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SYMPOSIUM

The Landscape of Evolution: Reconciling Structural and Dynamic Properties of Metabolic Networks in Adaptive Diversifications

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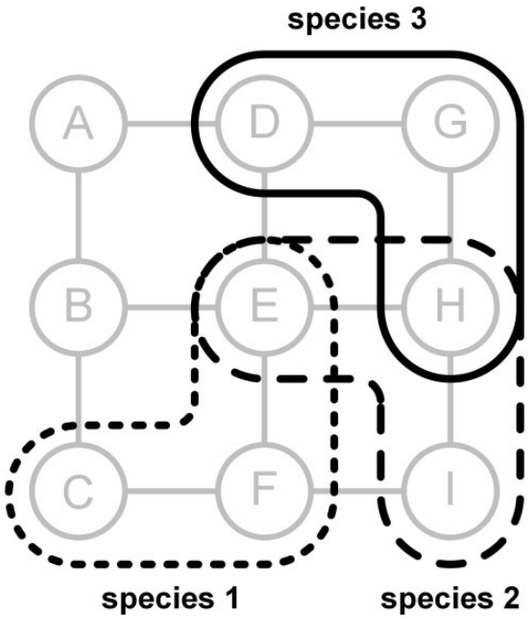
Synopsis The network of the interactions among genes, proteins, and metabolites delineates a range of potential phenotypic diversifications in a lineage, and realized phenotypic changes are the result of differences in the dynamics of the expression of the elements and interactions in this deterministic network. Regulatory mechanisms, such as hormones, mediate the relationship between the structural and dynamic properties of networks by determining how and when the elements are expressed and form a functional unit or state. Changes in regulatory mechanisms lead to variable expression of functional states of a network within and among generations. Functional properties of network elements, and the magnitude and direction of evolutionary change they determine, depend on their location within a network. Here, we examine the relationship between network structure and the dynamic mechanisms that regulate flux through a metabolic network. We review the mechanisms that control metabolic flux in enzymatic reactions and examine structural properties of the network locations that are targets of flux control. We aim to establish a predictive framework to test the contributions of structural and dynamic properties of deterministic networks to evolutionary diversifications.

Assessing the role of regulatory mechanisms in phenotypic diversification

The link between the topology of genomic, proteomic, and metabolic network elements and the dynamic properties of their interactions is crucial for the stability of a phenotype and opportunities for evolutionary diversification. The structure of all of the possible functional relationships between genes, proteins, enzymes, and metabolites defines a deterministic network, in which each distinct functional state corresponds to a potential phenotype (Box 1; Schuster et al. 2000; Covert and Palsson 2002; Alon 2003; Barabási and Oltvai 2004; Covert et al. 2004). During diversifications within a lineage, some elements and interactions of deterministic networks remain unchanged, whereas others vary widely across taxa (Fraser et al. 2002; Almaas et al. 2005; Hahn and Kern 2005; Light et al. 2005; Bernhardsson et al. 2011; Badyaev et al. 2015). The difference in evolutionary conservation of elements and interactions may be related either to their roles in maintaining global structural properties, defined by the topology and connectivity of the entire network (Albert et al. 2000; Jeong et al. 2001; Schmidt et al. 2003; Vitkup et al. 2006), or to the distinct functional roles of elements and interactions independent of their structural positions (Papp et al. 2004; Almaas et al. 2005; Mahadevan and Palsson 2005; Vitkup et al. 2006). These contrasting explanations reflect the debate as to whether selection acts on structure of deterministic networks or on distinct functional states within a network (Wagner 2007; Papp et al. 2009). This distinction is the focus of our review.

Changes in structural properties of a network—such as in element connectivity and pathway length (Box 1; Costa et al. 2007)—are determined by the physical gain or loss of elements and interactions as a result of gene duplications (Wagner 2001; Vázquez et al. 2003; Kondrashov 2012), mutations in existing genes (Wagner 2003; Berg et al. 2004), or horizontal gene transfers (Light et al. 2005; Pál et al. 2005; Klassen 2010). In contrast, functional states—such as the rate of chemical reactions and the levels of



Box. 1 Structural and dynamic properties of deterministic networks.

Deterministic network represents all possible interactions (gray lines) among elements such as genes, proteins, enzymes, and metabolites (gray circles) that could underlie a phenotype.

Structural network properties describe the organization and location of the interactions among elements in a network.

Pathway length is the number of interactions between elements (e.g., pathway length between A and I is four interactions), and **connectivity** is the number of interactions per element (e.g. connectivity of B is three interactions).

Dynamic network properties define interactions and elements that are more likely to be co-expressed and the strength of this expression under different conditions. This can be due to abiotic factors or internal regulation. Co-expressed elements and interactions represent **functional states**. In the figure, the black patterned outlines represent functional states of the network that are expressed in different species.

Calibrating phenotypic differences on a deterministic network: The relationship between structural and dynamic properties of a deterministic network can be used to calibrate differences between its functional states. In the figure, the network of species 2 differs from both of the networks of species 1 and 3 by two interactions and elements, whereas the networks of species 1 and 3 differ by three interactions and elements. The current dynamic properties of the network establish that the interaction between D and G is more likely than other interactions between the elements expressed in these species, such as the interaction between D and E, which is not present in any of the species' networks.

gene expression (Fell 1997; de la Fuente et al. 2002; Kuznetsov et al. 2002; Farkas et al. 2003)—are determined by the physical and chemical properties of elements and interactions in a deterministic network (Westerhoff et al. 1984; Ibarra et al. 2002). Due to fundamental constraints placed on the chemical and physical properties of elements and interactions in the network, the efficacy of functional states varies across environments: Some states are more locally optimal than others in dynamic properties such as energy consumption or reaction rates (Westerhoff et al. 1984; Ortega and Acerenza 1998; Ibarra et al. 2002; Price et al. 2004). The dynamic properties of a deterministic network determine how the elements and interactions in the network are utilized in a particular environment (Box 1). Thus, knowledge of the relationship between dynamic and structural properties of the network is needed to assess their roles in evolutionary diversification. Regulatory mechanisms control expression of functional states in a deterministic network (Almaas et al. 2004; Papp et al. 2004; Price et al. 2004; Reed and Palsson 2004; Almaas et al. 2005; Nam et al. 2012) and, therefore, the evolutionary potential of changes in functional states and network expression across taxa (Westerhoff et al. 1984; Heinrich et al. 1991; Fell 1997; Edwards et al. 2001; Ibarra et al. 2002; Davidson and Erwin 2010). The efficacy of regulatory changes in functional states in the network, however, depends on topological locations of the regulatory mechanisms within a deterministic network (Erwin and Davidson 2009).

Here, we examine whether some topological positions within a network are more likely to be regulated than others to determine whether regulatory changes related to structural properties produce distinct phenotypic changes. We focus on metabolic networks, because complete topologies of these networks are now available for many species (Edwards and Palsson 1999, 2000a; Kanehisa et al. 2014). The study of metabolic flux—the rate of enzymatic reactions across a network—provides an opportunity to relate changes in enzyme activity at particular topological positions to phenotypic plasticity in the use of a network within an individual and to phenotypic change across generations. We integrate knowledge of topological properties of optimal flux control with studies that examine variation in flux caused by diverse abiotic and biotic conditions (Kacser and Burns 1981; Fell 1997; Ibarra et al. 2002; Segrè et al. 2002; Dekel and Alon 2005).

We first present an overview of the mechanisms that can control flux in metabolic pathways. We then review structural properties in metabolic networks that are associated with locations of optimal flux control, and assess the impact of differences in the topological locations of regulatory controls on expression of functional states.

Mechanisms of metabolic flux control

Flux through a pathway is regulated by enzyme activity and production (Fell 1992, 1997; Rossell et al. 2006). Differences in the availability of the initial substrates of metabolic pathways and the affinity of enzymes for these substrates contribute to flux variation. Substrate concentrations over certain threshold levels activate enzymes, followed by an increase in reaction rates until enzymes become saturated (Matsuno et al. 1978; Bongaerts and Vliegenthart 1988). Flux in a metabolic network can change rapidly and reversibly, caused by changes in the availability of initial, often external, substrates (Nasution et al. 2006; Wu et al. 2006; Taymaz-Nikerel et al. 2011; Taymaz-Nikerel et al. 2013), or due to short-term fluctuations in the enzyme affinity for the substrate (e.g., caused by temperature or pH changes; Dixon 1953; Szasz 1974; Bongaerts and Vliegenthart 1988; Saavedra et al. 2005; Sørensen et al. 2015). Flux can be permanently changed, however, as the result of irreversible modifications to the enzyme that changes its affinity for a substrate, often caused by changes in the physical structure of the enzyme, such as due to mutation (Lamb et al. 1997).

Metabolic flux is also affected by the allosteric regulation of enzymes, in which enzymes are activated or deactivated by reversible covalent modifications. Allosteric regulation adjusts enzyme activity to changes in abiotic and biotic environments of the metabolic network (Ralser et al. 2009; Link et al. 2013). Feedback inhibition of enzymes by other metabolites is one of the ways allosteric regulation can be accomplished (Umbarger 1956; Yates and Pardee 1956). In these cases, metabolites produced at the end of pathways bind to the enzymes at the beginning of pathways and deactivate the enzymatic reactions to limit the further production of downstream compounds. Alternatively, covalent modifications might be caused by protein complexes binding to specific enzymes. For example, protein kinases and phosphoprotein phosphates activate or inhibit enzymes via phosphorylation and dephosphorization (Krebs and Beavo 1979). Protein complexes themselves can be regulated by abiotic factors (Kaufmann et al. 1999; Jarmuszkiewicz et al. 2015), hormones (Cohen 1988; Strålfors and Honnor 1989), growth factors (Lee et al. 1991; Kholodenko et al. 1999), or neural impulses (Wang et al. 1988; Bauerfeind et al. 1997). Variation in these factors can underlie adaptive responses of metabolic flux without permanently altering pathway structure or the structure of the enzyme itself (ter Kuile and Westerhoff 2001; Heinemann and Sauer 2010; Chubukov et al. 2013; Schwender et al. 2014; Machado et al. 2015).

In addition to changes to enzyme activity, metabolic flux can be regulated by transcriptional and translational controls involved in enzyme production. Transcription rates of specific enzymes in pathways can vary widely in response to metabolite concentrations (Goelzer et al. 2008; Bradley et al. 2009), signaling molecules (Cho et al. 2008), or abiotic environmental perturbations (Gasch et al. 2000; Causton et al. 2001; Enjalbert et al. 2006). The regulation of translation is also dependent on the relative stability of mRNA transcripts (Smolke et al. 2000, 2001; Bennett et al. 2008; Wang et al. 2015), such that heritability of transcription rates can contribute to the evolutionary stability of flux control (Emilsson et al. 2008; Gordon and Ruvinsky 2012; Schaefke et al. 2013). Mechanisms of flux control can thus underlie both short-term, reversible changes and more permanent, evolutionary changes in the expression of metabolic pathways. Below we review the optimal placement of flux control in a metabolic network and assess how topological locations of regulatory mechanisms affect short- and long-term evolutionary diversification.

Integration of flux control mechanisms into the static structure of metabolic networks

Relationship between functional modularity and flux control in metabolic networks

Groups of enzymes and compounds that are interlinked by stronger regulatory mechanisms than other elements in the network form a functional module (Hartwell et al. 1999). The coordinated regulation of enzymatic reactions within functional modules could be the result of optimizing flux control for a metabolic network functioning in a wide range of environments. Indeed, species that are frequently exposed to many substrates and abiotic factors tend to have networks with greater structural and functional modularity (Borenstein et al. 2008; Kreimer et al. 2008). Alternatively, the coordinated regulation of enzymes in a pathway could evolve to prevent their unnecessary buildup in the limited volume of a cell, which could occur if enzymes were regulated independently (Ellis 2001; Minton 2001; Wessely et al. 2011; de Hijas-Liste et al. 2015).

Several models have been proposed to specifically link flux control to network topology and functional modularity of metabolites and enzymes. One model proposes that the coordination of flux through multiple enzymes is controlled by a single rate-limiting

enzyme at the beginning of a pathway to prevent the buildup of intermediate metabolites (Blackman 1905; Krebs 1957). Indeed, enzymes located at the beginning of pathways tend to evolve greater flux control than downstream enzymes (Eanes et al. 2006; Wright and Rausher 2010; Olson-Manning et al. 2013; Olson-Manning et al. 2015). Another model posits that rate-limiting enzymes are uncommon, such that the regulation of flux is distributed across the enzymes along a pathway (Kacser and Burns 1973; Heinrich and Rapoport 1974; Fell 1992; Fell and Thomas 1995; Rossell et al. 2006). In this case, changes in the activity of a single enzyme do not affect the flux in a pathway (Van Hoek et al. 1998; Nilsson et al. 2001; Daran-Lapujade et al. 2004) and multiple enzymes are all controlled by the same regulatory mechanisms (Thomas and Fell 1998; Wessely et al. 2011; de Hijas-Liste et al. 2015). Although the targets of regulatory mechanisms differ between these models, both ultimately result in the coordinated control of groups of multiple enzymes that are not associated with any structural property of a network (Pfeiffer et al. 1999; Ravasz et al. 2002; Schuster et al. 2002; Spirin et al. 2003; Ihmels et al. 2004; Kharchenko et al. 2005; Çakir et al. 2006; Seshasayee et al. 2009; Zelezniak et al. 2014).

In this case, functional modules, and not enzymes in specific topological positions, are therefore the source of metabolic diversification on a biochemical network (Wagner and Altenberg 1996; Nagy 1998; von Dassow and Munro 1999; Raff and Raff 2000; Badyaev and Foresman 2000; Badyaev 2007). Within an individual, changes in flux affect all metabolites in a functional module, but the relative proportions of the enzymes remain constant due to their coordinated activity and expression (Fell and Thomas 1995; Rossell et al. 2006). Thus, if the metabolic network is portioned into functional modules under different regulatory mechanisms, then changes in the flux of enzymes will be unrelated to their structural positions, because the relative changes in flux of enzymes in the same functional module will be constant (Fig. 1A). Targeted regulation of functional modules leads to environment-specific expression of these modules (Almaas et al. 2004; Papp et al. 2004; Reed and Palsson 2004). Indeed, the gain and loss of enzymes within a module co-occurred over evolutionary time, because proteins in the same functional module tended to co-evolve at the same rate (Campillos et al. 2006; Chen and Dokholyan 2006). When selection targets the coordinated regulation of a functional module, structural positions of enzymes should not be related to evolutionary conservation of enzymes across species (Fig. 1A).

Independent flux controls at the beginning and end of pathways

The coordinated regulation and expression of enzymatic reactions may not extend over the entire pathway. Uncoordinated regulation of flux along a pathway modulates the synthesizing capacity of a pathway, such that different parts of the network can respond to changing conditions independently and only the flux related to certain elements and interactions in a pathway is altered. Greater numbers of independent flux controls in a pathway are thus expected in fluctuating environments (Soyer and Pfeiffer 2010). Also, multiple regulatory mechanisms in the same pathway should be most optimal for longer, linear pathways, because of a time delay before changes in the flux of an upstream enzyme reaches the end of a pathway (Seshasayee et al. 2009; Wessely et al. 2011). Independent flux controls at the first and last enzymes in a pathway mitigates these metabolic time delays (Klipp et al. 2002; McAdams and Shapiro 2003; Zaslaver et al. 2004). For example, differences in the transcriptional regulation of the first and last enzymes in a pathway resulted in lesser co-expression of enzymatic reactions in relation to the distance between reactions (Spirin et al. 2003; Ma et al. 2004; Kharchenko et al. 2005; Yu and Gerstein 2006; Notebaart et al. 2008; Seshasayee et al. 2009; Wessely et al. 2011). Similarly, the initial and terminating enzymes can be regulated by different mechanisms, such as when the last enzyme is regulated by transcriptional or translational factors, whilst the first enzyme—via feedback inhibition based on the concentration of the last metabolite in the pathway (Moxley et al. 2009; de Hijas-Liste et al. 2015).

When distinct flux controls are located along a pathway, changes in flux depend on the topological positions of the enzymes in the pathways (Fig. 1B). For example, the flux of enzymes located in upstream positions should change at a different rate than the enzymes located further downstream when these enzymes are regulated by different control mechanisms. From an evolutionary perspective, the presence of several regulatory mechanisms may represent different ways in which the same pathway can be optimized to function in different environments. For example, in the aliphatic glucosinolate pathway of *Arabidopsis thaliana*, flux was controlled by the first enzyme in the pathway in almost all environments, but different regulatory factors governed enzymes located further downstream (Olson-Manning et al. 2015). Stabilizing selection was evident only in the first enzyme in the pathway that had the largest

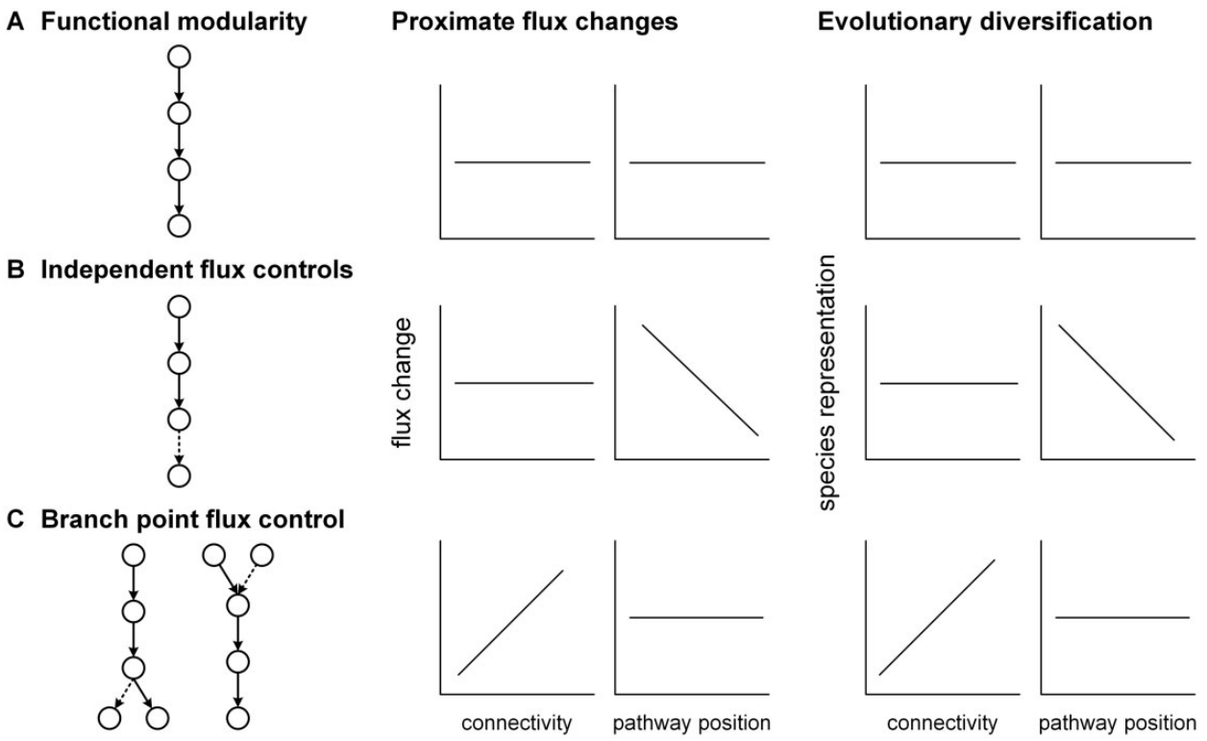


Fig. 1 Structural locations of the control of metabolic flux affect phenotypic changes in the rate of enzymatic reactions in a biochemical pathway and the evolutionary diversification of metabolic networks across species. The circles and arrows on the left represent metabolites and enzymes, respectively, in a biochemical pathway. The solid and dashed arrows denote structural locations of distinct regulatory mechanisms on enzymes in a pathway. In the graphs on the right, proximate flux changes are measured by changes in the expression level of compounds in a pathway (flux change), and the evolutionary diversification of a compound in a pathway is determined by the number of species in a lineage that express the compound (species representation). To capture the structural positions of a compound in a network, connectivity measures the number of reactions per compound and pathway position is the number of reactions that separate a compound from the beginning of a pathway (Box 1). (**A**) When one regulatory mechanism coordinates the flux of all of the enzymes in a pathway, then the structural position of a compound in a pathway does not matter, because all of the compounds in the pathway will experience the same magnitude of flux change. Compounds that are part of the same functional module will be targeted by selection as a unit and will thus be gained or lost together across species with no relation to their structural positions. (**B**) When independent regulatory mechanisms control flux in different locations of the same pathway, changes in the flux of compounds will be related to their pathway position. In pathways with multiple regulatory controls, upstream compounds that are located fewer reactions away from the beginning of pathways have a large impact on changes in flux, and tend to be under stabilizing selection. Compounds located several reactions from the starting point of a pathway do not have a significant influence on the overall flux, and thus divergent selection should be stronger on compounds located at the end of pathways. (**C**) When there are different regulatory controls for pathways that either converge or diverge from the same compound (branch point), flux changes should be related to the connectivity of a compound. Due to their participation in multiple pathways, branch points have the greatest connectivity in a biochemical network, and thus tend to have a greater influence on metabolic flux than less connected compounds only associated with one pathway. Therefore, over evolutionary time, we would predict that the most connected compounds in the network will be conserved while divergent selection would occur among compounds with fewer enzymatic reactions that contribute less to flux control in pathways.

influence on the overall flux in the pathway (Olson-Manning et al. 2013). Several studies have documented distinct selection on enzymes in different locations in a pathway: Central or upstream enzymes and metabolites that contributed more to the control of flux in pathways tended to be under stabilizing selection, while downstream or terminal enzymes that had less of an influence on the flux in pathways were under divergent selection (Rausher et al. 1999; Ramsay et al. 2009; Wright and Rausher 2010; Bernhardsson et al. 2011). It follows that when multiple regulatory controls in a pathway are under selection, the upstream elements of the pathways should be conserved across species and downstream elements should diverge (Fig. 1B).

Flux control at branching points in metabolic pathways

Locations within a metabolic network where separate pathways either converge to produce the same

metabolite or diverge from the same precursor can be targets of metabolic flux control. Metabolic control theory predicts that the interactions between the pathways at branching points should result in distinct patterns of flux control (Kacser 1983; Fell and Sauro 1985; Heijnen et al. 2004). At branching points, there is often a decoupling of regulation between incoming and outgoing reactions, and one pair of incoming and outgoing reactions from the shared metabolite tends to be more optimal than another potential reaction pair (LaPorte et al. 1984; Heinrich et al. 1991; Stephanopoulos and Vallino 1991; Vallino and Stephanopoulos 1994; Spirin et al. 2003; Ihmels et al. 2004; Notebaart et al. 2008; Seshasayee et al. 2009). When the flux is optimized in one of the branching pathways, it may shut down the expression of the other pathway (Kacser and Burns 1981) or, alternatively, the presence of two converging pathways can be advantageous when it allows for a greater flux into the following downstream enzyme (Heinrich et al. 1991). Metabolites at branching points have high connectivity, such that these metabolites are associated with more incoming and outgoing enzymatic reactions compared with the metabolites in less connected parts of the network. Given that many changes in flux are associated with branch points in pathways, changes in the regulatory control of highly connected enzymes and metabolites should contribute more to changes in flux than less connected metabolites and enzymes (Zhang et al. 2007; Fig. 1C).

Branching points in metabolic networks enable the robustness of metabolism to the loss of pathways due to environmental or genetic perturbations, or they can lead to specialization in the same environment (Edwards and Palsson 2000b; Zhang et al. 2007; Vogt 2010; Chen et al. 2011; Weng 2014). The redundancy inherent in convergent branch points of pathways that produce the same metabolite from different substrates buffers against the loss of one of the substrates in the external environment (Badyaev et al. 2015; Higginson et al. In press). For example, when *Escherichia coli* was exposed to fluctuating levels of glucose and acetate, some strains evolved a generalist phenotype that allowed them to use both substrates; whereas neither pathway was optimized compared with specialist strains, this strategy allowed the generalist strain to adapt to changing conditions (Herron and Doebeli 2011). Alternatively, the presence of divergent branching points allows the expression of different pathways from the same starting metabolite in different environments, leading to diversification and specialization (Lavington et al. 2014). The key role of branching points in the adaptive evolution of metabolism is supported by the finding that branching point enzymes tend to occur in locations of optimal flux control (Eanes 1999; Flowers et al. 2007; Rausher 2013). The metabolites that anchor these branch points tend to be conserved over evolutionary time, whereas the less connected compounds within the pathways that either converge or diverge from the same highly connected branch point metabolite often experience divergent selection (Fig. 1C; Fraser et al. 2002; Hahn and Kern 2005; Bernhardsson et al. 2011; Badyaev et al. 2015).

Implications of the relationship between structural and dynamic properties in metabolic networks

Examination of flux regulation in relation to the structural properties of deterministic networks provides a way to understand proximate mechanisms of phenotypic change from a more global perspective. Instead of only being able to see where and how changes occurred with respect to a current phenotype, we can begin to understand why certain phenotypic changes are recurrent, whereas others are rarely realized. As such, this approach links microevolutionary and macroevolutionary changes. The effect of network structure on the delineation of diversification opportunities depends on the integration of regulatory mechanisms into the network structure. When entire modules of a metabolic network are under the same regulatory mechanism, the network structure is an emergent property in the evolutionary change of metabolism. In this case, the metabolism in the biochemical network is optimized to current abiotic and biotic factors and does not depend on the topology of enzymes. When there are multiple regulatory controls within pathways, the static structure becomes predictive of the potential for evolutionary change of certain enzymes and compounds, because these regulatory controls target different structural locations to optimize metabolic flux.

Linking the evolutionary stability of changes in regulatory mechanisms to their corresponding phenotypic changes within a deterministic network has implications for understanding the plasticity of a phenotype and its role in diversification. We can predict both how and when a phenotype should change, as well as the relative stability of the change. For example, establishing topology of the enzymes that are targets of hormonal signals gives insight into changes in functional properties of the network in response to hormonal signaling. Transient properties of hormonal control, in which hormonal signaling changes in

response to environmental perturbations (Schulte 2013), can lead to phenotypic plasticity in a metabolic network. Selection on functional properties of a metabolic network can produce adaptive radiations in functional states (Kitano et al. 2010) as a result of exposure to more stable environments or genetic assimilation of hormone production in regulatory mechanisms (Rissman et al. 1997; Flurkey et al. 2001; Ellis et al. 2003; Badyaev 2009).

To test our predictions (Fig. 1), intraspecific changes in regulatory mechanisms need to be compared with interspecific patterns of diversification in expressed metabolites and enzymes. Determining the topological locations of changes in flux would require comparisons of the dynamic properties of the same metabolic network in different environments (Almaas et al. 2004; Papp et al. 2004; Price et al. 2004; Reed and Palsson 2004; Lavington et al. 2014). The next step would be to assess the relationship between structural properties of regulatory controls and evolutionary patterns of metabolic diversification. Comparing how the metabolic network is used across species in a lineage establishes conservation of compounds and enzymes over evolutionary time; these evolutionary differences should correspond to the structural positions of changes in flux on the metabolic network.

More work is needed to assess how functional states of metabolic networks change in multicellular organisms. Many of the studies reviewed here examine flux in microbes and the mode of evolution often differs between unicellular and multicellular organisms. For example, in unicellular organisms pathways and elements can be gained independently of their functional properties during horizontal gene transfers from other organisms (Lawrence and Roth 1996; Pál et al. 2005; Kreimer et al. 2008). This mode of evolution can result in indistinguishable evolutionary patterns to changes in regulatory mechanisms, because connected compounds would be conserved due to the preferential attachment of the horizontal transmission of acquired enzymes to the same initial compounds (Eisenberg and Levanon 2003; Light et al. 2005), or the divergence among downstream enzymes across species is due to the horizontal gene acquisition of enzymes at the end of pathways (Bernhardsson et al. 2011).

A greater focus on metabolic network divergence in multicellular species is also an opportunity to evaluate the dynamics of metabolic networks for variable functional properties. Almost all of the empirical studies discussed in this review examine the targets of flux control in relation to optimal growth rate under different conditions (e.g., Ibarra et al. 2002; Almaas et al. 2004; Almaas et al. 2005; Herron and Doebeli 2011). Different dynamics of regulatory control may characterize other functional states, such as in floral pigmentation (Rausher et al. 1999; Rausher et al. 2008) and insect flight performance (Eanes et al. 2006).

Establishing how the topology of regulatory mechanisms in deterministic networks is linked to functional and evolutionary changes gives us a quantitative perspective on the underlying mechanisms of phenotypic change and stability. Not only can we pinpoint the specific differences between phenotypes, but we can also assess both the magnitude of these changes and possible sources of the variation based on differences across functional states in relation to the structure of their deterministic network. In short, linking structural and dynamic properties of genetic, protein, and metabolic networks offers an opportunity to apply a predictive structure to observed evolutionary patterns.

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