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Exploration of Histidine Kinases in Green Algae: Domain and Structural Analysis

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Histidine kinases (HKs) constitute a large family of proteins that play a central role in signal transduction, enabling cells to sense and respond to a wide range of both intercellular and intracellular signals [1]. Although their structural features and distribution are well-studied in prokaryotes, the understanding of histidine kinases in eukaryotes remains incomplete, despite several phylogenetic studies on the subject [2]. Of particular interest are histidine kinase rhodopsins (HKRs), discovered in Green Algae, whose precise biological roles have yet to be fully determined [3].

Green Algae, an important group of autotrophic eukaryotes, are critical to ecosystems by providing habitats, serving as a food source for various organisms, facilitating nutrient cycling, and increasing oxygen levels in aquatic environments through photosynthesis. The ability to sense and respond to light is vital for the photosynthetic processes of Green Algae. Although some of the mechanisms behind these processes have been uncovered [4], further investigation is required.

The main aim of this study was to perform a bioinformatics analysis and explore the structural organization of histidine kinases (HKs) in Green Algae, with a focus on the domains present to infer their possible functions. Amino acid sequences were obtained from the UniProt database using the search query "txid1148[Organism] AND (Histidine[All Fields] AND Kinase[All Fields])"and sequences classified under the EC code for HKs (2.7.13.-). After removing redundant sequences, the remaining data were analyzed using InterProScan-5.66-98.0 [5] to identify the domains within the Pfam collection. Proteins that lacked domains characteristic of HKs were filtered out to minimize the inclusion of incorrect entries. This process resulted in the identification of 458 proteins likely to be HKs.

Within this dataset, two important subgroups were identified. The first subgroup, known as hybrid histidine kinases, consists of proteins that not only possess the histidine kinase domain (HK domain) but also include a receiver (REC) domain. A total of 269 such proteins were identified, representing 58% of all HKs analyzed. The second subgroup, termed histidine kinase rhodopsins (HKRs), consists of hybrid histidine kinases that begin with a rhodopsin-like (Rhd) domain. This subgroup includes 97 proteins, accounting for over a quarter of the HKs studied. We propose that the bacteriorhodopsin-like segment in this subgroup functions as a sensor for external signals, which are then transmitted via the conserved histidine residue to the response regulator. The most prevalent domains and domain architectures were identified for both subgroups. Notably, more than a quarter of the HKRs feature two REC domains instead of one. To better understand the relationships among these proteins, a phylogenetic tree was constructed, offering a visual representation of their evolutionary relatedness within this subgroup.

For the HKRs, three-dimensional protein structures were predicted using AlphaFold3 [6], which largely confirmed the domain identifications. However, in some cases, additional domains were detected that had not been identified by InterProScan. Further analysis with FoldSeek suggested these additional domains could represent secondary REC domains, indicating that the Rhd-HK-REC-REC architecture may be more widespread than previously thought.

In conclusion, our work provides information on the possible and most common domain architectures of histidine kinases. Furthermore, identification of rhodopsin-like domains in a significant proportion of histidine kinases in photosynthetic Green Algae may imply an important functional role for proteins with this architecture in these microorganisms. Additionally, the presence of two REC domains may be a characteristic feature of these proteins, warranting further investigation.

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