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PROSPECTS

Gene Expression Monitoring for Gene Discovery in Models of Peripheral and Central Nervous System Differentiation, Regeneration, and Trauma

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Abstract Gene expression monitoring using gene expression microarrays represents an extremely powerful technology for gene discovery in a variety of systems. We describe the results of seven experiments using Incyte GEM technology to compile a proprietary portfolio of data concerning differential gene expression in six different models of neuronal differentiation and regeneration, and recovery from injury or disease. Our first two experiments cataloged genes significantly up- or down-regulated during two phases of the retinoic acid-induced differentiation of the embryonal carcinoma line Ntera-2. To identify genes involved in neuronal regeneration we performed three GEM experiments, which included changes in gene expression in rat dorsal root ganglia during the healing of experimentally injured sciatic nerve, in regenerating neonatal opossum spinal cord, and during lipopolysaccharide stimulation of primary cultures of rat Schwann cells. Finally we have monitored genes involved in the recovery phase of the inflammatory disease of the rat spinal cord, experimental allergic encephalomyelitis, as well as those responsible for protection from oxidative stress in a glutamate-resistant rat hippocampal cell line. Analysis of the results of the approximately 70,000 data points collected is presented. J. Cell. Biochem. 80:171–180, 2000.

The development of nonviral gene delivery technology has been a major research effort at the Immune Response Corporation for the past 6 years. The initial focus of this work was gene delivery targeted to the liver, specifically exploring the utility of the asialoorosomucoid receptor to deliver two genes licensed from large pharmaceutical companies: (1) the human Factor VIII gene for Hemophilia A and (2) the human Interferon-alpha gene for hepatitis B therapy. To expand our gene delivery potential to disorders of the human central nervous system (CNS) and to identify and obtain proprietary genes for further gene therapy applications, we have begun to monitor differential gene expression in a series of models of development, differentiation, regeneration, and recovery from injury in the vertebrate central and peripheral nervous systems using state-of-the-art gene expression microarray technology [Shalon et al., 1996; Schena, 1996; Schena et al., 1998]. This technology allows the simultaneous monitoring of thousands of genes and their differential expression patterns in distinct cell populations in a expeditious and efficient manner.

Treatment of disorders of the human central nervous system remains a key issue facing modern science. The inability of the mammalian CNS (as opposed to its peripheral counterpart) to repair itself by regeneration of functional neurons [Ramon y Cajal, 1928] accounts in large part for the millions of patients suffering from varying degrees of impairment due to a variety of causes including spinal cord injury (SCI), stroke, and neurodegenerative diseases such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis (ALS). There exist a variety of well-described animal and tissue culture cell model systems mimicking normal growth,

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development, differentiation and regeneration of neuronal cells as well as response to and recovery from neurotrauma and neurologic disease. We have begun extensive analysis of messenger RNA (mRNA) populations to monitor changes in levels of expression of the approximately 10,000 cDNA sequences present on the Incyte UniGEM 1 gene expression microarray (GEM) in six pertinent model systems: differentiation of the embryonal carcinoma cell line NTera 2 (NT2) into mature neurons, regenerating neonatal monodelphis domestica spinal cord, dorsal root ganglia (DRG) during sciatic nerve regeneration, lipopolysaccharide (LPS) activation of primary rat Schwann cells, protection from oxidative stress in a glutamate resistant hippocampal cell line, and recovery from the paralysis-inducing inflammatory disease experimental allergic encephalomyelitis (EAE). In this manuscript we describe our preliminary efforts to monitor and catalog differential gene expression in these diverse models of neuronal differentiation, regeneration, and recovery from injury and disease.

As the initial model system in which to monitor changes in gene expression during differentiation to a neuronal phenotype we chose the human embryonal carcinoma cell line NTera-2 (NT2). Treatment of NT2 cells with retinoic acid and mitotic inhibitors causes cessation of the cell cycle and differentiation of these cells into mature neurons that express a variety of molecular markers specific to the neuronal cell lineage, including the neurofilament proteins NF195, NF170 and NF70, vimentin, microtubule-associated protein 2, and the neural cell adhesion molecule NCAM [Lee and Andrews, 1986; Pleasure and Lee, 1993]. Retinoic-acid-treated NT2 cells also demonstrate electrophysiological properties characteristic of neurons, including functioning glutamate receptor channels [Younkin et al., 1993]. Furthermore, untreated NT2 cells acquire the molecular phenotype of fully mature neurons within 6 months of transplantation into the central nervous systems of either adult or neonatal mice [Kleppner et al., 1995]. To investigate and compile information concerning specific changes in gene expression during different stages of this well-described model of neuronal differentiation, mRNA was harvested from three distinct populations of cells: (1) undifferentiated, dividing NT2s; (2) NT2s treated with retinoic acid for 4 weeks and mitotic inhibitors for 3 days, and (3) NT2 cells after 4 weeks of retinoic acid and 6 days of mitotic inhibitor exposure. These mRNA samples were analyzed in two separate experiments on the UniGEM 1, resulting in the compilation of differences of levels of expression of approximately 10,000 genes during both the initial phase of differentiation from proliferating cells to the preliminary development of the neuronal phenotype as well as the secondary phase of differentiation into a more mature neuronal phenotype.

The second major emphasis in our development of a proprietary catalog of genes potentially important for the ultimate restoration of damaged CNS tissues has involved the use of three animal model systems, which allows us to identify genes involved in neuronal regeneration. Regeneration, a phenomenon that is common in repair of injury within the peripheral nervous systems (PNS) does not readily occur in the adult mammalian central nervous system [Cajal, 1928]. To address specifically the differences in regenerative potential between the PNS and CNS we have developed in-house two models: (1) regenerating peripheral nerve after crush injury in the rat and (2) lipopolysaccharide-mediated stimulation of primary cultures of rat Schwann cells. In the first set of experiments we performed bilateral crush injury of sciatic nerves of a collection of adult rats and harvested mRNA from L4 and L5 dorsal root ganglia at 7 days postinjury as well as at the same timepoint from a series of sham-operated animals to compare directly the variation in gene expression in regenerating and uninjured tissue. To carry this analysis further we examined a particularly interesting component of the regeneration of peripheral nerves, which is the role played by the Schwann cell that when activated appropriately will function as during normal PNS development, secreting a variety of proteins, including neurotrophic factors and stimulating extracellular matrix molecules, to establish an environment permissive for axonal growth. To begin to assess which genes may be responsible for these properties we have treated primary cultures of rat Schwann cells with the stimulatory agent lipopolysaccharide [Skundric, 1997] and consequently monitored differences in gene expression during Schwann cell activation.

As a further effort to identify mRNA transcripts whose levels are changing significantly in populations of neurons in "regeneration" mode, we have been studying the remarkable ability of the spinal cord of the newborn South American opossum monodelphis domestica to heal completely following severe injury with concomitant functional recovery [Treherne et al., 1992; Saunders et al., 1995]. In collaboration with the laboratory of Dr. John Nicholls we have prepared mRNA from spinal cords of animals injured at 9 days old (a timepoint at which they possess this extraordinary CNS regenerative capability) and have compared this profile with that of mRNA from the similarly injured cords of 13-day-old animals (which in the four day interval have lost this regenerative capability) on the UniGEM 1. This collection of genes displaying significant changes in gene expression during this crucial window in the CNS development of monodelphis domestica allows us to begin to unravel the cellular and molecular factors responsible for inducing neurite growth in the spinal cord of this neonatal opossum with the ultimate hope of finding application to disorders of the mature vertebrate CNS.

Two model systems have been utilized specifically to identify genes involved in neuronal protection and recovery from disease of, or injury to, the CNS. We have begun to dissect molecular pathways involved in defense of neurons from oxidative stress, which is implicated in several neurodegenerative disorders as well as neurotrauma and stroke, [reviewed in Coyle and Puttfarcken, 1993; and Busciglio et al., 1998] using a murine cell line model which compares the susceptibility to glutamate toxicity of a hippocampal-neuron-derived cell line, HT-22, to the glutamate-resistance of an HT-22 subclone that exhibits 10-fold higher survival upon treatment with the neurotransmitter [Sagara et al., 1998]. To characterize the mechanisms conferring this invulnerability by identifying the genes whose expression is changing during and are therefore potentially responsible for the protection of neurons from oxidative stress, we have monitored differences in gene expression resulting from glutamate insult of the parental HT-22 in comparison to the reaction to such treatment of its resistant subclone HTR-10.

The final experiment we describe using gene expression monitoring to identify genes, which may have significance in recovery from disease in CNS tissue, involved the experimental allergic encephalomyelitis (EAE) model representing recovery from inflammation-induced disease of the rat spinal cord generated by injection of foreign myelin basic protein (mbp) [Lisak and Behan, 1975]. Rats receiving mbp derived from guinea pigs display a characteristic disease course after inoculation with symptoms including weight loss, tail flaccidity, ataxia, hind limb paresis or paralysis, and urinary incontinence. Symptoms disappear in a typical recovery period of 2 weeks. To determine which genes are involved in the recovery phase of this experimental model of the human disease multiple sclerosis we monitored differential gene expression in mRNA populations isolated from the spinal cords of rats at days 8 and 14 post-mbp injection.

METHODS

Preparation of Total and Messenger RNA

Total RNA was prepared from all samples using the standard Trizol (Gibco BRL) protocol. mRNA was isolated from total RNA preparations using the oligo dT columns and the standard Oligotex (Qiagen) protocol. Spinal cord tissue was homogenized in the Trizol reagent with the Brinkmann Polytron PT homogenizer 4 times for 15–20 sec each time prior to RNA isolation procedures.

Retinoic Acid-Induced Differentiation of NT2 Embryonal Carcinoma Cells

NT2 precursor cells were purchased from Stratagene (catalog #204101) and grown routinely in DMEM F12 complete media. Cells were induced to differentiate by the introduction of 10 μM retinoic acid to DMEMF 12 media and grown under these conditions for 4 weeks and then for 3 or 6 days in the presence of mitotic inhibitors. Cells were collected and frozen at -70°C in Trizol and total and mRNA was prepared as described.

Induction of Experimental Allergic Encephalomyelitis in Lewis Rats

Six- to eight-week-old Lewis rats were injected with 5 μg of guinea pig myelin basic protein (gp-mbp) and 5 mg/ml complete Freund's adjuvant in each of their two front foot pads. Half of the animals were sacrificed under anesthesia 8 days after treatment by

perfusion with 4% paraformaldehyde in phosphate buffered saline, and spinal cords were removed. The remaining rats were similarly treated at day 14 following gp-mbp treatment. Specifically, the T12, T13, and L1 spinal segments were isolated and stored in 1 ml of Trizol per 50–100 mg of tissue and total and mRNA was prepared as described.

Spinal Cord Crush Injury in Neonatal Monodelphis Domestica

Animals were removed from the pouches of their mothers at postnatal day 9 or postnatal day 13, anaesthetized by cooling and Metofane, and their intact central nervous systems dissected out under Eagles BME media as previously described [Nicholls et al., 1990; Stewart et al., 1991]. The CNS preparations prepared for culture were comprised of dorsal root ganglia, trigeminal ganglia, and in some cases an eye. After dissection the preparations were crushed at spinal segments C5 and C6, and grown in Eagles MEM medium or in BME containing 0.2% fetal calf serum (GIBCO), 30 ng/ml NGF, 10 μg/ml insulin, and 0.1 mg/ml gentamycin sulfate, bubbled with CO2 in oxygen and kept at room temperature (24–25°C). After 3 days in culture, the cords minus sacral regions and dorsal root ganglia were flash frozen in liquid nitrogen and then processed for mRNA isolation as described above.

Glutamate Toxicity in HT-22 and HTR-10 Cells

Fetal bovine serum (FBS) and dialyzed FBS were from Irvine Scientific (Irvine, CA). Dulbecco's Modified Eagle's Medium (DMEM) was made according to the original procedure [Vogt and Dulbecco, 1963]. HT-22 cells [Davis and Maher, 1994; Maher and Davis, 1996] were derived from the immortalized mouse hippocampal cell line, HT-4 [Morimoto and Koshland, 1990]. The derivation of the glutamate resistant cell line HTR-10 was described in Sagara et al. [1998]. Both HT-22 and HTR-10 cells were grown on tissue culture dishes (Falcon, Indianapolis, IN) in DMEM supplemented with 10% FBS. Pancreatin (GibcoBRL, Rockville, MD) was used to dissociate these cells from culture dishes for passaging and total and mRNA was prepared as described.

Sciatic Nerve Injury and DRG Isolation

Female Sprague-Dawley rats (200–250 g) were anaesthetized with Ketamine/xylazine/acepromazine and the sciatic nerves were exposed bilaterally and crushed twice for 20 sec. In control animals the sciatic nerves were exposed only (no crush). Seven days later, the animals were again anaesthetized and the L4 and L5 dorsal root ganglia were removed, snap frozen in liquid nitrogen, and stored at -70°C and total and mRNA was prepared as described.

Maintenance of Primary Schwann Cell Cultures and Activation by Lipopolysaccharide

Three-day-old rat pups were anaesthetized by cooling on crushed ice, and the sciatic nerves were removed and transferred to DME + 1% pen/strep. The nerves were digested enzymatically using trypsin/collagenase and plated onto polylysine-coated plastic flasks. After incubation for 2 days in media containing cytosine arabinoside, to inhibit fibroblast growth the cultures were passaged and incubated in media containing bovine pituitary extract and forskolin to stimulate proliferation of Schwann cells. Pure cultures were incubated in DME + 10% FBS for 3 days to induce differentiation to the mature phenotype and then activated with 0.5 mg/ml bacterial lipopolysaccharide for 6 hr. The cultures were rinsed with Trizol and stored at -70°C and total and mRNA was prepared as described.

RESULTS

Compilation of Data from Seven Initial UniGEM Experiments

The numbers of cDNA sequences with biologically significant (greater than twofold) changes in expression in each of the seven experiments described is displayed graphically in Figure 1A, grouped into the specific categories of (1) total number of genes changing significantly (genes); (2) number of genes showing significant up-regulation (up); (3) total number of genes showing greater than twofold down-regulation (down); (4) total number of cDNAs of unknown identity changing significantly (ESTs); (5) total number of cDNAs of unknown identity showing significant up-regulation (ESTs up); (6) total number of cDNAs of unknown identity showing significant down-regulation (ESTs down); and (7) total number of cDNAs of unknown identity but with a degree of homology to known genes changing significantly (EST sim). In all, 2,588 genes show

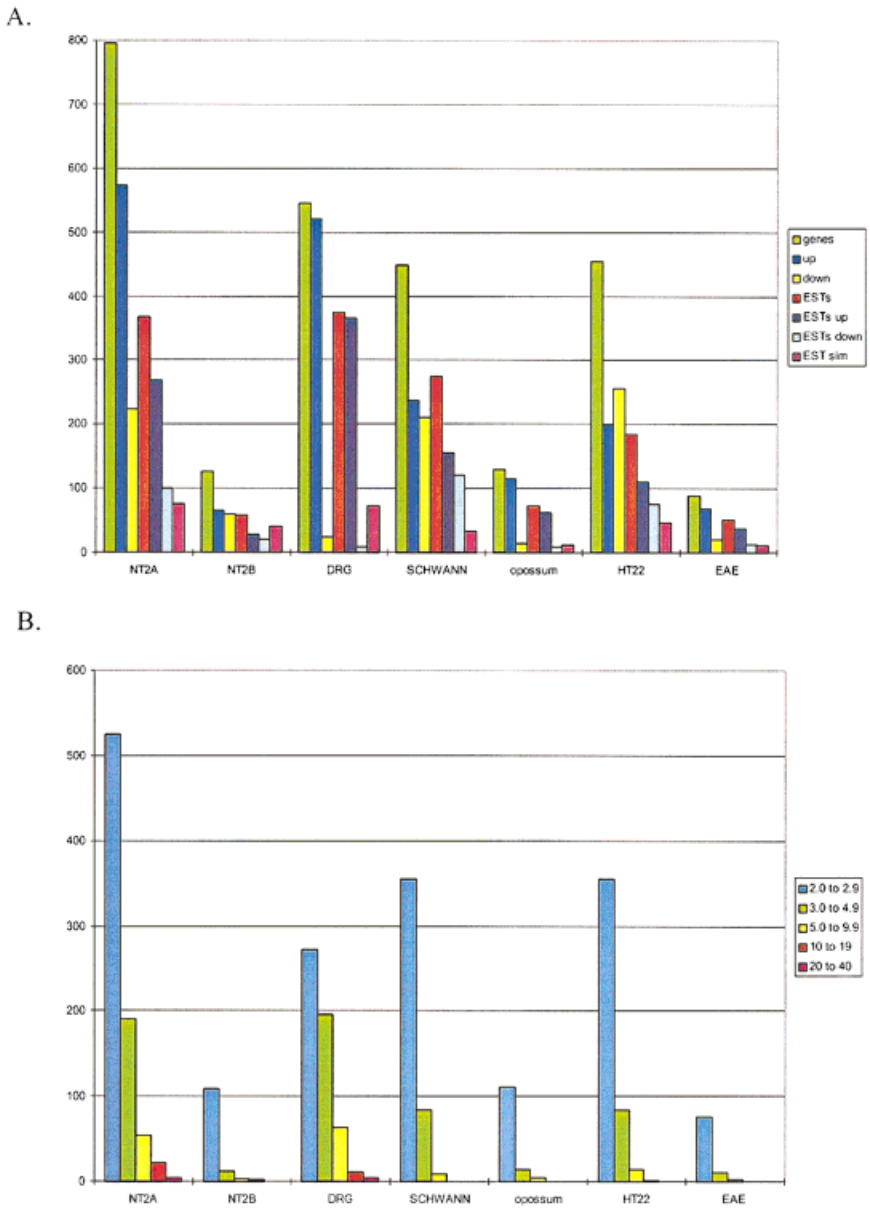

Fig. 1. Differential gene expression in seven models of neuronal differentiation, regeneration, and recovery from injury or disease. **(A)** The specific categories are labeled: (1) genes, total number of genes changing significantly (greater than twofold); (2) up, number of genes showing significant up-regulation; (3) down, total number of genes showing greater than twofold down-regulation; (4) ESTs, total number of cDNAs of unknown identity changing significantly; (5) ESTs up, total number of cDNAs of unknown identity showing significant up-regulation. (6) ESTs down, total number of cDNAs of unknown identity showing significant down-regulation; and (7) EST sim, total number of cDNAs of unknown identity but with a degree of homology to known genes changing significantly. The specific experiments are labeled (1) NT2A, initial time points in neuronal differentiation of NTera2 cells; (2) NT2B, second time points in neuronal differentiation of NTera2 cells; (3) DRG, dorsal root ganglia during peripheral nerve regeneration; (4) Schwann, lipopolysaccharide-induced activation of primary cultured Schwann cells; (5) opossum, spinal cord regeneration in the marsupial monodelphis domestica; (6) HT22, response to oxidative stress of a glutamate-resistant and glutamate-sensitive hippocampal neuron cell line; and (7) EAE, recovery of the spinal cord from the inflammatory disease experimental allergic encephalomyelitis. **(B)** Specific categories are labeled (1) 2.0 to 2.9, genes whose levels of expression are changing between two- and threefold; (2) 3.0 to 4.9, genes changing between three- and fivefold; (3) 5.0 to 9.9, genes changing between five- and 10-fold; (4) 10 to 19, genes changing between 10- and 20-fold; and (5) 20 to 40, genes changing between 20- and 40-fold. The specific experiments are labeled as described in Fig. 1A.

biologically significant differential gene expression.

Several interesting observations concerning this initial compilation of data are immediately apparent from this straightforward graphical analysis. For example, comparison of the results from the experiment labeled NT2A (which monitors differential gene expression in undifferentiated, dividing NT2 cells as compared to cells treated with retinoic acid for 4 weeks and mitotic inhibitors for 3 days) with the results of experiment NT2B (which monitors differential gene expression) in the following 3 days (i.e. after 6 days of exposure to mitotic inhibitors) of neuronal differentiation of this cell population exhibits an obvious decrease in the numbers of genes whose expression is changing in the later time frame (specifically 124 as compared to 796). This result is consistent with the observation that the major morphological and functional changes in this differentiating cell line occur in the first phase of response to retinoic acid and mitotic inhibitor treatment, during which time the cells display the major changes of cessation of the cell cycle as well as development of a neuronal phenotype, while the cells in the second phase monitored simply exhibit further neuronal differentiation.

Comparatively lower levels of changes in gene expression are also observed in the opossum spinal cord crush injury (labeled "opossum") and in the recovery phase of experimental allergic encephalomyelitis (data group EAE). We hypothesize that the former result is due in part to the evolutionary distance between the probe mRNA (derived from the marsupial monodelphis domestica) and the human cDNA sequences present on the UniGEM. Cross-species hybridization is probably not responsible, however, for the lower numbers of affected genes in the EAE experiment in which the probe mRNA is derived from the rat (note the relatively high numbers of genes differentially expressed in the rat DRG, Schwann, and HT22 experiments) but rather this less robust result may imply that the response to this experimentally induced inflammatory disease is indeed very specific in nature. Finally, it is of interest to note in the DRG experiment that greater that 95% (521/546) of genes with significant variation in levels of expression are expressed at higher levels (up-regulated) during this instance of peripheral nerve regeneration, which is very similar to the 88% (114/129) of genes up-regulated during the regeneration of the neonatal opossum spinal cord. Only in these two experiments, which involve regeneration (of both the peripheral and central nervous system), is the percentage of genes being up-regulated so high.

Range of Differential Gene Expression in Initial GEM Experiments

Figure 1B shows the graphical representation of the range of differential expression in each of the seven experiments previously described, specifically displaying the tabulation of the numbers of genes: (1) changing between two- and threefold (2.0 to 2.9); (2) changing between three- and fivefold (3.0 to 4.9); (3) changing between five- and 10-fold (5.0 to 9.9); (4) changing between 10-and 20-fold (10 to19); and (5) changing between 20- and 40-fold (20 to 40). The widest range of differential expression (specifically with two genes being up-regulated and two genes down-regulated more than 20-fold) is seen in the experiment NT2A, in which Ntera 2 cells are changing from their undifferentiated, dividing state through cessation of the cell cycle to exhibit a neuronal phenotype. In contrast, the experiment NT2B shows much less dramatic differential gene expression in the 10,000 genes monitored which is not unexpected due to the less extreme biological change (further neuronal differentiation) being examined. Furthermore, the response of dorsal root ganglia to peripheral nerve crush injury is similar in magnitude to the first phase of NT2 differentiation with four genes showing higher than 20-fold up-regulation.

Determination of Biological Relevance of GEM Data

In order to examine more closely the biological relevance of data obtained via GEM analysis we performed a thorough and comprehensive literature search to compile a list of genes known to be differentially regulated during various stages of retinoic acid-induced differentiation of NT2 cells to a neuronal phenotype. We observed concordance of data we obtained experimentally with respect to the genes present on the UniGEM 1 with available published data concerning the direction of changing gene expression. Two specific examples are the neurofilament light chain (L) gene, and the

neural cell adhesion molecule NCAM which are standard markers for differentiation of NT2 cells [Lee and Andrews, 1986; Pleasure and Lee, 1993] and which are shown by GEM analysis to be up-regulated 3.8- and 2.1-fold, respectively, in the NT2 experiments described herein. To further assess the significance of the GEM data, we determined that more than 40 genes known to be expressed in nervous tissue are indeed shown to be up-regulated in differentiating NT2 cells in the first 3 days of exposure to mitotic inhibitors and that most of these genes continue to be expressed during the next 3 days of differentiation. Finally, as represented in Figure 2, we observed that 21 genes known to be involved in control of the cell cycle are changing significantly during the initial phase of NT2 differentiation (in which the first major phenotypic event is cessation of the cell cycle) while the levels of expression of only two such genes change significantly during the following period in which the sole phenotypic change observed is differentiation to a more mature neuronal phenotype.

DISCUSSION

The wealth of data compiled after our initial eight experiments should prove to be invaluable for our ultimate goal of treatment of disorders of the human central nervous system. We are pursuing many different lines of experimentation using data arising directly from the chip studies to advance both methods of gene delivery in the CNS as well as to identify potentially efficacious genes to deliver. The potentially most valuable source of information derived from the gene expression monitoring experiments performed is the population of genes with biologically interesting patterns of expression whose specific function remains undetermined. Figure 3 shows a flowchart of steps to separate known genes from those whose sequence is truly novel. One initial approach we have undertaken to directly and immediately accumulate more information about these ESTs has been to obtain more complete DNA sequence information than was originally available. Once the available clones have been sequenced completely it will be of further benefit to identify longer (ultimately full-length) cDNA copies of these genes. Once full-length coding sequence is available there is an extensive body of Internet-based and other software available for analysis of both nucleotide and amino acid sequence to help determine biological properties of genes of unknown identity. Finally, it is of interest to note that a large percentage of the ESTs present on the UniGEM 1 have already been found to be associated with known gene families, with different levels of similarity ("highly", "moderately," or "weakly" similar as classified by the IMAGE consortium) to other members of these groups.

An obvious preliminary step to analyze GEM data to identify genes of potential therapeutic applicability is to simply examine those genes whose differential gene expression is most extreme—that is, to ask which sequences exhibit the most dramatic changes during, for example, different time points of neuronal regeneration, differentiation and/or recovery from injury or disease. Genes of known function previously not known to be involved in the basic neurobiological processes mimicked by the model systems we are studying as well as genes of unknown function (ESTs), which are strongly up-regulated are immediately flagged as being potentially efficacious for repair of damaged or diseased CNS tissues. More detailed computational analysis can point to ESTs of biological interest and potential therapeutic importance by organizing gene expression data by grouping together sequences with similar patterns of expression. Exploitation in this manner of the vast amounts of information obtained from gene expression microarray experiments can be accomplished using the technique of "cluster analysis" [Eisen et al., 1998; Michaels et al., 1998; Wen et al., 1998], which provides putative identification of the function of EST clones by comparing their levels of expression to those of genes in known functional families.

A second valuable source of information coming from the GEM data compiled thus far is the identification of groups of genes of known function with expression patterns of interest in the models studied. We have identified, for example, a collection of secreted proteins not previously associated with neuronal development that are very highly up-regulated during the retinoic acid induced differentiation of NT2 cells to a neuronal phenotype. We have obtained full-length copies of the genes for these secreted proteins and are testing their biological function in this particular model system using the genes themselves as well as their expressed products to determine their poten-

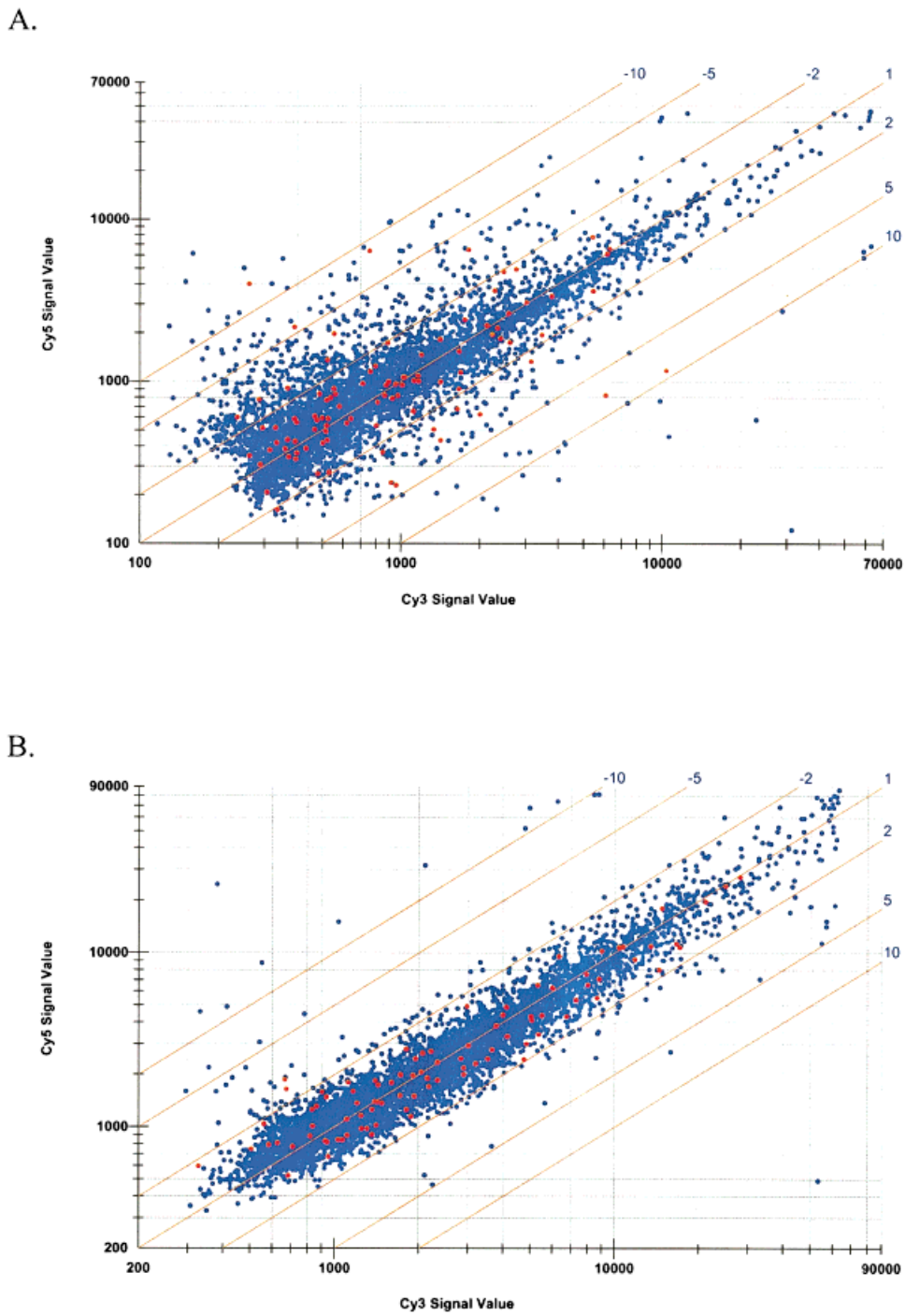

Fig. 2. Differential expression of genes involved in cell cycle control during two phases of the neuronal differentiation of NT2 cells. **(A)** Log-log graph of the Cy5 and Cy3 signal values of all genes present on the microarray at day 0 and day 3 of the neuronal differentiation of NTera2 cells. Highlighted (red) spots represent the differential expression of genes known to function in the control of the cell cycle.

tial neurite-outgrowth inducing capability. Further analysis of genes with neurotrophic properties can be accomplished by overexpressing these genes in fibroblast cells, transplanting these recombinant cells into an injured rat spinal cord and measuring neurite outgrowth [McTigue et al., 1998].

Effective delivery of genes which can act as therapeutic agents for disorders of the central nervous system also remains an important

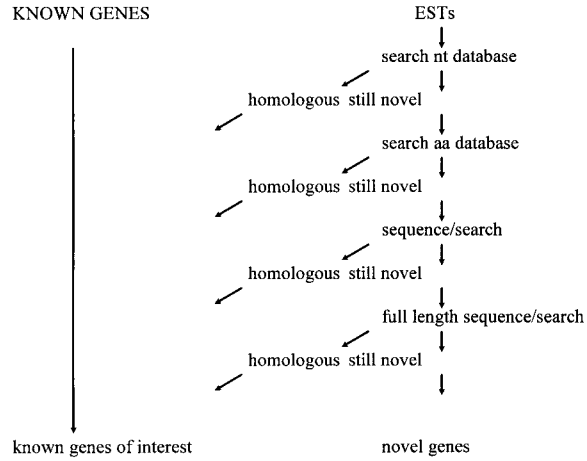

Fig. 3. Identification of novel gene sequences of clones represented on biochips. Partial nucleotide sequence information available for ESTs with expression patterns of interest is searched against database nr using the local alignment search tool BLASTN (Altschul et al., 1997). ESTs with no significant homology in nucleotide databases are subject to BLASTX search to compare its putative amino acid sequence against database nr. Genes whose sequence remains novel are subjected to further sequencing and reanalyzed by BLASTN and BLASTX. Ultimately, full-length cDNA clones of ESTs are identified and compared to available online databases at both nucleotide and amino acid levels.

area to which data collected from biochip experiments can be applied. Analysis of genes with expression patterns of interest will be of great benefit in enhancing the proprietary gene delivery technology of the Immune Response Corporation by distinguishing novel regulatory elements that will direct genes to be delivered to cells of the central nervous system. From the GEM data we have selected a series of more than 200 EST clones whose expression is either constitutively high in neuronal cell populations or whose expression is strongly up-regulated during neuronal differentiation and have identified a subgroup of these clones for which there is extensive genomic DNA sequence available (i.e. those associated with previously sequenced bacterial or P1-derived artificial chromosome clones-BACs and PACs) and are analyzing this sequence and working to obtain the regulatory elements responsible for the observed neuron-specific gene expression.

In summary, we have presented the initial results obtained from a pilot study of gene expression monitoring using GEM technology of various pertinent animal models of neuronal differentiation, regeneration, and recovery from trauma or disease, and have described some of our ongoing efforts to enhance our program for gene discovery and delivery for disorders of the central nervous system. Data compiled from gene expression microarray analysis should prove to be invaluable for similar efforts throughout all fields of basic and applied scientific research.

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