to be required for endogenous twist-1 expression. Nevertheless, further evidence is presented for a feedback loop between PPARδ and twist-1: PPARδ, but not PPARα or PPARγ, bind to the twist-1 promoter and direct twist-1 expression in brown adipocytes. Given that PPARδ acts as a lipid sensor, these findings raise the possibility that nutrients may influence this transcriptional network. Additionally, energy sensors such as the AMP-activated protein kinase (AMPK) may lie upstream of PPARδ. AMPK activators increase expression of PGC-1α target genes, and siRNAs against either PPARδ or AMPK subunits block the effects of PPARδ agonists on fatty acid oxidation (Kramer et al., 2007). However, any requirement for AMPK signaling in the PPARδ-inducible, negative feedback loop of twist-1 on PGC-1α transcriptional regulation is speculative.

Given the unexpected evidence for brown adipose tissue in humans (Nedergaard et al., 2007), the role of twist-1 in mitochondrial biogenesis and thermogenic programs in brown adipose tissue may have important implications for human obesity. Bearing in mind that the physiological role of brown fat in adult humans remains unclear, do alterations in the twist-1 protein level influence PGC1-α-dependent gene expression and metabolism in humans? Evidence from clinical genetics implicates twist-1 haploinsufficiency in Saethre-Chotzen syndrome. Hence, caution should be exercised when considering pharmacological approaches to target twist-1 expression in humans. Furthermore, the role of twist-1 in other tissues expressing PPARδ and PGC1-α, such as cardiac and oxidative skeletal muscle, should be considered to fully appreciate whether twist-1 has a role beyond that described for brown adipose tissue. The identification of twist-1 as a regulator of programs in brown adipose tissue reveals new insight into the mechanisms controlling cellular and whole-body energy homeostasis in obesity.

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Systems Biology Strikes Gold

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Integrating synthetic biology and systems biology efforts can advance our understanding of biomolecular systems. This is illustrated in this issue by Cantone et al. (2009), who construct a synthetic gene network in yeast and use it to assess and benchmark systems biology approaches for reverse engineering endogenous gene networks.

There is an old story about five blind men who are presented with an elephant and asked to identify what is before them. Each touches only a portion of the animal, and offer guesses ranging from a rope to a tree branch. It is likely that the men would have done better had they first practiced on another large animal, such as a camel, knowing a priori what was before them. In that way, they could have benchmarked their approaches, that is, assessed their relative performances using a common standard.

There is much interest in both systems biology, that is, the use of integrated experimental and computational approaches for studying endogenous biomolecular networks, and synthetic biology, that is, the use of engineering techniques to model, design, and construct artificial biomolecular networks. One of the cornerstones of systems biology research is the identification of network structures from experimental data through reverse engineering (inference) approaches. In many ways, those of us working in this area are like the five blind men confronted with an elephant—we are not sure what the actual biomolecular networks look like, and we lack accurate benchmarks for our reverse-engineering approaches.

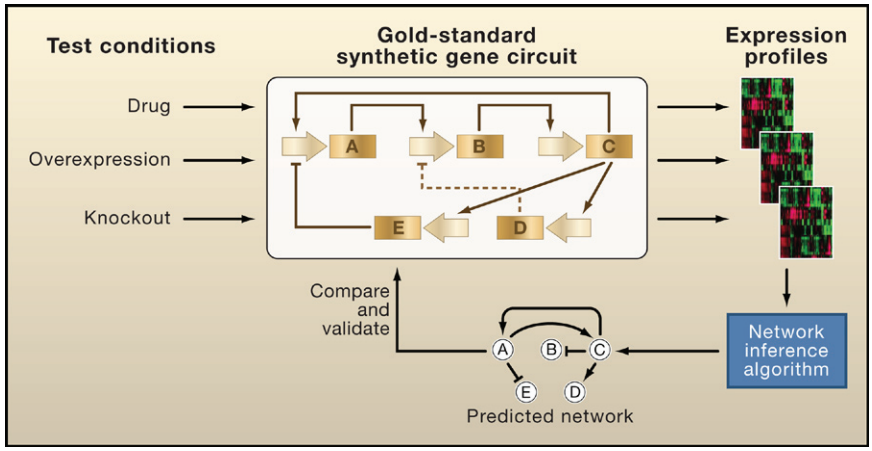

Figure 1. Benchmarks for Reverse Engineering of Networks

The development of a gold-standard synthetic gene circuit allows for the validation of network inference algorithms. The methodology involves collecting expression profiles for the synthetic circuit (e.g., from drug profiles, and overexpression or knockout experiments) and applying inference algorithms to these data. As the topology of the gold-standard circuit is known a priori, one can assess the validity and power of the inference tools by comparing the predicted networks to the known network.

In this issue, Cantone et al. (2009) address this problem and present us with the systems biology equivalent of a camel. Specifically, they develop a synthetic gene network in the budding yeast *Saccharomyces cerevisiae* that they use as an in vivo gold standard to assess and benchmark network inference approaches.

Until now, the available benchmarks have been in silico, essentially computational models of gene networks (e.g., see Camacho et al., 2007). Although these models offer desired levels of control and robustness and constitute a gold standard in their own right, they lack the full intricacies of in vivo biological networks. The system presented by Cantone et al. is an important step toward the development of a comprehensive, biology-driven and synthetic biology-controlled, standardized platform for validating reverse-engineering approaches.

The authors generated a complex gene regulatory network, with an intricate network structure (topology), by carefully selecting a small number of yeast genes and integrating these with promoter sequences that enabled a variety of regulatory interactions. The network was composed of five genes (*ASH1*, *CBF1*, *GAL4*, *GAL80*, and *SWI5*, all transcription factors), and included multiple feedback loops and protein-protein regulatory interactions. The system was designed so that each gene controlled the transcription of at least one other gene in the network.

The authors were careful to select promoter/gene pairs that belong to distinct, nonredundant pathways, so as to effectively eliminate the influences that other genes in the yeast genome could have on the synthetic network. They also coupled the network to a galactose-sensing promoter (*GAL1-10*) and used a background yeast strain that does not express *GAL10*, enabling them to switch the network into an "on" or "off" state depending on the available carbon source (galactose or glucose, respectively). These design features allowed for the careful collection of data that relate solely to the functioning of the network and, therefore, could provide in vivo measurements of gene expression that can be used for network inference or modeling applications.

To investigate the applicability of their gold standard platform for systems biology (Figure 1), Cantone et al. collected time series and steady-state expression data from their network after multiple perturbations, such as overexpression of each of the five network genes, one by one. They then compared the effectiveness of different reverse-engineering algorithms on the collected expression data. They considered inference methods based on ordinary differential equations or ODEs (Gardner et al., 2003; Lorenz et al., 2009), Bayesian networks (Yu et al., 2004), and information theory (Margolin et al., 2006; Faith et al., 2007). Methodologies based on ODEs strive to uncover network structures by estimating the parameters in a differential equation model. In contrast, Bayesian network methods reconstruct network architectures on the basis of probabilistic graphical models, and information-theoretic approaches rely on extracting the network features on the basis of the probability that a pair of genes is coexpressed across a data set. Each of these strategies has its own benefits and pitfalls, which are described in the extensive literature on network inference (e.g., see Bonneau, 2008).

The authors found that the best performers on their synthetic gene network were the ODE-based methods and Bayesian network methods, with the former being superior in cases where known genetic perturbations are used in the data-generating experiments. Information-theoretic methods did not perform well on the small synthetic circuit; these approaches were hindered by their inability to infer the directionality of interactions. However, as the authors note, information-theoretic approaches (Margolin et al., 2006; Faith et al., 2007) are quite effective for studying large networks, given that they can infer undirected gene-gene interactions from a relatively small amount of expression data.

Network-driven biological research has illustrated the power of systems biology, e.g., for uncovering the mode of action of drugs (di Bernardo et al., 2005; Kohanski et al., 2008). However, systems biology approaches that are not appropriately validated or benchmarked can lead to erroneous results and misguided experiments, particularly given the complexity of the systems being considered and the noisy, limited nature of typical experimental data sets. The work by Cantone and colleagues is an important step forward in the quest for developing accurate, effective systems biology approaches for network inference.

So, are we done? Can we now reliably infer the elephant-like biomolecular networks that are out there? Well, one has to question whether small networks of the type considered by Cantone and coworkers are all that is needed for a

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,