J. Eukaryot. Microbiol., 55(5), 2008 pp. 365–373

DOI: 10.1111/j.1550-7408.2008.00335.x

Action Mechanisms of the Secondary Metabolite Euplotin C: Signaling and Functional Role in *Euplotes*

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ABSTRACT. Among secondary metabolites, the acetylated hemiacetal sesquiterpene euplotin C has been isolated from the marine, ciliated protist *Euplotes crassus*, and provides an effective mechanism for reducing populations of potential competitors through its cytotoxic properties. However, intracellular signaling mechanisms and their functional correlates mediating the ecological role of euplotin C are largely unknown. We report here that, in *E. vannus* (an *Euplotes* morphospecies that does not produce euplotin C and shares with *E. crasssus* the same interstitial habitat), euplotin C rapidly increases the intracellular concentration of both Ca^{2+} and Na^{+}, suggesting a generalized effect of this metabolite on cation transport systems. In addition, euplotin C does not induce oxidative stress, but modulates the electrical properties of *E. vannus* through an increase of the amplitude of graded action potentials. These events parallel the disassembling of the ciliary structures, the inhibition of cell motility, the occurrence of aberrant cytoplasmic vacuoles, and the rapid inhibition of phagocytic activity. Euplotin C also increases lysosomal pH and decreases lysosomal membrane stability of *E. vannus*. These results suggest that euplotin C exerts a marked disruption of those homeostatic mechanisms whose efficiency represents the essential prerequisite to face the challenges of the interstitial environment.

Key Words. Action potentials, cation homeostasis, ciliated protists, lysosomes, marine microorganisms, oxidative stress, phagocytosis, sesquiterpenoids.

EUKARYOTIC, marine microorganisms produce unique secondary metabolites as adaptative tools to survive and defend against environmental challenges. Some of these compounds are known to function as intracellular signal transducers and regulators of complex processes (Blunt et al. 2007; Pietra 1997; Zhang et al. 2005). The sesquiterpenoids euplotin A, B, and C and their putative biogenetic precursor preuplotin are a class of lipophilic metabolites uniquely produced by the strains characterizing the cosmopolitan, marine, interstitial, ciliate morphospecies *Euplotes crassus* (Dini et al. 1993; Guella et al. 1994). The structure of euplotin C has been elucidated at the absolute configuration level (Guella, Dini, and Pietra 1996; Guella et al. 1994, 2004). Euplotin C represents the most abundant secondary metabolite along the biosynthetic pathway leading to terpenoids in *E. crassus*, and it is the most powerful agent among the other related products. In particular, euplotin C mediates its cytotoxic effects in non-producer *Euplotes* strains, including *E. vannus*, by altering the cell cycle, ciliary motility, and cell shape, and thus allowing *E. crassus* to broaden its niche size (Dini et al. 1993; Guella et al. 1994, Guella, Dini, and Pietra 1996; Savoia et al. 2004). However, intracellular signaling mechanisms mediating the ecological role of euplotin C are largely unknown. In recent years, the spectrum of cytotoxic actions of euplotin C has been broadened to microorganisms other than ciliates, including parasitic protists, yeasts, and some bacterial strains (Savoia et al. 2004). In addition, signaling mechanisms (i.e. Ca^{2+}) coupled to the cytotoxic actions of euplotin C have been elucidated in neuroendocrine tumoural cells of mammals (Cervia et al. 2006, 2007). A similar scenario may also occur in the marine ecosystem because intracellular systems in bacteria, unicellular eukaryotes, and metazoa share common features (Christensen et al. 1998).

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In ciliated protists, the importance of Ca^{2+} as an intracellular mediator has been well established. Indeed, Ca^{2+} plays a role in the regulation of many processes, such as exocytosis/endocytosis (phagocytic activity), chemosensory responses, ciliary motility/regeneration, cell contraction, and nuclear migration/mitotic division (Plattner and Klauke 2001). Ciliated protists are excitable cells displaying Ca^{2+}-dependent membrane action potentials (Machemer 1995; Plattner and Klauke 2001). A correlation between Ca^{2+} and Na^{+} homeostasis has been demonstrated in *Euplotes* (Burlando et al. 1999; Kruppel 1993; Kruppel and Lueken 1990). The notion that, in higher eukaryotes, Ca^{2+} mediates cell damage has been well established, as well as its cross-talk with different pathways regulating oxidative stress (Camello-Almaraz et al. 2006; Orrenius, Zhivotovsky, and Nicotera 2003). In this respect, imbalance in the steady-state of reactive oxygen species (ROS), mostly formed as side products of oxidative phosphorylation in mitochondria, are deleterious and even lethal (Ryter et al. 2007). Interaction between Ca^{2+} and ROS also occurs in protists (Ridgley, Xiong, and Ruben 1999). At the functional level, the stability of lysosomes, the cell organelles which are involved in compartmentalization and degradation of nutrients, particulate material, and xenobiotics ingested during phagocytosis, may be affected by different signals, including Ca^{2+} and ROS (Andrews 2002; Dondero et al. 2006; Plattner and Kissmehl 2003; Plattner and Klauke 2001; Roussi et al. 2007; Zhao, Wang, and Zhang 2005). In protists, including *Euplotes*, different compounds with ecological activity, including many toxic substances, appear to influence lysosome homeostasis which is, therefore, used as a valuable indicator of cellular damage and of compromised biotic integrity (Dondero et al. 2006; Trielli et al. 2007).

In the present study, we investigated intracellular signaling mechanisms and their functional correlates mediating the ecological role of euplotin C in *E. vannus* as a representative of a sample of marine, interstitial ciliate diversity. Among *Euplotes* morphospecies, which do not produce euplotin C, *E. vannus* is one of the most sensitive to euplotin C (Guella et al. 1994, Guella, Dini, and