Evolving promiscuously

Dan I. Andersson[1]

Department of Medical Biochemistry and Microbiology, Uppsala University, SE-751 23 Uppsala, Sweden

Of the various types of gene rearrangements that have been found in living organisms, gene duplication-amplification (GDA), a process that alters gene dosage, appears to be especially common and biologically important. GDA is significant from a fundamental evolutionary perspective because it serves as a primary genetic source for genetic innovation (i.e., evolution of new genes) and also plays an important role in the generation of genomic variability for cellular adaptation to conditions in which growth is constrained by various external (e.g., presence of toxic drugs) or internal (e.g., deleterious mutations) limitations (1–3). Furthermore, recent discoveries in medical genetics show that gene copy number variation in the human genome is an important contributor to many human diseases, phenotypic variability among individuals, and human susceptibility to infectious diseases (4, 5).

Increased Gene Dosage Can Confer Antibiotic Resistance

As shown by studies in eubacteria, eukaryotic microorganisms, insects, plants, and human tumors, a common and effective adaptive mechanism in response to toxic drug exposure is amplification of preexisting bona fide resistance genes (6–12). Bacteria adapt to antibiotics through several types of responses, including genetic changes that lead to the degradation or sequestration of the antibiotic, prevent its uptake or binding to the target molecule, or pump it out of the cell (13). GDA can, for example, confer resistance to antifolates, tetracyclines, and b-lactams by increasing the dosage of antibiotic-modifying enzymes, target molecules, and efflux pumps (2). Differently from previous work, the report by Soo et al. (14) in PNAS addresses how bacteria may adapt to toxic compounds by overexpression of proteins that are part of the proteome of a nonpathogenic antibiotic-susceptible *Escherichia coli* bacterium and without any known role in conferring drug resistance. Using a clever high-throughput screen, the authors identified 61 ORFs, from an *E. coli* library containing over 4,000 inducible genes (15), that could confer partial resistance to 86 of 237 antibiotic/toxin-containing environments when overexpressed from an inducible plasmid. Of the tested ORFs, most conferred moderate but significant increases (up to 16-fold) in the minimal inhibitory concentration (MIC) against such diverse antibiotics as tetracyclines, b-lactams, antifolates, aminoglycosides, and macrolides, which, in some cases, reached the clinical break points for resistance.

What types of proteins were identified in the screen? A priori, one might expect broad and nonspecific mechanisms to be common. Indeed, of the 61 ORFs identified, 18 were found to confer multidrug resistance (MDR) and several were identified as efflux pumps and transporters based on sequence similarities. Furthermore, several regulators, stress proteins, and capsule biosynthetic genes were found to increase the MICs in several toxin-containing environments, and the resistance-conferring effect could be rationalized in certain cases by the pleiotropic downstream effect on efflux and transport functions. It has been suggested that overexpression of efflux pumps

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contributes to the MDR observed in clinical isolates of several Gram-negative bacteria, for example, *Pseudomonas aeruginosa*, *E. coli*, *Salmonella enterica*, and *Campylobacter jejuni* (16). Furthermore, recent evidence suggests that efflux pumps of the resistance-nodulation-division (RND) type are important for pathogenicity and survival in various host niches for pathogens such as *S. enterica*, *Neisseria gonorrhea*, and *C. jejuni* (16). The mechanisms that give rise to increased efflux in clinical isolates are typically mutations in local repressor genes, global regulators, promoter mutations, or insertion mutations upstream of the transporter gene, but it is also conceivable that overexpression attributable to gene amplification and increased gene dosage of pump systems, as observed by Soo et al. (14), might be a significant contributor to altered resistance and pathogenicity in clinical isolates. Because tandem gene amplifications are generally highly unstable when selection is relaxed (17, 18), however, the contribution of GDA to antibiotic resistance and host adaptation could be difficult to detect because the GDA might already have been lost when the clinical isolates are genetically characterized. Thus, detection of GDA and its potential contribution to MDR might require analysis of the strains immediately after isolation from patients, without any preceding growth in the laboratory.

Evolution of New Genes

In 43 of 61 cases, a specific ORF improved growth in the presence of a single antibiotic/toxin. Among these compound-specific ORFs, 12 appeared to be cases in which metabolic enzymes showed catalytic substrate-binding promiscuity. These cases are especially interesting from the perspective of genetic innovation and the potential mechanisms involved in creation of new genes. The innovation-amplification-divergence (IAD) model posits that selection acts on weak promiscuous activities present in existing proteins (19). By duplication and subsequent higher order gene amplification, the promiscuous activity is increased and maintained in the population. The increase in copy number increases the likelihood of mutations that improve the secondary activity while also maintaining the primary parental activity in at least one gene copy. Thus, two central requirements of this model are that (*i*) gene amplifications are common and (*ii*) promiscuous activities are widespread among proteins.

The first requirement appears accurate because gene duplications are among the most common type of mutation found in eubacteria and many other organisms. For example, in a growing population of *S. enterica* bacteria, the frequency of tandem duplications ranges from 3×10^{-2} down to $\sim 10^{-5}$ depending on the particular gene and genomic region (20); with the exception of the replication terminus, duplications have been found in all regions of the chromosome showing a wide size range (from base pairs to several megabase pairs). From these frequencies and amplicon sizes, it has been estimated that at least 10% of all cells contain a duplication somewhere in the genome at any given time in a nonselectively growing culture (21). Considering the relative frequencies of GDA vs. point mutations, it is apparent that variants with an increased level of a promiscuous enzyme activity are much more likely to owe that increase to a change in gene copy number rather than a point mutation. Thus, up-regulation of a specific gene via a promoter mutation is perhaps five to eight orders of magnitude less frequent than a GDA event that increases the gene copy number.

With regard to the second requirement, until recently, most cases of promiscuity

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[1]E-mail: dan.andersson@imbim.uu.se.

have been discovered adventitiously (22) and systematic large-scale studies to explore a complete proteome for the prevalence of promiscuous activities have been lacking. The present study, as well as a similar study by Patrick et al. (23), fills an important gap and comprehensively shows that multifunctionality and promiscuity in proteins are common and that gene overexpression can allow access to this reservoir of new activities. The multicopy suppression approach provides a powerful methodology to access this reservoir, and it is likely to be an important future tool to define the "promiscuome" in various organisms. In conclusion, the work by Soo et al. (14) shows that the IAD model is biochemically feasible. Nevertheless, it remains to be shown by which mutational routes and rates the amplified copies diverge and acquire efficient novel enzymatic functions during continued selection.

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