Research Report

Monitoring of Representational Difference Analysis Subtraction Procedures by Global Microarrays

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ABSTRACT

Various approaches to the study of differential gene expression are applied to compare cell lines and tissue samples in a wide range of biological contexts. The compromise between focusing on only the important genes in certain cellular processes and achieving a complete picture is critical for the selection of strategy. We demonstrate how global microarray technology can be used for the exploration of the differentially expressed genes extracted through representational difference analysis (RDA). The subtraction of ubiquitous gene fragments from the two samples was demonstrated using cDNA microarrays including more than 32 000 spotted, PCR-amplified human clones. Hybridizations indicated the expression of 9100 of the microarray elements in a macrophage/foam cell atherosclerosis model system, of which many were removed during the RDA process. The stepwise subtraction procedure was demonstrated to yield an efficient enrichment of gene fragments overrepresented in either sample (18% in the representations, 86% after the first subtraction, and 88% after the second subtraction), many of which were impossible to detect in the starting material. Interestingly, the method allowed for the observation of the differential expression of several members of the low-abundant nuclear receptor gene family. We also observed a certain

background level in the difference products of nondifferentially expressed gene fragments, warranting a verification strategy for selected candidate genes. The differential expression of several genes was verified by real-time PCR.

INTRODUCTION

Most studies investigating the regulation of metabolic and pathological conditions at the level of gene expression have traditionally used a candidate gene approach. However, this strategy is limited in scope because only a single gene and its expression are investigated. It is now possible to apply powerful gene expression technologies to monitor global expression patterns to elucidate the magnitude and complexity of many genes simultaneously. Several cDNA subtraction methods have been reported to identify differentially expressed genes and are based on the hybridization of cDNA from one population (tester) to the excess of cDNA from the other population (driver). The unhybridized fraction (target) is then separated from the hybridized common sequences by various means. Two PCR-based subtractive methods, representational difference analysis (RDA) (16) and suppression subtractive hybridization (SSH) (11), have been proposed to increase the sensitivity in the subtraction to facilitate the identification of rare transcripts. The cDNA RDA technique employs a positive selection approach in which tester unique cDNA fragments are sequentially enriched and amplified by PCR, while material ubiquitous in both populations

is eliminated. Multiple rounds of subtraction are usually performed. SSH involves a single subtraction round, and the normalization of sequence abundance is achieved by hybridization kinetics combined with long, inverted terminal repeats attached to the investigated cDNA population. Both of these techniques are commonly used and have been used on microarrays to a limited degree to facilitate rapid screening (4,12,23,24). The selective techniques are important for the detection of rare transcripts, especially considering that several of the regulating genes can be expressed at low levels (10,13).

Here we describe the use of global cDNA microarrays, containing more than 32 000 PCR-amplified human cDNA clones, to identify differentially expressed genes obtained by a cDNA RDA. Differentially expressed genes are identified in a THP-1 macrophage cell line that is transformed into foam cells through treatment with oxidized low-density lipoprotein (LDL) (2). This well-established system for the study of atherosclerotic plaque formation is screened for both expected changes in gene expression and novel findings, including the differential expression of rare transcripts enabled by RDA.

MATERIALS AND METHODS

Cell Cultivation and cDNA Synthesis

THP-1 cells were cultured as previously described (1). To establish a macrophage phenotype, THP-1 cells were treated with 0.2 μ M phorbol 12-myristate-13 acetate (PMA) (Sigma, St.

Louis, MO, USA) for 24 h. To further stimulate the cells into a foam cell phenotype, 50 µg/mL oxidized LDL (oxidized by exposure to copper for 24 h) were added to the PMA-treated THP-1 cells for 24 h. Total RNA was isolated using TRIzol® (Invitrogen, Paisley, Scotland, UK). The mRNA was isolated from total RNA using oligo-dT paramagnetic beads, and cDNA synthesis was performed essentially according to Reference 14, using a biotinylated oligo-dT primer. The dsDNA was digested with *DpnII* (New England Biolabs, Beverly, MA, USA) and ligated to complementary R-Bgl-24 linkers as previously described (16).

RDA Procedure

The solid-phase RDA procedure has been described in detail in previous publications (5,19). Briefly, the initial RDA representations were generated by the PCR amplification and *DpnII* digestion of the cDNA. Twenty micrograms of *DpnII*-digested tester representation were purified using streptavidin-coated magnetic beads, and 2 µg purified tester were annealed and ligated with biotinylated J-Bgl-24 linkers. The subtractive hybridization of tester and driver and PCR amplification of the hybridization product were performed to generate the first difference product (Dp1). The Dp1 PCR products were *DpnII*-digested, solid-phase purified, ligated to a new set of N-Bgl linkers, and the procedure was repeated as described earlier to generate difference product 2 (Dp2).

PCR Labeling of RDA Representations and Difference Products

RDA representations and difference products were labeled by the direct incorporation of labeled nucleotides into the PCR amplification steps in the RDA. A PCR mastermixture was prepared as described previously (5), except where 1.75 mM dCTP was labeled with Cy3 or Cy5 dye (Cyanine 3- or Cyanine 5-dCTP; NEN® Life Science Products, Brussels, Belgium). The extension time at 72°C was changed to 10 min/cycle (compared to the original protocol in which 3 min/cycle is used)

to compensate for the slower incorporation of the labeled nucleotides.

Target Preparation and Microarray Hybridization

Spin column purification of labeled PCR products was performed using a QIAquick™ PCR purification kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. The samples were dried and resuspended in 26 µL hybridization buffer (50% formamide, 5× SSC, and 0.1% SDS), 20 µg human COT1-DNA, and 20 µg poly(A) DNA, and the mixture was denatured by heating at 95°C for 3 min and snap-cooled on ice for 30 s. The cDNA microarrays containing more than 32 000 PCR-amplified human cDNA clones (Research Genetics, Huntsville, AL, USA) together with negative controls from *Arabidopsis thaliana* were printed, and hybridizations were performed as previously described (15). All experiments were performed in duplicates.

Slides were scanned using a GenePix™ 4000 scanner, and the images were analyzed using the GenePix Pro 3.0 software (both from Axon Instruments, Union City, CA, USA). The inspection of negative controls and a quality-control report generated for each hybridization ensured sequence-specific hybridizations overall, low background levels, and an even hybridization quality on all parts of the array. The final editing of the grid used for signal calculations and the removal of microarray elements lacking a signal and elements with aberrant spot morphology were performed manually. The resulting signal intensity data were checked for quality. A median signal higher than the local median background plus two times the standard deviation in at least one of the channels in both duplicates was required for an element to be considered for the subsequent analysis. For elements in which one of the channels yielded a high signal and the other was below the threshold, the low signal intensity was set to the threshold value (the local background plus two times the standard deviation) to avoid negative ratios. The median normalization factors generated by GenePix were used for data normalization. A 2-fold signal ratio in individual

experiments was required to determine element over- or underrepresentation, and the results were required to be consistent between duplicates (i.e., an element should be identified as differentially expressed in both experiments to be considered). Further analyses and comparisons of the data were performed using a beta version of the gene expression analysis software, GeneWeaver™ (Visual Bioinformatics, Stockholm, Sweden), which uses the GenBank® clone accession number to map the elements on the microarray to the entries in the UniGene database (<http://www.ncbi.nlm.nih.gov/UniGene>) and provides links to further functional annotation on the genes and gene products.

Real-Time PCR Verification of Microarray Analysis

Gene-specific real-time PCR primers for 32 microarray elements indicating differential expression were designed using the PrimerExpress™ software (Applied Biosystems, Foster City, CA, USA). The analysis was performed in triplicates, using the *DpnII*-cleaved cDNA (diluted 1:5) as template and the β-glucuronidase gene as an internal standard. A PCR master mixture was prepared using SYBR® Green PCR Core Reagents (Applied Biosystems) and aliquoted into microplate wells together with 2 µL template and 10 pmol each primer for a final volume of 50 µL per reaction. The iCycler iQ™ Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) was used for PCR and the detection of the fluorescent signal. For data evaluation, the comparative cycle threshold (C_T) method was used, and the level of significance was set to a 2-fold relative difference between samples ($C_T < 0.5$ or $C_T > 2.0$ for down-regulated and up-regulated gene fragments, respectively).

RESULTS

Analysis of RDA Product Diversity

We have previously reported on the isolation of differentially expressed genes during the atherosclerosis-related foam cell formation using an RDA

strategy (1). The aim was to compare the gene expression in macrophages and foam cells (macrophages treated with oxidized LDL). To isolate both the up- and down-regulated gene fragments, the subtractions were performed in successive rounds with both cell types used as tester and driver. A large number of distinct gene fragments were present in the resulting subtraction products (difference products), as determined by random sequencing. Sampling of more than 300 sequences per subtraction product yielded between 130 and 250 independent sequences per data set, which indicated that a significant number of fragments remained undiscovered in the difference products. We have further analyzed the difference products by performing microarray analysis. The 32 448-element microarrays were constructed using 32 256 unique cDNA clones from the Research Genetics sequence-verified human clone collection and 192 negative controls that represented genes from *A. thaliana*. The RDA starting material (representations) and the two successive difference products were fluorescently labeled by the incorporation of labeled dCTPs into the amplification steps (Figure 1A). The amplification had been optimized with regard to template concentration and number of cycles to maximize PCR product yield without adversely affecting the size range of the DNA fragments (5). The labeled PCR products were then used in microarray hybridizations to compare element presence in the two representations and difference products from the two rounds of RDA subtractive hybridizations. Because all labeled samples were amplified at least once before microarray hybridization, the reproducibility between experiments was investigated by comparing ratios in measured duplicate experiments (Figure 2). Approximately 9% of the elements in the hybridized representations have ratios that vary more than 2-fold between duplicate experiments (Figure 2A), as compared with 13% in the hybridizations of Dp2, which had been subjected to two additional rounds of amplification (Figure 2B). To determine the statistical significance of these results, the $\log_2$ (ratio1/ratio2) was calculated for each element, and the distribution of the values was determined.

The 95% confidence intervals were found to be between -1.28 and 1.13 for the representations and between -1.61 and 1.56 for Dp2 (data not shown).

Visual inspection of the scanned images showed that the representations gave images resembling those expected from RNA hybridizations, with a high percentage of elements giving a detectable signal and a high number of equally expressed elements (Figure 1B). When the hybridization of a representation and its first difference product are compared, the disappearance of a large number of elements after subtraction is clearly seen. A significantly

smaller number of elements yield a signal and, of these, the majority was identified as being differentially expressed in the subtracted samples (Figure 1C).

The hybridization data were used to generate scatter plots of signal intensities of each step in the RDA procedure (Figure 3, A–C). The elements from duplicate experiments with a signal intensity lower than the local background plus two standard deviations in both channels were disregarded, and a 2-fold ratio in the normalized median signal intensity was used as a cut off in the sorting of elements into over- or underrepresented in either sample. The relative enrichment and depletion of gene fragments during

Figure 1. Labeling of targets for microarray hybridization performed through the addition of fluorescently labeled dCTPs in the RDA amplification steps. (A) The labeled representations and successive difference products were then used for pairwise microarray comparisons of samples enriched for genes up- and down-regulated by oxidized LDL, respectively. Typical hybridization patterns on the same 19×19 grid area after hybridization with (B) nonsubtracted and (C) subtracted (once) cDNA populations on a global microarray. This initial visual comparison reveals that, after RDA subtraction, there is an overall decrease in the number of elements yielding a detectable signal on the array.

Figure 2. $\log_2$ ratios from duplicate experiments plotted to investigate the reproducibility in the RDA amplification steps. Comparisons between (A) the representations and (B) Dp2 were performed, and the frequency of the elements varying more than 2-fold, indicated by the dash, is shown to be slightly higher in the Dp2 (13%) than in the representations (9%).

subsequent subtraction cycles using data from experiments was analyzed (Figure 3D). The total number of measurable elements above background was 9100 in the representations, 5324 in Dp1, and 3391 in Dp2. The relative frequencies of differentially expressed elements were

18% in the representations, 86% in Dp1, and 88% in Dp2.

To compare the number of shared genes between the representation and the subtraction cycles and to identify the total number of differentially expressed genes, we performed a Venn di-

agram analysis (Figure 4) of the UniGene-mapped expression data. Initially, we compared nondifferentially expressed genes (Figure 4A). Of the 5343 genes, 85% were found to be present exclusively in the representation, an additional 7% were shared with the first difference product and less than 4% with the second difference product. Approximately 6% of the nondifferentially expressed genes were present in the second difference product, indicating the level of experimental noise. We then compared genes showing differential expression by 2-fold or more (Figure 4B), and a total of 4577 genes were found in this category. Seventy-eight percent of the differentially expressed genes were found exclusively in the difference products, while only 10% were found only in the representation. This estimate may reflect the total difference in gene expression in this experimental system. The relatively small number of genes shared between cycles probably reflects differences in the subtraction stringency between subtractions. The first subtraction had an 100-fold excess of driver to tester, while the second subtraction used a 800-fold excess of driver-to-tester ratio.

Gene Function and Real-Time PCR Verification

Mapping of the microarray elements to UniGene entries allowed for the identification and real-time PCR confirmation of 32 gene fragments that either appear exclusively or are shared between subtractions and that were considered of high interest to the study of macrophage differentiation in atherosclerosis (Table 1). Twenty-one of the selected genes could be confirmed by analysis of the original non-amplified cDNA, six were below the cut off for differential expression (2-fold difference between samples), and five gave conflicting results in the real-time PCR.

DISCUSSION

Here we have evaluated cDNA microarrays as a tool to monitor and describe the differential expression in a biological system using material obtained from a cDNA RDA. Several reports

Figure 3. Scatter plots of the Cy5 (y-axis) and Cy3 (x-axis) signal intensities for each element in the individual hybridizations analyzed. Examples of scatter plots are shown for the successive hybridizations with (A) representations and (B and C) Dp1 and Dp2, respectively. The comparison provides an overview of the elements' distribution of up-regulated (green), down-regulated (red), and nondifferentially expressed (yellow) elements during the RDA. (D) The distribution of the elements that were identified in duplicate assays to be underrepresented, overrepresented, and nondifferentially expressed in the oxidized LDL-treated data set are shown. A 2-fold signal ratio was used as a cut off to define the differential expression.

have previously shown that cDNA RDA is a relatively reliable method to obtain differentially expressed genes. It is clear that the amplification in the protocol is one of the critical steps in achieving usable information. Thus, the initial titration of the number of amplification cycles is essential. In this study, we showed that the amplification was performed robustly, with a relatively small fraction of spurious amplification products. The labeling was achieved by the same amplification procedure used to generate the representations and the difference products. The scatter plots shown in Figure 2 compare two experiments (two separate amplifications) and demonstrate the expected linear regression curve and the 2-fold interval used in this study to define differential expression. The distribution of signal ratios in cluster around the $\log_2$ ratio of 0 for the representations (Figure 2A) is more scattered along the diagonal, with a

somewhat depleted area around the $\log_2$ ratio of 0 for the Dp2 hybridizations (Figure 2B). This could be an indication of successful RDA subtractions. The total number of differentially expressed elements, identified as those present in the

RDA data sets, was approximately 5000 in the first difference product and 3000 in the second difference product. A similar estimate of 3969 ± 400 differentially expressed gene fragments was obtained using a complementary sample coverage

Figure 4. The overlap is shown between representations (Repr), Dp1, and Dp2 and includes elements with matches in UniGene. The nondifferential portion of the signals, a total of 5343 genes, is represented in part A and the 4577 differentially expressed genes (both up- and down-regulated) are shown in part B.

Table 1. Real-Time PCR Verification

Gene	Sequence (GenBank Accession Nos.)	Under- or Overexpression in Microarray Hybridization (ratio)			Real-Time RT-PCR (C _T)
		Representations	Dp1	Dp2	
ABC1, member 3	H29521	—	↓ (5.29)	↓ (8.74)	↓
ABC1, member 4	AI335447	—	↓ (5.5)	N.S.	↓
ABC1, member 8	AA634308	—	↓ (6.93)	↓ (5.69)	↓
Adipose differentiation-related protein	AA700054	—	↑ (0.39)	N.S.	↑
Apolipoprotein B	H93837	↑ (0.17)	↑ (0.001)	↑ (0.04)	—
Carboxypeptidase D	AA428209	↓ (3.92)	↓ (10.59)	↓ (16.21)	↓
CCAAT/α enhancer binding protein	AI669881	N.S.	N.S.	↑ (0.09)	↓
CD14 antigen	AA701476	N.S.	—	↑ (0.23)	↓
CD34 antigen	AA434483	—	↑ (0.02)	↑ (0.10)	↓
Clathrin (Light Polypeptide)	AA055475	—	↑ (0.13)	↑ (0.07)	↑
Coagulation factor VII	AI247880	N.S.	↑ (0.15)	N.S.	—
Cofactor required for Sp1 transcriptional activation, subunit 2	R40567	↑ (0.28)	↑ (0.003)	↑ (0.20)	↓
Cofactor required for Sp1 transcriptional activation, subunit 9	AA459244	↑ (0.36)	↑ (0.06)	—	—
Dimethylarginine dimethylaminohydrolase 1	AA478470	↑ (0.33)	↑ (0.04)	↑ (0.22)	—
FLJ20303	N24538	—	↓ (45.69)	↓ (14.14)	↓
Granulin	AA496452	N.S.	↑ (0.26)	↑ (0.16)	↑
L3 pigment	AA699770	N.S.	↑ (0.06)	↑ (0.06)	↑
Nuclear receptor 0B1, (DAX1)	AI681514	↑ (0.46)	—	—	—
Nuclear receptor 1D1, (REVERB α)	AA453202	—	↑ (0.24)	—	↑
Nuclear receptor 1H2, (LXR β)	AA629265	↓ (0.50)	—	↓ (28.39)	↓
Nuclear receptor 2C2, (TR4)	AI218732	—	↓ (5.15)	—	↓
Nuclear receptor 3C1, (GR)	AA664219	—	↓ (9.13)	—	↓
Nuclear receptor 5A2, (LRH-1)	N59115	—	↓ (26.40)	↓ (9.70)	↓
Nuclear receptor 4A2, (NURR1)	AA598611	—	↑ (0.25)	↑ (0.25)	↓
Phospholipase A2	R32409	N.S.	↓ (5.39)	N.S.	↓
Phospholipase A2 activating protein	AI675369	—	↓ (6.42)	N.S.	↓
Prosaposin	N72215	—	↓ (3.84)	↓ (4.61)	↓
Retinoblastoma binding protein 2	AA460756	—	↓ (5.05)	↓ (6.03)	↓
Transcription factor CA150	AA045180	—	↓ (2.57)	↓ (9.96)	—
Vascular cell adhesion molecule 1 (VCAM1)	H07071	↑ (0.39)	↑ (0.15)	↑ (0.17)	↑
Vascular endothelial growth factor B (VEGF B)	AA630120	—	↓ (2.72)	↓ (12.54)	↓
Vascular endothelial growth factor C (VEGF C)	H07991	↑ (0.40)	↑ (0.23)	↑ (0.39)	↑

↓ down-regulation by oxidized LDL and ↑ up-regulation by oxidized LDL. The dash (—) symbolizes a hybridization ratio (Cy5/Cy3) or an RT-PCR measured fold expression difference between 0.5 and 2.0.
N.S., no significant signal

analysis (8) of the previous RDA sequence data sets (data not shown). These are relatively high numbers compared to previous reports using SAGE, which indicated gene expression differences between a normal and tumor cell to be slightly more than 500 transcripts (25) and could either indicate that the difference products still contained a background of spurious fragments or that the RDA approach is more sensitive for identifying rare, but differentially expressed genes. The false-positive rate was approximately 15% (5 in 32), as detected with real-time PCR. This discrepancy may be due to inherent features in the method, although previous RDA experiments have indicated a false-positive rate of less than 5% (5,19). Alternatively, the RDA fragments may not be directly comparable to the sequence of the spotted cDNA elements because alternate splicing may play a role. The widely used data normalization strategies may also be difficult to use with difference products in which the enrichment of extreme spots with high signals in one channel and “no” signal in the other may skew the results. However, our results indicate that the use of duplicates to identify differently expressed genes can eliminate false negatives and suggest that, as a minimum, duplicate assays should be performed. Nevertheless, care should be taken to confirm results with real-time PCR or Northern blot analysis and to sequence-verify the spotted cDNA elements.

The disadvantage of using microarrays is that the identification of novel transcripts, the original goal of RDA, will not be possible because the genes and gene fragments that are not represented on these arrays will not be seen. A previous study using this specific model system, based on the random sequencing of 2000 RDA difference product fragments, identified 921 differentially expressed gene transcripts (1), of which only 400 were represented on the microarray we used. This indicates the compromise between surveying many gene transcripts on a global microarray assay compared to obtaining fewer, but potentially novel transcripts, by the relatively tedious sequencing of RDA products. Of the 400 RDA transcripts present on the microarray, 210 resulted in a significant

signal. Missing signals are likely to result when the RDA gene fragments (derived from regions between two *DpnII* restriction sites in the cDNA sequence) do not overlap with the spotted cDNA sequence or if the spotted cDNA corresponds to a splice variant.

The redundant microarray clone collection used in this study includes 23 178 distinct UniGene (release 146) entries and 2186 unknowns. The relatively large coverage of the human transcriptome represented on these microarrays allowed for the extraction of much additional biological information contained within the RDA difference products, as compared to the previous sequencing of 2000 gene fragments from the difference products. Of special interest is the observed differential regulation of several members of the family of nuclear receptors. The nuclear receptor gene family members are ligand-induced transcription factors, of which several have been demonstrated to regulate different pathways involved in lipid homeostasis (9,18,20,22).

One of the more well-studied lipid activated transcription factors is the peroxisome proliferator-activated receptor- γ (PPAR γ), which has been shown to induce a number of genes involved in lipid metabolism and storage. Here we see the effects of PPAR γ on a gene regulatory level as the up-regulation of CD36 (accession no. N39161) and adipose differentiation-related protein (accession no. AA700054). We also observe an up-regulation of RXR β (accession no. H422-47) implicated in PPAR γ activation as well as the differential expression of several other nuclear receptors. Up-regulation was observed for the nuclear receptors REVERB α (NR1D1; no. AA453202), MR (NR3C2; no. AA447079), NURR1 (NR4A2; no. AA598611), and DAX1 (NR0B1; no. AI681514), whereas the more well-studied LXR β (NR1H2; no. AA629265), which is known to heterodimerize with RXR and mediate the effects on lipid metabolism, was indicated to be down-regulated together with the nuclear receptors TR4 (NR2C2; no. AI218732), GR (NR3C1; no. AA664219), LRH1 (NR5A2; no. N59115), and SHP (NR0B2; no. W90085). The individual roles of the majority of these receptors in the oxidized LDL metabolism remain to be investigated.

The RXR/LXR-mediated effects on lipid homeostasis include the activation of the ATP-binding cassette protein, gene subfamily A (ABC1), which is a group of lipid transporter proteins responsible for sterol influx and efflux in macrophages (20). The elucidation of the ABC1 gene function attracted much attention when discovered recently, since the malfunction of this protein could explain the symptoms in Tangiers disease (3,6,17). The inefficient efflux of cholesterol from the cells results in very low cholesterol levels in the plasma, accompanied by the accumulation of cholesterol particles inside cells, which leads to a foam cell-like phenotype. The ABC1 members analyzed in this study have previously been shown to be induced by acetylated LDL (acLDL) in macrophages (21). The down-regulation in response to oxidized LDL that we observed (Table 1) is in accordance with the simultaneous down-regulation of their regulator LXL and may contribute to the lipid-laden phenotype observed in the foam cells of the THP-1 model system, in which the efflux of cholesterol is clearly insufficient. Compared to the study by Schmitz et al. (21), the discrepancy, seen as opposite gene expression regulation of ABC1, could be explained by differences in the acLDL and oxidized LDL lipoprotein composition and may be the consequence of the depletion of ATP after the 24-h exposure to highly oxidized LDL. In macrophages, there is a hierarchy in which cellular functions are shut down when energy supply decreases (7). The initial expected induction of ABC1 activity, a process likely to be largely dependent on free ATP, would therefore be one of the first mechanisms to be shut down in response to energy deficiency, resulting in the disease cellular phenotype.

Here we were able to detect the differential expression of several nuclear receptors, a group of genes with assumed low abundance and high importance in a number of gene regulatory pathways, including atherogenic processes. The results are supported by the literature because a few of these receptors and their downstream targets discussed previously have already been demonstrated in macrophage response to oxidized LDL, but the majority of

these receptors were only recently discovered. The appearance of these receptors in this study strongly suggests their implications in lipid metabolism and encourages further investigation of their functional roles and ligand specificities. In conclusion, the combination of RDA followed by global microarray analysis allows for the efficient analysis of gene expression at different abundance levels, which yield starting points for studies of regulatory mechanisms in model systems. Future developments will yield an even more comprehensive view of the gene expression attained by microarrays, comprising full-length cDNA clones of the entire transcriptome.

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