The following resources related to this article are available online at
www.sciencemag.org (this information is current as of November 11, 2008):

Updated information and services, including high-resolution figures, can be found in the online
version of this article at:
http://www.sciencemag.org/cgi/content/full/305/5687/1107d

A list of selected additional articles on the Science Web sites **related to this article** can be
found at:
http://www.sciencemag.org/cgi/content/full/305/5687/1107d#related-content

This article **cites 13 articles**, 6 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/305/5687/1107d#otherarticles

This article has been **cited by** 2 articles hosted by HighWire Press; see:
http://www.sciencemag.org/cgi/content/full/305/5687/1107d#otherarticles

This article appears in the following **subject collections**:
Computers, Mathematics
http://www.sciencemag.org/cgi/collection/comp_math

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce
this article** in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl

Response to Comment on "Network Motifs: Simple Building Blocks of Complex Networks" and "Superfamilies of Evolved and Designed Networks"

Our previous work (*1*) presented a phenomenological observation on real-world networks: They show distinct subgraph significance profiles (SP) when compared with randomized networks with the same degree sequence as the real networks. This observation calls for a theory—a model that prescribes evolutionary dynamics or constraints that, once used to evolve a network, yield the observed SPs. The SP method also provides a way to test whether a given theoretical model actually reproduces the local structure of the real network. Selection of network motifs for their function is one such possible theory, which, as we mention below, can and should be tested experimentally; it is certainly not the only possible theory (*1, 2*).

Along these lines, Artzy-Randrup *et al.* (*3*) comment that the observed network motifs (*2*) can arise by various different mechanisms, not only by evolutionary selection for function. They proposed two such theoretical models ("toy networks") as counterexamples, showing some of the network motifs found in the neuronal synaptic network of *Caenorhabditis elegans* and in the transcription network of *Escherichia coli*. The models were (i) a random-lattice (geometrical) model, in which neurons that are close in space tend to form synapses and (ii) a preferential-attachment (PA) model for transcription networks, in which networks are grown so that genes preferentially link to genes that already have

many connections. These models were shown in the comment to display some of the same network motifs (overrepresented subgraphs) as do the real-world networks. Here, we demonstrate that if one wishes to test whether these toy mechanisms can explain the real-world networks, one may compare the structure they produce more fully to the real networks, using the SP approach. We demonstrate that both models give SPs that are quite different from the SPs of the real networks: They produce many strong motifs that do not appear in the real networks.

We begin with the random-lattice model for the neuronal network. The random-lattice model (*1*) yields feedforward loops, just as does the real neuronal network. However, the SP of lattice models shows two three-node subgraphs that are not found in the real net-

work (Fig. 1A). One is the 3-loop, a cycle made of three nodes (subgraph 8), and the second is a 3-loop with one mutual edge (subgraph 11). These subgraphs are generally overrepresented in random-lattice models, regardless of the dimensions of the lattice. For example, based on symmetry, the ratio of feedforward loops and 3-loops can be generally shown to be 3:1 in lattice models. In the real neuronal network, the ratio is 22:1 (about 1500:70). Thus, geometry or clustering alone does not seem to explain the structure of the neuronal network of *C. elegans*. It seems more likely to us that the spatial arrangement and the connectivity of the neurons coevolved to supply the needed, highly designed circuitry (for example, the scarcity of 3-loops in the real neuronal network may be the result of evolutionary selection against unwanted designs within a geometrically constrained neural architecture). The hypothesis that the neuronal network motifs function as recurring circuitry elements should be tested experimentally.

We now consider the PA model, which can produce feedforward loops, a common motif in the *E. coli* transcription network. However, when considering four-node subgraphs, one finds that the PA model shows many subgraphs that are not found in the real network (Fig. 1B). This includes feedforward loops connected to form four-node patterns in different ways (Fig.
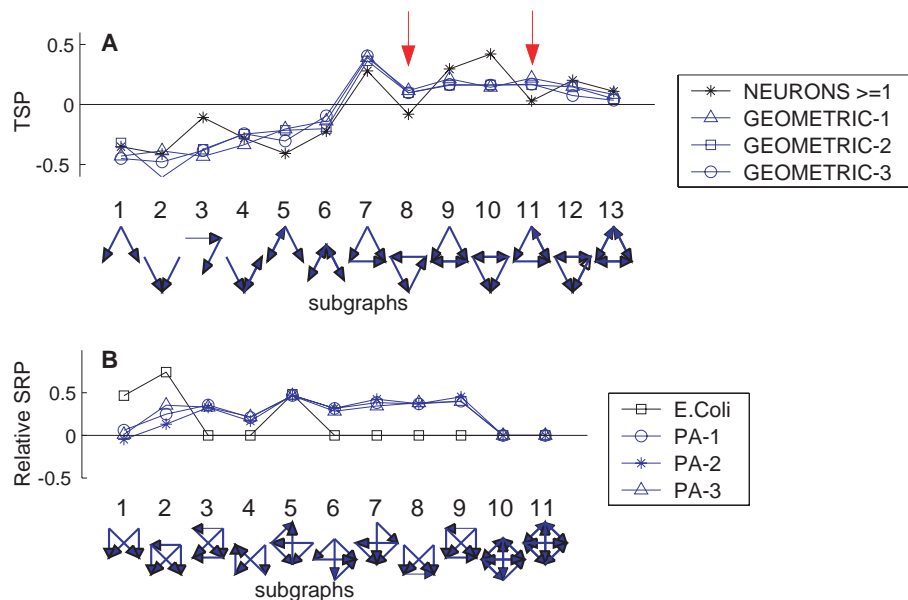
1B, subgraphs 3 and 6 to 9) that are not realized in the natural network. These subgraphs are generally found in other variants of PA models that generate feedforward loops. Thus, PA processes do not seem sufficient to explain the evolution of this real transcription network. Again, it seems likely that transcription networks rapidly rewire over evolutionary time scales to adapt to the environment (*4, 5*), and thus that their connectivity is not just a vestige of frozen history based on attachment rules.

It is notable that thinking of biological network motifs as information-processing units is not an assumption of the present approach, but rather a

Fig. 1. Comparison of the local structure of real-world networks and theoretical model networks. (**A**) Triad significance profile (TSP) of the *C. elegans* neural network (*13*) (black) and of three instances of random-lattice networks (blue). In the lattice networks, directed connections were formed at random between neighboring nodes arranged on a two-dimensional lattice (*1*). Red arrows indicate subgraphs that occur in the random-lattice network much more often than in the real network. (**B**) The four-node subgraph ratio profile (SRP) for the *E. coli* transcriptional network (*14*) (black) and three instances of model networks created by a directed preferential-attachment (PA) process (*15*) (blue). The PA networks are grown by adding new nodes, such that the probability of connecting a directed edge to an existing node increases with the number of edges it already has.

hypothesis that is, in principle, experimentally testable. Indeed, experimental and theoretical work on network motifs in transcription networks has yielded support for their role as information-processing units, performing tasks such as asymmetric filtering [coherent feedforward loop motif (6)], response acceleration [negative autoregulation motif (7)], pulse production [incoherent feedforward loop motif (8, 9)], and temporal pattern generation [single-input module motif (10, 11)]. It is therefore possible to suggest that these wiring patterns were selected based on their functions.

We believe that the search for theoretical models to explain the observed SPs will be a fruitful one and will help identify the mechanisms or evolutionary principles that lead to the observed local structure (or at least rule out evolutionary hypotheses that do not). Care should be taken, because distinct models could give rise to very similar structures; SPs of higher order subgraphs as well as topological generalizations of motifs (12) may help give increasing resolution to distinguish between models and real networks.

The present approach based on degree-preserving randomized networks is a simple first step for comparing networks and for discovering potentially interesting overrepresented and underrepresented patterns for further analysis. More elaborate null-hypothesis models could in principle be used to help highlight interesting patterns and to test models for their origin.

Ron Milo
Shalev Itzkovitz
Nadav Kashtan
Reuven Levitt
Uri Alon*
*Departments of Molecular Cell Biology
and Physics of Complex Systems,
Weizmann Institute of Science
Rehovot 76100, Israel
*To whom correspondence should be
addressed. E-mail: urialon@weizmann.ac.il*

References

1. R. Milo *et al.*, *Science* **303**, 1538 (2004).
2. R. Milo *et al.*, *Science* **298**, 824 (2002).
3. Y. Artzy-Randrup, S. J. Fleishman, N. Ben-Tal, L. Stone, *Science* **305**, 1107 (2004); www.sciencemag.org/cgi/content/full/305/5687/1107c.
4. S. A. Teichmann, M. M. Babu, *Nature Genet.* **36**, 492 (2004).
5. G. C. Conant, A. Wagner, *Nature Genet.* **34**, 264 (2003).
6. S. Mangan, A. Zaslaver, U. Alon, *J. Mol. Biol.* **334**, 197 (2003).
7. N. Rosenfeld, M. B. Elowitz, U. Alon, *J. Mol. Biol.* **323**, 785 (2002).
8. S. Mangan, U. Alon, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 11980 (2003).
9. S. Basu, R. Mehreja, S. Thiberge, M. T. Chen, R. Weiss, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 6355 (2004).
10. A. Zaslaver *et al.*, *Nature Genet.* **36**, 486 (2004).
11. M. Ronen, R. Rosenberg, B. I. Shraiman, U. Alon, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 10555 (2002).
12. N. Kashtan, S. Itzkovitz, R. Milo, U. Alon, *Phys. Rev. E.*, in press.
13. J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London Ser. B* **314**, 1 (1986).
14. S. Shen-Orr, R. Milo, S. Mangan, U. Alon, *Nature Genet.* **31**, 64 (2002).
15. A. L. Barabasi, R. Albert, *Science* **286**, 509 (1999).