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Review

Molecular cold-adaptation of protein function and gene regulation: The case for comparative genomic analyses in marine ciliated protozoa

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ABSTRACT

Euplotes focardii is a marine ciliated protozoan discovered in the Ross Sea near Terra Nova Bay, Antarctica. This organism is strictly psychrophilic, survives and reproduces optimally at 4–5 °C, and has a genome rich in A/T base pairs. Like other ciliated protozoans, *Euplotes* spp. are characterized by nuclear dimorphism: 1) the germline micronucleus contains the entire genome as large chromosomes; and 2) the somatic macronucleus (~50 megabases, or 5% of the micronuclear genome) contains small linear DNA nanochromosomes [1–12 kilobases], each of which constitutes a single genetic unit. These characteristics make *E. focardii* an ideal model for genome-level analysis to understand the evolutionary mechanisms that determine the adaptation of organisms to cold environments. Here we describe two examples that are controlled by phylogenetically appropriate comparison with mesophilic and psychrotolerant *Euplotes* species: 1) the genes and encoded proteins of the *E. focardii* tubulin superfamily, including α-, β-, and γ-tubulins; and 2) the genes of the heat-shock protein (Hsp) 70 family. The tubulins provide particular insight into protein-level structural changes that are likely to facilitate microtubule nucleation and polymerization in an energy poor environment. By contrast, the *hsp70* genes of *E. focardii* and of its psychrotolerant relative *E. nobilii* reveal adaptive alterations in the regulation of gene expression in the cold. The unique characteristics of the *E. focardii* genome and the results that we present here argue strongly for a concerted effort to characterize the relatively low complexity macronuclear genome of this psychrophilic organism.

Contents

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1. Introduction

1.1. Temperature as a driver of evolution

Temperature is one of the principal environmental variables that drive adaptive evolution at multiple biological levels. Small thermal changes cause profound alterations to many essential molecular and cellular phenomena, such as energy metabolism, protein stability and transport, mitosis and cytokinesis, assembly of macromolecular complexes, membrane fluidity, and secretory processes (Hochachka and Somero, 2002; Feller and Gerday, 2003). Polar marine ectotherms, most of which derive from temperate ancestors, have evolved compensatory restructuring of many of their biomolecular systems to preserve appropriate biological activity at low temperatures. The molecular bases of such restructuring remain poorly understood, due to the limitations of traditional "single-target" approaches that have largely focused on "adaptive changes" to individual enzymes/proteins [lactate dehydrogenase; (Fields and Somero, 1998; Johns and Somero, 2004; Somero, 2004), cytoskeletal proteins (Swezey and Somero 1982, Detrich et al., 1989; 1992; 2000)], or cellular organelles (Romisch et al., 2003)]. Rather less is known regarding temperature compensation of gene transcription and protein translation. Parker and Detrich (1998) reported the duplication of tubulin genes in the Antarctic yellowbelly rockcod, *Notothenia coriiceps*, which suggested that transcription at low temperature is facilitated by provision of additional templates for RNA polymerase. Chen et al. (2008) reported a large-scale transcriptional up-regulation of 177 gene families in the Antarctic toothfish, *Dissostichus mawsoni*, with respect to the orthologous families of temperate and tropical fish, much of which appears to be due to extensive gene duplication. Thus, gene family expansion may contribute to cold adaptation of cellular and physiological function. Smith and Haschemeyer (1980) have reported that Antarctic notothenioid fishes exhibit rates of polypeptide chain elongation at cold temperature that substantially exceed values inferred from temperate fishes. In fact, compensatory maintenance of gene expression at low temperature probably acts at a multitude of levels, including modification of transcription factors, of promoter and enhancer elements, and of initiation as well as elongation of protein synthesis. A substantial body of literature on plant adaptation and acclimatization indicates that both cis-acting gene regulatory motifs and their cognate transcription factors are involved in evolutionary accommodation to environmental stressors, including temperature (Sharma et al., 2005; Zhu et al., 2007).

The optimal strategy to identify molecular adaptations in psychrophilic (cold-loving) organisms should be based on a phylogenetically controlled, genome-scale comparison of protein and gene regulatory sequences from cold-adapted, mesophilic, and psychrotolerant species. These approaches are absolutely critical to obtaining: 1) a sufficient overview and identification of the *molecular features* related to *protein* thermal adaptation; 2) the identification of new genes and regulatory elements for more in-depth molecular analyses; and 3) sufficient sample sizes of nucleotide and amino acid sequence that can be evaluated via statistical and other computational approaches (e.g., three-dimensional protein homology modeling). Such data sets can only be effectively derived by genome sequencing analyses. In this context, the protozoan genus *Euplotes* provides several ideal model species for analysis, including the Antarctic psychrophile *E. focardii* [optimal growth at 4–5 °C (Valbonesi and Luporini, 1993)] and the mesophilic species *E. aediculatus, E. crassus,* and *E. otocarinatus*. We have undertaken a pilot *Euplotes* genome project to evaluate the efficacy of this approach.

Pending the outcome of our pilot program, we focus here on two gene families that illustrate the potential of genomic analysis of thermal adaptation in *E. focardii*. Our comparison of the amino acid sequences of the α-, β-, and γ-tubulins of *E. focardii*, derived from the corresponding genes, with those of orthologous tubulins from mesophilic *Euplotes* has enabled us to develop hypotheses regarding the molecular mechanisms responsible for efficient protein function (i.e., nucleation and polymerization of microtubules) at low temperature. Our studies of the *hsp70* gene family, in contrast, have generated insights into gene regulation at cold temperature.

1.2. What is Euplotes focardii?

E. focardii is a hypotrichous ciliated protozoan that was first isolated at Terra Nova Bay, Antarctica. Among *Euplotes* species, *E. focardii* has attracted special interest because its adaptation to cold appears to have evolved much earlier than for the psycrhotolerant Antarctic species *E. nobilii* (La Terza et al., 2001; Pucciarelli et al., 2003). Directly exposed to a constantly cold marine environment (−1.8 to +1 °C), *E. focardii* grows and reproduces optimally at 4–5 °C and is a strict stenotherm that cannot survive above 10 °C (Valbonesi and Luporini, 1993; La Terza et al., 2001). Thus, *E. focardii* is well suited as a model for genomic analysis of the evolutionary mechanisms that adapt organisms to cold environments.

E. focardii and all hypotrichs possess two nuclei: 1) the germline micronucleus (MIC) contains the entire genome in the form of large chromosomes; and 2) the somatic (working) macronucleus (MAC) contains small linear DNA molecules [nanochromosomes of 1–12 kilobases (kb)], each of which constitutes a single genetic unit flanked by regulatory regions and capped by telomeres (Klobutcher and Prescott, 1986). The MAC nanochromosomes, which represent only 5% of the sequence complexity of the MIC genome, are formed by fragmentation of the MIC chromosomes and subsequent amplification of retained sequences. Furthermore, MAC nanochromosomes contain almost no repetitive elements, a feature of considerable value in genome sequencing projects (Doak et al., 2003). Thus, the genomes of *E. focardii* and congeneric species are enriched in protein-coding and gene regulatory sequences, depauperate in repetitive elements, and of manageable size (~50 megabases). These characteristics make *E. focardii* and other species of the genus *Euplotes* ideal models for genome analysis. The comprehensive sequencing of MAC nanochromosomes from *Euplotes* species will yield thousands of homologous protein-coding and gene-regulatory sequences that can be systematically mined for molecular adaptations to cold temperatures.

2. Tubulins of the Antarctic ciliate *Euplotes focardii*: insights into cold adaptation of microtubule nucleation and polymerization

Microtubules are of fundamental importance in many eukaryotic cellular processes, including cell motility, maintenance of cytoskeletal architecture, intracellular transport, and mitosis (Hyams and Lloyd, 1993). Based on our extensive knowledge of cytoplasmic microtubule proteins from mesophilic vertebrates and invertebrates, one might naively predict that the psychrophilic eukaryotes of the Antarctic and other polar regions cannot form microtubules at temperatures near 0 °C, but of course they do. Our research on the tubulin gene family of *E. focardii* has provided important insights into cold adaptation of microtubule assembly.

The microtubules of metazoan cells polymerize from αβ-tubulin heterodimers, and nucleation of these polymers requires microtubule-organizing centers (MTOCs). MTOCs (centrosomes, basal bodies, etc.) contain a third member of the tubulin superfamily, γ-tubulin (Oakley and Oakley, 1989; Oakley et al., 1990; Sunkel et al., 1995), whose activity is required for their formation, maintenance (Dammermann et al., 2004; Ruiz et al., 1999; Shang et al., 2002), and capacity to nucleate microtubules (Moritz and Agard, 2001). One of our long-term goals has been to determine the evolved molecular features of *E. focardii* tubulins that facilitate microtubule nucleation and elongation in the cold.

Microtubule elongation is entropically driven, predominantly via hydrophobic interactions, and therefore is sensitive to environmental