Isolation of poly-A(+)-RNA from Single Cells Using the mRNA Capture Kit

Hans-Werner Breiner[1], Martin C. Simon[1,], and Helmut J. Schmidt*
Division of Ecology, Research Group Molecular Ecology
University of Kaiserslautern, Kaiserslautern, Germany
**Corresponding author: msimon@rhrk.uni-kl.de*
[1]Authors HWB and MCS contributed equally to this work and are listed in alphabetical order


Martin C. Simon

Introduction

The genome of the free-living ciliate *Paramecium* contains a multigene family, each member coding for a high molecular weight protein of 250–300 kDa. These proteins are called surface antigens, because they are located on the outer membrane of the protist and are fixed by glycosylphosphatidylinositol (GPI) anchors [1].

Paramecium has the ability to exhibit alternative types of surface antigens on its cell surface. In general, only one gene of the whole gene family is expressed, and the protein layer on the cell surface consists of a single protein species called a serotype. The comparison of these proteins with surface antigens of parasitic protists such as *Plasmodium* reveals several protein families that share the presence of internal tandem repeats and a conserved cysteine periodicity. Furthermore, the exclusive expression of only one type of surface antigen at constant environmental conditions is a common feature in protists. Whereas the reason for serotype expression in *Paramecium* is not understood, parasitic protists use serotype-shifting as a defense mechanism against the host's immune system. Serotypes also seem to be essential in *Paramecium*: In deletion mutants and in RNAi knockdown experiments alternative serotypes are expressed [2], (Simon, unpublished).

Surface antigen expression can be easily detected in *Paramecium* on the protein level by the addition of corresponding antisera to the culture medium. However, this traditional immunological test cannot be used in *Paramecium tetraurelia*, as there are cross-reactions between several serotypes (for reviews see [3, 4]). As an alternative approach, we used the mRNA Capture Kit to isolate mRNA from a single *Paramecium* cell in order to detect serotype-specific transcripts using RT-PCR.

Materials and Methods

Cell culture and serotype shifting

Strains 51-d48 (*P. tetraurelia*), 60 and 156 (*P. primaurelia*) were cultured in wheat grass powder (WGP) medium inoculated with *Klebsiella minuta* and supplemented with β-sitosterol (0.8 mg/l). The culture media of S-expressing stocks 60 and 156 was diluted 1:1 with water (no β-sitosterol) to decrease the fission rate of cells. Cells expressing serotypes 60S and 156S were cultured at 10°C with a fission rate lower than once a day. Serotype shifting from 51H to 51D was induced by rapidly increasing the temperature from 15°C (stable expression of serotype H, *P. tetraurelia* [5]) to 28°C (specific temperature for serotype D [6]). Samples for RNA isolation were taken from the beginning (0 hours) at intervals of three hours.

Slides

Isolation, washing, and lysis were performed in Sonneborn depression slides (glass bars with three separated depressions). The slides were washed three times in chloroform

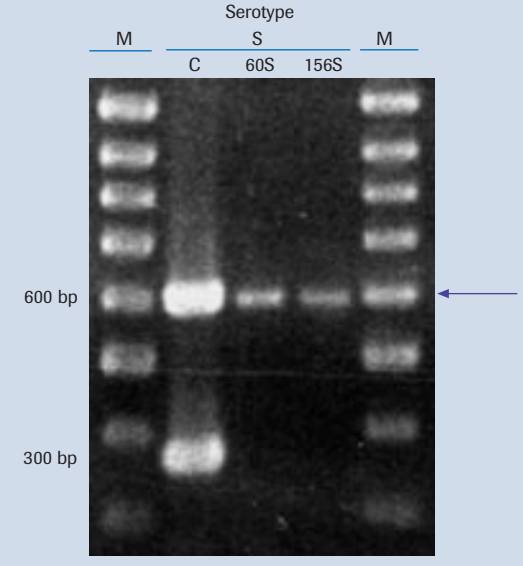

Figure 1: RT-PCR of serotype S-expressing cells of *P. primaurelia*, cultured at 10°C. Three cells were used for template isolation. In each sample an additional primer pair specific for an untranscribed region was used to show possible DNA contaminations. An artificial DNA contamination of 15 ng genomic DNA is responsible for the 300-base pair control fragment in *lane C*. The expression of serotype S is shown by the presence of the 600-base pair internal fragment produced with specific primers.

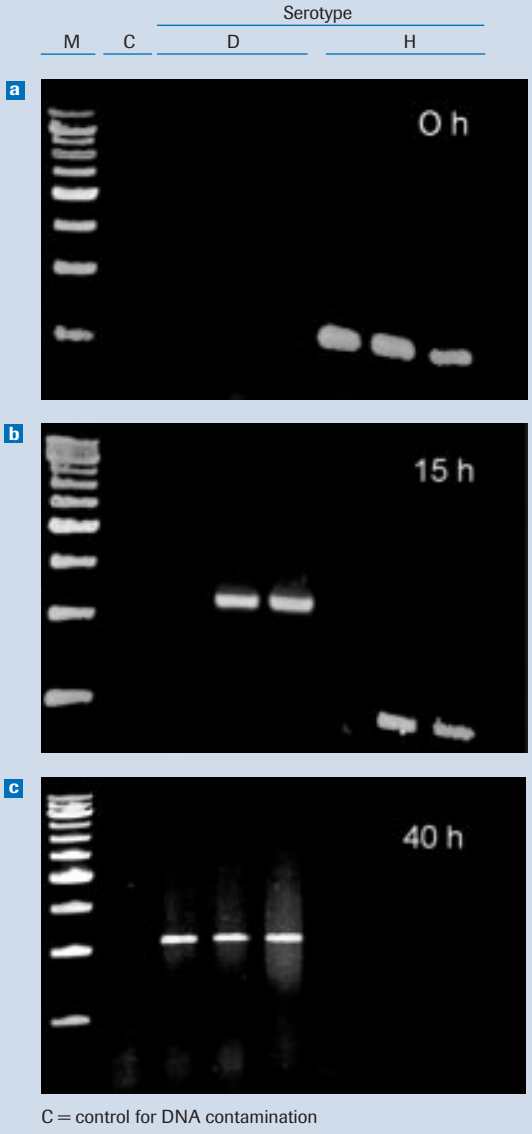

Figure 2: Serotype-shifting in *P. tetraurelia* from serotype H (expressed at 15°C) to D (expressed at 28°C) is demonstrated for a whole culture. A suitable number of single cells were analyzed using specific primers for the two serotype genes. RNA was isolated from single cells of serotype H at 0 hours, 15 hours, and 40 hours after the temperature shift to 28°C (*Lane C* is a control for DNA contamination).

and incubated at 200°C for 48 hours to destroy enzyme contaminations.

Cell isolation

The cell isolation was carried out using a binocular microscope. Living cells were washed three times in sterile-filtered mineral water. Single cells were isolated in a volume of 1-2 µl and placed between two drops of 1 µl 150 mM 1,4-dithiothreitol (DTT) and 0.5 µl RNase Inhibitor (40 units/µl) without mixing the three drops. Mixing was carried out in 47.5 µl lysis buffer of the mRNA Capture Kit.

RNA isolation

DNA was sheared by passing the single cell in lysis buffer several times through a 21-gauge needle. Subsequently, 0.2 µl (0.02 nmol) biotin-labeled oligo dT probe of the kit was added to the mixture and incubated for 10 minutes at 37°C for hybridization of the probe with the poly(A) tail of mRNA. After being transferred to a streptavidin-coated PCR tube, the sample was incubated again to immobilize the captured mRNA. Finally, three washing steps were performed using 250 µl ice-cold washing buffer.

Results and Discussion

Gene detection

Using the mRNA capturing strategy, we were able to isolate sufficient template RNA from a single *Paramecium* cell for RT-PCR analysis. The ability to analyze single cells is extremely useful, as northern blotting allows only the description of the status of a whole cell culture, whereas individual cells can differ in their expression behavior.

We analyzed *Paramecium* for the expression of surface antigens, which is strongly influenced by environmental conditions such as cultivation temperature. It is particularly difficult to detect gene expression at the RNA level at low temperatures due to the low metabolic rate. Therefore, an extremely sensitive system is required.

We were able to show the expression of a previously unknown gene coding for serotype S of *P. primaurelia.* After isolating the putative gene from macronuclear DNA, we linked its expression to the emergence of the S protein, which was detected simultaneously by indirect immunofluorescent staining (data not shown). Serotype S was characterized as a low-temperature serotype. At the same time we found that the division rate of the culture has more influence on serotype expression than expected. Using the mRNA Capture Kit, we were able to isolate mRNA for serotype S from starving cells cultured at low temperatures. The 600-base pair internal fragment is shown in Figure 1. Absence of the 300-base pair control fragment in each sample shows quantitative removal of DNA during mRNA isolation.

With the gene for serotype S the sequence data for regularly expressed serotypes of *P. primaurelia* is now complete. Sequence comparison with other gene family members demonstrated that only the central parts – built of nearly perfect tandem repeats – carry the information for putative epitopes that are responsible for immunological detection. This is interesting for understanding the role of tandem repeats in serotype function.

Serotype shifting

Subsequent serotype-shifting experiments with *P. tetraurelia* showed that in spite of the relatively early appearance of "new" mRNA, translation and protein processing took more time than reorganization of gene expression. Whereas mRNA of the new serotype was detected within 12–15 hours after triggering (Figure 2), the protein was