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COMMENTARY

Horizontal Gene Transfer and the Evolution of Secondary Metabolite Gene Clusters in Fungi: An Hypothesis

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A remarkable property of filamentous fungi is their ability to synthesize an immense variety of complex secondary metabolites, a trait that they share with few other groups of organisms, notably higher plants and certain prokaryotes. In the past few years a number of fungal genes involved in secondary metabolism have been identified. An emerging commonality is that these genes tend to be clustered, often being separated by less than ~2 kb from each other. Examples of secondary metabolite clusters in fungi include those for AK-toxin (Tanaka *et al.,* 1999), ergot alkaloids (Tudzynski *et al.,* 1999), gibberellins (Tudzynski and Holter, 1998), HC-toxin (Ahn and Walton, 1996), lovastatin (Kennedy *et al.,* 1999), penicillin (Smith *et al.,* 1990), sterigmatocystin and aflatoxins (Brown *et al.,* 1996; Keller and Hohn, 1997), and trichothecenes (Hohn *et al.,* 1993). Most fungal genes for other biosynthetic pathways follow the normal eukaryotic model of dispersion throughout the genome. The self-contained nature of fungal secondary metabolite clusters is striking: in addition to the genes for the biosynthetic enzymes proper they often contain regulatory genes that control the entire pathway and genes conferring autoresistance (Ahn and Walton, 1998; Brown *et al.,* 1996; Proctor *et al.,* 1995; Woloshuk *et al.,* 1994). Clustering is much appreciated by those who study them because the entire pathway can, at least in theory, be obtained in a single cloning step, but from an evolutionary point of view it is not clear why fungal secondary metabolite genes should tend to be clustered. There is probably some selective pressure that drives and maintains clustering of secondary metabolite genes, because whereas there are no known selective pressures or known internal genomic mechanisms that promote or maintain clustering, there are known mechanisms that act to disperse genes, e.g., translocation, inversion, and unequal crossing over. Therefore, it is reasonable to ask why so many fungal secondary metabolite genes are found in this particular genomic arrangement.

(It is important to distinguish between secondary metabolite gene clusters and other types of "clusters," i.e., those containing globin, rRNA, and plant disease-resistance genes. The latter type is composed of paralogous genes of high nucleotide sequence identity and arises by gene duplication caused by unequal crossing over. The genes in a secondary metabolite cluster, on the other hand, encode proteins that catalyze sequential steps in a biochemical pathway. They therefore have a variety of diverse biochemical functions and show little sequence similarity.)

Several hypotheses have been put forth to rationalize clustering of fungal secondary metabolite genes. One is that clustering optimizes coregulation of the constituent genes either by *cis* regulatory elements or by having them all in a similar chromatin environment. Coregulation

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seems an unlikely explanation for two reasons. First, housekeeping gene pathways in fungi and secondary metabolite pathways in other eukaryotes (e.g., anthocyanin biosynthesis in plants) are typically dispersed, which argues that chromatin context is not, at least in general, important for correct coregulation in eukaryotes. Second, genes for secondary metabolite pathways in fungi are known to be regulated by *trans*-acting transcription factors, which can control the expression of dispersed genes as effectively as clustered genes (Keller and Hohn, 1997; Ahn and Walton, 1998). Another reason that has been proposed for clustering is that fungal secondary metabolite genes were acquired from prokaryotes by horizontal gene transfer and that clustering reflects their original organization in operons. However, this seems unlikely because the genes of fungal secondary metabolite pathways have typical fungal introns and individual promoters and are not transcribed as polycistronic mRNAs.

This commentary proposes a novel explanation for clustering of secondary metabolite genes in fungi: clustering has evolved and is maintained because it confers selective advantage to the cluster itself, above and beyond any selective advantage that the pathway, manifested as the secondary metabolite produced by that pathway, confers on the producing organism. That is, the evolutionary pressure that maintains the pathway as a cluster is distinct from the pressure that maintains the capacity to produce the secondary metabolite. Furthermore, it is proposed that the reason that clustering favors survival of secondary metabolite genes is because these kinds of genes depend, at least in part, on horizontal gene transfer for their dispersal and survival.

Any mechanism that favors the survival of genes when they are clustered, as opposed to when they are dispersed, would lead over time to the evolution and maintenance of the genes of a pathway as a cluster. Survival of a gene depends on its successful transmission, since all individual organisms are ultimately ephemeral. Normal ("vertical") gene transmission from one generation to the next, either sexually or asexually, involves transmission of the entire genome as a unit. Clustering would therefore provide no advantage during vertical transmission. However, horizontal gene transfer involves the movement of relatively small, but contiguous, fragments of DNA. Therefore, a clustered pathway can be transmitted as a pathway by either horizontal or vertical transmission, whereas dispersed pathways can be transmitted only vertically. Movement of the entire pathway confers the capacity to make the relevant secondary metabolite to the recipient, which thus acquires a new trait that might endow it with a significant selective advantage over isolates that lack it. If so, the genes of the pathway will continue to be propagated and survive. On the other hand, movement of a single gene of a pathway, which would be more likely if the genes were dispersed, would be unlikely to confer any advantage to the new host and that gene therefore would tend to lose function through random mutation. Therefore, genes of a pathway are more likely to persist if they move together, and during horizontal transfer this would be more likely to occur if they are clustered.

The "selfish cluster" hypothesis presented here thus contains two parts: one, that clusters exist because of the selective advantage conferred by clustering on the genes that comprise them, apart from any advantage that the product of that cluster confers on the host organism, and, two, that the advantage of clusters for the persistence of the constituent genes derives from the fact that horizontal gene transfer is an important mechanism by which they propagate and hence persist.

Horizontal gene transfer is the process by which genetic information of one organism is incorporated into the genome of another organism. The other organism could be the same or a different species. The importance of horizontal transfer in the evolution of prokaryotes is now widely acknowledged (Doolittle, 1999; Jain *et al.,* 1999; Woese, 1998). There are many well-supported cases of horizontal transfer of fungal mitochondrial genes (Collins and Saville, 1990; Goddard and Burt, 1999; Hoekstra, 1994; Holst-Jensen *et al.,* 1999; Kempken, 1995), but many fewer cases of horizontal transfer of fungal nuclear genes. One of the few examples comes from the work of Manners and co-workers, who experimentally demonstrated the movement of an entire dispensable chromosome between two isolates of the same species (He *et al.,* 1998). Despite the scarcity of direct experimental evidence in fungi, horizontal transfer has not infrequently been suggested as an explanation for the discontinuity in distribution of some fungal secondary metabolite genes, when they are present only in some isolates of a species and are completely absent from others (e.g., Kimura *et al.,* 1998; Tanaka *et al.,* 1999; Yang *et al.,* 1996).

The ultimate driving force for the survival of secondary metabolite genes must be the selective advantage that they confer on the organisms that have them, although the basis of this selective advantage is known in only a few cases (Panaccione *et al.,* 1992; Tanaka *et al.,* 1999; Yang *et al.,* 1996). Whereas the persistence of secondary metabolite pathways depends on the selective advantage that they confer to the organism in which they exist, in this Commentary it is being proposed that clusters are subject to an

additional level of selection pressure, which acts to promote and maintain the pathway in a particular subgenomic arrangment. This pressure acts on the cluster directly and is neutral in regard to its effects on the organism. So, on the one hand, the biosynthesis of a secondary metabolite is subject to natural selection for the benefits that it confers on the fungus that makes it. On the other hand, clustering of the necessary genes is promoted by selection forces that act at the level of the cluster itself, as a fragment of DNA that, like all DNA, has as its "prime directive" its own continued existence.

An implicit assumption of this argument is that natural selection can act at the subgenomic level, in this case at the level of supragenic organization. However, traditional theories of evolution hold that the individual organism is the unit of selection, in which case any hypothesis about the selective advantage of clustering would have to address its utility to the organism. However, some evolutionary biologists have argued that the unit of natural selection can be suborganismal, even as small as the individual gene (or, more precisely, a loosely delineated small unit of genetic material termed the "active replicator") (Dawkins, 1982). The idea that natural selection can act on subgenomic entities, such as clusters, is therefore not heresy to all evolutionary biologists. Along the continuum of "selfishness," secondary metabolite clusters lie between truly selfish fragments of DNA, like transposable elements, and essential housekeeping genes, which function and evolve within a tightly integrated metabolic network.

The hypothesis of the "selfish cluster" does not require that clusters propagate solely by horizontal transfer. Nor does it require that horizontal transfer be overall more common than vertical transfer; it requires only that it be more than an occasional aberration. Resolution of the question of horizontal transfer in fungi will ultimately depend on direct experimental evidence and on comparative genomics, but two unique characteristics of fungi are consistent with horizontal transfer being a significant mode of gene transfer. The first is that a reasonable mechanism exists. Fungi readily undergo hyphal anastamoses with other, even unrelated fungi, and heterokaryon incompatibility is probably insufficient to prevent concomitant gene flow (Hoekstra, 1994). The second is the relative inefficiency of vertical transmission due to the instability of fungal genomes. Translocations, deletions, and inversions are common in fungi. Many studies have documented highly variable karyotypes among field populations (e.g., McCluskey and Mills, 1990; McDonald and Martinez, 1991; Morales *et al.*, 1993), as well as high rates of spontaneous mitotic and meiotic instability (Pitkin *et al.*, 1999; Sweigard *et al.*, 1995). Many mycologists can attest anecdotally to the instability of ascomycetous fungi under laboratory conditions. Insofar as it leads to the loss of essential genes, a high level of genomic instability would cause the rapid extinction of a species or lineage, regardless of whether it reproduces sexually, asexually, or both. Genomic instability makes vertical transfer risky relative to horizontal transfer, which would favor the evolution of processes to exploit horizontal tranfer in fungi. Here it is proposed that gene clustering is one such process.

Gene clusters are well known in prokaryotes in the form of operons, which are clusters of genes with related biochemical functions driven by a single promoter and transcribed as a single mRNA. The received wisdom has been that operons evolved because they optimize coordinate regulation of the constituent genes. However, recently this theory has been questioned and an alternative, called the "selfish operon" theory, has been proposed (Lawrence and Roth, 1996; Lawrence, 1997, 1999). This theory states that the organization of genes into operons confers no advantage to the organism, but only to the operons themselves. The reason that clustering is advantageous to the operon is because the genes of the operon can thereby exploit horizontal transer to optimize their long-term survival. In other words, the selfish operon and the selfish cluster theories propose the same explanation for the same phenomenon in prokaryotes and eukaryotes, respectively.

The theory of the selfish operon was developed in part to explain the observation that genes for nonessential functions, for which selection pressure is relatively weak, are more often organized into operons than are housekeeping genes. Because selection for nonessential genes is almost neutral, they will tend to accumulate mutations relatively quickly and thus be short-lived if restricted to a particular lineage. However, if genes for a novel metabolic process can move by horizontal transfer, they have a reasonable chance of conferring a new selective advantage to a "naïve" genome, which would promote the survival of the new host and therefore also of the genes. As discussed above, clustered pathways are more likely than dispersed pathways to be transmitted horizontally. Because "physical proximity" provides "no selective benefit to the donor organism" but only a "strong advantage to the genes themselves," "the cluster can be considered a selfish property" (Lawrence and Roth, 1996).

Characteristic selfish operons for nonessential functions include those needed for the "degradation of unusual compounds" and "those employed only under specific, rarely encountered environmental conditions" (Lawrence

and Roth, 1996). In regard to unusual compounds, it is consistent with the selfish cluster theory that genes for catabolism of rare compounds such as proline and quinate are also clustered in fungi (Keller and Hohn, 1997). Although not mentioned explicitly by Lawrence and Roth (1996), secondary metabolite genes are also by definition nonessential and are presumed also to have only sporadic utility, for example, during parasitism of a rare sensitive host (Panaccione *et al.*, 1992; Yang *et al.*, 1996).

What would be predicted to happen to a secondary metabolite gene cluster after it had moved into a new host by horizontal transfer? Known genomic forces such as unequal crossing over and translocation would be predicted to cause dispersal of the individual pathway genes. There is no apparent reason why this should adversely affect production of the secondary metabolite, because coordinate regulation of the genes should still be maintained by *trans*-acting pathway-specific transcription factors. The genes for HC-toxin biosynthesis in *Cochliobolus carbonum* might present an example of what happens to a horizontally transferred cluster that is persisting in a stable lineage. A plausible scenario for the evolution of the genomic arrangement of the genes for HC-toxin production is that they arose in *C. carbonum* by horizontal transfer and were originally present in a single cluster on a single chromosome of 2.2 MB. In extant isolates, however, the genes are duplicated, dispersed, and only partially clustered. In some isolates the genes are dispersed locally (within ~600 kb), but in others a reciprocal translocation has resulted in most of the genes residing on a 3.5-MB chromosome but with at least one copy of one of the genes being on a different chromosome of 0.7 MB. There is no reason to believe that this trend will not continue, unless the HC-toxin-producing isolates become extinct, resulting eventually in the HC-toxin biosynthesis genes becoming completely dispersed throughout the genome. Regardless of the genomic organization of the genes, effective coregulation would be maintained by the pathway-specific transcription factor encoded by TOXE (Ahn and Walton, 1996, 1998).

Both the selfish operon and the selfish cluster theories assume that horizontal transfer not only occurs but is a significant evolutionary process in prokaryotes and fungi, respectively. Appreciation of the role of horizontal gene transfer in prokaryote evolution has, in large part, developed from comparative analyses of the complete sequences of many prokaryotic genomes. Similar resources for the mycological community would allow this, and other critical evolutionary issues, to be addressed in fungi.

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