comprehensive assessment of inference algorithms. As the authors point out, information-theoretic approaches are not ideal for reconstructing small-scale networks, but seem to do quite well for reconstructing large-scale networks. An obvious development along these lines would be to increase the size and complexity of the gold-standard synthetic gene network by including additional genes and interactions. Ideally, it would be useful to have a library of diverse, gold-standard synthetic gene networks, including ones consisting of 25–100 genes and varied network architectures. As DNA synthesis capabilities become less error prone and more cost effective, creating such a library will become feasible.

It would also be useful to expand the gold-standard synthetic networks to include additional components, such as small RNAs and microRNAs, and to take account of pre- and posttranscriptional and translational modifications. These developments would enable one to consider multiple levels of regulation and to integrate different types of data in network inference studies. These enhanced capabilities could lead to the development of new systems biology techniques and analysis tools.

The work by Cantone and colleagues nicely illustrates the value of integrating the bottom-up network construction approaches of synthetic biology with the top-down network inference methodologies of systems biology. These efforts will be applicable to many different organisms, and may one day enable us to reverse engineer the gene regulatory networks that make up an elephant.

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# A Nurr1 Pathway for Neuroprotection


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**Mutations in the gene encoding the orphan nuclear receptor Nurr1 are linked to a rare familial form of Parkinson's disease. By examining the function of its mouse homolog, Saijo et al. (2009) provide evidence that Nurr1 protects dopaminergic neurons by suppressing inflammatory gene expression in astrocytes and microglia.**

Parkinson's disease is a neurodegenerative disorder, characterized by tremors and rigidity, that results from the progressive loss of dopamine-producing neurons in the substantia nigra of the brain. Among the genetic factors contributing to the disease are rare mutations in the orphan nuclear receptor Nurr1 (also known as NR4A2) that are associated with a familial late-onset form of the disease (Le et al., 2003). Prior work on Nurr1 is consistent with the view that this protein might mediate its neuroprotective effects primarily through its function in neurons. Nurr1 was initially characterized in rats as a transcription factor that regulates expression of the gene encoding tyrosine hydroxylase, a key enzyme in dopamine synthesis (Sakurada et al., 1999). Genetic deletion of *Nurr1* in mice inhibits the development of midbrain dopamine-producing neurons (Zetterstrom et al., 1997), and the dopaminergic neurons of heterozygous null mice are more susceptible to neurotoxic challenge than those of wild-type mice (Le et al., 1999). In this issue, Saijo et al. (2009) present evidence for an unexpected mechanism by which Nurr1 mediates neuroprotection. These authors show that mouse Nurr1 acts in microglia and astrocytes to suppress the production of inflammatory mediators that trigger the death of dopaminergic neurons.

The NR4A subfamily of nuclear receptors consists of three members: NR4A1, NR4A2, and NR4A3 (also known as Nur77,

Nurr1, and Nor1) (Maxwell and Muscat, 2006). Unlike many other nuclear receptors, the NR4As do not possess ligand-binding cavities. Instead, NR4As are immediate early genes whose expression is induced by various stimuli including cyclic AMP, growth factors, inflammatory signals, and hormones. The activity of NR4A is thought to be controlled primarily at the level of protein expression and posttranscriptional regulation. NR4A receptors positively regulate the expression of target genes by directly binding to response elements in their promoters (Maxwell and Muscat, 2006).

In addition to positively regulating gene expression, nuclear receptors can also inhibit transcription. Recent work from several groups has begun to elucidate the transcriptional mechanisms through which nuclear receptors repress inflammatory gene expression. For example, the ligand-activated nuclear receptors PPAR and LXR inhibit inflammation through protein-protein interactions with the inflammatory transcription factor NF-κB (Bensinger and Tontonoz, 2008). Importantly, this so-called "transrepression" mechanism does not depend on direct binding of the receptors to response elements in target promoters. Instead, ligation of PPAR and LXR inhibits inflammatory gene expression by preventing the inflammatory signal-specific removal of the corepressor complexes containing SMRT and NCoR from inflammatory gene promoters (Ghisletti et al., 2007; Pascual et al., 2005).

Saijo et al. now reveal a previously unappreciated function for Nurr1—the repression of inflammatory gene expression. This repression provides protection from the damaging effects of neuroinflammation and suggests new potential mechanisms linking Nurr1 function and Parkinson's disease. The authors set the stage for their discovery by administering lipopolysaccharide (LPS) to the substantia nigra of mice using stereotaxic injections (a technique that uses a coordinate system to precisely target an injection needle to particular regions of the brain). This treatment triggers local inflammation and leads to a loss of neurons expressing tyrosine hydroxylase ($TH^+$). They demonstrate that inflammation induces Nurr1 expression and that local knockdown of Nurr1 (by injection of lentiviral vectors expressing short-hairpin RNAs) enhances the loss of $TH^+$ neurons. Interestingly, the primary targets for the neuroprotective effects of Nurr1 appear to be the neighboring microglia and astrocytic cells rather than the neurons themselves. In vitro studies with microglia and astrocytes implicate these support cells in the release of neurotoxic factors that induce neuronal death.

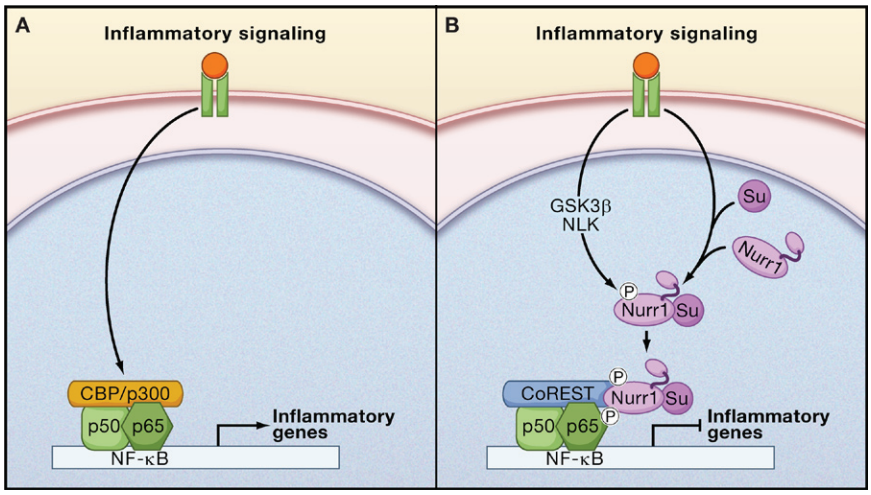


**Figure 1. A Nurr1 Pathway for Transrepression of Inflammatory Gene Expression**

A model for Nurr1 transrepression based on the work of Saijo et al. (2009).

(A) Inflammatory signals promote the expression of inflammatory genes through activation of NF-κB signaling and the recruitment of coactivator complexes, such as p300/CBP, by the NF-κB subunit p65 to inflammatory promoters.

(B) In cells expressing Nurr1, such as microglia, inflammatory signals also trigger Nurr1 phosphorylation (by Nemo-like kinase; NLK) and sumoylation. This promotes its interaction with p65 leading to the recruitment of the CoREST complex to the promoters of inflammatory genes.

Building on their previous studies of the anti-inflammatory action of PPAR and LXR, the authors investigated the mechanisms underlying the protective effects of Nurr1. Examination of isolated microglia and astrocytes demonstrates that Nurr1 is a potent repressor of inflammatory gene expression in these cells. Chromatin immunoprecipitation (ChIP) analysis indicates that Nurr1 is recruited to the promoters of LPS-responsive genes. Interestingly, the ability of Nurr1 to inhibit these promoters is signal specific but does not require direct binding to a specific DNA sequence. Thus, Nurr1 appears to act as a transrepressor, similar to the ligand-dependent nuclear receptors PPAR and LXR. The authors provide evidence from coimmunoprecipitation experiments that Nurr1-mediated transrepression involves the physical association of Nurr1 with the p65 subunit of NF-κB (Figure 1). Mutations in key phosphorylation sites, coupled with the use of kinase inhibitors, identify GSK3β-mediated phosphorylation of p65 as a signal for recruitment of Nurr1 to p65. Phosphorylation of serine 468 drives the interaction of Nurr1 and p65, resulting in the attenuation of inflammatory gene transcription by NF-κB. Analogous to PPAR- and LXR-mediated transrepression, Nurr1 repression of inflammatory promoters also requires sumoylation of Nurr1 at key lysine residues (Ghisletti et al., 2007; Pascual et al., 2005). Treatment of cells with the inflammatory cytokine interleukin 1β (IL-1β) promotes Nurr1 sumoylation.

Another important piece in this puzzle is the CoREST repressor complex, which the authors identify as an essential player in Nurr1-mediated transrepression. Previous work has shown that CoREST represses the expression of neural-specific genes in non-neural cells via binding to specific silencing elements (Ballas et al., 2005). CoREST recruits an array of proteins to target promoters in order to effect repression. These include histone deacetylases (HDACs), the histone methyltransferase G9a, histone demethylase, and lysine-specific demethylase 1 (LSD1). The authors show that Nurr1 interacts directly with CoREST and find that recruitment of G9a, LSD1, and HDAC1 is required for Nurr1/CoREST-

dependent repression. The authors also report that the Nurr1-CoREST interaction is stimulated by phosphorylation of Nurr1 by Nemo-like kinase. Lastly, ChIP analysis of iNOS promoter occupancy following stimulation by LPS suggests a temporal model of transrepression in which p65 binding precedes Nurr1 association, which is followed by recruitment of CoREST to the complex.

The Saijo et al. study provides important insights into the ability of Nurr1 and CoREST to modulate neuroinflammation. The work unravels molecular mechanisms that may underlie human neurological disease and opens the door for future work on inflammatory signaling in the brain. These studies also provide a better understanding of how transrepression is achieved at the molecular level. Interestingly, certain components of the transrepression mechanism appear to be conserved between different nuclear receptors, such as the requirement for sumoylation and the interaction with NF-κB proteins and corepressors. At the same time, Saijo et al. illustrate how different nuclear receptors utilize distinct corepressor complexes to repress gene expression in a signal- and context-dependent manner.

Finally, the work prompts a number of interesting questions to be addressed in future studies. For example, the three NR4A receptors (Nurr77, Nurr1, and Nor1) regulate overlapping target genes in some cell types. Do Nurr77 and Nor1 also interact with CoREST in response to inflammatory signals? What is the relative contribution of transrepression and direct gene activation in NR4A-dependent regulation of inflammation? Sumoylation appears to be a critical component of the transrepression mechanism. In the PPAR and LXR transrepression pathways, sumoylation of the receptor is triggered by ligand binding. Is Nurr1 sumoylation responsive to other cellular signals in addition to IL-1β? Is the Nurr1/CoREST transrepression pathway intact in monocytes and macrophages, and if so, does this repression pathway also have a role in peripheral inflammation? Lastly, there is strong evidence that neuroinflammation contributes to the pathogenesis of a number of diseases, including Alzheimer's disease and multiple sclerosis. It will be important to determine if this repression pathway is altered in these diseases and whether NR4A receptors might represent potential therapeutic targets.

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# β-tting on p63 as a Metastatic Suppressor


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**Although much is known about the genes that promote metastasis, few suppressors of metastasis have been found. Adorno et al. (2009) now identify p63 as a potent suppressor of metastasis and uncover an intricate mechanism for the inactivation of metastasis in cancer cells in response to transforming growth factor β.**


Cancer progression from the primary phase to the metastatic phase represents one of the key determinants of prognosis and outcome for cancer patients. However, the mechanistic details behind the metastatic spread of cancer remain obscure. Although the cytokine transforming growth factor β (TGFβ) has emerged as a major player in the metastatic process, it has seemingly contradictory functions. Ordinarily a tumor suppressor that mediates growth arrest and apoptosis, TGFβ appears to take on the opposite role in end-stage tumors where it promotes metastasis (Padua and Massague, 2009). Reporting in this issue of *Cell*, Adorno et al. (2009) identify a mechanism in cells expressing mutant p53, which enables TGFβ to switch to an oncogenic role by promoting mutant p53-mediated suppression of