Chapter 2

Application of Differential Display and Serial Analysis of Gene Expression in the Nervous System

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Introduction

Every biological process in both plant and animal species is associated with changes in gene expression. In the central nervous system (CNS), changes in gene expression are not only causally linked to the development of the CNS but also to complex and as yet not well-understood phenomena such as memory formation, learning and cognition. In addition, changes in gene expression underlie the pathogenesis of many acute and chronic CNS-related disorders such as ischemia, epilepsy, Alzheimer’s disease and Parkinson’s disease. Thus, insight into and characterization of gene expression profiles, and in particular the changes occurring therein, are crucial for understanding how the brain functions at the molecular level and how malfunction will lead to disease.

Given its complexity, i.e. tens of thousands of genes each expressed at a different level, characterization of gene expression profiles is not a straightforward task. The set of genes expressed and the stochiometry of the resulting messenger RNAs, together called a “transcriptome”, determine the phenotype of a cell, tissue and whole organism. The human genome is thought to contain 50,000–100,000 genes of which a subset of approximately 15,000–20,000 genes is expressed in an individual cell. Therefore, gaining insight into gene expression profiles in a particular tissue or cell is a major enterprise, and the identification of a limited set of differentially expressed genes resembles searching for a needle in a haystack.

In the 1980s, several methods aimed at the identification of differentially expressed genes were described, including plus/minus screening and subtractive hybridization methods. Although these methods have proven to be useful in isolating differentially expressed genes, they are technically difficult and labour-intensive, relatively slow and require large amounts of RNA (see e.g. Kavathas et al., 1984; Vreugdenhil et al., 1988).

In the beginning of the 1990s, the sensitivity, speed and accuracy of differential screening techniques were boosted by two major developments: first, polymerase chain reaction techniques were introduced resulting in the possibility to amplify minimal amounts of starting material and making the monitoring of expression of thousands of genes simultaneously possible. Second, the increasing knowledge of DNA sequences of a large number of genes and corresponding transcripts necessitated and resulted in the establishment of nucleotide sequence databases. In addition, different genome projects were initialised to unravel complete nucleotide sequences of several species including several bacterial species, yeast, the nematode, drosophila, mouse and human (McKu-
sick, 1997; Rowen et al., 1997; Duboule, 1997; Levy, 1994). At present, the complete genomes of *E. coli* (10⁶ bp) and yeast (2x10⁷ bp) are known, while those of nematode (10⁸ bp) and human (2x10⁹) are partially sequenced; respectively 80% and 5% are known. These known DNA sequences are publicly available and as a consequence application of screening techniques has only to result in a small portion of a particular gene to unambiguously identify it as up- or down-regulated.

The introduction of PCR and the establishment of databases have revolutionized differential screening strategies and resulted in a number of highly sensitive techniques. Here we will discuss two of these: differential display (DD) and serial analysis of gene expression (SAGE).

### Differential Display

DD was first described in 1992 by Liang and Pardee (Liang and Pardee, 1992). The tremendous impact of DD is probably best illustrated by the number of approximately 1700 DD-related articles which have been published since its introduction. Many genes linked to numerous CNS-related processes such as neurodegeneration and apoptosis have been identified by DD (Kiryu et al., 1995; Livesey et al., 1997; Tsuda et al., 1997; Imazumi et al., 1997; Su et al., 1997; Shirvan et al., 1997).

The principle of DD is based on the random amplification and subsequent size separation of cDNA molecules. To this end, total RNA is isolated from a cell or tissue of interest and reverse-transcribed into cDNA. Instead of a single oligodT primer, four different anchored oligodT primers are used (oligodT-MC, oligodT-MG, oligodT-MT and oligodT-MA; M=G/A/C) in four separate cDNA synthesis reactions. Basically, this modified cDNA synthesis divides the original mRNA population into four different cDNA pools. Subsequently, a fraction of each pool of cDNA is randomly amplified using a randomly chosen primer in combination with the same anchored oligodT primer. The PCR conditions, in particular the annealing temperature, are chosen such that approximately 60–100 cDNA fragments are amplified. These cDNA fragments, derived from “stimulated” and “non-stimulated” tissues, are size-separated in parallel on gels. Differentially expressed products are identified by comparing the presence (upregulation) or absence (downregulation) of cDNA fragments in the two situations. This process is repeated with other randomly chosen primers, resulting in the amplification of another portion of the cDNA pool. Finally, differentially expressed cDNA fragments can be excised from gel and further characterized by, e.g., Northern blot analysis, *in situ* hybridization and DNA sequence analysis (see below). The major advantage of this DD approach is its simplicity, its extreme sensitivity and the possibility to identify both up- and downregulated genes in the same experiment. Disadvantages of DD are its labour-intensive character and the generation of many false positives.

Since its introduction, many modifications and improvements of the DD technique have been described. For example, instead of the originally described radioactive DD cDNA fragments, different labels, e.g. fluorescent labels, have been used to monitor DD fragments (Bauer et al., 1993; Ito et al., 1994; Rohrwild et al., 1995; Vreugdenhil et al., 1996b). Consequently, automated DNA sequencers could be used to facilitate the monitoring and analysis of DD fragments. Other efforts have focused on primer design (Liang et al., 1994; Liang et al., 1993; Malhotra et al., 1998). These latter studies have led to the use of extended 20-nucleotide-long primers in more recent reports. Several excellent review articles on the principles of differential display have been published (Liang and Pardee, 1995; Livesey and Hunt, 1996; Vreugdenhil et al., 1996b; Liang and Pardee, 1997).