Are Tissues a Patch Quilt of Ectopic Gene Expression?

The report by Gobinda Sarkar and Steve S. Sommer (*1*) indicates that there are low levels of tissue-specific mRNA in tissues which would not be expected to express these products. These authors suggest that many "tissue-specific" genes might be expressed at a basal rate in a variety of cell types. Such a phenomenon could have important implications for understanding the process by which T cells become tolerant to self.

There is good experimental evidence (*2*) indicating that tolerance to self antigens is mediated by specific deletion of T cell clones in the thymus. But how is the totality of self proteins presented to T cells in this organ? Soluble self proteins might diffuse into the thymus and be processed and presented there [presumably by class II MHC (major histocompatability complex) molecules], but the majority of cellular-, tissue-, or organ-specific antigens are hardly expected to do that. A second mechanism of antigen presentation involves intracellular peptide products derived from protein synthesized by the same cell, with the use of, as a rule, class I MHC. On the basis of this, peptide antigens presented by class I MHC molecules on thymus cells would also have to be synthesized intracellularly. We suggest that, in fact, thymus cells might synthesize and present the majority of tissue-specific products expressed within the body. Such tissue-unrestricted antigen presentation could be a functional consequence of the tissue-unrestricted transcription detected by Sarkar and Sommer.

Synthesis of random heterotopic proteins in thymus cells, followed by rapid catabolism to peptides, would be sufficient for antigen presentation. However, if we assume that thymus cells bear an average of 50,000 class I MHC molecules each, it appears unlikely that any one thymus cell will be able to present more than a fraction of the possible genomic products. Rather we propose that random regions of chromatin might become open during the development of individual thymus cells and that, as a consequence of this stochastic chromatin activation, the majority of genomic products will be expressed by the population of thymus cells. This would explain how the transplantation into chicken embryos of the bud of a quail wing together with quail thymus allows the adult birds to be tolerant of the whole wing (*3*). So the definition of immunological self would become: "self is what you have a gene for."

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Response: A low level of tissue-specific gene expression has recently been shown in many cell types (*1, 2*). We prefer to call this phenomenon "ectopic expression" because it is not necessarily "illegitimate (*2*)," nor is it necessarily due to "leaky" expression (This Week in Science, 21 April 1989, p. 271). In general, the amount of ectopic mRNA averages to much less than one molecule per cell, since consecutive and nested polymerase chain reaction (PCR) generally was necessary in our experiments to obtain enough amplified material to visualize by staining with ethidium bromide, while Chelly *et al.* (*2*) relied on Southern blots for detection after one round of PCR. Ectopic gene expression could be due to one or more of at least three different mechanisms.

1) *Stochastic or "leaky" gene expression*. In this model, an occasional cell produces one or a few transcripts. The resultant mRNAs and their protein products are degraded with time, returning the cell to its initial condition. In the meanwhile, a few transcripts appear in another cell. In this way, a low level of gene expression occurs in occasional cells. The expression is transient and not localized over time.

2) *Nonheritable gene activation*. Due to an alteration in the structure of chromatin or to other events that do not change the genomic sequence, the promoter is activated over a period of time in a given cell. Such an event may produce high levels of protein in that cell.

3) *Heritable gene activation*. A mutation can activate expression. If division occurs, the daughter cells will both have the mutant activated gene.

Quantitative PCR, in situ hybridization, and flow cytometry will be helpful in distinguishing among these possibilities. A variation of the Luria-Delbruck fluctuation experiment can distinguish between hereditary and nonhereditary mechanisms (*3*).

While leaky gene expresson will result in transient, diffuse expression of very low levels of the gene product, heritable or nonheritable gene activation can produce localized areas containing significant amounts of gene expression. Such localized areas are more likely to accumulate enough product to allow antigen presentation of tissue-specific genes in the thymus. This potentially could mediate self-tolerance, as suggested by Linsk *et al.*

In addition, ectopic expression could predispose cells to neoplasia or to metastasis, if for example, they were in the neighborhood of an area that inappropriately expresses high levels of a growth factor. Likewise, cells shed from a primary tumor might form metastases only if lodged in a microenvironment that produces the necessary level of growth factor.

Another likely consequence of ectopic expression is a certain rate of endogenous tissue injury due to the expression of genes that are either lethal to the cell or injurious to neighboring cells. If ectopic expression increases with age, the rate of endogenous tissue injury would be expected to increase.

In addition to the ectopic expression of tissue-specific genes, the same mechanisms could produce foci that express inappropriate levels of housekeeping genes. Since there are more than 50,000 genes, each tissue may well be a patch quilt of many microenvironments with inappropriate gene expression. While we can conceive of potential approaches to test these speculations, the euphoria of speculation has yielded to the pain of designing critical and unambiguous experiments.

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