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STUDIES OF GENE EXPRESSION IN HUMAN LYMPHOCYTES USING HIGH-RESOLUTION TWO-DIMENSIONAL ELECTROPHORESIS

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Introduction

The introduction of high-resolution two-dimensional electrophoresis (1-4) and its subsequent development to allow large-scale systematic studies (5,6) have made it possible to consider cataloging a large proportion of the protein products of the estimated 30-50,000 human structural genes (9). Such a catalog, or Human Protein Index (10), is a necessary prerequisite to the detailed understanding of cellular function and malfunction. It will perform the same basic service for biology that the periodic table performs for chemistry or that a complete list of parts serves in the maintenance of a 747.

Initially, two major problems arose concerning the feasibility of constructing a Human Protein Index: (i) the two-dimensional patterns might contain too much uninterpretable data and (ii) there were no methodologies available for adequately characterizing the thousands of proteins resolved and for gaining useful insight into their biologically relevant properties. Lately, it has become clear that computerized systems of several types can quantitate and organize the data obtained from 2-D gels, greatly facilitating the interpretation of results (11-16). The way is now open for the construction of large and useful data bases containing a wide variety of information about a large number of molecules. It is also apparent that a new spectrum of techniques can be devised

which yield specific information about many proteins simultaneously (i.e., without the need for the classical approach of biochemical isolation). The variety of such techniques currently in use is illustrated in Table I. Taken together, these developments suggest that detailed information on a large number of cellular proteins can be obtained and usefully managed. This type of data base, in association with the growing library of DNA sequence information, forms the core of a major expansion in our knowledge of human biology.

Categorization of 2-D Gel Spots into Functional Sets

A major objective in the systematic analysis of cells is, of course, to know what each of the thousands of cellular proteins is doing and how its synthesis is controlled. As it is currently used, the concept of a protein's function is not very rigorous. Some proteins such as actin or calmodulin are known to have a variety of functions, and there seems to be no justification for assuming that evolution would not package several useful properties in each gene product. Even a single function is known for no more than 2 to 3% of the cellular proteins. Likewise, the current notions of gene regulation are rather vague, except in the cases of exceptional molecules like the globins, immunoglobulins, or ovalbumin (whose control may not be at all representative). It can therefore be concluded that very little is known or understood concerning the properties or control of the vast majority of cellular proteins. In this situation, the principal advantage of two-dimensional mapping as a means of exploring gene regulation and gene-product function is the ability of the technique to look in detail at a large part of the whole system. The major effects of manipulating various control systems can be determined by inspection.

Table I

Technique	Information Obtained
Physico-chemical	
1. Various amino acid radiolabels	Partial amino acid composition (ratios)
2. $^{32}PO_4$ labeling	Identification of phosphoproteins
3. ^{125}I surface labeling	Identification of surface proteins
4. ^{14}C-iodoacetamide labeling	Identification of reactive vs total SH
5. Thermal denaturation cofactors	Thermostability, interaction with cofactors
6. Affinity chromatography	Identification of proteins binding to immobilized high or low MW molecules
7. Immunoprecipitation	Identification of proteins reacting with a given antibody
Cell Manipulation	
8. Treatment with mitochondrial inhibitors	Mature mitochondrial proteins not produced
9. Treatment with tunicamycin	Mature glycoproteins not produced
10. Extraction with digitonin	Soluble proteins released
11. Extraction with NP-40	Cytoskeletal and nuclear proteins remain
12. Pulse-chase labeling	Rate of synthesis and degradation
13. Treatment with α-amantin, actinomycin D	Message lifetimes
14. Cell fusion	Assignment of protein genes to chromosomes
15. Gene amplification	Identification of amplified gene product and co-amplified genes
Post-electrophoretic	
16. Nitrocellulose or DBM transfers	Identification of proteins binding given antibodies, etc.
17. Proteolytic digestion mapping of 2-D spots	Comparative positions of proteolytic cleavage sites (detection of protein homology)
18. Microsequence analysis of individual spots	Partial amino-acid sequence
Use as Part of Assay System	
19. Assay for monoclonal antibody specific to given spots	Identify antibody specific to desired protein(s)
20. Assay for message (or gene) for specific spots after reticulocyte translation	Identify nucleotide sequence associated with desired protein

As a first step toward characterization of some of the major regulation systems, a broad survey of possible regulatory effectors was undertaken. Three types of human cells have been used: fresh peripheral blood lymphocytes, a lymphoblastoid cell line (GM607), and a fibroblast line. Gene control in fresh lymphocytes, cultured in vitro for 18 hr or less, represents the closest approximation we now have to regulation under normal physiological conditions. Even so, there are some substantial differences in the patterns of gene expression in lymphocytes isolated in Ficol-Paque compared to lymphocytes labeled briefly in whole heparinized blood at 37°C. Lymphoblastoid cells represent a transformed (presumably by E-B virus) version of the lymphocyte, and fibroblasts a nontransformed, but also nonphysiological, cell for comparison. The survey of regulatory effectors has so far encompassed about 60 compounds or treatments. The majority do not appear to alter gene expression, even though at high enough levels most can kill the cells. Table II lists some of the treatments that I have found to alter gene expression in the lymphocyte.

The results of these exploratory experiments identify sets of proteins that are controlled in various ways. The different groups of spots are given six letter acronyms ("Group name" in Table II; 17) referring to the properties that define them. Interf:1 is, for example, the first protein of the set induced by interferon, while Calgon:3 is the third member of the set repressed by raised intracellular calcium. Each protein within a group has a unique number in that group. A particular protein may have several names in addition to its spot number and biochemical name. For example, Mitcon:1 is a protein that belongs to the set whose synthesis stops when cells are treated with mitochondrial poisons; its uncleaved precursor is Mitpro:1; its master spot number in the lymphocyte pattern is 10 and it is probably the α-subunit of the F_1ATPase.

Table II. A List of Some Treatments Producing Changes in Gene Expression in Human Cells

Treatment	Group Name	No. of Proteins Affected
1. Antimitochondrial agents (nonactin, oligomycin, DNP etc.)	Mitcon	40
2. Chloramphenicol	Mitcod	1
3. Heat shock	ShockH	6
4. Sulfhydryl poisons (iodoacetamide, etc.)	ShockS	8
5. Amino acid analogues	ShockA	6
6. Cyclosporin A	CycspA	2
7. Tunicamycin	Tglyco	10
8. Colchicine	ColchA	3
9. Dexamethasone	Dexmet	1
10. Interferon	Interf	7
11 Poly I:C	PolyIC	7
12. Phorbol esters	Phorbl	30
13. A23187 (Ca^{++} ionophore)	Calgon	10
14. Ouabain	Ouaban	3
15. $CdCl_2$	Hmetal	5
16. Isolation (altered from rate of production in blood)	VitroA	20
17. Urinary proteins	Urocon,Urocoff	30
18. Variable (unknown control)	Varble	3

Interesting relationships exist among some of these regulation sets. The set of proteins induced in human lymphocytes by human interferon is substantially the same as the set induced by poly I:C. However, human interferon does not produce the effect in cells of other species, while poly I:C still does. This is apparently an example of the control of a single set of genes at two levels, one level specific for cell type and species, the other nonspecific. The relationship between

some peptide hormones and cAMP is formally similar. There are overlapping sets, however, which share only one or a few elements. The Mitcon group, which appears to contain all the mitochondrial matrix and inner membrane proteins (17), shares only one major spot with the set of lymphocyte proteins affected by treatment with the phorbol ester tumor promoters (the Phorbl group). This protein (Mitcon:5) is absent from lymphoblastoid and fibroblast cells, and therefore is a transformation-sensitive mitochondrial protein, possibly lymphocyte specific. Mitcon:5 is among the three or four most abundant proteins of lymphocyte mitochondria, and its complete repression by transformation must make a major difference to cellular metabolism. Further entirely unsuspected set relationships may be found. An example is the effect of colchicine in shifting a particular group of fibroblast spots (probably forms of a single protein) to a higher apparent SDS-molecular weight. This group happens to be one that is shifted to lower SDS-MW by tunicamycin treatment (i.e., when glycosylation is inhibited). It seems likely therefore that the effect of colchicine in this case is to cause hyperglycosylation of the affected protein. Since this protein is known also to be a member of the class of spots that are relatively invariant through the mammals, its function (and any disturbance of it) is likely to be important to the cell.

Discussion

The examples I have given here of results derived from protein set comparisons are few, and as yet do not solve the major problems of gene regulation. However, they do begin to demonstrate that the complex pattern of proteins produced by living cells can be broken down into tractable, inter-related subsets based on regulatory behavior. Proteins at the intersection of several sets may possess sufficiently unique properties as to suggest aspects of function in some cases.

Once sets are established in one cell type, interesting questions arise concerning the preservation of these sets unbroken in other cell types and other animal species. So far it appears that a number of sets (including Mitcon, ShockH, poly I:C) can be considered constant throughout the mammals and perhaps further. Such homologies, and the associated ability to identify analogous proteins in widely separated species, offer interesting opportunities for the comparative study of evolutionary variations on a constant functional theme.

A rudimentary, but self-consistent, picture of gene expression in some human cells is beginning to take shape. It provides sufficient insight into the "state of the cell" to determine objectively whether a number of major systems are functioning properly, and in some cases to differentiate various forms of cellular damage. Although the major goal is to understand the basic cellular systems almost in chemical engineering terms, the ultimate diagnostic usefulness of a Human Protein Index data base can not be ignored.

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