Ci/mmol) and 10 nM circular pTEC100, a pUC19 derivative containing a single T7 consensus promoter, which served as template. After different incubation times ranging from 0 (background value) to 3600 s after enzyme addition, parallel aliquots were subjected to Whatman 3MM paper (Clifton, NJ, USA) and either dried (total amount of radioactivity present in the aliquot) or incubated for 30 min in 10% TCA, washed twice in 5% TCA and washed once in ethanol (incorporated radioactivity). The radioactivity retained on the filters was counted in an LSC device (Model LS6000LL; Beckman Instruments, Fullerton, CA, USA).

In this assay, the specific activity was 613 U/mg, 1 U corresponding to the amount of enzyme that incorporates 1 nmol [α-32P]UTP in TCA-precipitable form in 1 h. The specific activity of the 6-histidine-tagged T7 RNA polymerase was 1.7 times higher than the specific activity of commercially available T7 RNA polymerase (MBI Fermentas, Vilnius, Lithuania), which had been tested in the same assay. Additionally, in vitro run-off transcription experiments using the fusion protein resulted in the same product pattern as with the commercially available polymerase, indicating that the 6-histidine tag did not influence the activity of the enzyme. The total amount of pure RNA polymerase isolated from a 3-L culture was 32.62 mg or 2 × 107 U.

As established by incubation of labeled double-stranded and single-stranded DNA with 3 U/mL (final concentration) of the polymerase preparation for one hour at 37°C in transcription buffer and by the specific product pattern of in vitro transcription reactions, the enzyme was virtually free of disturbing nuclease activities and could serve as RNA polymerase in 3SR reactions in which RNA and DNA intermediates occur (4,5). The addition of DTT to a final concentration of 1 mM every 6 months allows the long-term storage of the preparation over more than one year with no detectable loss of activity.

REFERENCES

We thank Prof. F.W. Studier for the generous gift of the T7 RNA polymerase overexpression system and A. Wagenhaus and A. Heller for their technical assistance. This work was supported by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie Grant No. 0310799. Address correspondence to Dr. Thomas Ellinger, IMB Jena, Dept. Molecular Information Processing, Beutenbergstrasse 11, 07745 Jena, Germany. Internet: ellinger@imb-jena.de

Received 8 December 1997; accepted 23 February 1998.

Thomas Ellinger and Ralf Ehricht
Institute of Molecular Biotechnology
Jena, Germany

Differential mRNA Display at the Single-Cell Level


The differential mRNA display technique represents a powerful tool for the identification of genes that are over- or underexpressed in one cell population relative to another (5). To perform differential mRNA display, total RNA is isolated from the two cell populations to be compared, and first-strand copies of both RNAs are made by reverse transcription polymerase chain reaction (RT-PCR) amplification. Not only known differentially expressed genes are detected by this approach, but also previously unknown genes can be detected using short, random PCR primers. Differential mRNA display has already been successfully applied for the identification of genes that are differentially expressed in patients with malignancies (e.g., brain tumors), heart disease and diabetes (6). In the mammary gland, several cDNA fragments have been characterized that are up-regulated in tumor tissue in comparison to normal tissue, with the role of the respective genes in tumorigenesis remaining undetermined so far (4). A disadvantage of the differential display technique is the fact that whole-tissue material obtained from malignant tissues does not consist exclusively of tumor cells but is always contaminated by a variety of nonmalignant cells, such as fibroblasts and peripheral blood cells. Therefore, the use of this technique by itself does not allow for any conclusions regarding the cellular origin of differentially expressed genes because they can not be attributed to a defined cellular subpopulation of the tissue from which the amplified cDNA had been made. To circumvent these limitations, we modified the mRNA display technique for single cells. Single cells can be picked under defined conditions (3) and serve as a distinct source for the isolation and PCR amplification of mRNA. Single-cell PCR has facilitated the characterization of tumor cells, which make up a minority of involved tissue; e.g., single Hodgkin and
for fluorescence detection. Five CD3 streptavidin-phycoerythrin (1:100; Di-
mL; Dako, Hamburg, Germany), and
cDNA generation, 2
as recommended by the manufacturer
with immobilized DNase I (MoBiTec)
(NP40). DNA digestion was performed
oligo(dT)
min with 200 U (1
action) in reaction buffer (0.04 M Tris-
cells were incubated with biotinylated
analysis. Then, resting and activated T

tehesis was performed at 37
upstream primer (10 mM, 1
TCTCAT and TCTGGAGGCA) for
was performed as described by Bauer

tion and one of the four decamers (CT-
was cloned into pCR
scribed above. Purified cDNA frag-
ment covering a part of the coding
region of the T-cell receptor (TCR)-as-
associated ζ chain was used as control for
equal RNA loading per lane.

Five CD3+ resting or activated T
cells were picked from cell suspensions
in triplicate. Five cells served as the
source for each cDNA because dilution

Figure 1. Differential mRNA display pattern
in activated T cells. One (left) or five (right) acti-
ated CD3+ T cells were picked, and the cDNA
expression was analyzed by differential mRNA
display. Five cells were chosen for analysis in
further experiments because they provided a
more homogeneous cDNA expression pattern.
experiments had shown that five cells of a given cell type are the minimal cell number that provides a homogeneous and reproducible cDNA expression pattern (Figure 1). Integritiy of the generated cDNA was checked by β-actin PCR for all samples as described (10). Differential display was performed with one of the four decamer primers. Three cDNA fragments (F1–F3) were isolated because of their expression restricted to activated T cells. The differential expression pattern was confirmed by Northern blot analysis of T cells that had been purified by magnetic cell sorting. All genes that had been identified by single-cell differential display were shown to be strongly up-regulated because of their expression restricted to activated T cells. The differential expression pattern was confirmed by single-cell differential display and cloning of messenger RNAs from human breast cancer versus mammary epithelial cells. Cancer Res. 52:6966-6968.


The authors thank Birgit Bette and Natalie Fadle for expert technical assistance. This work was supported in part by Grants No. Re 1271/1-1 from the Deutsche Forschungsgemeinschaft to C.R. and No. 10-0918 Tr 2 by the Deutsche Krebshilfe to L.T. Address correspondence to Dr. Christoph Renner, Med. Klinik I, Universität des Saarlandes, D-66421 Homburg, Germany. Internet: inren@med-rz.uni-sb.de

Received 9 June 1997; accepted 18 February 1998.

Universität des Saarlandes
Homburg, Germany

REFERENCES


5. Liang, P. and A.B. Pardee. 1992. Differential mRNA display has the important advantage that it allows for the attribution of the identified genes to defined cellular subpopulations because the cells that serve as the source for the mRNA are picked from cell suspensions under the fluorescence microscope after staining with specific antibodies. Single-cell differential mRNA display is therefore an important step forward in the molecular analysis of single cells of a defined origin. Single-cell differential display will be especially valuable where minor subpopulations within a diseased tissue are to be analyzed at the RNA level. It can be used to study a large spectrum of inflammatory diseases as well as malignant disorders in which it not only allows for the comparison of the malignant cell with its benign counterpart of origin but also between defined cellular subpopulations of a given tumor, e.g., cells at the primary site and in different metastases. Single-cell differential display should therefore be helpful in the elucidation of molecular mechanisms that are involved in tumorigenesis or metastasis.