The EMBO Journal vol.3 no.8 pp.1849–1855, 1984

Spontaneous redistribution of cell-surface glycoproteins in lymphoid cells during cytokinesis

Stefanello de Petris

Department of Zoology, University College London, Gower Street, London WC1E 6BT, UK

Communicated by S. de Petris

The 'unperturbed' distribution of plasma membrane glycoproteins during cytokinesis has been examined by immunofluorescence and electron microscopy on dividing mouse and rat lymphoid cells fixed before being labelled with the appropriate reagents. Two groups of molecules which cap 'spontaneously' to the uropod of non-dividing cells, i.e., the common receptors for *Helix pomatia* (HPA) and peanut agglutinin (PNA) (and in particular the thymocyte glycophorin-like glycoprotein) and membrane immunoglobulins, redistribute spontaneously to the cleavage furrow during cytokinesis. By electron microscopy, the redistributed molecules (HPA receptors) appear to be aggregated in clusters. Other glycoproteins, such as Concanavalin A receptors and Thy.1 antigens, which do not cap spontaneously on interphase cells, remain uniformly distributed or are somewhat depleted over the cleavage furrow. The results suggest that a spontaneous 'transport' of certain membrane molecules from the nuclear pole to the cleavage furrow occurs normally during cytokinesis by a mechanism analogous to that of uropod formation and spontaneous capping in interphase cells. The existence of redistribution phenomena in dividing cells imposes some restrictions on the possible mechanisms of redistribution and on certain aspects of the cleavage process.

Key words: cytokinesis/lymphocyte membrane glycoproteins/spontaneous redistribution

Introduction

Particular surface glycoproteins can redistribute and cap 'spontaneously' (i.e., in the absence of external ligands) on the uropod of morphologically polarized leucocytes. Examples of such behaviour are membrane immunoglobulins (m-Ig) on B lymphocytes (Schreiner *et al.*, 1976) and common receptors for the lectins *Helix pomatia* agglutinin (HPA) and peanut agglutinin (PNA) on mouse and rat thymocytes, T lymphocytes and granulocytes (de Petris and Baumgartner, 1982). During the course of the investigation on the spontaneous capping of HPA and PNA receptors, I observed that in dividing thymocytes the receptors concentrate spontaneously over the cleavage furrow.

Berlin, Oliver and collaborators (Berlin *et al.*, 1978; Koppel *et al.* 1982) have shown that plasma membrane proteins cross-linked by a ligand [in the particular case, concanavalin A (Con A)-Con A receptor complexes] are able to redistribute to the cleavage furrow of dividing cells. The redistribution is analogous to capping as it is due to directional flow and not to diffusion and is strictly dependent on cross-linking (Koppel *et al.* 1982). Mitotic cells labelled in the cold show little or no redistribution. Berlin and Oliver suggested that the region of the cleavage furrow, characterised by the microfilament of the contractile ring, is functionally equivalent to the 'protuberance' (uropod) of interphase capping cells.

The (unpublished) observation that HPA receptors redistribute spontaneously during cytokinesis suggested that certain surface macromolecules can be transported to the cleavage furrow even in the absence of cross-linking by a ligand. An obvious hypothesis is that the molecules which show this behaviour are the same which cap spontaneously on the uropod of polarized lymphocytes. This would support the view of a substantial identity of the cytomechanical processes during cleavage and uropod formation. To test this hypothesis, I have examined the distribution on dividing lymphoid cells of two types of molecules known to cap spontaneously, i.e., the HPA/PNA receptors (on mouse and rat thymocytes) and m-Ig [on lipopolysaccharide (LPS)-stimulated mouse spleen cells and on Ig^+-positive hybridoma

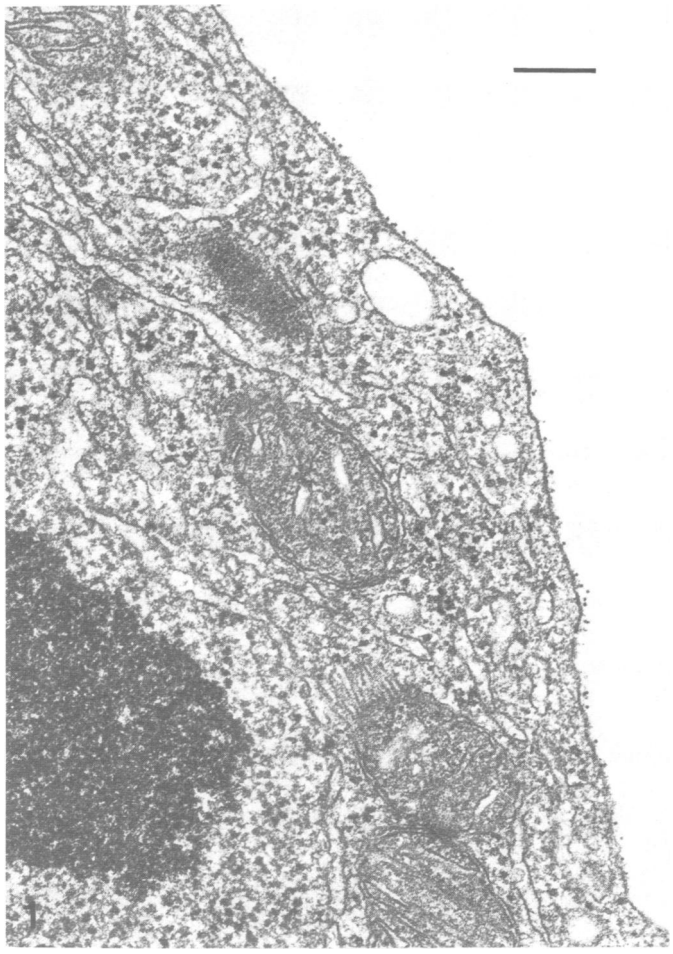

Fig. 1. Detail of a fixed mitotic mouse thymocyte in metaphase labelled with HPA-ferritin. The ferritin distribution is essentially dispersed. x 54 000 (bar: 0.2 μm).

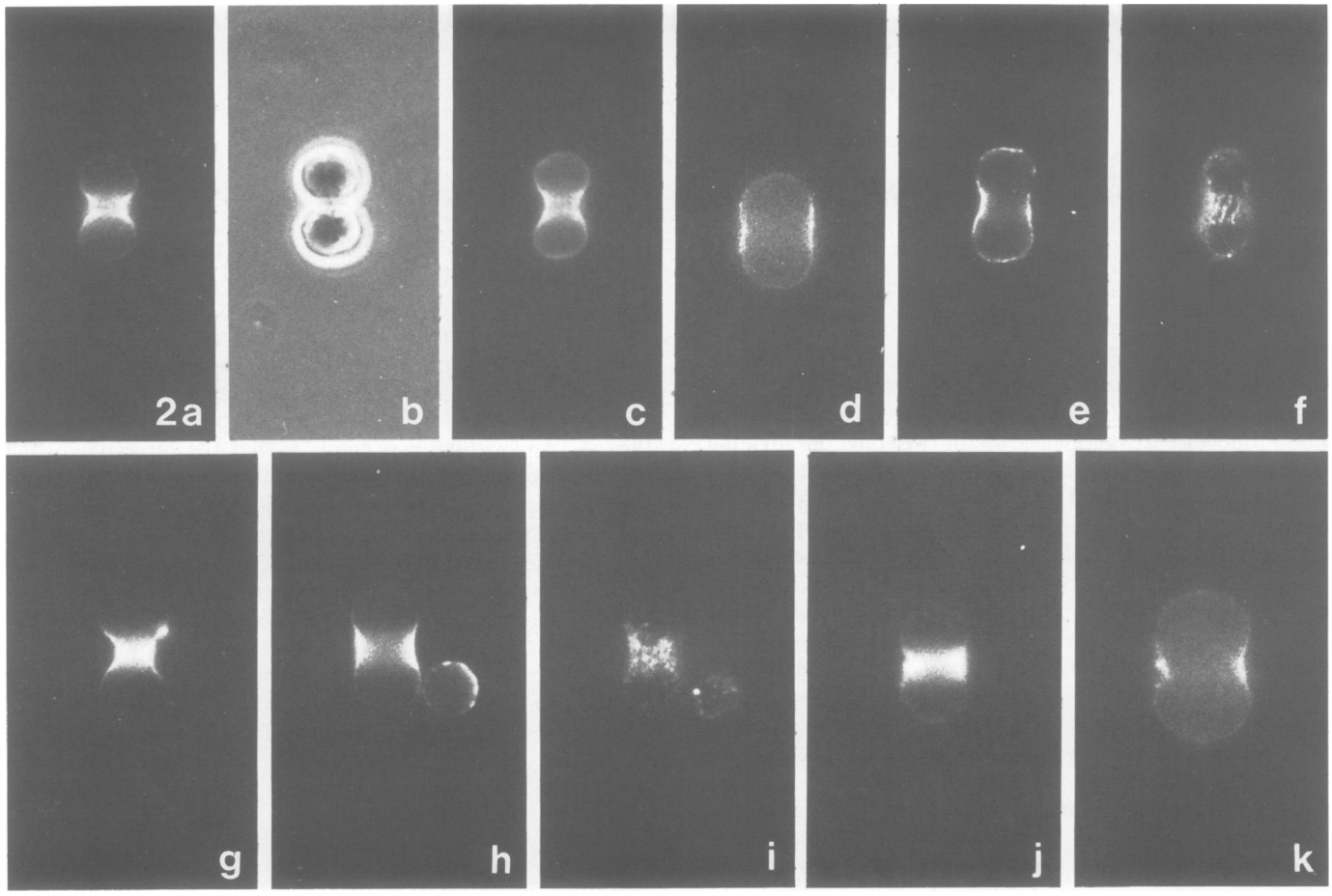

Fig. 2. Spontaneous (**a**–**f**, **j**–**k**) and ligand-induced (**g**–**i**) redistribution of HPA receptors and m-Ig on dividing lymphoid cells. All cells were incubated at 37°C, except the cell in **d** (28°C). x 1300. (**a**–**d**) Dividing mouse thymocytes, fixed and stained with TRITC-HPA [**a**,**b**: almost complete redistribution (**b**: phase contrast); **c**: incomplete redistribution; **d**: redistribution at an early stage of division]. (**e**–**f**) Dividing rat thymocytes, fixed and stained with antibody W3/13, followed by $(Fab')_2$ RaMIg-ferritin and TRITC-RaFer. The broken staining is an effect of the three-layer procedure. In **f**, the upper plane of the cleavage furrow is in focus, showing a structured pattern (ridges?). (**g**–**i**) HPA-induced redistribution. Unfixed dividing mouse thymocyte incubated with TRITC-HPA at 37°C. In **i**, the upper plane of the furrow is in focus, showing TRITC-HPA in patches. (**j**–**k**) Dividing LPS-stimulated mouse spleen cells (**j**) and WEHI 231 hybridoma cells (**k**), fixed and stained with $(Fab')_2$ RaMIg-ferritin, followed by TRITC-RaFer.

cells] and compared it with that of two non-spontaneously capping molecules, i.e., Con A receptors and Thy.1 antigen on thymocytes and a tumour cell line. All the cells were briefly fixed with glutaraldehyde (generally followed by formaldehyde) to ensure complete immobilization of the receptors, and were then labelled with the appropriate reagents for immunofluorescence or electron microscopy.

Results

Distribution of PNA/HPA receptors (and glycophorin-like glycoprotein)

The distribution of HPA receptors (as well as that of all the other molecules examined in this study) on fixed metaphase mouse and rat thymocytes was uniform by immunofluorescence, in agreement with the observations of Berlin *et al.* (1978) and Oliver and Berlin (1982). By electron microscopy the distribution of HPA-ferritin was dispersed with occasional small clusters (Figure 1).

In contrast, on cells fixed during cytokinesis, in which a cleavage furrow was already visible or well developed, the fluorescent HPA (or PNA, data not shown) was preferentially concentrated on the furrow (Figure 2). The staining intensity on mitotic cells varied considerably, but was generally weaker than on non-dividing cells. The redistribution was more striking in well stained cells, but was noticeable also in the weakly stained ones. The redistribution was not obviously correlated with the stage of development of the furrow (Figure 2a–d) and was usually incomplete, as some stain remained on the nuclear poles (Figure 2a–c). This pattern could not be attributed entirely to the presence of minor non-capping HPA-binding components, as it was obtained also with monospecific reagents, such as the monoclonal antibody W3/13 (Standring *et al.*, 1978). The latter recognizes the rat thymocyte glycophorin-like glycoprotein (Standring *et al.*, 1978; Brown *et al.*, 1981), which is probably the major spontaneously capping HPA/PNA-binding component (de Petris, 1984). W3/13 staining on fixed mitotic cells was very weak, and had to be enhanced by a three-layer procedure (see Materials and methods), a process which led to some detachment and clustering of the fluorescent complexes. Although most of the antigen was concentrated on the furrow, clearly some remained on the nuclear poles (Figure 2e,f).

As shown by electron microscopy, the increased HPA staining on the furrow reflected a real increase in the surface concentration of the molecules, and was not due to geometrical or structural factors such as accumulation of microvilli (which are in fact rare or absent in most thymic lymphocytes) (Figure 3). The ferritin molecules were mainly dispersed

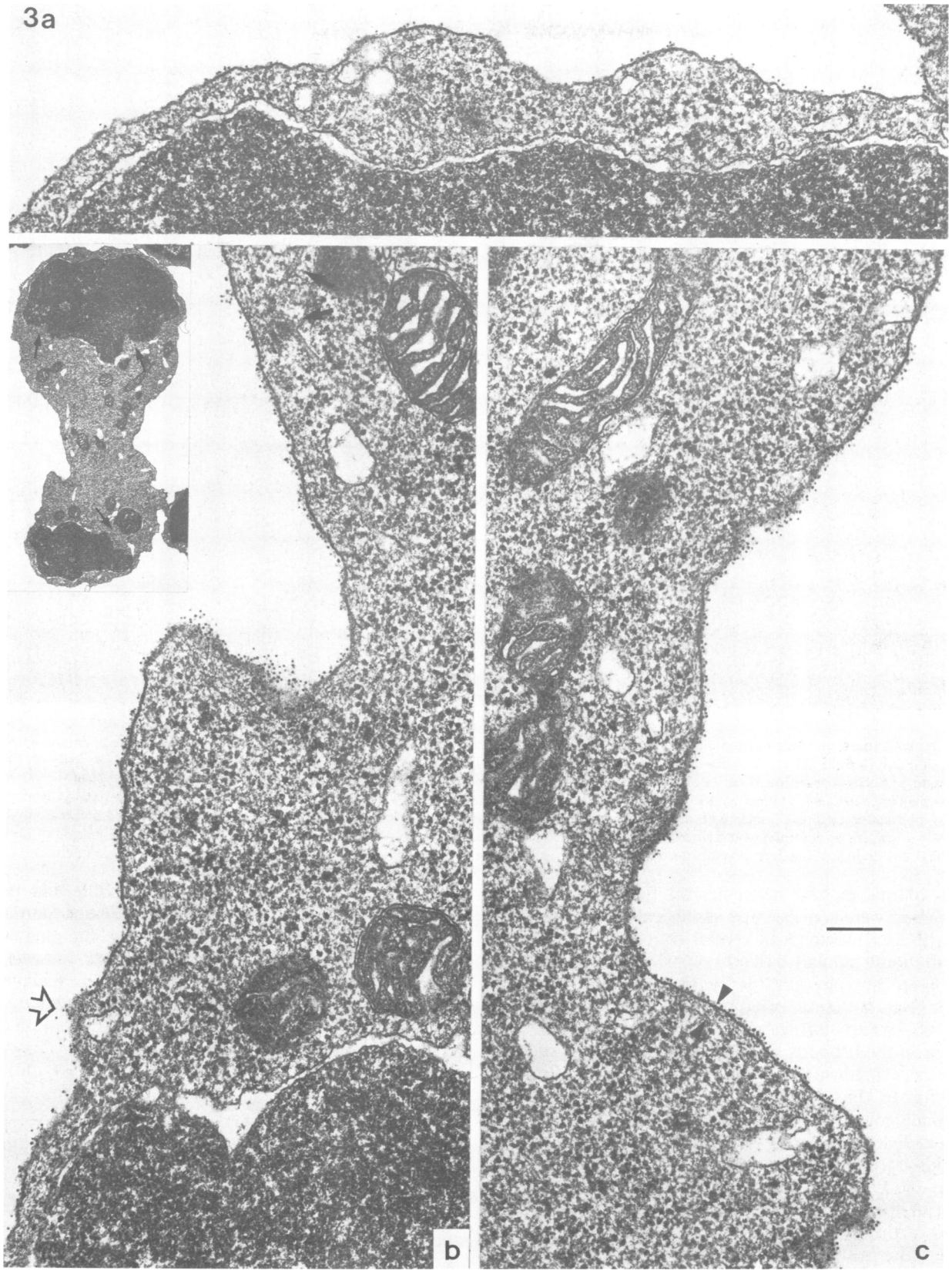

Fig. 3. Dividing mouse thymocyte (telophase), fixed and labelled with HPA-ferritin. Details **a**–**c**: x 47 500 (bar: 0.2 μm). **Inset:** general view. The perinuclear membrane is completely reformed over the poles (cf. detail in **a**), but is still incomplete (small arrows) on the side facing the furrow. x 5300. **(a)** Detail of the (upper) nuclear pole. The ferritin molecules are mostly dispersed. **(b)** Transition (empty arrow) from the nuclear region with dispersed ferritin molecules (bottom; compare with similar area in **c**) to the cleavage furrow area (centre and top) with ferritin in clusters and patches. Arrow (top) points at microtubules apparently ending at a dense area immediately underneath the cortical layer. **(c)** Detail of the cleavage area (right side). The ferritin is mainly concentrated in clusters and patches (average concentration over the two sides of the furrow: 51 molecules/μm as against 11 molecules/μm over the two poles). Arrowheads point to the cortical microfilament layer (obliquely sectioned in places).

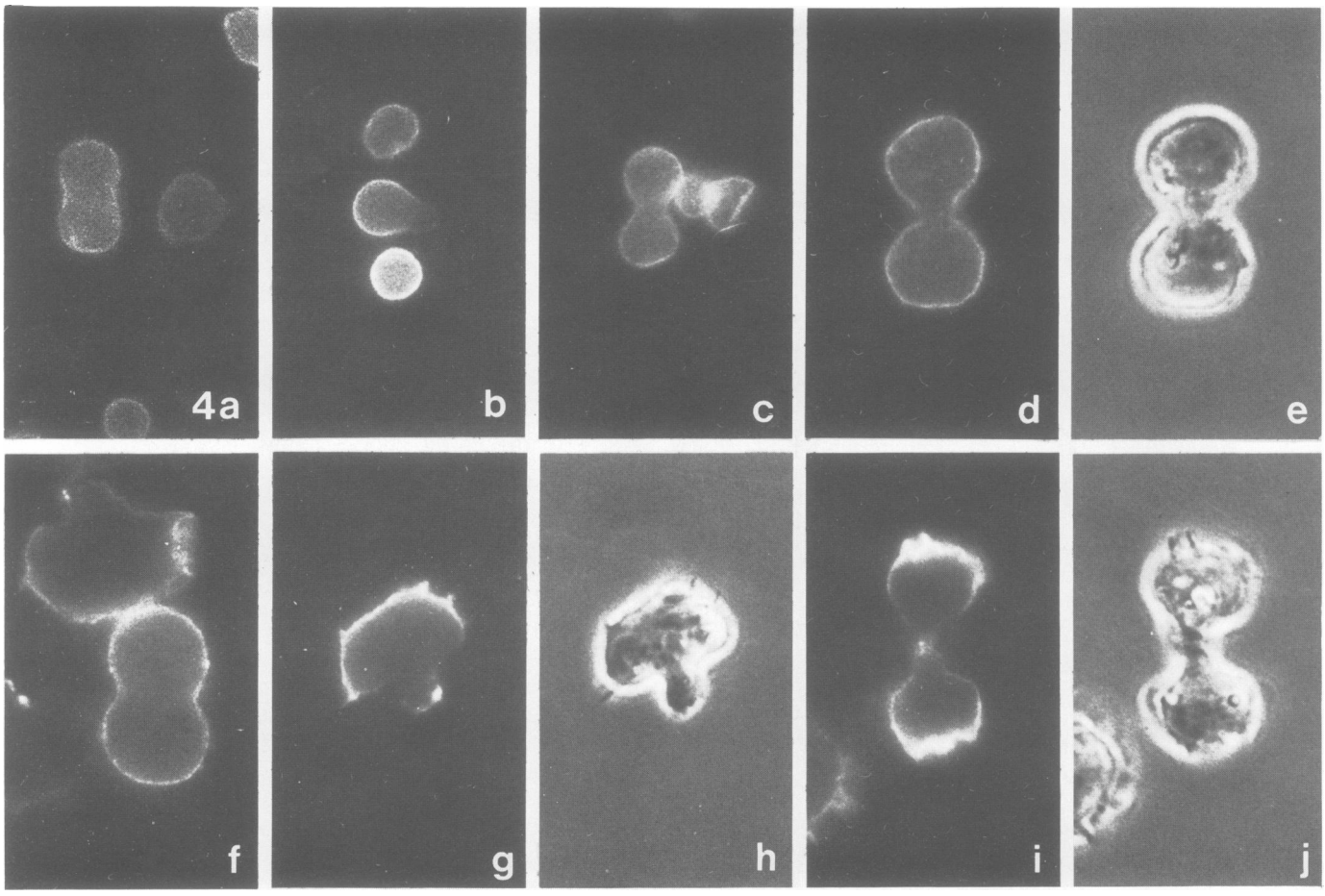

Fig. 4. Distribution of non-spontaneously capping molecules on dividing lymphoid cells. x 1300. (**a,b**) Mouse thymocytes incubated with an anti-Thy 1.1 monoclonal antibody (10 min, 37°C), then fixed and stained with TRITC-GaMIg (**a**: uniform distribution on a dividing cell; **b**: two spherical cells and one cell with uropod with stain concentrated on the front of the cell). (**c**) Dividing mouse thymocyte, fixed and stained with FITC-Con A. Uniform distribution. (**d,e**) EL4 lymphoblast, fixed and stained with FITC-Con A (**d**: uniform distribution, with reduced staining on the cleavage furrow; **e**: phase contrast). (**f–i**) EL4 lymphoblasts, fixed and stained with an anti-Thy 1.2 monoclonal antibody followed by TRITC-GaMIg [**f**: one dividing cell and one motile cell; **g,h**: interphase cell (**h**: phase contrast) with stain concentrated on the front of the cell, and with constriction and most of the uropod depleted of antigen; **i,j**: pair of ruffling cells (**j**: phase contrast), possibly a dividing cell still joined at the midbody, with a 'reverse' distribution typical of motile interphase cells].

as groups of one or two molecules on the nuclear poles (Figure 3a) and were concentrated in clusters in the cleavage furrow, with larger patches in central region (Figure 3b,c). This distribution is similar to that observed on the uropod of spontaneously capping cells (de Petris and Baumgartner, 1982). No special structures could be identified in this area except for a 60–70 nm cortical microfilament layer similar to that present in the uropods, and, more internally, arrays of microtubules (not shown), some of which apparently terminate at the furrow (Figure 3b).

The spontaneous redistribution pattern of HPA receptors on pre-fixed dividing cells was very similar to that induced on neuraminidase-treated cells by TRITC-HPA before fixation (Figure 2g–i). Ligand-induced capping was more complete, with most of the lectin clearly concentrated (in patches, see Figure 2h) in the centre of the furrow, although in several cells some still remained over the nuclear poles (Figure 2h,i). The shape of the dividing cells was not apparently modified by ligand binding and redistribution.

Distribution of membrane Ig

The distribution of m-Ig, another spontaneously capping component, was examined on fixed B lymphoid cells. Most of the stain was concentrated on the cleavage furrow of mitotic LPS-stimulated mouse spleen cells (Figure 2j). Also in this case the fluorescence intensity was variable and relatively weak on mitotic cells, and the redistribution partial, with some stain remaining on the nuclear poles. Staining was even weaker on fixed WEH1 231 hybridoma cells, but even in this case most of the membrane IgM appeared to have 'capped' spontaneously to the cleavage furrow (Figure 2k).

Distribution of Con A receptors and Thy.1 antigen

Unlike the distribution of HPA receptors on mouse and rat thymocytes, the distribution of two classes of molecules which do not cap spontaneously on the same cells or on EL4 lymphoma cells, i.e., the Con A receptors [on at least the majority of them (de Petris, 1978a)] and the Thy.1 antigen (de Petris and Raff, 1974) remained essentially uniform on fixed dividing cells, or the staining was possibly even somewhat depleted over the furrow (Figure 4a,c–f). In several cases, the fluorescent rim, as observed by focussing up and down over the cleavage furrow, appeared thinner and less bright than on the poles (although this was difficult to reproduce photographically because of the stronger background halo generated by the out of focus fluorescent cell surfaces which are closer to the focussed plane in this region). This decrease in staining was clearly less marked than that observed on polarized non-dividing cells of the same samples, especially in the case of Thy.1 antigen, which, as previously reported (de

Petris and Raff, 1974) was strongly depleted on the uropod and in particular on the constriction region. This 'reversed' distribution was not perturbed if a monoclonal anti-Thy.1.1 antibody was added to the mouse thymocytes at 37°C 10 min before the cells were fixed (Figure 4b) [although patches and caps could be induced by the further addition of a second cross-linking layer (data not shown)]. The 'reverse' pattern was even more striking in the EL4 cells (60–70% of which were motile) stained with an anti-Thy.1.2 antibody after fixation (Figure 4g,h). The Thy.1.2 antigen was uniformly distributed, or was possibly slightly depleted on the cleavage furrow of dividing cells, whereas it was strongly concentrated on the leading edge of the motile cells and absent or almost completely absent from the post-nuclear constriction and from the uropod (except for isolated 'patches' on the distal part of the latter) (Figure 4g,h). This polarized pattern was apparently acquired by the dividing cells (some still attached by the mid body) as they regained motile activity at the completion of mitosis (Figure 4i,j).

Discussion

The present observations show that during lymphoblast cytokinesis there is a ligand-independent redistribution of certain surface components to the cleavage furrow. The molecules which redistribute, such as surface m-Ig and the lymphocyte glycophorin-like glycoprotein, appear to be the same molecules which cap spontaneously on the uropod of polarized lymphocytes. Other molecules (probably including the majority of surface glycoproteins) such as the majority of Con A receptors and the Thy.1 antigen are not transported to the furrow. The nature of the molecules which redistribute, together with the ultrastructural characteristics of the distribution (practically identical to that on lymphocytes with uropods), strongly suggests that spontaneous redistribution in dividing and non-dividing cells occurs by the same mechanism, and that the mechanical processes during cleavage and uropod formation are basically equivalent. This is consistent with the observations of Berlin and Oliver (1980) and Berlin *et al.* (1978) on Con A receptors and with their view that the constriction ring region of dividing cells is functionally analogous to the 'protuberance' [uropod, or perhaps more properly, post-nuclear constriction and uropod (de Petris, 1978)] of non-dividing capping cells, with the cleavage furrow corresponding to the uropods of the two daughter cells joined together with opposite polarity. Berlin and his colleagues showed that the redistribution of Con A receptors was induced by cross-linking by the ligand, an event which by itself could have triggered the process. The present results, however, indicate that a unidirectional transport of certain membrane molecules from the nuclear pole to the cleavage furrow takes place normally in dividing cells in the absence of external stimuli. This normal process could be further stimulated by cross-linking, although it cannot be excluded that the latter simply makes membrane molecules more susceptible to transport by the formation of surface aggregates. Although the evidence for a spontaneous transport is limited to lymphoid cells for which suitable markers are available, such a transport is likely to be a general feature of dividing cells. One of its visible manifestations is probably the membrane shrinkage and the accumulation of membrane-attached particles into the furrow, well documented in classical studies of cytokinesis (see, for example, reviews in Dan, 1948; Wolpert, 1960).

As shown by the distribution of HPA-ferritin [which on glutaraldehyde-fixed cells probably mostly labels the glycophorin-like glycoprotein (de Petris and Takacs, 1983; de Petris, 1984)], the HPA receptors remaining on the nuclear poles are mainly dispersed, whereas those transported to the cleavage furrow are aggregated in clusters. This is in agreement with the previous observation that these molecules have a tendency to aggregate spontaneously, and suggests that the aggregates, rather than the single molecules, are preferentially redistributed. As the clusters on the cleavage furrow are more numerous and larger than those seen on metaphase cells (cf. Figure 1), it seems likely that the size of the aggregates increases during transport to the furrow, or after they have accumulated there. The fact that the clustered molecules do not redisperse could indicate that the aggregation process has a strong concentration dependence or that the mobility of the molecules is restricted (for example, by interaction with cortical structures). These observations do not provide unambiguous evidence for a particular mechanism of redistribution, as aggregation would favour redistribution according to any of the proposed models for ligand-dependent and -independent redistribution. However, the particular characteristics of mitotic cells impose some restrictions on the possible redistribution mechanisms in dividing and (by analogy), in non-dividing cells. Among these mechanisms, the overall membrane (or lipid) recirculation mechanism (Bretscher, 1976) faces the greatest difficulty as it would require massive membrane internalization at the cleavage furrow in order to maintain a steady flow. Pinocytosis, however, appears to be dramatically reduced (30-fold) during mitosis, including cytokinesis, and resumes only at the telophase-G1 transition (Berlin and Oliver, 1980). Some 'new' membrane from an intracellular pool is probably inserted at the cell surface during cytokinesis (Warren *et al.*, 1983), but insertion alone could not generate a flow. Mechanisms involving direct interactions between cortical layer and membrane molecules [anchorage/entrapment models (Bourguignon and Singer, 1978; de Petris, 1977; de Petris and Baumgartner, 1982), with the molecules being dragged by moving cortical structures] are more consistent with the experimental observations and also with some proposed mechanisms of cleavage (White and Borisy, 1983). As regards spontaneous redistribution, a 'surf-riding' model (Hewitt, 1979), as modified by Oliver and Berlin (1982) (who suggest that molecules with a spontaneous tendency to redistribute to areas of high curvature would follow the moving crest of a surface wave) is not incompatible with the characteristics of spontaneously capping molecules such as m-Ig or HPA receptors which tend to concentrate on high curvature regions such as microvilli (de Petris and Baumgartner, 1982; de Petris, 1978b). The free energy changes connected with changes in curvature [as estimated from observed m-Ig concentration ratios on microvilli and cell body, $c_{mv}/c_{cb} \leq 10$ (de Petris, 1978b and unpublished data)] are probably small ($\leq 1-2$ kcal/mol), but over a short range (say, 0.1 μm), they could generate forces possibly strong enough ($\sim 10^{-8}$ dyne) to keep the molecules entrained with the hypothetical waves.

Although the redistribution on dividing and non-dividing cells appears to have the same basic characteristics, there are clearly some differences. Spontaneous redistribution to the furrow is, to a variable extent, incomplete (especially in weakly stained cells). A lower redistribution efficiency could be related to a somewhat lower transport rate, to differences in

the characteristics of the transported molecules (for example, in the degree of spontaneous clustering) or in the organization of the cytoskeleton [which might vary even between individual dividing cells (Nunnally *et al.*, 1980)]. The differences are even more obvious in the case of the 'reverse' redistribution of Con A receptors and, in particular, of the Thy.1 antigen. In most interphase cells (EL4 or normal thymocytes) with a uropod the Thy.1 antigen is completely or almost completely excluded from the narrow post-nuclear region and concentrated on the ruffling front of the cell and in a few spots over the uropod, whereas in dividing cells the reduction in labelling over the cleavage area is (by immunofluorescence) only marginal. If, as previously suggested (de Petris, 1978a), the Thy.1 (and Con A receptor) molecules are progressively excluded from the constriction at the same time as specific (but as yet unknown) cytoskeleton-bound molecules which apply tension to the membrane become progressively concentrated in that area, it would follow that the degree of constriction in the cleavage furrow is less than in highly polarized interphase cells. The normal motility and redistribution characteristics are apparently restored at the end of mitosis (possibly in concomitance with the resumption of pinocytosis). On the whole, it looks as if in dividing cells the mechanism for unidirectional displacement of surface molecules (which probably depends on the activity of cortical structures) is operating, but other manifestations of motility are suppressed. One possibility is that this is due to a stabilizing effect of microtubules of the mitotic apparatus on the cell cortex. However, as in the case of interphase cells (de Petris and Baumgartner, 1982), microtubules probably do not play any active role in the actual mechanism of redistribution, although the mitotic apparatus apparently determines the position of the cleavage furrow (Wolpert, 1960; Rappaport, 1965) and hence the polarity of the redistribution. The similarity with redistribution in interphase cells is also relevant to the possible role of the asters in the initiation of cleavage, as postulated in the astral relaxation theory (e.g., Wolpert, 1960; Rappaport, 1965; White and Borisy, 1983). According to this theory (supported by considerable evidence) cleavage is initiated by a relaxation of surface tension at the nuclear poles under a direct or indirect influence of the asters. However, if polarization and surface redistribution mechanisms are essentially the same in dividing and non-dividing cells, it is unlikely that the asters (and in particular their microtubules) are directly involved in the generation of the signal for cleavage, as analogous morphological and molecular events can be stimulated in interphase cells in the absence of microtubules (de Petris, 1978a; de Petris and Baumgartner, 1982). It seems more likely that the signal for the initiation of cleavage, like that for uropod formation and spontaneous capping, is generated at the nuclear pole(s) by non-microtubular structures, and that the position of the asters (or, in general, of the mitotic apparatus) at anaphase mainly determines the localization and organization of these structures, which in interphase cells can in fact remain functional for several hours after microtubule depolymerization.

Materials and methods

Techniques and reagents were as in previous studies (de Petris and Baumgartner, 1982; de Petris and Takacs, 1983) with a few modifications.

Cells

Thymocytes were obtained from 2–4 week old Wistar rats and from AKR and CBA 1.1 BC 10 F_4 congenic mice. Mice from both these strains carry the Thy 1.1 antigen.

Spleen cells from BALB/c mice were stimulated with 50 μg/ml of LPS for 72 h. The mouse hybridoma cell line WEHI 231 (which has IgM molecules on its surface) and a subline of EL4 mouse lymphoma cells (which carry the Thy.1.2 antigen), were grown in modified Iscove medium supplemented with 10% foetal calf serum. LPS-stimulated spleen cells and cell lines were kindly provided by Dr. M. Ratcliffe.

The cells were collected by centrifugation, and 4–8 x 10^6 tumour cells resuspended in 1 ml of the original medium, or 20 x 10^6 thymocytes in 1 ml of Hepes-buffered Minimal Essential Medium (H-MEM) containing 0.25% bovine serum albumin (BSA) were further incubated for 30–45 min at 37°C (occasionally, at 28–30°C) and then fixed (see below).

Reagents

Tetramethylrhodamine-conjugated HPA (TRITC-HPA), PNA (TRITC-PNA), goat anti-mouse Ig immunoglobulin (TRITC-GaMIg) and rabbit anti-ferritin immunoglobulin (TRITC-RaFer), and fluorescein-conjugated concanavalin A (FITC-Con A) were previously described (de Petris and Baumgartner, 1982; de Petris, 1975). A ferritin-conjugate of $(Fab')_2$ rabbit anti-mouse Ig fragments [$(Fab')_2$-RaMIg], prepared as described (de Petris and Raff, 1972), was a kind gift of Dr. D. Lawson. The anti-Thy 1.1 antibody was a mouse IgG monoclonal originally produced by Dr. P. Lake in our Department by a described method (Lake *et al.*, 1979), the anti-Thy 1.2 antibody, the mouse IgM monoclonal HO.13.4 (Marshak-Rothstein *et al.*, 1979), subcloned by Dr. G. Köhler, and kindly provided by Dr. M. Ratcliffe. The mouse monoclonal W3/13 antibody against rat glycophorin-like glycoprotein (Standring *et al.*, 1978) was obtained from Seralab (Crawley Down, Sussex, UK).

Formaldehyde (4%, FA) and glutaraldehyde (6%, GA) in 0.12 M sodium phosphate buffer pH 7.4 were prepared freshly before use from paraformaldehyde powder (Fluka, Buchs, CH) or 25% GA stock solution (Elmskop Labs., Ashford, Kent, UK).

Fixation and staining

Immediately before fixation the top 0.5 ml of essentially cell-free supernatant was removed from the cell samples, and the cells in the bottom 0.5 ml were fixed by addition of 0.1 ml of 6% GA for 30 or 60 s or 0.2 ml for 30 s, followed by dilution with 12 ml of 4% FA. After 5 min the cells were centrifuged, resuspended in 4% FA (for a total fixation time of 15–30 min) and finally washed once with phosphate buffered saline (PBS) and twice with PBS/0.2% BSA. The cells were treated with sodium borohydride (20 mM, 30 min at 37°C, in isotonic carbonate-buffered saline at pH 9.0) to quench GA autofluorescence. Cells to be stained with HPA or PNA were additionally treated with 0.025 IU neuraminidase (de Petris and Takacs, 1983). Brief GA fixation was employed routinely despite its adverse effect on the antigenic characteristics of many surface molecules because FA fixation alone apparently does not immobilize completely some membrane glycoproteins, such as the HPA receptors. The redistribution effects described in the Results section were, however, also detectable in cells fixed with FA alone (data not shown).

Fixed cells were stained according to standard procedures with fluorescent lectins, or in the case of the Thy.1 antigens, with the monoclonal antibody followed by TRITC-GaMIg. In some experiments the anti-Thy1.1 antibody (ascites, diluted 1:500) was added to the unfixed cells during the last 10 min of incubation at 37°C. The cells were then fixed, treated with borohydride, and stained with TRITC-GaMIg. In the case of surface Ig, to compensate for the decreased staining resulting from GA fixation, the fluorescence was amplified by staining the cells with a $(Fab')_2$-RaMIg-ferritin conjugate followed by TRITC-RaFer (de Petris and Baumgartner, 1982). Similarly, the W3/13 antibody was followed by $(Fab)_2$-RaMIg-ferritin and TRITC-RaFer (de Petris, 1984).

To examine the effect of ligand-induced redistribution of HPA receptors on unfixed thymocytes, the cells in H-MEM/BSA were incubated for 20 min at 37°C in the presence of neuraminidase (0.025 IU) centrifuged, incubated for 10 min in fresh medium without neuraminidase, and for additional 5 min in the presence of 100 μg/ml of HPA-TRITC. The cells were then fixed with GA/FA.

All cells were examined and photographed with a standard Leitz fluorescence microscope with epi-illumination. Mitotic cells were identified by phase contrast, and then analysed for the distribution of fluorescence. For electron microscopy, mouse thymocytes incubated at 37°C were fixed with glutaraldehyde and labelled with HPA-ferritin as described (de Petris and Baumgartner, 1982).

References

Berlin,R.D. and Oliver,J.M. (1980) *J. Cell Biol,* **85**, 660-671.
Berlin,R.D., Oliver,J.M. and Walter,R.J. (1978) *Cell,* **15**, 327-341.
Bourguignon,L.-Y. and Singer,S.J. (1977) *Proc. Natl. Acad. Sci. USA,* **74**, 5031-5035.

Bretscher,M.S. (1976) *Nature,* **260**, 21-23.
Brown,W.R.A., Barclay,N., Sunderland,C.A. and Williams,A.F. (1981) *Nature,* **289**, 456-460.
Dan,J.C. (1948) *Physiol. Zool.,* **21**, 191-218.
de Petris,S. (1975) *J. Cell Biol.,* **65**, 123-146.
de Petris,S. (1977) in Poste,G. and Nicolson,G.L. (eds.), *Dynamic Aspects of Cell Surface Organization*, North Holland, Amsterdam, pp. 643-728.
de Petris,S. (1978a) *J. Cell Biol.,* **79**, 235-251.
de Petris,S. (1978b) *Nature,* **272**, 66-68.
de Petris,S. (1984) *Exp. Cell Res.,* **152**, 510-519.
de Petris,S. and Raff,M.C. (1972) *Eur. J. Immunol.,* **2**, 523-535.
de Petris,S. and Raff,M.C. (1974) *Eur. J. Immunol.,* **4**, 130-137.
de Petris,S. and Baumgartner,U. (1982) *J. Ultrastruct. Res.,* **80**, 323-338.
de Petris,S. and Takacs,B. (1983) *Eur. J. Immunol.,* **13**, 831-840.
Hewitt,J.A. (1979) *J. Theor. Biol.,* **80**, 115-127.
Koppel,D.E., Oliver,J.M. and Berlin,R.D. (1982) *J. Cell Biol.,* **93**, 950-960.
Lake,P., Clark,E.A., Khorshidi,M. and Sunshine,G.H. (1979) *Eur. J. Immunol.,* **9**, 875-886.
Marshak-Rothstein,A., Fink,P., Grindley,T., Raulet,D.H., Bevan,M.J. and Gefter,M.L. (1979) *J. Immunol.,* **122**, 2491-2497.
Nunnally,M.H., D'Angelo,J.M. and Craig,S.W. (1980) *J. Cell Biol.,* **87**, 219-226.
Oliver,J.M. and Berlin,R.D. (1982) *Int. Rev. Cytol.,* **74**, 55-94.
Rappaport,R. (1965) *J. Theor. Biol.,* **9**, 51-66.
Schreiner,G.F., Braun,J. and Unanue,E.R. (1976) *J. Exp. Med,.* **144**, 1683-1688.
Standring,R., McMaster,W.R., Sunderland,C.A. and Williams,A.F. (1978) *Eur. J. Immunol.,* **8**, 832-839.
Warren,G., Featherstone,C., Griffiths,G. and Burke,B. (1983) *J. Cell Biol.,* **97**, 1623-1628.
White,J.G. and Borisy,G.G. (1983) *J. Theor. Biol.,* **101**, 289-316.
Wolpert,L. (1960) *Int. Rev. Cytol.,* **10**, 163-216.

Received on 7 May 1984