Lysosomes and Membrane Recycling

A HYPOTHESIS

By ROGER T. DEAN

Division of Cell Pathology, Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, U.K.

(Received 26 September 1977)

A mechanism for intralysosomal membrane recycling is proposed. After invagination of the lysosomal membrane during autophagy, intralysosomal vesicles are formed. It is suggested that membrane can bleb out from these internal vesicles, probably in the form of very small vesicles, and return to the external lysosomal membrane by membrane fusion. This mechanism would conserve lysosomal membrane during autophagy, and is analogous to current models of plasma-membrane recycling. Its relationship to turnover of lysosomal-membrane proteins and other proteins is discussed.

The endocytic rates of several types of cell seem to be much greater than the rates of degradation of most plasma-membrane proteins of those cells (Steinman *et al.*, 1976). This discrepancy has been rationalized by the suggestion that, after endocytosis, some plasma-membrane components may return intact to the plasma membrane. In principle, such 'recycling' might occur by exocytosis, in which intracellular vesicles fuse with the plasma membrane and contribute material to it (Fig. 1) or via soluble cytoplasmic pools of plasma-membrane components. Indeed Bretscher (1973) has suggested that membrane proteins may be incorporated from a soluble pool. Elegant evidence for plasma-membrane recycling has been obtained by Tulkens *et al.* (1977, 1978).

A similar recycling process is sometimes induced when depletion of intracellular membrane is triggered by initiation of secretion, as in the case of insulin secretion by pancreas (Orci *et al.*, 1973). Similarly secretion of lysosomal enzymes may normally be accompanied by increased endocytosis [and this is true when certain lymphokines stimulate macrophages (Meade *et al.*, 1974; Pantalone & Page, 1975), though it has not yet been shown with a single purified stimulant]. In such cases endocytosis compensates for exocytosis.

The intracellular vacuolar system may also be depleted during autophagy. In the case of autophagy by lysosomal invagination, cytosol proteins are sequestered (Dean, 1975a), but this process should also result in degradation of lysosomal membrane, apparently as a unit. However, it is clear that lysosomal-membrane proteins have quite heterogeneous half-lives (Dean, 1975b; Wang & Touster, 1975). If invagination and unit degradation accounted for a large part of lysosomal-membrane turnover, this observation would be difficult to explain.

In the absence of such invagination, lysosomal-membrane turnover might occur mainly *in situ*. As

it is known that, with lysosomal suspensions, lysosomal proteins of short half-life *in vivo* are the most susceptible to autolysis *in vitro* (Bohley *et al.*, 1976; Schon & Bohley, 1977), degradation *in situ* would allow turnover of the kind observed. However, since lysosomes do seem to play a substantial part in turnover of cellular protein (Dean, 1975c), it seems likely that at least a substantial rate of invagination also obtains. Thus the discrepancy between the apparent unit-degradation mechanism (invagination) and the observed heterogeneous turnover of lysosomal-membrane proteins remains to be explained.

This discrepancy may possibly be resolved by an intralysosomal recycling mechanism (Fig. 1): an intralysosomal vesicle may give rise by budding to several small vesicles with a high surface-to-volume ratio from which cytosol proteins are mainly excluded, leaving one or more larger vesicles containing cytosol proteins. If much of the cytosol protein is actually internalized while bound to the membrane, such molecules may be clustered on the membrane of the large retained vesicles, and excluded from that of the small recycling vesicles. Similar processes of selective exclusion or inclusion of proteins from phagocytic vesicles have been described (Dunham *et al.*, 1974; Oliver & Berlin, 1976). Selective clustering of membrane proteins might be an energy-requiring process, but could occur before or after entry to the lysosomal system. In the former case, cytoskeletal involvement in clustering would be quite feasible. The unequal distribution of soluble materials between the two vesicles formed by division would be a simple consequence of vesicle geometries.

The small vesicles lacking in cytosol proteins might fuse again with the lysosomal membrane, recycling lysosomal membrane, whereas the larger retained vesicles would undergo degradation. This process is highly analogous to that which represents the simplest explanation of plasma-membrane recycling. The proportion of each lysosomal-membrane protein

Fig. 1. *Mechanisms of membrane recycling*

Plasma-membrane recycling is shown on the left of the diagram: after endocytosis (A) of soluble materials (●), the endocytotic vesicle may divide to form one large and several smaller vesicles (B; see Duncan & Pratten, 1977). Alternatively, a similar vesicle division may occur after the endocytotic vesicle has fused (C) with a lysosome (carrying lysosomal enzymes, L). In both cases the soluble materials are largely retained in the large vesicle. The small vesicles may then fuse with the plasma membrane (D), thus recycling membrane. The right side of the diagram illustrates lysosomal-membrane recycling: the lysosome may invaginate to take in material from the cytoplasm (E). This material is at first in an intralysosomal vesicle, and so separated by a membrane from the lysosomal enzymes (L), and at this stage the vesicle may divide (F), in a manner analogous to the division in plasma-membrane recycling (B). The small vesicles formed may rejoin the lysosomal membrane by fusion (G), while most internalized material is retained within the lysosome, and after disintegration of the intralysosomal membrane (H) becomes accessible to lysosomal enzymes and undergoes degradation.

recycled by this mechanism might depend on how much was degraded during the intralysosomal period (i.e. on their respective proteolytic susceptibilities), and on the characteristics of division of the intralysosomal vesicle. Since lysosomal proteins of short half-life are the most susceptible to autolysis *in vitro*, this mechanism would be likely to result in most substantial degradation of short-half-life lysosomal proteins *in vivo*, and least extensive degradation of long-half-life lysosomal proteins. Selectivity of degradation should be most pronounced on membrane proteins that are solely on the interior surface of the lysosomal membrane, as these will be available to lysosomal enzymes both normally and after invagination, whereas those on the cytoplasmic surface of the lysosomal membrane may show unit degradation.

This description of the turnover of lysosomal-membrane proteins can also accommodate the observation that they do not show a clear correlation between subunit size and half-life (Dean, 1975b; Wang & Touster, 1975), unlike endoplasmic-reticulum-membrane proteins. During digestion *in vitro* of soluble proteins by lysosomal or other proteinases, those of short half-life *in vivo* (which are enriched in

large-subunit proteins) are degraded fastest. But during autolysis of endoplasmic-reticulum membranes *in vitro*, in contrast, proteins that have long half-lives *in vivo* (and that on average have small subunits) are degraded preferentially (Bohley *et al.*, 1976; Schon & Bohley, 1977). Thus proteolysis of membrane proteins *in situ* has quite different characteristics from proteolysis of soluble proteins, and does not show the normal correlation between subunit size and proteolytic susceptibility. The lack of correlation between subunit size and half-life of lysosomal-membrane proteins is thus a corollary of the suggestion that they are degraded *in situ*.

A similar hypothesis has been discussed by Segal (1976) to explain the characteristics of degradation of cytoplasmic proteins by a lysosomal route. He suggests that specificity of degradation is determined by intralysosomal proteolytic susceptibility. For proteolysis to be the rate-limiting step in lysosomal degradation it seems probable that uptake of proteins into the lysosomal internal-degradation pool would need to be reversible so that exchange between the two pools could occur. This requirement is in complete opposition to a vast body of literature on lysosomes (see Dean & Barrett, 1976); and, although the intra-

lysosomal recycling route proposed above allows exchange of membrane proteins between an intralysosomal degradation pool and a pool on the external membrane of lysosomes, presumably at least partly protected from degradation, it does not allow the required exchange of cytosol material with an intralysosomal degradation pool. For the only internalized cytoplasmic proteins that would be able to leave the lysosome again would be those which had not reached the degradation pool (Fig. 1), being separated from it by an intact membrane (before stage H in Fig. 1).

A method for the measurement of autophagy by supplying labelled non-degradable material to the cytoplasm of cells (by means of liposomes) and following its progressive association with sedimentable structures has been proposed (Dean, 1977, 1978). This method should be applicable to the demonstration of intralysosomal recycling. It may be expected that such recycling will be increased under conditions of induced autophagy to allow preservation of intracellular membranes.

References

- Bohley, P., Kirschke, H., Langner, J., Wiederanders, B., Ansoerge, S. & Hanson, H. (1976) in *Intracellular Protein Catabolism* (Hanson, H. & Bohley, P., eds.), pp. 201–208, Martin-Luther-Universität, Halle
- Bretscher, M. S. (1973) *Science* **181**, 622–629
- Dean, R. T. (1975a) *Biochem. Biophys. Res. Commun.* **67**, 604–609
- Dean, R. T. (1975b) *Biochem. Soc. Trans.* **3**, 250–252
- Dean, R. T. (1975c) *Nature (London)* **257**, 414–416
- Dean, R. T. (1977) *Acta Biol. Med. Germ.* in the press
- Dean, R. T. (1978) in *Protein Turnover and Lysosomal Function* (Segal, H. L., ed.), Academic Press, London and New York, in the press
- Dean, R. T. & Barrett, A. J. (1976) *Essays Biochem.* **12**, 1–40
- Duncan, R. & Pratten, M. K. (1977) *J. Theor. Biol.* **66**, 729–735
- Dunham, P. B., Goldstein, I. M. & Weissman, G. (1974) *J. Cell Biol.* **63**, 215–226
- Meade, C. J., Lachmann, P. J. & Brenner, S. (1974) *Immunology* **27**, 227–239
- Oliver, J. N. & Berlin, R. D. (1976) in *Immunobiology of the Macrophage* (Nelson, D. S., ed.), Academic Press, New York
- Orci, L., Malaisse-Lagae, F., Ravazzola, M., Amherdt, M. & Remold, A. E. (1973) *Science* **181**, 561–562
- Pantalone, R. M. & Page, R. C. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2091–2094
- Schon, E. & Bohley, P. (1977) *Acta Biol. Med. Germ.* in the press
- Segal, H. L. (1976) *Curr. Top. Cell. Regul.* **11**, 183–201
- Steinman, R. M., Brodie, S. E. & Cohn, Z. A. (1976) *J. Cell Biol.* **68**, 665–687
- Tulkens, P., Schneider, Y. J. & Trouet, A. (1977) *Acta Biol. Med. Germ.* in the press
- Tulkens, P., Schneider, Y. J. & Trouet, A. (1978) in *Protein Turnover and Lysosomal Function* (Segal, H. L., ed.), Academic Press, London and New York, in the press
- Wang, C.-C. & Touster, O. (1975) *J. Biol. Chem.* **250**, 4896–4902