Tissue and Cell xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Turnover of pigment granules: Cyclic catabolism and anabolism of ommochromes within epidermal cells

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ARTICLE INFO

Article history:
Received 11 March 2009
Received in revised form 22 May 2009
Accepted 25 May 2009
Available online xxx

Keywords:
Pigment turnover
UV-damage
Ommochromes
Autophagy
Melanin
Spiders
Misumena vatia

ABSTRACT

Ommochromes are end products of the tryptophan metabolism in arthropods. While the anabolism of ommochromes has been well studied, the catabolism is totally unknown. In order to study it, we used the crab-spider *Misumena vatia*, which is able to change color reversibly in a few days, from yellow to white and back. Ommochromes is the only pigment class responsible for the body coloration in this animal. The aim of this study was to analyze the fine structure of the epidermal cells in bleaching spiders, in an attempt to correlate morphological changes with the fate of the pigment granules. Central to the process of bleaching is the lysis of the ommochrome granules. In the same cell, intact granules and granules in different degradation stages are found. The degradation begins with granule autolysis. Some components are extruded in the extracellular space and others are recycled via autophagy. Abundant glycogen appears associated to granulolysis. In a later stage of bleaching, ommochrome progranules, typical of white spiders, appear in the distal zone of the same epidermal cell. Catabolism and anabolism of pigment granules thus take place simultaneously in spider epidermal cells. A cyclic pathway of pigment granules formation and degradation, throughout a complete cycle of color change is proposed, together with an explanation for this turnover, involving photoprotection against UV by ommochromes metabolites. The presence of this turnover for melanins is discussed.

1. Introduction

Ommochromes are end products of the tryptophan metabolism in arthropods, which are either excreted or stored until the death of the animal (Linzen, 1974; Needham, 1974). Female crab-spiders of the family Thomisidae are capable of changing their color reversibly in a few days, from white to yellow and back (Gabritchevsky, 1927; Weigel, 1941; Holl, 1987). In particular, the pigmentation of the species of *Thomisus* has been studied since the pioneering work by Heckel (1891). The process of bleaching, i.e. the color reversion from yellow to white, has not been elucidated. The loss of color may involve three possibilities: (1) the dilution of epidermal pigment granules, for instance by growth and cell multiplication; (2) their degradation; or, (3) their translocation. Reversibility of the change is furthermore possible by pigment dilution. In the insect *Carausius morosus*, where a genuine morphological color change occurs, the role of ommochromes in the process has been quantitatively analyzed (Bückmann and Dustmann, 1962; Dustmann, 1964; Bückmann, 1977). However, the ommochrome pigmentation appeared to be irreversible in that insect. Only by "dilution" of ommochromes, which once formed in the integument are never removed, the color change was reversible (Dustmann, 1964). As for degradation, there are only a few histological studies mentioning ommochrome lysis. For example, degradation of ommochrome pigment granules was observed in the compound eyes of some insects and other arthropods (Fahrenbach, 1969; Wachmann, 1969; Perrelet et al., 1971) and in the epidermal cells of insects during a moulting cycle (Lhonoré et al., 1980; Bouthier and Lhonoré, 1984). Physiological color change involves translocation of existing pigment within chromatophores (pigment cells) or muscular movement of pigment cells and can occur over very short time periods (Booth, 1990). Movements of ommochrome granules due to light–dark adaptation processes and circadian rhythms have been observed in insect eyes (Stavenga, 1989). However, the translocation was never related to morphological color change.

The basic metabolic pathway and enzymes for the anabolism of ommochromes are partially identified (Butenandt et al., 1954a,b, 1958; Linzen, 1967). The yellow coloration in *Misumena vatia* is produced by ommochrome pigment granules located in the epidermal cells, immediately beneath the transparent cuticle (Seligy, 1972; Insausti and Casas, 2008). The formation of ommochrome pigment granules proceeds in three distinctive steps. Translucent progranules (*type I*) are first produced by a dense network of rough endoplasmic reticulum associated with numerous mitochondria and glycogen rosettes. These progranules are present in white spiders only, and regularly distributed in the cytoplasm.

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doi:10.1016/j.tice.2009.05.002

The biochemical transformation of progranules of *type I* into a transient state (progranule *type II*) leads then to the formation of ommochrome granules (*type III*) (Insausti and Casas, 2008). By contrast, the catabolic pathway was never studied, causing the lack in understanding of the reversible color change. *In vitro* chemical reactions have provided some insight into the degradation process of ommochromes. By *in vitro* ommatin degradation in acid or alkaline media a mixture of fluorescent compounds containing xanthurenic acid and 2-amino-3-hydroxy-acetophenone has been isolated. Under very mild conditions the decomposition of xanthommatin results in 3-hydroxy-kynurenine (Butenandt et al., 1954a,b, 1958; Linzen, 1974). Analysis of insect excretion, of a number of Lepidoptera such as *Manduca sexta*, yielded granules of xanthommatin as the only ommochrome in the larval epidermis. This xanthommatin is excreted with feces at the end of larval life in the form of water-soluble ommochromes, rhodommatin and ommatin D. Red rhodommatin and ommatin D are furthermore present in the meconium of newly emerged butterflies and moths (Kayser, 1985).

In the present work, we analyze the fine structure of the epidermal cells in bleaching spiders. We describe the ultrastructural changes associated with ommochrome degradation, in an attempt to correlate morphological changes with the turnover of pigment granules. By integrating our findings with previous work (Insausti and Casas, 2008) we are able to describe the entire cycle of pigment granules formation and degradation, throughout a complete cycle of color change. We end up this work by discussing the pigment granules turnover and the implications for other pigment classes.

2. Materials and methods

Yellow colored, adult females of crab-spiders *M. vatia* (Araneae: Thomisidae) (Clerck, 1757) were collected on flowers in the surroundings of Tours, France, during the spring and summer 2007 and 2008. Upon capture, they were maintained in clear plastic vials (7 cm high, 5 cm diameter) containing pieces of damp cotton, and were fed on houseflies (about one a week). We removed discarded prey items and cleaned the vials weekly.

To induce the color change, the spiders were maintained in an environment with diffuse solar light and a white background. To quantify the color change, we measured the reflectance of the opisthosoma of each female twice a week with an Avaspec 256 spectroradiometer (Avantes, Eerbeek, the Netherlands) using a deuterium/halogen light source. We averaged three measurements on each female to establish spider coloration. Once the desired color state was attained, the spiders were processed for histology.

For the morphological analysis, transmission electron (TEM) and light microscopy (LM) were performed, following the technique described by Ribi (1987). Briefly, a spider was fixed for 3 h in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in phosphate buffer (pH 7.3) with glucose and $CaCl_2$ added. Subsequently, the pieces were post-fixed with buffered 1% osmium tetroxide for 1–2 h. After dehydration, they were embedded via propylene oxide in Durcupan ACM (Fluka–Sigma). Blocks were serially sectioned at 1.5–5 μm thickness, using glass knives mounted in a microtome (Leica RM 2265). The sections were stained on a hot plate with Toluidine Blue–Basic Fuchsin or were mounted unstained on a slide with DPX (Sigma–BioChemika). The unstained sections were investigated by transmission and fluorescence light microscopy (Olympus microscope with a USH102D burner, DM400 dichroic mirror, a BP330-385 excitation filter and a BA420 barrier filter). For electron microscopy, ultrathin sections, cut with a diamond knife, were doubly stained by uranyl acetate and lead citrate and observed using a JEOL 1010 transmission electron microscope.

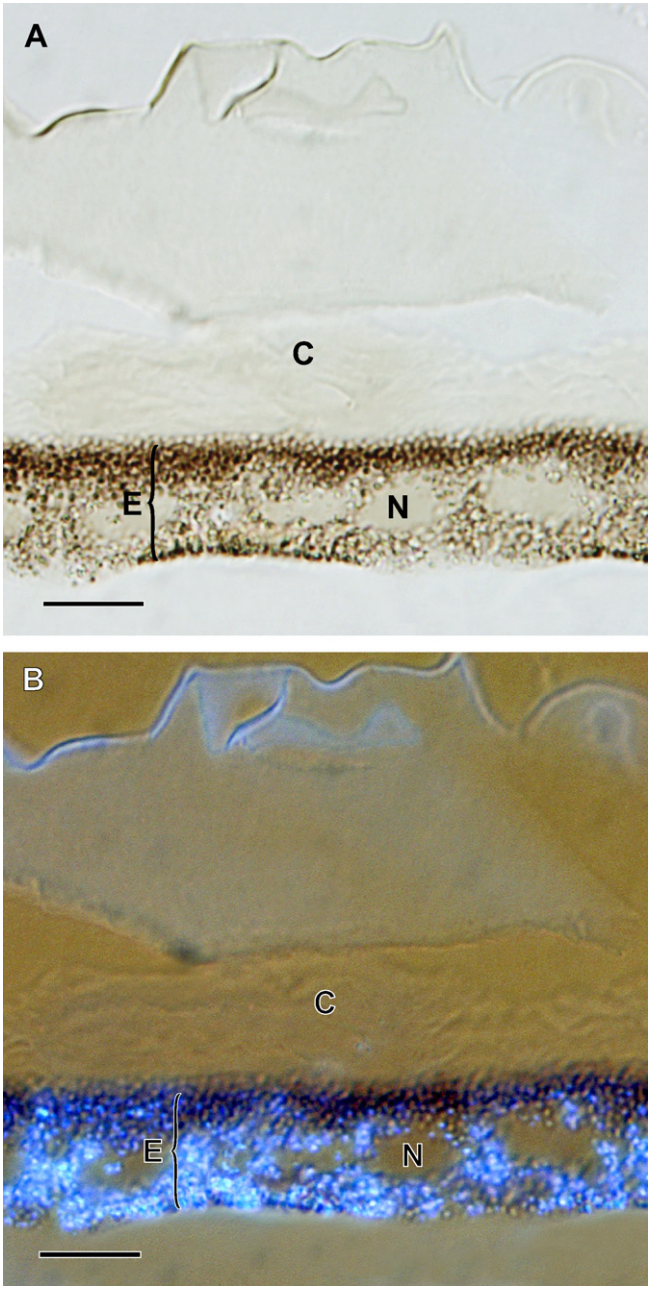

Fig. 1. Micrographs of cross-sections of the tegument of a spider *M. vatia* turning white. (A) Unstained section. Two types of inclusions can be observed: brown and translucent types. (B) Same region as in A, observed under white and UV-light mixed. The translucent inclusions show a strongly auto-fluorescence, whereas the brown granules emit no fluorescence. E, epidermis; C, cuticle; N, nucleus. Bars, 10 μm.

3. Results

We analyzed the epidermal cells of yellow spiders at different phases of color change from yellow to white. Light microscopy of unstained sections enabled us to distinguish two kinds of inclusions, translucent and brown ones (Fig. 1A). The translucent type emits light-blue fluorescence under UV excitation light, whereas the brown type remains dark (Fig. 1B).

3.1. Ultrastructural characteristics of the epidermis

The epidermal cells in bleaching spiders are held together near their apices by zonulae adhaerens and lower down by septate

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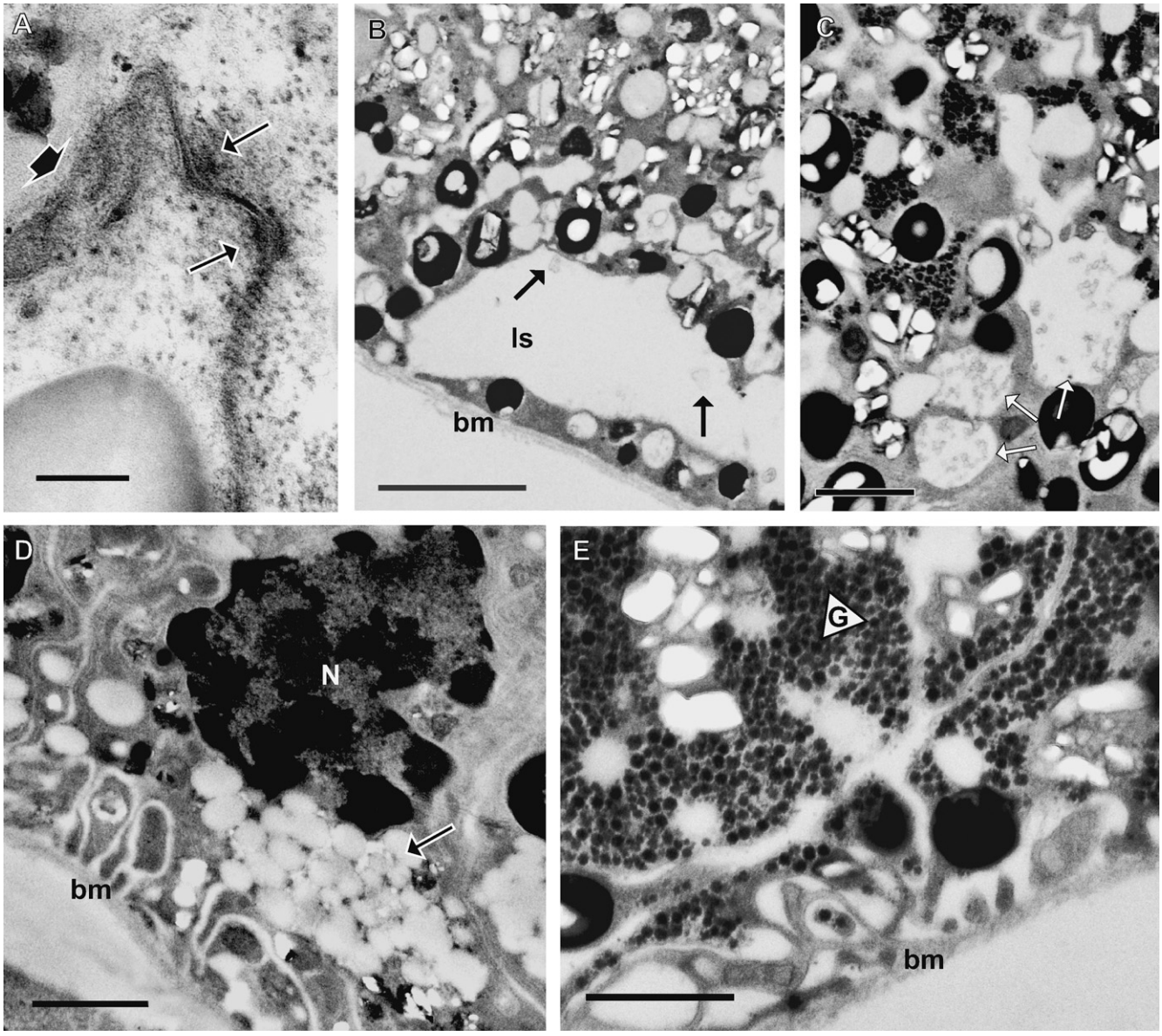

Fig. 2. (A) Attachment devices of the epidermal cells. The cells are held together near their apices by zonulae adhaerens (short thick arrow) and lower down by septate desmosomes (long narrow arrows) (bar, 0.2 μm). (B and C) Details of the cellular basal region. In B, large intercellular lacunary space (ls) with secretion vesicles (arrows) are marked. Granules in different stages of degradation can be seen in the cell cytoplasm (bar, 2 μm). In (C) the lacunary spaces (arrows) with abundant vesicles of secretion are shown (bar, 1 μm). (D and E) Details of the basal reticular meshwork formed from the folded plasma membrane. (D) Vacuole aggregation present at the base of the cell (arrow) (bar, 2 μm). In (E) the large amount of glycogen filling the cell cytoplasm is marked (bar, 1 μm). bm, basal membrane; G, glycogen rosettes; N, nucleus.

desmosome (Fig. 2A). The cell cohesion diminishes in the lower two thirds and lacunary spaces appear between them. The presence of abundant secretion vesicles is frequent in these intercellular spaces (Fig. 2B and C). In the basal region close to the basal lamina, the cell shows a well-developed reticular meshwork. This reticular system is produced from basal plasma membrane invaginations and intercellular infolds (Fig. 2D and E). The cell cytoplasm contains abundant vacuoles of different kinds, different types of granules, dictyosomes, lysosomes and mitochondria. In the basal zone, rosettes of glycogen fill up the cytoplasmic space, in close association with granules and aggregations of vacuoles (Fig. 2E).

We found abundant electron-lucent vacuoles clustered together at the base of the epidermal cell in spiders in an advanced state of color change (Figs. 2D; 3A and B). These aggregations of vacuoles correspond to the basal fluorescent inclusions observed with UV-light. The rough endoplasmic reticulum (RER) is greatly developed and parallel cisternae looped to envelope the aggregations of vacuoles (Fig. 3A–C). We also observed the cisternae of RER integrated into a whorl enclosing one vacuole, sometimes two. Mitochondria and debris of cytoplasmic material were occasionally encountered (Fig. 3D). Lysosomes are found in close proximity to these structures. Furthermore, arrays of concentric cisternae of RER are found scattered in the cell cytoplasm in close association with mitochondria (Fig. 3E).

3.2. The epidermal granules

Two types of homogeneous granules can be distinguished in the cell cytoplasm. The first class of granules consists of poorly osmiophilic, ellipsoidal granules, 0.8 μm × 1.4 μm in cross-section, that are enclosed by a unit membrane (Figs. 3A and F). These granules correspond to the translucent-fluorescent inclusions observed by transmission light microscopy in the distal region of the cell. The second class of granules consists of osmiophilic structures, diam-

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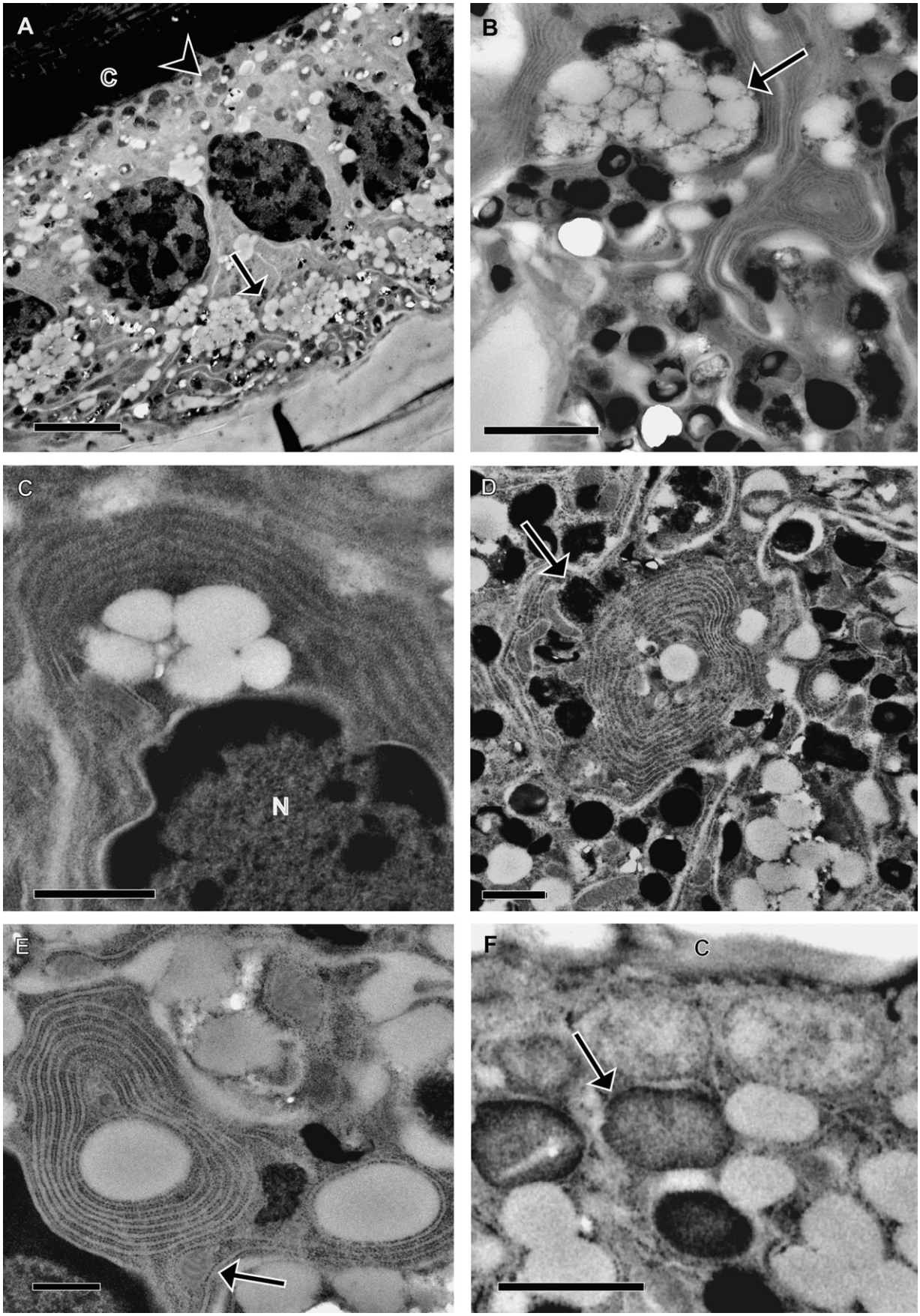

Fig. 3. (A–F) The epidermal cells of a spider in an advanced state of bleaching. (A) General view of the epidermis showing the aggregations of electron-lucent vacuoles (long arrow) in the basal region and progranules I (arrowhead) in the distal region of the cell (bar, 5 μm). (B) Details of a cluster of vacuoles (arrow). The abundant rough endoplasmic

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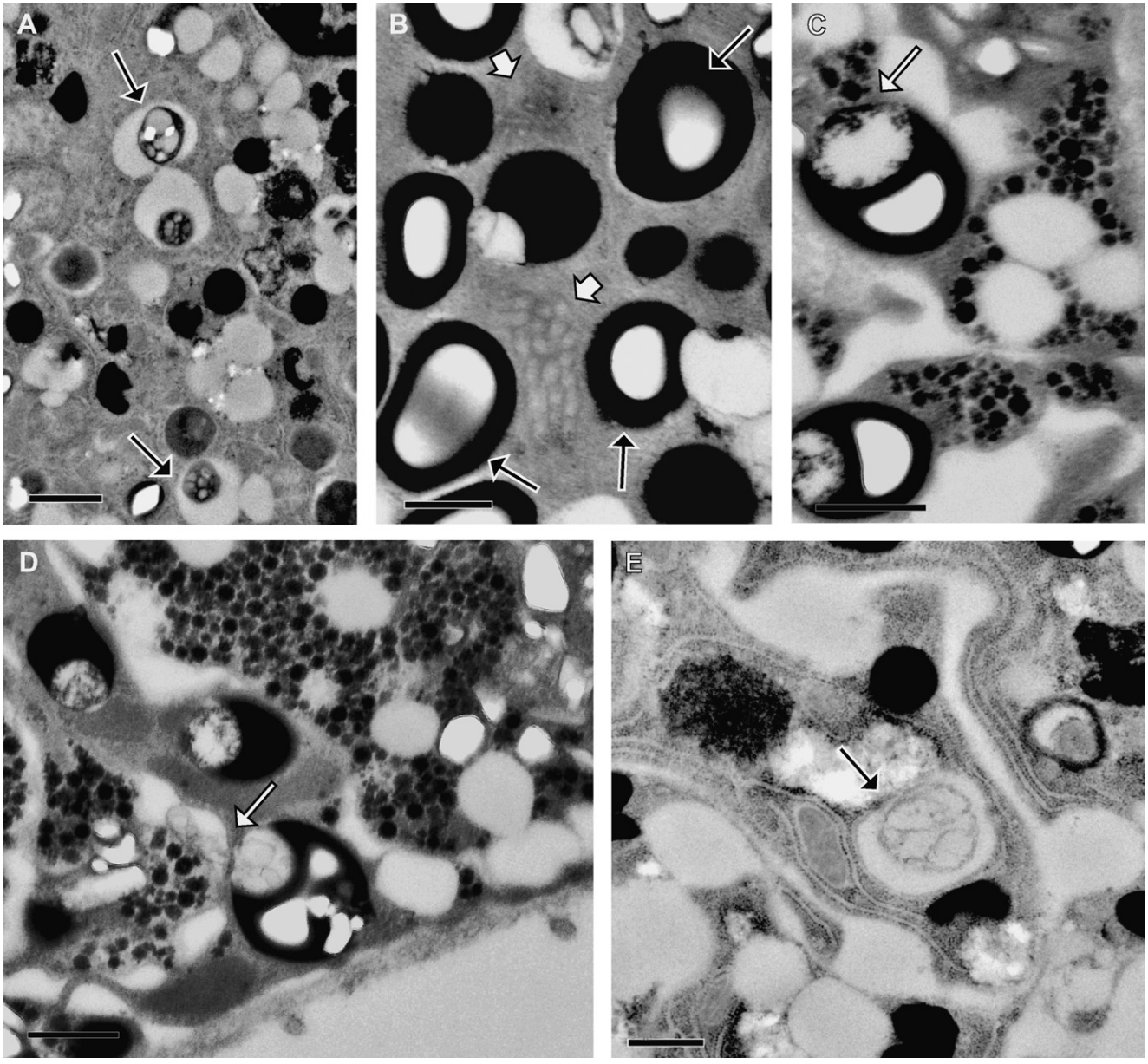

Fig. 4. (A–E) Ommochrome granules in the process of degradation. (A and B) Granules in the first stages of degradation (black arrows). The Golgi apparatus close to granules is marked with white arrows in B. Bars: A, 1 μm; B, 0.5 μm. (C) Glycogen rosettes are evacuated into the cytoplasm as the result of the degradation of the matrix of granules (arrow). (D) The granules are in relation with secretion vesicles present in the intercellular spaces (arrow). Note the large amount of glycogen filling the cell cytoplasm (bar, 0.5 μm). (E) Granule in advanced state of degradation. The granule turns into an electron-lucent vacuole with a central flocculent material (arrow) (bar, 0.5 μm).

eter 0.8–1 μm, that are enclosed by a membrane (Fig. 4A and B). These granules correspond to the brown inclusions observed by transmission light microscopy.

In addition to the granules described above, the cells contained abundant atypical granules surrounded by a membrane. Some granules resemble a vacuole with a core of nonhomogeneous electron-dense material and larger or smaller electron-lucent compartments (Fig. 4A). Others show an homogeneous electron-lucent central or semicircular area with an electron-dense region surrounding the clear centre. We consistently observed an association of granules with a Golgi region (Fig. 4B). Glycogen rosettes appearing in the semicircular electron-lucent area of granules and in the cytoplasmic area adjacent to them were often observed (Fig. 4C and D). In an advanced state of bleaching, electron-lucent granules with a central flocculent material were frequently seen (Fig. 4E).

4. Discussion

The results of this study show that the process of bleaching in *M. vatia* involves the degradation of the ommochrome granules. Thus, we are able to discard their dilution or translocation in this case. The degradation of the ommochrome granules in the epithelial cells is a progressive process. Full granules as well as granules

reticulum begins to wrap them (bar, 2 μm). In (C) the rough endoplasmic reticulum encircles the vacuoles to initiate the formation of a multilamellar autophagosome (bar, 1 μm). (D) An example of an autophagosome. The core of this structure contains cytoplasmic debris surrounding a vacuole. A lysosome can be seen in the proximity (arrow) (bar, 1 μm). (E) Detail of a multilamellar structure of rough endoplasmic reticulum associated with the genesis of progranules *type I*. Note the presence of mitochondria in close association with it (arrow) (bar, 0.5 μm). (F) Details of progranules *type I* (arrow) present in the distal region of the cell (bar, 1 μm). C, cuticle; N, nucleus.

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in different degradation stages can be found in the same cell. In the following, we first describe the different stages of granules. Subsequently, we attempt an identification of the lytic processes leading to the appearance of the granules of different stages and the fate of the granules constituents during lysis. We then propose an ordered cyclic sequence implicating all recognized stages and of cyclic nature. Finally, we discuss the simultaneous anabolism and catabolism within the same cell in epidermal cells and the hypothesis of UV protection by the ommochrome metabolites.

4.1. Characterization of the different granule states

On the basis of the results of our previous work (Insausti and Casas, 2008), we interpret the osmiophilic homogeneous granules (brown inclusions observed under light microscopy) as ommochrome granules (*type III*). The poor osmiophilic homogeneous granules (the distal translucent-fluorescent inclusions) are *type I* progranules and contain kynurenine. In addition, the cells harbor abundant atypical granules surrounded by a membrane with electron-dense material and electron-lucent areas. They represent granules in different degradation phases. The ommochrome pigment granules belong to a group of cell type-specific organelles called "lysosome-related organelles". This group includes melanosomes produced in melanocytes and retinal pigment epithelial cells, platelet-dense granules, lytic granules of cytotoxic T lymphocytes, MHC class II compartments of antigen-presenting cells, and neutrophil primary granules (Schraermeyer and Dohms, 1993; Lloyd et al., 1998; Mullins and Bonifacino, 2001). According with Dell'Angelica et al. (2000) "Lysosomes are membrane-bound cytoplasmic organelles involved in intracellular protein degradation. They contain an assortment of soluble acid-dependent hydrolases and a set of highly glycosylated integral membrane proteins. Most of the properties of lysosomes are shared with a group of cell type-specific compartments referred to as 'lysosome-related organelles'. In addition to lysosomal proteins, these organelles contain cell type-specific components that are responsible for their specialized functions". Lysosome-related organelles are cell type-specific modifications of the post-Golgi endomembrane system which have a variety of functions, but which all share some characteristics with lysosomes (Cutler, 2002). Due to the lysosomal character of ommochrome granules, it seems probable that the atypical granules found in the bleaching spider are autolytic profiles resulting from the breakdown of dense pigment granules. The Golgi apparatus often observed close to such granules could provide the necessary elements to start the transformation.

4.2. Identification of the genesis of the different states of granules during catabolism

The few studies of ommochrome degradation (Fahrenbach, 1969; Wachmann, 1969; Perrelet et al., 1971) describe large autophagic vacuoles into which the intact granules are degraded. Lhonoré et al. (1980) reported detailed ommochrome degradation in *Pieris brassicae* larvae, in the context of cellular lysis during a moulting cycle. Bouthier and Lhonoré (1984) observed a similar breakdown of ommochromes granules, related to integumental mitotic activity in locust. They did not observe a total breakdown, but the granules transformed into uric microcrystal inclusions.

The processes of granulolysis that we have described here in *M. vatia* seem to be rather different from those reported up to now. First, the breakdown develops in two stages: a granule autolysis, followed by autophagy for recycling the remained products. We postulate that the granule matrix of ommochromes bound to proteins is gradually disintegrated down to a clear vacuole. The vacuoles generated by this process would then be eventually degraded by means of autophagy. The term autophagy groups indeed a series of intracellular pathways that lead to the removal of cytosolic components in lysosomes (Baba et al., 1994; Dunn, 1990; Levine and Klionsky, 2004).

The process that leads to the autophagic vacuoles involves two steps. First, parts of the cell are sequestered by membranes of the endoplasmic reticulum. The formed organelle is an autophagosome. By definition, the autophagosome contains intact and recognizable parts of the cell: mitochondria, small vesicles, and debris of cytoplasm (Amenta and Brocher, 1981; Glaumann et al., 1981; Holtzman, 1989). As a consequence of the wrapping around portions of cytoplasm during the engulfment process, a multi-membrane structure is formed. In the second step of the autophagic process, the autophagosome membrane fuses with a lysosome, forming an autophagolysosome. The lysosomal enzymes are rapidly delivered into the organelle lumen where their contents are degraded. Finally the digested material is recycled (Lawrence and Brown, 1992; Shintani and Klionsky, 2004; Fader and Colombo, 2008). In the late bleaching spider, the vacuoles, which are built from the autolysis of pigment granules together with portions of cytoplasm, are sequestered by several concentric layers of RER. The structure resulting from this process is a typical autophagosome. The presence of lysosomes in their proximity suggests the second step of the autophagic process, during which the content of vacuoles is recycled. In the spider in an advanced state of bleaching, we found multilamellar RER structures, related to the genesis of progranules (Insausti and Casas, 2008), as well as the progranules themselves. The presence of these elements in the same cell with granules in different stages of degradation supports the hypothesis that the final result of the granule degradation is a new progranule.

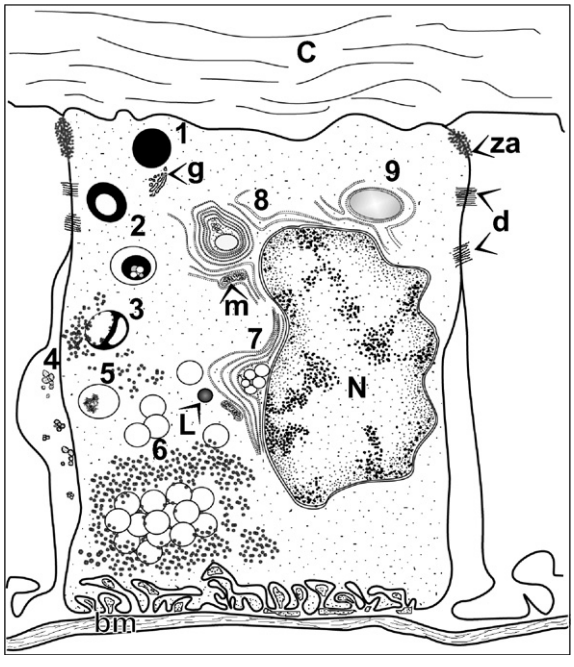

Fig. 5. Diagram of the epidermal cell summarizing the steps of ommochrome degradation and development of progranules as described in the text. 1, ommochrome granule. 2, granules in degradation process. 3, glycogen rosettes appear as consequence of granulolysis. 4, secretion vesicles appear in the intercellular spaces. 5, the granules become clear with central flocculent material. 6, electron-lucent vacuoles clustered together at the base of the cell. Abundant glycogen is present in this region. 7, the cisternae of RER are integrated into a whorl enclosing these vacuoles. 8, arrays of concentric cisternae of RER, closely associated with mitochondria, are scattered in the cell cytoplasm. 9, progranules *type I* appear in the distal zone of the epidermal cell. C, cuticle; bm, basal membrane; d, desmosome; g, Golgi region; L, lysosome; m, mitochondria; N, nucleus; za, zonulae adhaerens.

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4.3. Fate of the constituents of the granules

Ommochrome-binding proteins (OBPs) have been described in various insect tissues (Martel and Law, 1991; Yepiz-Plascencia et al., 1993; Sawada et al., 2000). Ommochromes are bound to proteins to form the matrix of the pigment granule (Langer, 1967; Ajami and Riddiford, 1971; Ishiguro and Nagamura, 1971; Linzen, 1974; Langer, 1975; Kayser, 1979). During the granulolysis process, the ommochrome and the proteins ought to be degraded. We presume that the degraded proteinic components of the granule matrix are eliminated from the cell to extracellular spaces, whereas the remaining fluorescent metabolites of ommochrome catabolism are recycled via autophagy in the same cell. Our observation that the vacuoles resulting from the granulolysis show auto-fluorescence under UV-light is characteristic for ommochrome derivates, in particular the indole group (Linzen, 1974). The fluorescence suggests that the products of ommochrome degradation in *M. vatia* cannot be the colored and non-fluorescent ommochromes rhodommatin or ommatin D, which are excreted by insects (Kayser, 1979, 1985), but they could be some of the fluorescent compounds found in the *in vitro* chemical reaction of these pigments (Butenandt et al., 1954a,b, 1958; Linzen, 1974). Biochemical analyses of the epidermal cell throughout the process of color change, as well as of the spider excreta, are necessary to corroborate the hypothesis proposed.

Associated to granule breakdown, large amounts of glycogen rosettes appear. A similar fact was reported during the moulting cycle in the epidermis of *Pieris brassicae* and *Locusta migratoria* larvae (Bouthier and Lhonoré, 1984; Lhonoré et al., 1980). However, these authors could not establish a direct relationship between the disappearance of acridiommatins and the appearance of glycogen rosettes. In *M. vatia*, the relationship between the granule degradation and the glycogen appearance is clear. The matrix of the granules becomes electron-lucent and glycogen rosettes appear in its place. Large amounts of glycogen surround vacuoles produced by the degradation of the granules. In insects, the glycogen accumulated in the epidermal cell has been interpreted as a store of energy necessary for the synthesis of a new cuticle (Jungreis, 1979). This is not the case in an adult spider. The glycogen accumulation can still be related to energy needs, but for other physiological process, such as the build up of new granules.

4.4. Evolutionary pressures for the turnover of the indole group

We proposed in our previous work that the transparency of the epidermal cell could explain the presence of ommochrome granules, in order to photoprotect against UV-damage (Insausti and Casas, 2008; Théry and Casas, 2009). *M. vatia* has indeed a transparent cuticle exposing epidermal cells to direct solar radiation. This

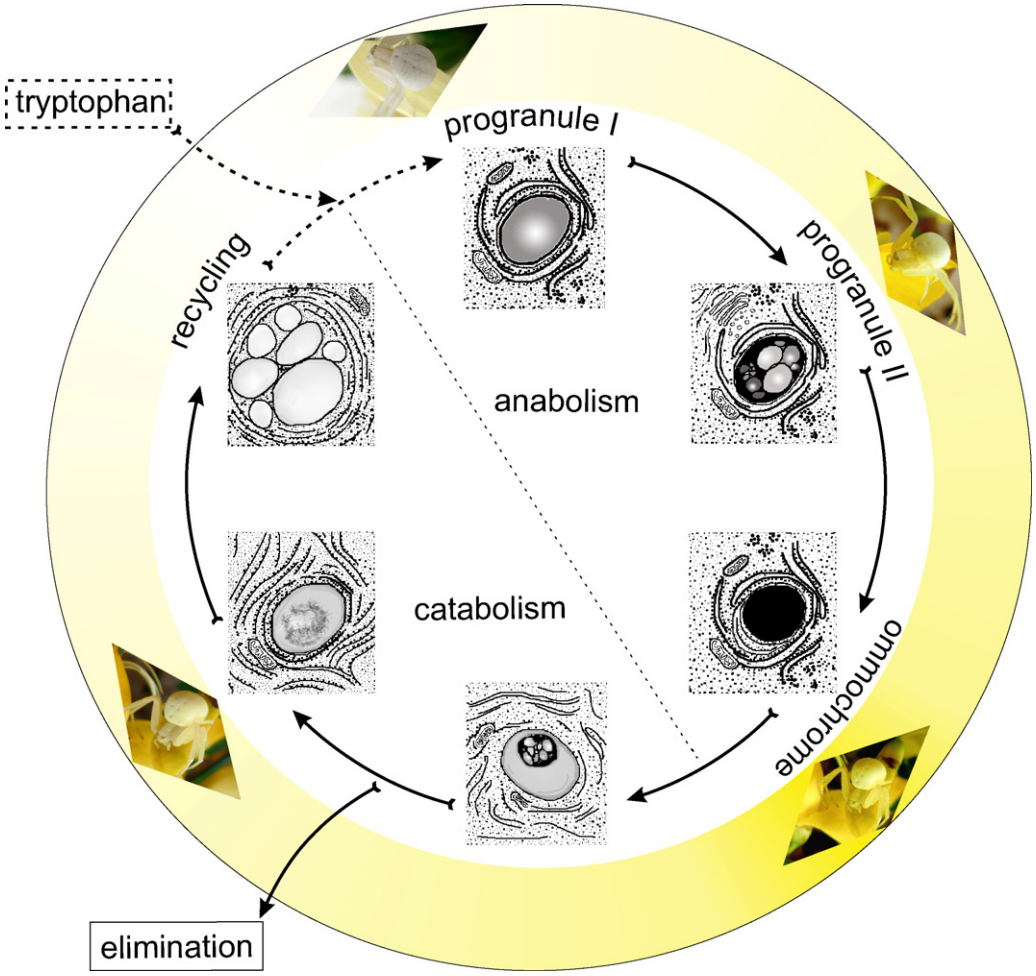

Fig. 6. Diagram summarizing the cyclic successive stages of the anabolism and catabolism of the ommochrome pigment granules in relationship to spider color change. The formation of ommochrome granules proceeds in three distinctive steps, starting with a white spider. Translucent progranules, present in white spiders only, are produced by a dense network of endoplasmic reticulum. The biochemical transformation of progranules into a transient state leads to the formation of ommochrome granules. In the process of reverting to white, the degradation of pigment granules begins with the granule autolysis. Some components are extruded in the extracellular spaces and others remain in electron-lucent vacuoles. The vacuolar compounds will be recycled via autophagy. At this time, external tryptophan might be incorporated. In the final stage of bleaching, ommochrome progranules appear again in the distal zone of the epidermal cell.

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transparency implies a need for protective means in the tissues situated beneath the cuticle. Ommochromes as well as their precursors might act as such (Dontsov, 1999; Dontsov et al., 1984; Ostrovsky et al., 1987; Sakina et al., 1987; Stavenga, 1989). The catabolism of ommochrome granules in bleaching spiders put then at risk of UV damage, as it leaves the cells unprotected. The epidermis of white spiders with transparent cuticle is however always full of granules containing ommochrome precursors, most likely kynurenine. The simultaneous anabolism of kynurenine progranules thus allows the epidermal cell to be continuously protected.

4.5. Synthesis: cyclic anabolism and catabolism within epidermal cells

Our combined analysis of color change and ultrastructural changes enables us to propose a pathway for the different stages of ommochrome granulolysis (Fig. 5):

1. Electron-dense ommochrome granules are presents in the epidermal cell of a yellow spider.
2. Different atypical granules appear in the early bleaching spider. They are autolytic profiles, products originating from the breakdown of dense pigment granules. Golgi regions are consistently associated to these granules.
3. The glycogen rosettes appear in the semicircular electron-lucent area of the granules during the degradation process and are evacuated to the cytoplasm.
4. Secretion vesicles appear in the intercellular spaces. The degrading granules are observed in connection with the secretion.
5. In the late bleaching spider, the granules become electron-lucent with central flocculent material.
6. The granules develop then into electron-lucent vacuoles, which cluster together at the base of the cell. Abundant glycogen is associated with these structures. At this time, parallel cisternae of RER loop in such way as to envelope the clusters of vacuoles.
7. The cisternae of RER are integrated into a whorl enclosing a vacuole (sometimes two). This structure has a typical autophagic profile. Lysosomes are present in their proximity.
8. Arrays of concentric cisternae of RER closely associated with mitochondria are scattered in the cell cytoplasm.
9. Progranules *type I* appear in the distal zone of the epidermal cell.

Based on the above scheme, we conclude that the bleaching epidermal cells degrade the ommochromes granules at the same time as they form new progranules. This means that the catabolism and anabolism of ommochromes take place simultaneously in a single epidermal cell (Fig. 6). While the ultrastructural evidence is strong, studies with radioactive tryptophan should be performed to confirm our results. Such simultaneous turnover of pigment granules within the same cell is an unrecorded physiological process in animals and begets the question of its generality. A similar process might actually occur for melanin. It is know that melanin degradation occurs (Garcia et al., 1983; Schraermeyer, 1993; Schraermeyer and Dohms, 1996; Sichel et al., 1997; Borovansky and Elleder, 2003) and in an order reverse of their manner of synthesis (Keefe, 1973). Sichel et al. (1997) have furthermore reported that the amphibian Kupffer cells are "melanosome producing cells" and that they are also able to "demolish" melanosomes by heterophagocytosis and autophagocytosis. Finally, the presence of premelanosome-like structures during melanin degradation is another indicator of within-cell melanin turnover (Schraermeyer, 1992, 1993). Thus, while pigment granule turnover within the same cell is still unproven for melanins, our work on ommochromes is yet another piece of information supporting the likelihood of such phenomenon.

Acknowledgements

We thank U. Schraermeyer, D.G. Stavenga and an anonymous referee for their comments on the manuscript.

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Please cite this article in press as: Insausti, T.C., Casas, J., Turnover of pigment granules: Cyclic catabolism and anabolism of ommochromes within epidermal cells. Tissue Cell (2009), doi:10.1016/j.tice.2009.05.002

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