Journal of Cell Science 108, 3645-3647 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995
JCS4076

COMMENTARY

Regulation of yolk degradation, or how to make sleepy lysosomes

François Fagotto

Cellular Biochemistry and Biophysics Program, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA

(e-mail: f-fagotto@ski.mskcc.org)

INTRODUCTION

Yolk is the major internal food supply on which most embryos rely. Strict regulation of its utilization is essential to provide nutrients at the right time to the developing tissues, and to ensure survival of the embryo until it becomes a free-living organism able to feed. Yolk proteins are derived from a maternal serum protein, vitellogenin (Vg). Vg is internalized by the growing oocyte via receptor-mediated endocytosis, partially processed in an endosomal compartment, and then stored in specialized organelles, the yolk granules (YGs or yolk platelets) (Wallace, 1985; Opresko et al., 1980; Opresko and Karpf, 1987).

YGs appear to be modified lysosomes, containing the whole set of hydrolases necessary for yolk degradation. Yet, paradoxically, YGs do not degrade their content until specific developmental stages, sometimes weeks after their formation, in striking contrast to classical lysosomes, which are voracious degradative organelles, capable of rapidly reducing almost any protein down to free amino acids, sugars and other small products. The lysosomal nature of the YGs and their latency was hypothesized more than a quarter of century ago by Pasteels (1966), who first detected acid phosphatase activity in the YGs of a molluscan egg. His observations have since been confirmed in many other species, but the regulation of yolk degradation remained a puzzle. A recent series of studies on both vertebrate and invertebrate models has brought new light on this phenomenon. Two factors appear to be responsible for the regulation of yolk degradation: pH and enzymatic latency.

pH REGULATION

One major characteristic of classical lysosomes is their acidic internal milieu, which is essential for the activity of the lysosomal degradative enzymes, typically acid hydrolases. The key observation for the understanding of the regulation of the YGs has been that these organelles can modulate their pH: in the four models examined so far (the tick *Ornithodoros moubata* (Fagotto, 1991), the cockroach *Blattella germanica* (Nordin et al., 1991); the sea urchins *Strongilocentrotus purpuratus* and *Lytechinus pictus* (Mallya et al., 1992); and the frog *Xenopus laevis* (Fagotto and Maxfield, 1994a,b)) the initial YG pH is neutral, or at most mildly acidic, which is very unfavorable for normal lysosomal function. Developmentally regulated acidification then occurs during embryogenesis, which triggers yolk degradation.

The simplest version of this regulation is found in the two arthropod models, *Ornithodoros* and *Blattella* (Fagotto, 1991; Nordin et al., 1991), where YG pH, estimated by the accumulation of the fluorescent weak base Acridine Orange, is close to neutral in early developmental stages, and becomes strongly acidic in the course of embryonic development (*Blattella*) or just before hatching (*Ornithodoros*), concomitant with the appearance of structural and biochemical signs of yolk degradation. In both species, in vitro studies have shown that such low pHs are required for the proteolysis of the yolk proteins (Fagotto, 1990a,b; Nordin et al., 1991). *Ornithodoros* YGs contain typical acid hydrolases, including a cysteine proteinase very effective in digesting yolk polypeptides (Fagotto, 1990a,b) and a similar enzyme is probably present in *Blattella* (Nordin et al., 1991). Direct correlation between the internal pH and the state of degradation of the YGs has been demonstrated in *Ornithodoros*: YGs in the course of degradation are lighter than intact YGs, and can be separated on density gradients. Only the light YGs were found to be acidic (Fagotto, 1991).

In sea urchins, the YG pH, estimated by immunolocalisation of another weak base, DAMP, is also initially near neutral (Mallya et al., 1992). Weak acidification occurs early during development, at a stage where yolk proteins undergo limited processing. Total yolk degradation, however, only takes place in larval stages in these species. The possibility that lower pHs are reached at these later stages has not been investigated.

Quantitative pH determinations have been performed in *Xenopus* oocytes (Fagotto and Maxfield, 1994a,b) using three different microscopic techniques (photometry of fluorescein-labeled yolk, dual emission confocal fluorescence microscopy, and quantitation by confocal microscopy of Acridine Orange accumulation). The three methods gave a consistent YG pH around 5.6. A similar pH was found in the YG precursors, which are smaller vesicles where Vg is proteolytically processed into yolk proteins. These conditions are thus sufficient for partial proteolysis of Vg, but do not promote more extensive degradation. Extracts of *Xenopus* YGs do have pro-

Key words: cysteine proteinase, lysosomal acidification, vitellogenin, embryonic development

teolytic activity, but yolk degradation in vitro requires pHs <5 (unpublished observations).

YG pH in *Xenopus* oocytes was found to be extremely stable over days (and even weeks). Using semipermeabilized oocytes, we were able to demonstrate the activity of a vacuolar proton pump in the YGs, and we found that YG pH is sensitive to cytoplasmic factors, since addition of a cAMP analogue caused stronger acidification under certain ionic conditions (Fagotto and Maxfield, 1994a). In vivo, YGs become more acidic during embryonic development (Fagotto and Maxfield, 1994b). This enhanced acidification occurs at defined stages, specific for each tissue, and again strictly correlates with the appearance of morphological signs of YG digestion. During this phase of intense acidification and degradation, YGs reach quite low pHs, clearly in the range of lysosomal pH and compatible with the hydrolytic activity observed in vitro. Consistently, yolk utilization does not take place when acidification is experimentally inhibited with bafilomycin, a specific inhibitor of the vacuolar proton pump.

These data therefore strongly support YG acidification as the trigger for yolk degradation. However, other regulatory mechanisms must reinforce the latency of the YGs, since controlling the luminal pH alone is probably not sufficient to completely prevent premature YG proteolysis during the long period of oogenesis. Indeed, most lysosomal enzymes function under mild acidic conditions and display residual activity even at pH close to neutrality, while digestion of yolk polypeptides only occurs at quite low pHs.

ENZYMATIC REGULATION

Evidence for additional modes of regulation of yolk degradation has been inferred from biochemical studies on *Ornithodoros* eggs. The major yolk proteinase, a cathepsin L-like cysteine proteinase (Fagotto, 1990a), is stored as a latent proenzyme, which is completely inactive at mildly acidic pHs (Fagotto, 1990b). Low pH in vitro, and YG acidification at late developmental stages in vivo, induce the maturation of the enzyme, which is then active at the full range of acidic pHs. It is not clear whether a low pH is required directly for the cleavage of the proenzyme, or, by analogy with the cathepsin-cystatin complexes, to detach the proenzyme from an inhibitor. This latter possibility is supported by the observation that the procathepsin L appeared to be bound to a larger protein, probably the yolk protein (Fagotto, 1990a). Cysteine proteinases are also the major degradative enzymes in the yolk of sea urchins (Mallya et al., 1992), *Blattella* (Nordin et al., 1991) and several other species (Kageyama et al., 1981; Medina and Vallejo, 1989; Perona and Vallejo, 1982). Although the latency and regulation of yolk cathepsin have been studied only in one species, the control mechanism found in *Ornithodoros* eggs may be widespread.

While a short treatment at low pH is sufficient to activate the Ornithodoros cathepsin L, yolk degradation, unlike hydrolysis of other proteins or synthetic substrates, still requires quite low pHs even after enzyme activation (Fagotto, 1990a). Such resistance to degradation may be achieved by exposing only a minimal number of cleaving sites at the surface of the protein, and/or protecting them by modifications such as glycosylation. As observed with other proteins, acid denaturation would cause more sites to be accessible. In addition, Vg, or one of the products of its partial degradation, may act more specifically by binding and inhibiting proteolytic enzymes. Interestingly, yolk proteins display trypsin-chymotrypsin inhibitor activity (Salisbury et al., 1980; Ezquieta and Vallejo, 1986). It remains to examine whether yolk proteins can inhibit a wider range of proteinases, including cysteine proteinases. If this is the case, yolk proteins may play a determinant role in the regulation of their own degradation.

While usually considered as a mere generic food supply, Vg has been in fact remarkably well conserved during evolution (Byrne et al., 1989). Vg is a complex phospho-lipo-glycoprotein which also binds hormones, vitamins and ions, and it has been proposed that these nutritional functions have exerted evolutionary constraints on the Vg sequence. Considering that the mode of YG degradation is quite similar in the various models examined, it is conceivable that resistance to degradation is also a conserved property of Vg.

CONCLUSION AND FUTURE DIRECTIONS

Resistance to proteolysis and proteinase latency combined with regulation of YG pH may therefore be sufficient to describe both the biogenesis and the degradation of the YGs: once internalized by the oocyte, Vg would be at most partially cleaved at mild acidic pH, but these proteolytic products would fail to be degraded further under these conditions. In consequence, they would accumulate, forming unusually large organelles instead of normal lysosomes. Vg might even dictate the enzymatic composition of the YG, since hydrolases that bind to the yolk polypeptides would be enriched. The very high concentrations of Vg in the YG may also participate in setting or stabilizing the resting pH by acting as an internal buffer (Fagotto and Maxfield, 1994a). Modulation of the YG pH would then be sufficient to precisely regulate yolk processing during embryonic development: small decreases in pH would cause partial processing and stronger acidification would finally allow full activity of the proteolytic enzymes and total degradation of the yolk.

Variations in the time course of YG acidification and differences in the resistance of various Vgs to proteolysis would combine to produce the diverse patterns of Vg processing found among different species. These differences are likely to reflect specific adaptations of the yolk functions: for instance in *Xenopus* oocytes, where YG are mildly acidic, the pH gradient across the YG membrane seems to be used to drive accumulation of sodium ions through a Na^+/H^+ exchanger (Fagotto and Maxfield, 1994a). Storage of sodium is important for the embryonic development of this fresh water species (Slack et al., 1973).

It is likely that the apparent complexity and diversity in the composition of the yolk and its digestion results from subtle modulations of simple conserved regulatory mechanisms.

Future biochemical experiments should confirm the latency and activation of the *Ornithodoros* proteinase in other species. They should address directly the resistance to degradation and the potential protease inhibitor properties of yolk proteins, and eventually define the sequence(s) responsible for such peculiar features.

Most importantly, the molecular mechanism of pH regulation

in YGs remains to be determined. We already know that changes in YG pH are almost certainly not caused by addition of new pumps or channels at their membrane: the fact that *Xenopus* YG pH could be experimentally decreased in permeabilized oocytes is compelling evidence that the regulatory mechanisms are intrinsic to the 'original' YG membrane (Fagotto and Maxfield, 1994a). Thus YG pH is likely to be directly under the control of cytoplasmic signals, which must integrate yolk acidification and degradation into the developmental program of the embryo. A future challenge will consequently be the characterization of the intracellular signaling pathways involved and their targets at the YG membrane. By virtue of their large size, the membrane properties of YGs may be amenable to patch clamp analyses, which have been so successful with plasma membranes. pH regulation is quite poorly understood in endosomes and lysosomes, though it is essential for the various functions of these compartments. YGs, being much larger, stable, easily identifiable organelles, may then become a model of choice to study the physiology of degradative compartments.

I thank Dr Alpha Yap for critical reading of the manuscript, and I am greatly indebted to Dr Fred Maxfield for support and advice. This work is dedicated to Marie, born on 31 March 1995.

REFERENCES

Byrne, B. M., Gruber, M. and Ab, G. (1989). The evolution of egg yolk proteins. *Progr. Biopys. Mol. Biol.* **53**, 33-69.

Ezquieta, B. and Vallejo, C.G. (1986). Lipovitellin inhibition of Artemia trypsin-like proteinase: a role for storage protein in regulating proteinase activity during development. *Arch. Biochem. Biophys.* **250**, 410-417.

Fagotto, F. (1990a). Yolk degradation in tick eggs: I. Occurrence of a cathepsin L-like acid proteinase in yolk spheres. *Arch. Insect Biochem. Physiol.* **14**, 217-235.

Fagotto, F. (1990b). Yolk degradation in tick eggs: II. Evidence that cathepsin L-like proteinase is stored as a latent, acid-activatable proenzyme. *Arch. Insect Biochem. Physiol.* **14**, 237-252.

Fagotto, F. (1991). Yolk degradation in tick eggs: III. Developmentally regulated acidification of the yolk spheres. *Dev. Growth Differ.* **33**, 57-66.

Fagotto, F. and Maxfield, F. R. (1994a). Yolk platelets in Xenopus oocytes maintain an acidic internal pH which may be essential for sodium accumulation. *J. Cell Biol.* **125**, 1047-1056.

Fagotto, F. and Maxfield, F. R. (1994b). Changes in yolk platelet pH during *Xenopus laevis* development correlate with yolk utilization. A quantitative confocal microsc opy study. *J. Cell Sci.* **107**, 3325-3337.

Kageyama, T., Takahashi, S. Y. and Takahashi, K. (1981).Occurrence of thiol proteinases in the eggs of the silk worm, Bombix mori. *J. Biochem.* **90**, 665-671.

Mallya, S., Partin, J. S., Valdizan, M. C. and Lennarz, W. J. (1992). Proteolysis of the major yolk glycoproteins is regulated by acidification of the yolk platelets in sea urchin embryos. *J. Cell Biol.* **117**, 1211-1221.

Medina, M. and Vallejo, C. G. (1989). The maternal origin of acid hydrolases in Drosophila and their relation with yolk degradation. *Dev. Growth Differ.* **31**, 241-247.

Nordin, J. H., Beaudoin, E. L. and Lin, X. (1991). Acidification of yolk granules in Blattella germanica eggs coincide with proteolytic processing of vitellin. *Arch. Insect Biochem. Physiol.* **18**, 177-192.

Opresko, L., Wiley, H. S. and Wallace, R. A. (1980). Differential postendocytotic compartmentation in Xenopus oocytes is mediated by a specifically bound ligand. *Cell* **22**, 4-57.

Opresko, L. K. and Karpf, R. A. (1987). Specific proteolysis regulates fusion between endocytic compartments in Xenopus oocytes. *Cell* **51**, 557-568.

Pasteels, J. J. (1966). Les corps multivésiculaires de l'oeuf de Barnea candida (Mollusque bivalve) étudiés au microscope électronique. Activité phosphatasique et accumulation du rouge neutre. *J. Embryol. Exp. Morphol.* **16**, 301-310.

Perona, R. and Vallejo, C. G. (1982). The lysosomal proteinase of Artemia: Purification and characterization. *Eur. J. Biochem.* **124**, 357-362.

Salisbury, N., Calaprice, N. and Triplett, E. (1980). Amphibian embryo protease inhibitor. VI: Maternal origin and identity with lipovitellin heavy subunit. *Cell Differ.* **9**, 219-227.

Slack C., Warner, A. and Warren, A. E. (1973). The distribution of sodium and potassium in amphibian embryos during early development. *J. Physiol.* **232**, 297-312

Wallace, R. A. (1985). Vitellogenesis and oocyte growth in nonmammalian vertebrates. In *Developmental Biology: A Comprehensive Synthesis* vol. 1: *Oogenesis* (ed. W. L. Browder), pp. 127-166. Plenum Press, New York.