Volume 210, number 2, 165–168 FEB 04328 January 1987

Degradation of nuclear proteins: studies on transplanted B82 cell karyoplast proteins

David G. Fernig and R. John Mayer

Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, England

Received 19 September 1986; revised version received 29 October 1986

Karyoplasts were prepared from B82 cells (thymidine kinase deficient mouse L cells) by cytochalasin B mediated enucleation. Morphological measurements show that the nucleus constitutes 89% of a karyoplast by volume. Homokaryons were obtained by Sendai virus mediated karyoplast-B82 cell fusion. Transplanted nuclei were not destroyed catastrophically but were maintained intracellularly for at least 140 h. Transplanted nuclear proteins were degraded with an average half-life of 84 ± 7 h by processes partially sensitive to inhibition by NH_4Cl (50%) and leupeptin (30%). The data therefore suggest that some nuclear proteins are translocated to the cytoplasm for lysosomal degradation.

Karyoplast; Protein degradation; Cell nucleus; Lysosome; (L cell)

1. INTRODUCTION

Most previous degradation studies on nuclear proteins have concentrated on establishing the rates of degradation of specific classes of proteins, e.g. histones [1]; non-histone chromosomal proteins [2]; high-mobility group proteins 1 and 2 [3,4]. There are few data on the pathways involved in the degradation of nuclear proteins. This study attempted to determine if there are any translocative events involved in the degradation of nuclear proteins (cf. [5]). Our approach was to transplant radiolabelled nuclei into unlabelled cells. To transplant a functional nucleus into cells, karyoplasts, i.e. nuclei surrounded by a thin rim of cytoplasm, enclosed by plasma membrane [6–10] were used as vectors. Nuclei were microinjected into recipient cells by karyoplast-cell fusion mediated by Sendai virus.

Correspondence address: R.J. Mayer, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, England

2. MATERIALS AND METHODS

2.1. *Karyoplast preparation and transplantation*

Karyoplasts were prepared from B82 cells (a thymidine kinase deficient L cell subclone) and fused to B82 cells as described in [7]. 3 h post fusion, the growth medium from the culture dishes was removed, the cells were washed twice with 2 ml Ca^{2+}/Mg^{2+} free phosphate buffered saline (PBS) to remove unfused karyoplasts and dead cells, and 3 ml growth medium was added to each dish. This point was taken as zero time (t_0) in all experiments.

2.2. *Measurement of karyoplast cytoplasm*

B82 cells were dual labelled as follows: cytoplasm was labelled with 1 μm latex beads (Sigma, Poole, Dorset, England) [8] and cell plasma membranes were then labelled with fluorescein isothiocyanate as described by Russell and Mayer [11]. Both transplanted dual labelled karyoplasts and cells were cultured on sterile coverslips. Coverslips were collected over 72 h, and processed as before [11]. The number of beads

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

in each fluorescent cell was counted 24 h after plating out. This was the earliest time at which the cells were sufficiently flattened out to allow the number of beads per cell to be precisely determined.

For autoradiographic measurements, transplanted, L-[^{3}H]leucine labelled karyoplasts were cultured on sterile coverslips. Coverslips were collected over 140 h and processed for autoradiography according to standard techniques and photographed with a Zeiss standard microscope.

Cells and karyoplasts were prepared for electron microscopy as described in [12]. Photomicrographs were taken with a Philips 410 electron microscope.

2.3. *Measurement of whole cell and transplanted nuclear protein degradation*

Cells were grown for 36 h in growth medium (Dulbecco's modified Eagles medium supplemented with 10% newborn calf serum, 100 μg/ml streptomycin, and 100 U/ml penicillin G) containing only 0.1 mM L-leucine and 1 μCi/ml L-[^{3}H]leucine (spec. act. 63 Ci/mmol, Amersham, Bucks, England). The radiolabelled cells were either enucleated and the karyoplasts fused to unlabelled B82 cells to measure the degradation of transplanted nuclear proteins, or they were subcultured to measure endogenous protein degradation. In either case cells were plated out in growth medium supplemented with 10 mM leucine to minimize isotope reutilization and washed as described above. All measurements of cell and growth medium trichloroacetic acid-soluble and -insoluble radioactivity were made as described in [11].

3. RESULTS

3.1. *Transplantation of B82 karyoplasts*

The karyoplast preparation had a purity of 95% as determined by analysis of karyoplasts cultured for 72 h [7]. 24 h after transplantation of karyoplasts prepared from L-[^{3}H]leucine labelled cells into unlabelled cells, between 10 and 15% of the total initial radioactivity was retained as cell-associated trichloroacetic acid-insoluble radioactivity (10 experiments), indicating that stable transplantation was between 10 and 15% efficient.

The amount of cytoplasm co-transplanted with the karyoplast nucleus was measured in order to determine the usefulness of this technique for measuring the degradation of nuclear proteins. Electron micrographs of karyoplasts (30) and cells (20) were taken at random and the cytoplasmic and nuclear volumes determined with a MOP Kontron image analyser. The cytoplasm constitutes only 11% of the karyoplast volume. A similar value was obtained from the latex bead distribution in homokaryons. Therefore the decrease in cell trichloroacetic acid-insoluble radioactivity (table 1) represents predominantly the degradation of nuclear proteins in B82 homokaryons.

Autoradiographs of transplanted B82 homokaryons show that only 4.4% of the radiolabelled nuclei 24 h post fusion are in contaminating mononucleate cells (table 1) and that the silver grains are concentrated over the transplanted nucleus in the homokaryons. Few silver grains were observed over the cytoplasm, or the unlabelled nuclei in the homokaryons for up to 140 h. Quantitative analysis of the autoradiographs (table 1a) at intervals after fusion shows that contaminating whole cells divide whilst the homokaryons do not divide and are therefore quiescent. Sendai virus does not affect the degradation of endogenous or microinjected pro-

Table 1

Quantitative examination of autoradiographs

Time (h) post fusion (a)	Mononucleate cells (%)	Homokaryons (%)
24	4.4	95.6
48	8.1	91.9
72	16.9	83.1

(b)	Number of homokaryons in 65 fields
15	157
48	174
140	184

(a) Mononucleate cells and homokaryons with at least one radiolabelled nucleus as a percentage of the total number of radiolabelled cells observed. (b) The number of labelled homokaryons in 65 microscope fields does not alter with time. The apparent increase from t_{15} to t_{48} is due to a greater ease of counting at the latter time because the cells have flattened out

teins [2–4] except by fusing cells and forming quiescent homokaryons. Thus table 2a shows that the rate of endogenous protein degradation increased 30% after treatment of cells with Sendai virus as in a normal fusion [7], commensurate with the proportion of homokaryons produced by the fusion. Table 1b shows that the transplanted nuclei in B82 homokaryons are stable over 140 h, i.e. the transplanted nuclei are not catastrophically destroyed.

Table 2

Average half-life of transplanted karyoplast and metabolically labelled protein in B82 cells

Cell growth condition	Protein half-life (h)
(a) Metabolically labelled cell protein	
growing	161 ± 6 (2)
growing, treated with Sendai virus as for a fusion	130
confluent	66 ± 8 (3)
(b) Transplanted karyoplast protein	
transplanted	84 ± 7 (10)
transplanted + 10 mM NH_4Cl	169 ± 25 (3)
transplanted + 0.2 mM leupeptin	103

Half-lives are calculated from the loss of cell trichloroacetic acid-insoluble radioactivity over 120 h. Each determination of protein half-life involved the analysis of six time points (t_0 to t_{120}) in triplicate. The errors (calculated as SD) were less than 10% of the mean. When the half-life was determined more than once, the half-life is expressed as the mean ± SD of all available values, with the number of determinations in parentheses. Drugs, if included, were present at the concentration stated for the 120 h of the experiments. Cell death was monitored by light microscopy and was negligible. In a typical experiment the medium trichloroacetic acid-soluble radioactivity rose to 1644 dpm at t_{120} (from 0 dpm at t_0). The total radioactivity in the medium at each time point was equal to that lost from cells. 'Latent' radioactivity [23] was never observed

3.2. *Effects of inhibitors on the degradation of transplanted B82 karyoplast protein*

The half-life of transplanted B82 nuclear proteins is 84 ± 7 h (table 2b) which is similar to the average rate of degradation of all endogenous proteins in confluent cells (table 2a). The data in table 2b show that the lysosomotropic inhibitor NH_4Cl [13,14] inhibits the degradation of transplanted nuclear protein by 50%. Leupeptin, an inhibitor of lysosomal thiol proteases [15], inhibited the degradation of transplanted B82 karyoplast proteins by 30%.

4. DISCUSSION

The results in this paper and in [7] show that B82 karyoplasts prepared by cytochalasin B mediated enucleation are successfully transplanted into recipient B82 cells when Sendai virus is used as a fusogen. The 5% whole cells contaminating the karyoplast preparation do not make a significant contribution to the fusion products (table 1). Morphological techniques illustrate that the nucleus occupies 89% of the karyoplast volume. The autoradiographs show that silver grains, i.e. radiolabelled protein, are concentrated over the transplanted nucleus, and few grains are seen over the cytoplasm or the unlabelled nucleus of the homokaryon (not shown). Therefore when we measure the degradation of transplanted karyoplast protein (table 2) it is predominantly the degradation of transplanted B82 nuclear proteins that is being followed. Since catastrophic destruction of nuclei over 140 h did not occur, the decrease of cell-associated trichloroacetic acid-insoluble radioactivity observed in B82 homokaryons may represent the normal pathway(s) of nuclear protein degradation for these multinucleate cells. We do not know whether these same pathways are identical with those employed by nuclear proteins in mononucleate B82 cells.

The effects of both NH_4Cl [13,14] and leupeptin [15] on lysosomes are well documented. Since lysosomes are located exclusively in the cytoplasm [18] the results suggest that some transplanted nuclear proteins, whose degradation is inhibited by NH_4Cl and leupeptin, are degraded in lysosomes, which implies that they must translocate through the nuclear pore (the site for nucleo-cytoplasmic

exchange (e.g. [19]) to the cytoplasm before being degraded.

We cannot rule out the possibility that both NH_4Cl and leupeptin directly affect nuclear proteases, although we could measure little or no degradation of nuclear proteins in isolated whole or disrupted karyoplasts (not shown). The observation that two inhibitors of lysosomal protein degradation, which act through different mechanisms, both inhibit the degradation of some transplanted nuclear proteins strongly suggests that these proteins are degraded by a lysosomal mechanism. Furthermore since non-histone chromosomal proteins of mitogen stimulated lymphocytes are degraded by a lysosomal mechanism [20–22] it is possible that lysosomes play a hitherto unrecognized role in the breakdown of nuclear proteins.

REFERENCES

[1] Djondjurov, L.P., Yancheva, N.Y. and Ivanova, E.C. (1983) Biochemistry (USA) 22, 4095–4102.
[2] Yamaizumi, M., Uchida, T., Okada, Y., Furusawa, M. and Mitsui, H. (1978) Nature 273, 782–784.
[3] Rechsteiner, M., Kuel, L. and LeRoy, P. (1979) Cell 16, 901–908.
[4] Wu, L., Rechsteiner, M. and Kuehl, L.J. (1981) J. Cell Biol. 91, 488–496.
[5] Doherty, F.J. and Mayer, R.J. (1986) FEBS Lett. 198, 181–193.
[6] Poste, G. and Lyon, N.C. (1978) in: Cytochalasins: Biochemical and Cell Biological Aspects (Tanenbaum, S.W. ed.) Frontiers of Biology 46, pp.161–185, North-Holland, Amsterdam.
[7] Fernig, D.G. and Mayer, R.J. (1985) in: Microinjection and Organelle Transplantation Techniques (Celis, J.E. et al. eds) pp.237–249, Academic Press, New York.
[8] Veomett, G.E., Prescott, D.M., Shay, J.W. and Porter, K.R. (1974) Proc. Natl. Acad. Sci. USA 71, 1999–2004.
[9] Brown, R.L., Wible, L.J. and Brinkley, B.R. (1980) Cell Biol. Int. Rep. 4, 453–458.
[10] Shay, J.W. and Clark, M.A. (1979) J. Supramolecular Structure 11, 33–49.
[11] Russell, S.M. and Mayer, R.J. (1983) Biochem. J. 216, 163–175.
[12] Jeffrey, I.W., Flint, D.J., Vernon, R.G. and Mayer, R.J. (1985) FEBS Lett. 182, 451–454.
[13] Amenta, J.S., Hlivko, T.J., McBee, A.G., Shinojuka, H. and Brocher, S. (1978) Exp. Cell Res. 115, 357–366.
[14] Seglen, P.O. (1975) Biochem. Biophys. Res. Commun. 66, 44–52.
[15] Umezawa, H. and Aoyagi, T. (1983) in: Proteinase Inhibitors (Katunuma, N. et al. eds) pp.3–16, Springer, Berlin.
[16] Poole, B., Ohkuma, S. and Warburton, M. (1978) in: Protein Turnover and Lysosomal Function (Segal, H.L. and Doyle, D.J. eds) pp.43–58, Academic Press, New York.
[17] Berg, T. and Tolleshaug, H. (1980) Biochem. Pharmacol. 29, 917–926.
[18] Pfeifer, U. (1978) J. Cell Biol. 78, 152–167.
[19] Feldherr, C.M., Kallenbach, E. and Schultz, N. (1984) J. Cell Biol. 99, 2216–2222.
[20] Polet, H. (1983) Exp. Cell Res. 148, 345–362.
[21] Polet, H. (1985) J. Cell. Physiol. 122, 415–423.
[22] Polet, H., Molnar, J. and Goral, J. (1986) Biochim. Biophys. Acta 886, 33–39.
[23] Silverman, J.A. and Amenta, J.S. (1984) Anal. Biochem. 141, 538–544.