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Hox genes from the parasitic flatworm *Schistosoma japonicum*

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ABSTRACT

Hox genes are characterized by a highly conserved peptide domain and contribute to antero-posterior axis patterning during embryogenesis. These genes have been widely studied in a variety of animal species due to their central role in evolutionary developmental biology. Based on the published genome assembly and unpublished re-sequencing project data, we present the first genome-wide characterization and comparative genomic analysis of the *Hox* gene family within *Schistosoma japonicum*. Eight *Hox* genes were identified and validated in our investigation. Phylogenetic analysis revealed that these genes are distributed among seven orthology groups of the *Hox* gene family. Our study further suggested that differences in the *Lox5* gene copy number existed between the two closely related species, *S. japonicum* and *Schistosoma mansoni*. Semi-quantitative real-time polymerase chain reaction experiments revealed that *Lox5* and *Hox4* gene expression was high in the schistosomulum stage, and all four genes investigated showed highest expression within the eggs.

1. Introduction

The *Hox* gene family is a class of fundamental transcription factors involved in antero-posterior axial patterning during animal morphogenesis [1–3]. Since it was first reported in 1984 [4], the family has been identified in almost all metazoan phyla. Members of this gene family are characterized by a highly conserved deoxyribonucleic acid (DNA) binding domain, the homeodomain, which has been important in the study of evolutionary developmental biology. Expression patterns of *Hox* genes are highly associated with their spatial and temporal genomic arrangement, features that are phylogenetically highly conserved [5,6]. Thus the co-linearity rules of *Hox* genes have facilitated our understanding of body plan evolution in animals.

Members of the phylum Platyhelminthes have a simple bilateral body plan and at one time were considered to represent basal bilaterians [5,7]. It is now clear that the phylum belongs within the Lophotrochozoa, one of three major clades of bilaterian phyla [6]. The *Hox* gene family of Platyhelminthes may therefore provide important insights into the evolution of lophotrochozoans and of early bilaterians. [6]. Ongoing investigations into the origin of *Hox* genes have consequently included studies on several platyhelminths (e.g. turbellarians, such as *Polycelis nigra*, *Dugesia japonica* and *Girardia tigrina* [8–11]; cestodes, such as *Echinococcus granulosus*, *Mesocestoides vogae* and *Taenia asiatica* [12]; and trematodes, such as *Echinostoma trivolvis* and *Schistosoma mansoni* [5,13]). However, most previous studies on platyhelminths have only reported partial gene information and lacked details of genomic organization. Studies on *S. mansoni* [13,14] provided the first genome-wide perspective on the *Hox* gene family within the phylum. Nine *Hox* genes in seven orthologous groups were identified. Four genes were dispersed among two chromosomes of *S. mansoni* based on fluorescence *in situ* hybridization (FISH). It was supposed that remaining *Hox* genes were dispersed across the genome [13].

Our study aimed to perform genome-wide characterization of the *Hox* gene family in *Schistosoma japonicum* using the published genome assembly [15], and unpublished data from an ongoing re-sequencing project conducted by the Chinese National Human Genome Center, Shanghai. All *Hox* genes of *S. japonicum* were identified and phylogenetically characterized, and the expression patterns of four were assessed.

2. Results

2.1. Genome-wide identification of S. japonicum Hox genes

Nomenclature for the *S. japonicum Hox* genes follows that for the lophotrochozoans, with the prefix Sj used to denote this species. *S. mansoni* was reported to possess nine *Hox* genes, two of these (*Smox1* and *SmLox5*) being paralogs of the *Lox5* genes [13,14]. Although our BLAST and peptide domain searches of the *S. japonicum* data generated a large number of homeodomain-like fragments, only eight showed high sequence similarity and intron–exon architectures consistent with the homeodomain of the *S. mansoni Hox* genes. DNA level comparisons of homeodomains between *S. japonicum* and *S. mansoni*

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is provided in Supp. Fig. 5. The sequences found were homologous to the *Hox1*, *Hox2*, *Hox3*, *Hox4*, *Lox5*, *Lox4* and *Post-2* genes in other platyhelminths. *Hox5*, *Lox2*, *Post-1* and *Hox7* genes were not found in *S. japonicum*. A single *Lox5* gene (*SjLox5*) was identified in *S. japonicum* The *Hox* genes identified were spread across the scaffolds of the primary genome assembly, with only *SjHox2* and *SjHox4* located close to each other on the same scaffold, SJC_S000052 (Fig. 1B). The *S. japonicum* intron–exon homeodomain architecture is presented in Fig. 1, and is consistent with that of *S. mansoni*.

2.2. Hox gene alignment and phylogenetic analysis

To generate a high confidence catalogue of gene predictions, we used protein sequence alignments and phylogenetic analysis to subdivide the *Hox* gene family into subfamilies, each containing orthologous genes. Alignment of the *S. japonicum* homeodomains against the corresponding genes of *S. mansoni* revealed variable amino acid identities among the different subfamilies (Fig. 2). The homeodomains of the anterior genes (*SjHox1*, *SjHox2* and *SjHox3*) had lower identities to those of *S. mansoni* than did the central and posterior genes. Homeodomains of the central genes (*SjLox5* and *SjLox4*) and posterior gene (*SjPost-2a*) exhibited 100% amino acid similarity to the corresponding genes of *S. mansoni*.

Phylogenetic analysis (Fig. 3) revealed seven subfamilies containing all of the *S. japonicum Hox* genes and corresponding to the subfamilies indicated by sequence comparisons. In addition, the homeodomains of the *S. japonicum Hox* genes consistently clustered with the corresponding genes of *S. mansoni* for all analyses.

2.3. Quantitative analysis of Hox genes of S. japonicum

Semi-quantitative PCR was used to study *Hox* expression in *S. japonicum* (Fig. 4, Supp. Fig. 2). Using total extracted ribonucleic acid, variable expression patterns were observed between the egg, miracidium, schistosomulum, adult female and adult male developmental stages. Four *Hox* genes (*SjHox2*, *SjHox4*, *SjLox5* and *SjLox4*) were successfully assessed, with the highest expression levels for all four observed in the eggs. Expression of *SjHox4* and *SjLox5* was higher in schistosomulum. *Hox4*, *Lox5* and *Lox4* expression in *S. japonicum* did not follow the typical rule of temporal co-linearity. Instead, expression of these genes within the eggs was initiated at the same time as the anterior genes. These results suggested that a serial expression pattern of *Hox* genes might not be strictly observed in *S. japonicum*.

3. Discussion

Our study is the first reported identification of the *Hox* genes in the parasitic flatworm *S. japonicum* and characterization of their orthologous relationship to other lophotrochozoans. As done in other studies [14,16] we based this analysis on homeodomain sequence conservation and phylogenetic relationships. Previous study has suggested that the *Hox5*, *Lox2*, *Hox7* and *Post-1* genes are absent from the platyhelminth lineage [14], and was further confirmed by our investigation. A total of eight *Hox* genes and seven ortholog groups were identified, including a duplication of the *Post-2* posterior gene. Previous reports suggested [13,14] that another schistosome, *S. mansoni*, possessed two *Lox5* genes (*Smox1* and *SmLox5*, the latter located within the unplaced reads of *S. mansoni* genome assembly). All triclad flatworms also possess two *Lox5* genes [14]. However, only one *Lox5* gene (*SjLox5*) was found in *S. japonicum*. Although the DNA sequence alignment (Supp. Fig. 4B) of the *Lox5* genes revealed that *Smox1* and *SjLox5* are highly conserved between *S. japonicum* and *S. mansoni*, no homolog of *SmLox5*, another member of *Lox5* in *S. mansoni* was found in the primary genome assembly, re-sequencing contigs, or raw Solexa reads of *S. japonicum*. This gene in *S. mansoni* is very different from orthologs in other lophotrochozoans in possessing two non-conserved intron positions and a divergent parapeptide [13] (Supp. Fig. 4A). Considering the high quantity of data for the primary genome assembly and the re-sequencing project used in our study, we doubt that a homolog of *SmLox5* exists in *S. japonicum*.

The lophotrochozoan *Capitella* possesses 11 *Hox* genes and the ancestral *Hox* genes was well-organized within the ancestral *Hox* cluster

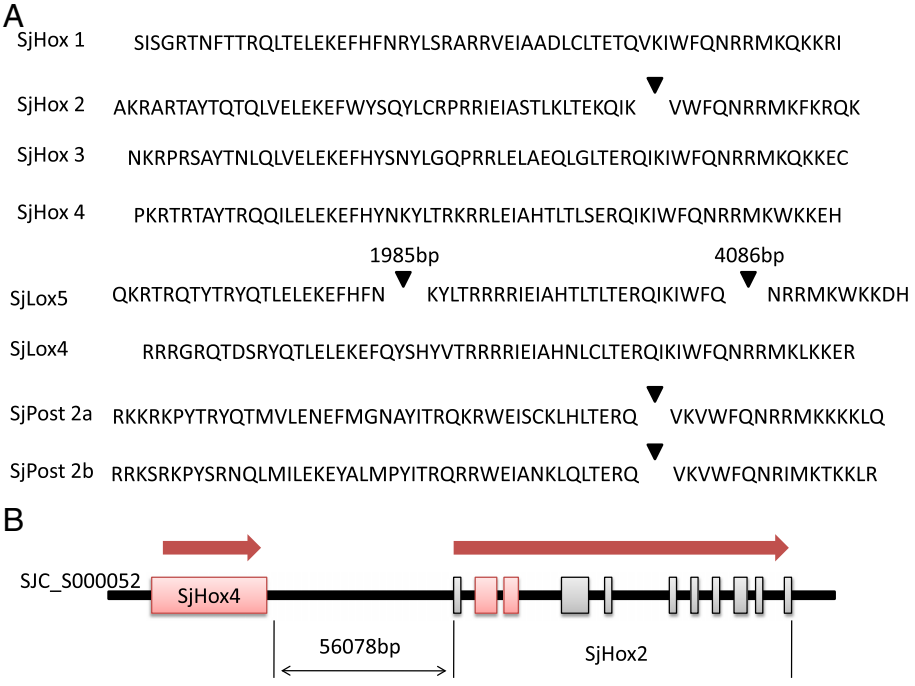

Fig. 1. Homeodomain structure for *S. japonicum*. (A) Intron positions are marked as a black triangle. (B) Locations of *Hox2* and *Hox4* genes in the primary genome assembly. Exons of the *Hox*2 and *Hox*4 genes are shown as boxes. The red arrow indicates the transcription orientation and the exons containing homeodomains are presented as pink boxes.