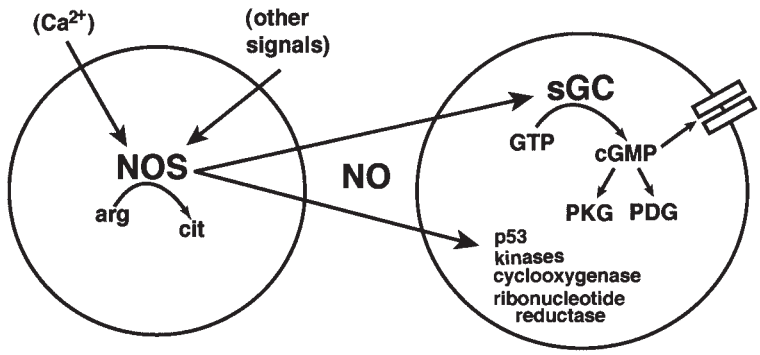

Fig. 2. Cellular targets of NO. NO can diffuse into cells and stimulate soluble guanylate cyclase to produce cGMP, which can interact with ion channels, protein kinases, and phosphodiesterases. Other potential molecular targets for NO include p53, cyclooxygenases, and ribonucleotide reductases.

ized as inducible and calcium-independent *(24).* Further research has revealed that expression of the different NOS isoforms is not restricted to the tissue type in which they were first localized, and single cells can actually contain several NOS isoforms that may be either constitutive or inducible *(30).* These findings create a more complex and diverse view of the biological functions of NO.

An unconventional signaling molecule, NO can diffuse from the point of synthesis to enter any neighboring cell. In biological tissue, this distance has been predicted to be up to 100 μm *(31).* How then is the specificity of NO action conferred within a cell (Fig. 2)? In many cases, the effects of NO are mediated by means of soluble guanylate cyclase (sGC), a dimeric enzyme consisting of alpha and beta subunits and a NO-binding heme moiety *(32).* The binding of NO to sGC stimulates the production of the second-messenger molecule 3′,5′-cyclic guanosine monophosphate (cGMP) *(33).* cGMP can interact with a variety of downstream molecular targets, including cGMP-dependent protein kinases *(34,35),* phosphodiesterases *(36),* and ion channels *(37,38).* Some of these pathways can transmit signals to the nucleus, and have been shown to affect gene transcription *(39),* thus leading to long-lasting or permanent changes in cellular function. Other cellular targets for NO include cyclooxygenases, ribonucleotide reductase, and some mitochondrial enzymes *(40,41).* Of interest to this article are recent studies showing that NO can change the expression and activity of cell-cycle regulatory proteins *(42–45).* In addition, NO can modify proteins through the nitrosylation of reactive thiol groups. Through this mechanism, NO has been shown to affect neurotransmitter vesicle docking and release at presynaptic terminals *(46–48).* It is likely that other molecular targets for NO will be discovered, thus augmenting the already diverse range of potential cellular changes effected by this unique molecule.

NO Regulates Cell Proliferation In Vitro

Shortly after endothelium-derived relaxing factor was identified as NO, the effects of NO on mitogenesis and proliferation in vascular endothelium and the overlying vascular smooth-muscle cells (VSMCs) became an area of intense clinical research, as they are known to proliferate in response to injury *(49).* NO donors were found to inhibit the proliferation of the vascular endothelium *(50,51)* and VSMCs in culture *(52).* This effect could be mimicked by the addition of a cGMP analog, suggesting that the antiproliferative effect of NO in these cells was mediated through the stimulation of SGC *(52–54).* Inhibition of endogenous iNOS expression using anti-

sense RNA also decreases the proliferative response of cultured endothelial cells *(55).* Gene-transfer techniques and transgenic models have provided even more persuasive evidence for the role of NO signaling in vascular proliferation. Mice carrying a disrupted eNOS (NOS3) gene have thicker arterial walls than wild-type siblings *(56),* and virus-mediated eNOS and nNOS expression inhibits VSMC proliferation *(57,58).* The role of sGC and cyclic-GMP-dependent protein kinase (PKG) in this process has been further supported by recent studies using gene-transfer techniques to increase sGC and PKG expression in cultured VSMCs, thus augmenting their antiproliferative response to NO *(59,60).* Taken together, the results of these studies suggest that therapeutic techniques manipulating the NO/cGMP pathway may someday facilitate patient recovery following procedures such as angioplasty and vein grafting.

The discovery that NO can also inhibit proliferation during development is relatively recent, and the clinical implications of these findings are still unexplored. However, these studies provide exciting new insights into the regulation of cellular patterning in the developing embryo and nervous system. A role for NO in development was demonstrated in *Drosophila,* where it was shown to inhibit proliferation during embryogenesis *(61),* and in the imaginal discs during metamorphosis *(62).* In several descriptive studies, the NADPH-diaphorase histochemical technique revealed putative NOS expression in the developing brain and spinal cord, correlating with regions of neurogenesis. These included the cerebellum *(63,64),* olfactory neurons *(63,65),* the cerebral cortex, hippocampus, and other brain regions *(66–68),* as well as proliferative zones in the spinal cord *(69).* Tanaka et al. *(70)* provided direct *in situ* evidence for the role of NO in neural proliferation by exposing cultured slices of neonatal rat cerebellum to inhibitors of NO synthesis and activity. Inhibition of NO led to increases in granule-cell proliferation and prevented glial-cell differentiation, suggesting that NO regulated both termination of proliferation and initiation of differentiation of these cells in the developing cortex. The expression and activity of eNOS and nNOS isoforms were subsequently described in the granular-cell layer of neonatal rats during the period of cell proliferation in this layer of the cortex *(71).* However, chronic inhibition of NOS in vivo during this same developmental window has not led to an increase in proliferation *(72),* and in one case appeared to produce a decrease in granule-cell number *(71).* Further studies on the role of NO in the developing cerebral cortex should be conducted to resolve these conflicting results.

An additional link between NO and neuronal proliferation was established by an examination of the response of PC12 cells to nerve-growth factor (NGF) *(73).* Used as an in vitro model of neural differentiation, PC12 cells exposed to NGF undergo a period of division, followed by growth arrest and the acquisition of differentiated neuronal morphology. It was observed that in the presence of NGF, PC12 cells showed strong NADPH-diaphorase staining. The presence of NOS was confirmed with antibody staining for the three NOS isoforms, which were all expressed. Interestingly, NOS expression was initiated at a low level in the cells prior to differentiation, and gradually increased as the cells extended neurites. In the absence of NGF, the cells did not express NOS or extend neurites. To test whether NOS expression and neurite outgrowth were linked, PC12 cells were cultured with NGF and an inhibitor of NOS. Under these conditions, the cells failed to cease proliferation and remained in an undifferentiated state, an effect that could be partially overcome by the addition of the precursor L-arginine, and completely reversed by the addition of exogenous NO. Finally, the addition of NO and NGF together, but not separately, led to the cessation of proliferation and the initiation of neurite outgrowth in a line of PC12 cells that ordinarily do not express the differentiated phenotype. This important study provided the basis for a model in which endogenous NO modulates the response of developing neurons to mitogenic factors, serv-

Table 1
Extracellular Factors Modulated by NO in Proliferating Cells

Factor	Effect on/of NO signaling
NGF	Increases expression of NOS *(73,82,83)*
	Inhibits proliferation *(73,82,83)*
	Promotes differentiation *(73,83,102)*
VEGF	Increases nNOS expression *(75–77)*
	Inhibits proliferation (non-neuronal; *76–78*)
EGF	Increases nNOS expression *(75)*
BDNF	Increases NADPH-diaphorase staining *(74)*
bFGF	Increases nNOS expression *(75)*
NT-3/-4	Increases NADPH-diaphorase staining *(74)*
Ecdysone	High levels decrease NOS synthesis and promote proliferation *(86)*
	Low levels increase NO synthesis and inhibit proliferation *(86)*
SHH	Mitogenic effects decreased by NO *(87)*
	Downstream signals inhibited by NO *(SMG, unpublished results)*
Serotonin	Expression increased after NOS inhibition *(98)*
	Promotes cell proliferation *(98–100)*
Steroids	Decreased steroids lead to increased NOS expression in hippocampus, *(97)*
	Decreased steroids and NOS inhibition cause increased proliferation *(97,98)*
Angiotensin II	Stimulates NO synthesis *(81)*
	Induces differentiation *(80,81)*
Retinoic acid	Stimulates NO synthesis *(79)*
	Induces differentiation *(79)*

ing to stop proliferation and initiate a program of differentiation.

Since the publication of Peunova and Enikolopov's work *(73)*, the response of cultured neuronal cells to the combined effects of NO and various growth factors has been the subject of many studies (Table 1). Rat spinal cord neurons were shown to respond in culture to brain-derived neurotrophic factor (BDNF) and neurotrophins NT-3 and NT-4 with an increase in NADPH-diaphorase staining *(74)*, and the expression of nNOS was induced in PC12 cells by basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and vascular endothelial cell-growth factor (VEGF) *(75)*. Interestingly, endogenous NO release was also shown to increase in vascular endothelia cells after exposure to VEGF *(76,77)*, an effect that leads to cessation of proliferation *(76,78)*. An increase in NO synthesis is associated with the differentiative response of human neuroblastoma cells to retinoic acid *(79)*, and may modulate the effects of angiotensin II on neuroblastoma-cell differentiation *(80,81)*. Finally, the induction of NOS expression in response to NGF appears to be mediated through a combination of the TrkA neurotrophin receptor and Ras-Erk members of the MAP kinase cascade *(82,83)*.

NO Regulates Cell Proliferation In Vivo

The influence of NO on neuronal proliferation and the modulation of developmentally relevant factors have recently been demonstrated in vivo. These relatively few studies have demonstrated the relevance of NO in coordinating the processes of proliferation and differentiation in the developing nervous system. In *Manduca* and other holometabolous insects, the transition from larval life to metamorphosis is triggered primarily via steroid

hormones known as ecdysteroids *(84).* One effect of ecdysteroids is to trigger neurogenesis in the central nervous system (CNS) of the animal, thus leading to expansion of the brain and nerve cord in preparation for complex adult behaviors. The temporal correlation of the coordinated proliferative and differentiative response in different regions with specific developmental events suggests that additional factors, produced locally, may regulate the response of groups of cells to the ecdysteroid signal. This appears to be true for the developing optic lobe region of the *Manduca* brain, where neural precursors show an all-or-none group proliferative response to ecdysteroids in vitro *(85).* The coordination of this response appears to be mediated by NO, which is expressed in the optic anlage during the time that proliferating neuroblasts begin producing optic lobe neurons *(86).* Culturing isolated nervous systems with various NOS inhibitors and donors, coupled with the addition of ecdysteroids, has revealed low levels of hormone-stimulated NO production and the inhibition of proliferation in the optic lobe. However, when ecdysteroid levels were increased to metamorphic levels, NO synthesis rapidly declined and neurogenesis resumed. The picture that emerges from this study is one in which low levels of ecdysteroid induce NO synthesis and inhibition of neuroblast proliferation until the appropriate time in metamorphosis, when NO production and cell proliferation cease in response to increasing ecdysteroids, thus coordinating the differentiation of optic lobe interneurons with the arrival of incoming photoreceptor axons from the developing eye.

NO may also regulate proliferative and differentiative events in the developing spinal cord. In zebrafish embryos, NADPH-diaphorase staining was observed in the notochord and ventral neural tube following primary neural induction, and decreases after secondary patterning of ventral motor neurons is complete *(87).* During the same temporal window, cells of the floor plate at the ventral midline of the neural tube showed that cGMP production that could be stimulated with exogenous NO. Pharmacological inhibition of NO or cGMP production in whole embryos during this developmental period led to expansion of the floor plate and the disorganization of motor neurons *(87).* It is not clear whether this results from increased proliferation or induction of floor plate cells; however, cell division in the ventral neural tube was increased with NOS and sGC inhibition (S. M. Gibbs, unpublished observations). In addition, the domain of *patched* expression, which is induced by the morphogenetic molecule Sonic Hedgehog and mediates its activity in SHH-responsive cells *(3,88,89)* was increased in both the ventral neural tube and floor plate after inhibition of NOS and sGC (S. M. Gibbs, unpublished observations). In this system, NO from the ventral neural tube and notochord may act via cGMP to limit floor-plate size by directly preventing proliferation of these cells, or by downregulating the response of floor-plate cells to the inductive effects of Shh.

As in *Manduca* and zebrafish, NO appears to serve an important function by controlling proliferation in the developing brain of the frog, *Xenopus laevis (27).* The gene for a neuronal-type isoform was cloned from *Xenopus* tadpoles *(XNOS),* and *in situ* hybridization demonstrated XNOS expression in cell bodies of the developing optic tectum, a pattern that was also detected by NADPH-diaphorase staining. Double labeling with BrdU revealed a band of proliferating cells lying just adjacent to the region of *XNOS* expression, but no overlap between the two domains. The role of NO in this system was investigated in vivo by inserting a pharmacological reagent containing gel matrix into the ventricle of tadpoles for one or three days, and then analyzing the optic tectum for changes in cell proliferation, cell death, and overall brain-cell number and volume. Within 24 h after NO donors were supplied, brain volume and the number of dividing cells in the tectum were dramatically reduced. Proliferation levels returned to control levels after three days, which likely reflected a decrease of NO production by the donor, and demon-

strated that the effect of NO was specific for cell proliferation and not survival. In the converse experiment, brains exposed to pharmacological inhibitors of NOS showed increased cell proliferation in the optic tectum. Labeling with neuron-specific antibodies revealed these extra tectum cells to be neuronal in nature. Although this study does not address the question of whether the antiproliferative effects of NO are direct or involve other endogenous morphogenetic factors in the *Xenopus* tectum, it provides further evidence that NO can regulate the switch between neuronal proliferation and differentiation in vivo, and that this effect is specific for certain cell types.

Evidence for Regulation of Proliferation by NO in the Adult Brain

The regulation of neurogenesis in the adult brain is a topic of great interest to researchers and clinicians seeking new therapeutic avenues for treating age and injury-related impairment of neural functioning. Progenitor cells that can give rise to both neurons and glia have been described in adult brain regions of several species, including humans *(90–93).* In addition to the regulation of neuronal proliferation in the developing brain, NO may play a role in controlling the generation of new neurons from these stem cells in the adult. In adult rats and mice, neuronal progenitors and stem cells have been identified in the subventricular zone (SVZ), which lies inside the external wall of the lateral ventricles *(90,91,93).* These progenitors migrate tangentially to the olfactory bulb, where they divide and differentiate into granular and periglomerular neurons *(94,95).* This region of migration is referred to as the rostral migratory stream (RMS). NADPH-diaphorase and NOS immunostaining have revealed a putative source of NO in the SVZ and RMS, intermingled with regions of proliferating and undifferentiated progenitor cells *(96).* NO may then serve to prevent the division and differentiation of neural progenitors as they migrate along the RMS, confining these events to their destination in the olfactory bulb. The elimination of adrenal steroids by adrenalectomy leads to increases in NOS expression in the dentate gyrus of the adult rat hippocampus *(97),* and pharmacological inhibition of NOS in adrenalectomized rats leads to a more than fivefold increase in dentate gyrus-cell proliferation over controls *(98).* Serotonin expression in the dentate gyrus was also found to be increased after NOS inhibition in this study *(98),* suggesting a functional correlation between NO and the previously demonstrated mitogenic effects of serotonin *(99,100).*

How NO Signaling Affects Cell-Cycle Progression

NO can inhibit cell-cycle progression in many cell types; however, the molecular mechanisms underlying this effect appear to be conserved. Interestingly, cGMP does not appear to mediate this role for NO in most systems *(101–104).* Instead, studies have focused on the influence of NO on various proteins known to promote or inhibit cell-cycle progression, which regulate the transition between cell proliferation and differentiation (Fig. 3). During the cell cycle, advancing from G_1 phase into S phase is mediated by a set of cyclin-dependent kinases (cdks), which form complexes with cyclins A, D and E. Cdks phosphorylate the retinoblastoma protein Rb late in G_1, which triggers entry into S phase. In the unphosphorylated state, Rb acts as a cell-cycle inhibitor *(105).* Cyclin E expression increases during G_1 and peaks at the G_1-S transition, forming a complex with cdk2. Cyclin A is expressed late in G_1, and inhibition of cyclin A kinase prevents the entry into the S phase. Both cyclin E and cyclin A kinases can be inhibited by the cyclin-dependent kinase inhibitors *p27* and *p21* (Waf1/Cip1/Sdi1). *p21* is present in proliferating cells during all phases of the cell cycle, in complex with cdks. When the ratio of *p21* relative to cdk is increased, entry into S phase is

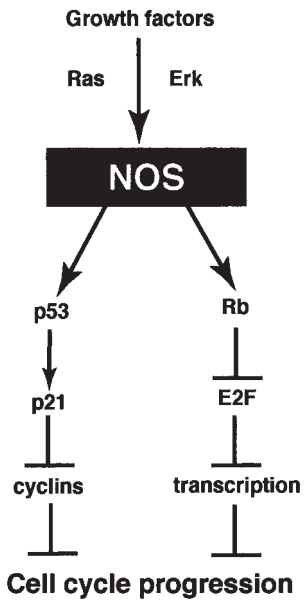

Fig. 3. Two potential pathways through which NO could inhibit cell division. NO has been shown to prevent cell-cycle progression through activation of p53 as well as the Rb signaling pathways.

blocked and cells become quiescent. Extracellular and intracellular signals can thus mediate the switch between proliferation and differentiation by acting on many potential targets: cyclin expression, and cdk expression and/or activation, Rb phosphorylation, and expression of ckis.

Ishida et al. *(42)* performed an extensive study of cellular events in VSMCs after exposure to NO donors. The effects of NO included inhibition of cdk2 and phosphorylation of the Rb protein, which resulted from an increased expression of the cdk inhibitor *p21.* NO also enhanced the amount of *p21* that was associated with cdk2. The upregulation of *p21* expression and subsequent inhibition of cdk2 activity was confirmed in VMSCs transfected with a NOS expression construct *(44).* A similar induction of *p21* was soon revealed as the basis of combined effects of NGF and NO on the differentiation of PC12 phenochromocytoma cells *(43).* It had been previously shown that NGF increased *p21* expression in PC12 cells by activating the transcription factor Sp1 *(106),* and that *p21* was required to promote PC12 cell survival during NGF-induced differentiation *(107).*

Poluha et al. *(43)* found that NOS expression and NO synthesis, induced by NGF *(73),* led to increased expression of the tumor-suppressor protein *p53. p53* acts to induce cell-cycle arrest following cell stress *(108),* and may repress the DNA-damaging effects of NO in tumor cells by inhibiting expression of NOS2 *(109). p53* is also a potent transcriptional activator of *p21 (110).* Inhibition of NOS in NGF-treated PC12 cells prevented acquisition of the differentiated phenotype (assayed by neurite outgrowth), and induction of a *p21* expression vector restored neurite extension in these cells *(43).* The induction of *p21* by NO in VSMCs was also shown to result from sustained expression of *p53 (103).* In an attempt to further elucidate the antiproliferative effects of NO and *p53,* Nakaya et al. *(45)* examined a suite of cell-cycle regulatory genes expressed in cultured fibroblasts. They showed that *p53* and many of its target cell-cycle regulatory genes were upregulated in the presence of NO, which inhibited fibroblast proliferation. However, when fibroblasts from *p53*-knockout mice were exposed to NO, cell division was only partially inhibited. In addition, the expression of some but not all the cell-cycle genes induced by NO in wild-type fibroblasts was unaltered in fibroblasts that lacked the *p53* gene. Finally, NO was shown to induce a unique pattern of *p53* phosphorylation that changed with the length of NO exposure. This study demonstrates that NO can modulate *p53* phosphorylation, activity, and expression, and that this interaction leads to the transcription of specific cell-cycle regulatory genes. However, the results also suggest that *p53* is responsible for some but not all the effects of NO on cell proliferation. The authors propose other potential mechanisms by which NO could affect the cell cycle, such as the inhibition of ribonucleotide reductase, which would prevent DNA synthesis and progression through the S phase.

Using an elegant combination of pharmacology and genetics, Kuzin et al. *(111)* examined the interaction between NO and cell cycle in the developing *Drosophila* eye. The adult eye of the fly is a highly organized and invariant array of different cell types, a system that is especially amenable to studying cell proliferation and patterning in vivo. NOS activity was manipulated in transgenic flies that expressed the *Drosophila* NOS gene *(dNOS); (26)* under the control of an eye-specific promoter. Another line of flies was developed with eye-specific expression of RBF, a negative regulator of cell proliferation and the *Drosophila* homolog of Rb *(112).* The eyes of adult flies carrying the dNOS or RBF genes alone had not apparent defects in cell number or organization. However, when the NOS and RBF flies were crossed, the adult progeny possessed defective eyes with fewer cells. When the gene dosage of RBF alone was increased, the phenotype of the adult eye was similar to that seen with the combination of RBF and NOS expression. Amazingly, the normal eye phenotype was almost restored to the extra-RBF flies when they were fed a pharmacological inhibitor of NOS as larvae, when the patter of cells in the developing eye is established. These reciprocal experiments demonstrate that NO and RBF can act in a synergistic fashion to suppress cell division in the developing *Drosophila* eye. The authors go on to show that the effect of NO on RBF may be mediated through interactions with the transcription factors E2F and dDP, homologs of transcription factors whose expression is inhibited by Rb in mammalian cells *(112,113).* In contrast to the flies that express extra RBF, in which inhibition of NOS was required to increase precursor proliferation in the eye, flies that overexpressed E2F and dDP developed eyes with extra cells, an effect that was rescued with addition of the *dNOS* gene. Thus, in *Drosophila,* NO appears to augment RBF-mediated inhibition of E2F-dependent transcription, which is required for entry into S phase and cell-cycle progression. It will be interesting to determine whether similar interactions between NO and E2F-family proteins are revealed in mammalian cells.

Conclusion

During the past 10 years, the scientific community has witnessed an explosion in the number of studies related to NO. This research has established NO as a ubiquitous and promiscuous molecule, capable of modulating such divergent processes as vasodilation and LTP. The most recent incarnation of NO as a regulator of cell proliferation reveals that the role of NO in neural development is not restricted to later events such as synaptogenesis. Instead, the studies summarized in this article suggest that NO may serve to inhibit cell division in specific regions of the developing nervous system, thus coordinating the processes of proliferation and differentiation during critical developmental periods. The NO signaling pathway is ideal for modulating such finely tuned events. NO can be directly and rapidly synthesized in response to myriad growth factors and other extracellular signals, thus circumventing gene expression. Once it is made, NO simply diffuses into neighboring cells, thus obviating the need for packaging, secretion, or membrane-bound receptors. It can interact with a variety of intracellular proteins, but is also quickly degraded, thus allowing it to exert specific localized effects within the tissue. This rapid and reversible action of NO allows it to serve a status-quo function during development, either preventing cells from proliferating at the wrong time or initiating their differentiation in response to other inductive factors. The ability of NO to modulate other signals may help to explain how a ubiquitous morphogenetic molecule like Shh is capable of inducing many distinct cell types in the embryo at different times. Considering the number of studies that describe NOS expression in the developing nervous system, it is reasonable to assume that the role of NO in coordinating early induction and patterning, especially in the intact embryo, is just beginning to be understood.

The preliminary studies on the effects of NO on cell division in the postnatal rodent brain may serve as a foundation for further research into therapeutic applications for NO in adult neurogenesis. These findings may also call for a reexamination of the potentially harmful effects of inhaled NO, which is often supplied to premature infants as a pulmonary vasodilator *(114).* NO is inactivated by circulating hemoglobin, however, it is also known to travel outside the lungs *(114).* Although it has not been demonstrated, a potentially harmful antiproliferative effect of exogenous NO on the developing neonatal brain is an avenue worthy of future exploration.

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