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Bow ties, metabolism and disease

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Highly organized, universal structures underlying biological and technological networks mediate effective trade-offs among efficiency, robustness and evolvability, with predictable fragilities that can be used to understand disease pathogenesis. The aims of this article are to describe the features of one common organizational architecture in biology, the bow tie. Large-scale organizational frameworks such as the bow tie are necessary starting points for higher-resolution modeling of complex biologic processes

Systems biology is a multidisciplinary approach for describing, understanding and controlling the properties and dynamics of whole biological networks and systems that combines high-throughput data generation with new computational and mathematical tools [1]. The long-term goal in systems biology – that is, the integration of information from varied sources into models that are in some sense 'complete' – will require the connection of data and models at several levels of abstraction and detail [2].

Without an organizational framework, however, the sheer complexity of whole cells and organisms can be overwhelming, and model-based predictions about the nature of rare events of greatest interest (i.e. disease states) will be difficult to extract. Thus, complex systems can be understood only by identifying their organizing principles, theories, design rules and, in particular, protocols. By protocols, we mean the rules and interfaces by which modules interact; these protocols are organized within a global framework referred to as the 'architecture' [3].

Engineering approaches to understand the organizational principles of biological networks have had both a rich, successful history and a recent revival in interest [4]. Engineering tools have been used to identify important biological motifs and modules [5], as well as their regulation by universal principles of robustness [3]. Even in engineering, however, complex networks such as very-large-scale integrated circuits are not modeled simultaneously at the level of whole-chip or 'device physics', but instead are modeled with a hierarchy of schemes of various resolution.

Here we address global architectures and protocols that complement the device physics of local circuit motifs [6]. The natural language in which to describe these universal principles is, of course, mathematical. Unfortunately, theory for the type of distributed and asynchronous global control used in biology is relatively new [7]. Nevertheless, from existing concepts it is possible to distill insight into candidate universal architectures that can be tentatively confirmed by comparing biological systems with one another and with technological systems.

'Bow-tie' structures and protocols in metabolism

Although bacterial metabolism is probably the best-studied biological network, little is known about the detailed kinetics necessary to model quantitatively the vast regulatory feedbacks that control metabolism. Yet much progress has been made by taking stoichiometry, which is known in some detail, as a prerequisite and by simply assuming that the unmodeled control actions are optimal (i.e. they rapidly create controlled equilibrium that are perfectly adapted to environmental conditions and cellular demands) [8–10]. In this article, we consider in what sense the global architecture of metabolism itself, including stoichiometry and regulation, can be thought of as 'optimal', and we argue that this remarkable, evolved optimal architecture, together with its protocols, reflects universal organizational principles of complex networks.

Bacterial metabolic networks are a striking example of 'bow-tie' organization and illustrate the flexibility that such a structure provides. As shown in Figure 1, a myriad of nutrient sources are catabolized, or 'fan in', to produce a handful of activated carriers (e.g. ATP, NADH and NADPH) and 12 precursor metabolites (e.g. glucose 6-phosphate, fructose 6-phosphate, phosphoenolpyruvate and pyruvate), which are then synthesized into roughly 70 larger building blocks (e.g. amino acids, nucleotides, fatty acids and sugars). The precursors and carriers can be thought of as two 'knots' of separate bow ties that are both fed by catabolism, but whereas the former 'fan out' locally to the biosynthesis of universal building blocks, the latter fan out to the whole cell to provide energy, reducing power and small moieties. The building blocks then further fan out into the complex assembly of macromolecules by general-purpose polymerases. Although this description of the bow-tie structure of metabolism is an engineering interpretation of familiar textbook biochemistry, it also has been derived from the computational analysis of genomic data from 65 microorganisms [11].

The transcription and translation ('trans') processes also have a bow-tie architecture. A few universal polymerase modules that make up the 'knot' of the trans bow-tie machinery function efficiently with a universal codon usage protocol, facilitating the fan in of a large variety of genes and the fan out of an even larger variety of proteins. Nested together, the bow ties of core metabolism and the trans machinery create a larger 'metabolism bow tie' that

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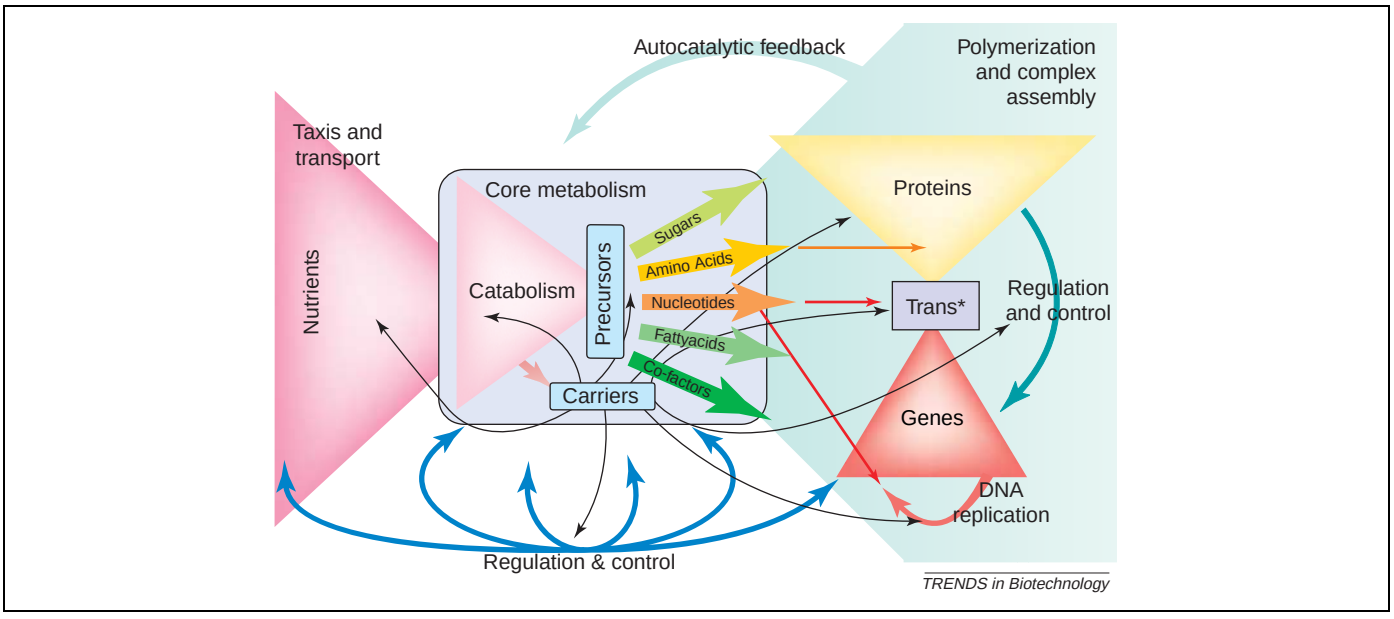

Figure 1. The nested bow-tie architectures of metabolism input a wide range of nutrients and produce a large variety of products and complex macromolecules using a relatively few intermediate common currencies. The common currencies and their enzymes form the knot of the bow tie. The overall bow tie can be decomposed into three principal subsidiary bow ties. One produces the activated carriers, such as ATP, NAD and NADP, that globally supply the cell with energy, reducing power and small moieties. In parallel, catabolism produces a standard group of 12 precursor metabolites, among them glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), phosphoenolpyruvate (PEP), pyruvate (PYR), α-ketoglutarate (AKG) and acetyl-coenzyme A (ACCOA), which are the starting points for the biosynthesis of amino acids, nucleotides, carbohydrates, fatty acids and cofactor building blocks. These building blocks are then used by general-purpose polymerases, particularly in the transcription and translation (trans*) bow tie, to assemble complex macromolecules. This architecture uses selective homogeneity at the knot to facilitate control, organization and management of the enormous heterogeneity in enzyme specificity, action and regulation, and in substrate size, flux and concentration. All modern technologies, from manufacturing to the power grid to the Internet, are organized with bow ties.

produces all cellular macromolecules. Modularity and shared protocols also facilitate the recycling of building blocks within the system.

This robust design has inherent fragilities. In a bow-tie structure, a chief source of fragility is that the universal common currencies responsible for robustness can be easily hijacked by parasites or used to amplify pathological processes. For example, tumor survival is enhanced by hijacking and upregulating processes that are part of normal physiological homeostasis. The efficiency and adaptability of metabolism, coupled with its fragilities, illustrate a highly/heterogeneous optimized/organized trade-off/tolerance (HOT) architecture [12]; in other words, the metabolism bow-tie architecture and associated protocols allow highly optimized trade-offs between numerous requirements, such as reaction complexity (number of substrates in a reaction), genome size, efficiency (energy required for each reaction) and particularly adaptability, through tolerance to various perturbations and evolvability on longer timescales.

Some general consequences of a HOT architecture are clear. For example, if every nutrient–product combination had independent pathways without shared precursors and carriers, the total genome would be much larger and/or its encoded enzymes would be vastly more complex. In both cases, adaptation to fluctuating environments on any timescale would be difficult. Only an organization such as the bow tie facilitates the type of extreme heterogeneity that allows for robust regulation, manageable genome sizes and biochemically plausible enzymes. Bow-tie structures and protocols are found throughout biology in parallel or convergent systems, as well as in homologous systems. Furthermore, the basic framework of bow ties described here is used throughout advanced technologies. Taken together, the convergent evolution in biology and developments in technology suggest that these structures and protocols are universal.

Control, robustness and evolvability over multiple timescales

The robustness of the bow-tie structure with its small knot of common currencies (carriers and precursors) is that it facilitates control, accommodating perturbations and fluctuations on many timescales and spatial scales. Although metabolism allows large fluctuations in nutrients and products, relatively small fluctuations in ATP are lethal. But the very architecture that creates this fragility also helps to alleviate it, because ATP concentrations are tightly regulated and not easily changed. The structure also allows great heterogeneity or 'self-dissimilarity' throughout metabolism: every possible extreme in enzyme complexity, specificity, control mechanism, and metabolite size, concentration and turnover is accommodated.

On fast timescales, fluxes and concentrations of carriers and precursors are regulated primarily by allostery and competitive inhibition, which is effected by the bow-tie structure. With a few high-flux currencies, fast transient fluctuations in supply and demand have more opportunity to average out. If a change in fluxes is required, it is typically easier to regulate a small number of large fluxes with a few enzymes than a large number of small fluxes with many enzymes. Thus, the enzymes closest to the 'knot' of metabolism are highly efficient and specialized, with most catabolism and basic biosynthesis pathways involving one or several enzymes devoted to each reaction.

Typically, the more central the reactions are to the core precursor metabolites, the more specialized the enzymes, the smaller the metabolites, the higher the ranges of possible fluxes and turnover, and the lower the concentrations. With tight regulation at the knot, downstream processes involving more complex assembly do not need to be so robust to variations in their building block concentrations and fluxes. Similarly, a minimal set of building blocks, low concentrations (inventory), highly variable fluxes and their rapid control in the face of external perturbations are familiar management features of highly adaptive 'just-in-time' manufacturing.

At the downstream edge of the metabolism bow tie, additional robustness and flexibility are created by general-purpose polymerases and other trans machinery plus the degradation machinery. These general-purpose machines are extraordinarily adaptable and controlled by regulated recruitment [13], a mechanism of feedback control with a very different biochemical implementation from that of allostery. On the intermediate timescale of transcriptional regulation and translation, the bow tie facilitates feedback regulation, because whole pathways can come and go on demand, plugging into the common currencies in the knot of the bow ties with minimal effect on other pathways. The protocol of shared currencies creates a 'plug-and-play' modularity, such that fluctuating supply and demand are met with the minimal synthesis of new enzymes. At the edges of the bowtie turnover and fluxes can still be highly variable, although they are less so than near the knot. Most different from the knot is the enormous variation in molecular size and concentration, but these are closely controlled to meet cellular demands.

A striking feature of bow-tie architectures is that they make robustness and evolvability completely compatible objectives [14,15]. On long-time horizons, evolvability can be considered as the robustness of lineages to potentially large (environmental and/or internal) changes. Universal carriers, precursors, core polymerases and codon usage facilitate lateral gene transfer, because shared universal protocols enable swapped modules to be functional in novel settings with minimal modification. Gene copying and the evolution of genes through the accumulation of point mutations can effect new functionality more efficiently in shared systems than in a less structured system. Regulated recruitment is an extremely evolvable regulatory mechanism [13] and is made possible by a bow-tie architecture encompassing a few general-purpose enzymes.

Bow-tie structures accommodate the flow of information and/or control, as well as the flow of materials and energy. Bacterial signal transduction features a bow-tie structure for the flow of information via two-component systems using the shared protocol of a histidine autokinase transmitter and aspartyl phospho-acceptor receiver. Various biological responses are organized and coordinated through these protocols, and the universality of the structure makes it evolvable because new functionality can be easily generated through gene duplication and divergence or lateral transfer. Molecular phylogenies of signal transduction systems support claims for the robustness, universality and evolvability of these designs. In eukaryotic signal transduction, G proteins are conserved examples of flexible bow-tie structures that mediate a wide range of cellular functions.

The 'robust yet fragile' trade-offs of the bow-tie architectures also extend to evolutionary timescales. Much of the core of a bow tie is often highly conserved, yet this conservation facilitates high variability nearby. The core is preserved by selection on three levels: first, the whole system is fragile to short-term changes in the core, because the common currencies and other functions provided by the core are used systemically, making an exploration of alternatives difficult; second, the structure facilitated by the core provides robustness elsewhere, typically by managing regulation; and third, the architecture facilitates evolution in the long term by providing a plug-and-play modularity around the core. Crucial protocols and modules are standardized and not easily changed because, in turn, standardization optimizes robustness and evolvability in the rest of the network. There is also a trade-off in the size of the knot in the bow-tie architecture: a large knot can provide flexibility in the short term by delivering a rich set of common components or services, but only at the risk of constraining short-term controllability and long-term evolvability.

Universality and technology

The ubiquity of bow-tie structures in advanced technologies supports the large amount of biological evidence indicating that these structures are universal and fundamental organizing principles, rather than frozen accidents of evolution. In the power grid, several different energy sources are used to make a universal 60-Hz AC common carrier, which in turn is widely disseminated to provide power to a large and rapidly changing variety of uses.

In general, manufacturing is typically a bow tie with numerous raw materials, which are transformed into relatively few building blocks, which are then assembled into many different products. The most complex assemblies involve more fixed or low-flux substrates (such as aircraft or buildings) and machines controlled by regulated recruitment. The fixed machine on the factory floor that rapidly makes rivets from raw materials is analogous to the enzymes of core metabolism. By contrast, the riveting machine that moves rapidly around a building or aircraft undergoing construction parallels the general-purpose polymerases of complex assembly. Universal standards (at the knot) both freeze out and facilitate change.

Perhaps the most famous technological bow tie is the Internet protocol stack 'hourglass' (a bow tie on its side). Here, the application layer that includes email and the Internet sits above hardware or link layers that provide raw packet delivery. Between these highly heterogeneous extremes are the universal, homogeneous, but hidden transmission control protocol/Internet protocol (TCP/IP) layers that provide routing, reliable transport and congestion control. All layers are decentralized and asynchronous, but the TCP/IP protocol suite ensures robust and coherent behavior. For example, readers accessing this article on the Internet will use various ancillary protocols at all layers of the protocol stack,

including standard languages for document display (PDF and HTML). Unfortunately, the same hidden mechanisms that facilitate the transparent delivery of this article also enable the propagation of spam, viruses and denial of service attacks.

It is not only biology and technology that use bow-tie architectures. Money can be thought of as a common carrier that implements a bow-tie protocol for the exchange of varied goods and services. As compared with a barter system, money greatly facilitates trade and economic growth, but it increases the risk of fragilities in the form of theft, counterfeiting, creative accounting and financial market collapses. This example further shows that the most severe fragilities created by bow-tie architectures involve hijacking or manipulating the universally used central protocol and carriers, rather than simple destruction. Systemic collapse owing to these fragilities can result from attack, as in pathogen infections, or from cascading failure events triggered by the breakdown of regulatory mechanisms, as in power grid failures.

Implications for disease states

The consequences of fragilities in bow-tie architectures are rarely observed: catastrophic failure events do not usually occur in well-designed systems under typical circumstances. Nevertheless, these fragilities have some predictability if the architecture is understood.

Bow-tie structures dominate complex mammalian physiology. Although ATP is the energy carrier for fast intracellular processes, organism-wide glucose homeostasis is also configured as a bow-tie structure, where glucose is the common carrier in the knot. The sources, quality, availability and percentages of energy sources in diets (the incoming 'fan in' to the bow-tie knot) vary considerably both within and between individuals. Regulation of glucose in the bloodstream involves coordinated, nested feedback loops at cellular and tissue levels.

At the inter-organ level, these loops include short- and long-term satiety feedback circuits in the brain, driven by signals from the gut and from adipocytes, that are responsive to global metabolic demand. At the cellular level, insulin function drives glucose into cells with counterregulatory balance from glucagon signaling; independently, glucose affects transcriptional profiles, generating diverse changes in cellular function. At the downstream end of the bow tie, the demand for glucose also varies widely, depending on acute and chronic activity levels, age, circadian rhythms and the substrate use or energetic status of individual organs.

The bow-tie architecture of glucose homeostasis is a necessary organization that protects cells from the devastating short-term effect of too little glucose (to which the brain is exquisitely sensitive) or chronic hyperglycemia (leading to microvascular failure). But a dependence on glucose as a common energy currency exposes the whole organism to failure if the glucose regulatory system is subverted.

Another fragility in the organization of internal homeostatic protocols is that they create a welcoming environment for microorganisms. The tight regulation of glucose, oxygen, pH and temperature is ideal for microorganism replication, enabling some parasitic microorganisms to streamline their genomes. With such a stable environment for microorganisms to inhabit, the boundaries between innocuous and pathological colonization must be controlled by robust immune surveillance. Although the immune system supports maintenance of our complex internal state, its power can be turned against us when self-antigens are misread as foreign. An example of such fragility (autoimmunity) that can lead to cascading failure is type 1 diabetes, which is initiated by an immune system attack on insulin-producing pancreatic β cells. The catastrophic disease that follows is the consequence of one (immune) control system disrupting another integrated (glucose) bow tie.

Similar to type 1 diabetes, type 2 diabetes is an inherent fragility of a robust system in which common currencies are disabled. Complex mammalian homeostasis requires a coordinated transport system (vasculature) through which regulatory proteins and gases are distributed and partitioned, mediating feedback control of the cellular environment. In this respect, the vasculature is a common currency that is vulnerable to predictable fragilities inherent in the architectural design of organism homeostasis. Thus, type 2 diabetes is a fragility of robust systemic homeostasis, because the common currency of transport is disabled by endothelial dysfunction.

Both forms of diabetes show that near-perfect robustness to typical perturbations has a trade-off in systemic fragilities. These fragilities might be rare at the cellular or organismal level, but their nature is often highly structured and thus predictable. The common carrier (glucose) that creates the opportunity for extremely robust regulation and function also creates – through its very universality – the potential for catastrophic cascading failure events in type 1 and type 2 diabetes. Although type 2 diabetes represents a catastrophic failure that occurs over a longer time horizon than the failure occurring in type 1 diabetes, systems architecture is relevant in both diseases to the failure mode, which depends on specific inherent fragilities in the organization of glucose regulation.

With this elaborate, multilayered control over glucose levels, why is diabetes now so rampant? In fact, diabetes is rare when viewed over centuries of human history. The glucose-control bow-tie architecture is robust to the enormous changes in diet and demand that have characterized most of human history. Obesity in nature is unusual and was probably uncommon in humans until recent generations. The ability to store fat (and lose less fat) in times of food shortage probably confers a survival advantage, such that this evolved safeguard is part of a normal genetic background. In this respect, all humans are at 'genetic risk' for obesity. Acute stresses that cause transient loss of weight or weight gain (which are both associated with malnutrition) are usually tolerated in terms of glucose homeostasis and regulation, but sustained obesity leads to maladaptive metabolic changes that reinforce diabetic pathways (insulin resistance).

The architecture that controls glucose homeostasis is not, then, robust to the extremes of modern energy-dense diets that contribute to obesity. (Analogies to the power

grid are clear. The United States power grid provides reliable electricity on most days, but it is not robust to the extremes of uncontrolled population growth combined with rare weather events that boost demand.) This framework suggests that therapeutic approaches targeted only to bring glucose levels back into the normal physiological range are insufficient therapy for modern metabolic syndrome: unless obesity is also treated, the global system regulation cannot capitalize on the evolved control protocols, and pathological compensatory changes in organ function are inevitable.

Conclusion

Though modern technologies have provided scientists with enormous amounts of biologic data, interpretation of the data is limited by analytical approaches to modeling the interactions and functions of biologic components and larger systems. Prediction of function and responses of complex biologic processes, including pathologic processes requires an organizational framework on which mathematical models can be built. The simple architecture described here, the bow tie, is ubiquitous in biologic and engineered systems, and is distinct from other architectures proposed for complex systems. Evolved bow tie structures facilitate robust biologic function, and based on their design, also have inherent but predictable fragilities. Identification of large-scale architectures such as the bow tie is a necessary prerequisite for progress in the modeling and understanding of complex biologic processes.

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