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Review

Two dimensional gel human protein databases offer a systematic approach to the study of cell proliferation and differentiation

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Introduction

Proteins, which are accurately synthesized from coded information contained in DNA, orchestrate most of the cellular functions. The total number of proteins in the human body is believed to be quite large and estimates range from 30,000 to 50,000. There are many different cell types in the body and the protein composition of one type differs partially from another, with at least 3,000-6,000 proteins present in any given cell type. To date, only a small proportion of the total set of human proteins have been identified, and very little is known concerning the actual protein composition of differentiated human cell types.

A biochemical technique such as high resolution 2D gel electrophoresis (O'Farrell, 1975; O'Farrell *et al.*, 1977) can separate about 2000 [^{35}S]-methionine labeled proteins from a few hundred somatic cells (O'Farrell, 1975; Garrels, 1979; Celis and Bravo, 1984) and has therefore provided a unique tool to examine the global patterns of gene expression of a given cell type. The technique, which was originally described by O'Farrell (1975) separates proteins in terms of their isoelectric points and molecular weights. Under carefully controlled conditions, protein separation is reproducible to the extent that the position and quantity of each protein can be compared under various experimental conditions. Much of the information that has reached the scientific community so far, however, has been incomplete, and only recently, thanks to the development of appropriate computer software (Garrels, 1989, and references therein), has it been possible to scan, assign numbers to individual polypeptides, compare, quantitate and store the wealth of information contained in the gels (Phillips *et al.*, 1987; Celis *et al.*, 1988, Celis *et al.*, 1989a, b, c; Garrels and Franza, 1989a, and references therein). It is now possible therefore to use this technology to accurately compare protein patterns between various cell types of normal and abnormal origin and to establish comprehensive databases containing information concerning *physical* (Mr, pl, etc.), *biochemical* (activity, modifications, turnover, purification procedures, partial and complete amino acid sequences, etc.), *physiological* (function, behavior under various physiological conditions, groups of coregulated proteins, etc.), *biological* (proliferation and transformation sensitive, cell cycle regulated, etc.), *genetic* (gene mapping, assignment of structural genes to gel maps, etc.) and *architectural* (cellular localization, etc.) of all the detected proteins of a given human cell. These databases are expected to have a great impact on basic and clinical research as they will provide an easy, standardized medium for storing and communicating protein information. Once a protein is identified in a given database (comigration with purified proteins, immunoblotting using specific antibodies, comparison of peptide sequences with sequences stored in protein databanks), all of the information accumulated can be easily retrieved and made available to the researcher. Databases will reach the scientific community by means of standard publications (maps and annotations) and in the future through computer networks.

Human 2D gel protein databases

Establishment of comprehensive, computerized 2D gel human protein databases for the study of cell proliferation, differentiation, cancer and other disease states.

To date, three comprehensive, computerized protein databases of human cellular proteins (transformed epithelial amnion cells (AMA), peripheral blood mononuclear cells (PMBC), and embryonic lung MRC-5 fibroblasts) (Celis *et al.*, 1988, 1989b) have been established. A database of total normal epidermal human keratinocytes has recently been published (Celis *et al.*, 1990). In these databases, each polypeptide (Fig. 1, NEPHGE gel of AMA cellular proteins; upper panel) has been assigned a number (Fig. 1, lower panel; small area of the gel shown in the upper panel) and various categories or entries (Table 1) have been created to store qualitative (annotations) and quantitative data. In general, categories or entries have been created to compile information concerning physical, biochemical, physiological, biological, genetic and architectural properties of proteins. Much of the information stored so far has been gathered in our laboratory. Some annotation categories available in the human AMA protein database are listed in Table 1, and examples of entries for the proliferation sensitive protein cyclophilin (*prolifsen*, Fig. 2, upper panel; *microseq*, Fig. 2, lower panel) are given in Fig. 2.

A total of 138 transformation sensitive cellular polypeptides have been revealed so far by the analysis of the overall patterns of protein synthesis of pairs of normal and transformed cells (Bravo and Celis, 1982; Celis *et al.*, 1989b). Some have been identified and these correspond to *cytoskeletal* (actin (IEF 7423); profilin (NEPHGE 3561); gelsolin (IEF 5714); tropomyosins (IEF's 9109, 9213, 9215, 9226); lipocortins I, II and V (IEF 3217, NEPHGE 4306 and IEF 8214), *nuclear* (cyclin/PCNA (IEF 9218); dividin (NEPHGE 2610); primatin (IEF 5703), nucleolar protein B23 (IEF 6318) and hnRNP's A1 (NEPHGE 308), C4 (NEPHGE 305), B1a (NEPHGE 1311) and C (IEF 8318)), *protein synthesis factors* (EF-2, IEF 2714) and *others* (hsx 70 (IEF 6610); cyclophilin (IEF 3004); stathmin (IEF 5001); ß-subunit of prolyl hydroxylase (IEF 8505), progressin (NEPHGE 3201) and Golgi proteins 1 and 2 (IEF's 8110 and 8108)). Six

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Abbreviations used in this paper: 2D, two-dimensional; NEPHGE, non equilibrium pH gradient electrophoresis.

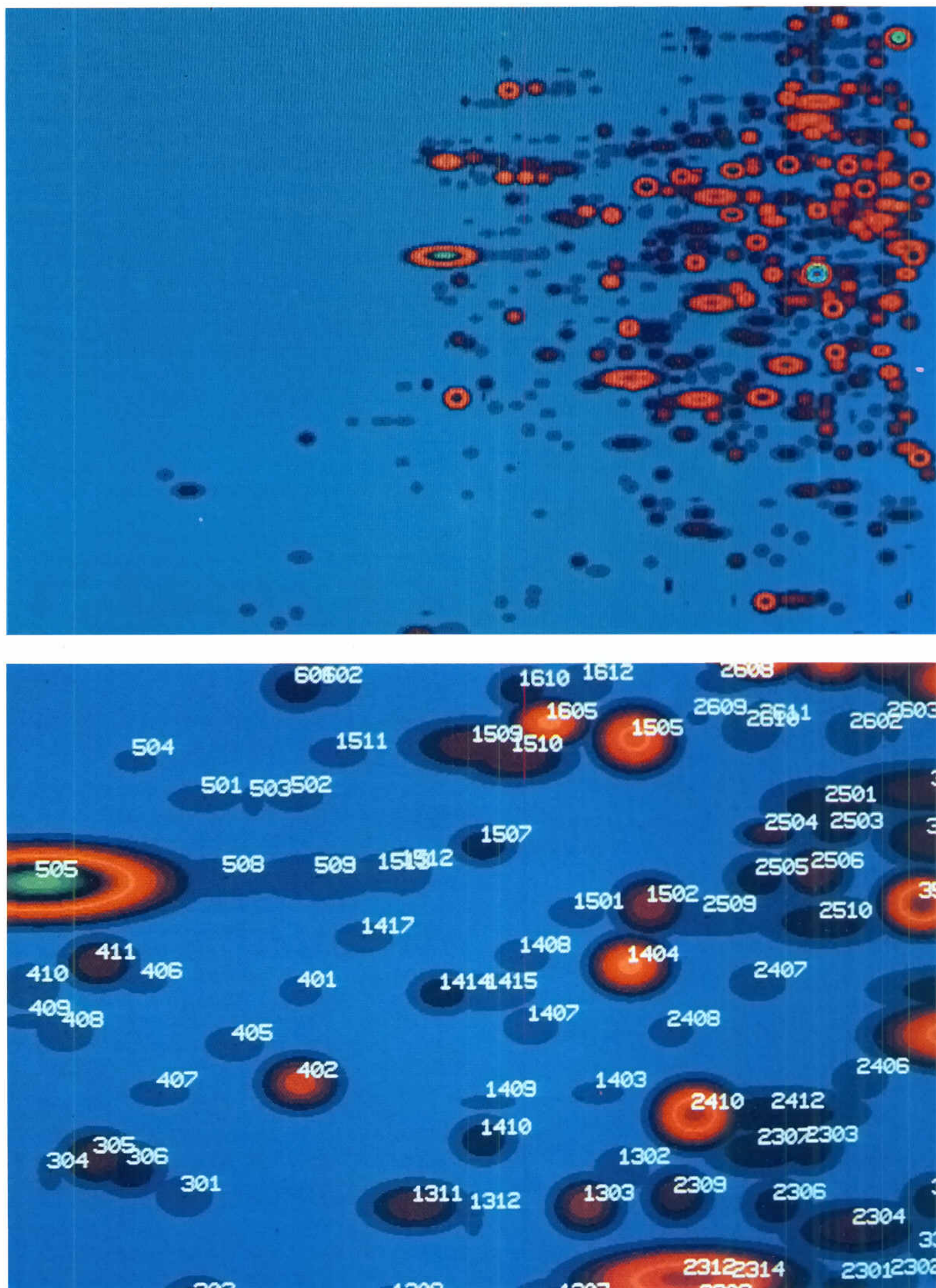

Fig. 1. Synthetic images of NEPHGE gels *(O'Farrell et al., 1977) of [^{35}S]-methionine labeled AMA cellular proteins. (Upper panel) total pattern. (Lower panel) small area of the gel shown above depicting protein numbers.*

TABLE 1

ANNOTATION CATEGORIES AVAILABLE IN THE COMPREHENSIVE 2D GEL PROTEIN DATABASE OF HUMAN AMA CELLS

CATEGORY NAME	DESCRIPTION
aaseq	Amino Acid Sequence
antibod	Antibodies to Protein
cellcycle	Cell Cycle Specific Proteins
cellspecif	Protein Marker for a Cell Type
coreg	Co-Regulation with Other Proteins
cpm	Manual CPM
cytosk	Cytoskeletal Proteins
dnaseq	DNA Sequence
downreg	Down Regulated Proliferation Sensitive Proteins
drugfact	Drugs and Factors that Affect Protein
endopret	Endoplasmic Reticulum Proteins
golgi	Golgi Proteins
halflife	Half-Life
heatshock	Heat Shock Proteins
helacat	HeLa Protein Catalogue Number
highlevel	Cell Type in which Protein is Present or Synthesized in High Levels
hnRNP	Heterogeneous Nuclear RNA Proteins
humansera	Human Sera that React with AMA Antigens
humantiss	Presence in Adult Human Tissues
ifprot	Proteins Present in Intermediate Filaments
interferon	Proteins Sensitive to Interferons
lupusant	Lupus Antigens
microseq	Spots Cut for Microsequencing
mitoch	Mitochondrial Proteins
modificat	Modifications
mousecat	Mouse Protein Catalogue Number
mrpi	Mr and pl
numocell	Number of Molecules per Cell
nuclear	Nuclear Proteins
nucleolar	Nucleolar Proteins
phospho	Phosphorylated Proteins
postranmod	Post-Translational Modifications
primsp	Primate Specific Proteins
prolifsen	Proliferation Sensitive Proteins
prosynfac	Protein Synthesis Factors
proteasome	Proteasome Proteins
protfunc	Protein Function
protname	Protein Name
purific	Purification Methods
rabbitinj	Proteins Injected in Rabbits
ribosompro	Ribosomal Proteins
secretprot	Secreted Proteins
spespec	Species Specificity
subcelloc	Subcellular Location(s)
synculcell	Levels in Cultured Cells
synquies	Synthesis in Quiescent Cells
tritonext	Proteins Extracted by Triton X-100
upreg	Up Regulated Proliferation Sensitive Proteins

of these proteins (cyclin/PCNA, increased synthesis at the G_1/S border; dividin, increased synthesis at the G_1/S border; progressin, increased synthesis at the G_1/S border; hsx70 increased synthesis during mitosis; EF-2, preferentially phosphorylated during mitosis and nuclear protein B23, increased phosphorylation during mitosis) are cell cycle regulated (Celis *et al.*, 1987; Celis *et al.*, unpublished observations), and of these, the DNA replication protein cyclin/PCNA has been the most studied both in our laboratory (Celis *et al.*, 1987 and references therein) as well as in other laboratories (Miyachi *et al.*, 1978; Mathews *et al.*, 1984; Bravo, 1986; Bravo *et al.*, 1987; Prelich *et al.*, 1987; Tan *et al.*, 1987; Garrels and Franza, 1989b, and references therein). All available information indicates that this protein is a central component of the pathways leading to DNA replication and cell division. The precise function of cyclin/PCNA is at present unknown although it has been proposed that it coordinates leading and lagging strand synthesis (Prelich *et al.*, 1987, and references therein).

Examples of NEPHGE gel fluorograms of [^{35}S]-methionine labeled proteins from human MRC-5 fibroblasts and their SV40 transformed counterparts (MRC-5 V2) are shown in Fig. 3. Spots corresponding to the proliferation sensitive protein cyclophilin (Handschumacher *et al.*, 1984) (decreased synthesis in transformed cells; see also Fig. 2, upper panel; Celis *et al.*, 1989b) are indicated in this Fig. It has been proposed that this cytosolic protein, which has binding activity for cyclosporin A (CSA), plays a role in signal transduction from surface receptors to the nuclei. Indirect immunofluorescence pictures of methanol fixed MRC-5 and MRC-5 V2 cells reacted with polyclonal antibodies produced against cyclophilin (Celis *et al.*, unpublished observations) are shown in Figures 4A and B respectively. In the normal cells most of the staining is cytoplasmic and localized in focal adhesion plaques. (Fig. 4A). Staining of the Golgi apparatus is also observed (Figs. 4A and B). Transformed cells exhibited much reduced staining in the cytoplasm as well as in the focal adhesion plaques.

Protein and DNA information can be linked thanks to the advent of microsequencing: identification of new proteins

The systematic 2D gel protein analysis approach has recently gained a new dimension with the advent of techniques to microsequence major proteins recorded in the databases (Hewick *et al.*, 1981; Hunkapillar *et al.*, 1983; Vandekerckhove *et al.*, 1985; Aebersold *et al.*, 1986, 1987; Bauw *et al.*, 1987,1989; Matsudaira, 1987; Eckerskorn *et al.*, 1988; Kennedy *et al.* 1988; Moos *et al.*, 1988; Celis *et al.*, 1989c). Partial protein sequences can be used to search for protein identity as well as for preparing specific DNA probes for cloning. Furthermore, since these sequences can be stored in the database (Fig. 2, lower panel), they offer a unique opportunity to link protein information with the forthcoming DNA sequence information on the human genome (Fig. 5).

In a collaborative effort between our laboratories, we have started a long-term program to microsequence all of the major human proteins recorded in the AMA database. So far, 18 Coomassie blue stained polypeptides have been microsequenced using a simple procedure described by Bauw *et al.* (1989) (Fig. 6; see also Fig. 2, lower panel). Dry protein spots are collected from multiple gels, rehydrated, concentrated by stacking into a new gel, electroblotted onto inert membranes and *in situ* digested with trypsin. Peptides eluting from the membrane are then separated by HPLC (Fig. 6) and sequenced. Partial protein sequences of

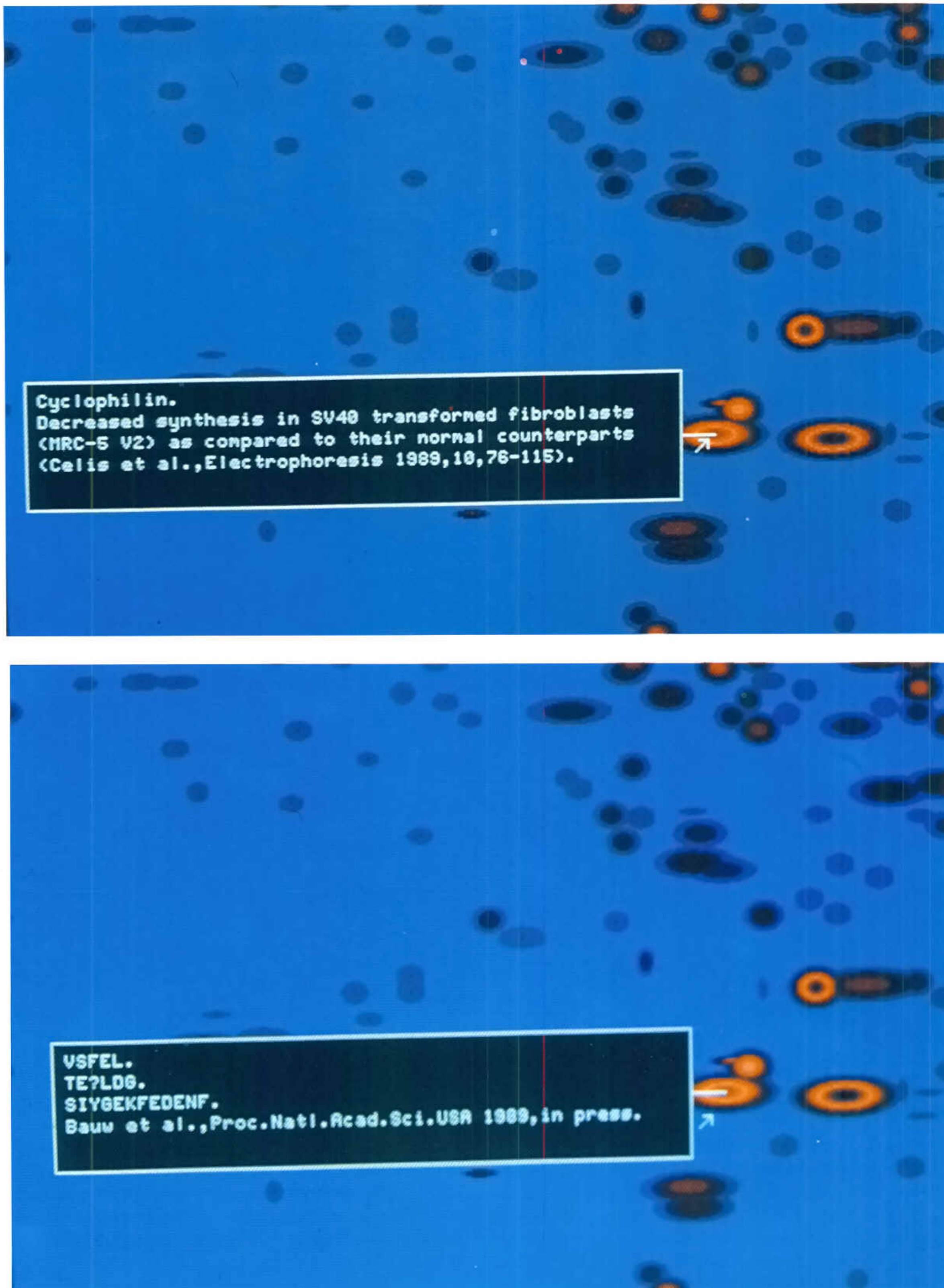

Fig. 2. Synthetic images of NEPHGE gels *(O'Farrell* et al., *1977) of [^{35}S]-methionine labelled AMA cellular proteins showing information contained in the entries* prolifsen *(upper panel) and* microseq *(lower panel).*

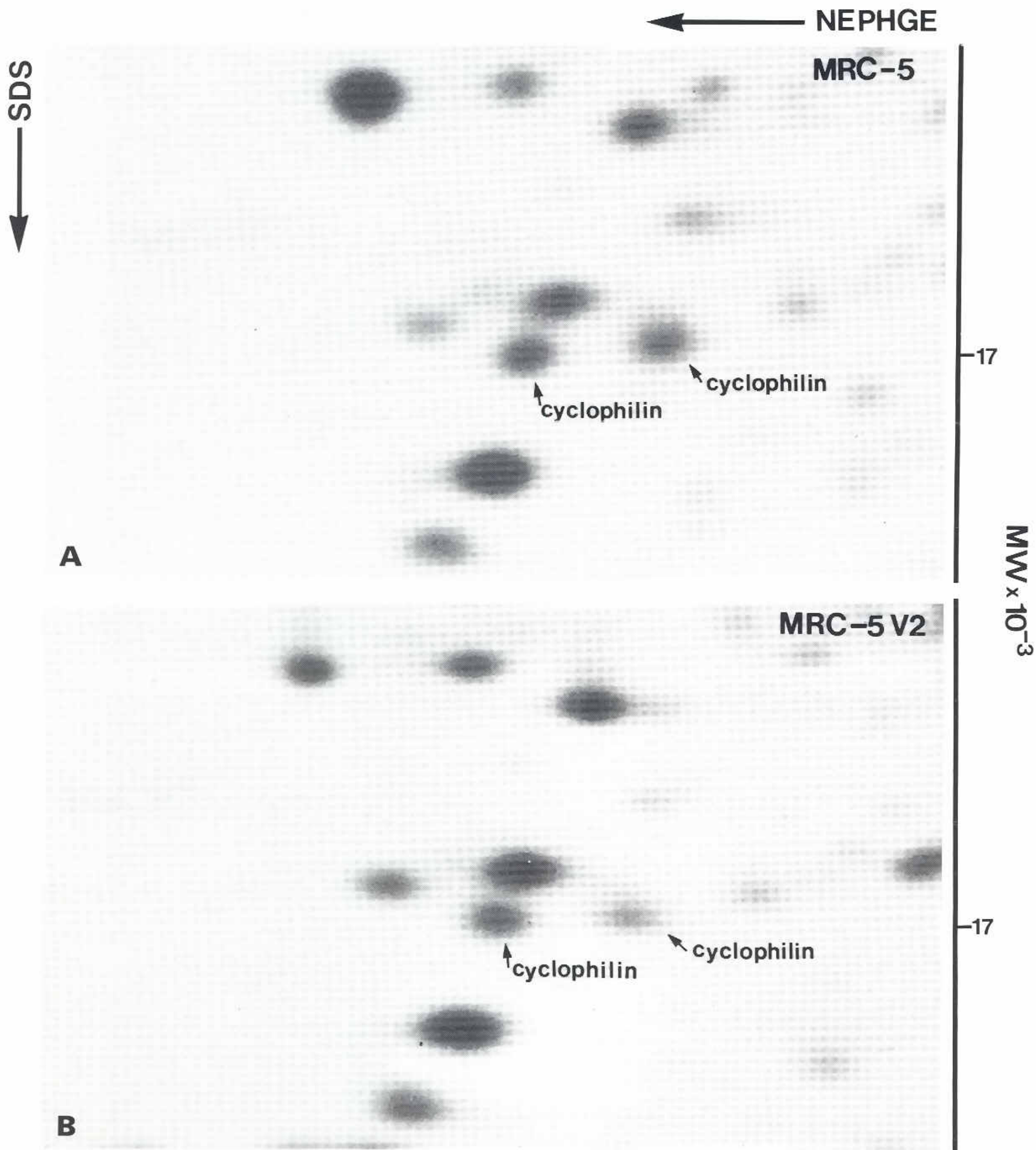

Fig. 3. Synthesis of cyclophilin in normal and transformed human fibroblasts. *2D gel electrophoresis (NEPHGE) of [^{35}S]-methionine labeled proteins from* **(A)** *normal and* **(B)** *SV40 transformed human MRC-5 fibroblasts (MRC-5 V2). Only a fraction of the gels is shown.*

ten of the proteins microsequenced so far matched those of proteins stored in databases and some corresponded to transformation sensitive polypeptides (gelsolin, cyclophilin (Fig. 6; see also Fig. 2, lower panel), hnRNP C, nucleolar protein B23, lipocortin V, ß-subunit of prolyl-4 hydroxylase and a tropomyosin) (Bauw *et al.*, 1989; Celis *et al.*, 1989b). All available information indicates that it should be possible to obtain partial protein sequences from most of the proteins resolved by 2D gels that can be visualized by Coomassie blue staining. Furthermore, by making use of the information stored in the comprehensive 2D gel protein databases, it may be possible to analyze proteins of interest by selecting human tissues or cultured cell types where a particular protein is expressed in higher amounts, thus increasing considerably the versatility of the technique.

Concluding Remarks

Although still at an initial stage, our studies as well as those of others have clearly underlined the value of esta-

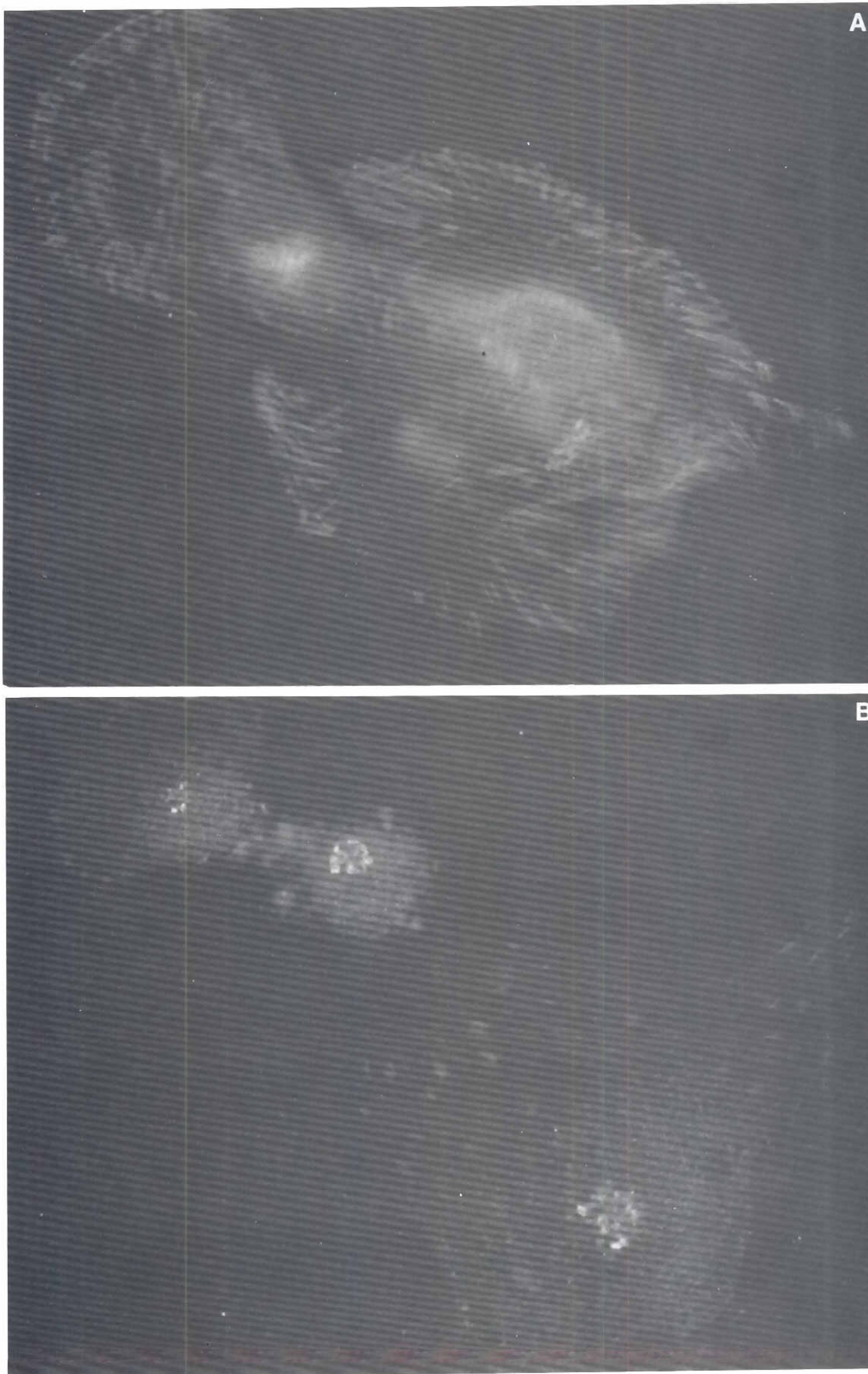

Fig. 4. Indirect immunofluorescent staining *of* **(A)** *normal and* **(B)** *SV40 transformed human MRC-5 fibroblasts (MRC-5 V2) fixed with methanol and reacted with rabbit polyclonal antibodies prepared against human cyclophilin (Celis* et al., *unpublished observations).*

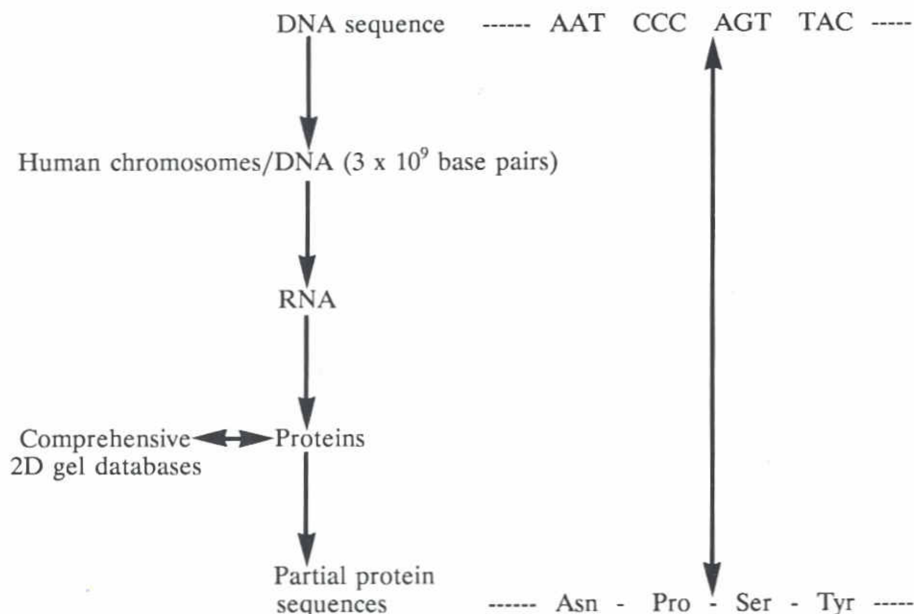

Fig. 5. Interface between partial protein sequence databases, comprehensive 2D gel databases, and the human genome sequencing project. *Both the partial protein sequence and the DNA sequence (B. Honoré* et al., *unpublished observations) are from human protein IEF 9806 (Celis* et al., *1988).*

blishing comprehensive databases of human protein information for the study of cell proliferation, differentiation, cancer and other disease states. The proteins we have recorded are probably the most abundant components of the cells and, thus, further studies involving the analysis of purified preparations or subcellular fractions may be necessary to reveal minor proteins. Also, the gel systems used do not resolve very basic proteins or proteins having M_r > 200,000 or < 8,500. In the future, databases will yield a wide variety of biological information that can be used to support collaborative research projects in basic and clinical research. Furthermore, by displaying global patterns of genome function, databases may help to pinpoint proteins or groups of coregulated proteins that could be selected for further study. For example, once a change in a given protein is correlated to a particular disease, a simple radioimmunoassay can be devised and used in the clinics.

In the long run as the human genome sequence data becomes available, it will be possible to assign partial protein sequences to genes for which the full DNA sequence and the chromosomal location is known (Fig. 5). In our laboratories efforts are being devoted to the study of transformation and/or proliferation sensitive proteins (cytoskeletal proteins included) that are coregulated under various physiological conditions, cell differentiation included. Antibodies raised against some of these proteins as well as oligonucleotide probes prepared from partial protein sequences will be used to clone the cDNAs. These in turn will be used to quantitate message expression and to screen genomic libraries in an effort to identify common regulatory sequences in their upstream regions. So far, we have been able to clone and partially sequence cDNAs fished out with specific monoclonal antibodies prepared in our laboratory as well as with oligonucleotide probes synthesized from information derived from partial protein sequences (see legend to Fig. 5).

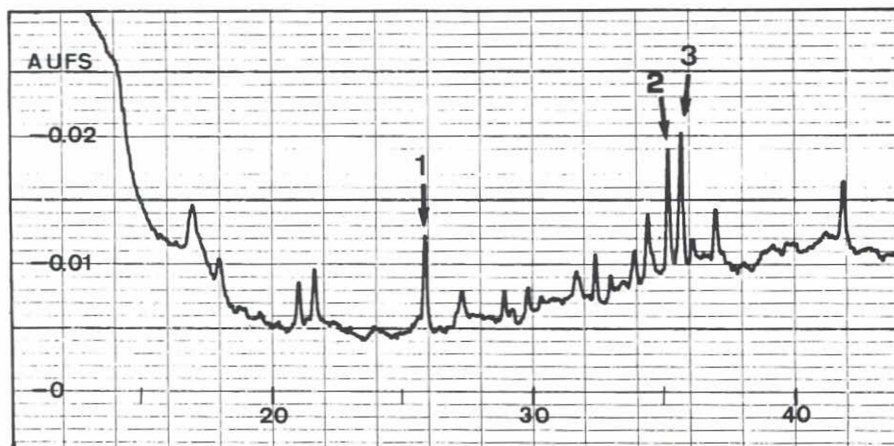

Fig. 6. Partial amino acid sequence of a protein recovered from a 2D gel. *Tryptic peptides obtained by membrane* in situ *cleavage of cyclophilin were separated on a C4 (4.6 x 250mm) reverse phase column (Vydac Separations Group, USA). Solvent A consisted of 0.1% TFA, and solvent B was 70% acetonitrile in 0.1% TFA. A gradient from 0% solvent B to 100% solvent B was applied in a linear mode over 70 min. The eluate (1ml/min) was recorded by absorbance at 214 mm (0.2 AUFS). 0 indicates the start of the chromotogram. Peptides were collected manually in Eppendorf tubes. Those indicated by arrows were subjected to gas-phase sequence analysis using a 470 A Applied Biosystems Inc. (USA) gas-phase sequenator equipped with a 120 A on-line PTH-amino acid analyzer (see Fig. 2, lower panel).*

Summary

Human cellular protein databases have been established using computer-analyzed 2D gel electrophoresis. These databases, which include information on various properties of proteins, offer a global approach to the study of regulation of cell proliferation and differentiation.

Furthermore, thanks to the advent of microsequencing the databasesmake it possible to directly link protein and DNA information.

KEY WORDS: *human genome, DNA, proteins, 2D gel databases, gene expression*

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