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REVIEW ARTICLE

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Ciliate cryptobiosis: a microbial strategy against environmental starvation

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Abstract This review outlines the main features of ciliate resting-cyst formation or encystment. It represents a strategy against several environmental stresses (such as starvation), which involves a highly gene-regulated cell differentiation process and originates a more resistant, differentiated form or resting cyst. This process is mainly characterized by drastic cytoplasmic dehydration that induces a general metabolic rate decrease, intense autophagic activity, the formation of a permeable cyst wall protecting the cell against the adverse environmental conditions, and a gene-silencing mechanism after opening the specific encystment genes.

Keywords Ciliate encystment · Resting cysts · Dehydration · Cyst wall · Gene silencing

Introduction to ciliate cryptobiosis

The survival of living systems depends on their ability to sense alterations in the environment and to respond appropriately to new situations. Starvation is the most habitual stress which micro-organisms must face in any environment on Earth. Eukaryotic and prokaryotic micro-organisms have solved this crucial environmental situation by two different and exclusive strategies:

1. Mechanisms that allow micro-organisms to survive such periods in a metabolic hold-on situation. This involves new starvation-induced activities, such as the production of degradative enzymes, and morphological and physiological changes to cope with starvation conditions [49]. This strategy is characteristic of non-differentiating micro-organisms.
2. Mechanisms involving complex cell differentiation processes, which are highly gene-regulated and originate a more resistant, differentiated state to maintain viability during starvation.

In the first microbial strategy, low metabolic rates are maintained under stress conditions, with the option of rapidly regaining full metabolism and cell division as soon as nutrients are plentiful. In contrast, the second strategy consists of surviving by shutting off almost all metabolic activity. Ciliated protozoa present either of these types of strategies, depending on the ciliate species [6].

According to the definition supplied by Keilin in 1959 [25], the second microbial strategy is generally called cryptobiosis. The cryptobiotic process involves the formation of a resting form or cryptobiotic state, which may be identified by different names depending on the type of micro-organism, e.g., bacterial spores and cysts, conidia and spores of fungi, and resting cysts of protozoa.

In this review, we report the best studied aspects of the differentiation process that, in many species of ciliates, exists as the second strategy against environmental starvation. This facultative cell differentiation process, named the encystment–excystment (E-E) cycle, is linked to the growth–division (G-D) cycle of many soil and aquatic ciliates [19, 21]. The E-E cycle consists of two antagonist processes: encystment (cryptobiosis), which involves the formation of a resting cyst from the vegetative cell, and excystment, which involves the vegetative cell emergence from the mature resting cyst (Fig. 1). When starvation appears, ciliates with an E-E cycle open a specific genetic program that involves progressive and drastic morphological and physiological changes, concluding with the resting cyst formation. Therefore, it is a strategy by which ciliates escape temporally from nutritionally unfavorable local conditions via dormancy or cryptobiosis. Besides, ciliate resting cysts can also be relocated spatially by wind, water, living hosts, etc., to environments potentially

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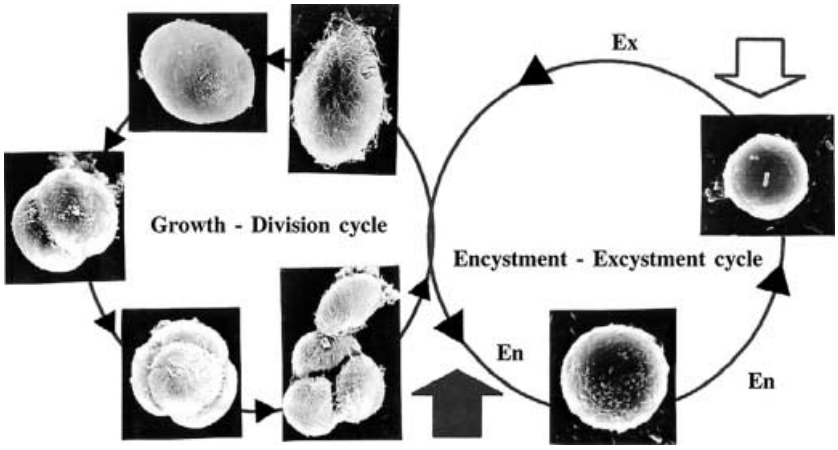

Fig. 1 Growth–division cycle and encystment (*En*)–excystment (*Ex*) cycle of the ciliate *Colpoda inflata*, elaborated using scanning electron micrographs from different phases of the life cycle of this ciliate. *Black arrow* Encystment induction, *white arrow* excystment induction

favorable for excystment and resumption of vegetative G-D cycle. As a result, ciliate resting cysts can be found in environmental samples from almost all regions of the Earth; and, as such, they represent a highly successful strategy for the survival and widespread dispersal of these eukaryotic micro-organisms.

Dehydration and autophagy: metabolic rate decrease

Starvation is the most universal exogenous inducer of ciliate encystment [21], but this cell-differentiation process can also be induced by desiccation of the medium. In fact, both inducers can be successively present in many ecosystems and ciliate resting cysts are also microbial desiccation-tolerant forms. Besides, strategies against environmental starvation and desiccation tolerance show very similar characteristics.

During ciliate encystment, one of the most remarkable changes is a drastic decrease in cellular volume. In some ciliates, this volume loss represents 60–80% and is a consequence of the loss of intracellular water [21, 42]. According to a comparative study on 14 different ciliate species [42], a relatively smaller standard deviation of the cyst diameters supports the possible existence of a certain cytoplasmic "quantum", not further reducible without severely affecting the survival potential of the cell. Starvation, like desiccation, induces cytoplasmic dehydration to form an anhydrobiotic cell (resting cyst), characterized by its singular deficiency in water. Some general consequences of drying cells are: (1) changes in volumes of the cell compartments (e.g., ciliate macronuclear volume decreases in proportion to the cell volume decrease) [21], (2) reduced fluidity (increasing cytoplasmic viscosity; which can be manifested in the different sedimentation rates of resting cysts and vegetative cells on a discontinuous density gradient of Percoll) [19], (3) changes in biophysical properties (e.g., loss of electrical excitability) [13], and (4) crowding of organelles and macromolecules (e.g. mitochondrial clusters, ribosomal crystal-like arrangements, nucleolar fusion, macronuclear chromatin condensation) [18, 21, 34].

In general, desiccation-tolerant cells accumulate large amounts of either one or both of the disaccharides, trehalose and sucrose [40]. A water-replacement hypothesis has been developed to explain how cellular components may be protected during extreme drying. Essentially, the hypothesis is that polyhydroxyl compounds, such as trehalose, replace the shell of water around macromolecules, circumventing any damaging effects during drying [12].

Photoprotective pigments are important in desiccation-tolerant organisms. Some ciliates show a characteristic pigmentation in the cryptobiotic state. Hypotrich ciliates grown on unicellular green algae as food usually form yellowish resting cysts. The pigment responsible for this coloration showed characteristics of α-carotene [16]. This cystic pigment may be obtained from the algae, which is the habitual food of these protozoa, and then its accumulation can occur in the encysting cell. Therefore, pigmentation of some ciliate resting cysts appears to be a protective mechanism against photooxidation, which increases in a desiccating medium.

Before cytoplasmic dehydration becomes an ametabolic state, a high autophagic activity is present during several encystment phases. This activity then gradually decreases and ceases in the last phases of encystment and in mature resting cysts. Autophagy is a universal strategy linked to starvation tolerance in any cell with or without cryptobiotic capacity. In cells with a non-differentiating starvation strategy, autophagy is the way to maintain a minimum metabolic rate. For instance, in ciliates such as *Paramecium* and *Tetrahymena thermophila* (both without an E-E cycle in their life cycles), starvation induces a high level of cellular autophagy (auto-cannibalism), which involves a very high decrease in cell volume [29]. Under starvation conditions, a real cannibalism process can exist in ciliate populations, with the biggest cells feeding on smaller ones.

Autophagic activity during ciliate encystment can degrade many cellular components such as ribosomes, mitochondria, membranes, cortical elements (cilia), and a part of the nuclear apparatus (both micronuclei and/or macronuclear extrusion bodies). This autophagic activity is evident by the abundance of autolysosomes and/or

autophagosomes in precystic cells, representing a highly controlled, degradative process which eliminates some cellular elements not required in the differentiated state (resting cyst). Thus, in the majority of ciliates, cortical structures (cilia) that are involved in mobility and/or uptake of food in the vegetative form are totally or partially destroyed; and only in a few ciliates are these structures maintained almost intact. At present, ciliate resting cysts are classified into three different groups according to the resorption level of cortical elements: kinetosome-resorbing, partially kinetosome-resorbing, and non-kinetosome-resorbing cysts [32, 50].

Ciliate encystment is clearly a RNA and protein synthesis-dependent process [21]. As in other differentiation processes induced by starvation, cyst formation probably depends completely upon endogenous materials, because an exogenous energy source is absent. These endogenous materials, originated by autophagy, are used extensively for synthesizing the new macromolecules directly or indirectly involved in cyst formation. Evidence for protein turnover, by pulse-labeling experiments using [^{35}S] methionine, has been reported during ciliate encystment [15]. This process involves amino acid recycling from vegetative proteins to cystic proteins. A protein turnover has also been described in other eukaryotic cells undergoing differentiation induced by starvation, e.g., sporulation in *Dictyostelium discoideum* [52] and *Saccharomyces cerevisiae* [26]. The newly synthesized proteins could be protected against proteolysis by different cell mechanisms, one of which might be glycosylation [3, 38].

In summary, autophagy and cytoplasmic dehydration play crucial roles in ciliate encystment (Fig. 2); and both are involved in obtaining metabolic inactivation (ametabolism), which is the main feature of any cryptobiotic state.

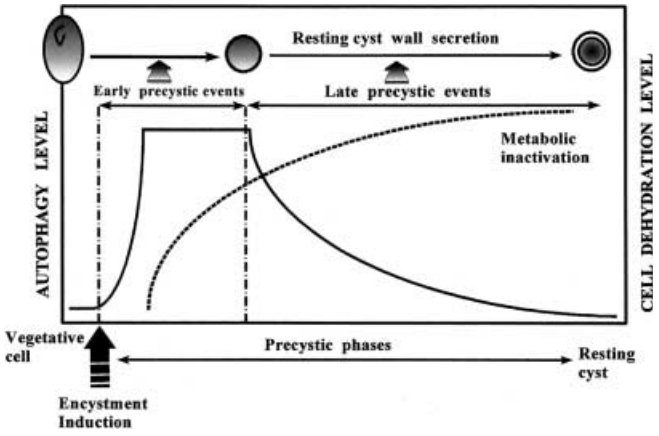

Fig. 2 A theoretical schematic representation of both autophagic and cell dehydration levels during ciliate encystment. After encystment induction (*black arrow*), the vegetative cell increases its lysosomal activity (data shown for in situ acid phosphatase enzymatic activity during *C. inflata* encystment), which coincides with the presence of a high number of cytoplasmic autophagosomes. This autophagic level is maintained during the early precystic events (about 24 h in *C. inflata*). Then, it gradually decreases during the late precystic events and becomes null in mature resting cysts. In contrast, the cytoplasmic dehydration level gradually increases during both early and late encystment phases, reaching its highest level during the resting-cyst phase. During early precystic events, specific encystment transcriptions and translations take place, among which the different cyst wall precursors are synthesized. Cyst wall secretion occurs after the precystic cell reaches a spherical form. This secretion process gradually continues during the further precystic phases, until the resting cyst is completed

Building a permeable barrier (the cyst wall)

Another universal feature of any cryptobiotic form is the presence of partially permeable barriers (walls, envelopes, cuticles, other rigid structures) protecting the cell against adverse environmental conditions. The essential characteristics of a protective extracellular biopolymer forming a wall in microbial cryptobiotic states are: (1) high water retention, probably with an ordered structure and intricate network of fibers, (2) a complex repeating structure that requires several enzymes for dissolution (during excystment or germination) and therefore presents a poor substrate for utilization by competitors, (3) toxicity to prevent grazing by other eukaryotic or prokaryotic cells, (4) absorption properties that provide scavenging of cations, metals, etc., (5) stringent regulation of induction, biosynthesis, and secretion of the polymer, and (6) an intimate contact with the cell surface, in order to provide molecular information coming from the extracellular medium. Ciliate resting-cyst walls show several of these ideal properties. Many authors have studied the fine structure of the different cyst wall layers, using both transmission and scanning electron microscopy. For an update review on ciliate cyst wall ultrastructure, number of cyst wall layers, and cyst wall precursors see the paper by Martín-González et al. [33].

One of the most important questions related with the ciliate cyst wall is to know how it is built. This process involves at least two phases: (1) the synthesis and intracellular transport of the cyst wall components and (2) the assembly of the extracellular cyst wall polymers. In the majority of ciliates, the different cyst layers originate from different cyst wall precursors or encystment-specific vesicles, which are formed de novo during the early phases of encystment [17]. These secretory vesicles release their contents only after the cell becomes spherical; and it is a regulated secretion mechanism (exocytosis) in a controlled pathway. In higher eukaryotes, proteins destined for secretion are folded, assembled, and glycosylated as they are transported from the endoplasmic reticulum (ER) through the Golgi complex to the cell surface. In ciliates, cytochemical evidence supports the idea that the origin of these cyst wall precursors is from the ER and/or Golgi complex [10]. Mucocysts or structures like-mucocysts have also been involved in the resting-cyst wall formation in some ciliates [36].

Both the kinetics of the appearance of cyst wall precursors in the precystic cytoplasm and the degree of cyst wall formation are the most significant encystment

markers. For this reason, they have been very useful tools in analyzing ciliate encystment kinetics [4, 17]. An immunocytochemical method using a polyclonal antiserum against isolated cyst walls from the ciliate *Colpoda inflata* [4] has been an excellent tool for identifying encystment phases and analyzing the encystment kinetics of this ciliate.

Little is yet known about the macromolecular composition of the different cyst wall layers of ciliates. In general, the main molecular ciliate cyst wall components are proteins, glycoproteins, and carbohydrates [21]. Among carbohydrates, the polysaccharide chitin has been detected in numerous ciliates [7], but at present no any other known polysaccharide has been reported as the main component of the ciliate cyst walls. Proteins are usually among the main components of ciliate resting-cyst walls. However, the study of these molecules has been incomplete and far from sufficient, which might be due to the insolubility of these cyst wall components making analysis difficult. The electrophoretic pattern analysis of these proteins from isolated and homogenized cyst walls has been carried out in very few ciliates [23, 35, 43]. As has been reported [23, 43], the average number of electrophoretic cyst wall protein bands depends on solubilization conditions, such as the addition of urea, sodium dodecyl sulfate, and β-mercaptoethanol. This probably indicates that both hydrogen bonds and disulfide bridges are usually present in ciliate cyst wall proteins. Several of these proteins are periodic acid Schiff (PAS)-positive, indicating the presence of glycol groups in them [23, 35, 43]. Glycoproteins represent about 66.6% of the total electrophoretic protein bands in *C. maupasi*, 50% in *C. inflata*, 31.5% in *C. cucullus*, and 42.8% in *Cyrtolophosis elongata* [23], whereas lower percentages are detected in hypotrichous ciliates: 12.5% PAS-positive bands in *Histriculus muscorum* [35] and 20% in *Paraurostyla* sp. [43]. The characterization of the carbohydrate moiety of cyst wall glycoproteins has been carried out in some ciliates, using specific lectins [9, 23]. In general, authors conclude that ciliate resting-cyst wall glycoproteins are principally N-glycans with a high mannose content, although some O-glycans may exist in some ciliate glycoproteins [9, 23]. The role of protein glycosylation during encystment has been studied in colpodid ciliates [3], using specific inhibitors acting at different levels in the glycosylation process. Results obtained by lectins were confirmed by those using inhibitors, such as tunicamycin, which blocks the formation of all N-linked, high mannose and complex-type oligosaccharides [38]. Tunicamycin inhibits the encystment process of *Colpoda inflata* [3], so colpodid encystment depends on N-linked glycoproteins. Likewise, in *C. inflata* [2], 1-deoxymanojirimycin (dMAN) did not block the encystment process, but some important alterations in the resting-cyst morphology have been reported. This inhibitor blocks the first steps in the processing of N-linked, high-mannose oligosaccharides to complex oligosaccharides (removal of four α-1,2-linked mannose residues). Thus, glycoproteins synthesized in the presence of dMAN carried only high-mannose oligosaccharides (which are abundant in colpodid cyst wall glycoproteins) and, therefore, it is evident that their transformation into complex oligosaccharides must be very important for the correct formation of the cyst wall.

Recently, we isolated a cDNA from mRNA stored in mature resting cysts of the ciliate *Oxytricha nova* (unpublished data), which encodes the lysosomal enzyme α-mannosidase. This glycosil hydrolase is involved in the catabolism of N-linked carbohydrates, catalyzing the hydrolysis of terminal, non-reducing α-D-mannose residues; and it can cleave all known types of α-mannosidic linkages. The presence of this mRNA in resting cysts might have a special meaning during ciliate excystment, because glycoproteins with a high content of mannose oligosaccharides are present in the cyst wall. Therefore, this enzymatic activity could be useful (by cleaving any type of cyst wall α-mannosidase linkage) to make it easy for the cell to emerge from the mature resting cyst.

At yet, no amino acid sequence of ciliate cyst wall polypeptides has been reported. However, some information on cyst wall protein amino acid composition from four ciliates is available [24, 43, 46]. Two glycoproteins isolated from the cyst wall protein pattern of two colpodid ciliates were analyzed for their amino acid composition [24]. Both glycoproteins are very rich in glycine and have a relatively high hydrophobicity, additionally containing many leucine and alanine residues. Their amino acid compositions are similar to those found in the hydrolyzed cyst wall total proteins from the ciliates *C. steinii* [46] and *Paraurostyla* sp. [43], in which glycine is also an abundant amino acid. It is rare to find proteins with a high content of glycine; and, among those which are glycine-rich, keratin (a cytoplasmic intermediate filament in vertebrate cells), collagens (the major proteins of the extracellular matrix), and elastin (another fiber-forming protein of the extracellular matrix) should be mentioned. Glycine-rich proteins have been found in the fruiting body stalk of the ciliate *Sorogena stoianovitchae* [5]. The mixospore coat of *Myxococcus xantus* [27] contains glycine, but it is not a part of the protein, which suggests that glycine might form bridges within polysaccharide polymer chains. This possibility has also been suggested in the cyst wall of *Paraurostyla* sp. [43].

The third most abundant amino acid in the two colpodid cyst wall glycoproteins is leucine [24] as it occurs in two cyst wall proteins of the intestinal parasitic protozoon *Giardia lamblia* [31].These proteins contain five tandem copies of a 24-residue, leucine-rich repeat, a motif implicated in protein–protein interactions.

The biosynthesis and assembly of eukaryotic extracellular superstructures such as plant and fungal cell walls and microbial cysts or spore walls are not completely understood. How ciliates generate a highly

ordered, supramolecular cyst wall is a very interesting problem that could be taken as a model for the study of wall morphogenesis in microbial cryptobiotic forms. Furthermore, ciliate cyst wall formation presents interesting topological problems, because precursors are synthesized intracellularly, but deposition of the components and all macromolecular organization must occur outside the cell [17]. Which molecules are present in the ciliate cyst wall? How do these molecules interact to give rise to the final cyst wall architecture? What is the function of the individual components in the resting-cyst wall? These are really important questions and their future solution may help to understand better the ciliate cryptobiotic state and its ecological significance.

Opening and silencing genes: modifications of encystment macronuclear chromatin

Every microbial cryptobiotic state is a direct result of the opening and closing of specific genes. Nuclear changes, macronuclear chromatin reorganization and DNA modifications during ciliate encystment have been recently revised in several update reviews [18, 20, 21]. Nuclear dualism is a general feature of ciliates; and changes in both types of nuclei have been reported during ciliate encystment. These changes include: macronuclear (Ma) chromatin condensation, Ma-DNA loss, changes in the Ma-DNA methylation pattern, nucleolar changes, and an extensive rearrangement of the Ma-chromatin. All of these modifications probably interfere with gene expression and are indicative of transcriptional inactivity, which is the main genetic characteristic of any cryptobiotic form [18].

Ciliates with several macronuclei in the vegetative state fuse during encystment, to form only one cystic macronuclear mass. This Ma fusion causes a drastic volume reduction and chromatin condensation. In those ciliates with only one Ma-mass in the vegetative form, a volume reduction and chromatin condensation also exists. How does Ma fusion occur?. At present, we know very little about how Ma fusion works. In both ciliate division and encystment, microtubules are present during Ma division; and they, via their insertions into the nuclear envelope and chromatin, could provide the motive force responsible for the chromatin condensation [51]. However, Ma-chromatin condensation can apparently take place in the absence of microtubules [48]. Therefore, other factors, such as cell dehydration, as well as microtubules may be involved in the encystment Ma fusion. Generally, an increase in the size of Ma-chromatin bodies is reported during ciliate encystment [18], which is due to the fusion of smaller chromatin bodies. This compaction is especially high in some colpodid resting cysts, such as *Bursaria truncatella* [44], *B. ovata* [45], and *C. inflata* [34]. In these species, crystal-like hexagonal chromatin structures (liquid crystal types) have been detected in the Ma-chromatin of mature resting cysts, after the application of chromatin-spreading procedures (Fig. 3). A spontaneous ordering of DNA into liquid crystal phases at high concentration has been hypothesized as an important mechanism in chromatin packaging [28]. Recently [53], a prokaryotic DNA protection by stress-induced (starvation) biocrystalization was reported in *Escherichia coli*. A cocrystalization of the DNA with the stress-induced protein Dps is considered in this study as a generic defense strategy against stress conditions.

We must also consider, as an additional factor involved in chromatin packing, the presence of specialized basic nuclear proteins. A study on the ciliate, *Gastrostyla steinii* [14], suggested that the high Ma-DNA condensation in resting cysts could be due to the presence of arginine-rich proteins (such as protamines), as in metazoan cells, where substitution of protamines for histones in spermatogenesis is correlated with an extremely dense chromatin packing and loss of capacity for RNA synthesis. Also, we recently isolated, from mature resting cysts of *O. nova*, a cDNA encoding a high mobility group (HMG)-like protein (unpublished data). HMG proteins are a family of relatively low molecular weight, non-histone components in chromatin. These basic, non-histone proteins have been referred to as architectural transcription factors, whose role in gene transcriptional regulation appears to be the recognition and modulation of both DNA and chromatin structure [41]. As architectural components of chromatin, the HMG proteins serve as structural elements that, by interacting with other nuclear proteins and certain types of DNA substrates, facilitate the formation of specific multicomponent complexes that perform a particular biological function [41]. In this sense, the presence of this protein (mRNA) in resting cysts could have a role in the Ma-chromatin condensation or as a future element involved in the opening of gene expression during ciliate excystment.

At present, Ma-DNA methylation pattern changes during encystment of *C. inflata* [20, 39] is the only Ma-DNA modification reported in encysting ciliates. In this study, authors obtained experimental evidence for Ma-DNA demethylation during encystment, supporting

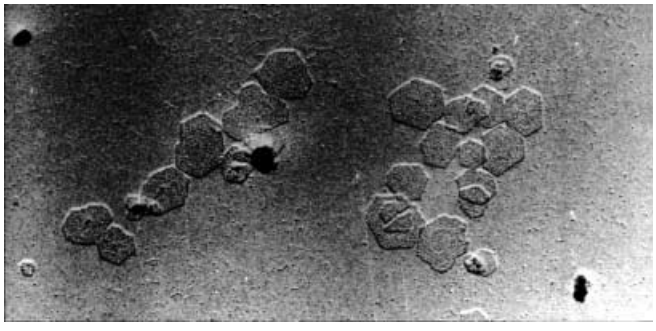

Fig. 3 Macronuclear chromatin isolated from the resting cyst of the ciliate, *C. inflata*, showing crystal-like hexagonal bodies (approx. 28,000×)

the idea that new gene expression is necessary to elaborate the resting state.

mRNA in mature resting cysts: saving for the future?

In 1997, for the first time in ciliates, we reported [2] the presence of accumulated mRNAs in the resting cysts of two ciliates (*C. inflata*, *O. nova*). The accumulation of specific mRNA molecules associated with the cryptobiotic process has been reported in both eukaryotic [11] and prokaryotic cells [1]. Several fungal species [30] produce dormant spores containing stored mRNA, which is translated upon germination. *D. discoideum* spores contain mRNA-specific species not found in the later stages of germination [11]. *S. cerevisiae* sporulation is accompanied by the accumulation of RNAs corresponding to at least 27 different genes [22]. Also, in *Acanthamoeba* [8], mRNA has been isolated from resting cysts that was capable of actin translation in vitro. This stored mRNA can be supposedly maintained in the metabolically inactive microbial cryptobiotic form for indefinite periods of time.

In the hypotrichous ciliate *Sterkiella histriomuscorum* [47], transcripts have also been isolated from resting cysts, confirming the presence of a mRNA pool in the dormant state of ciliates. Very recently (unpublished data), we found transcripts from both *C. inflata* and *O. nova* resting cysts encoding very diverse proteins, such as actin, α-tubulin, HS70 (a heat-shock protein), glyceraldehyde-3-phosphate dehydrogenase, and metallothioneins, among others.

A very important point to discuss is the meaning of stored mRNAs in microbial cryptobiotic states. At least two possible interpretations might be established:

1. They represent remaining molecules, which are encystment-specific; and therefore they are required during the encystment process. The high dehydration that precystic cytoplasm undergoes during encystment may play an important role in the accumulation of macromolecules. If this hypothesis were true, the resting cyst cytoplasm could be a good source for encystment transcripts.
2. They represent transcripts, biosynthesized during encystment, but only required during the excystment process of the cell. In *C. inflata*, excystment depends on both RNA and protein synthesis; and therefore both transcriptional and translational processes are involved in the emergence of vegetative cells from the resting cysts.

Our second interpretation is not completely invalidated by the transcriptional dependency of excystment. To elucidate between these hypotheses, a study on the expression kinetics of these stored mRNAs should be carried out during both encystment and excystment processes.

Concluding remarks

In this review, we have outlined the main features of ciliate encystment or resting cyst formation, which is a general strategy against several environmental stresses, such as starvation. In summary, a considerable reduction in cell volume and a high cytoplasmic dehydration, involving a general metabolic inactivation, the presence of a cyst wall (a permeable barrier allowing molecular communication with the environment), and gene-silencing are the main features of ciliate resting-cyst formation. In ciliates, many questions related to the encystment process are still without an appropriate solution; and we hope that future molecular, physiological, and ecological studies can be carried out to increase our currently incomplete knowledge on this exciting microbial, eukaryotic differentiation process. Besides, another very interesting point, related to microbial cryptobiotic forms, is to analyze their resistance to both extreme terrestrial and extraterrestrial environments. Bacterial spores are good candidates for interplanetary travel [37] and, among eukaryotic micro-organisms, fungal spores and ciliate resting-cysts could be potential living models to be considered in exobiology.

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References

1. Albertson NN, Nyström T, Kjelleberg S (1990) Functional mRNA half-lives in the marine *Vibrio* sp. S14 during starvation and recovery. J Gen Microbiol 136:2195–2199
2. Benitez L, Gutiérrez JC (1997) Encystment-specific mRNA is accumulated in the resting cysts of the ciliate *Colpoda inflata*. Biochem Mol Biol Int 41:1137–1141
3. Benitez L, Palacios G, Martín-González A, Gutiérrez JC (1992) Protein glycosylation in ciliated cryptobiosis. In: Wegmann L, Wegmann R (eds) Recent advances in cellular and molecular biology, vol 4. Peeters Press, Brussels, pp 357–364
4. Benitez L, Martín-González A, Gutiérrez JC (1994) Identification of precystic stages and encystment kinetics analysis of *Colpoda inflata* by using an immunofluorescent method. J Eukaryot Microbiol 41:387–391
5. Blanton RL, Warner SA, Olive LS (1983) The structure and composition of the stalk of the ciliated protozoan *Sorogena stoianovitchae*. J Protozool 30:617–624
6. Bradbury PC (1987) Protozoan adaptations for survival. In: Henis Y (ed) Survival and dormancy of microorganisms. Wiley, New York, pp 267–299
7. Bussers JC, Jeuniaux CH (1974) Recherche de la chitine dans les productions methaplasmatiques de ciliès. Eur J Protistol 10:43–46
8. Byers TJ, Kim BG, King LE, Hugo ER (1991) Molecular aspects of the cell cycle and encystment of *Acanthamoeba*. Rev Infect Dis 13:373–384
9. Calvo P, Miguel M de (1995/96) Study of *Histriculus cavicola* cyst wall using different lectins. Arch Protistenkd 146:329–339
10. Calvo P, Torres A, Perez-Silva J (1986) Ultrastructural and cytochemical study of the encystment in the hypotrichous ciliate *Histriculus similis*. Arch Protistenkd 132:201–211

11. Camonis J, Julien J, Ayala J, Jaequet M (1982) Polyadenylated RNA population present in dormant spores of *Dictyostelium discoideum*. Cell Differ 11:55–61
12. Clegg JS (1986) The physical properties and metabolic status of *Artemia* cysts at low water contents: the "water replacement hypothesis." In: Leopold AC (ed) Membranes, metabolism and dry organisms, 1st edn. Cornell University Press, Ithaca, pp 169–187
13. Deitmer JW (1987) Loss of electrical excitability during encystment of the hypotrichous ciliate *Stylonychia mytilus*. Naturwissenschaften 74:140–141
14. Gutiérrez JC (1985) Quantitative cytochemical study of chromatin and histones on isolated macronuclear masses from the resting cysts of *Gastrostyla steinii*. Microbios 43:43–51
15. Gutiérrez JC, Martín-González A (1990) Evidence for protein degradation and synthesis during encystment of the ciliate *Colpoda inflata*. Microbios Lett 43:57–63
16. Gutiérrez JC, Serrano A, Parra F (1982) Spectrophotometric identification of a carotenoid pigment in the resting cysts of a hypotrichous ciliate, *Laurentiella acuminata*. Acta Protozool 21:89–94
17. Gutiérrez JC, Torres A, Pérez-Silva J (1983) Structure of the cyst wall precursors and kinetics of their appearance during the encystment of *Laurentiella acuminata* (Hypotrichida, Oxytrichidae). J Protozool 30:226–233
18. Gutiérrez JC, Martín-González A, Callejas S (1998) Nuclear changes, macronuclear chromatin reorganization and DNA modifications during ciliate encystment. Eur J Protistol 34:97–103
19. Gutiérrez JC, Martín-González A, Matsusaka T (1990) Towards a generalized model of encystment (cryptobiosis) in ciliates: a review and a hypothesis. BioSystems 24:17–24
20. Gutiérrez JC, Callejas S, Borniquel S, Martín-González A (2000) DNA methylation in ciliates: implications in differentiation processes. Int Microbiol 3:139–146
21. Gutiérrez JC, Izquierdo A, Martín-González A, Callejas S (1998b) Cryptobiosis of colpodid ciliates: a microbial eukaryotic cell differentiation model. In: Pandalai SG (ed) Recent research and development in microbiology, vol 2. Research Signpost, Trivandrum, pp 1–15
22. Holaway BL, Lehman DJ, Primerano DA, Magee PT, Clancy MJ (1985) Sporulation-regulated genes of *Saccharomyces cerevisiae*. Curr Genet 10:163–169
23. Izquierdo A, Martín-González A, Díaz S, Gutiérrez JC (1999) Resting cyst wall proteins and glycoproteins of four colpodid ciliates: a comparative analysis. Microbios 99:27–43
24. Izquierdo A, Martín-González A, Gutiérrez JC (2000) Resting cyst wall glycoproteins isolated from two colpodid ciliates are glycine-rich proteins. Cell Biol Int 24:115–119
25. Keilin D (1959) The problem of anabiosis or latent life: history and current concept. Proc R Soc Lond B 150:149–191
26. Klar AJS, Halvorson HD (1975) Proteinase activities of *Saccharomyces cerevisiae* during sporulation. J Bacteriol 124:680–685
27. Kottel RH, Bacon T, Clutter D, White D (1975) Coats from *Myxococcus xanthus*: characterization and synthesis during myxospore differentiation. J Bacteriol 124:550–557
28. Leforestier A, Livolant F (1994) DNA liquid crystalline blue phases electron microscopy evidence and biological implications. Liq Cryst 17:651–658
29. Levy MR, Elliott AM (1968) Biochemical and ultrastructural changes in *Tetrahymena pyriformis* during starvation. J Protozool 15:208–222
30. Linz JE, Orlowski M (1982) Stored mRNA in sporangiospores of the fungus *Mucor racemosus*. J Bacteriol 150:1138–1144
31. Lujan HD, Mowatt MR, Conrad TJ, Bowers B, Nash TE (1995) Identification of a novel *Giardia lamblia* cyst wall protein with leucine-rich repeats. J Biol Chem 270:29307–29313
32. Martín-González A, Benítez L, Gutiérrez JC (1992) Ultrastructural analysis of resting cysts and encystment in *Colpoda inflata*: II. Encystment process and a review of ciliate resting cyst classification. Cytobios 72:93–106
33. Martín-González A, Palacios G, Gutiérrez JC (1994) Cyst wall precursors of *Colpoda inflata*: a comparative ultrastructural study and a review of the ciliate cyst wall precursors. Cytobios 77:215–223
34. Martín-González A, Palacios G, Gutiérrez JC (2001) Macronuclear chromatin changes during encystment in the ciliate *Colpoda inflata*: formation of crystal-like structures in the resting cyst chromatin and nucleolar condensation. Eur J Protistol 37:121–136
35. Matsusaka T, Hongo F (1984) Cytochemical and electrophoretic studies on the cyst wall of a ciliate *Histriculus muscorum* Kahl. J Protozool 31:471–475
36. McArdle EW, Bergquist BL, Ehret CF (1980) Structural changes in *Tetrahymena rostrata* during induced encystment. J Protozool 27:388–397
37. Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 64:548–572
38. Olden K, Bernard BA, Humphries MJ, Yeo TK, Yeo KT, White SL, Newton SA, Bauer HC, Parent JB (1985) Function of glycoproteins and glycans. Trends Biochem Sci 2:78–82
39. Palacios G, Martín-González A, Gutiérrez JC (1994) Macronuclear DNA demethylation is involved in the encystment process of the ciliate *Colpoda inflata*. Cell Biol Int 18:223–227
40. Potts M (1994) Desiccation tolerance of prokaryotes. Microbiol Rev 58:755–805
41. Reeves R (2000) Structure and function of the HMG (Y) family of transcription factors. Environ Health Perspect 108:803–809
42. Ricci N, Verni F, Rosati G (1985) The cyst of *Oxytricha bifaria* (Ciliata, Hypotrichida) I. Morphology and significance. Trans Am Microsc Soc 104:70–78
43. Rios RM, Sarmiento R, Torres A, Fedriani C (1989) Solubilization and electrophoretic studies of cyst wall proteins of a hypotrichous ciliate. Biol Cell 67:271–279
44. Sergejeva GI, Bobyleva NN (1988) The formation of crystal-like structures in somatic nucleus chromatin of *Bursaria* (Ciliophora, Protozoa) upon their preparation for a long-term resting stage (in Russian with English summary). Tsitologiya. 30:1291–1299
45. Sergejeva GI, Bobyleva NN (1995) Polynemic structures in the differentiated macronucleus of the ciliate *Bursaria ovata* Beers 1952. Acta Protozool 35:115–124
46. Tibbs J (1982) Covalent binding of protein in polyamine in the cyst coat of the protozoan *Colpoda steinii*. Eur J Biochem 122:535–539
47. Tourancheau AB, Morin L, Yang T, Peraso R (1999) Messenger RNA in dormant cells of *Sterkiella histriomuscorum* (Oxytrichidae): identification of putative regulatory gene transcripts. Protist 150:137–147
48. Verni F, Rosati G, Ricci N (1984) The cyst of *Oxytricha bifaria* (Ciliata, Hypotrichida) II. The ultrastructure. Eur J Protistol 20:87–95
49. Wai SN, Mizunoe Y, Yoshida S (1999) How *Vibrio cholerae* survives during starvation. FEMS Microbiol Lett 180:123–131
50. Walker GK, Maugel TK (1980) Encystment and excystment in hypotrich ciliates. *Diophrys scutum* and remarks on comparative features. Eur J Protistol 16:525–531
51. Walker GK, Maugel TK, Goode D (1980) Encystment and excystment in hypotrich ciliates. I *Gastrostyla steinii*. Eur J Protistol 16:511–524
52. Watts DJ (1984) Protein synthesis during development and differentiation in the cellular slime mould *Dictyostelium discoideum*. Biochem J 220:1–14
53. Wolf SG, Frenkiel D, Arad T, Finkel SE, Kolter R, Minsky A (1999) DNA protection by stress-induced biocrystallization. Nature 400:83–85