Biochem. J. (1981) **198**, 235–237
Printed in Great Britain

Increased phosphorylation of ribosomal protein S6 in hamster fibroblasts transformed by polyoma virus and simian virus 40

Iain M. KENNEDY* and David P. LEADER
Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

(*Received 25 February 1981/Accepted 8 April 1981*)

The extent of phosphorylation of ribosomal protein S6 was compared in normal hamster fibroblasts and in fibroblasts transformed by polyoma virus or simian virus 40. In both strains of transformed cells the protein was more highly phosphorylated than in the normal cells.

In eukaryotic cells under normal conditions the 40S ribosomal subunits contain a single phosphorylated protein, designated S6 (Gressner & Wool, 1974; Leader, 1980). This phosphorylation is evolutionarily highly conserved, but its function is not yet known. Although in certain animal tissues cyclic AMP can cause the degree of phosphorylation of this protein to be increased (Gressner & Wool, 1976; Leader, 1980), extensive phosphorylation of the protein can occur in cultivated cells in which the concentration of cyclic AMP is quite low (Leader *et al.*, 1976). This latter point was demonstrated in work in which we found that ribosomal protein S6 was more highly phosphorylated in pre-confluent BHK cells (baby-hamster kidney fibroblasts) than in cells that had reached confluence (Leader *et al.*, 1976). However, it was not clear in that study whether the difference in cell density, growth rate, or some other factor was responsible for the difference in phosphorylation. To gain more information about the control of the phosphorylation of ribosomal protein S6 in BHK cells, we decided to examine certain papova-virus-transformed cell lines, which have different growth characteristics from the untransformed parent. This was of especial interest in view of both the importance of protein kinase activity in the transformation of cells by papova viruses (Tijan & Robbins, 1979; Eckhart *et al.*, 1979), and previous reports of the increased ^{32}P labelling of uncharacterized 'ribosomal' proteins in a wide spectrum of transformed cells (Segawa *et al.*, 1977, 1978).

Experimental

The origins of the normal BHK21/C13 cells and the polyoma-virus-transformed BHK21/C13/PyY cells have been described previously (Leader *et al.*, 1976). The BHK21/C13/SV28 cells were a variant transformed by SV40 virus (Wiblin & MacPherson, 1972). The cells were cultivated as described previously (Leader *et al.*, 1976) and harvested after 3 days' growth, by which time they had reached confluence. Both untransformed and transformed cells are still growing at this stage, because BHK cells do not give good contact inhibition, and overgrow the monolayer. The density of the virus-transformed cells was greater than that of the normal cells at this time. They were labelled for 3h with [^{32}P]P_i, their ribosomes isolated, dissociated into subunits, and the proteins extracted and analysed by two-dimensional gel electrophoresis, as described previously (Leader *et al.*, 1976; Leader & Coia, 1978). The specific radioactivity of the cellular [γ-^{32}P]ATP was determined in each experiment (Rankine *et al.*, 1977).

Abbreviation used: SV40 virus, simian virus 40.

* To whom reprint requests should be addressed.

Results

Changes in the extent of phosphorylation of ribosomal protein S6 are most unequivocally detected by analysis of the ribosomal proteins by using the method of two-dimensional gel electrophoresis described by Kaltschmidt & Wittmann (1970), as this resolves the phosphorylated derivatives of protein S6 from the parent protein (Gressner & Wool, 1974). When this method was used to compare ribosomal proteins from normal BHK cells with those from cells transformed by polyoma virus, it was found that in the latter case there was more stained material migrating in the first dimension to the anodic side of the parent S6 protein (Figs. 1*a* and 1*b*). Similar results were obtained with cells transformed by SV40 virus (Figs. 1*c* and 1*d*).

Although individual discrete species were not observed in the stained material at the anodic side of

0306-3283/81/070235-03$01.50/1

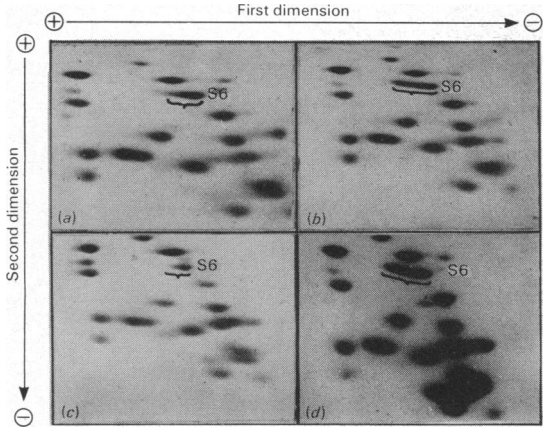

Fig. 1. *Two-dimensional gel electrophoresis of protein extracted from the 40S subunit of normal and virus-transformed hamster fibroblasts*

Cells: (*a*) BHK/21/C13, (*b*) BHK/21/C13/PyY, (*c*) BHK/21/C13, (*d*) BHK/21/C13/SV28. The photographs are of stained gels. The proteins shown in (*a*) and (*b*) were from ribosomes prepared in parallel, as were the proteins shown in (*c*) and (*d*). The results are typical of three and two separate preparations respectively. Although small differences in other proteins may be seen, they were not reproducibly observed, unlike that of protein S6.

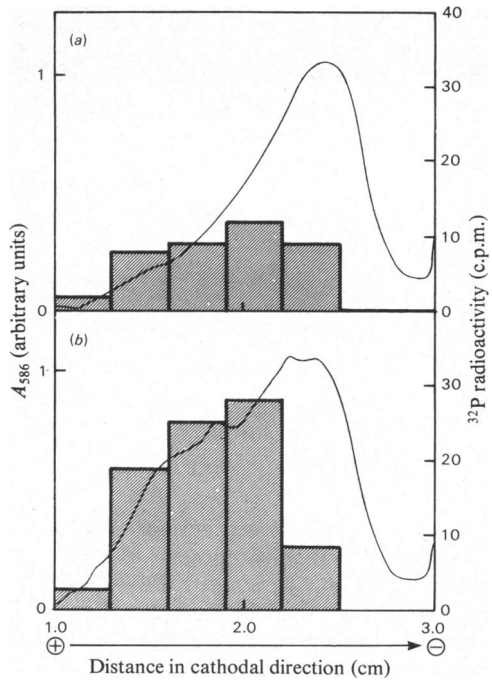

Fig. 2. *Analysis of the distribution of ^{32}P in ribosomal protein S6 from normal and polyoma-virus-transformed hamster fibroblasts*

Segments of rectangular cross-section containing protein S6 were excised from gels similar to those shown in Figs. 1(*a*) and 1(*b*), scanned densitometrically at 586nm (——) by using the linear transport accessory to the Gilford 240 spectrophotometer, then cut into slices with an assembly of razor blades, and the radioactivity in the slices was determined (▨). (*a*) Protein S6 from BHK/21/C13 cells; (*b*) protein S6 from BHK/21/C13/PyY cells. The sum of the radioactivity recovered from the gel slices of (*a*) and (*b*) was 35c.p.m. and 83c.p.m. respectively.

protein S6, there is no doubt that this latter does represent more phosphorylated derivatives of the protein. Thus it coincided with the majority of the radioactive phosphate, which was quantitatively greater (by a factor of approx. 2.4) in the protein from the transformed cells (Fig. 2). After correction for the different specific radioactivities of the [γ-^{32}P]ATP in the cells, we calculated that there was 3.2 times as much phosphate in ribosomal protein S6 from PyY cells than from normal cells.

We also examined transformed cells at later stages of growth, where we found that, like normal cells (Leader *et al.*, 1976), the extent of phosphorylation declined to very low values (I. M. Kennedy and D. P. Leader, unpublished work).

Discussion

As in our previous experiments with normal cells at different stages of growth (Leader *et al.*, 1976), the differences in phosphorylation of protein S6 described here cannot be due to increased concentrations of cyclic AMP. This is because the concentration of cyclic AMP is generally lower in virus-transformed cells, including those transformed by polyoma and SV40 viruses (Ryan & Heidrick, 1974). Both in this study, and in our previous one with pre-confluent and confluent untransformed cells, the greater phosphorylation of ribosomal protein S6 was found in the more rapidly growing cells. However, in the previous study these were the ones having the lower cell density (the pre-confluent cells), whereas here they are the ones having somewhat higher cell density (the transformed cells). This rules out cell density as the correlate with phosphorylation of ribosomal protein S6, in these two studies. It seems likely, although not proved by the present studies, that the difference in growth rate is the factor determining the difference in phosphorylation. It should perhaps be pointed out that even greater phosphorylation of ribosomal protein S6 is observed immediately after resting cells are stimulated to grow (Lastick *et al.*, 1977; Haselbacher *et al.*, 1979).

In view of the uncertainty over the physiological role of the phosphorylation of ribosomal protein S6, its importance in relation to the transformed phenotype is difficult to assess. It would clearly be of interest to determine its relationship to other phosphorylations occurring in transformed cells. However, we recognize that it may reflect an exaggeration of normal metabolism in, for example, the more rapid growth of the cells.

We thank John Connelly for technical assistance and the Cancer Research Campaign for financial support.

References

Eckhart, W., Hutchinson, M. A. & Hunter, T. (1979) *Cell* **18**, 925–933

Gressner, A. M. & Wool, I. G. (1974) *J. Biol. Chem.* **249**, 6917–6925

Gressner, A. M. & Wool, I. G. (1976) *J. Biol. Chem.* **251**, 1500–1504

Haselbacher, G. K., Humbel, R. E. & Thomas, G. (1979) *FEBS Lett.* **100**, 185–190

Kaltschmidt, E. & Wittmann, H. G. (1970) *Anal. Biochem.* **36**, 401–412

Lastick, S. M., Nielsen, P. J. & McConkey, E. H. (1977) *Mol. Gen. Genet.* **152**, 223–230

Leader, D. P. (1980) in *Protein Phosphorylation in Regulation* (Cohen, P., ed.), pp. 203–233, Elsevier/North-Holland, Amsterdam

Leader, D. P. & Coia, A. A. (1978) *FEBS Lett.* **90**, 270–274

Leader, D. P., Rankine, A. D. & Coia, A. A. (1976) *Biochem. Biophys. Res. Commun.* **71**, 966–974

Rankine, A. D., Leader, D. P. & Coia, A. A. (1977) *Biochim. Biophys. Acta* **474**, 293–307

Ryan, W. L. & Heidrick, M. L. (1974) *Adv. Cyclic Nucleotide Res.* **4**, 81–116

Segawa, K., Nobuo, Y. & Oda, K. (1977) *J. Virol.* **22**, 679–693

Segawa, K., Kinichiro, O., Tuasa, Y., Shiroki, K. & Oda, K. (1978) *J. Virol.* **27**, 800–808

Tijan, R. & Robbins, A. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 610–614

Wiblin, C. N. & MacPherson, I. A. (1972) *Int. J. Cancer* **10**, 296–304