Minireview

Redrawing compartmental boundaries in the exocytic pathway

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Abstract Compartments can be defined both morphologically as well as biochemically. The former relies on a direct visualisation of membrane boundaries and the latter by the characterisation of enzymatic functions taking place within the compartment. The combination of morphology and biochemistry has led to the identification of several compartments within the exocytic pathway, each assumed to carry out independent functions in a logical succession [Palade, G. (1975) Science 189, 347–358]. However, recent findings show that resident enzymes are confined to not one but at least two adjacent compartments suggesting that morphological and biochemical definitions do not coincide. We will here discuss these findings and propose a model to explain how compartments are organised and maintained along the exocytic pathway.

Key words: Protein transport; Compartmental boundary; Retention; Retrieval

1. Introduction

Proteins destined for the exocytic pathway are imported into the ER where they undergo folding and oligomerisation. This maturation process is facilitated by resident proteins, some of which mediate post-translational modifications such as glycosylation, acylation, phosphorylation, rearrangement of disulphide linkages or isomerisation of prolines [2]. Providing maturation is successful, the protein is allowed to leave the ER and enter the Golgi apparatus where it undergoes additional modifications such as processing of oligosaccharides and sulphation. At the *trans*-Golgi network (TGN), the protein continues to the plasma membrane unless it displays signals for sorting to other compartments (e.g. lysosomes, secretory granules). The vectorial movement of the protein through the exocytic pathway is thought to occur by default. This non-selective transport, often referred to as 'bulk flow', is carried out by vesicles bringing cargo from one compartment to the next [3]. While bulk flow cargo is allowed to diffuse freely through the compartment and to become incorporated into budding transport vesicles, enzymes which carry out modifications must be held in place. Mechanisms exist to ensure this and these can be divided into two types based on their mode of action. The first, referred to as retrieval, brings back protein from downstream compartments after they have escaped. This mechanism depends on retrograde transport and on the recognition of retrieval motifs displayed by the escaped proteins. The second type is referred to as retention and prevents residents from leaving the compartment in the first place. Both retention and retrieval co- operate to ensure fidelity in maintaining residents along the pathway [4].

2. Retrieval

Examples of retrieval signals can be found in the ER, the intermediate compartment and in the TGN. Lumenal ER proteins have a short motif, KDEL, at their carboxy terminus which is sufficient for ER localisation [5]. The KDEL motif is recognised by a receptor, erd2 [6], which upon binding relocates to the ER to release its ligand [7]. Membrane proteins of the ER and the intermediate compartment display retrieval motifs in their cytoplasmic domains consisting of positively charged amino acids [8–10]. These have been shown to bind specifically to subunits of the coatomer [11,12] and to drive the polymerisation of microtubuli [13], in vitro. Resident membrane proteins of the TGN, such as furin and TGN38 display tyrosine-based motifs in their cytoplasmic tails and mutation of these motifs leads to accumulation on the plasma membrane [14–17].

3. Retention

The first retention signals to be identified were in the membrane spanning domains of Golgi resident proteins [18]. It has been suggested that retention by the membrane-spanning domain is a direct consequence of its length. This is borne out from the observation that membrane-spanning domains of intracellular proteins are shorter than those of plasma membrane proteins [19]. This difference correlates well with the increased thickness of the plasma membrane due to its relatively high concentration of cholesterol. Since a gradient of cholesterol has been shown to exist in the exocytic pathway [20], it was postulated that this would mediate the maintenance of residents along the pathway. Indeed, increasing the length of the membrane spanning domain of the TGN resident α2,6-sialyltransferase results in the appearance of this enzyme on the cell surface [21].

A second possibility is that the membrane-spanning domain aids in the formation of oligomeric structures which are too large to be incorporated into transport vesicles [22]. There are several lines of evidence for the existence of oligomeric structures. The Golgi resident M proteins of both IBV (avian infectious bronchitis virus) and MHV (mouse hepatitis virus) form oligomeric structures and this is correlated with them being retained in the *cis*-cisternae and the TGN, respectively [23,24]. Furthermore, *medial* enzymes have been shown to interact spe-

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cifically with each other demonstrating the existence of hetero oligomers or 'kin oligomers' [25]. Residents of this part of the Golgi apparatus can be isolated biochemically as a proteinaceous structure by detergent extraction [26], suggesting that kin oligomers constitute large and stable structures. Similar detergent-resistant structures can also be isolated from the rough ER and these consist mainly of resident proteins involved in the translocation of nascent polypeptides [27]. Consistent with the presence of oligomeric structures in the ER is the notion that removal of retrieval signals from residents does not result in a significant loss of ER localisation [5,9,28]. In this review, we will propose that oligomeric structures not only constitute means for retention but also that they contribute to the structural maintenance of compartments in which they reside (see below).

4. Morphological and biochemical definitions of a compartment are out of register

The ER, itself consisting of several subdomains, is continuous with the intermediate compartment [29], a tubular reticular network extending a considerable distance away from the ER [30]. The intermediate compartment, first identified and defined as the so-called 15°C compartment [31], is the compartment from which vesicles destined for the Golgi apparatus bud. It has been shown to move in an anterograde fashion towards the Golgi apparatus [32] allowing for the formation of the CGN. Residents of the ER such as protein disulphide isomerase (PDI) and BiP occupy the rough and the smooth ER as well as the intermediate compartment [33–36]. Calreticulin and the calcium-binding protein CaBP1 are located in the ER [28,37] and the latter has also been shown to reside in the intermediate compartment [38]. CaBP1 does not, however, co-localise with the lectin-binding protein p53 [39] but rather coincides with p63 by subcellular fractionation [37]. Both p53 and p63 are established markers for the intermediate compartment [40,41], however, they show different distributions arguing for subdomains within this compartment (D. Mundy, personal communication). The small GTP-binding proteins, rab1 and rab2, both locate to the intermediate compartment, the *cis*-Golgi network (CGN) as well as to the *cis*-cisternae of the Golgi apparatus [34,42–44]. A similar distribution is also observed with the KDEL receptor, erd2 [34], as well as with the t-SNARE, syntaxin 5 [35].

It is evident that residents in the early part of the pathway occupy more than one compartment or subcompartments. This pattern becomes more striking in the Golgi apparatus when examining the distribution of glycosylation enzymes involved in the construction of complex *N*-linked oligosaccharides. Two enzymes, α1,3-1,6-mannosidase II and β1,2-*N*-acetylglucosaminyltransferase I, reside in both the *medial* and the *trans*-cisternae with a more or less equal distribution over these two compartments. They share the *trans*-cisternae with β1,4-galactosyltransferase and α2,6-sialyltransferase which are also found in the TGN [45–48]. A similar pattern is seen in the TGN where residents display patterns of overlapping distribution between different subcompartments (C. Rabouille and T. Nilsson, manuscript in preparation).

We would like to propose that the Golgi enzymes are but one of a population of Golgi residents that have an overlapping distribution. Others would include coat-binding proteins and v- and t-SNAREs. Discrete membrane domains would be formed within compartments and these would be made up of specific sets of residents through the formation of kin oligomers. We envisage that membrane domains facing each other in adjacent compartments are composed of the same set of residents and that direct interactions exist across the intercompartmental space. These paired membrane domains define a basic biochemical unit and we draw the new boundaries of a biochemical compartment around these units (see Fig. 1). This new definition separates the morphological compartment (physical compartment) from the biochemical one and this has implications both for the organisation of the exocytic pathway and for vesicular transport between compartments.

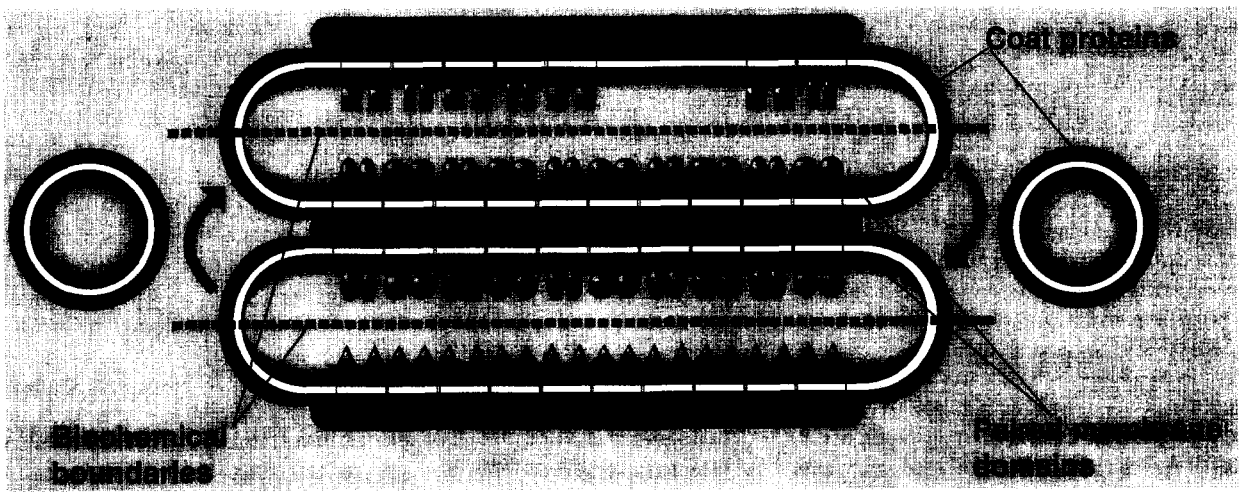

Fig. 1. Formation of a biochemical compartment by paired membrane domains. Discrete membrane domains are formed through lateral interactions between resident proteins such as enzymes, lectins, coat binding proteins as well as v- and t-SNAREs. Two domains, having the same set of residents, face each other in adjacent compartments forming templates for stacking. These domains define the biochemical compartment. Transported proteins (not depicted) have access both to anterograde and retrograde vesicles as do residents. The latter as a consequence of breaking down the oligomer at the dilated rims. Biochemical boundaries are shown as dashed lines.

5. Transport between compartments – heterotypic or homotypic?

Vesicular transport between physical compartments relies on the incorporation of resident proteins into the vesicle along with bulk flow proteins. For example, coat-binding proteins used in the formation of the vesicle must be incorporated as are those molecules involved in docking and fusion (v- and t-SNAREs) [49]. It has always been assumed that fusion of the transport vesicle with the next compartment is heterotypic. However, having redefined the boundaries of the compartment to include paired membrane domains in two adjacent compartments, we propose that transport between two physical compartments involves *only* those molecules which exist within the biochemical unit as defined above. In this view, all transport steps within the pathway are homotypic, not heterotypic. The same principle applies for both anterograde as well as for retrograde transport. This ensures a steady-state distribution of residents involved in transport and removes the need for an intricate sorting machinery. Resident proteins which escape the biochemical compartment, however, are brought back by the retrieval machinery mentioned above.

Bulk flow proteins would in this model have access both to anterograde as well as to retrograde transport vesicles. To ensure a directional flow, it is possible to envisage various means of selective incorporation of transported proteins into vesicles. For example, resident lectins of a given biochemical compartment could selectively recognise glycoproteins at particular stages in their maturation and incorporate them into transport vesicles [50]. This can either be in a retrograde or in an anterograde fashion depending on what side of the biochemical unit they happen to be. Following modification by glycosylation enzymes, the lectin no longer recognises the glycoprotein and the latter is free to move to the next biochemical unit. This results in a vectorial flow of glycoproteins (as well as for glycolipids) and ensures that transported proteins are properly modified before leaving the biochemical compartment. Such facilitated incorporation is not inconsistent with the ideas of bulk flow if viewed as a means of maintaining proteins which have not yet been properly modified (or in the case of newly synthesised proteins, properly folded [51]).

6. The role of residents in maintaining the biochemical compartment

Resident molecules play important roles in the structural maintenance of the compartment in which they reside. We envisage they do so in three ways: The first is by forming large oligomers (kin oligomers) which can regulate the extent of fenestration and tubulation of the compartment. The second by serving as anchors to the cytoskeleton and the third by serving as templates for the adjacent membrane allowing for stacking.

Whereas anchoring to the cytoskeleton helps to maintain the physical compartment, formation of oligomers and serving as templates for stacking allows residents to define and maintain the biochemical compartment (see Fig. 1). Each cisterna is divided into functional zones which can be distinguished morphologically [52]. In the centre, the cisterna is relatively non-fenestrated compared to the dilated rims where vesicles bud. Through the formation of kin oligomers, residents act as endo- and exoskeletons protecting the central core of the cisternae against fenestration and vesicular budding.

By serving as templates for the adjacent membrane domain, Golgi residents mediate stacking of the cisternae allowing for the stacking of multiple cisternae, even for those extreme cases where 30 or more cisternae are found within the stack. The role of residents in stacking is also seen in the ER where high levels expression of HMG-CoA reductase leads to the appearance of stacked ER cisternal membranes [53]. Similar structures have also been observed in the rough ER of HMV infected cells where the viral M protein interacts with the viral nucleocapsid to form stacked cisternae [54].

7. Conclusions

Transport between compartments has always been viewed as a process which needs to be tightly controlled in order for the compartments to maintain their distinct set of residents (read functionality) and to enable vesicles to move forward in a vectorial fashion. In the above model, redefining the biochemical compartment allows for homotypic transport which provides an explanation for the sorting of resident proteins such as v- and t-SNAREs. The steady state of resident proteins is determined by their ability to interact with each other to form biochemical units. Such units can exist between compartments which are separated physically as well as within compartments. Having biochemical compartment out of register with the physical one also removes much of the need for an argument over whether or not particular compartments are continuous with each other (e.g. the ER and the intermediate compartment(s) and the cisternae of the Golgi apparatus).

Acknowledgements: Due to the limited size of this review, the focus has been exclusively on the exocytic pathway in mammalian cells and we have therefore not included the equally important body of work dealing with the exocytic pathway in yeast and other systems. In spite of this, we hope that this review will stimulate the ongoing debate among scientists in this exciting field of intracellular transport. We thank Drs. Graham Warren, Birte Sönnichsen, Dorothy Mundy, Tim Levine and Norman Hui for critical reading and helpful suggestions.

References

[1] Palade, G. (1975) Science 189, 347–358.
[2] Hurtley, S.M. and Helenius, A. (1989) Annu. Rev. Cell Biol. 5, 277–307.
[3] Pfeffer, S.R. and Rothman, J.E. (1987) Annu. Rev. Biochem. 56, 829–852.
[4] Nilsson, T. and Warren, G. (1994) Curr. Opin. Cell Biol. 6, 517–521.
[5] Munro, S. and Pelham, H.R. (1987) Cell 48, 899–907.
[6] Lewis, M.J. and Pelham, H.R. (1990) Nature 348, 162–163.
[7] Lewis, M.J. and Pelham, H.R. (1992) Cell 68, 353–364.
[8] Nilsson, T., Jackson, M. and Peterson, P.A. (1989) Cell 58, 707–718.
[9] Jackson, M.R., Nilsson, T. and Peterson, P.A. (1990) EMBO J. 9, 3153–3162.
[10] Schutze, M.P., Peterson, P.A. and Jackson, M.P. (1994) EMBO J. 13, 1696–1705.
[11] Cosson, P. and Letourneur, F. (1994) Science, 263, 1629–1631.
[12] Letourneur, F., Gaynor, E.C., Hennecke, S., Demolliere, C., Duden, R., Emr, S.D., Riezman, H. and Cosson, P. (1994) Cell 79, 1199–1207.
[13] Dahllöf, B., Wallin, M. and Kvist, S. (1991) J. Biol. Chem. 266, 1804–1808.
[14] Bos, K., Wraight, C. and Stanley, K.K. (1993) EMBO J. 12, 2219–2228.
[15] Humphrey, J.S., Peters, P.J., Yuan, L.C. and Bonifacino, J.S. (1993) J. Cell Biol. 120, 1123–1135.

[16] Wong, S.H. and Hong, W. (1993). J. Biol. Chem. 268, 22853–22862.
[17] Molloy, S.S., Thomas, L., VanSlyke, J.K., Stenberg, P.E. and Thomas, G. (1994) EMBO J. 13, 18–33.
[18] Machamer, C.E. (1993) Curr. Opin. Cell Biol. 5, 606–612.
[19] Bretscher, M.S. and Munro, S. (1993) Science 261, 1280–1281.
[20] Orci, L., Montesano, R., Meda, P., Malaisse-Lagae, F., Brown, D., Perrelet, A. and Vassalli, P. (1981) Proc. Natl. Acad. Sci. USA 78, 293–297.
[21] Munro, S. (1991) EMBO J. 10, 3577–3588.
[22] Nilsson, T., Lucocq, J.M., Mackay, D. and Warren, G. (1991) EMBO J. 10, 3567–3575.
[23] Weisz, O.A., Swift, A.M. and Machamer, C.E. (1993) J. Cell Biol. 122, 1185–1196.
[24] Krijnse Locker, J., Opstelten, D.J., Ericsson, M., Horzinek, M.C. and Rottier, P.J.M. (1995). J. Biol. Chem. 270, 8815–8821.
[25] Nilsson, T., Hoe, M.H., Slusarewicz, P., Rabouille, C., Watson, R., Hunte, F., Watzele, G., Berger, E.G. and Warren, G. (1994) EMBO J. 13, 562–574.
[26] Slusarewicz, P., Nilsson, T., Hui, N., Watson, R. and Warren, G. (1994) J. Cell Biol. 124, 405–413.
[27] Hortsch, M., Crimaudo, C., and Meyer, D.I. (1987) in: Integration and Control of Metabolic Processes: Pure and Applied Aspects (Kon, O.L. Ed.) pp. 3–11.
[28] Sönnichsen, B., Füllekrug, J., Nguyen, V.P., Diekmann, W., Robinson, D.G. and Mieskes, G. (1994) J. Cell Sci. 107, 2705–2717.
[29] Krijnse-Locker, J., Ericsson, M., Rottier, P.J.M. and Griffiths, G. (1994) J. Cell Biol. 124, 55–70.
[30] Hauri, H.-P. and Schweizer, A. (1992) Curr. Opin. Cell Biol. 4, 600–608.
[31] Saraste, J. and Kuismanen, E. (1984) Cell 38, 535–549.
[32] Saraste, J. and Svensson, K. (1991) J. Cell Sci. 100, 415–430.
[33] Sitia, R, and Meldolesi, J. (1992) Mol. Biol. Cell. 3, 1067–1072.
[34] Griffiths, G., Ericsson, M., Krijnselocker, J., Nilsson, T., Goud, B., Söling, H.D., Tang, B.L., Wong, S.H. and Hong, W.J. (1994) J. Cell Biol. 127, 1557–1574.
[35] Banfield, D.K., Lewis, M.J., Rabouille, C., Warren, G. and Pelham, H.R.B. (1994) J. Cell Biol. 127, 357–371.
[36] Hammond, C. and Helenius, A. (1994) J. Cell Biol. 126, 41–52.
[37] Füllekrug, J., Sönnichsen, B., Wünsch, U., Arseven, K., Nguyen Van, P., Söling, H.-D. and Mieskes, G. (1994) J. Cell Sci. 107, 2719–2727.
[38] Schweizer, A., Peter, F., Nguyen, V., Soling, H.D. and Hauri, H.P. (1993) Eur. J. Cell Biol. 60, 366–370.
[39] Arar, C., Carpentier, V., Lecaer, J.P., Monsigny, M., Legrand, A. and Roche, A.C. (1995) J. Biol. Chem. 270, 3551–3553.
[40] Schindler, R., Itin, C., Zerial, M., Lottspeich, F. and Hauri, H.P. (1993) Eur. J. Cell Biol. 61, 1–9.
[41] Schweizer, A., Ericsson, M., Bachi, T., Griffiths, G. and Hauri, H.P. (1993) J. Cell Sci. 104, 671–683.
[42] Tisdale, E.J., Bourne, J.R., Khosravifar, R., Der, C.J. and Balch, W.E. (1992) J. Cell Biol. 119, 749–761.
[43] Chavrier, P., Parton, R.G., Hauri, H.P., Simons, K. and Zerial, M. (1990) Cell 62, 317–329.
[44] Saraste, J., Lahtinen, U., and Goud, B. (1995) J. Cell Sci. 108, 1541–1552.
[45] Dunphy, W.G., Brands, R. and Rothman, J.E. (1985) Cell 40, 463–472.
[46] Roth, J. and Berger, E.G. (1982) J. Cell Biol. 92, 223–229.
[47] Nilsson, T., Pypaert, M., Hoe, M.H., Slusarewicz, P., Berger, E.G. and Warren, G. (1993) J. Cell Biol. 120, 5–13.
[48] Rabouille, C., Hui, N., Hunte, F., Kieckbusch, R., Berger, E.G., Warren, G. and Nilsson, T. (1995) J. Cell Sci. 108, 1617–1627.
[49] Söllner, T., Whitehart, S.W., Brunner, M., Erdjumentbromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) Nature 362, 318–324.
[50] Fiedler, K. and Simons, K. (1995) Cell 81, 1–20.
[51] Hebert, D.N., Foellmer, B. and Helenius, A. (1995) Cell 81, 425–433.
[52] Weidman, P., Roth, R. and Heuser, J. (1993) Cell 75, 123–133.
[53] Pathak, R.K., Luskey, K.L. and Anderson, R. (1986) J. Cell Biol. 102, 2158–2168.
[54] Tooze, J., Tooze, S.A. and Warren, G. (1985) Eur. J. Cell Biol. 36, 108–115.