PERSPECTIVES: GENOMICS

Mix and Match in the Tree of Life

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The evolutionary relationship between prokaryotes and eukaryotes has long been viewed from the perspective of a single molecule: ribosomal RNA (rRNA). Analyses of rRNA from many different organisms provided the basis for the clonal theory of the evolution of eukaryotic genomes from prokaryotes. This theory holds that genes have been passed directly from generation to generation, with modifications in the genes resulting in the appearance of new organisms. But like a color-blind friend who admires your ability to observe the nearly invisible little "green" flowers on a rose bush, rRNA genes cannot be used to distinguish genomes that are mosaics (mixtures) of genes from different sources. By relying too heavily on rRNA, scientific attention has been diverted away from considering the impact of gene acquisition from other species (horizontal gene transfer) on the evolution of eukaryotic genomes. Viewed now from the vista of completed genome sequences for a number of bacteria and for the yeast *Saccharomyces* (a eukaryote), the clonal theory of eukaryotic genome evolution contains evident flaws (*1*).

The clonal theory began to crumble a decade ago when scientists started analyzing a variety of genes from different organisms and found that their relationship to each other contradicted the evolutionary tree of life derived from rRNA analysis alone. To explain the differences between the evolutionary trees reconstructed from eukaryotic rRNAs and from proteins, Sogin (*2*) proposed a chimeric origin for eukaryotic genomes, with rRNA genes coming from one organism and genes encoding proteins coming from another. Analyses of DNA-dependent, RNA polymerases (*3*) and heat shock protein (hsp70) gene sequences from different organisms (*4*) supported theories of chimeric evolution (*5–10*).

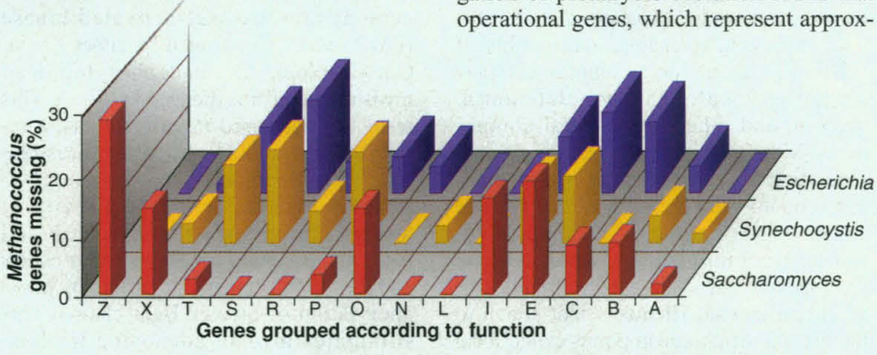
As genomes contain large numbers of genes from different functional classes, it is now possible to analyze the evolutionary history of groups of genes that do similar jobs. Until recently, phylogenetic conclusions were based on the analysis of one or a few genes; now they are based on the analysis of hundreds. Thus, it is possible to ask questions about genome evolution that could never have been answered by analysis of only one gene. For example, have the enzymes of intermediary metabolism evolved as a single group? Are lipids good markers of prokaryotic lineages?

Koonin and his co-workers (*11*) were one of the earliest groups to appreciate the strengths of analyzing functional groups of proteins. Examining a variety of genomes including those of *Methanococcus*, a methane-producing archaeabacterium that thrives in deep-ocean thermal vents, and *Saccharomyces*, they observed that *Methanococcus* genes for translation, transcription, replication, and protein secretion closely resembled their orthologs in yeast but not their orthologs in eubacteria (true bacteria). In contrast, *Methanococcus* genes encoding metabolic enzymes, metabolite-uptake systems, and enzymes for cell wall biosynthesis were more closely related to those of their eubacterial relatives. They concluded that the *Methanococcus* genome is a chimera composed of genes for translation and transcription "borrowed" from *Saccharomyces*, and genes for small-molecule transport and intermediary metabolism "borrowed" from eubacteria. Although their interpretation was not subsequently confirmed by molecular phylogenetics, the selective transfer of functional gene groups between organisms has been established.

In a recent analysis of the complete genome sequences of *Escherichia coli* (a proteobacterium), *Synechocystis* (a cyanobacterium), *Methanococcus* (an archaeabacterium), and *Saccharomyces* (a eukaryote), we found that genes fell into two functional superclasses: informational genes (those involved in transcription, translation, and related processes) and operational genes (those involved in housekeeping) (*10*). Eukaryotes appear to have obtained their informational genes from an organism that is more closely related to *Methanococcus* than to either the proteobacterium or the cyanobacterium, whereas their operational genes seem to have come principally from an *Escherichia* relative (see the figure). These new results begin to explain the mystifying, mixed origins of eukaryotic genomes.

The prevailing wisdom holds that even though eukaryotic genomes may be chimeras, prokaryotic genomes have evolved clonally and are not chimeric. Findings that challenge this viewpoint have been dismissed as exceptions, the result of investigating idiosyncratic genes or organisms (*12*). Now, there is growing evidence that in prokaryotes, too, horizontal gene transfer and chimerism prevail. An investigation of prokaryote evolution found that operational genes, which represent approximately two-thirds of the prokaryotic genome, have been transferred laterally many times, whereas informational genes do not show characteristics in keeping with horizontal transfer (*10*). These results suggest that horizontal gene transfer is an important evolutionary mechanism in prokaryotes as well as in eukaryotes.

A new book (*13*) sets out to demonstrate that horizontal gene transfer is not an accident, but is the product of highly



Gene transfer and the tree of life. Classifying genes according to what they do reveals that many genes in the genome of *Methanococcus* (an ancient bacterium) are not found in the genomes of its eubacterial relatives. Almost all *Methanococcus* genes for translation and transcription are present in the yeast *Saccharomyces* (a eukaryote), whereas nearly 20% of these genes have no known orthologs in the eubacteria *Escherichia coli* and *Synechocystis*. Genes are classified according to function: amino acid synthesis (A), biosynthesis of cofactors (B), cell envelope proteins (C), energy metabolism (E), intermediary metabolism (I), fatty acid and phospholipid biosynthesis (L), nucleotide biosynthesis (N), other (O), cell processes (P), replication (R), transcription (S), translation (T), transport (X), and regulatory genes (Z).

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selective evolutionary events. The probability that a specific gene will be successfully transferred to a new host depends on the exact mechanism of gene transfer (transformation, transduction, or conjugation) (*13*), on the relationships of these mechanisms to the types of nucleic acids that are being transferred (single-stranded, double-stranded, linear, or circular) (*14*), and even on such factors as the intracellular distribution of integrases, the enzymes that integrate DNA into the genome (*15*). Furthermore, horizontal gene transfer does not seem to be a minor, obscure element of prokaryotic evolution—evidence suggests that it still continues today (*16*).

The transfer of DNA among bacteria plays a major part in continuous prokaryotic horizontal gene transfer. In a recent study, Lawrence and Ochman demonstrated that in the 100 million years following the divergence of *E. coli* from *Salmonella*, 755 genes from many sources have been introduced into the *E. coli* chromosome by more than 230 lateral transfer events and these insertions often occur close to transfer RNA (tRNA) loci (*17*). As lysogenic coliphages (viruses that infect bacteria) are known to insert nucleic acid preferentially at tRNA loci, the *E. coli* insertions are likely to have been mediated by this group of bacteriophages. Moreover, the genome of *Bacillus subtilis*, a well-characterized Gram Positive bacterium, also contains a large number of genes that resemble bacteriophage-like inserts (*18*), lending further credence to the view that the transfer of genes between bacteria by bacteriophages has been an important mechanism of horizontal gene transfer.

Completion of genome sequencing projects for prokaryotes such as *Deinococcus radiodurans* (a radiation-resistant bacterium) and for eukaryotes such as *Drosophila* are sure to spur the dissolution of old paradigms and yield a new wave of revelations about the evolutionary tree of life.

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PERSPECTIVES: SIGNAL TRANSDUCTION

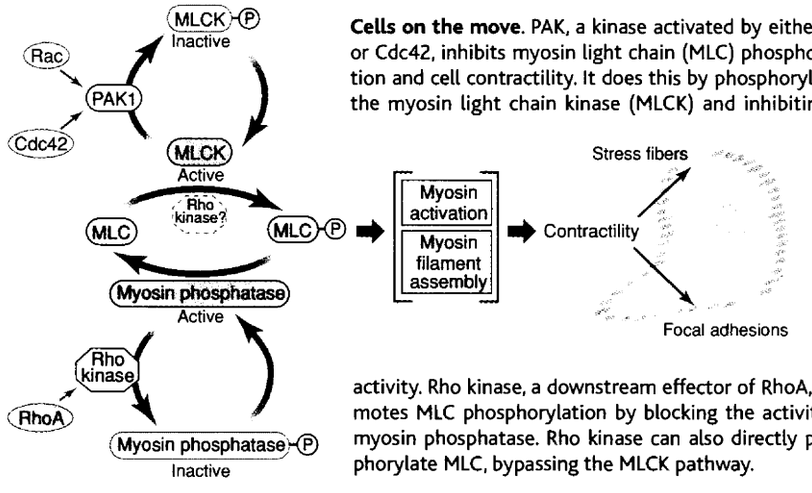
Crosstalk Between Rac and Rho

Keith Burridge

The cytoskeletal changes that alter the adhesion, spreading, and motility of cells depend on a complex interplay among molecules that regulate actin, myosin, and other cytoskeletal components. Members of the Rho family of guanine nucleotide triphosphate (GTP)–binding proteins—including RhoA, Rac, and Cdc42—are important regulators of cytoskeletal organization (*1*). RhoA controls the assembly of stress fibers (bundles of actin and myosin filaments that attach to the plasma membrane at points called focal adhesions), Rac regulates the formation of membrane ruffles, and Cdc42 governs the extension of slender membrane protrusions (filopodia or microspikes) (*1*). Although a hierarchy has been established in which activated Cdc42 stimulates Rac activity and Rac stimulates RhoA activation (*1*), during cell movement these proteins work antagonistically. Rac and Cdc42 promote protrusive events at the leading edge of cells, whereas RhoA induces retraction of the leading edge. Little is known about how Rac or Cdc42 oppose the action of RhoA. On page 2083 of this issue, Sanders *et al.* identify a possible pathway through which Rac may counteract RhoA (*2*). The authors demonstrate that p21-activated kinase (PAK), which is activated by either Rac or Cdc42, blocks the phosphorylation of myosin light chains induced by RhoA. This results in decreased myosin activity, a reduction in contractility, and the disassembly of stress fibers (*3*).

In nonmuscle cells, the activity of myosin II (the myosin found in all cell types that is composed of two heavy chains and four light chains) is regulated by phosphorylation of myosin light chains. This stimulates myosin adenosine triphosphatase activity and induces a conformational change in myosin that promotes its assembly into bipolar filaments. The activated myosin generates tension on the actin filaments and bundles them into stress fibers. Myosin light chain phosphorylation is regulated both by kinases (which add a phosphate group) and phosphatases (which remove a phosphate group).

Historically, most attention has been paid to the myosin light chain kinase (MLCK), an enzyme regulated by calcium and calmodulin. But a new player arrived on the scene with the discovery that Rho kinase (a downstream effector of RhoA) inhibits a myosin phosphatase that removes phosphate groups from myosin light chains and blocks myosin activity (see the figure) (*4*). Thus, a pathway emerged in which RhoA elevates myosin light chain phosphorylation by inhibiting its dephosphorylation. This scheme became even more



Cells on the move. PAK, a kinase activated by either Rac or Cdc42, inhibits myosin light chain (MLC) phosphorylation and cell contractility. It does this by phosphorylating the myosin light chain kinase (MLCK) and inhibiting its activity. Rho kinase, a downstream effector of RhoA, promotes MLC phosphorylation by blocking the activity of myosin phosphatase. Rho kinase can also directly phosphorylate MLC, bypassing the MLCK pathway.

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