Convergence of autophagic and endocytic pathways at the level of the lysosome

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Introduction

Radioactive sucrose may be readily introduced into the cytosol of isolated rat hepatocytes by means of electroporation-mobilization (Gordon & Seglen, 1982; Seglen & Gordon, 1984; Gordon & Seglen, 1986). A significant portion of the electroinjected [14C]sucrose eventually accumulates in lysosomes having entered them via autophagy, i.e. the way in which intracellular substances destined for autophagic-lysosomal degradation are initially sequestered by membranous entities termed phagophores (Seglen et al., 1985) into non-digestive vesicles (autophagosomes) and later delivered to lysosomes for digestion, by a process involving fusion between these two types of vesicle.

It is well established that certain substances enter the lysosomal compartment as a result of heterophagy, i.e. the process by which extracellular substances are taken up into the cell via endosomes which eventually deliver their contents to lysosomes, probably after fusing with them. The question has arisen whether there exist separate populations of lysosomes whose function is to receive material from these distinct pathways or whether there is but a common population of lysosomes capable of receiving material from both pathways. We investigated this question by following the fate of autophagically sequestered sucrose in hepatocytes which were allowed to endocytose the sucrose-cleaving enzyme invertase.

Results and discussion

The net accumulation of autophagically sequestered sucrose was practically abolished in the presence of invertase (Fig. 1). The very small amount of sucrose accumulating above the background may possibly represent a steady-state level of sequestered sucrose present in autophagosomes in transit to the lysosomes. It should be noted that the experiment shown in Fig. 1 does not display the initial, transient accumulation of sequestered sucrose previously demonstrated in the presence of invertase (Seglen et al., 1986), probably because of the higher concentration of enzyme presently used.

The fact that no net accumulation of sucrose occurred beyond 20 min indicated that all autophagically sequestered sucrose eventually reached invertase-containing lysosomes, in other words that a complete intermixing of autophagically and endocytically delivered material occurred. However, we needed to exclude the possibility that invertase might affect the autophagic process per se. Hepatocytes were therefore allowed to autophagically sequester the radioactive sugar [14C]lactose (which is degraded within lysosomes) under conditions in which it could not reach the lysosomes, i.e. in the presence of vinblastine (0.05 mm). During such experiments, the sequestered [14C]lactose accumulated in pre-lysosomal vesicles (autophagosomes) at exactly the same rate in the absence or presence of invertase, showing that the enzyme did not interfere with autophagy per se.

To see whether the autophagic and endocytic pathways converged at the lysosomal level or pre-lysosomally, the lysosomes were loaded autophagically with [14C]sucrose, and then the autophagic pathway was shut off before the cells were allowed to endocytose invertase. This was achieved by allowing the hepatocytes to sequester sucrose for 1 h before their autophagic activity was blocked using the specific inhibitor 3-methyladene (Seglen & Gordon, 1982). As shown in Fig. 2, 3-methyladene stopped all further sequestration of sucrose, and the pre-sequestered radioactivity (known to reside in lysosomes) remained at a constant level in the absence of invertase. In the presence of invertase, on the other hand, the remaining amount of pre-sequestered sucrose fell gradually until all radioactivity had disappeared. This latter result would tend to indicate that the endocytic pathway delivers invertase directly to the lysosome, independently of whether the autophagic pathway is open or shut (active or inactive). Thus, autophagic-endocytic rendezvous would seem to take place at the level of the lysosome.

In view of the fact that our data point to a rather complete

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Abbreviations used: 3-MA, 3-methyladene; INV, invertase.
convergence of the autophagic and endocytic pathways (all autophagically sequestered sucrose is accessible to endocytosed invertase) it would seem imprudent to make any distinction between autolysosomes (autophagic vacuoles) and heterolysosomes.

Stability of urogastrone and some fusion derivatives and the induction of stress proteins in *Escherichia coli*

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The synthesis of aberrant or foreign protein in *Escherichia coli* can result in the rapid, selective degradation of the polypeptide, aggregation of the aberrant molecule to form inclusion bodies and the induction of the heat-shock response (Goff & Goldberg, 1985). In this communication, we have compared the ability of four related foreign gene products — urogastrone (Uro) (epidermal growth factor, a 53 amino acid peptide with asparagine as amino-terminal amino acid); TrpE-Uro (i.e. urogastrone with a 13 amino acid residue sequence (QTSKPTPSKLKK) attached to the amino terminus); Uro-Arg, and TrpE-Uro-Arg, (i.e. urogastrone with five extra arginine residues attached to the carboxy-terminus and a 14 amino acid residue sequence (QTKPPTPSKLKENG) at the amino terminus) to invoke these effects.

Protein synthesis in cells (*E. coli* AB1157) grown in M9 minimal medium was detected by pulse labelling for 5 min with [35S]Met (10 μCi/ml) following heat-shock (10 min at 45°C) or induction (60 min) of urogastrone synthesis with indoleacrylic acid. Proteins were then separated by SDS-PAGE (Laemmli, 1970) and visualized by autoradiography.

Native urogastrone was the least stable of the four proteins (t1/2 < 5 min); TrpE-Uro and Uro-Arg, showed increased stability of urogastrone and some fusion derivatives and the induction of stress proteins in *Escherichia coli*