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Cosuppression Comes to the Animals

Minireview

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A remarkable series of investigations in plants and fungi during the last eight years has revealed a set of phenomena, under diverse names, that will be referred to here collectively as cosuppression. In all cases of cosuppression, the presence of supernumary (two or more) copies of a gene in the nuclear genome results in specific repression of expression of some or all copies of that gene. (This use of the term cosuppression is broader than the original term. This broader use may be appropriate as discussed below.) While silencing of tandemly repeated gene copies has been demonstrated in animals (Dorer and Henikoff, 1994), silencing of dispersed copies—as frequently seen in plant and fungal cosuppression—had not been clearly shown until the studies of Pal-Bhadra et al. (1997) published in this issue of *Cell*. I focus here on mechanistic and theoretical questions posed by cosuppression from the expanded perspective provided by recent work in animals.

Cosuppression in an Animal

Pal-Bhadra et al. (1997) show that increasing numbers of copies of a *white-Adh* fusion construction introduced at dispersed locations in the Drosophila genome result in substantial repression of expression of both the fusion and the endogenous *Adh* gene. The authors go on to show that this repression requires the well-known *Polycomb* group (*Pc*-G) genes. The products of these genes are chromatin-associated proteins implicated in the maintenance of somatically heritable, transcriptionally repressed states of developmentally regulated genes (Orlando and Paro, 1993). The authors show that partial mutational disability of the *Pc*-G system reduces cosuppression. They further show that a *Pc*-G protein-containing chromatin complex is selectively formed on a copy of the *white-Adh* fusion when it is subject to cosuppression but is not when it is not.

These observations represent compelling evidence that cosuppression occurs in an animal. Moreover, they strongly suggest that the presence of supernumary gene copies is detected early in development of the animal and results in the incorporation of copies of the gene into a repressed chromatin state that is subsequently inherited somatically through the remainder of development.

Cosuppression in Plants and Fungi

The numerous, diverse studies of cosuppression in plants provide a rich source of comparative information. This work has been extensively reviewed recently (see Depicker and Van Montagu, 1996, and Metzlaff et al., 1997, and references therein) and two general observations are relevant here. First, the plant work indicates that cosuppression is a generic phenomenon—it can be triggered by a number of different transgenes (including exogenous genes) and by unusual duplications of endogenous genes. Second, repression associated with cosuppression appears to occur by different mechanisms in different cases (below).

Several well-documented cases of cosuppression and related phenomena are known in fungi (Selker, 1990; Rossignol and Faugeron, 1994; Cogoni et al., 1996) and mechanisms of repression in these cases are likewise apparently heterogeneous (below).

In addition to mechanistic heterogeneity, these phenomena show variability in developmental timing. Cosuppression in plants occurs somatically, though the repressed state can apparently sometimes be transmitted meiotically. In fungi the cosuppression-like phenomenon referred to as "quelling" (Cogoni, et al., 1996) occurs in vegetative tissue while other silencing phenomena, MIP (Rossignol and Faugeron, 1994) and RIP (Selker, 1990), are initiated during sexual reproduction with resulting inactivated states being retained in vegetative tissues of offspring.

Actualization of Cosuppression

Cosuppression poses two potentially separate mechanistic problems. The first problem is initial detection of supernumary gene copies and the second is their subsequent repression.

There is now very strong evidence that cosuppression-associated repression is mechanistically heterogenous. On the one hand, repression of transcription initiation is implicated in several cases. Transcriptional repression might result from somatically heritable repressed chromatin states as indicated for Drosophila (Pal-Bhadra et al., 1997). In several cases in plants (Matzke and Matzke, 1995; Depicker and Van Montagu, 1996; references therein) and fungi (Rossignol and Faugeron, 1994), DNA methylation is seen in conjunction with transcriptional repression. It is currently unknown whether repressed chromatin states might cooccur with this methylation.

On the other hand, posttranscriptional silencing is implicated in a number of other cases in plants (English et al., 1996; Goodwin et al., 1996; Metzlaff et al., 1997; references therein) and fungi (Cogoni et al., 1996). In these cases, transcription initiation appears to occur normally but the RNA products of cosuppressed genes are subsequently eliminated. Perhaps the most decisive evidence for this class of mechanisms comes from transgene-induced virus resistance (English et al., 1996; Goodwin et al., 1996; references therein). In these cases the presence of multicopy, but not single copy, genomic transgenes confers strong resistance to infection by cytoplasmically replicating RNA viruses. This resistance requires that the nuclear transgenes be experiencing cosuppression in at least some cases and is specific to viruses with homology to the cosuppressed transgene.

Further supporting the hypothesis of diverse mechanisms for actualization of cosuppression are the observations that the chromatin proteins producing somatically heritable repressed states in Drosophila are apparently distinct for tandemly (Dorer and Henikoff, 1994) and dispersed (Pal-Bhadra et al., 1997) repeated sequences.

Initiation of Cosuppression

Several sets of observations indicate that cosuppression might be initiated in at least two distinct ways.

First, there is abundant evidence that generic systems exist in complex organisms allowing homologous genomic DNA sequences to find one another. This includes the phylogenetically diverse occurrence of polytene chromosomes and apparent premeiotic chromosome pairing (Weiner and Kleckner, 1994). It is thus likely that dispersed (or tandem) multicopy genes can associate—in general, probably transiently and only at some stages of the cell cycle. Cosuppression might then require detection of such transient multicopy association and initiation of repression in response to it. The widespread occurrence of locus synapsis-dependent *trans* regulatory effects in Drosophila (reviewed in Wu, 1993), for example, indicates that such a response is within the technical capabilities of eukaryotic cells.

Second, there is strong evidence from plant systems that RNA products, rather than simply the presence of multiple genomic copies of DNA sequences, can trigger cosuppression in some cases. The existence of posttranscriptional actualization of cosuppression and the apparent requirement in some cases for active transcription to initiate cosuppression (reviewed in Depicker and Van Montagu, 1996) are consistent with this possibility but do not demonstrate that it is correct (see following section).

However, two elegant recent studies are persuasive. Specifically, Wassenegger et al. (1994) show that RNA:RNA viroid replication in plants leads to methylation, and presumptive transcriptional silencing, of engineered chromosomal DNA copies of the viroid genome. If this methylation is related to that seen in cosuppression these studies clearly indicate that RNA-mediated events can trigger cosuppression. Further, Goodwin et al. (1996) demonstrate that infection with a cytoplasmically replicating virus can induce nuclear transgene-dependent virus resistance (delayed recovery) in plants where the transgene dose is not high enough to produce a priori resistance.

The Question of a Single Universal System for Cosuppression

Superficially, this mechanistic complexity of both initiation and actualization suggests that cosuppression might be a polyglot phenomenon—a group of independent processes artificially grouped by outcome. However, it is important to notice that the evidence for this suggestion is not decisive. It is possible that current cosuppression systems share common ancestry and are more similar than they so far appear.

I draw on several previous mechanistic suggestions (Dorer and Henikoff, 1994; Matzke and Matzke, 1995; English et al., 1996; Depicker and Van Montagu, 1996; Metzlaff et al., 1997; Pal-Bhadra, et al., 1997, and references therein) to provide an example of this as follows. Detection of supernumary gene copies at the DNA level could be universal and frequently the event initiating cosuppression. Given the likely function of cosuppression (below), the search leading to this detection might be expected to be restricted to transcribing sequences—on the basis of their altered chromatin structure, for example—in at least some cases. This detection would commonly result in formation of altered chromatin and/or preinitiation complexes, as well as DNA methylation in some systems, on detected multicopy genes. These altered structures could then result in modified transcriptional behavior. Depending on context, this alteration could consist of failure of initiation or alteration of a subsequent event—for example, premature transcription termination. In cases of premature termination, the resulting aberrant RNA products could be identified and used to generate a posttranscriptional repressor—for example, an antisense RNA. In addition, one or more of these specialized RNA products could also be used to search for homologous genomic DNAs with detection provoking modification of chromatin structure and/or DNA methylation. This last step would thus provide feedback extending and reinforcing cosuppression. In the presence of such self-reenforcing feedback, cosuppression could be initiated by multicopy DNA segments, specialized RNA products homologous to nuclear DNA segments, or some combination of the two depending on the case and the system in question.

While some of the steps in this process are hypothetical, it has been pointed out previously that none is farfetched in light of available information (see Depicker and Van Montagu, 1996, and references therein). Most or all of the widely noted idiosyncracies of different cosuppression phenomena could be accounted for by a model of this general form.

In light of the substantive reasons to suspect a single, ancient origin for cosuppression (below), a universal mechanism is an attractive possibility worthy of continued experimental attention. On such a unified view, the quantitative importance of distinct steps in the process could have undergone some evolutionary change in different lineages leading, in turn, to the observed differences in behavior of cosuppression in these lineages. Thus, on this unified view, future study is expected to reveal otherwise unexpected common features shared by disparate cosuppression systems.

Evolutionary Implications

As discussed above, generic detection and repression of multicopy genes presents formidable logistical problems. The existence of the highly elaborate cosuppression system(s) indicated by available evidence thus suggests strong selection for this capability. It has been argued by several investigators that cosuppression might be a mechanism for control of transposon parasites (Matzke and Matzke, 1995; Pal-Bhadra et al., 1997, and references therein). Such parasites are a ubiquitous adaptive challenge to complex organisms. Transposons are commonly present at multiple dispersed genomic locations and cosuppression would thus likely be an effective control strategy.

I argue here for a slightly expanded version of this view as follows. Genic selection models predict that conflicts of ''interest'' will arise between the genes making up complex genomes. Given the bias inherited from Darwin toward viewing the entire genome (the individual organism) as the smallest unit of selection, such predictions were shocking when originally made (Hamilton, 1964; Williams, 1966; Dawkins, 1976). However, subsequent experimental analysis of diverse examples of genes that are ''outlaw'' with respect to the genome as

a whole—including those involved in mammalian imprinting, sex chromosome evolution, and segregation distortion—have clearly shown such genic conflicts of interest to be a significant process shaping complex genomes (see Hurst et al., 1996, for a recent review).

Adaptation to single copy outlaws—for example, segregation distorters—apparently involves selection for case-specific second-site modifiers or suppressors as the interests of the rest of the genome contra such outlaws are expressed (reviewed in Hurst et al., 1996). Until this adaptive response is complete, the fitness of the remainder of the genome and of individual organisms can be significantly reduced.

The case of outlaws that are capable of dispersing multiple copies of themselves throughout the genome can be even more extreme. The establishment and spread of such multicopy outlaws are generally more reliable and rapid than for single copy outlaws. As a result, multicopy outlaws can impose a higher fitness burden on the remainder of the genome and still successfully invade a sexual gene pool. However, multicopy outlaws are vulnerable to a generic control strategy that represses expression of transcribing genes present in multiple copies. Cosuppression appears to have precisely the properties required of such a generic policing strategy.

The occurrence of cosuppression-like phenomena in germ cells (Selker, 1990; Rossignol and Faugeron, 1994) is, of course, consistent with this interpretation. However, it is important to note that the observed cosuppression in somatic tissues is also expected. Modification of the physiology or behavior of parents through somatic outlaw expression to allow preferential production or survival of outlaw-inheriting offspring is an expected parasitic strategy requiring control.

As complex genomes initially evolved, each adaptive advance at the organismic level would have provided new opportunities for outlaws—presumably limiting attainable levels of organismic adaptation. It is thus likely that development of cosuppression or equivalent law enforcement was essential before cooperative genomes of modern complexity could arise and sustain extensive selection at the genomic (organismic) level (Buss, 1987). Given these considerations, it is very attractive to suppose that cosuppression arose early in the common lineage ancestral to modern multicellular forms.

It is particularly noteworthy in this context that currently known multicopy outlaws capable of encoding protein are usually highly adapted, ancient specialists (transposons). The ubiquity of such specialists suggests that only the rare survivors of a lengthy, lethal host/parasite arms race can long occupy this parasitic niche in contemporary complex genomes. Cosuppression has the earmarks of the host side of this arms race.

Lastly, although cosuppression may have arisen as an adaptation to multicopy outlaws, its subsequent exploitation as a source of regulatory variation for selection at the organismic level during the long history of multicellular lineages is an attractive possibility. For example, it is tantalizing in this context that Pal-Bhadra et al. (1997) implicate a system—the *Pc*-G genes—in cosuppression that also functions in ''normal'' developmental control of gene expression in contemporary animals.

Concluding Remarks

Discovery that a process might be ancient and phylogenetically widely distributed usually leads to dramatic increases in insight as diverse, idiosyncratic sources of experimental power are simultaneously brought to bear. Addition of the Drosophila genetic system to the power of plant and fungal genetics can be expected to produce substantive new understanding of cosuppression in the next several years. Moreover, it will be of considerable interest and potential technical value to establish whether cosuppression occurs in other animal lineages, including vertebrates, as the newly expanded body of evidence clearly predicts. In this context it will be of great interest to see further analysis of the recently reported transgene silencing of a normally imprinted mammalian gene to determine if this represents cosuppression or a more specialized mechanism associated with imprinting (Hatada et al., 1997).

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