Chapter 11

The Evolution of Protein Material Costs

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11.1 INTRODUCTION

We here survey evidence that natural selection on reducing the material cost of making proteins can profoundly change the chemical composition of proteins and of the genomes that encode them.

Organisms take nutrients from their environment and use them as substrates for generating energy and for making the tissues, cells, and molecules that compose their cells. Different nutrients are often required in specific ratios to support balanced growth. However, the relative availability of nutrients can vary sharply across different environ-

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ments, or over time within environments, leading to growth limitation by one or more nutrients (Sterner and Elser, 2002).

Growth limitation can lead to strong selective pressure for an improved ability to acquire a limiting nutrient, or for increases in the efficiency with which a nutrient is used for growth once acquired. Accordingly, adaptations to nutrient scarcity can be observed on almost all levels of biological organization, from the sizes of whole cells (e.g., Aksnes and Egge, 1991; Button, 1991; Chisholm, 1992; Yoshiyama and Klausmeier, 2007), to the numbers and types of atoms that are used to make molecules (e.g. Baudouin-Cornu and Bragg, 2006; Van Mooy et al., 2006). Here, we discuss protein atomic (material) costs in relation to nutrient availability. This discussion includes the evolution of protein expression costs in response to transient and chronic nutrient shortages, as well as the evolution of genome composition—with respect to both nucleotide and gene content—as it relates to the costs of protein expression.

11.2 PROTEIN MATERIAL COSTS

The presence, expression, and functional characteristics of specific genes and their protein products are fundamentally important for evolutionary responses to nutrient limitation. For example, when the yeast *Saccharomyces cerevisiae* evolves in the laboratory under glucose limitation (Paquin and Adams, 1983), chromosomal rearrangements result in amplification of high-affinity hexose transporters, leading to greater affinity of yeast cells for glucose (Brown et al., 1998; Dunham et al., 2002). In addition to performing essential functions, proteins constitute a large proportion of cellular biomass, and their expression carries significant material costs. Specifically, proteins are composed of amino acids that contain different numbers of atoms of carbon (2–11 atoms), nitrogen (1–4 atoms), and sulfur (1 atom in Cys and Met only). In many environments, these elements are ecologically limiting. Amino acids also contain oxygen and hydrogen, although these elements are probably rarely growth limiting (but see Acquisti et al. (2006)). Some proteins also require metal cofactors, including iron, copper, zinc, molybdenum, magnesium, manganese, or nickel. These metals can potentially limit growth, sometimes by limiting the activity of enzymes needed to assimilate macronutrients (Saito et al., 2008).

It has been suspected for some time that the costs of synthesizing and incorporating different amino acids into proteins might be evolutionarily significant. For instance, Richmond (1970) argued that few amino acid substitutions are completely neutral, since different amino acids tend to vary in their abundance and in their costs. Since then, numerous studies have highlighted patterns in protein amino acid and elemental composition that are consistent with selection for reduced energetic or material costs. It is important to note that the function of a protein will place large constraints on its primary structure. In certain positions along the length of a protein, there will be no opportunity to replace an expensive amino acid with a cheaper one, without compromising the functional integrity of the protein. However, in other positions, it may be possible to use one of several different amino acids. This suggests that beyond protein functional constraints, there is room for variation in amino acid composition, and hence elemental composition. In discussing research on the evolution of protein material costs, we begin by describing the response of yeast to limitation by sulfur. The reason is that yeast's response to sulfur stress highlights concepts that are central to evolution of protein material costs in general. In subsequent sections, we consider these patterns and concepts in greater detail. Finally, we consider the evolution of protein elemental composition on a whole genome scale.

11.3 AN EXAMPLE: PROTEOMIC SULFUR SPARING

It was observed more than 25 years ago that sulfur starved bacteria produced protein containing less sulfur per unit mass than cells that were not sulfur starved (Cuhel et al., 1981). At that time, it was not known whether this was an "active" response by the sulfur stressed cells, or whether it simply reflected the inability of sulfur starved cells to make proteins that required large quantities of sulfur.

Sulfur-depleted proteins are not only produced in sulfur-stressed bacteria, but also in sulfur-stressed yeast. In a study by Fauchon et al. (2002), yeast cells were exposed to cadmium, which causes the synthesis of the sulfur-rich detoxifying compound, glutathione. The diversion of sulfur to glutathione synthesis leads to sulfur stress. Major changes in gene expression occur in response to these conditions, at both the mRNA and protein levels. These changes in protein expression led to a reduction in the sulfur content of expressed proteins by approximately 30% (Fauchon et al., 2002). Two main processes were responsible for this reduction. First, several abundant proteins were downregulated, and sulfur-poor isozymes were upregulated to replace them (Fauchon et al., 2002). This pattern was observed for several proteins that function in glycolysis. The glycolysis-specific functions of these upregulated isozymes make their direct role in detoxification or sulfur assimilation unlikely. It is therefore probable that these isozymes were upregulated to reduce the sulfur costs of protein expression. Second, sulfur metabolism genes that were upregulated during cadmium exposure tended to have low sulfur content (Fauchon et al., 2002). The induction of sulfur metabolism genes and of sulfur-poor glycolytic isozymes were linked to the yeast transcriptional activator, Met4p, demonstrating that the observed "sulfur sparing" during sulfur limitation was transcriptionally regulated (Fauchon et al., 2002). These observations elegantly showcase several important processes related to the evolution of protein material costs. Below, we will discuss these processes with further examples, and also the types of selective pressure that may underpin them.

11.4 EPISODIC NUTRIENT SCARCITY CAN SHAPE PROTEIN MATERIAL COSTS

In the example of sulfur-stressed yeast, the replacement of abundant proteins by sulfur-poor isozymes led to a substantial reduction in the total amount of sulfur needed to express proteins (Fauchon et al., 2002). Similar observations have been reported for sulfur and for other elements in a variety of microbes.

In cyanobacteria, phycobiliproteins are highly expressed photosynthesis proteins that can account for a substantial proportion of total cellular protein. The cyanobacterium *Calothrix* encodes a duplicate set of phycobiliproteins whose protein products contain substantially fewer sulfur atoms than the phycobiliproteins that are typically expressed (Mazel and Marlière, 1989). During sulfur limitation, the sulfur-poor versions of the phycobiliproteins are specifically upregulated (Mazel and Marlière, 1989). The sulfur-poor phycobiliproteins do not have an obvious functional connection to sulfur metabolism, suggesting that their induction is related to their smaller demand for sulfur containing amino acids during protein synthesis.

A number of ribosomal proteins contain zinc binding domains. Panina et al. (2003) identified four ribosomal proteins that typically contain zinc binding domains, but that have paralogous copies without zinc binding domains in a substantial number of bacterial genomes. These paralogues appear to be induced during shortages of zinc, which may

allow the bacteria to reduce their use of zinc in ribosomal proteins when zinc is scarce (Panina et al., 2003).

A variety of microbes use the proteins ferredoxin, flavodoxin, or both, for electron transfer in metabolic pathways. Ferredoxin contains iron, but flavodoxin does not. Iron is a key limiting nutrient in large regions of the ocean (Martin et al., 1991). In several microbes, both ferredoxin and flavodoxin are known to be encoded by the same genome and to be differentially expressed according to iron availability. Specifically, during iron limitation, the expression of ferredoxin is suppressed, and iron-free flavodoxin is induced (Knight and Hardy, 1966; La Roche et al., 1993; Mayhew and Massey, 1969). Interestingly, the relative levels of ferredoxin and flavodoxin expression by marine microbes have been used as an index for the intensity of iron limitation in the ocean (e.g., Erdner et al., 1999).

In the above examples, the genes differentially regulated during nutrient stress may not be required for the acquisition and assimilation of an element, but their protein products may merely contain much of the element. Not surprisingly, genes encoding proteins necessary to assimilate an element may also contain less of the element than other proteins (Baudouin-Cornu et al., 2001). As mentioned above, sulfur-stressed yeast upregulate sulfur metabolism genes that encode sulfur-poor protein products (Boer et al., 2003; Fauchon et al., 2002). In fact, it was noted as long ago as 1966 that a bacterial sulfate binding protein had the "unusual feature" of containing no cysteine or methionine residues (Pardee, 1966; p5888). It has since been observed that proteins involved in sulfur uptake and assimilation are sulfur poor in bacteria and yeast (e.g., Baudouin-Cornu et al., 2001; Van der Ploeg et al., 1996). Furthermore, for yeast and *Escherichia coli*, proteins used for the assimilation of carbon contain fewer carbon atoms compared to the rest of the proteome (Baudouin-Cornu et al., 2001).

Two possible and subtly different types of selective mechanisms could account for the expression of proteins that are depleted in a specific element when the element is scarce. First, selection might favor the induction of such proteins to reduce cellular demand for the limiting element. Second, selection could favor reduced use of an element in specific proteins to ensure that those proteins can be translated when the element is strongly limiting. In the case of assimilatory proteins, it has been suggested that the latter mechanism is more likely, since assimilatory proteins probably contain a relatively small proportion of the total cellular budget of an element, but their translation and activity are critically important during conditions of strong limitation (Baudouin-Cornu et al., 2001). Currently, there is little direct evidence to distinguish between the two hypotheses. A potentially relevant observation is that yeast grown under carbon limitation over short evolutionary timescales exhibit a relaxation in their tendency to upregulate carbon-poor proteins. That is, during adaptive evolution to carbon limitation, the tendency to upregulate carbon poor proteins is not elaborated as part of an evolved response to carbon limitation (Bragg and Wagner, 2007).

11.5 HIGHLY EXPRESSED GENE PRODUCTS OFTEN EXHIBIT REDUCED MATERIAL COSTS

Evidence that cells economize on gene expression costs is not restricted to transient periods of acute limitation by specific elements. Specifically, genes that are constitutively expressed at high levels have low expression cost according to several criteria. For instance, highly expressed genes tend to encode shorter proteins (Brocchieri and Karlin, 2005), and contain fewer introns (Castillo-Davis et al., 2002), than less highly expressed genes. Proteins encoded by highly expressed genes also contain fewer energetically expensive or heavy amino acids (Akashi and Gojobori, 2002; Heizer et al., 2006; Seligmann, 2003). Similarly,

highly expressed genes encode proteins with relatively low material costs for nutrients that are commonly limiting. For instance, highly expressed yeast genes encode proteins that are depleted in sulfur, carbon, and nitrogen (Bragg and Wagner, 2007; Fauchon et al., 2002). Similar observations have been made for plants that are commonly limited by nitrogen, but may rarely face carbon limitation. Consistent with this observation, highly expressed plant genes tend to encode proteins that are poor in nitrogen, but do not exhibit any significant bias in carbon content (Elser et al., 2006).

11.6 MATERIAL COSTS AND THE EVOLUTION OF GENOMES

Material costs of gene expression can influence the evolution of whole genomes. One pertinent line of evidence relates to metal cofactor use over geological time (Dupont et al., 2006). During the history of life, dramatic changes occurred in the availability of metals that are commonly used as cofactors. In particular, the availability of iron, zinc, manganese, and cobalt probably changed greatly when ocean geochemistry was transformed after the oxygenation of the atmosphere some 2 billion years ago. Extant representatives of lineages that went through major expansions before and after these events show patterns in the use of metal cofactors that reflect likely changes in metal availability. For example, Dupont et al. (2006) characterized the use of different metal binding domains in the proteomes of organisms in the superkingdoms Archaea, Bacteria, and Eukarya. Specifically, these authors fitted curves of the form $y = mx^a$ to relationships between the number of predicted metal binding domains (y) and the total number of structural protein domains (x), across the predicted proteomes in each superkingdom. This yielded estimates for the value of the scaling exponent, a, which was used to make inferences about the change in the prevalence of metal binding proteins in proteomes over evolutionary history. Specifically, values of the scaling exponent greater than one ($a > 1$) were taken to imply that the metal binding domains were preferentially retained during the evolution and growth of proteomes, while values of the scaling exponent smaller than one ($a < 1$) indicate the opposite (Dupont et al., 2006). After the oxygenation of the earth, the availability of zinc to marine organisms probably increased drastically. Eukaryotes probably originated in an oxygenated earth, and the number of zinc binding domains in eukaryotic proteomes scales with an exponent greater than one ($a > 1$). Conversely, Bacteria and Archaea evolved prior to the oxygenation of earth, and the numbers of zinc binding domains in their proteomes scales with exponents smaller than one ($a < 1$, for both Archaea and Bacteria) (Dupont et al., 2006).

Metal cofactor use can also be biased in the genomes of individual species that are adapted to environments where a specific metal is scarce. The human body provides an iron-poor environment for microbes, in part because humans produce and secrete proteins that bind iron, and reduce its availability to pathogens. Some bacteria counter this "withholding" of iron by producing specialized molecules that help extract iron from their hosts (Ratledge and Dover, 2000). In contrast, the bacterial pathogen *Borrelia burgdorferi* has drastically reduced its iron requirements for growth by encoding very few, if any, iron binding proteins (Posey and Gherardini, 2000).

Limited evidence suggests that the quantities of macronutrients used in whole genomes and in the proteins they encode respond adaptively to nutrient availability. Aerobic, nitrogen-fixing bacteria tend to have higher genomic GC content (proportion of guanine plus cytosine base pairs) than their non nitrogen fixing congeners (McEwan et al., 1998). This phenomenon may be related to the greater nitrogen content of GC base pairs (8 N atoms) than AT base pairs (7 N atoms) (McEwan et al., 1998). That is, bacteria that

are capable of fixing atmospheric nitrogen might have encountered relaxed selective pressure for low nitrogen content in their DNA (and possibly in their mRNAs) and may thus use a greater proportion of the more nitrogen-rich GC base pairs. Alternatively, bacteria with higher DNA (and mRNA) nitrogen content might be subjected to greater selective pressure to fix atmospheric nitrogen (McEwan et al., 1998). However, DNA and messenger RNA account for a relatively small proportion of cellular nitrogen, meaning that the reduction in cellular nitrogen content afforded by low GC content is relatively small. Observations like these motivated a study of protein material costs among diverse prokaryotes that found a positive association between genomic GC content and the average nitrogen content of predicted proteins (per amino acid) (Bragg and Hyder, 2004). Therefore, it is possible that nitrogen-fixing bacteria with high DNA nitrogen content often also have high average protein nitrogen costs and that this association underpins an adaptive association between GC content and atmospheric nitrogen fixing (McEwan et al., 1998).

The correlation between the elemental content of genomes and proteins may stem from the structure of the genetic code. For instance, nitrogen-rich amino acids, such as Arg and His, have relatively GC-rich codons (Bragg and Hyder, 2004). Protein carbon content is associated negatively with GC content among organisms (Baudouin-Cornu et al., 2004; Bragg and Hyder, 2004), probably because carbon-rich amino acids often have AT-rich codons. The average sulfur content of proteins tends to be low in organisms with very high and very low GC content, and higher in organisms with intermediate GC content (Bragg et al., 2006). This association may exist because cysteine and methionine codons collectively have moderate GC content. Additional variation among species in average protein sulfur content can be explained by environmental conditions to which different species are adapted. Prokaryotes adapted to high temperatures tend to have lower average protein sulfur content than those adapted to lower temperatures, and anaerobic species tend to have greater average protein sulfur content than nonanaerobes (Bragg et al., 2006). While these analyses do not indicate that protein sulfur content evolves in response to the availability of sulfur, they do suggest that environmental conditions may affect the demand for specific nutrients.

11.7 MATERIAL COSTS AND OTHER COSTS OF MAKING PROTEINS

Costs other than material costs can influence the evolution of protein composition. As mentioned previously, energetic costs have been linked to the frequencies with which different amino acids occur in proteins (Akashi and Gojobori, 2002; Craig and Weber, 1998). Similar observations have been made for the size of amino acids (e.g., molecular weight) as a surrogate for biosynthetic cost (Dufton, 1997). The use of such a surrogate is convenient when biosynthetic costs are compared for several organisms, since it does not require detailed information on the metabolic pathways of amino acid biosynthesis in each organism (Heizer et al., 2006; Seligmann, 2003).

In some cases, protein material costs and energetic costs can be strongly related. In yeast, protein carbon content per amino acid is related strongly and positively to the energetic cost per amino acid (Bragg and Wagner, 2007). However, in other cases, material costs and energetic costs may interact in more complex ways. Microbial organisms often obtain inorganic nitrogen and sulfur in oxidation states that are too high to incorporate into organic compounds, and must reduce them before they can be used in proteins. The interacting demands for materials and reducing power, along with metabolic differences among organisms and variation in the availability of elements in different oxidation states,

may significantly increase the number of potential ways in which protein material costs could evolve. For example, two species of prokaryotes that perform dissimilatory sulfate reduction—where sulfate acts as a terminal electron acceptor in anaerobic respiration—have high average protein sulfur content (Bragg et al., 2006). The reason may be that cells of these species produce reduced sulfur as a by-product of their metabolism.

11.8 CONCLUSIONS

Nutrient limitation has profound effects on the evolution of microbial genomes, including the kinds of genes a genome encodes, and their regulation. Many important adaptations to nutrient limitation promote the uptake and assimilation of limiting nutrients, such as the acquisition and retention of genes that encode high affinity transporters (Dunham et al., 2002). Organisms may also evolve to use smaller quantities of scarce nutrients in making proteins. For instance, specific proteins may evolve to contain relatively small quantities of an element. This has been observed for proteins that are expressed specifically during nutrient limitation (Baudouin-Cornu et al., 2001; Boer et al., 2003; Bragg and Wagner, 2007; Fauchon et al., 2002), and for proteins that are highly expressed in general (Bragg and Wagner, 2007; Elser et al., 2006; Fauchon et al., 2002). In a growing number of cases, organisms respond to nutrient limitation by down-regulating specific proteins, and by replacing them with proteins of similar function but smaller amounts of the limiting element (e.g., Fauchon et al., 2002; Knight and Hardy, 1966; Mazel and Marlière, 1989; Panina et al., 2003). Over much longer time scales, the availability of specific metals appears to have influenced the rate at which metal binding domains proliferated in proteomes (Dupont et al., 2006). The costs of protein expression can thus evolve in diverse ways (i.e., through changes in protein composition, expression, or both), and in response to nutrient shortages that occur over vastly different time scales. Undoubtedly, many more instances where protein material costs have evolved in response to nutrient limitation await discovery. Taken together, these instances will help to illuminate many aspects of genome evolution.

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