when complexed with GTP binds any aminoacyl-tRNA formed and prevents its hydrolysis (Leder, 1973). An average of 0.97 mol of tRNA\textsuperscript{IIe} was charged per mol of ATP added to the reaction mix.

It may be calculated from the relative reactivity of the isoleucyl-tRNA synthetase towards valine and isoleucine in the presence of tRNA and ATP (Fersht, 1977) and the relative concentrations of valine and isoleucine in the cell (Raunio & Rosenquist, 1970), that 1 mol of ATP is wastefully hydrolysed in rejecting valine for every 16 mol of isoleucyl-tRNA successfully charged. Only 6\% of the ATP is ‘spent’ on editing. This is the most costly situation. For the valyl-tRNA synthetase, only 1 mol of ATP is hydrolysed in rejecting threonine for about 1300 mol of valyl-tRNA formed. The overall cost of editing is thus very low.


---

Deoxyribonucleic Acid Methylases in Normal and Polyoma-Virus-Transformed BHK-21 Cells

ANDREW C. B. CATO and ROY H. BURDON

Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

The ratio of 5-methylcytosine residues to those of cytosine is higher in the DNA of polyoma-virus-transformed BHK-21 cells\* than in the DNA of non-transformed BHK-21 cells (Rubery & Newton, 1973; Nass, 1973; Browne et al., 1976). The 5-methylcytosine residues result from the transfer of methyl groups from S-adenosyl-L-methionine to cytosine residues in DNA, which is catalysed by DNA methylases located in nuclei.

In view of these differences observed between transformed and non-transformed cells, the nuclear DNA methylases from such cells were investigated. Nuclei from BHK-21/PyY (polyoma-transformed BHK cell line) and BHK-21/C13 (non-transformed) cells were prepared by first allowing these cells to swell in RSB medium [10 mm-NaCl/10 mm-Tris/HCl (pH 7.4)/1.5 mm-MgCl\textsubscript{2}] for 10 min. The cells were then resuspended in 1% Tween 80 in water, homogenized, and the resulting nuclei collected by centrifugation. These were washed three times with RSB medium, and suspended in buffer M [50 mm-Tris/HCl (pH 7.8)/1 mm-EDTA/1 mm-dithiothreitol/10% (v/v) glycerol]. This suspension was made 0.4M with respect to NaCl, stirred gently for 15 min at 4°C and then centrifuged for 1 h at 12000g. The resulting supernatant was dialysed extensively against buffer M to yield a preparation henceforth termed ‘nuclear supernatant fraction’.

Nuclear supernatant fractions from both the BHK-21/C13 and BHK-21/PyY cell lines were then used as crude sources of DNA methylase activity to modify cytosine residues in added Escherichia coli DNA. Subsequent analysis by depurination and homochromatography (Browne et al., 1976) of the methylated E. coli DNA produced by these fractions indicated that although the nuclear supernatant fraction from BHK-21/C13 cells was able to methylate cytosine residues in oligonucleotide sequences characteristic of vertebrate DNA (Browne et al., 1976), that fraction from BHK-21/PyY cells showed some differences.

\* Abbreviation: BHK cells, baby-hamster kidney cells.
Further purification of the DNA methylase activity from the nuclear supernatant fractions was performed by (NH₄)₂SO₄ precipitation followed by chromatography on DEAE-cellulose and hydroxyapatite. When the properties of these more extensively purified DNA methylase preparations were examined, differences between the transformed and the non-transformed line were again found.

Whereas concentrations of 0.1 M-NaCl tend to inhibit the ability of the BHK-21/C13-cell preparation to modify E. coli DNA, such a salt concentration stimulated the BHK-21/PyY-cell preparation. Examination of the pattern of oligonucleotide sequences methylated by the BHK-21/PyY-cell preparation were notably different from the characteristic pattern for vertebrate produced by the BHK-21/C13-cell preparation. Only in the presence of 0.1 M-NaCl (i.e. at maximum stimulation) is the BHK-21/PyY-cell preparation able to produce a pattern similar to that characteristic of the BHK-21/C13-cell preparation. However, whether such differences in enzymic properties can be related to the presence or absence of the polyoma genome as part of the BHK-cell genome must await further experimentation.


---

The Relationship Between the Activity of Ribonuclease H and Synthesis of Deoxyribonucleic Acid in Synchronously Growing BHK-21/C13 Cells

PATRICIA M. DUFF and HAMISH M. KEIR

Department of Biochemistry, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

BHK-21/C13 cells grown in culture contain four ribonucleases H distinct from any mitochondrial ribonuclease H. Ribonuclease H activity was separated into two fractions, HA and HB, by chromatography of a cell extract on phosphocellulose. On DEAE-Sephadex A25, ribonuclease HA activity was separated into two fractions, HA1 and HA2. By gel filtration on Sephadex G-100 or on Sepharose 6B, ribonuclease HB activity was separated into two fractions, HB1 and HB2 (Cooper et al., 1974). By using these methods, ribonucleases HA1, HA2, HB1 and HB2 were purified 99-fold, 85-fold, 20-fold and 320-fold respectively. The enzymes had distinct properties and were classified as true ribonucleases H by their abilities to degrade only the RNA component of a RNA–DNA hybrid.

Since the enzymes require a RNA–DNA hybrid as substrate, they have been assigned a postulated biological role in eukaryotic cells either in the regulation of transcription or in the replication of DNA for the removal of RNA primers. The suggested role in replication of DNA was supported by the observation that ribonucleases HB1 and HB2 co-fractionate with BHK-cell DNA-dependent DNA polymerases α and β respectively. Indirect evidence for a function(s) of ribonuclease H in the replication of DNA in vivo may be adduced if a positive correlation between enzyme activity and the rate of DNA synthesis in the cells exists.

BHK-21/C13 cells were grown in culture to the resting or quiescent (G₀) state by the method of serum depletion (Bürk, 1970; Craig et al., 1975). DNA synthesis was monitored by pulse-labelling the cells with [³H]thymidine. The cells were in the G₀-condition after 4 days in the 'low-serum' medium; by this time the rate of synthesis of DNA had declined almost to zero. In general, after an initial increase, the activity of ribonuclease H appeared to decrease only slightly as the cells entered the quiescent state. However, after separation and purification of the four ribonuclease H species, it was found that the activity of ribonuclease HA1 had decreased 13-fold relative to its activity in exponentially growing cells. Although there was very little change in the activities of