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Genome-wide, whole mount *in situ* analysis of transcriptional regulators in zebrafish embryos

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ABSTRACT

Transcription is the primary step in the retrieval of genetic information. A substantial proportion of the protein repertoire of each organism consists of transcriptional regulators (TRs). It is believed that the differential expression and combinatorial action of these TRs is essential for vertebrate development and body homeostasis. We mined the zebrafish genome exhaustively for genes encoding TRs and determined their expression in the zebrafish embryo by sequencing to saturation and *in situ* hybridisation. At the evolutionary conserved phylotypic stage, 75% of the 3302 TR genes encoded in the genome are already expressed. The number of expressed TR genes increases only marginally in subsequent stages and is maintained during adulthood suggesting important roles of the TR genes in body homeostasis. Fewer than half of the TR genes (45%, $n=1711$ genes) are expressed in a tissue-restricted manner in the embryo. Transcripts of 207 genes were detected in a single tissue in the 24 h embryo, potentially acting as regulators of specific processes. Other TR genes were expressed in multiple tissues. However, with the exception of certain territories in the nervous system, we did not find significant synexpression suggesting that most tissue-restricted TRs act in a freely combinatorial fashion. Our data indicate that elaboration of body pattern and function from the phylotypic stage onward relies mostly on redeployment of TRs and post-transcriptional processes.

Introduction

Vertebrate embryogenesis is believed to be crucially dependent on differential gene expression. Moreover, development is organised in a hierarchical fashion, in which, in a stepwise manner, more complex structures are derived from simpler structures laid down during earlier phases of ontogeny. It is thus assumed that the employed regulatory machinery in the developing animal becomes progressively more complex. The establishment of specific transcriptional expression programs leading to specific cell fate determination is controlled by the selective expression and/or activity of transcriptional regulators (TRs), as exemplified by the role of Myod in muscle differentiation (Weintraub et al., 1991). Among these, transcription factors (TFs) bind to DNA in a sequence-specific manner. DNA regions bound by TFs form gene regulatory elements also referred to as enhancers, repressors, silencers and promoters. Many TRs are downstream effectors of signalling pathways and integrate different signalling inputs that control cell behaviour. Although the concept of master regulators with unique transcriptional functions in the organism has been suggested (Halder et al., 1995), a growing body of evidence indicates that TFs act in a combinatorial fashion to control specific regulatory output (Davidson et al., 2002; Ravasi et al., 2010). Indeed, TFs have frequently multiple roles in multiple organs and it is the particular combination of TRs expressed or repressed at a particular time and space that dictates cellular morphology

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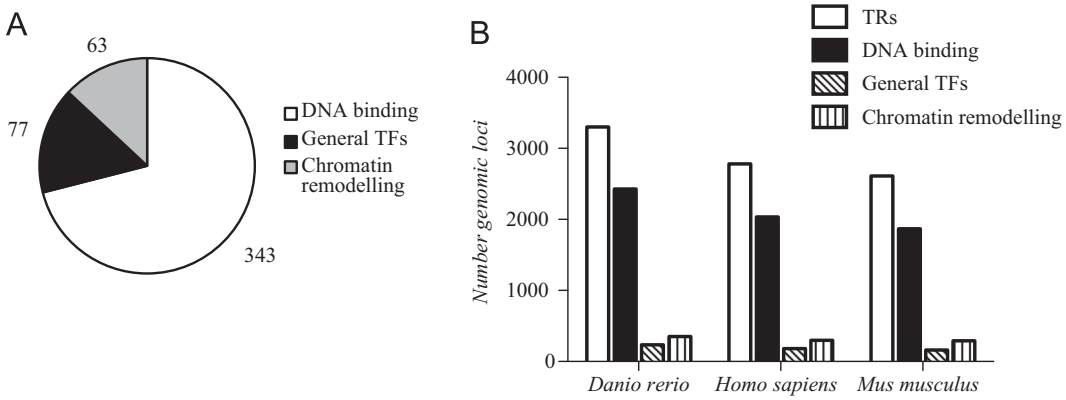

Fig. 1. Gene loci encoding transcriptional regulators. (A) Categorisation of InterPro domains into distinct functional groups specific to transcriptional regulators. The number of protein domains belonging to each group is indicated. (B) Number of genomic loci encoding transcriptional regulators in the zebrafish (Zv9), human (GRCh37.p2) and mouse genome (NCBIM37). The categorisation into families is based on their predicted protein domains.

and function. Expression of certain TRs can be sufficient to drive cells into a specific differentiation programme (Vierbuchen et al., 2010) or to induce a pluripotent stem cell state (Takahashi and Yamanaka, 2006). Estimations based on the analysis of known DNA-binding domains suggest that 1500–2000 genomic loci of the mouse and human genome encode transcription factors (Tupler et al., 2001; Vaquerizas et al., 2009; Venter et al., 2001). In addition, transcription is regulated at a higher order by modification of the chromatin structure. Chromatin modifications can affect gene expression by changing the accessibility of genes to transcription factors or modifying promoter and enhancer activity, in either a positive or a negative manner. The activity and/or expression of these chromatin-modifying enzymes need to be carefully orchestrated with that of the TFs and factors of the general transcriptional machinery.

Although many systematic expression studies have been performed in various vertebrate models (Belgard et al., 2011; Fu et al., 2009; Gray et al., 2004; Hunt-Newbury et al., 2007; Ravasi et al., 2010), comprehensive genome-scale data on the spatiotemporal expression of TR genes in the developing vertebrate embryo is not available. This information is a prerequisite for a systematic elucidation of transcriptional regulatory networks during development. The zebrafish (*Danio rerio*) embryo represents a promising model to obtain such a genome-scale description of TR gene expression as it allows the combination of transcriptome studies with large scale *in situ* expression analysis. We report here a comprehensive analysis of TR gene expression in zebrafish. We profiled the relative abundance of TRs by microarray analysis over different developmental stages and adult body parts, and compiled a genome-wide analysis of gene expression states by RNA sequencing (RNAseq) during organogenesis, larval maturation and adult homeostasis. We cloned 2149 gene probes and provided a comparative atlas of 1711 TR genes, including 746 new patterns of expression in the 24 hpf (hour post-fertilization) embryo. The 24 hpf stage is of particular importance as it represents the evolutionarily conserved phylotypic stage of this model organism (Domazet-Loso and Tautz, 2010). At this stage, the embryos of all the different vertebrate subclasses look very similar. Organogenesis and the vertebrate-subclass specific elaboration of the body pattern have begun at this stage, but is far from complete. The majority of TR genes is already expressed at the phylotypic stage. For example the anlage of the telencephalon expresses more than 1100 different TR genes at this early stage. Expression of these factors is largely maintained in the adult zebrafish suggesting roles of TR genes in tissue and body homeostasis. Quite unexpectedly, we find that 55% TR genes are expressed ubiquitously. Our comprehensive study of the TR gene expression state in the zebrafish embryos uncovers the complexity of the expression state of TR genes at the immature phylotypic stage and points at differential redeployment of TR genes and post-transcriptional modifications as fundamental regulatory processes in the further elaboration of body pattern.

Results

Characterisation of the repertoire of transcriptional regulatory genes

To obtain a comprehensive representation of gene loci involved in transcriptional regulation, we mined the InterPro database (Hunter et al., 2009) and the literature to systematically identify protein domain families specific to TRs. We scored 483 InterPro protein domains that fell into 3 distinct functional groups: (i) DNA-binding domains, (ii) chromatin remodelling domains and (iii) domains specific to factors of the general transcriptional machinery (Fig. 1A, Supplementary Table T1).

We searched the zebrafish genome (Zv9) for loci encoding proteins with at least one of these domains. We additionally mined 24,386 zebrafish Refseq transcripts (Refseq, NCBI, Nov 2010) with InterProscan (v4.6) (Zdobnov and Apweiler, 2001). We identified 3302 unique genomic loci encoding potential TRs, representing 11,6% of the 28,491 genes annotated in the zebrafish genome (Fig. 1B, Supplementary Table T2). When sorted according to potential function, 2677 (81%) of the zebrafish TR genes encode TFs with a DNA-binding domain, and 488 (15%) genes code for proteins with chromatin remodelling domains. Proteins with a putative function in general transcription are represented by 137 loci (4%). In comparison to the human (2782 genes) and mouse (2612 genes) genome, the zebrafish genome encodes more TR genes (Fig. 1B), presumably reflecting gene retained after the genome duplication at the base of the evolution of actinopterygian fish (Taylor et al., 2003).

Most transcriptional regulators are expressed throughout development

We next wished to assess the expression state of the TR genes, during embryogenesis. First, we determined the developmental profile of TR gene expression by employing a custom-designed microarray with probes representing 1565 TR genes, to which we hybridised cDNA from six different developmental stages. cDNA samples from 3 to 6 independent RNA preparations from each stage were analysed (Supplementary Fig. S1). Among the 1565 TRs genes present on the microarray, 225 are novel genes which were