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Synthesis of surface sphingomyelin in the plasma membrane recycling pathway of BHK cells

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Abstract

Sphingomyelin, which has been degraded at the BHK cell surface by exogenous sphingomyelinase, is converted back into sphingomyelin with kinetics similar to those of plasma membrane recycling. Resynthesis of sphingomyelin under these conditions proceeds at a rate about 4-fold higher than normal biosynthesis of sphingomyelin. Neither resynthesis of sphingomyelin nor its return to the surface is inhibited by brefeldin A (BFA), which is a potent blocker of vesicular transport through the Golgi but has no effect on plasma membrane recycling. However, resynthesis of plasma membrane sphingomyelin is greatly decreased in cells undergoing mitosis or energy depletion, where endocytosis is inhibited. We conclude that the main site of surface sphingomyelin synthesis in BHK cells could be in recycling endosomes and not in the Golgi apparatus as proposed previously. We also suggest a model pathway by which cholesterol may reach the plasma membrane via recycling endosomes.

Key words: Sphingomyelin; Endocytosis; Brefeldin A; Cholesterol

1. Introduction

It has been known for some years that sphingomyelin is synthesised by an unusual process which depends on transfer of the phosphocholine headgroup of pre-existing phosphatidylcholine to ceramide [1–4]. Although it seems clear that all the steps in ceramide biosynthesis occur in the endoplasmic reticulum (ER) [5], the precise location of the site of sphingomyelin synthesis has been the subject of some dispute. Originally it was thought to be the plasma membrane [3,6] but recent work has suggested that sphingomyelin is synthesised in the early Golgi [7–10] and is delivered to the cell surface by a vesicular transport process which follows the secretory pathway [11–13]. Consistent with this interpretation, inhibitors of vesicular transport such as monensin and BFA interfere with the normal movement of sphingomyelin to the surface [13–15].

We have recently found evidence for a site of synthesis of internal sphingomyelin which could be in the ER or early Golgi [14–16], but this does not appear to be the site responsible for the synthesis of cell surface sphingomyelin. From evidence that monensin inhibits the synthesis of cell surface sphingomyelin from ceramide, we have suggested that sphingomyelin destined for the cell surface is synthesised not in the early Golgi but at a site distal to the medial Golgi [15]. We show here that in BHK cells there is a major site of sphingomyelin synthesis which is not in the Golgi, based on its insensitivity to BFA and monensin, and which appears to be part of the plasma membrane endocytic recycling pathway. This site may be responsible for the de novo synthesis of plasma membrane sphingomyelin.

2. Methods

Incubation of cells and measurement of sphingomyelin resynthesis. BHK 21 cells were cultured in 3.5-cm dishes using 2 ml of Glasgow MEM supplemented with 5% foetal calf serum and tryptose phosphate as described previously [14,15,17]. In some experiments cells were labelled for 4 h with 20 μCi of [^{3}H]acetate (NEN-Dupont) with and without addition of 5 μg/ml BFA. Half of the samples were then treated with 1 μl (0.1

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IU) *B. cereus* sphingomyelinase (Sigma) for 20 min to degrade surface sphingomyelin. The medium was removed and then all of the samples were extracted with 1.9 ml of methanol/chloroform (2:1 v/v).

In most experiments cells labelled to equilibrium (48 h) with 5 μCi of [^{3}H]acetate (NEN Dupont) were washed with cold MEM, reincubated at 37°C with serum-free MEM and then treated with 1 μl (0.1 IU) *B. cereus* sphingomyelinase (Sigma) for 20 min to degrade surface sphingomyelin. They were then washed three times with ice-cold serum-free MEM and returned to the same medium at 37°C for up to 3 h to allow them to resynthesise sphingomyelin. At various time points triplicate samples of cells were washed with 2 ml ice-cold saline and extracted with 1.9 ml methanol/chloroform (2:1 v/v). Some samples which had been allowed to resynthesise sphingomyelin for 3 h were treated for a second time with sphingomyelinase before extraction to determine if the resynthesised sphingomyelin had returned to the surface. Experiments were carried out in which the cells were allowed to resynthesise sphingomyelin in the presence of *N*-ethylmaleimide (100 μM), digitonin (50 μM), BFA (5 μg/ml), or nocodazole (10 μM) (all from Sigma). Nocodazole was added 30 min prior to addition of sphingomyelinase. In other experiments cells were energy-depleted by treatment for 2 h with 0.1 mM KCN and 50 mM deoxyglucose or with 30 mM NaF prior to breakdown and resynthesis of sphingomyelin as above.

Preparation of mitotic cells. A modified version of the method of Featherstone et al. [18] was used for preparation of mitotic cells. BHK21 cells were grown as monolayers to subconfluence in 75-cm^2 Falcon flasks. Cells from one flask were seeded out into 850-cm^2 Falcon roller bottles containing 50 ml growth medium plus 500 μCi [^{3}H]acetate. The bottles were incubated under constant rotation for 2 days at 37°C. To synchronise the cells in interphase, thymidine was added to a final concentration of 5 mM at the end of day one. Following incubation overnight for 10–12 h at 37°C the bottles were rotated for 5 min at 200 rpm to remove loosely-attached cells and cell debris. The medium was discarded and the cells washed twice with cold growth medium. Subsequently the cells were reincubated for 3 h in 50 ml radioactive growth medium (10 μCi [^{3}H]acetate/ml) plus 0.1 μM nocodazole. This treatment blocks the cells in metaphase after passing G2. Mitotic cells were harvested by rotating the roller bottles for 15 min at 200 rpm. The cells were sedimented by centrifugation of the medium at 1000 rpm for 5 min and resuspended in 20 ml of serum-free growth medium. About $1.5–3 \cdot 10^7$ cells were thus obtained from each roller bottle, equivalent to a mitotic yield of 15–30%.

Sphingomyelin resynthesis in mitotic cells. Triplicate 0.5-ml aliquots were taken from the resuspended mitotic cells for lipid extraction before and immediately after treatment of the cells with *B. cereus* sphingomyelinase (0.1 units/ml) for 20 min at 37°C. The remainder of the sample after treatment with sphingomyelinase was divided into two portions and washed twice with cold growth medium either with or without nocodazole (0.1 μM). The cells were suspended to a volume of 12 ml in growth medium with or without addition of nocodazole and triplicate 0.5-ml aliquots were incubated at 37°C for up to 4 h, before extraction and analysis of lipids.

Lipid analysis. Lipids were separated by tlc as described previously [14] and identified by comparison with standards (Sigma). Spots identifiable by iodine-staining were transferred to Pico Prias counting vials (Canberra Packard) to which were added 0.2 ml of methanol/water/acetic acid (5:3:2 v/v) and 2 ml of Ultima Gold scintillation fluid. Samples were counted in a Canberra Packard TC2500 scintillation counter.

ATP determinations. These were carried out on perchloric acid extracts of cells which had been allowed to resynthesise sphingomyelin as above. The method [19] employed firefly luciferase (Sigma).

3. Results and discussion

BFA prevents delivery of newly synthesised sphingomyelin to the plasma membrane but has no effect on resynthesis and surface localisation of sphingomyelin degraded at the cell surface

Fig. 1 shows that normal delivery of endogenously synthesised sphingomyelin to the cell surface is prevented by BFA [14]. Thus in the absence of BFA about 40% of newly synthesised SM can be degraded by

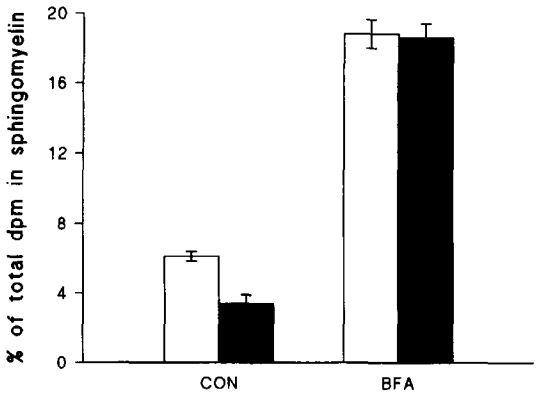


Fig. 1. BFA increases de novo synthesis of sphingomyelin but prevents it from reaching the cell surface. Triplicate dishes of cells were labelled with [^{3}H]acetate for 4 h in the absence (CON) or presence of BFA (5 μg/ml) and then incubated with (filled bars) or without (open bars) sphingomyelinase. Extraction and analysis of lipids was as described in Section 2. Results are expressed as mean ± S.D. of percentage of total lipid radioactivity in sphingomyelin. Similar results were obtained in three other experiments.

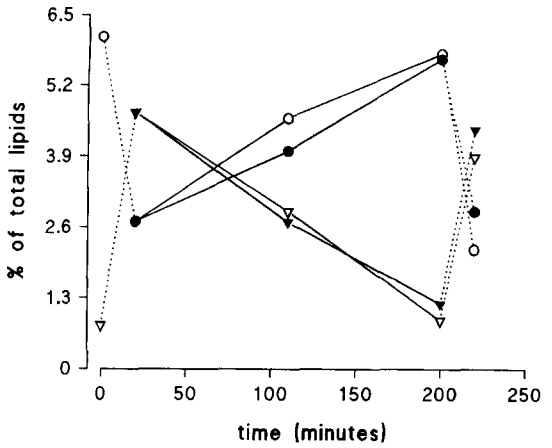


Fig. 2. BFA does not affect resynthesis and surface localisation of sphingomyellin of BHK cells exposed temporarily to sphingomyelinase. Cells were labelled to equilibrium with [^{3}H]acetate, treated with sphingomyelinase and then allowed to resynthesise sphingomyelin after removal of the enzyme, as described in Section 2. After 3 h, a further aliquot of sphingomyelinase was added to some samples in order to determine the proportion of surface sphingomyelin after resynthesis (dotted lines). Parallel determinations were carried out with 5 μg/ml BFA present during resynthesis (solid symbols). Values are expressed as the percentage of total lipid radioactivity present as sphingomyelin (circles) or ceramide (triangles) and represent the means of triplicate determinations in one experiment which gave results representative of three similar experiments.

external sphingomyelinase whereas none can be degraded in the presence of BFA. BFA also caused the characteristic increase in de novo synthesis of sphingomyelin [14,20,21] which has been considered to be diagnostic of its action in fusing Golgi cisternae with endoplasmic reticulum (ER), inducing retrograde movement of Golgi components (up to and including the *trans* Golgi) back to the ER [22].

In marked contrast, when BHK cells were exposed to exogenous sphingomyelinase and then allowed to resynthesise sphingomyelin after removal of the enzyme [17,23], BFA had no effect on this resynthesis (Fig. 2). The resynthesised sphingomyelin was also found to be on the cell surface, since a second treatment with sphingomyelinase degraded the same amount of sphingomyelin as it did originally. Similar results were obtained with monensin [15], another inhibitor of vesicular transport through the Golgi.

In BHK cells it has been reported that BFA prevents export of viral proteins from the *trans* Golgi network (TGN) to the surface but does not affect plasma membrane recycling [24] or the budding of clathrin-coated vesicles from the plasma membrane [25]. The inability of BFA to prevent plasma membrane recycling fits in with the reported failure of monensin to stop recycling of NBD-sphingomyelin introduced into the plasma membrane [26], and indicates that neither the Golgi cisternae nor the TGN are involved in recycling of plasma membrane. The absence of an effect of BFA on sphingomyelin resynthesis or its localisation at the cell surface thus strongly suggests that resynthesis occurs at a site which is distal to the TGN, perhaps even at the plasma membrane itself as suggested previously [6,8].

Resynthesis of surface sphingomyelin depends on endocytosis

For the following reasons we believe that resynthesis of sphingomyelin occurs not at the plasma membrane but at some internal site. Firstly, resynthesis appeared to depend on vesicular transport, since mitotic cells (whose vesicular transport mechanisms are inoperative [18,27]) showed no ability to resynthesise sphingomyelin (Fig. 3, solid triangles). In contrast, cells released from mitosis (open triangles) resynthesised up to 60% of the originally degraded sphingomyelin, although this was clearly less than asynchronous cells (circles) which had not been exposed to nocodazole. It should be emphasised that the cells released from mitosis in Fig. 3 were mitotic up to the end of the treatment with sphingomyelinase and were only released gradually from mitosis as the nocodazole diffused out from them during the course of the incubation in nocodazole-free medium. Thus it is likely that the absence of any resynthesis during the first hour relates to the time taken for these cells to emerge from mitosis. It is important to note also that nocodazole at up to 10 μM had no perceptible effect on resynthesis of sphingomyelin in normal cells and neither did cytochalasin D (10 μM), despite its very marked effect in

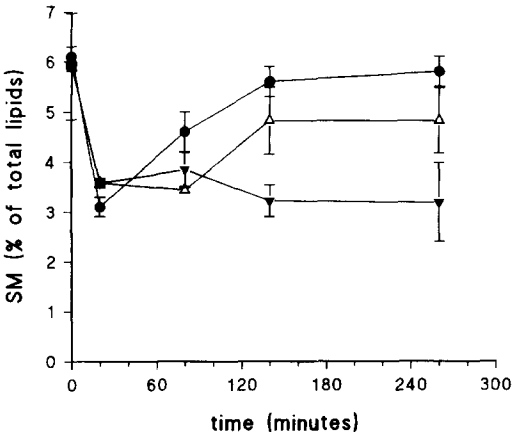


Fig. 3. Mitotic cells are unable to resynthesise sphingomyelin. Labelled mitotic cells were prepared and incubated with sphingomyelinase as described in Section 2. After removal of the enzyme, the resynthesis of sphingomyelin was measured in the presence (▼) or absence (△) of nocodazole. A parallel sample of interphase cells which had not been treated with nocodazole was also allowed to resynthesise sphingomyelin after exposure to sphingomyelinase (●). The experiment shown is one of three which gave similar results. Values represent the means ± S.D. of triplicate determinations at each time point.

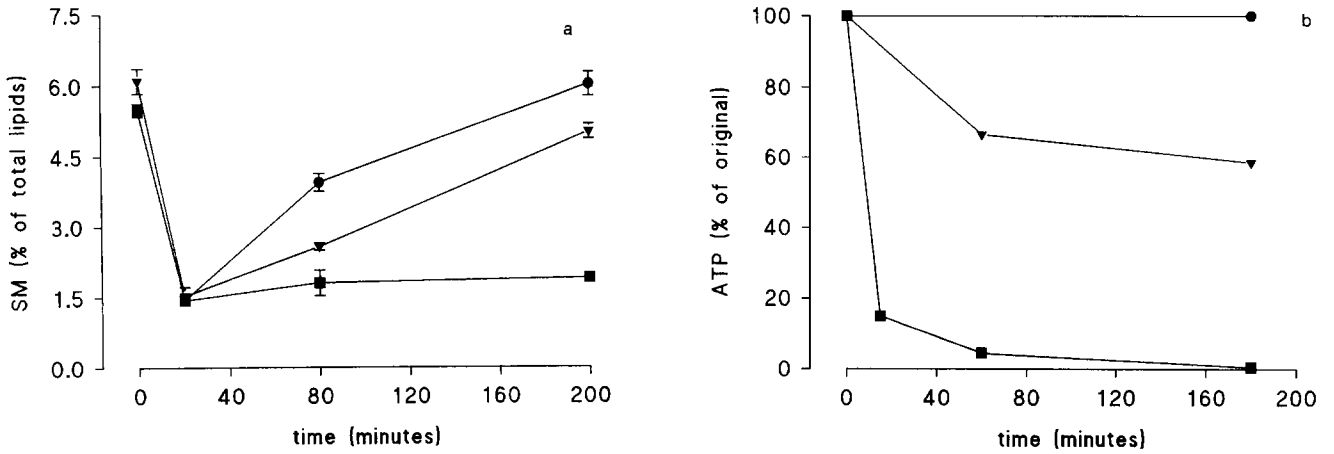
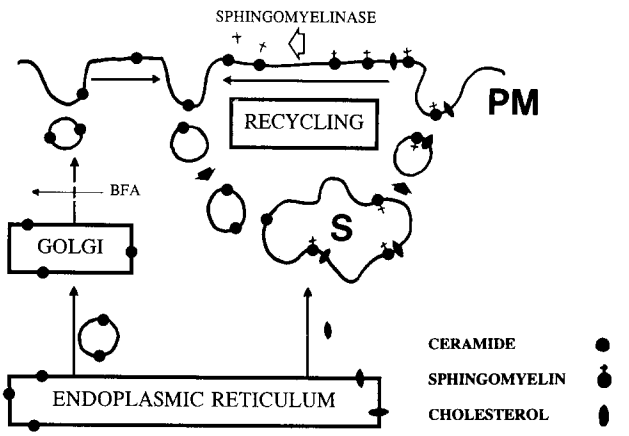


Fig. 4. Time course of resynthesis of sphingomyelin in energy-depleted cells. Cells labelled to equilibrium with [^{3}H]acetate were treated with sphingomyelinase and then allowed to resynthesise sphingomyelin after removal of the enzyme either alone (circles) or in the presence of 100 μM KCN + 50 mM deoxyglucose (triangles) or 30 mM NaF (squares). Sphingomyelin resynthesis (a) or ATP content (b) was measured in the cells at the beginning of the incubation and at various times subsequently. ATP measurements represent the means of duplicate determinations which differed by less than 5%.

causing the cells to round up (results not shown). Thus the integrity of microtubules or of actin filaments are not necessary for resynthesis of cell surface sphingomyelin.

Secondly, energy depletion of the cells by treatment with cyanide + deoxyglucose or with 30 mM NaF, which is known to inhibit endocytosis [28,29] inhibited resynthesis by 50% and 90% respectively (Fig. 4) even though the ceramide-phosphatidylcholine phosphocholinetransferase itself has no known energy requirement. Under these conditions, ATP levels were reduced by 40% in the presence of cyanide + deoxyglucose and by more than 95% in the presence of NaF. Treatment with *N*-ethylmaleimide or digitonin also caused almost complete inhibition of sphingomyelin resynthesis, but this might also be explained in terms of the ATP depletion promoted by these agents. *

We conclude from the above experiments that restoration of surface sphingomyelin after its degradation by sphingomyelinase involves endocytosis of surface membrane containing the newly-formed ceramide, movement of endocytic vesicles to a site where ceramide is converted to sphingomyelin and then transport of sphingomyelin back to the surface. Although this interpretation is consistent with previous indications that lipids inserted into the cell surface are internalised and recycled back to the surface by a pathway similar to that utilised by receptors [30,31], our results show that the site of sphingomyelin resynthesis must



CONSEQUENCES OF AN ENDOSOMAL SITE (S) OF PLASMA MEMBRANE SPHINGOMYELIN SYNTHESIS

Fig. 5. A model for the synthesis of plasma membrane sphingomyelin in BHK cells. The diagram illustrates the proposed pathway of ceramide transport from its site of synthesis in the ER to the plasma membrane (PM) and thence to recycling vesicles where it is converted into sphingomyelin, which is then delivered to the surface. This model also suggests a route by which cholesterol undergoes non-vesicular transfer from the ER to recycling vesicles in response to sphingomyelin synthesis at site S in recycling endosomes.

* Some authors [25] have ascribed morphological effects on cells incubated with 65 μM aluminium chloride in the presence of 30 mM NaF to the action of aluminium fluoride on heterotrimeric GTP-binding proteins. Clearly, under these conditions which are similar to those employed in Fig. 4b, it would be difficult to differentiate effects on GTP-binding proteins from effects due to energy depletion. Without precise determinations on ATP levels in intact cells treated with aluminium fluoride, claims for specific effects on G proteins must be regarded with caution.

also be on the recycling pathway. This is shown diagrammatically in Fig. 5.

The site of sphingomyelin synthesis could be the perinuclear area associated with the centrioles where NBD-sphingomyelin accumulates on its way through the membrane recycling pathway [30] and may correspond to the perinuclear or 'juxtanuclear' endosomes referred to by Hopkins [32] which appear to be similar to the recycling compartment described by Maxfield and colleagues [31,33]. If so, then sphingomyelin synthetic activity could be an enzymatic marker for these endosomes. Perinuclear endosomes appear to have a luminal pH which is only slightly acid (pH 6.5) [33] and interestingly, the pH optimum of the ceramide-phosphatidylcholine phosphotransferase is also 6.5 [34].

Kinetics of sphingomyelin resynthesis and plasma membrane recycling

The rate of resynthesis of sphingomyelin which had been broken down at the surface was about 50% in the first hour (Figs. 1, 4, 5) or about 30% of the original total sphingomyelin per hour. Quantitative analysis of endocytosis has shown that BHK cells internalise 0.6 μm^3 of fluid per min in about 1000 coated vesicles [35], giving a value for the vesicle surface area internalised of about 30 μm^2 per min. With a total surface area of 2200 μm^2 [35], this means that BHK cells should internalise half of their surface in about 40 min, in broad agreement with the kinetics of sphingomyelin resynthesis and recycling [31]. Thus sphingomyelin resynthesis could be limited by the rate of endocytosis and indeed, may represent a novel way of quantifying endocytosis. It should be noted that the rate of endocytosis is not affected by treatment with sphingomyelinase [17].

BHK cells grown under our conditions had a doubling time of 12 h, so that the normal rate of synthesis of all cell components including sphingomyelin was about 8% of the total per hour or about a quarter of the maximum rate of the resynthesis pathway. This difference is similar to the relative rates of de novo synthesis and recycling of NBD-sphingomyelin in CHO cells, although in these cells the recycling pathway seems faster than in BHK cells [13].

The observation that ceramide produced at the plasma membrane is so completely converted back to surface sphingomyelin indicates that little of the endocytosed membrane lipid is lost to other intracellular compartments. Thus endocytosed membrane appears to be largely directed to the site of sphingomyelin resynthesis (S in Fig. 5) and thence back to the surface. Little change was observed in the amount of glucosylceramide or GM3 (sialyllactosylceramide, the major BHK cell glycolipid [36]) during resynthesis of sphingomyelin (Fig. 6), suggesting that the ceramide produced at the cell surface was not available for conversion into

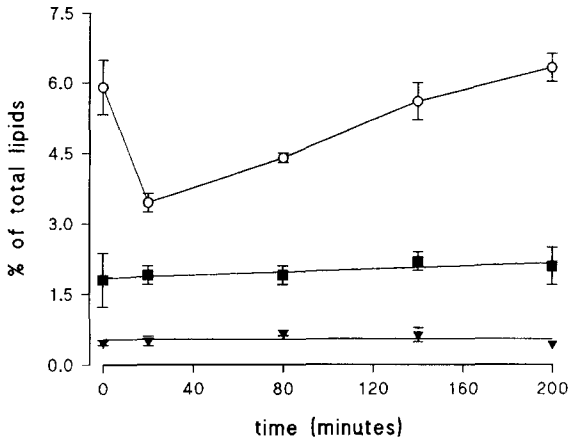


Fig. 6. Changes in glycosphingolipids during resynthesis of sphingomyelin. In an experiment similar to that shown in Fig. 1, measurements were made of sphingomyelin (○), glucosylceramide (▲) and ganglioside GM3 (■) after alkaline methanolysis of total lipids [14]. A small amount of label, equivalent to about 0.1% of the total was found in lactosylceramide and in triosylceramide, but no change was seen in either of these species in the course of this experiment. Values represent the means ± S.D. of three experiments in which triplicate determinations were carried out.

glycolipid. Since synthesis of GM3 depends on transfer of ceramide to the early Golgi [37–39], this result signifies that most of the endocytosed membrane is recycled directly and not routed through the early Golgi. That would be consistent with an estimate based on uptake of endocytosed ricin into the Golgi apparatus of BHK cells which suggests that no more than 5% of endocytosed membrane was routed through the Golgi [40]. The data also indicate that ceramide, like glucosylceramide [30] and sphingomyelin [31] inserted into the plasma membrane, follows non-selective (bulk) membrane flow through the recycling pathway.

Our observations regarding the fate of natural ceramide generated in the plasma membrane contrast with those of Pagano and co-workers [13,26] who concluded that exogenous NBD-ceramide could be converted into both glycolipids and sphingomyelin (probably in the cis-Golgi), even in cells which were in mitosis and where consequently vesicular transport processes were inoperative. This suggests that unlike endogenous ceramide, NBD-ceramide introduced into the cell surface rapidly gains access to the intracellular site of glycolipid synthesis in the early Golgi. These observations emphasise that results obtained using short-chain NBD-ceramide, which is more likely to diffuse across aqueous partitions between membranes, are not necessarily a reliable guide to the fate of endogenous plasma membrane ceramide.

The cis / medial-Golgi site for sphingomyelin synthesis

Despite the conclusion from the present work that the major site of sphingomyelin synthesis is endosomal,

there is evidence for another site of sphingomyelin synthesis in the cis/medial Golgi [7–10]. We have shown recently that BHK cells possess an internal pool of sphingomyelin which does not mix with the plasma membrane sphingomyelin pool but labels more rapidly with [^{3}H]choline [16]. The short-term labelling of this pool with [^{3}H]acetate is not inhibited by monensin [15] so that the internal pool of sphingomyelin could be synthesised at an early Golgi site. However it should be noted that the localisation of sphingomyelin synthesis to the early Golgi has only been claimed in liver, and the results obtained may not be typical of less specialised cells. Also, the liver fractionation schemes employed are unlikely to have separated early Golgi elements from other vesicular smooth membranes including the recycling vesicles, where we propose that sphingomyelin is resynthesised.

Is the endosomal site of sphingomyelin resynthesis also responsible for de novo synthesis of plasma membrane sphingomyelin?

Considering the large capacity of the recycling pathway for resynthesising sphingomyelin, it is tempting to suggest that it represents part of the normal pathway which is responsible for de novo biosynthesis of plasma membrane sphingomyelin. We have recently shown that the de novo synthesis of cell surface sphingomyelin from ceramide is blocked by monensin, demonstrating that monensin prevents the transport of ceramide to a site which is distal to the medial Golgi where ceramide can be converted into sphingomyelin destined for the plasma membrane [14]. The present work using brefeldin A suggests that this site is distal to the TGN. If the distal site of sphingomyelin biosynthesis is the same as the site of resynthesis on the plasma membrane endocytic recycling pathway, then this implies that ceramide must make its way to an endosomal site in order to be converted into plasma membrane sphingomyelin. Our hypothesis that sphingomyelin synthesis may occur in recycling endosomes represents the first suggestion that endosomes have any lipid biosynthetic capacity at all, and clearly differs from previous ideas that sphingomyelin synthesis occurs in the cis/medial-Golgi [7–10] and that sphingomyelin reaches the surface by a process involving vesicular transport through the Golgi cisternae. This hypothesis explains why monensin and BFA block the appearance of newly-synthesised sphingomyelin at the surface (Fig. 1) [10,14] whereas they have no effect on sphingomyelin resynthesis and return to the surface after treatment of cells with exogenous sphingomyelinase (Fig. 2). It also explains why monensin causes a build-up of ceramide (presumably in the medial Golgi) which can partly be converted into glucosylceramide [10], whereas most of the ceramide introduced into the recycling pathway through the action of external sphingomyelinase does not reach the Golgi and is consequently not converted into glycolipids (Fig. 6).

Although some products of the exocytic pathway can enter endosomes [41], the bulk of exocytic flow probably passes directly to the plasma membrane. Rather than invoking another site of sphingomyelin synthesis between the TGN and the plasma membrane, we conclude that most of the ceramide in the exocytic pathway is normally transported all the way to the plasma membrane and is only converted into sphingomyelin when it enters the endosomal pathway, where the sphingomyelin synthesis site appears to be localised (Fig. 5). Thus the characteristic orientation of sphingomyelin on the external leaflet of the plasma membrane would be defined by its synthesis in the lumen of endocytic vesicles involved in membrane recycling. Such a tortuous route for surface sphingomyelin synthesis would be consistent with the relatively slow rate at which newly-synthesised sphingomyelin appears at the plasma membrane [8].

Does cholesterol reach the cell surface by cotransport with sphingomyelin in endosomes?

Finally, we would like to suggest the possibility that cholesterol transport to the cell surface could take place utilising the same recycling endosomal vesicles which carry newly-synthesised sphingomyelin (Fig. 5). Such a model would explain why cholesterol transport to the cell surface is not affected by monensin [42] or BFA [43] (and thus does not involve the Golgi apparatus) but is blocked by energy depletion [42] (and thus depends on vesicular transport, probably through the endosomal pathway). Our explanation for this apparent enigma of cholesterol transport does not need to assume a vesicular bypass to the secretory pathway as proposed recently by van Meer [44]. It would also explain why purified trans-Golgi membrane vesicles [45] or exocytic vesicles [46] are not enriched in cholesterol or in sphingomyelin, as would be expected if these lipids were added in the early Golgi. The model would fit in with the evidence that sphingomyelin and cholesterol interact specifically [47] and that the synthesis and delivery to the cell surface of sphingomyelin and cholesterol are intimately connected [23]. Thus the rate of delivery of cholesterol to the plasma membrane would be determined by the rate of synthesis of sphingomyelin in the recycling pathway. The putative endosomal vesicles which carry cholesterol and sphingomyelin could correspond to the vesicles rich in newly-synthesised cholesterol which have been described by other workers [42,48]. In this case there would have to be a direct route for cholesterol transport from its site of synthesis in the endoplasmic reticulum to the recycling endosomal vesicles as shown in Fig. 5.

This proposed mechanism for the cotransport of sphingomyelin and cholesterol to the cell surface

through the endosomal pathway (which is described in more detail in a recent review [49]) has the advantage of making it easier to understand how the characteristic lipid composition of the plasma membrane is conserved, since these major plasma membrane lipid constituents are added in a closed vesicular loop which is largely independent of the secretory pathway.

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5. References

[1] Diringer, H., Maagraf, W.D., Koch, M.A. and Anderer, F.A. (1972) Biochem. Biophys. Res. Commun. 47, 1345–1352.
[2] Ullman, M.D. and Radin, N.S. (1974) J. Biol. Chem. 249, 1506–1512.
[3] Voelker, D.R. and Kennedy, E.P. (1982) Biochemistry 21, 2753–2759.
[4] Eppler, C.M., Malewicz, B., Jenkin, H.M. and Baumann, W.J. (1987) Lipids 22, 351–357.
[5] Mandon, E., Ehses, I., Rother, J., Van Echten, G. and Sandhoff, K. (1992) J. Biol. Chem. 267, 11144–11148.
[6] Marggraf, W.-D. and Kanfer, J.N. (1987) Biochim. Biophys. Acta 897, 57–69.
[7] Van Meer, G., Stelzer, E.H.K., Wijnaedents-van-Resandt, R.W. and Simons, K. (1987) J. Cell Biol. 105, 1623–1635.
[8] Futerman, A.H., Stieger, B., Hubbard, A.L. and Pagano, R.E. (1990) J. Biol. Chem. 265, 8650–8657.
[9] Jackel, D., Karrenbauer, A., Birk, R., Schmidt, R.R. and Wieland, F. (1990) FEBS Lett. 261, 155–157.
[10] Van 't Hoff, W. and Van Meer, G. (1990) J. Cell Biol. 111, 977–986.
[11] Pagano, R.E. (1988) Trends Biochem. Sci. 13, 202–205.
[12] Kobayashi, T. and Pagano, R.E. (1989) J. Biol. Chem. 264, 5966–5973.
[13] Lipsky, N.G. and Pagano, R.E. (1985) J. Cell Biol. 100, 27–34.
[14] Kallen, K.-J., Quinn, P. and Allan, D. (1993) Biochem. J. 289, 307–312.
[15] Kallen, K.-J., Quinn, P. and Allan, D. (1993) Biochim. Biophys. Acta 1166, 305–308.
[16] Quinn, P. and Allan, D. (1992) Biochim. Biophys. Acta 1124, 95–100.
[17] Allan, D. and Quinn, P. (1988) Biochem. J. 254, 765–771.
[18] Featherstone, C., Griffiths, G. and Warren, G. (1985) J. Cell Biol. 101, 2036–2046.
[19] Leach, F.R. and Webster, J.J. (1986) Methods Enzymol. 133, 51–70.
[20] Hatch, G.M. and Vance, D.E. (1992) J. Biol. Chem. 267, 12443–12451.
[21] Brüning, A., Karrenbauer, A., Schnabel, E. and Wieland, F.T. (1992) J. Biol. Chem. 267, 5052–5055.
[22] Lippincott-Schwartz, J., Donaldson, J.G., Schweizer, A., Berger, E.G., Hauri, H.-P., Yuan, L.D. and Klausner, R.D. (1990) Cell 60, 821–836.
[23] Slotte, J.P., Härmälä, A.-S., Jansson, C. and Pörn, M.I. (1990) Biochim. Biophys. Acta 1030, 251–257.
[24] Miller, S.G., Carnell, L. and Moore, H.-P.H. (1992) J. Cell Biol. 118, 267–283.
[25] Robinson, M.S. and Kreis, T.E. (1992) Cell 69, 129–138.
[26] Koval, M. and Pagano, R.E. (1989) J. Cell Biol. 108, 2169–2181.
[27] Berlin, R.D. and Oliver, J.M. (1980) J. Cell Biol. 85, 660–671.
[28] Kok, J.W., Eskelinen, S., Hoekstra, K. and Hoekstra, D. (1989) Proc. Natl. Acad. Sci. USA 86, 9896–9900.
[29] Slotte, J.P., Lundberg, B. and Björkerud, S. (1984) Biochim. Biophys. Acta 793, 423–428.
[30] Kok, J.W., Hoekstra, K., Eskelinen, S. and Hoekstra, D. (1992) J. Cell Sci. 103, 1257–1269.
[31] Mayor, S., Presley, J.F. and Maxfield, F.R. (1993) J. Cell. Biol. 121, 1257–1269.
[32] Hopkins, C.R. (1986) Trends Biochem. Sci. 11, 473–477.
[33] Yamashiro, D.J., Tycko, B., Fluss, S.R. and Maxfield, F.R. (1984) Cell 37, 789–800.
[34] Marggraf, W.-D. and Kanfer, J.N. (1984) Biochim. Biophys. Acta 793, 346–353.
[35] Griffiths, G., Back, R. and Marsh, M. (1989) J. Cell Biol. 109, 2703–2720.
[36] Renkonen, O., Gahmberg, C.G., Simons, K. and Kääriänen (1972) Biochim. Biophys. Acta 255, 66–78.
[37] Trinchera, M. and Ghidoni, R. (1989) J. Biol. Chem. 264, 15766–15769.
[38] Schwartzmann, G. and Sandhoff, K. (1990) Biochemistry 29, 10865–10871.
[39] Young, W.W. Jr., Lutz, M.S., Mills, S.E. and Lechler-Osborn, S. (1990) Proc. Natl. Acad. Sci. USA 87, 6838–6942.
[40] Van Deurs, B., Sandvig, K., Petersen, O.W., Olsnes, S., Simons, K. and Griffiths, G. (1988) J. Cell Biol. 106, 253–267.
[41] Gruenberg, J. and Clague, M.J. (1992) Curr. Opin. Cell Biol. 4, 593–599.
[42] Kaplan, M.R. and Simoni, R.D. (1985) J. Cell Biol. 101, 446–453.
[43] Urbani, L. and Simoni, R.D. (1990) J. Biol. Chem. 265, 1919–1923.
[44] Van Meer, G. (1993) Curr. Opin. Cell Biol. 5, 661–673.
[45] Taylor, J.A., Limbrick, A.R., Allan, D. and Judah, J.D. (1984) Biochim. Biophys. Acta 769, 171–178.
[46] Helmy, S., Porter-Jordan, K., Dawidowicz, E.A., Pilch, P., Schwartz, A.L. and Fine, R.E. (1986) Cell 44, 497–506.
[47] Lund-Katz, S., Laboda, H.M., McLean, L.R. and Phillips, M.C. (1988) Biochemistry 27, 3416–3423.
[48] Lange, Y. (1991) J. Lipid Res. 32, 329–339.
[49] Allan, D. and Kallen, K.-J. (1993) Prog. Lipid Res. 32, 195–219.