The effects of lectin transformation on cytoplasmic polyadenylated RNA from human lymphocytes

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Summary

The translational activity of cytoplasmic poly(A)+RNA from resting human lymphocytes was approximately 20% of that from phytohemagglutinin-transformed lymphocytes in a rabbit reticulocyte lysate assay. Translation assays in the presence of cap analogues suggested that the mRNA from resting cells was relatively deficient in functional 5′-terminal cap structures. Neither mRNA fraction inhibited the translation of globin mRNA in the cell-free assay, and both preparations were essentially pure as shown by hybridisation with [³H]poly(U). The size distribution and poly(A) tail length of poly(A)+RNA was similar in the resting and transformed cell and both preparations directed the synthesis of peptides of molecular weight 15 000 to 90 000. Two dimensional gels of total proteins from resting and transformed lymphocytes showed predominantly quantitative changes. However cross-hybridising cDNA and mRNA from resting and transformed cells after the common sequences have been removed by hydroxylapatite chromatography showed that about 4% of the cytoplasmic poly(A)+RNA from transformed lymphocytes was not present in resting cells. This difference may result from transformation-specific gene expression.

Introduction

The normal human peripheral blood lymphocyte is a quiescent cell which on exposure to antigens or lectins can be stimulated to enter the mitotic cell cycle. Phytohemagglutinin (PHA) transformation of lymphocytes provides a useful model for investigating the molecular events controlling areas such as the immune response, cell growth and gene expression. Blastogenic transformation of the normally quiescent lymphocyte results in increased protein and nucleic acid synthesis and several studies have been directed at determining the mechanism by which the resting lymphocyte maintains a low level of protein synthesis and how this changes on transformation. These studies have stressed the importance of translation initiation factors (1, 2), of double-stranded RNA content (3), or of processing of 45S ribosomal precursor RNA (4) in regulating protein synthesis during lymphocyte transformation. Other evidence suggests that there is stabilisation of cytoplasmic poly(A)+RNA upon activation with phytohemagglutinin (5, 6).

A possible expectation of transformation is gene activation. However early DNA-RNA hybridisation experiments, using methods by which only highly repetitive sequences could be detected, suggested that resting and transformed lymphocytes possessed similar mRNA species (7, 8). Torelli et al. (9) using more sophisticated cDNA-poly(A)+RNA hybridisations recently suggested that the complexity of total cellular poly(A)+RNA is decreased on lymphocyte transformation (which they considered to be a nuclear event) while many sequences increase in abundance with respect to resting lymphocytes (which they concluded was a cytoplasmic event), but were unable to separately analyse the nuclear and cytoplasmic poly(A)+RNA fractions. Willard and Anderson (10) have shown by two-dimensional gel electrophoresis that lymphocytes do
not produce any unique proteins during the first 18 hr of lectin transformation.

In this study our aim was to determine if there were any differences between the poly(A)$^+$RNA populations present in the cytoplasm of resting and transformed lymphocytes. The cytoplasmic poly-(A)$^+$RNA from resting and transformed cells was examined with respect to size distribution, length of poly(A) tails, translational efficiency and peptide synthesis. Complementary DNA synthesised from cytoplasmic poly(A)$^+$RNA by reverse transcriptase was used in cross-hybridisation experiments to detect subtle differences between the mRNA populations present in resting and transformed cells.

**Methods**

**Lymphocyte culture**

Human peripheral blood lymphocytes were isolated by Ficoll-Paque (Pharmacia) technique from buffy coats supplied by the N.S.W. Red Cross Blood Transfusion Service. Cells were cultured at a density of $10^6$ per ml in RPMI 1640 medium containing Hepes buffer 20 mM pH 7.6, sodium bicarbonate 25 mM, penicillin 50 000 units per litre, streptomycin 50 000 units per litre, streptomycin 50 mg per litre and 10% fetal calf serum (dialysed against 0.9% saline for one week and heated at 60 °C for 5 hr). The cells were transformed by the addition of purified phytohemagglutinin (PHA16, Burroughs Wellcome) 0.5 µg per ml. Cell culture was carried out at 37 °C in sealed 250 ml (75 cm² side surface area) angle-neck flasks (Corning) for 72 hours. Cells were harvested by centrifugation at room temperature at 100 × g for 10 min. Resting cells were either isolated directly from peripheral blood or after culture in the absence of phytohemagglutinin.

**Isolation of total cellular RNA**

Lymphocytes were homogenised by 10 strokes of a Dounce homogeniser in 7.8 M guanidine HCl pH 7.0 containing β-mercaptoethanol 5% (v/v) and sodium lauroyl sarcosine (sarkosyl) 2% (w/v) at a concentration of 10$^8$ cells per ml. The cell homogenate (10 ml) was layered onto a 3 ml cushion containing 5.7 M CsCl and 0.1 M EDTA and centrifuged at 100 000 × g for 20 hr in a Beckman SW-41 Ti rotor (28 500 rpm) at 20 °C (11). The RNA, pelleted on the bottom of the tube, was resolubilised in 7.8 M guanidine HCl, pH 5.0, then precipitated with two volumes of ethanol at −20 °C.

**Isolation of total cytoplasmic RNA**

Lymphocytes were resuspended at 0 °C at a concentration of 2.66 × $10^6$ cells per ml in a buffer containing 20 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM magnesium acetate and 5 mM of each of the four ribonucleoside-vanadyl complexes prepared as described by Berger and Birkenmeier (12). Lysis was then accomplished by adding 0.33 volume of the resuspension buffer supplemented with 5% sucrose (w/v) and 1.2% Triton X-100 (w/v). The cells were allowed to stand at 0 °C for 15 min with occasional gentle stirring. The lysate was then centrifuged at 600 × g for 3 min at 0 °C to remove the nuclei. The supernatant solution was decanted and to this was added an equal volume of 7.8 M guanidine HCl pH 7.0 containing β-mercaptoethanol 5% (v/v) and sarkosyl 2% (w/v). The solution was homogenised and the cytoplasmic RNA isolated by centrifugation through a dense CsCl cushion as described above except that centrifugation was reduced to 100 000 × g for 16 hr.

**Isolation of polysomal RNA**

Polysomal RNA was isolated by the method of Palmiter (13) in the presence of ribonucleoside-vanadyl complex as described by Berger and Birkenmeier (12).

**Isolation of poly(A)$^+$RNA**

A polyadenylated RNA fraction was isolated from the total RNA by two cycles of oligo(dT)-cellulose chromatography similar to published methods (14, 15) except that the RNA was incubated at 60 °C for 15 min in 50% (v/v) formamide followed by rapid cooling in ice prior to chromatography. Sodium chloride was then added to a concentration of 0.5 M and the sample diluted 1 in 5 with a high salt buffer containing 0.5 M NaCl, 0.1% (w/v) sarkosyl, 1 mM EDTA and 10 mM Tris-HCl pH 7.4 for sample loading. Unbound RNA was washed off with the high salt buffer and poly(A)$^+$RNA was eluted with a low salt buffer containing 0.1% sarkosyl, 1 mM EDTA and 10 mM Tris-HCl pH 7.4.