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Dual-histidine kinases in basidiomycete fungi

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ABSTRACT

Dual-histidine kinases (HKs) are complex hybrid HKs containing in a single polypeptide two HK transmitter modules (T) and two-response regulator received domains (R) that are combined in a TRTR geometry. In fungi, this protein family is limited to some particular species of the phylum Basidiomycota and absent in the other phyla. This study extends the investigation of dual-HKs to 80 fully sequenced genomes of basidiomycetes, analyzing their distribution, domain architecture and phylogenetic relationships. Moreover, similarly to dual-HKs of basidiomycetes, several species of bacteria were found that contain hybrid HKs with a TRTR domain architecture encoded in a single gene.

1. Introduction

Two-component systems (TCS) are signal transduction mechanisms based on phosphotransfer reactions between histidine and aspartate residues that are used by living cells to sense and respond to extracellular or intracellular processes [1,2]. TCSs are prevalent in prokaryotes, and these signaling cascades are also present in eukaryotes, such as fungi and plants. The prototypical prokaryotic TCS contains a simple phosphotransfer mechanism composed of two signaling proteins, the transmembrane sensor histidine kinase (HK) and the effector response regulator (RR) proteins [3,4]. The modular nature of TCS proteins has originated a wide range of sensory and signaling domain architectures in prokaryotes [1,2,5].

In a typical prokaryotic TCS system, the sensor HK protein generally contains a functional transmitter module (T) characterized by two structurally conserved domains: a dimerization/histidine phosphotransfer domain (P) that typically is a HisKA, and an adjacent HATPase domain [5]. The HATPase domain binds ATP and catalyzes the autophosphorylation of the conserved His residue found within the dimerization/histidine phosphotransfer domain in response to a perceived signal. The phosphoryl groups are subsequently transferred to the Asp residue in the receiver domain (R) of the RR protein. On the other hand, classical hybrid HKs carry both the transmitter module (T) and the receiver domain (R) within a single polypeptide, and are involved in consecutive phosphorylations between the conserved His and Asp residues, known as phosphorelay TCS [6,7].

Fungal TCS signaling cascades generally are more complex with a multi-step His–Asp phosphorelay composed of 3 functional modules: a hybrid HK, a histidine-containing phosphotransfer (HPt) protein, and a RR protein [8,9]. Fungal TCS proteins are components of complex signal transduction pathways with essential roles in the regulation of several cellular functions and responses [10].

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Basidiomycetes are a large and diverse fungal phylum that comprises 3 subphyla: *Agaricomycotina*, *Pucciniomycotina* and *Ustilaginomycotina* [11–13]. Studies of the TCS pathways in the human pathogen *Cryptococcus neoformans* have identified the unusual domain architecture of Tco2 and Tco4 hybrid HKs containing 2 HK and 2 RR domains [8,14]. The function of *C. neoformans* Tco4 remains unclear, and Tco2 is responsible for drug sensitivity and osmotic and oxidative stress and has a redundant role with Tco1 in activating the high osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) pathway [8,14]. In previous comparative genomics studies of basidiomycetes, we have shown that the presence of Tco2 and Tco4 orthologs is limited to some particular species of basidiomycetes and they are absent in the other fungal phyla [15,16]. Considering the domain structure of this protein family of hybrid HKs (2 HK and 2 RR domains), we named them dual-HKs. In the present manuscript, we extend the analysis of dual-HKs to 80 fully sequenced genomes of basidiomycetes, and we show that the atypical domain architecture of dual-HKs is similar to that of some other bacterial hybrid HKs.

2. Materials and methods

2.1. Sequence data

Proteomes encoded in 80 fully sequenced genomes of basidiomycetes were obtained from MycoCosm (as of June 2013) (http://jgi.doe.gov/fungi) [17]. For consistency, we used in our analysis the species names as provided by the database source.

2.2. Identification and analysis of dual-HKs

Genes coding for dual-HKs in the complete genome sequences of basidiomycetes were identified using the pipeline web server BASID2CS (http://bioinformatics.unavarra.es:1000/B2CS/BASID2CS.htm) [16]. Recently, we have developed this bioinformatics platform for the identification, classification and functional annotation of putative TCS proteins from any genome of basidiomycetes. A Hidden Markov Model (HMM) targeting dual-HKs was used, and hits with an E-value below a defined cutoff (10^{-20}) were extracted. In addition, genome sequences were also analyzed using TBLASTN [18]. Functional domains of dual-HKs were identified by searching the Conserved Domain Database (CDD) with Reversed Position Specific BLAST [19] or the PROSITE database with the ScanProsite tool [20]. Multiple-sequence alignments were performed using MUSCLE [21] or Clustal Omega [22]. Prediction of transmembrane spanning regions was carried out using TMHMM v 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) [23].

2.3. Phylogenetic analysis

Phylogenetic analysis was performed on whole-protein sequences using the Phylogeny.fr platform (http://www.phylogeny.fr) [24] and comprised the following steps. A multiple-sequence alignment of dual-HK protein sequences was generated using MUSCLE v3.7 [21] configured for highest accuracy (MUSCLE with default settings). After alignment, ambiguous regions (i.e. containing gaps and/or poorly aligned) were removed with Gblocks v0.91b [25] using the default settings and a minimum length of a block after gap cleaning of 10. A phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program v3.0 aLRT [26,27] using the default settings. Reliability for internal branch was assessed using the bootstrapping method with 100 bootstrap replicates. Graphical representation and edition of the phylogenetic tree were performed with TreeDyn v198.3 [28].

3. Results

3.1. Dual-HKs in basidiomycetes

In this work, we have analyzed the presence of genes coding for dual-HKs in 80 complete genome sequences of species belonging to the phylum Basidiomycota using the web server BASID2CS [16]. This study includes 71 genome sequences of species from the subphylum *Agaricomycotina*, six from the subphylum *Pucciniomycotina* (*Cronartium quercuum*, *Melampsora laricis-populina*, *Mixia osmundae*, *Puccinia graminis*, *Rhodotorula graminis* and *Sporobolomyces roseus*) and two from the subphylum *Ustilaginomycotina* (*Malassezia globosa* and *Ustilago maydis*), as well as one species classified as Basidiomycota *incertae sedis* (*Wallemia sebi*) (Table S1, Supplementary data). Dual-HKs are only present in 16 of the 71 *Agaricomycotina* and in all the *Pucciniomycotina* species analyzed. However, they are absent in *M. globosa*, *U. maydis* and *W. sebi*. When present, there is a single or two dual-HKs in each species (Table 1). Dual-HK genes are mainly scattered across the genomes of basidiomycetes. However, it is noteworthy that *Rickenella mellea*, *Piriformospora indica*, *Sebacina vermifera*, *R. graminis* and *Sistotremastrum suecium* contain two dual-HK genes oriented in the same direction and mapping relatively close in the chromosome. The distance between the pair of dual-HK genes is 882 bp in *S. vermifera*, 10,430 bp in *S. suecium*, 10,726 bp in *P. indica*, 30,868 bp in *R. graminis* and 135,241 bp in *R. mellea*. Moreover, both dual-HK proteins are highly similar in *S. vermifera* (86%) and *P. indica* (80%). Analysis of the *P. indica* genome has provided evidence for a high number of expanded protein families, which are typically present in clusters of 2 to 7 genes within the genome, and it has been proposed that expansion of these genes is likely to be due to local duplication events [29].

3.2. Domain structure of dual-HKs

The prokaryotic hybrid HKs have diverse and complex domain architectures and, therefore, have been classified according to the number and order of their T module and R domain [5,30]. In bacteria and fungi, the most common and simple domain architecture of hybrid HKs is the TR geometry containing a single T module and R domain (Fig. 1) [5,7,14,15,30]. However, analysis of the domain structure of the 34 dual-HKs identified in basidiomycetes