IMMUNOLOGY

Expansion of the Allelic Exclusion Principle?

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Cells normally have two copies of autosomal genes, one inherited from the mother and one from the father. For most genes, both copies (or alleles) are used by the cell, but for certain genes, cells randomly select only one allele to encode RNA and protein for that gene. For various cells of the immune system and for olfactory neurons, this so-called monoallelic expression is one of the mechanisms ensuring that a single kind of receptor is displayed on the surface of each cell. For example, each B cell must produce a single antibody from among the extremely large number of possible antibodies that could be made. A well characterized, complex DNA rearrangement mechanism leads to the expression of this single, specific antibody; a key aspect of this mechanism is that it results in the monoallelic expression of each expressed immunoglobulin gene. This phenomenon is called allelic exclusion, a particular type of monoallelic expression where not only is the other allele not expressed, but other genes from the same family are turned off as well (*1*). Similarly, T cells and certain cells expressing natural killer (NK) cell receptor genes (*2*) use allelic exclusion to maintain each cell's specificity. Individual neurons of the olfactory system express only one of a family of olfactory receptors using mechanisms that also result in allelic exclusion (*3*). Now, a report on page 2118 of this issue by Holländer *et al.* (*4*) describes monoallelic expression of the mouse gene encoding interleukin-2 (IL-2), an important immunomodulatory cytokine expressed and secreted by most activated $CD4^+$ T cells (*5*). In contrast to the diverse protein which are monoallelically expressed, IL-2 is not a receptor and its expression does not confer a cell-specific phenotype. How does monoallelic transcription fit into the picture of the regulation of the IL-2 gene and the biological function of IL-2?

The IL-2 gene is coordinately regulated by T cell receptor signaling and other signals from accessory receptors. Because its transcriptional regulation depends on multiple signals, the IL-2 gene has been extensively studied as a model for how distinct signal transduction pathways can be integrated into a specific transcriptional response (*5*). The extremely tight regulation of the IL-2 gene has been explained by the cooperative binding of transcription factors regulated by a variety of signaling pathways. All of the necessary factors must be present for any of them to bind, leading to an all-or-nothing effect on transcription (*6*). There is also evidence that changes in the chromatin structure of the IL-2 gene are important for its regulation (*7*).

Holländer *et al.* present a number of observations that are consistent with monoallelic expression of IL-2. Only half of stimulated $CD4^+$ cells from heterozygous IL-$2^{+/-}$ mice express IL-2, whereas nearly all $CD4^+$ cells from wild-type mice do so. In addition, Holländer *et al.* used interactive laser cytometry to compare the relative levels of IL-2 production in individual, activated $CD4^+$ cells from IL-$2^{-/-}$, IL-$2^{+/-}$, and IL-$2^{+/+}$ mice. The IL-$2^{+/-}$ cells fall into a bimodal distribution; half the cells have a fluorescence profile similar to the IL-$2^{+/+}$ cells and the other have a profile similar to the IL-$2^{-/-}$ cells. These observations are consistent with monoallelic expression of the IL-2 gene, but they could also be explained in the context of bi-allelic expression. A well-established positive feedback loop regulates IL-2 expression (*5*) and can likely stabilize expression if a critical threshold level of IL-2 is reached. It is therefore possible that even if monoallelic expression was not occurring, the absence of a contribution from one allele could lead to failure of a significant fraction of cells to reach the threshold necessary to establish IL-2 expression.

MONOALLELICALLY EXPRESSED GENES

Gene	Chromosome	Parental strand
Immunoglobulin genes	Autosomal	Random
T cell receptor genes	Autosomal	Random
NK cell receptor genes	Autosomal	Random
Olfactory receptor genes	Autosomal	Random
Interleukin-2 gene	Autosomal	Random
X-inactivated genes	X-linked	Random
Imprinted genes	Autosomal	Nonrandom

The most compelling evidence for monoallelic expression of the IL-2 gene comes from an allele-discriminating reverse transcriptase–polymerase chain reaction (RT PCR) analysis of the IL-2 mRNA in single, activated $CD4^+$ T cells. In these experiments, the two alleles are distinguished by a polymorphism. Holländer *et al.* observe that individual cells contain either maternal transcripts or paternal transcripts, and do not observe any cells expressing both alleles. It will be interesting to see whether future experiments demonstrate that monoallelic expression is an absolute phenomenon in $CD4^+$ T cells. Alternatively, certain T cells may express both alleles. This would be similar to the case of the LY49 NK cell receptor gene, which was first observed to be strictly monoallelic but subsequently proved to be expressed from both alleles in some cells (*8*).

Also consistent with monoallelic expression of the IL-2 gene is the observation that the gene replicates asynchronously. Asynchronous replication is associated with various monoallelically expressed genes including the olfactory receptor genes, imprinted genes, and X-inactivated genes (*3*, *9*). In imprinted genes and the inactive X chromosome, a correlation can be made between which allele is replicated earlier and which allele is transcribed. In the olfactory receptor genes, which allele is transcribed and which allele is replicated first are both random with respect to parental legacy. However, it has not been possible to determine whether there is a correlation between early (or late) replication and transcription because the olfactory receptor genes are only transcribed in postmitotic cells. Whether the replication asynchrony of the IL-2 gene is random with respect to parental legacy and correlated with transcription is not yet known.

The timing of replication for the lymphocyte antigen receptor genes has not been extensively analyzed. The mouse immunoglobulin heavy chain (IgH) constant region gene is synchronously replicating (*9*). This could reflect the predominance of the well-characterized negative feedback mechanism in regulating allelic exclusion in immunoglobulin genes. Alternatively, other areas of the IgH locus may reveal asynchronous replication. For genes where asynchronous replication is observed, the asynchrony is observed in embryonic cells and in adult cells where the genes are not expressed. This indicates that the asynchrony of replication reflects a distinct marking of the two alleles

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in all cell types, irrespective of tissue-specific gene expression. If the asynchrony of replication of the IL-2 gene is also present in cells other than T cells, this would indicate that before T cell development, one IL-2 allele is rendered unavailable for future activation.

All other known examples of random monoallelic expression of autosomal genes (immunoglobulins, T cell receptors, olfactory receptors, and LY49 NK cell receptors) involve genes encoding diverse receptors in systems in which receptor expression is restricted so that cells have distinct specificities. In all of these cases, monoallelic expression is a fundamental aspect of the transcriptional restriction of receptor expression. Why would the cytokine IL-2, which is expressed in most activated $CD4^+$ T cells be expressed monoallelically? Interestingly, both IL-2 and the IL-2 receptor are expressed during thymocyte development around the time of establishment of allelic exclusion in T cell receptor genes. For mature T cells, the well-characterized integration of signal transduction pathways by the transcription factors could account for the observed regulation without having to invoke monoallelic expression (6). Thus, monoallelic expression may reflect a new aspect of the regulation of IL-2 gene expression, perhaps one involving an interplay between nuclear architecture and chromatin structure (*10*).

References

1. B. Pernis, G. Chiappino, A. S. Kelus, P. G. Gell, *J. Exp. Med.* **122**, 853 (1965).
2. W. Held, J. Roland, D. H. Raulet, *Nature* **376**, 355 (1995).
3. A. Chess, I. Simon, H. Cedar, R. Axel, *Cell* **78**, 823 (1994).
4. G. A. Holländer *et al.*, *Science* **279**, 2118 (1998).
5. R. H. Schwartz, *Curr. Opin. Immunol.* **9**, 351 (1997).
6. P. A. Garrity, D. Chen, E. V. Rothenberg, B. J. Wold, *Mol. Cell. Biol.* **14**, 2159 (1994).
7. U. Siebenlist *et al.*, *ibid.* **6**, 3042 (1986).
8. W. Held and D. H. Raulet, *Eur. J. Immunol.* **27**, 2876 (1997).
9. D. Kitsberg *et al.*, *Nature* **364**, 459 (1993).
10. K. E. Brown *et al.*, *Cell* **91**, 845 (1997).

ECOLOGY

Planning for Biodiversity

Stuart L. Pimm and John H. Lawton

At present, species are going extinct at a rate 100 times the natural background rates (*1*). The readily observable destruction of habitats such as the Amazon (*2*) and the now-calibrated relationship between habitat loss and species loss (*3*) predict that these rates will only get larger. Only ~5% of the planet's land surface is in reserves that are protected to one degree or another (*4*). If human activities destroy or greatly modify the remaining 95% of the land, only half the planet's species would survive in the protected 5%, the other half would go extinct. (See related commentary on page 2060.)

The most vulnerable species—those with the smallest geographical ranges—are not distributed randomly. Nature has put her eggs in a few baskets—hotspots—where these rare, endemic species are concentrated (*5*). By a cruel twist of fate, current rates of deforestation appear to be highest in the richest hotspots (*6*). If humanity placed reserves judiciously over these special places, could we save a greater fraction of species (*7*)? Two reports from southern Africa, one on page 2106 of this issue, and a third from North America, on page 2126, describe the challenges involved and conclude that the solution is not so simple.

Globally, reserves are allocated poorly. The reserves that are larger than 100,000 km^2 are high mountains, tundra, and the driest deserts, areas not particularly species-rich (*4*). Hotspots such as Madagascar and the Philippines protect less than 2% of their land (*4*). The same is true in the Algulhas Plain, the southern tip of Africa and one of the world's hottest spots for plants. Here, some 1500 km^2 (half the size of Rhode Island) house 1751 species; although most of the state forests and private nature reserves are coastal, most of this region's 99 endemic plants live inland.

Lombard *et al.* (*8*) asked: Where should new reserves be situated to protect the maximum number of species at minimum cost? One aspect of their analysis is purely a matter of biogeography. Computer algorithms select sets of cells (which represent subsections of land) according to their complementary species composition. These can be designed so that a set of cells captures either as many total species or as many rare species as possible (see the diagram) (*9*). Lombard *et al.* developed software similar to that widely available (*9*), but they included both endemic species and different kinds of classified vegetation types. Selecting complementary vegetation types is another way of setting conservation priorities.

Naïvely applied, these algorithms are not practical conservation tools. The selected sites may not be available for reserves. In addition, selection of too small a cell size can lead to the "Noah's Ark" effect. All the desired species can be captured in a collection of widely scattered, tiny cells of a small combined area, but in fact the populations protected in this seemingly efficient strategy are too small to persist. Like Noah, scattered tiny reserves protect everything in a small area, but only for a short time—and he had divine help.

Lombard *et al.* selected a grid size of 3 km by 3 km. Reserves of this size are politically feasible and represent a trade-off between efficiency and population viability. When other constraints are added to the biogeographical ones, these ecology and computer algorithms become a practical tool. Some species are already in reserves and do not need to be preserved again, and every species should be represented more than once as insurance against disasters. Some areas are unsuitable; alien weeds overrun others; and some selected sites are in mostly agricultural or urban areas. Whenever possible, algorithms should add areas adjacent to existing reserves. Combined, these constraints produce a variety of selections, but the results are broadly comparable in their priorities. As such, the methods outlined by Lombard *et al.* provide both local advice and an excellent case history that combines ecological patterns with practical and political considerations.

Value for money motivates Ando *et al.* (*10*). Dobson *et al.* (*11*) documented the distribution of endangered species in the United States, county by county, thus identifying the minimum number of counties needed to achieve a given coverage of endangered species. Were land prices broadly similar everywhere, the approach would be relatively straightforward. Unfortunately, areas with many endemic species include the counties encompassing San Diego, Santa Cruz, and San Francisco in California, Honolulu in Hawai'i, and counties in Florida, all of which contain some of the highest priced land in the United States. Ando and her colleagues modified this approach in two ways. The first seeks to minimize costs by taking into account land prices while including a fixed number of species; the second maximizes the number of species protected for a given cost.

Their results include a striking feature: The average cost per hectare fluctuates widely as more species are protected. The ef-

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