Detection of telomerase activity by combination of TRAP method and scintillation proximity assay (SPA)

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Numerous studies on telomerase, an enzyme which can elongate telomere ends (1,2), led to the increasing evidence that the presence of telomerase in cells that normally lack it may contribute to the uncontrolled cell growth of cancer (3–6). Those findings, together with the fact that the enzyme is not active in normal cells (7), make it an interesting target for cancer drugs, as well as an important marker for diagnostic of cancer. For these reasons, establishment of a high-throughput assay system would constitute a potent tool for screening large number of compounds or clinical samples.

A recently developed TRAP (telomeric repeat amplification protocol) assay, allowing amplification of the telomerase reaction product by PCR has considerably improved the conventional assay, in term of speed and sensitivity (8,9). However, this method still involves the analysis of 32P-labeled reaction products by polyacrylamide gel electrophoresis, which limits the number of samples to be examined, and presents problems related to 32P handling. Modifications such as introduction of an internal standard (10) have improved the reliability of the assay. However, quantification of telomerase activity from band intensities on gel patterns still remains a tedious process and reproducibility often depends on the operator.

In order to alleviate these inconveniences, we have constructed a system based on the combination of TRAP assay and scintillation proximity assay technology (SPA) developed by Amersham (11). A 5′-end biotinylated oligonucleotide is used as primer for amplification of the telomerase reaction product in the presence of tritiated thymidine. Upon addition of streptavidin coated fluorospheres, the biotinylated 3H-labeled product will bind to the beads which contain a scintillant stimulated by the close proximity of β-emitters. The incorporated tritiated nucleotides are thus able to stimulate the scintillant and produce a signal, whereas the unincorporated free nucleotides, whose energy is absorbed in the assay buffer, cannot.

Here, we describe the adaptation of conventional TRAP assay to SPA and show the possibility of using this system for high-throughput screening.

Extracts from human erythroleukemia cells (HEL) were prepared by the CHAPS detergent method as described previously (8), the lysate adjusted to 5 × 10^3 cells/µl was clarified by centrifugation for 30 min at 15 000 r.p.m. and the supernatant was divided into small aliquots and stored at –80°C. Telomerase assays were based on the one-tube TRAP method previously described (8,9) with some modifications. For detection of the reaction products by SPA, TRAP assay was modified as follows:

CX primer was replaced by its 5′-biotinylated version (Biot-CX), dATP, dCTP and dGTP concentration was kept at 50 µM, dTTP reduced to 2 µM for the standard assay and [α-32P]dTTP was replaced by 2 µCi [Me-3H]TTP (Amersham, 114 Ci/mmol). Other components remained unchanged except that T4g32 protein was usually omitted. In addition, in the case of larger number of samples, assays were performed in 96-well plates, without wax barrier, and Biot-CX was added just before starting amplification for 31 cycles in an oil-free thermal cycler. Reaction products (40 µl) were mixed with 50 µl of streptavidin-coated fluorospheres (1:4 solution in 0.56 M EDTA) and incubated for 10 min at 37°C to allow binding of the biotinylated, 3H-labeled reaction products to the streptavidin beads. Plates were then counted on a MicroBeta scintillation counter (Wallac).

The linearity and sensitivity of SPA detection was estimated by comparing it with the conventional TRAP assay (TRAP–PAGE) using serial dilutions of HEL CHAPS extracts. The linearity of both methods was comparable and dependent on the amount of protein in the reaction mixture. Whereas, for TRAP–PAGE, the detection limit was ∼100 cells per assay (Fig. 1B), in the case of TRAP–SPA, telomerase activity could be clearly detected with as few as 10 cells (Fig. 1A). Moreover, a very high signal/noise ratio of ∼40:1 was obtained at our standard conditions of 10^6 cells per assay. The incorporation observed corresponded to the amplification of telomerase elongation product since it depended on the presence of cell extracts and was RNase-sensitive, as for TRAP–PAGE. It also depended on the incorporation of the biotinylated primer in the amplification product and could be competed by an excess of non-biotinylated CX primer (data not shown). Similar results were obtained with other cell lines such as multiple myeloma, hepatomas, and Jurkat, all being previously screened by TRAP–PAGE, and the observed incorporation correlated almost perfectly with the respective intensities of band patterns on polyacrylamide gel (Fig. 2). The strongest signal was obtained with multiple myeloma cells in both systems, whereas no activity could be detected in keratinocytes from a primary culture used as control.

Coupling of telomerase product amplification with the scintillation proximity assay leads to an increased speed of assay, since after PCR, SPA detection yields results in <1 h, and two TRAP–SPA can be easily carried out within one day. Moreover, very good sensitivity and specificity, easy quantitation and easy handling of a large number of samples can be achieved in the

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Figure 1. Sensitivity and linearity of SPA detection. Serial dilutions of CHAPS extracts corresponding to $10^5$, $10^4$, $10^3$, $10^2$ and $10^1$ cells or lysis buffer were assayed for their telomerase activity. (A) Assay in the presence of $2 \mu M$ dTTP, $2 \mu Ci \cdot ^{3}H$-TTP and reaction products detected by SPA. Data are representative of at least two independent experiments; (B) assay in the presence of $25 \mu M$ dGTP, $2 \mu Ci \cdot \left[\alpha^{32}P\right]dGTP$ and analysis of reaction products by 10% native PAGE and exposure to imaging plate. For both methods, extract corresponding to $10^4$ cells was pre-incubated for 10 min at room temperature with $1 \mu g$ RNase before adding the nucleotides.

The presence of a low-energy $\beta$ emitter without the need for special containment, and any separation between reaction product and unincorporated radioisotope. Setting of TRAP–SPA parameters in comparison with gel electrophoresis overcomes the disadvantage of a lack of visual confirmation and establishes that the signal corresponds to the specific amplification of telomerase elongation products. We have also recently reduced the chances of non-specific incorporation due to primer–primer interaction by using a CX primer two nucleotides shorter at its 3′ end, rendering it unable to dimerize with the TS primer. Since this assay can be used for the screening of telomerase inhibitors, discrimination from a possible Taq inhibitor is required. Therefore, a control for Taq activity has been constructed that can also be used with SPA (Savoysky, pers. comm.).

Such an assay system is now used routinely in our laboratory for most experiments, in preference to the gel system, and has proved to be highly reproducible and quantitative. It has been adapted to 96-well plates and not only permits the screening of a large number of clinical samples for diagnostic, or natural or chemical compounds for telomerase inhibitors, but also provides a fast screening of active fractions during the steps of telomerase purification. The subsequent availability of a more concentrated enzyme will then allow for a further simplification of telomerase assay, eliminating the need for amplification and yielding results in <2 h.

Figure 2. Detection of telomerase activity of CHAPS extracts from several cell lines, corresponding to $10^4$ cells per assay. (A) Assay in the presence of $2 \mu M$ dTTP, $2 \mu Ci \cdot ^{3}H$-TTP and reaction products detected by SPA. Data are representative of at least two independent experiments; (B) assay in the presence of $25 \mu M$ dGTP, $2 \mu Ci \cdot \left[\alpha^{32}P\right]dGTP$ and analysis of reaction products by 10% native PAGE and exposure to imaging plate.

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