Advances in two-dimensional electrophoresis

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New developments in the first dimension step of two-dimensional electrophoresis have expanded the utility of the technique in cell and molecular biology.

WHILE two-dimensional electrophoresis is unsurpassed by any other technique for simultaneously resolving hundreds of polypeptides, its potential to contribute to our understanding of molecular processes remains largely untapped. Because of the difficulty in achieving adequate reproducibility and truly high resolution, the technique has successfully been practised in a relatively small number of laboratories. Those difficulties notwithstanding, it was evident at a two-dimensional electrophoresis meeting held in Vienna last November* that the technique has been used to identify a good number of novel proteins that play a role in cell activation, proliferation, differentiation and other functions[1]. Genes corresponding to proteins detected on two-dimensional gels have been cloned by several groups, including our own.

Immobilized pH gradients

A major contributor to the variability seen in two-dimensional gel patterns within and between laboratories is the carrier ampholyte (CA) which is the determinant of the first dimension separation, based on charge. Detailed comparisons of patterns generated for the same cell material in separate laboratories have been difficult, hampering efforts to establish collective protein databases of two-dimensional gel information. Problems with CA-based separations include: gaps in the gel patterns, resulting from discontinuities in the pH gradient, which varies between sources of CA; drift in the focusing pattern during electrophoresis (particularly severe for basic polypeptides); and difficulty in producing pre-cast gels suitable for two-dimensional electrophoresis.

These difficulties prompted us to develop a two-dimensional approach that utilizes immobilized pH gradients (IPG) for the first dimension separation[2,3]. With IPG, the pH gradient is an integral part of the polyacrylamide gel matrix[4]. The technique overcomes many of the problems encountered when using carrier ampholytes. The pH separation range can be defined from broad to extremely narrow, basic polypeptides can be resolved well, and precast gels can be used. IPG gels can also be loaded with preparative amounts of protein suitable for polypeptide sequencing, because they have higher loading capacities than CA gels.

In the IPG method, gradient slab gels of 0.5 mm in thickness are first cast on a GelBond PAG film in a large format (24 cm wide with a 17-cm pH gradient separation distance). For most applications, the gradient spans three pH units: 4–7 for acidic and neutral polypeptides, and 6.5–9.5 for neutral and basic molecules. Each slab gel yields roughly seventy 3.5-mm-wide first dimension strips. Following extensive washing with deionized water, the gels are dried and wrapped in thin plastic film, and can be stored at −20 °C for several months.

Prior to the second dimension electrophoresis step, the desired number of strips are cut and rehydrated, as a group, with a solution of urea, non-ionic detergent, dithioerythritol and dilute acetic acid. The strips are then placed on the cooling plate of a humidified, sealed electrofocusing apparatus, and sample applicators of between 40 and 80 μl in capacity are placed directly onto the surface of the gel.

The degree of sample entry into the gel is critical. We have found that the best results are obtained using dilute samples with a low salt concentration to which carrier ampholytes have been added, with

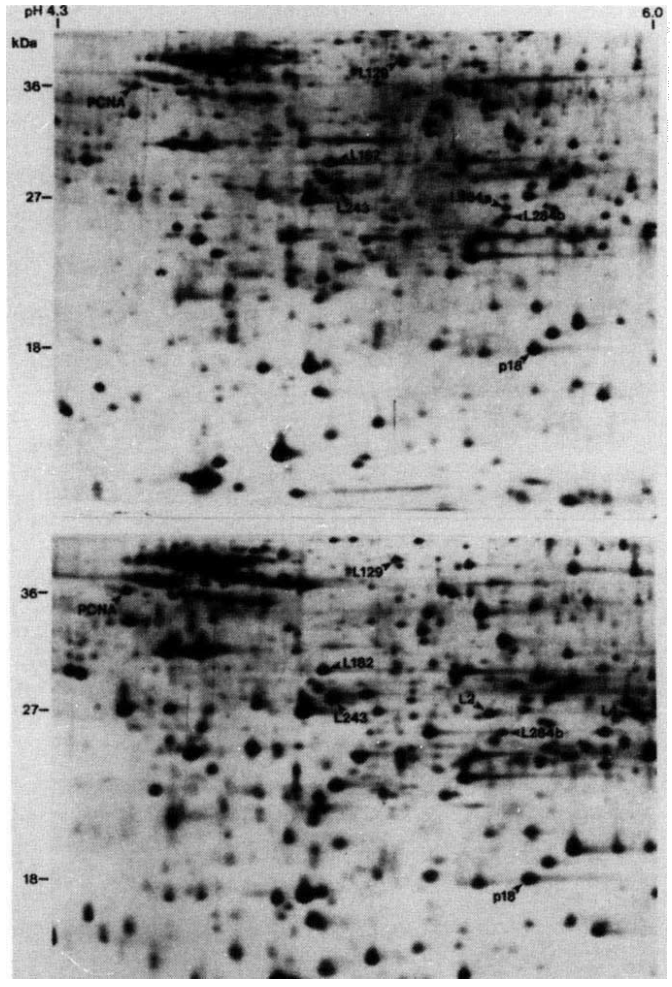

Fig. 1 Comparison of the polypeptide patterns of two separate Epstein–Barr virus-transformed lymphoblastoid cell lines, generated with two-dimensional gel electrophoresis using immobilized pH gradient gels prepared from separate castings. Arrows point to polypeptides of interest to the study of acute leukaemia, some of which have been sequenced and their corresponding genes cloned[6,7]. Some of the differences between the protein patterns are due to genetic polymorphisms, and others to stage of differentiation[7,8].

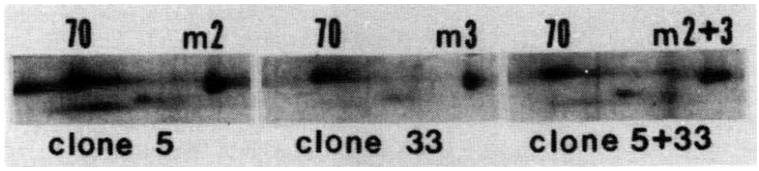

Fig. 2 Close-up sections of two-dimensional gels prepared with narrow immobilized pH gradients (pH 6.3–6.8) in the first dimension. Two mutants of polypeptide 70 prepared from separate mutagenized lymphoblastoid cell lines phenotypically identical in broad-range CA-based gels are clearly resolved by immobilized pH gradients. The mutants differ in charge by <0.003 pH units.

* The International 2-Dimensional Electrophoresis Meeting, Vienna, Austria, 8–11 November 1988.

a low voltage gradient across the gel (30 V cm^{-1}) for the first hour. The voltage can then be increased to 5,000 V (0.1 mA per strip, maximum) for 22–24 hours.

The second dimension electrophoresis step can be performed using vertical or horizontal slab gels. Figure 1 illustrates the reproducibility and resolution which can be achieved using relatively broad pH gradients. An example of the resolution which can be achieved using narrow gradients is shown in Fig. 2.

Other developments

It should be emphasized that in practice, no one technique can lead to the simultaneous visualization of all cellular polypeptides. Thus, for some applications, the selective analysis of protein subsets of interest (such as the low-abundance soluble nuclear proteins) by prior cell fractionation is advantageous.

Additional approaches include the use of separation modes other than isoelectric focusing for the first dimension separation. The direct coupling of microbore HPLC to SDS electrophoresis is particularly promising. In such a system, various HPLC separation modes, from reversed phase to ion exchange, could be used to perform the first dimension separation. In a previous study of plasma proteins, we observed that the resolving power of ion exchange HPLC is equivalent to isoelectric focusing with CA, based on the separation of transferrin variants[5].

Another promising development is the use of capillary electrophoresis as the first dimension separation mode. An effort is also being made to overcome some of the problems experienced with CA-based separations to eliminate gaps in the gels and to allow the production of precast focusing gels containing urea which can be stored for lengths of time.

It is becoming clear that, after an initial lag behind DNA technology, most of the practical knowledge is now in place to allow a major leap forward in our understanding of the organization, structure and function of the principal cellular components which mediate cell function, namely, the proteins. □

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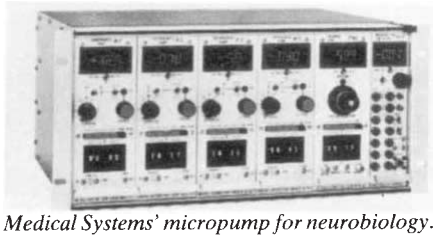
Of peptides and DNA

Neurobiology and neurochemistry will be the talk of the town in Miami next week during the Miami Bio/Technology *Winter Symposium. Besides an array of patch-clamps and recorders, the exhibits will feature products for studying peptides, and the nucleotides which code for them.*

MEDICAL Systems Corporation has a new **micropump system** for controlling the ejection of fluids from micropipettes in neurobiotechnological studies *(Reader Service No. 101)*. The company's Neuro-



Medical Systems' micropump for neurobiology.

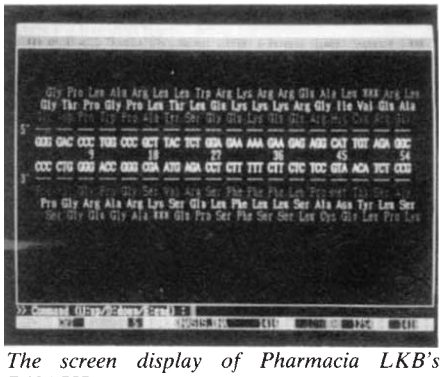
Phore BH-2 has both a pneumatic and an ionophoretic pump module, and can deliver intracellular or extracellular ejections of minute volumes using up to five pumps in parallel. The ejection schedule for each pump can be programmed independently. Medical Systems Corporation also offers multibarrel micropipettes for use with the NeuroPhore BH-2 system to enable researchers to eject several different fluid combinations for investigating cell responses. The company is exhibiting in booth 78.

United States Biochemical, exhibiting in booths 62 and 63, offers a range of **neuropeptides** which affect the central nervous system *(Reader Service No. 102)*. The company is noteworthy for producing Bombesin and MBHA-peptide resin, the preferred resin for producing alpha-carboxamide peptides, but they also sell endorphins, delta sleep-inducing peptide, alpha- melanocyte stimulating hormone, and enkephalins. Purified amino acids and derivatives for peptide synthesis can also be chosen from USB's product listing, which contains over 6,000 products. The company has recently been appointed by Perkin- Elmer Cetus as a co-distributor of Perkin-Elmer Cetus's GeneAmp reagents for performing the polymerase chain reaction.

Software smarts

Beckman has updated its popular MicroGenie **sequence analysis software** to include the capability to calculate circular restriction maps, including double and multiple digests *(Reader Service No. 103)*. The new feature allows the user to enter and store lists of restriction enzymes and their recognition sites, even before analysis, and to calculate restriction fragment sizes based on gel mobilities. The new version retains the old MicroGenie's shotgun DNA sequencing function, where sequences can be merged and scanned for overlaps, insertions, deletions and mismatches, and the homology search and alignment function, which draws upon the GenBank nucleic acid database and the National Biomedical Research Foundation protein sequence database. With MicroGenie, it is also possible to predict protein secondary structures and probabilities, determine residue and codon frequency, predict and plot protein hydrophobicity, and find inverted repeat regions and calculate the free energy. Beckman offers a free demonstration disk for MicroGenie, which runs on IBM-compatible computers. The company is exhibiting in booths 29 and 30.

Pharmacia LKB, exhibiting in booths 88 and 89, has just launched its PROSIS **protein analysis software** package for personal computers *(Reader Service No. 104)*. The software can perform secondary structure predictions, based upon the Chou-Fasman and Robson theories; calculate the maximum amino acid homology



The screen display of Pharmacia LKB's DNASIS.

between two sequences; perform molecular weight and homology searches; plot homologies; and perform hydrophobicity analyses. PROSIS has keyword search and database access functions, and can be used with an optional CD-ROM disk that contains both the GenBank and National Biomedical Research Foundation databases. Version 3.0 of Pharmacia-LKB's DNASIS DNA analysis software has also recently been released. DNASIS has been updated to perform homology searches in one-third of the time required by previous versions, and to incorporate improved colour graphics. The new version also allows the automatic entry of fragment migration distances from an optional sonic digitizer for the creation of restriction