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Conservation, relocation and duplication in genome evolution

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The advent of whole-genome sequencing and genome-wide transcriptional profiling has opened up new approaches to the resolution of questions that only a few years ago seemed unanswerable. At the same time they have revealed new and sometimes unexpected patterns of gene conservation and functional compensation, chromosomal clustering of transcriptionally related genes, relocation of genes to depopulate or overpopulate the X chromosome with certain functional classes of genes, and gene duplication and functional divergence. What makes molecular evolutionary genomics different from previous approaches is the generality of the results. Choice of genes, and the uncertainties of extrapolating from a sample of genes to the entire genome, is supplanted by direct genome-wide observations. In this article we examine some key recent experiments in RNA interference that illustrate some of the strengths and limitations of evolutionary genomic analysis.

Investments in high-throughput DNA sequencing and functional genomics were justified initially by the argument that large-scale investigations of gene structure, function and expression would ultimately pay off in the form of new insights into the organization, integration and evolution of biological systems. Those who advanced this argument have not had to wait long until being vindicated. Genomics has already had a major impact on how biologists think about cells, metabolism, development and evolution. The field of molecular evolutionary genomics has begun to be shaped more by experimental results than by conjecture. In this article we give a brief and selective review of some of key recent experiments from the perspective of genome evolution, and highlight some of the controversies.

Conservation and evolution of essential genes

We begin by reviewing experiments in the nematode *Caenorhabditis elegans* in which gene function was disrupted by feeding worms with bacteria encoding double-stranded RNA molecules that are homologous to one of 16 757 worm genes (~86% of the entire genome [1]). In genes that are sensitive to RNA interference (RNAi), the presence of double-stranded RNA results in destruction of the endogenous transcripts and often results in mutant phenotypes. In these experiments, the mutant phenotypes were grouped into three broad categories: (i) embryonic or larval lethal or sterile (ii) growth defects or (iii) visible post-embryonic phenotype. Altogether, 1722 of the tests (10.3%) yielded worms with mutant phenotypes. Among genes previously identified by means of their abnormal phenotypes when mutated, the RNAi procedure yielded a mutant phenotype for approximately two-thirds. If this is taken as a rough estimate of the efficacy of RNAi in disrupting gene function, then the 10.3% yield of mutant phenotypes implies that a large number of genes are either functionally redundant or else have only subtle phenotypic effects.

C. elegans genes with an ortholog in at least one other eukaryote are much more likely to have an RNAi phenotype than those without an ortholog (21% versus 6%). Furthermore, 52% of the *C. elegans* genes with nonviable RNAi phenotypes have an ortholog in another eukaryote. These results imply that genes with detectable orthologs are more likely not only to yield an RNAi phenotype but also to yield a nonviable RNAi phenotype. There is also a suggestion in the RNAi data that essential genes in *C. elegans* might evolve more slowly than nonessential genes, but as explained in Box 1, this interpretation had best be regarded as tentative.

The molecular data seem to support for the idea that mutations affecting early developmental events are likely to be the most deleterious because early events determine the multiple genetic and epigenetic processes that occur later [2]. Genes participating in the early stages of development might also be constrained because of the large number of gene interactions that occur and the requirements for precision in the spatial and temporal patterns of gene expression [3,4].

Relocating genes: the X chromosome

Among *C. elegans* genes with an RNAi phenotype, there is a highly nonrandom distribution of phenotypes among

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Box 1. Do Essential genes evolve more slowly than nonessential genes?

The hypothesis that essential genes might evolve more slowly than nonessential genes [27] is highly contentious. The seemingly plausible rationale that essential genes might be under stronger selective constraints than nonessential genes [27] includes hidden assumptions about effective population size. In any population, the fate of a new mutation is determined by the relative strength of natural selection (proportional to the selection coefficient) as against the effects of random genetic drift (proportional to the reciprocal of the effective population size). Hence organisms with large effective population size (e.g. most bacteria) can either eliminate or fix mutations with smaller selection coefficients than can organisms with relatively small population size (e.g. most mammals).

Slower evolution of essential genes at first seemed to be supported by a comparison of 67 essential with 108 nonessential genes in rodents, but Hurst and Smith [28] found that the disparity in evolutionary rate could be attributed entirely to a faster rate of evolution of genes associated with the immune system, which might be under directional selection. Another apparent confirmation is that yeast deletion mutations have more severe effects on growth rate the greater their similarity to their best-matching coding sequence in the nematode, *Caenorhabditis elegans* [29]. This was contested on several grounds [30] including the failure to account for the effects of gene expression [31]. Gene expression also complicates the interpretation of the finding that amino acid sequences of essential genes in two bacterial species evolve more slowly than those of nonessential genes [32]. The essential bacterial genes also have a much lower rate of synonymous nucleotide substitutions, suggesting greater codon usage bias implying a higher expression level. The issues are confounded further by reports of a slower rate of evolution of proteins with greater connectivity in the gene-expression network [33], although this conclusion is disputed [34,35]. A recent study introduces yet another variable: gene duplication [36]. Although the rate of amino acid replacement in yeast is slower among genes that are lethal when deleted, this phenomenon is because of genes that are members of duplicate pairs. Single-copy genes that are essential show no reduction in their rate of evolution. The authors [36] interpret the faster rate of evolution of nonlethal duplicate genes to a reduction in selective constraints on genes with partially overlapping functions.

In the RNAi data for *C. elegans* [1], genes with an embryonic-lethal RNAi phenotype have a slower rate of amino acid replacement than genes with no detectable RNAi phenotype, both for single-copy genes and for those with duplicates (Figure I). We caution that this finding might be because of covariation with other variables, such as gene expression level [26,31], proteins involved in multisubunit complexes [37] or metabolic connectivity [33,35]. As in the cases cited earlier in this box, what appears to be true on the surface might, on further analysis, be open to alternative interpretations.

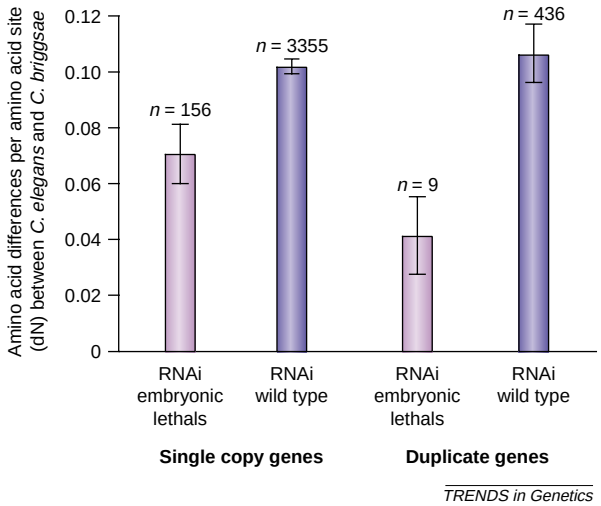

Figure I. Rates of amino acid replacement and 95% bootstrap confidence intervals (indicated by error bars) for genes in *Caenorhabditis elegans* showing an embryonic lethal RNA interference (RNAi) phenotype as compared with those showing no detectable RNAi phenotype. A significant difference is observed for single-copy genes as well as those with duplicates in the genome, but note that the number of duplicate genes (*n*) with a lethal RNAi phenotype is small. In these comparisons, the rates of amino acid replacement were estimated from best reciprocal BLAST hits against the *Caenorhabditis briggsae* genome sequence as described in Ref. [1].

chromosomes [1]. The greatest discrepancies are found between the autosomes and the X chromosome. The X chromosome has a much smaller proportion of lethal RNAi phenotypes (Figure 1). Kamath *et al.* [1] attribute the deficit to transcriptional silencing of the X chromosome that takes place in the germline during mitosis and early meiosis [5,6], because genes required for the viability of all cells (including germline cells) would be intolerant of silencing and so would be expected to be absent from the X chromosome. This does not, however, explain the selection pressure to remove essential genes from the X chromosome prior to the evolution of germline silencing. Perhaps it relates to dosage compensation, which in *C. elegans* XX hermaphrodites is mediated by the recruitment of specialized protein complexes to the X chromosomes that repress transcription of each X chromosome by half [7].

The RNAi data also show a significant but less dramatic excess of genes with visible post-embryonic phenotypes in the X chromosome [1]. The authors [1] attribute the excess to XX hermaphrodites acting as repositories for mutant alleles. The argument is that, under stressful conditions, the X chromosomes are prone to meiotic nondisjunction, and thus recessive alleles in the X chromosomes of XX hermaphrodites might be expressed in the nondisjunctiona XO males, thereby allowing the population to exploit the hidden genetic variation to adapt to a changing environment. On the whole, evolutionary biologists are likely to be skeptical of this hypothesis and others in which populations accumulate genetic variation for possible later use [8], because such models seem to invoke a foresight that is inconsistent with individual selection [9].

The *C. elegans* X chromosome also shows a striking deficit of genes that show biased expression in the germline or biased expression in sperm [5]. This is reminiscent of observations indicating that the *Drosophila* X chromosome has a relative deficiency of genes with male-biased sex expression [10,11]. However, the generality of this 'demasculinization' phenomenon and the underlying mechanisms are unclear (Box 2).

Gene clusters and coordinate regulation

Genes in *C.elegans* with similar RNAi phenotypes are also significantly clustered along the chromosomes [1]. Clustering of genes with similar functions has also been noted in *Drosophila* [12]. Among 1661 testes-specific genes in *Drosophila* expressed sequence tags (ESTs), one-third of the genes are clustered in groups with three or more members [12]. One obvious possibility is that clusters of

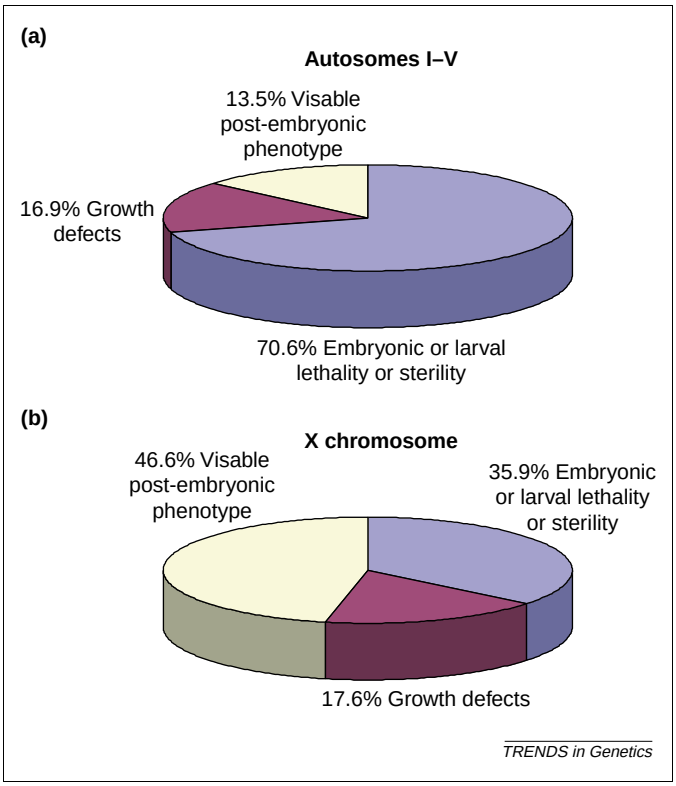

Figure 1. Percentage of each major RNA phenotype for genes in the autosomes (I–V) and the X chromosome of *Caenorhabditis elegans*. The phenotypes are classified as embryonic or larval lethal or sterile, growth defects, or visible post-embryonic phenotype. The total number of genes with RNAi phenotypes is 1591 for the autosomes and 131 for the X chromosome. Data from Ref. [1].

functionally related genes are the result of duplication and divergence of tandemly arrayed paralogs, but this cannot account for most of the clustering. Two or more paralogs were found in only 16% of 239 clusters in *Drosophila*, and although some of the smaller clusters consisted only of paralogs, the larger clusters also contained nonhomologous genes. The generality of such clustering is suggested by similar trends found for genes upregulated in the embryo and in the adult head [12].

Because genes with related functions show similar expression patterns, clustering of genes with related functions is expected because genes with similar expression profiles tend to be clustered in many eukaryotes, including yeast [13], worms [14], flies [12], humans [15] and even in the malaria parasite *Plasmodium falciparum* [16]. In *C. elegans*, the clustering has been attributed largely to operons [17], in yeast to reduced recombination between genes with similar expression patterns [18], in flies to chromatin domains [12], and in human to clustering of housekeeping genes [19]. In view of the variety of explanations for the clustering of genes with similar expression levels, it seems an understatement to describe the situation as unsettled.

Single-copy genes and multigene families

In *C. elegans*, genes lacking paralogous copies are more likely to have an RNAi phenotype than genes that have paralogs (31% versus 12%) [1]. This result is consistent with studies of targeted deletions in budding yeast *Saccharomyces cerevisiae*, in which the effects of deletion on growth rate under various conditions were compared between deletions of 1275 genes without paralogs and 1147 deletions of genes with one or more paralogs [20]. A significantly higher probability of phenotypic compensation was observed for deletions of genes that have paralogs, with a higher probability of compensation found among paralogous families that are more similar in sequence.

These results suggest that paralogous genes only gradually lose their ability to functionally complement one another. An elegant theory of how this might happen is that of 'subfunctionalization' of duplicate copies [21]. In this process, duplicate copies of a gene expressed in multiple tissues (in multicellular organisms) or having

Box 2. Sexually antagonistic selection of X-linked genes

Sexually antagonistic selection takes place when a genotype that is beneficial for one sex is deleterious for the other [38]. It is evidenced in natural populations of *Drosophila* by a strong negative correlation in adult fitness between males and females [39]. Although sexually antagonistic genes should eventually evolve to be expressed only in the favored sex to minimize the deleterious effects in the opposite sex [38], sex-biased gene expression appears to evolve very rapidly between species [11]. The X chromosome especially is a hot spot for sexually antagonistic interactions [40]. This might result from a hereditary asymmetry of the X chromosome in species with XX females and XY males (or X males, as in the case of *Caenorhabditis elegans*). In these species, although an X-linked gene spends two-thirds of its existence in females, rare X-linked recessive alleles are expressed primarily in males. (With random mating, an X-linked recessive with allele frequency q is expressed in a fraction q of males and q^2 of females, and if the allele is rare, $q^2 \ll q$.) The hereditary asymmetry of the X chromosome is the basis of an influential theory [38] outlining the conditions under which sexually antagonistic alleles will either accumulate or be removed from the X chromosome.

The theory of antagonistic selection [38] has elicited comment [41,42] in connection with the under-representation of genes with male-biased expression in the X chromosome of both *C. elegans* [5] and *D. melanogaster* [10,11]. By contrast, genes expressed in spermatogonia are over-represented in the mammalian X chromosome [43,44]. The difference is bothersome because the relocation of male-favoring genes from the X chromosome to the autosomes is expected only for dominant gain-of-function mutations [38], and it is difficult to argue that alleles favoring males are dominant in worms and flies but recessive in mammals. Alternative explanations on the basis of dosage compensation [41] and X-inactivation during spermatogenesis [5] have been suggested. There is also roughly an order of magnitude difference in effective population size between the lineages [45,46], which affects the efficacy of selection (Box 1). Alternatively, the explanation might lie in gene regulation. Betran *et al.* [47] have found that *Drosophila* genes created by retrotransposition have two intriguing characteristics: (i) they originate predominantly from retrotransposition of X chromosomal genes to autosomes; and (i) most of the retrotransposed copies acquire testes expression. This discovery raises the possibility that many testes-expressed genes might indeed be gain-of-function mutations that acquired their testes expression in being transposed from an X chromosome into an autosome. This model would help explain the relative under-representation of male-biased genes in the *Drosophila* X chromosome. In evaluating this model, comparable studies of retrotransposed genes in *C. elegans* and mammals should be highly informative.

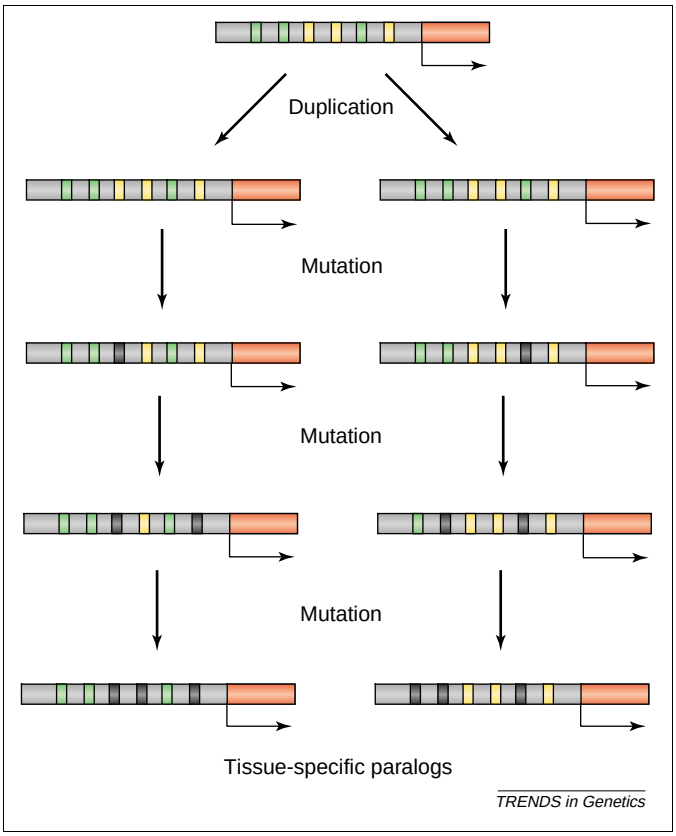

Figure 2. Model for gradual subfunctionalization by sequential inactivation of enhancers that interact cooperatively to drive transcription in either of two tissues. The right arrow denotes transcription of the coding sequence (red), and the region to the left of the transcription start site represents the promoter region. Functional enhancers are in color, tissue type I enhancers are in green, tissue type II enhancers are in orange, mutant enhancers are in dark gray. Based on a model of Lynch and Force [21].

multiple splice forms or functional domains each undergo mutations that either restrict or eliminate a different functional aspects of that gene. The mutations in each copy are tolerated because the other copy compensates for it, and both copies become subfunctional. Figure 2 shows a hypothetical example of a gene with multiple enhancer elements for tissue-specific expression in two types of tissues. Each copy might gradually lose the enhancers needed for expression in one of the tissue types, resulting in even greater tissue specificity. Until the cross-activation is completely lost, however, each gene might be able to partially compensate for the other. It is interesting to note that deletions of single paralogs of yeast genes tend to have more severe effects when the paralog with highest expression is deleted is deleted [20].

Studies of genome sequences in a wide variety of eukaryotes suggest that the dynamics of gene duplication seem to provide ample opportunity for the evolution of paralogous gene families [22]. The rate of gene duplication appears to be much greater than previously supposed (but for possible bias in the estimates, see [23–25]). Roughly speaking, a eukaryotic genome containing 15 000 genes might be expected to undergo 30 to 300 duplications per million years, any of which might undergo divergent processes such as subfunctionalization of the copies [21].

But even the establishment of gene duplications might be subject to developmental constraints. For example, in *C. elegans*, genes that peak in expression during embryogenesis show fewer paralogs than genes expressed post-embryonically, perhaps owing to greater negative consequences of two-fold dosage of genes that function in embryogenesis [26].

Conclusion

The strengths as well as the limitations of genomics in an evolutionary context are beginning to take shape. The strengths emerge from the ability to examine the genome comprehensively from the standpoint of DNA sequence and gene expression. Genome-wide studies have already revealed occasionally unexpected patterns of gene conservation, relocation and duplication in genome evolution. These insights are new in evolutionary biology and are the first fruits of molecular evolutionary genomics. Furthermore, the inferences based on genome-wide studies are not compromised by the need to extrapolate from small samples of genes to the whole genome.

Genomics approaches as applied at present also have limitations. The observed patterns of gene conservation, relocation and duplication challenge the imagination to propose testable evolutionary hypotheses. Why is the *C. elegans* X chromosome enriched for genes with visible post-embryonic RNAi phenotypes? Why are the X chromosomes of *C. elegans* and *Drosophila* deficient in genes that are male-biased in their expression, whereas the opposite is found in mammals? Why are genes with similar expression profiles apparently clustered in the genome? There are plausible explanations for each of these observations, but also wide disagreement and in some cases considerable controversy. Although the results seem solid, the explanations are tentative and require explicit experimental verification. Already a rich source of new data and hypotheses, evolutionary genomics now confronts the next challenge of hypothesis testing.

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Effects of reunited diverged regulatory hierarchies in allopolyploids and species hybrids

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The evolutionary fate of polyploids (especially plants) immediately following their formation has recently received renewed attention by several research groups. An increasing body of work demonstrates that genome rearrangements, epigenetic alterations, and changed gene expression levels often accompany polyploidization. New data indicate that altered gene expression patterns can occur in polyploids with few signs of genome reorganization, suggesting a mechanism involving interactions of diverged regulatory hierarchies.

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Glossary

Allopolyploid: A polyploid originating from the combination of two distinct genomes, for example, *Arabidopsis suecica*, which carries a full chromosome complement from *A. thaliana*, as well as a full complement of *A. arenosa* chromosomes.
Homoeologous: In an allopolyploid, referring to the corresponding genes from the two parental chromosome sets; for example, in *Gossypium hirsutum*, the A genome-derived copy of the *adhE* gene and the D genome-derived copy of *adhE* are homoeologous.
Hybrid incompatibility: Sterility or inviability occurring in species hybrids preventing gene flow.
Polyploidy: The state in which a species or individual has more than two chromosome sets.