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HISTORICAL PERSPECTIVE ESSAY

The Gene Balance Hypothesis: From Classical Genetics to Modern Genomics

The concept of genetic balance traces back to the early days of genetics. Additions or subtractions of single chromosomes to the karyotype (aneuploidy) produced greater impacts on the phenotype than whole-genome changes (ploidy). Studies on changes in gene expression in aneuploid and ploidy series revealed a parallel relationship leading to the concept that regulatory genes exhibited a stoichiometric balance, which if upset, would modulate target gene expression. The responsible regulatory genes for these types of effects primarily have been found to be members of signal transduction pathways or transcription factors of various types. Recent studies of retention of selected duplicate genes following diploidization of ancient polyplodization events have found that signal transduction and transcription factors have been preferentially maintained in a dosage-sensitive relationship. In this essay, we review the historical progression of ideas about genetic balance and discuss some challenges in this field for the future.

EARLY DEVELOPMENT OF THE CONCEPT OF GENETIC BALANCE

Changes in chromosome number played an important role in the early days of genetics, albeit in a misunderstood role. The mutations that inspired the mutation theory of deVries (1901), one of the rediscoverers of Mendel's laws, were most likely due to extra chromosomes present in his research material of *Oenothera lamarckiana*, the evening primrose, rather than mutations in the current sense of the word (Emerson and Sturtevant, 1931). Shortly thereafter, Alfred Blakeslee observed mutations in *Datura stramonium*, the Jimson weed (or the ''rank-smelling thorn apple'' in the words of Edna St. Vincent Millay [Crow, 1997]). These mutations behaved in an unusual fashion compared with others, in that they were transmitted primarily through the female parent and could never be made to be true breeding. Several different mutations with different phenotypic characteristics were found that behaved in the same fashion (Blakeslee and Avery, 1919). Eventually, when J. Belling examined these stocks cytologically, it was discovered that they were in fact carrying an extra chromosome and were thus designated as trisomics (Blakeslee et al., 1920). The derivation of additional such trisomics from starting material of a doubled haploid proved that the alterations in morphology that accompanied these changes in chromosome number were due to dosage effects of the chromosome rather than to genetic variation. Polyploid plants were known at the time, and their differences from the diploid phenotype were not as severe. Thus was born the concept of genetic balance (Blakeslee, 1921) (Figure 1).

As this work continued, trisomics were found for all of the possible 12 chromosomes of *Datura*, but additional forms continued to arise, each with different characteristics. Some of these new forms accentuated a portion of the phenotypic characteristics of the original set of primary trisomics but were missing other changes from the normal diploid. The basis of these forms was revealed to be an extra chromosome that possessed two identical chromosome arms derived from misdivision of the centromere, manifested by a break in the middle of a centromere with fusion of two sister chromatids, to produce mirror image chromosomes (isochromosomes) generated from a single chromosome arm (Belling and Blakeslee, 1924). In these cases, the extra chromosome, called a secondary trisome, would result in a total of four copies of the respective arm, thus providing an explanation for the accentuated partial phenotype compared with the respective trisomic from which it was derived (Figure 1). These results bolstered the concept that a balance of genes was important for the normal phenotype. These and other aneuploid types were summarized in an article entitled ''New Jimson Weeds from Old Chromosomes'' (Blakeslee, 1934), which cryptically conveys the message that the dosage of chromosomes is important in this case as opposed to genetic variation. Indeed, the more extreme the altered relative dosage of chromosomes, the greater the phenotypic change. This concept is aptly illustrated by the recovery of selected isochromosomes in otherwise haploid plants that had an extreme imbalance and severe phenotype (Satina et al., 1937) (Figure 1).

At about the same time as the early studies of *Datura* were conducted by Blakeslee, Richard Goldschmidt was studying the basis of sex determination in the gypsy moth (*Lymantria dispar*) (Goldschmidt, 1920). Crosses between different geographic isolates produced progeny that had differing levels of intersexuality, which is a mixture of male and female morphologies. To explain the various results, Goldschmidt hypothesized different male and female factors of varying strengths whose balance was critical for proper sex determination. Within a particular geographic isolate, the sex determination processes are normal, but crosses between different strains produced the intersexual individuals because the various factors had varied in dosage potential between the diverged populations and were no longer matched. In retrospect, this result foreshadowed the concept of Muller-Dobzhansky coevolving gene complexes that interact within a species but cause sterility or inviability in hybrids.

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HISTORICAL PERSPECTIVE ESSAY

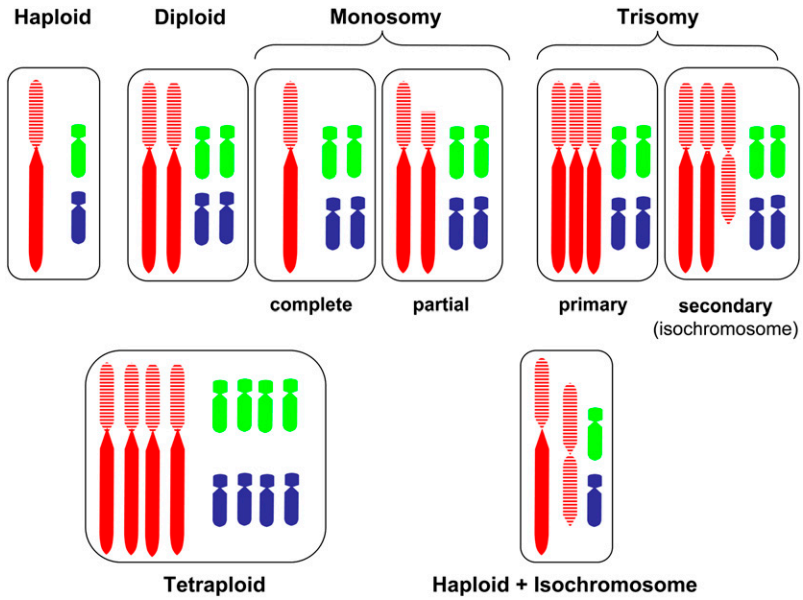

Figure 1. Chromosomal Imbalance.

Diagrammatic representation of chromosome variations with the varied chromosome shown in enlarged form. Full or partial monosomics with only one copy of a chromosome or chromosomal segment are usually more severely affected than haploid plants. Trisomic plants with an extra chromosome often show a greater morphological change from the diploid than observed in polyploid plants, such as tetraploids. Secondary trisomics carrying an isochromosome composed of two copies of a chromosome arm show enhanced phenotypic changes for some characteristics typical of the corresponding primary trisomic. When an isochromosome is recovered in an otherwise haploid plant, the imbalance is most severe and results in highly defective plants (Satina et al., 1937). These relationships led to the concept of genetic balance.

In further parallel studies, Calvin Bridges identified a triploid female *Drosophila* and among her progeny found intersexual flies that possessed a mixture of male and female parts (Bridges, 1925). The karyotype of these intersexual flies was composed of two X chromosomes with an otherwise triploid complement of chromosomes. However, flies with only one X chromosome that were otherwise triploid were male-like in phenotype. Of course, among diploid flies, the males have one X chromosome and the females have two, with all other chromosomes being present in duplicate in the two sexes. By comparing the various chromosomal constitutions of males, intersexes, and diploid and triploid females, Bridges concluded that a balance between the X and the autosomes (non-sex chromosomes) was the chromosomal basis of sex determination in *Drosophila*. This type of mechanism for sex determination has been postulated for many plant species as well, with the most well-documented case being *Rumex acetosa* (Ono, 1935). When the X-to-A ratio is 1, a female is produced, but when it is 0.5, a male occurs. In polyploid *Rumex*, X-to-A intermediate ratios produce intersexes and hermaphrodites (Dellaporta and Calderon-Urrea, 1993).

Similar results of chromosome additions to produce trisomics and tetrasomics as well as the reciprocal production of monosomic individuals have recapitulated the balance conclusions in scores if not hundreds of species in both the plant and animal kingdoms (e.g., Lee et al., 1996; see discussion in Birchler et al., 2001). Although great variation occurs, in general, both monosomics and trisomics cause detrimental effects on the phenotype, with the monosomics usually being more severe. Aneuploid studies in polyploids have further supported the balance idea because there is less effect of extra or missing chromosomes when the remainder of the genome is increased in copy number. The extensive studies of aneuploids in hexaploid bread wheat by Ernie Sears exemplify these conclusions (Sears, 1953, 1954).

Over the subsequent decades, the interpretation of genetic balance split into an interesting dichotomy of enzymatic/metabolic versus gene regulatory balance that lingers today. On the one hand, the involvement with sex determination mechanisms was viewed as a regulatory balance, while on the other hand, aneuploid syndromes were viewed as perturbations of metabolism resulting from altered relationships of enzymes involved with intermediary metabolism. Indeed, the basis of sex determination in *Drosophila* involves a dosage relationship between transcription factors on the X chromosome relative to those on the autosomes (Erickson and Cline, 1993). While aneuploid syndromes no doubt have a complicated basis, the emerging evidence suggests that it is likely that most of these balance phenomena reflect gene regulatory mechanisms in some form, although other gene products involved with macromolecular

complexes are likely to exhibit a balance as well, whether or not they are directly involved with gene expression (see below).

GENE EXPRESSION IN ANEUPLOIDS

Several decades ago, one of us (J.A.B.) examined the levels of enzyme activities and proteins in aneuploid series of maize. A popular exercise at the time was the localization of the cytological position of genes encoding various enzymes by screening the genome using a set of trisomics or segmental trisomics created by overlapping translocations (e.g., Carlson, 1972; O'Brien and Gethman, 1973). Work on *Datura* played an inspiring role in this trend (Carlson, 1972). The principle involved the concept that varying the dosage of a gene would produce a directly proportional amount of gene product (Grell, 1962). Thus, reversing the procedure in a screen of trisomics covering the genome should locate the position of the structural loci for various enzymes because the increased dosage of the encoding gene would produce a greater amount of gene product. This approach was successful in some cases; the unsuccessful attempts remain unknown. Thus, to test ideas about the regulation of the *Alcohol dehydrogenase-1* gene in maize (Schwartz, 1971), a dosage series of the chromosome arm on which this gene is located was examined for the levels of ADH activity (Birchler, 1979). Interestingly, the total amount of ADH present in a one to four dosage series was nearly equivalent to the diploid level. In other words, no gene dosage effect for *Adh* was found in the whole arm dosage series. However, a whole genome ploidy series involving monoploids, diploids, and tetraploids indicated that there was a directly proportional amount of ADH per cell through the series. When other enzymes encoded on other chromosomes were examined in the aneuploid series, their levels were modulated, either up or down, but the most common effect was an inverse correlation between the chromosomal dosage and the amount of activity present (Birchler, 1979). These studies were extended to other proteins and other aneuploid and ploidy series with similar results (Birchler and Newton, 1981). A hypothesis was formulated that the stoichiometry of regulatory genes was influential in modulating the levels of expression of the target genes studied (Birchler and Newton, 1981).

The failure to find a dosage effect for ADH was referred to as dosage compensation. The basis of this response was determined to be the result of a structural gene dosage effect of *Adh* itself being modulated in an inverse manner by a different part of the same chromosome arm involved in the aneuploid series (Birchler, 1981). In monosomics, the single *Adh* gene was upregulated approximately twofold. In trisomics, the three copies of *Adh* were each downregulated by two-thirds. Of course, dosage compensation of the X chromosome in males of *Drosophila* had been documented since the realization of its existence by Muller (1932). That is, males with only one X chromosome produce about the same amount of gene product as do the two X chromosomes present in females. However, Devlin et al. (1982) found that compensation would also occur for large trisomics of autosomal chromosome arms consisting of ~20% of the whole genome, in contrast with small trisomics surrounding a particular structural gene that vary only a very small fraction of the genome, as noted above. Later, they reported the extensive presence of the inverse dosage effect of these large trisomics on genes encoded on other chromosomes (Devlin et al., 1988). The basis of autosomal dosage compensation was also found to involve the combination of a structural gene dosage effect and an inverse effect being produced simultaneously by a large trisomic segment surrounding the *Adh* gene in flies. In other words, a structural gene dosage effect is cancelled via an inverse effect produced by another part of the varied region (Birchler et al., 1990).

In maize, a survey of aneuploids from many regions of the genome for six genes revealed that the RNA levels of any one gene could be modulated similarly by different dosage series (Guo and Birchler, 1994). In other words, changes in dosage of several different segments of the genome could have the same effect on the monitored gene. The magnitude of these modulations depended on whether the tissue examined was diploid or triploid. Adding or subtracting a chromosome arm to the genotype at different ploidy levels produced an effect whose severity was coincident with the degree of dosage imbalance. Changes in gene expression in a whole genome ploidy series were not as prevalent (Guo et al., 1996). Transcriptome studies of mammalian trisomics or highly aneuploid cancer cells indicate extensive transacting modulations of gene expression (e.g., Phillips et al., 2001; FitzPatrick et al., 2002; Saran et al., 2003; Tsafrir et al., 2006). Thus, the types and mode of effects parallel the classical phenotypic studies with regard to balance.

BALANCE AT THE GENE LEVEL

A simple interpretation of the cause of these transacting dosage effects is that they are caused by a gene or genes on the varied chromosome that exhibit a dosage effect themselves and that act in a regulatory fashion to modulate many targets. To screen for single gene mutations that would mimic the dosage effect, mutageneses were conducted in *Drosophila* to find mutations that could up- or downregulate the expression of the *white* eye color gene using a leaky phenotypic reporter called *white-apricot*. The eyes of these flies have a low level of pigment, so modulations of expression could be scored easily. An amazingly large number of mutations were recovered from these screens, totaling 47 at last count (Birchler et al., 2001). The first reported example was *Inverse regulator-a* (Rabinow et al., 1991). Mutations of this gene as a heterozygote upregulate the *white* gene twofold, mimicking how a monosomic condition would produce a similar aneuploid inverse effect. Trisomics of the region downregulate *white*. The presence of the mutation in an otherwise triploid fly upregulates *white* to ~150% of the control triploids. Thus, *Inr-a* serves as an example of a single gene that produces a balance effect, in this case with an inverse dosage response.

HISTORICAL PERSPECTIVE ESSAY

The large number of modifiers of a single target gene must be understood in the context that developmental regulators often operate in a hierarchy. That is, one early developmental regulator might affect a downstream regulator and so on. If each regulator is dosage sensitive, the effect could potentially be passed along through the hierarchy. One must also appreciate that any one regulator will affect many targets, so there would be significant overlap of modifiers for different traits.

A summary of the collection of the various modifiers of *white* revealed that their molecular basis fell into two major classes: members of signal transduction pathways and transcription/chromatin factors (Birchler et al., 2001). Thus, it was established that the dosage effects were in fact the result of regulatory processes. However, the diversity of regulatory molecules involved was mysterious at the time, and to some extent still is, but clues for why this is the case are emerging from studies of haploinsufficiency in yeast and humans (see below).

RELATIONSHIP TO QUANTITATIVE TRAITS?

Returning to the phenotypic effects of trisomics, it is noted that any one characteristic of an organism can be affected by different trisomics. This realization suggests that multiple dosage-sensitive genes might be capable of modulating a particular phenotypic characteristic. We cannot summarize the field of quantitative genetics, but we discuss some parallels as previously noted (Guo and Birchler, 1994; Birchler et al., 2001, 2005). For any one quantitative trait, there can often be multiple loci that affect its expression (Tanksley, 1993). Crosses between varieties that differ for such traits usually exhibit an intermediate (additive) phenotype to some degree (Tanksley, 1993). Thus, the multiple loci act as if they are dosage sensitive. An example involves the quantitative genetic differences between domesticated and wild sunflower (Burke et al., 2002). Many (78) loci were identified that were mostly of small effect and additive in mode of action. It seems reasonable, therefore, that the effects of aneuploids and of some quantitative trait loci (QTL) have a basis in common. QTL would be expected to be a heterogeneous group, but it is reasonable that variation in regulatory genes of sundry types would be expected to be a major contributor.

Within the past half decade, it became obvious that many clinical human conditions result not necessarily from a gain-of-function mutation or a homozygous recessive, but from haploinsufficiency of particular gene products. In other words, null mutations as a heterozygote would condition syndromes in a type of dosage effect. As the molecular basis of these conditions was revealed, they consisted primarily of transcription factors. To explain these results, one of us (R.A.V.) formulated mechanistic models of transcription factor assembly into molecular complexes, noting the importance of stoichiometry of the subunits for the action of the whole (Veitia, 2002). This concept has been extended to genetic and biochemical networks (Veitia, 2003, 2004, 2005). The balance between either the subunits of a complex or between proteins with opposing actions, such as transcriptional activators and repressors, needs to be maintained to some extent to avoid negative fitness consequences.

Experimental evidence of this type of relationship has not been systematically sought in plants. However, data have been obtained from the study of haploinsufficiency in yeast (Papp et al., 2003) and humans (Kondrashov and Koonin, 2004). Heterozygous gene knockouts were examined in diploid yeast for growth retardation. Those that produced a haploinsufficiency were overrepresented among classes of genes whose protein products are typically involved with molecular complexes. While these classes of genes extend beyond those involved with regulatory processes to some degree, regulatory gene products typically fall under this umbrella. The human study found that members of signal transduction and transcriptional functions were overrepresented among factors causing haploinsufficiency.

The concept of dosage balance predicts a relationship between the number of interactions (connectivity) of a component and to the possibility of dosage effects when under- or overexpressed. Lemos et al. (2004) have shown that the number of interactions a protein has within a network (i.e., connectivity) constrains genetic variation of gene expression in yeast and fruitfly populations. Specifically, they found a negative correlation between the variation of gene expression and the number of protein–protein interactions. As expected, the extent of variation in expression among genes encoding interactors was smaller than that of random pairs of genes, suggesting the existence of a balance relationship. Finally, the expression levels of interactors correlated positively across strains. High coexpression for proteins in the same complex has been reported independently several times (Jansen et al., 2002; Papp et al., 2003). These results suggest the existence of a dosage balance, which could be a force shaping gene expression even at a small evolutionary scale, that is, within populations.

Protein–protein connectivity in yeast complexes (and likely in other organisms) follows approximately a power law distribution (Hahn et al., 2004), which means that most components are poorly connected. Indeed, ~30% of genes involved in yeast complexes encode separable components (i.e., only one link with the rest of the complex, such as A or C in complex A-B-C). By comparison, only ~10% of components have 20 or more links (analysis of data from Fraser et al., 2003). Mutations in the poorly connected components are more likely to lead to less pleiotropic phenotypes, with higher chances of going undetected. Moreover, these separable components are expected to be less dosage-sensitive (unless they are represented several times per macromolecule as A in A-B-A) (Veitia, 2002, 2003). This concept might explain why overexpression of subunits of yeast complexes is usually well tolerated.

This is apparent from the study of Sopko et al. (2006), who analyzed overexpression phenotypes in a vast array of yeast strains, each containing an inducible copy of a different gene. They suggest that overexpression phenotypes in yeast reflect specific regulatory imbalances. Accordingly, they found that

overexpression of periodically expressed genes (i.e., during the cell cycle) is more likely to cause cell cycle arrest or abnormal morphology than constitutively expressed cell cycle genes. This is probably so because in many cases these factors participate in network modules involving opposing forces (i.e., a kinase and a phosphatase acting on a common though differentially modified substrate). Dosage effects in these networks are predicted by dosage balance. For instance, using a complex model of the cell cycle, Chen et al. (2004) found that >70% of the parameters can be changed at least 10-fold in either direction (i.e., under- or overexpression) without preventing cycling. However, the rest of the parameters do not exhibit this flexibility, and some are very sensitive to dosage changes. For example, for the synthesis of Cdc14, the boundaries are twofold up, otherwise there will be G1 arrest, and 0.5-fold down (i.e., heterozygous deletion), and the cell faces a telophase arrest. The contrary occurs for the degradation of Cdc14. The need for balance between synthesis (copy number or expression) of Cdc14 and its degradation (i.e., copy number or expression of a protease) must be reached to avoid cell cycle arrest. Note that altering these parameters within the relevant boundaries does not produce cell cycle arrest but does induce either faster or slower cycling (a quantitative character).

BALANCE IN SEX CHROMOSOME DOSAGE COMPENSATION?

As noted above, the same dosage effects on target gene expression that result from genomic imbalance are responsible for dosage compensation of various genes in maize (Birchler, 1979; Birchler and Newton, 1981; Guo and Birchler, 1994). The magnitude of expression modulation to account for dosage compensation of the various sex chromosome aneuploids in *Drosophila* is the inverse ratio of the X to autosome imbalance (Birchler et al., 2006). Recent studies of global gene expression between males and females of *Drosophila*, nematodes, and mammals indicates that in each species the single X chromosome in males is upregulated approximately twofold (Gupta et al., 2006), which would produce a total gene expression equivalent to the two X chromosomes in females (or hermaphrodites in nematodes). In an exhaustive comparison of average gene expression of X chromosomes and autosomes in various mammalian species and tissues (Nguyen and Disteche, 2006), a potential balance relationship was revealed. In female mammals, one of the two X chromosomes is inactivated in any one developmental lineage. Nevertheless, the active X in females is upregulated approximately twofold so that on average the total expression from the single X is more or less equivalent to the average expression of the equivalent length of a pair of autosomes. The single X in males is likewise upregulated. However, in haploid tissues, the average expression of the single X chromosome per unit length is basically the same as a single autosome. Thus, it appears that the X upregulation only occurs when an X-to-autosomal imbalance is present. Sex chromosome dosage compensation has been subjected to natural selection and thus is likely to involve some modifications in mechanism to those cases observed in laboratory-constructed aneuploids (Birchler et al., 2006), but some parallels do seem to exist.

BALANCE IN EVOLUTIONARY PROCESSES?

With the availability of whole-genome sequences, it has become obvious that repeated cycles of polyploidization followed by diploidization have occurred in the lineages leading to the evolutionary crown of eukaryotic organisms present today (Wolfe, 2001; Simillion et al., 2002; Bowers et al., 2003). Following the production of an allotetraploid from two related species, there is gene loss that leads back to the diploid level. An analysis of the functional classes of the genes retained in duplicate in *Arabidopsis* and rice indicates an overrepresentation of members of signal transduction components and transcription factors (Blanc and Wolfe, 2004; Maere et al., 2005; Chapman et al., 2006; Freeling and Thomas, 2006; Thomas et al., 2006). These classes of genes are similar to those that exhibit transacting dosage effects, as noted above. These findings led to the hypothesis that the duplicates are retained because they are in balance with each other and selection against deletion of one member of a pair would prevent their rapid loss (Birchler et al., 2005; Freeling and Thomas, 2006). In other words, deletion of one member of a balanced duplicate would mimic an aneuploid effect, which would diminish reproductive success. Genes not in an interacting balance relationship would be deleted at random over evolutionary time back to the diploid level.

Indeed, gene classes preferentially found in segmental duplications are the complement of those retained from whole-genome duplications (Davis and Petrov, 2005; Maere et al., 2005), suggesting that segmental duplications that upset a regulatory balance would be selected against because they also would mimic an aneuploid effect. Of course, genes retained in segmental duplications could be selected to condition greater increments of a particular gene product (Sharp et al., 2005; Redon et al., 2006). Not surprisingly, members of large gene families seldom encode components of macromolecular complexes in yeast and humans. Moreover, according to recent studies, duplicability of different genes decreases as the size of the complexes increases (Papp et al., 2003; Yang et al., 2003).

The studies on duplicate retention in *Arabidopsis* and rice by necessity must deal with genomes that are substantially returned to the diploid state. Thus, the possibility exists that directed elimination of members of the singleton classes occurred shortly after polyploidization to produce the observed genomic arrangement. However, a recent analysis of the genome of *Paramecium tetraurelia* illuminates the processes of whole-genome duplication and subsequent gene loss (Aury et al., 2006). The genomic content of this meager ciliate, with a predicted number of 39,642 genes, dwarfs that of humans. The reason for this number is that the whole genome has endured three duplications. By comparing the recent, intermediate, and

HISTORICAL PERSPECTIVE ESSAY

old events, insight into the processes of gene loss could be gained. Because many pseudogenes could be recognized in various stages of deterioration, it seems likely that the gene losses are the result of attrition over evolutionary time rather than a concerted elimination event immediately following polyploidization. Moreover, the retained duplicates tend to be classes of genes that are involved in macromolecular complexes. In addition, the retained genes show evidence of purifying selection, suggesting that mutation of one member of a duplicated pair is selected against because it upsets the correct stoichiometric relationships of subunits of such complexes. The authors thus proposed that the stoichiometry of duplicates is important for their retention.

Freeling and Thomas (2006) have suggested that the retention of duplicate regulatory genes following repeated polyploidization events holds such regulators in the evolutionary lineage for sufficient time to allow eventual divergence that fosters increasing complexity over evolutionary time. The polyploidization event proliferates the copy number of all loci. Deletions over time deteriorate the copy number of most target metabolic genes to the diploid level. However, the stoichiometric constraints on regulatory duplicates will preserve them in the lineage. Eventual divergence of function of different members of a duplicate pair of regulators will create developmental and biochemical complexity. While there is evidence that such divergence occurs, concepts of how a new balance is achieved have been little explored. However, given such changes, Freeling and Thomas argue that the repeated cycles of tetraploidization and diploidization are a contributor to driving increasing complexity during the evolution of eukaryotes.

WHY A BALANCE IN REGULATORY PROCESSES?

A regulatory system in which the components are sensitive to stoichiometric relationships provides a means for selection pressures to operate on new mutations in diploids and tetraploids while such mutations are still heterozygous. Also, a system of multiple genes affecting any one characteristic and subject to a dosage interaction will provide a means to modulate the phenotype in subtle ways via mutations in multiple genes. Because the regulatory system is dosage dependent, any new regulatory mutations have the potential to produce subtle effects as a heterozygote. If they are detrimental, they will be selected against rapidly. However, new semidominant mutations would be available in the heterozygous state for rapid adaptive changes as well. The detection of purifying selection on retained duplicates in Paramecium (Aury et al., 2006) suggests that this type of situation is operating. The balance relationship provides for evolutionary changes to be of small magnitude and to result from many possible modifiers of a single phenotype, as noted for the sunflower example above. Thus, a balance relationship of regulatory complexes would optimize purifying and adaptive selection for organisms with diploid and higher levels of ploidy.

FUTURE PROBLEMS TO ADDRESS

We have summarized above the historical and recent evidence suggesting a balance relationship of regulatory genes as a consequence of their membership in macromolecular complexes or networks for which the contributing members produce a stoichiometric effect on the function of the whole. There are several exciting research directions that such a hypothesis might inspire. One possibility is to examine the kinetic and interaction properties of macromolecular complexes in an attempt to understand the basis of how varying the dosage of individual components affects the function of the complex. This area will involve examining the association parameters of multiprotein regulatory complexes, the order in which they associate, and the topological connections between the different subunits. Such studies would test the proposition that highly connected proteins in stable complexes tend to display a higher dosage sensitivity and that this sensitivity can be modulated by the specific parameters of the association process.

Also, one would want eventually to understand the impact of overall gene expression on the phenotype. Such endeavors will no doubt be quite challenging. For example, using gene chip technology, it might be possible to assess correlations between patterns of modulation of thousands of genes and their corresponding phenotypic consequences. The known parallels between multigenic aneuploid effects and quantitative traits are noted above, but the nature of this relationship is quite obscure at present. Exhaustive studies of global patterns of gene expression will need to be conducted, both on aneuploid conditions and on quantitative trait variation, to test such potential connections. Indeed, modulations of morphologies by aneuploid syndromes and QTL might involve changes in cell division patterns as much as overall changes in gene expression, complicating the detection of the responsible genes. The major challenge for molecular quantitative genetics in the future is to tease out the relationship of total genomic expression patterns to phenotypic effects.

Such relationships will be important to gain a better understanding of the role of regulatory balance in evolution. One can imagine how this balance can provide many gradual changes in phenotype in succession, but what is needed for insight into this problem is to determine to what extent multiple regulatory mutations are additive, interactive, or epistatic to each other. The inclusion of regulatory genes among the retained duplicates on the return road from polyploidy to diploidy in diverse taxa has provided an exciting development for the study of evolutionary processes. In mammals, ultraconserved noncoding DNA elements are depleted among segmentally duplicated regions of the genome (Derti et al., 2006). It will be of interest to examine whether they are retained following polyploidization events and whether they are related to the balance characteristics of regulatory mechanisms.

If the stoichiometry of regulatory factors contributes to increasing complexity and speciation, the individual components

must be able to escape from a preexisting balance with other factors and establish a new balance. If such divergence is a contributing factor to evolutionary change, then the processes that allow shifts from one balance relationship to another must be explored. Many classical studies of species hybrid incompatibilities have suggested the presence of coevolving gene complexes (Dobzhansky, 1937; Muller, 1942; see discussion in Birchler et al., 2005). Whether interacting regulatory molecular complexes have a relationship to these mechanisms would be an interesting avenue of exploration.

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James A. Birchler
Division of Biological Sciences
University of Missouri
Columbia, MO 65211
birchlerj@missouri.edu

Reiner A. Veitia
Institut Cochin
Institut National de la Santé et de la Recherche Médicale
Centre National de la Recherche Scientifique
Université Paris 5 and 7
Paris 75005, France
veitia@cochin.inserm.fr

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