Identification of proliferation-sensitive human proteins amongst components of the 40S hnRNP particles. Identity of hnRNP core proteins in the HeLa protein catalogue

ARTICLE *in* FEBS LETTERS · FEBRUARY 1986

Impact Factor: 3.17 · DOI: 10.1016/0014-5793(86)80059-X · Source: PubMed

4 AUTHORS, INCLUDING:

Julio Celis
Danish Cancer Society
311 PUBLICATIONS **13,208** CITATIONS
SEE PROFILE

Wallace M Lestourgeon
Vanderbilt University
38 PUBLICATIONS **1,305** CITATIONS
SEE PROFILE

Identification of proliferation-sensitive human proteins amongst components of the 40 S hnRNP particles

Identity of hnRNP core proteins in the HeLa protein catalogue

Julio E. Celis*, Rodrigo Bravo°, Hartwig P. Arenstorf and Wallace M. LeStourgeon

*Division of Biostructural Chemistry, Department of Chemistry, Aarhus University, DK-8000 Aarhus C, Denmark, °European Molecular Biology Laboratory, 6900 Heidelberg, FRG and Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235, USA

Received 19 September 1985

The core proteins of HeLa 40 S hnRNP monoparticles have been identified in the HeLa protein catalogue. Human proteins previously indentified as proliferation-sensitive [NEPHGE 21 and 17; Bravo, R. and Celis, J.E. (1982) Clin. Chem. 28, 766], as well as two proteins characterized in this study (NEPHGE 16 W and 16 W1), are shown to be components of these particles. These basic nuclear polypeptides correspond to core proteins A_1, B_{1a}, B_2 and C_4, respectively. The significance of these results in terms of composition and function of hnRNP particles is discussed.

Proliferating cell *Resting cell* *Nuclear protein* *hnRNP function* *mRNA transport*

1. INTRODUCTION

The understanding of the mechanisms underlying cell transformation and cancer will be assisted by the identification of cellular proteins involved in the control of cell proliferation in normal cells ([1] and references therein). One approach for searching for such proteins amongst the thousands of polypeptides thought to be present in a somatic cell has been the analysis by means of high-resolution two-dimensional gel electrophoresis of the overall patterns of gene expression in pairs of normal and transformed cells as well as in normal cells grown under a variety of physiological conditions ([1–7] and references therein). To date, these studies have revealed several basic and acidic cellular proteins whose rate of synthesis is affected by changes in growth rate and/or cell transformation ([1–7] and references therein). The identity of only a few of these proteins is known: these include vimentin [5], tropomyosins [4,7], a heat-shock protein [1,5] and the nuclear protein cyclin [1,4,5], also termed PCNA [8.]

In this study, we have identified 4 proliferation-sensitive human proteins [5] amongst components of the 40 S hnRNP particles. The significance of these results in terms of composition and function of hnRNP particles is discussed.

This paper is dedicated to Professor S.P. Datta

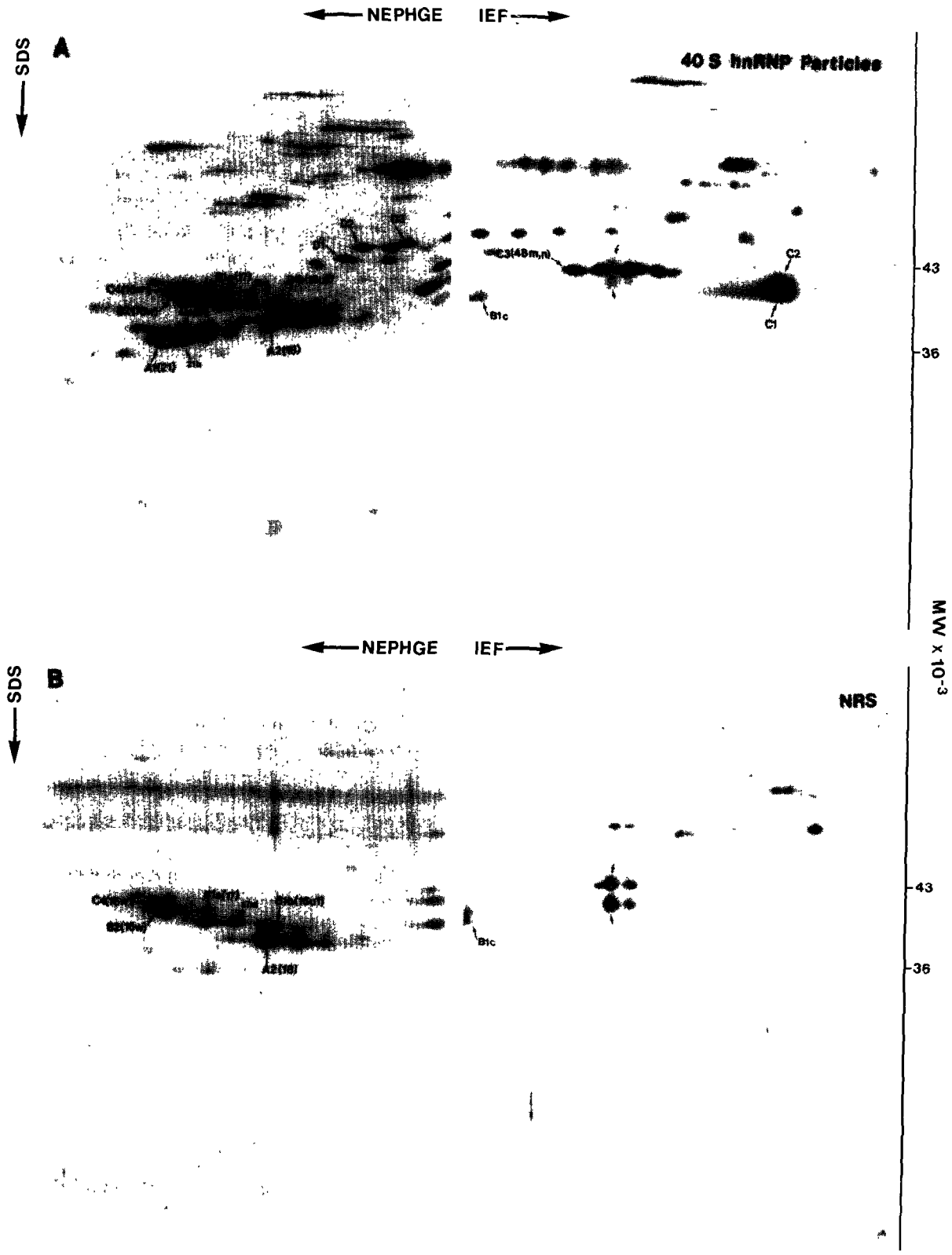
Abbreviations: IEF, isoelectric focussing; hnRNP, ribonucleoprotein complex containing heterogeneous nuclear ribonucleic acid; NEPHGE, non-equilibrium pH gradient electrophoresis

2. MATERIALS AND METHODS

2.1. *Cell culture and [^{35}S]methionine labelling*

HeLa cells free of mycloplasmas (Gibco Bio-Cult Ltd, Stock Source, American Type Culture Collection, catalogue no. H3002) were grown routinely as monolayer cultures in Dulbecco's modified Eagle's medium containing 10% (v/v)

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50


NEPHGE
IEF
SDS
A
40 S hnRNP Particles
C2
C1
B1c
43
36
MW x 10-3
NEPHGE
IEF
SDS
B
NRS
B1c
43
36

←

Fig.1. Two-dimensional gel electrophoresis (NEPHGE, IEF) of HeLa proteins extracted from (A) 40 S hnRNP particles and (B) NRS. Gels were stained with silver as described by Tunón and Johansson [18]. Core proteins are indicated with the nomenclature of Beyer et al. [11] and Wilk et al. [20] modified as described in the text. It should be noted that protein C_3 sometimes splits into 2 sets of proteins having slightly different M_r values. The HeLa protein catalogue number is given within parentheses. A few satellite spots are indicated with the HeLa protein catalogue number (small numbers and letters).

fetal calf serum and antibiotics (penicillin, 100 units/ml; streptomycin, 50 μg/ml).

Cells grown in 0.25 ml flat-bottomed microtiter plates (NUNC, Denmark) were labelled with 1 mCi/ml [^{35}S]methionine for 20 h as described [9,10].

2.2. *Isolation of 40 S hnRNP particles*

HeLa cells were grown in suspension culture in minimal essential medium supplemented with 5% calf serum and harvested in log-phase growth at an optimum density of $4–5 \times 10^5$ cells/ml. Nuclei were isolated in NHS buffer (20 mM Tris-HCl, pH 7.2, 1.0 mM $MgCl_2$, 0.02% Triton X-100) as described in [11]. Nuclear purity was monitored by phase-contrast microscopy. The nuclear pellet, containing $1.2–1.5 \times 10^9$ nuclei, was washed in 50 ml STM buffer (90 mM NaCl, 1.0 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.0), resuspended in 3.0 ml of the same buffer and ribonucleoprotein complexes were released by sonic disruption on ice [12]. The nuclear sonicate was incubated at 37°C for 10 min to increase the yield of 40 S monoparticles via the action of endogenous nucleases. Chromatin and nuclear debris were removed by centrifugation. The sonicate was placed in a 15 ml Corex tube and centrifuged for 10 min at 9500 rpm in a Sorvall RC2-B using the HB-4 rotor. The chromatin clarified supernatant was then layered over 15–30% sucrose density gradients (Schwarz-Mann Div., Becton, Dickson and Co., Orangeburg, NY; ribonuclease-free) at 4°C in STM buffer and centrifuged for 16 h at 25 000 rpm in an SW-27 rotor (Beckman Instruments, Fullerton, CA). The gradient fractions containing 40 S monoparticles were pooled and dialyzed against STM buffer at 4°C overnight to remove sucrose. The particles were then precipitated with 3 vols cold ethanol and the precipitate dried by evacuation for stable storage at ambient conditions.

2.3. *Preparation of nuclease-resistant structures*

The 43 S nuclease-resistant structure (NRS) which forms upon extensive RNA digestion [13] was prepared by incubating nuclear sonicates for 5 min at 37°C, followed by addition of 5 μg RNase A (Sigma, St. Louis, MO) per 10^8 nuclei and an additional 15 min incubation. After cooling to 0°C on ice the digests were prepared for sucrose gradient centrifugation as above.

2.4. *Other procedures*

The procedures for two-dimensional gel electrophoresis (NEPHGE, IEF) [9,14–16], fluorography [17], silver staining [18] and one-dimensional peptide mapping using V8 protease [19] have been described in detail elsewhere.

3. RESULTS

3.1. *Protein composition of HeLa 40 S hnRNP particles and nuclease-resistant structures (NRS)*

Fig.1A shows silver-stained two-dimensional gels (IEF, NEPHGE) of proteins extracted from purified HeLa 40 S hnRNP monoparticles. The 9 major core proteins described by Wilk et al. [20] are indicated in these gels using their nomenclature which is an extension of that originally proposed by Beyer et al. [11] to designate core hnRNP proteins separated by one-dimensional gel electrophoresis. We have, however, renamed the C-group of proteins to account for the fact that core proteins C_1 and C_2 (as defined by one-dimensional gel separation) were originally described as acidic proteins of low glycine content [11,21,22]. The equivalence between our numbering system for the C proteins and that of Wilk et al. [20] is as follows: $C_1 = C_3$; $C_2 = C_{3x}$; $C_3 = C_2$ and $C_4 = C_1$. In addition to the core protein, 40 S hnRNP particles contain several associated proteins that are not necessary for maintaining the hnRNP structure [20,23]: these include the $D_1–D_3$ group of proteins (fig.1A, NEPHGE) [20], and numerous high-M_r proteins of unknown identity (fig.1A).

Most associated proteins as well as a few core proteins are released from the 40 S particles following RNase A digestion (cf. fig. 1A and B). Only 6 core proteins are found in the nuclease-resistant structures (NRS) and these correspond to A_2, B_{1a}, B_{1b}, B_{1c}, B_2 and C_4 (fig.1B; table 1; see also [13]).

Besides the proteins mentioned above, there is a set of acidic spots in the gels of NRS particles (arrowheads in fig.1B) that is barely detected in similar gels of total particles (fig.1A). The identity of these spots is at present unknown.

3.2. *Identification of hnRNP core proteins in the HeLa protein catalogue*

Identity of the 40 S hnRNP core proteins with those in the HeLa protein catalogue [24–26] was determined by coelectrophoresis with [^{35}S]methionine-labelled HeLa cell proteins (fig.2). When possible, core proteins are indicated in fig.2 and table 1 with their corresponding number in the HeLa protein catalogue as well as with the nomenclature of Beyer et al. [11] and Wilk et al. [20] modified as described above (parentheses in fig.2). To facilitate comparison, the HeLa protein catalogue number has been included within parentheses in fig.1A and B. The identity of proteins B_{1c}, C_1 and C_2 has not been determined with certainty as they are minor [^{35}S]methionine-labelled proteins in HeLa cells (fig.2). The areas in which these proteins migrate have been enclosed within a small B_{1c}) and large box (C_1 and C_2) in fig.2 (IEF). The positions of actin, vimentin (v), α- and β-tubulin (αt, βt) are indicated as a reference.

Fig.3 shows one-dimensional peptide maps (V8 protease) of some basic [^{35}S]methionine-labelled core proteins (proliferation-sensitive, see below) extracted from two-dimensional gels [NEPHGE 21 (A_1), fig.3A; 17 (B_{1a}), fig.3D; 16 W (B_2), fig.3F and 16 W1 (C_4), fig.3G], and in a few cases of their satellite spots (NEPHGE 21b, fig.3B; 19l (A1x), fig.3C and 17a1, fig.3E; see also figs 1 and 2). The results agree in general with those of Wilk et al. [20], which showed that these are closely related

Table 1

Some characteristics of HeLa 40 S hnRNP core proteins

40 S hnRNP core proteins[a]	Hela protein catalogue number[b]	M_r ($\times 10^{-3}$)	Core proteins in whole HeLa cells		Core proteins in Hela Triton cytoskeletons		Core proteins in purified 40 S hnRNP particles		
			% of total protein label[c]	Ratio NEPHGE 21 (A_1)/core protein	% of total protein label[d]	Ratio NEPHGE 21 (A1)/core protein	Ratio A_1/core protein[e]	Ratio A_1/core protein[f]	Presence in NRS[g]
A_1	NEPHGE 21	35	0.14	1	0.11	1	1	1	–
A_2	NEPHGE 18	40	1.20	0.12	0.24	0.45	1	1	+
B_{1a}	NEPHGE 17	41	0.06	2.33	0.04	2.75			+
B_{1b}	NEPHGE 16_{u1}	41	nd	nd	nd	nd			+
B_{1c}	unidentified	41	nd	nd	nd	nd			+
B_2	NEPHGE 16w	41.5	0.03	4.66	0.03	3.7	6	3	+
C_1	unidentified	42	nd	nd	nd	nd			–
C_2	unidentified	42.5	nd	nd	nd	nd			–
C_3	IEF 48 m, 48 n	42.5	nd	nd	nd	nd			–
C_4	NEPHGE $16w_1$	42	0.03	4.66	0.03	3.7			+

[a] Termed according to the nomenclature of Beyer et al. [11] and Wilk et al. [20] with the modifications indicated in the text. [b] From Bravo and Celis [25,26]. [c] From Bravo and Celis [25,26], but corrected for methionine content [20]. [d] Calculated based on the percentages of each protein remaining in Triton cytoskeletons [25,26]. These values are: NEPHGE 21 (A_1), 81%: 18 (A_2), 80%; 17 (B_{1a}), 72%; 16w (B_1), 100%; $16w_1$ (C_4), 100%. The value of 90% previously calculated for NEPHGE 18 (A_2) should be corrected in the HeLa protein catalogue [25,26]. [e] From Wilk et al. [20]. [f] From Lothstein et al. [13]. [g] This study

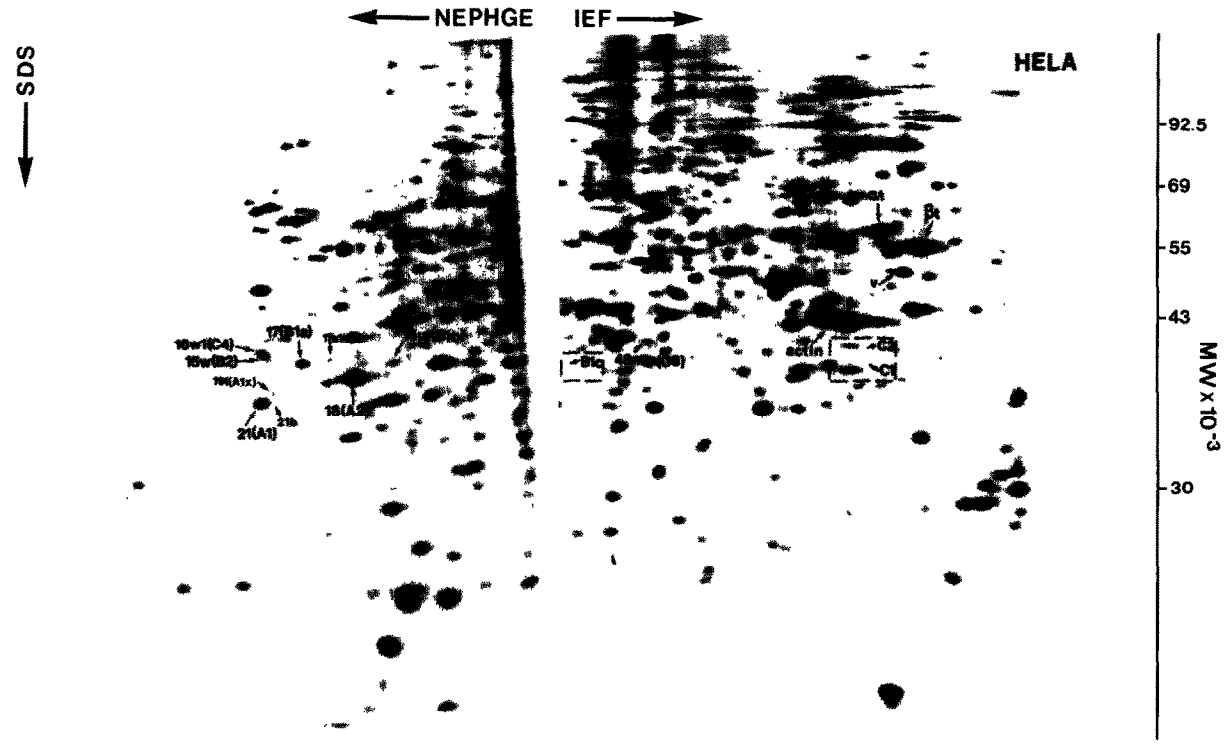


Fig.2. Two-dimensional gel electrophoresis analysis (NEPHGE, IEF) of [^{35}S]-methionine-labelled proteins from whole HeLa cells. HeLa cells were labelled with [^{35}S]methionine for 20 h (1 mCi/ml) as described [9]. When possible, core hnRNP proteins are indicated with the HeLa protein catalogue number. Spots are also indicated within parentheses with the nomenclature of Beyer et al. [11] and Wilk et al. [20]. These gels were not processed for fluorography.

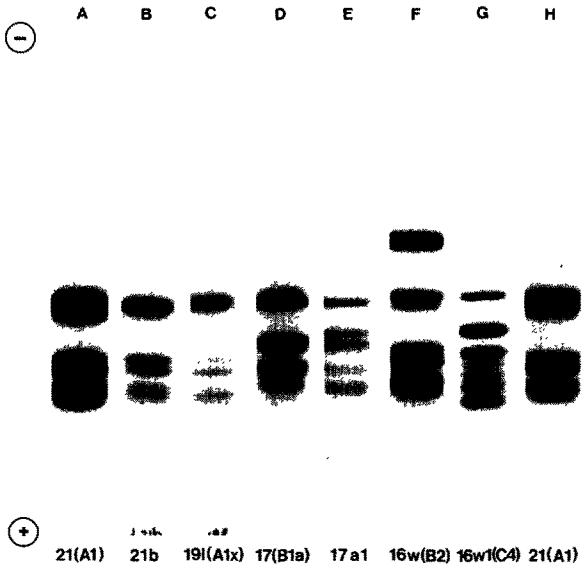


Fig.3. One-dimensional peptide maps (V8 protease) of some basic core proteins. [^{35}S]methionine-labelled proteins cut from two-dimensional gels were digested with V8 protease as described by Fey et al. [19]. Spots are indicated with the HeLa protein catalogue number and when possible with the nomenclature of Beyer et al. [1] and Wilk et al. [20] parentheses.

but distinct proteins. Both NEPHGE 19l (A1x) and 21b are related to NEPHGE 21 (A_1), while 17a1 seems to be a variant of NEPHGE 17 (B_{1a}).

Of the 9 core proteins, A_2 (NEPHGE 18) is the major [^{35}S]methionine-labelled polypeptide observed in vivo (fig.2), and it is an abundant component of these cells as judged by silver staining (fig.4, table 1). A considerable fraction of this protein can be extracted with Triton X-100 (Triton cytoskeleton, fig.5A; Triton supernatant, fig.5B;

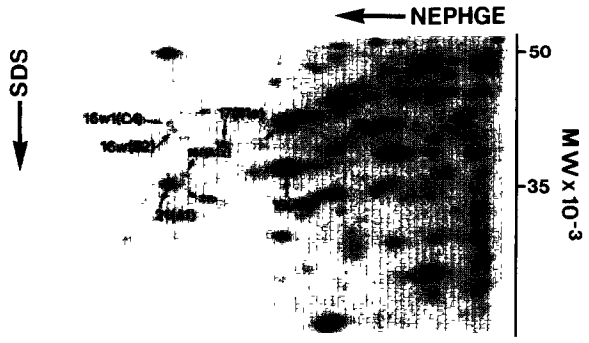


Fig.4. Two-dimensional map (NEPHGE) of HeLa basic proteins stained with silver nitrate. Only a fraction of the gel is shown.

see also fig.2) [25,26] under conditions in which most of the basic core proteins remain in the cytoskeleton (fig.5A and B); see also table 1). Table 1 gives the ratio A_1/core protein in both whole cells and Triton cytoskeletons. These ratios are significantly different to those reported for purified 40 S hnRNP particles [11,20].

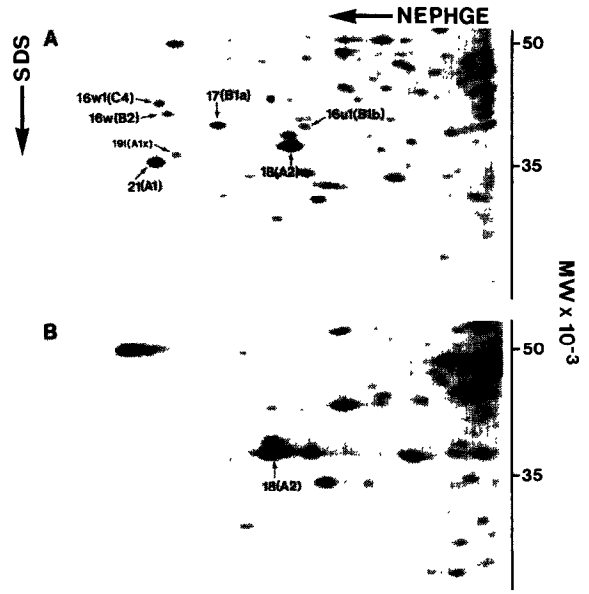


Fig.5. Two-dimensional gel electrophoresis (NEPHGE) of [^{35}S]methionine-labelled HeLa proteins from (A) Triton cytoskeletons and (B) Triton supernatants. Triton cytoskeletons were prepared as described [9]. Only the relevant region of the fluorograms is shown.

3.3. *Proliferation-sensitive proteins amongst 40 S hnRNP core proteins*

Studies by LeStourgeon et al. [21] first showed that the levels of core protein A_1 (NEPHGE 21) were high in proliferating cells but decreased as cells reached confluency and stopped dividing. Independent evidence supporting this observation has been obtained by Bravo and Celis, who analyzed the overall patterns of gene expression in mouse, hamster and human cultured cells by means of high resolution two-dimensional gel electrophoresis [1,3,5]. Their results showed a remarkable correlation between the levels of NEPHGE 21 (here shown to correspond to A_1), and the growth state of the cells. Fig.6 shows the appropriate regions of silver-stained gels (NEPHGE) of proteins extracted from resting human epidermal basal cells (fig.6A) and proliferating SV40 transformed human keratinocytes (K14, fig.6B). Clearly, there is a substantial increase in the levels of NEPHGE 21 (A_1) in the transformed

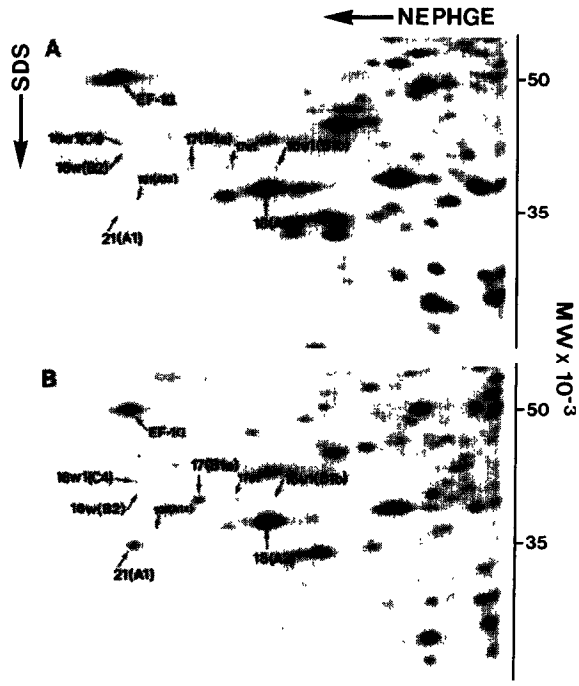


Fig.6. Levels of basic core proteins in resting human epidermal basal cells (A) and SV40 transformed human keratinocytes (B). Gels were stained with silver as described by Tunón and Johansson [18]. Only the appropriate region of the gel is shown.

keratinocytes which is also evident, albeit at a reduced rate, in proliferating normal epidermal cells (not shown). The increase in NEPHGE 21 (A_1) has also been observed in mouse cells exhibiting different growth rates. Fig.7 shows examples of fluorographs of two-dimensional gels

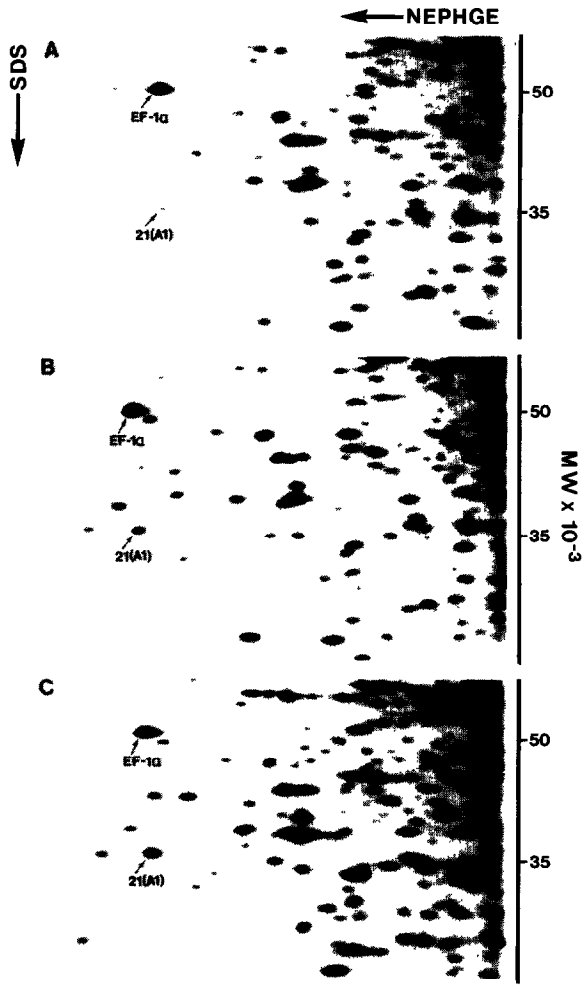


Fig.7. Two-dimensional gel electrophoresis of ^{14}C-amino acid-labelled proteins from whole mouse cells exhibiting different growth rates. (A) Resting eye fibroblasts, (B) proliferating kidney fibroblasts and (C) transformed mouse Sarcoma 180 cells. Cells were labelled with a mixture of 16 ^{14}C-amino acids (500 μCi/ml, CFB 104, Amersham) as described [34]. Only the pertinent region of the fluorograms is shown. Human core protein NEPHGE 21 (A_1) has the same number in the mouse protein catalogue, while NEPHGE 18 (A_2) corresponds to NEPHGE 19 [19].

(NEPHGE) of ^{14}C-labelled proteins obtained from resting eye fibroblasts (fig. 7A), proliferating kidney fibroblasts (fig.7B) and transformed sarcoma 180 cells (fig.7C). The identity of human and mouse NEPHGE 21 (A_1) has been confirmed by one-dimensional peptide mapping (cf. fig.3A and H).

Similar gel electrophoresis analyses of human cultured cells have shown that the levels of NEPHGE 17 (B_{1a}) are also modulated as a result of changes in growth rate (fig.6A and B) [1,3,5]. At present it is not clear which protein in the mouse corresponds to human NEPHGE 17.

Finally, a word should be said concerning human proteins NEPHGE 16 W1 (C_4) and 16 W (B_2). These proteins are minor [^{35}S]-methionine-labelled components of human cells and were not quantitated in previous studies [1,3,5]. However, re-examination of our library of gels from pairs of normal and transformed cells as well as results presented here (fig.6A and B) clearly show increased levels of these proteins in proliferating vs resting cells. Similar proteins can be seen in the gels of mouse cultured cells (fig.7A–C), but their identity has not been ascertained.

4. DISCUSSION

The most striking result presented in this article is the significant decrease in the levels of some hnRNP core proteins observed in resting cells as compared to their proliferating counterparts. In human cultured cells the proliferation-sensitive core proteins include NEPHGE 21 (A_1), 17 (B_{1a}) and most likely 16 W1 (C_4) and 16 W (B_2). So far, only NEPHGE 21 (A_1) has been identified as proliferation-sensitive in mouse cultured cells ([1,3,5] and this article). These results are in agreement with earlier findings of LeStourgeon et al. [21], which showed significant variations in the levels of core protein A_1 (NEPHGE 21) upon changes in growth rate. Similar results have been obtained by Coppard et al. [27], who have analyzed the methylated proteins of mouse 3T3B cells and their SV40 transformed counterparts.

It should be emphasized that our data concerning the levels of core proteins in resting and proliferating cells are based on the analysis of total proteins extracted from whole cells and do not reflect the actual levels of these proteins in the 40 S

hnRNP particles. We have shown that there is a pool of Triton-extractable core proteins that is particularly large in the case of NEPHGE 18 (A_2). About 80% of this protein can be extracted with 0.1% Triton X-100 under conditions in which a smaller but significant proportion of the other basic core proteins is extracted (fig.5A and B, table 1) [25,26]. The ratio NEPHGE 21 (A_1)/NEPHGE 18 (A_2) calculated for whole cells is different from that of Triton cytoskeletons (table 1), and both values differ from those reported for purified 40 S hnRNP particles [11,20] (see also table 1). Since Triton cytoskeletons may contain mainly large intact native hnRNP structures, it follows that the overall composition of these structures may be different from that of the 40 S hnRNP particles. These observations may explain why most of the proliferation-sensitive proteins described here have eluded identification, as most previous studies have analyzed partially purified cellular fractions whose composition may not necessarily reflect that of the whole cell.

In the light of the above observations, it would seem premature to discuss how variations in the overall levels of some core proteins may affect the particle's composition. However, the fact that resting human cells contain very low levels of core proteins A_1 (NEPHGE 21), B_{1a} (NEPHGE 17), C_4 (NEPHGE 16 W1) and B_2 (NEPHGE 16 W) raises important questions concerning the composition of the hnRNP particles in these cells. 40 S hnRNP particles have been isolated from non-dividing cells, and their protein composition has been analyzed [28-31]. Even though lack of standardization in the gel running conditions and in the nomenclature used to denominate the spot precludes precise comparisons, visual inspection of these gels has revealed a different protein composition for these particles as compared to those of proliferating HeLa cells (fig.1A). Taken together, these results emphasize a functional significance for the hnRNP particles [32].

Finally, a word should be said concerning the cellular localization of the hnRNP core proteins. Our studies of enucleated HeLa cells (cytoplasts) and karyoplasts have shown that all core proteins so far identified, except A_2 (NEPHGE 18), are present mainly in the nucleus [33]. About 60% of A_2 can be recovered in cytoplasts, a fact that may be taken to suggest a role for this protein in mRNA transport. Further studies will be necessary in order to assess this possibility.

ACKNOWLEDGEMENTS

We would like to thank S. Nielsen and P. Mose Larsen who participated at different stages of this work. We also thank M. Elbeck for expert technical assistance. This work was supported by grants from the Danish Medical and Natural Science Research Councils, the Danish Cancer Society and NOVO (to J.E.C.) and NSF grant PCM-8209420 (to W.M.L.).

REFERENCES

[1] Celis, J.E., Bravo, R., Mose Larsen, P., Fey, S.J., Bellatin, J. and Celis, A. (1984) in: Two-Dimensional Gel Electrophoresis of Proteins: Methods and Applications (Celis, J.E. and Bravo, R. eds) pp. 308-362, Academic Press, New York.
[2] Leavitt, J. and Moyzig, B. (1978) J. Biol. Chem. 253, 2497-2500.
[3] Bravo, R. and Celis, J. (1980) Exp. Cell Res. 127, 249-260.
[4] Bravo, R., Fey, S.J., Bellatin, J., Mose Larsen, P., Arevalo, J. and Celis, J.E. (1981) Exp. Cell Res. 136, 311-319.
[5] Bravo, R. and Celis, J.E. (1982) Clin. Chem. 28, 949-954.
[6] Celis, J.E., Bravo, R., Mose Larsen, P. and Fey, S.J. (1984) Leuk. Res. 8, 143-157.
[7] Garrels, J.I. and Franza, R. (1984) Cancer Cell 1, 137-146.
[8] Mathews, M.B., Bernstein, R.M., Franza, R. and Garrels, J.I. (1984) Nature 309, 274-276.
[9] Bravo, R., Small, J.V., Fey, S.J., Mose Larsen, P. and Celis, J.E. (1982) J. Mol. Biol. 154, 121-143.
[10] Celis, J.E. and Bravo, R. (1981) Trends Biochem. Sci. 6, 197-201.
[11] Beyer, A.L., Christensen, M.E., Walker, B.W. and LeStourgeon, W.M. (1977) Cell 11, 127-138.
[12] Walker, B.W., Lothstein, L., Baker, C.L. and LeStourgeon, W.M. (1980) Nucleic Acids Res. 8, 3639-3657.
[13] Lothstein, L., Arenstorf, H.P., Chung, S.-Y., Walker, B.W., Wooley, J.C. and LeStourgeon, W.M. (1985) J. Cell Biol. 100, 1570-1581.
[14] O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007-4021.
[15] O'Farrell, P.Z., Goodman, H.M. and O'Farrell, P.H. (1977) Cell 12, 1133-1142.

[16] Bravo, R. (1984) in: Two-Dimensional Gel Electrophoresis of Proteins: Methods and Applications (Celis, J.E. and Bravo, R. eds) pp. 3–36, Academic Press, New York.
[17] Laskey, R.A. and Mills, A.D. (1975) Eur. J. Biochem. 56, 335–341.
[18] Tunón, P. and Johansson, K.E. (1984) Biochem. Biophys. Methods 9, 171–179.
[19] Fey, S.J., Bravo, R., Mose Larsen, P., Bellatin, J. and Celis, J.E. (1981) Cell Biol. Int. Rep. 5, 491–500.
[20] Wilk, H.-E., Werr, H., Friedrich, D., Kiltz, H.H. and Schäfer, K.P. (1985) Eur. J. Biochem. 146, 71–81.
[21] LeStourgeon, W.M., Beyer, A.E., Christensen, M.E., Walker, B.W., Pourpore, S.M. and Daniels, L.P. (1978) Cold Spring Harbor Symp. Quant. Biol. 38, 921–932.
[22] LeStourgeon, W.M., Lothstein, L., Walker, B.W. and Beyer, A.L. (1981) in: The Cell Nucleus: Nuclear Particles (Busch, H. ed.) vol. 9, part B, pp. 47–87, Academic Press, New York.
[23] Choi, Y.D. and Dreyfuss, G. (1984) Proc. Natl. Acad. Sci. USA 81, 7471–7475.
[24] Bravo, R., Bellatin, J. and Celis, J.E. (1981) Cell Biol. Int. Rep. 5, 93–96.
[25] Bravo, R. and Celis, J.E. (1982) Clin. Chem. 28, 766–781.
[26] Bravo, R. and Celis, J.E. (1984) in: Two-Dimensional Gel Electrophoresis of Proteins: Methods and Applications (Celis, J.E. and Bravo, R. eds) pp. 445–476, Academic Press, New York.
[27] Coppard, N.J., Clark, B.F.C. and Cramer, F. (1983) FEBS Lett. 164, 330–334.
[28] Maundrell, K. and Scherrer, K. (1979) Eur. J. Biochem. 99, 225–238.
[29] Gallinaro-Matringe, H., Stevenin, J. and Jacob, M. (1977) Differentiation 9, 147–155.
[30] Stevenin, J. and Jacob, M. (1979) Mol. Biol. Rep. 5, 29–35.
[31] Suria, D. and Liew, C.C. (1979) Can. J. Biochem. 57, 32–42.
[32] Knowler, J.T. (1983) Int. Rev. Cytol. 84, 103–153.
[33] Bravo, R., Celis, A., Mosses, D. and Celis, J.E. (1981) Cell Biol. Int. Rep. 5, 479–489.
[34] Bravo, R., Fey, S.J., Small, J.V., Mose Larsen, P. and Celis, J.E. (1981) Cell 25, 195–202.