The evolution of molecular genetic pathways and networks

Jennifer M. Cork and Michael D. Purugganan*

Summary

There is growing interest in the evolutionary dynamics of molecular genetic pathways and networks, and the extent to which the molecular evolution of a gene depends on its position within a pathway or network, as well as over-all network topology. Investigations on the relationships between network organization, topological architecture and evolutionary dynamics provide intriguing hints as to how networks evolve. Recent studies also suggest that genetic pathway and network structures may influence the action of evolutionary forces, and may play a role in maintaining phenotypic robustness in organisms.
***BioEssays* 26:479–484, 2004.**

Introduction

Most biochemistry teaching laboratories (and indeed also research laboratories) display a wall-sized chart that depicts the complexities of organismal metabolism. This chart, known as the official International Union of Biochemsitry and Molecular Biology-Nicholson Chart of Metabolic Pathways, had modest beginnings nearly half a century ago.[1] Don Nicholson, a University of Leeds biochemist, sketched out all the known metabolic pathways and their intricate linkages in 1960 to use as a teaching aid; the 21st edition of the chart published in 2000 incorporates 550 enzymatic transformations. It is arguably the first large-scale molecular network assembled, a prelude to many of the molecular genetic networks currently under construction with contemporary genomics studies.

The metabolic pathways chart illustrates a central feature of biological organization at all hierarchical levels—no biological entity within our biosphere, whether metabolites, proteins, genes, cells or even species, exists in isolation. Instead, they are found as components of complex networks and pathways that together constitute organization at every level of biology.[2–5] Molecular genetic networks and pathways describe the molecular and/or genetic components that underlie cellular and organismal processes, and the functional interactions among them (see Fig. 1).[4,5] Pathways can be thought of as small linear components of larger global networks, and it might be useful to consider them as local networks in which interactions are depicted linearly or which possess simple unreticulated branch points. Network patterns are often more complex than simple linear pathways, and usually involve functional cross-connections that create webs of interactions.

It is clearly one of the objectives of biological research to identify and characterize these networks,[4,5] a task that is daunting, but increasingly possible. New high-throughput techniques of genomic analysis provide tools towards achieving these goals at molecular and cellular levels.[6] As the number of characterized molecular genetic networks increases, there are growing opportunities to dissect patterns of gene evolution within a network context.

Molecular evolutionary analyses have previously focused on individual genes outside of the interaction context. It is now clear that there is a need to understand how evolutionary forces act on multiple interacting genes that are components of molecular genetic pathways and networks. The network organization inherent to molecular genetic systems raises many questions regarding the evolutionary dynamics of interacting molecular systems.[7–9] How does the organization of genes as members of interacting pathways affect the rates of evolutionary change? How does the topology of molecular genetic networks constrain evolutionary forces acting on component genes? How do networks as entities evolve? Here we describe some recent studies on the molecular evolution of genetic networks, and how network structures can constrain or channel evolutionary processes, both at the microevolutionary and macroevolutionary levels.

Targets of selection in molecular genetic pathways

The organization of a molecular genetic pathway can provide a useful framework for the study of gene evolution, and the study of gene evolution in this context has been an important first step towards understanding how evolution acts differently on genes in a locally interacting system. Since pathways are, in essence, modular components of networks (see Fig. 1A), the study of pathways also provides a foundation for the study of global networks.

An area of interest has been how the position of genes in molecular genetic pathways can impact the levels and types of selective forces that act on these loci. One hypothesis

Department of Genetics, North Carolina State University.
Funding agency: In part by the US National Science Foundation.
*Correspondence to: Michael D. Purugganan, Department of Genetics, North Carolina State University, Box 7614, Raleigh, NC 27695.
E-mail: michaelp@unity.ncsu.edu
DOI 10.1002/bies.20026
Published online in Wiley InterScience (www.interscience.wiley.com).

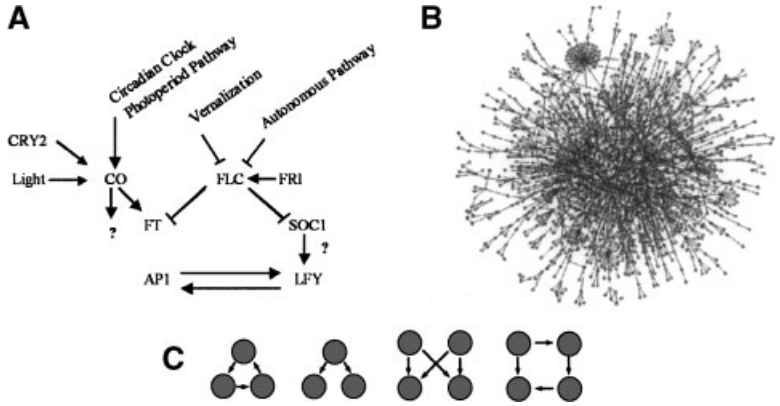

Figure 1. Three levels of organization at which the evolution of genes and their protein products have been studied in their interaction context. **A:** The *Arabidopsis thaliana* flowering-time pathway, shown as an example of a modular component of a genetic interaction network. Activation and repression interactions are depicted, along with several environmental signal inputs. **B:** A map of the yeast protein–protein interaction network from Ref. 31, as an example of global network organization (reproduced from Jeong H, Mason SP, Barabasi AL, Oltavi ZN. 2001. Lethality and centrality in protein networks. Nature 411:41–42 with permission of Nature Publishing Group). This network is represented by nodes (proteins) connected to one another by edges (interactions). Some proteins in the network have a large number of protein–protein interactions, while the majority of proteins have very few, resulting in a scale-free topology. **C:** Examples of network motifs (Refs. 43 and 44). Several three- and four-protein interactions motifs show types of recurring themes in network organization. Circles represents proteins and arrows show their interactions.

predicts that genes functioning early in a genetic pathway are subject to stronger stabilizing selection than downstream loci, since mutations in these genes are likely to have greater pleiotropic effects and affect all downstream phenotypes.

In a study on the rates of gene evolution in the plant anthocyanin biosynthetic pathway, differential rates of molecular evolution have been noted for enzymatic genes.[10] In comparisons between monocots and dicots, the nonsynonymous substitution rates of the downstream genes DFR, ANS and UF3GT evolve faster than the upstream loci CHS-D, CHI and F3H. The correlation between the protein evolutionary rate and the position of the enzyme in the anthocyanin pathway is significant ($P < 0.01$). This pattern has also been confirmed at the intragenic level between species in the genus *Ipomoea*.[11] In this pathway, upstream enzymes are positioned above major branch points, and mutational changes in these enzymatic loci are likely to have pleiotropic effects on the synthesis not only of anthocyanins but of flavonoids as well.[10] The pleiotropy of the upstream genes, and the greater specialization in biosynthetic functions of downstream enzymatic loci, appears to result in greater stabilizing selection on genes that act earlier in this biosynthetic pathway.

Greater constraint on earlier acting genes is also observed in studies of molecular variation in regulatory and signal transduction genes within populations. The *Ras*-mediated signal transduction pathway is an evolutionarily conserved genetic pathway that plays a central role in cell differentiation.[12] A molecular population genetic study suggests differential evolution among signal transduction loci in the *Ras* pathway in *Drosophila melanogaster*, indicating strong purifying selection on *Ras*, *Drk* and *polehole*, three upstream genes in the signaling pathway.[13] These three genes have only a few low-frequency replacement polymorphisms within *D. melanogaster* and no fixed amino acid differences between *D. melanogaster* and *D. simulans*. In contrast, there are several interspecific amino acid differences in downstream genes *Dsor1*, *corkscrew* and *Ksr*. In *Ras*-mediated signal transduction, the upstream genes act as control points, which may explain the greater degree of stabilizing selection on these loci. This study also suggests that the downstream components, which act as modifiers of *Ras* kinase signaling, are the most-likely source of quantitative phenotypic variation in this regulatory pathway.[13]

Other studies do not support the hypothesis that earlier-acting genes in genetic pathways are under strong stabilizing selection. In the *Arabidopsis thaliana* floral developmental pathway, upstream genes appear to be the targets of positive, not stabilizing, selection.[9] A molecular population genetic study of six genes in the *Arabidopsis* floral developmental pathway suggests that four downstream transcription factor genes (the floral meristem identity genes *AP1* and *CAL*, and the floral organ identity genes *AP3* and *PI*) have neutral patterns of molecular evolution. In contrast, the two earliest-acting genes in the study, the inflorescence architecture gene *TFL1* and the floral meristem identity gene *LFY*, show a significant reduction in silent site nucleotide variation consistent with a recent adaptive sweep.[9] Moreover, the promoter alleles of *TFL1* are differentiated into two distinct haplotype groups that

may be maintained by selective forces.[9] These results indicate that, in the floral developmental pathway, it is the earliest-acting genes that are likely to be the targets of recent selective forces.[9]

The difference in selective patterns between the *A. thaliana* floral regulatory genes and the *D. melanogaster Ras* signaling loci is noteworthy, but may be explicable in the context of the functions of these two developmental pathways. Mutations in the latter have potentially strong pleiotropic effects on fly development, since these loci affect several developmental processes from oogenesis to appendage morphogenesis.[12] In contrast, the floral developmental pathway is associated with a relatively specific and discrete developmental module (the flower), and the activity of *TFL1* and *LFY* function to specify when and where this entire module is established.[9] Thus, these genes form a discrete "gene net",[14] and any selection on evolutionary change in the spatial and temporal establishment of floral developmental modules would be more likely to act on these upstream loci in the regulatory hierarchy. Gene position within a molecular genetic pathway may indeed result in differential selection on component genes, but the specific nature of the selective forces on the component loci will largely depend on the function of the pathway.

Differential selective forces on different components of a pathway at a microevolutionary level may be taken to a macroevolutionary extreme in considering the diversification of sex-determination pathways.[15] In sex-determination cascades, downstream components appear to have conserved expression patterns (e.g. *Sox 9* between birds and mammals),[16] while upstream components, such as *Sry*, have a more circumscribed distribution even within mammals.[17] The observed plasticity of upstream components is also seen in other cases within the Diptera.[18,19] together, these observations suggest either retrograde evolution in pathway expansion or evolutionary flexibility in upstream (but not downstream) pathway genes.[15] The continued study of the evolution of sex-determination pathways may provide further insights into the nature of selective forces that not only impact on the evolution of genes, but also on the evolutionary construction of genetic pathways.

Metabolic networks and branch point evolution

The dichotomy between upstream and downstream genes in a pathway is a crude differentiation of function. For metabolic pathways, a theory of pathway fluxes provides a framework for developing more precise hypotheses regarding selection on component genes based on position within the pathway and/or specific biochemical functions. Metabolic control theory[20] describes how pathway architecture constrains evolutionary change by depicting how metabolic fluxes might be partitioned into alternate channels through selection.[21]

The evolution of genes in relation to their position in pathway branch points has been explored most explicitly in the context of population variation in the glycolytic pathway in *D. melanogaster*.[21] The PGM and G6PD enzymes sit at the top of the glycolytic pathway and partition glucose into glycogen and pentose shunt branches.[21] Nucleotide and allozyme variation at these two glycolytic loci show clinal variation across latitudes.[22,23] An excess of within-species amino acid changes is observed at the *PGM* gene, with 12 of 25 within-species coding region polymorphisms being replacement polymorphisms.[24] In contrast, none of the 19 fixed differences between *D. melanogaster* and *D. simulans* result in amino acid changes. This pattern suggests selection for the maintenance of protein variation in *D. melanogaster*. The *G6PD* locus shows a different pattern, with a significant excess of amino acid differences fixed between species. This pattern is consistent with positive selection favoring protein divergence between the two species.[25] Variation for these genes, as assayed by allozyme polymorphism, is also observed in species outside of *Drosophila*.[26,27]

Selection at enzymatic branch points is also observed in the evolution of starch biosynthesis in *Zea mays*.[28] Maize domestication has been accompanied by selection for altered starch content in kernels. A molecular population genetic study of six starch biosynthetic enzymes show reduced variation in three enzymatic loci—*bt2*, *ae1* and *su1*—associated with selective sweeps.[28] The latter two genes encode enzymes that function at branch points. Both are involved in amylopectin synthesis, partitioning ADP-glucose between amylose and amylopectin. As in *Drosophila* glycolytic pathway studies, selection on the maize starch biosynthetic pathway involved pathway branchpoints that function by partitioning substrates into alternate product pools.

Together, these results indicate that genes that act at metabolic pathway branch points are targets of adaptive forces. These studies also suggest multilocus responses to selection,[21] and provide clear examples of how a theoretical framework for pathway fluxes could yield insights into the nature of selective forces on molecular genetic pathways. More detailed studies on the evolution of molecular genetic networks will also benefit from more rigorous general theoretical models on network structure and dynamics, particularly on the phenotypic consequences of mutational change on network components. Development of these models for developmental regulatory systems,[8,29,30] for example, may provide the basis for a coherent study of the evolution of developmental phenotypes.

Global structure and the evolutionary growth of networks

Although there has been some progress in our understanding of pathway evolution, we are still left with several unanswered questions relating to the evolution of larger networks. Does

selection act differently at local (pathway) and global (network) scales? Is there selection for global network structure? How does the global network grow? Empirical studies of global network structure and evolution have only recently become possible and are beginning to address these and other related questions.

The identification of extensive protein–protein interaction networks in *Saccharomyces cerevisiae* has provided the basis for several early analyses of network evolution. One of the first examples of such a network consists of 1,870 proteins related to one another through a web of 2,240 interactions (see Fig. 1B). This protein interaction network is characterized by a scale-free topology, in which different proteins have varying numbers of interactions—a small number of proteins have a large number of interactions, while the majority of proteins have very few connections.[31] This scale-free topology has been shown to be a common characteristic of protein interaction networks,[31,32] metabolic networks[33] and *Caenorhabditis* expression networks determined from yeast, human, *Drosophila* and *C. elegans* microarray data.[34]

How does this scale-free network architecture originate? There have been some suggestions that this architecture may arise from selection for genetic robustness (see below). Others have suggested, however, that the emergence of a scale-free network topology can be accounted for without assuming the involvement of natural selection on global network structure.[35] Biological justification for the "growing network model"[35] reveals that there are only two requirements for the evolution of a scale-free network structure in the yeast protein interaction network: (1) the addition of new nodes to the network and (2) the preferential attachment of these new nodes to already highly connected nodes.[35,36,37] Although this model does not explicitly invoke positive selection in the large-scale organization of networks, it should be noted that selection may be implicitly involved since preferential attachment of new nodes may be driven by selective forces acting at the local level.

An analysis of divergent gene duplicates in the yeast protein interaction network provides some support for the preferential attachment assumption. The relationship between protein connectivity and the likelihood of gaining interaction partners was shown to be close to linear, indicating that proteins with larger numbers of interactions are more likely to gain new connections.[35] Examining the addition of new network interaction links through evolutionary time also permits testing of the preferential attachment hypothesis.[35] "Snapshots" in evolutionary time were generated for the yeast protein interaction network through genome-wide comparisons of yeast with *E. coli*, *A. thaliana* and *S. pombe*. Each protein was classified in one of four age groups based on its presence or absence within the comparison species, which allowed the observation of changes in protein connectivities over evolutionary time. It was demonstrated that new links in the network are more likely to be added through interactions with proteins that are already highly connected, which then leads to the emergence of network scale-free topology.[37]

Interestingly, Qin et al. advanced a slightly different view of network evolution for the yeast protein interaction network.[38] In their analysis, they suggested that proteins of similar ages (which they refer to as isotemporal categories) have a greater tendency to interact with each other than if they were in different age groups. Moreover, it appears that the networks tend to grow via addition of modules or groups of interacting proteins rather than single protein additions.[38] It would be instructive to examine whether network growth is driven by connectivity or evolutionary age, although it may be difficult to disentangle these two factors given that they may be related to some degree.

Gene evolution, network position and network motifs

The evolutionary rates of genes and/or proteins appear to be related in part to their network position. A negative correlation between connectivity and rate of protein evolution has been observed in the yeast protein interaction network (although this relationship has been debated[39]), with highly connected proteins in the network showing the slowest rates of evolution.[40] The authors suggest that this effect arises from highly connected proteins having most of their structure involved in functional interactions, and thus under greater selective constraint. Evolutionary changes at functionally important protein sites might thus be attributed largely to co-evolutionary diversification between interacting protein partners, and proteins that interact with one another have similar evolutionary rates.[40] Other correlations between protein age, connectivities and function have also been found.[37] Finally, essential proteins also appear to be older[37] and to have greater connectivities and slower rates of evolution;[31,40] however, this has also been questioned.[41]

These relationships are also buttressed by examining the evolution of network motifs, local patterns of interaction that recur at different positions within a network[42] (see Fig. 1C). Wuchty et al. classified topological motifs in a *S. cerevisiae* protein interaction database derived primarily from two-hybrid studies, which include 3,183 interacting yeast proteins.[43] These include all possible two-protein, three-protein, four-protein, and a few five-protein interaction motifs, and the database specifies between 10^3 and 10^6 copies of each motif. Conservation of motifs across evolutionary time was judged based on the presence of an orthologous protein across five eukaryotic species in plants, animals and fungi.[43]

The larger motifs with higher connectivities were conserved to a greater extent than smaller motifs; 47% of fully connected five-protein motifs were conserved, compared to less than 5% of linear three-protein motifs. The evolutionary retention rate of

every network motif was higher than expected by random chance, and the ratio of observed number of motifs over random expectation was higher the larger the motif. Interestingly, the composition and frequency of motifs is related to biochemical function. Large motifs had functionally homogenous components. In 95% of conserved fully connected five-protein motifs, all the protein components share at least one biochemical function class (i.e., regulation, protein fate, cell cycle, etc.). In contrast, only 10% of the two-protein motifs were functionally homogenous. Moreover, different functional classes have different characteristic motifs. Regulation, cellular transport and transport facilitation have only one or two characteristic motifs, while all 11 studied motifs were overrepresented in modules associated with subcellular localization, protein fate and transcription.[43] In addition, it should be noted that previous studies identified network motifs that also appear to be commonly involved with certain functional roles.[42,44,45]

Network connectivities and genetic robustness

The impact of the topological architectures of networks on their ability to withstand mutational perturbations is an exciting avenue of exploration, and could lead to refinement of hypotheses on selective targeting of genes during evolution. The observation that a large number of genetic changes may be buffered from expressing mutational variation has traditionally been ascribed to the presence of gene duplications that lead to genetic redundancies.[46–48] Recently, the role that genetic network organization may play in maintaining robustness is gaining increased attention.[46,49] It has been shown, for example, that highly connected proteins in the yeast protein interaction network are three times less likely to be tolerant to loss-of-function mutations than proteins with fewer connections.[31] This observation, coupled with the scale-free topologies in protein interaction networks, has led to suggestions that selection constructs a network that is phenotypically robust against mutations. If network structure does provide an organism with phenotypic robustness against mutation, then this may be one explanation for the phenomenon of genetic canalization.[50,51]

The identification of molecular genetic networks also provides avenues for exploring the molecular basis of evolutionary epistasis. It has always been recognized that network organization leads to genetic interactions that may result in epistasis.[8,52] Candidate epistatic interactions can now be systematically identified in the context of known genetic or physical interaction networks, and may provide a molecular basis for determining the extent and nature of evolutionary epistasis.

Conclusions

The study of the evolution of genes and genetic systems has entered a new phase. The advent of genomics technologies has provided opportunities for expanded analyses, which permit studies of evolutionary change at genome-wide scales. There is now the possibility of studying the evolution of whole genetic networks rather than single loci, and examining the implications of network organization on the dynamics of evolutionary change. Although much has been accomplished by our attempts to define molecular genetic networks, there is still a great deal of work to be done and many more details to consider.

There still remain several areas of network analysis that could help spur further evolutionary investigations, including the development of precise, quantitative models that relate network topologies and dynamics to their phenotypic consequences. Eventually the depiction of molecular genetic networks will have to allow for the incorporation of spatial and temporal regulatory mechanisms. The complete network of cellular interactions, after all, involves much more than just a depiction of who regulates and/or interacts with whom. The development of these models will provide evolutionary studies with a functional context and allow for the integration of molecular, population and quantitative genetic studies. Furthermore, analyses of global network structure will prove fruitful not only for understanding global evolutionary pressures, but also of forces acting at a local level and will allow us to consider pathway structure and evolution in a more realistic functional context.

Acknowledgments

We thank members of the Purugganan laboratory for a critical reading of the manuscript, as well as Dr. Adam Wilkins and two anonymous reviewers for insightful comments and ideas.

References

1. Dixon B. 1988. Metabolic pathways chart: an all-time bestseller. The Scientist 2:23.
2. Loomis WF, Sternberg PW. 1995. Genetic networks. Science 269:649.
3. Melian CJ, Bascompte J. 2002. Complex networks: two ways to be robust? Ecol Lett 5:705–708.
4. White KP. 2001. Functional genomics and the study of development, variation and evolution. Nat Rev Genet 2:528–537.
5. Slonim DK. 2002. From patterns to pathways: gene expression data analysis comes of age. Nat Genet 32:502–508.
6. DeRisi JL, Iyer VR, Brown PO. 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278: 680–686.
7. Wilkins AS. 2002. The evolution of developmental pathways. Sunderland, MA: Sinauer.
8. Frank SA. 1999. Population and quantitative genetics of regulatory networks. J Theor Biol 197:281–294.
9. Olsen KM, Womack A, Garrett AR, Suddith JI, Purugganan MD. 2002. Contrasting evolutionary forces in the Arabidopsis thaliana floral developmental pathway. Genetics 161:1641–1650.
10. Rausher MD, Miller RE, Tiffin P. 1999. Patterns of evolutionary rate variation among genes of the anthocyanin biosynthetic pathway. Mol Biol Evol 16:266–274.
11. Lu Y, Rausher MD. 2003. Evolutionary rate variation in anthocyanin pathway genes. Mol Biol Evol 20:1844–1853.
12. Khosravi-Far R, Rossman K, Clark G, Der C. 1998. Increasing complexity of Ras signaling. Oncogene 17:1395–1413.

13. Riley RM, Jin W, Gibson G. 2003. Contrasting selection pressures on components of Ras-mediated signal transduction in Drosophila. Mol Ecol 12:1315–1324.
14. Bonner JT. 1988. The evolution of complexity. Princeton, NJ: Princeton University Press.
15. Stothard P, Pilgrim D. 2003. Sex-determination gene and pathway evolution in nematodes. BioEssays 25:221–231.
16. Morais da Silva S, Hacker A, Harley V, Goodfellow P, Swain A, Lovell-Badge P. 1996. Aoz9 expression during gonadal development implies a conserved role for the gene in testis defferentiation in mammals and birds. Nat Genet 14:62–68.
17. Just W, Rau W, Vogel W, Akhverdian M, Fredga K, et al. 1995. Absence of Sry in species of the vole *Ellobius*. Nat Genet 11:117–118.
18. Saccone G, Peluso I, Artiaco D, Giordano E, Bopp D, Polito LC. 1998. The *Ceratitis capitata* homologue of the *Drosophila* sex-determining gene Sex-lethal is structurally conserved, but not sex-specifically regulated. Development 125:1495–1500.
19. Meise M, Hilfiker-Kleiner D, Dubendorfer A, Brunner C, Nothinger R, Bopp D. 1998. Sex-lethal, the master sex-determining gene in Drosophila, is not sex specifically regulated in Musca domestica. Development 125:1487–1494.
20. Kacser H, Burns JA. 1973. The control of flux. Symp Soc Exp Biol 27:65–104.
21. Eanes WF. 1999. Analysis of selection on enzyme polymorphisms. Ann Rev Ecol Syst 30:301–326.
22. Verrelli BC, Eanes WF. 2001. Clinal variation for amino acid polymorphisms at the Pgm locus in Drosophila melanogaster. Genetics 157:1649–1663.
23. Oakeshott JG, Chambers GK, Gibson JB, Eanes WF, Wilcocks DA. 1983. Geographic variation in G6pd and Pgd allele frequencies in Drosophila melanogaster. Heredity 50:67–72.
24. Verrelli BC, Eanes WF. 2000. Extensive amino acid polymorphism at the Pgm locus is consistent with adaptive protein evolution in Drosophila melanogaster. Genetics 156:1737–1752.
25. Eanes WF, Kirchner M, Yoon J. 1993. Evidence for adaptive evolution of the G6pd gene in the Drosophila melanogaster and D. simulans lineages. Proc Natl Acad Sci USA 90:7475–7479.
26. Schulte PM, Gomez-Chiarri M, Powers DA. 1997. Structural and functional differences in the promoter and 5′ flanking region of Ldh-B within and between populations of the teleost Fundulus heteroclitus. Genetics 145:759–769.
27. Watt WB. 1977. Adaptation at specific loci. I. Natural selection on phosphoglucose isomerase of *Colias* butterflies: biochemical and population aspects. Genetics 87:177–194.
28. Whitt SR, Wilson LR, Tenaillon MI, Gaut BS, Buckler ES. 2002. Genetic diversity and selection in the maize starch pathway. Proc Natl Acad Sci USA 99:12959–12962.
29. Mendoza L, Thieffry D, Alvarez-Buylla ER. 1999. Genetic control of flower morphogenesis in Arabidopsis thaliana: a logical analysis. Bioinformatics 15:593–606.
30. Johnson NA, Porter AH. 2001. Toward a new synthesis: population genetics and evolutionary developmental biology. Genetica 112–113: 45–58.
31. Jeong H, Mason SP, Barabasi AL, Oltavi ZN. 2001. Lethality and centrality in protein networks. Nature 411:41–42.
32. Rain JC, Selig L, De Reuse H, Battaglia V, Reverdy C, et al. 2001. The protein-protein interaction map of *Helicobacter pylori*. Nature 409:211–215.
33. Jeong H, Tombor B, Albert R, Oltvai ZN, Barabasi AL. 2000. The large-scale organization of metabolic networks. Nature 407:651–654.
34. Stuart JM, Segal E, Koller D, Kim SK. 2003. Gene-Coexpression network for global discovery of conserved genetic modules. Science 302:249–254.
35. Wagner A. 2003. How the global structure of protein interacion networks evolves. Proc R Soc Lond B 270:457–466.
36. Barabasi AL, Albert R. 1999. Emergence of scaling in random networks. Science 286:509–512.
37. Eisenberg E, Levanon EY. 2003. Preferential attachment in the protein network evolution. Phys Rev Lett 91:138701.
38. Qin H, Lu HH, Wu WB, Li WH. 2003. Evolution of the yeast protein interaction network. Proc Natl Acad Sci USA 100:12820–12824.
39. Jordan IK, Wolf YI, Koonin EV. 2003. No simple dependence between protein evolution rate and the number of protein-protein interactions: only the most prolific interactors tend to evolve slowly. BMC Evol Biol 3:1.
40. Fraser HB, Hirsh AE, Steinmetz LM, Scharfe C, Feldman MW. 2002. Evolutionary Rate in the protein interaction network. Science 296:750–752.
41. Pal C, Papp B, Hurst LD. 2001. Genomic function (communication arising): Rate of evolution and gene dispensability. Nature 411:1046–1049.
42. Shen-Orr SS, Milo R, Mangan S, Alon U. 2002. Network motifs in the transcriptional regulation network of *Escherichia coli*. Nat Genet 31:64–68.
43. Wuchty S, Oltavi ZN, Barabasi AL. 2003. Evolutionary conservation of motif constituents in the yeast protein interaction network. Nat Genet 35:176–179.
44. Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U. 2002. Network motifs: simple building blocks of complex networks. Science 298:824–827.
45. Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, et al. 2002. Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science 298:799–804.
46. Wagner A. 2000. Robustness against mutations in genetic networks of yeast. Nat Genet 24:355–361.
47. Smith V, Chou KN, Lashkari D, Botstein D, Brown PO. 1997. Functional analysis of the genes of yeast chromosome V by genetic footprinting. Science 274:2069–2074.
48. Kim SK. 2001. Functional genomics: The worm scores a knockout. Curr Biol 11:R85–R87.
49. Kitami T, Nadeau JH. 2002. Biochemical networking contributes more to genetic buffering in human and mouse metabolic pathways than does gene duplication. Nat Genet 32:191–194.
50. Hansen TF, Wagner GP. 2001. Modelling genetic architecture: A multilinear theory of gene interaction. Theor Pop Biol 59:61–86.
51. Gibson G, Wagner GP. 2000. Canalization in evolutionary genetics: a stabilizing theory? Bioessays 22:372–380.
52. Omholt SW, Plahte E, Oyehaug L, Xiang K. 2000. Gene regulatory networks generating the phenomena of additivity, dominance and epistasis. Genetics 155:969–980.