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Evolutionary conservation of TFIIH subunits: Implications for the use of zebrafish as a model to study TFIIH function and regulation

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ABSTRACT

Transcriptional factor IIH (TFIIH) is involved in cell cycle regulation, nucleotide excision repair, and gene transcription. Mutations in three of its subunits, XPB, XPD, and TTDA, lead to human recessive genetic disorders such as trichothiodystrophy and xeroderma pigmentosum, the latter of which is sometimes associated with Cockayne's syndrome. In the present study, we investigate the sequence conservation of TFIIH subunits among several teleost fish species and compare their characteristics and putative regulation by transcription factors to those of human and zebrafish. We report the following findings: (i) comparisons among protein sequences revealed a high sequence identity for each TFIIH subunit analysed; (ii) among transcription factors identified as putative regulators, OCT1 and AP1 have the highest binding-site frequencies in the promoters of TFIIH genes, and (iii) TFIIH genes have alternatively spliced isoforms. Finally, we compared the protein primary structure in human and zebrafish of XPD and XPB – two important ATP-dependent helicases that catalyse the unwinding of the DNA duplex at promoters during transcription – highlighting the conservation of domain regions such as the helicase domains. Our study suggests that zebrafish, a widely used model for many human diseases, could also act as an important model to study the function of TFIIH complex in repair and transcription regulation in humans.

1. Introduction

TFIIH is a general transcription factor essential for multiple processes, including initiation of transcription by RNA Polymerase II, DNA damage repair via the nucleotide excision repair (NER) (de Laat et al., 1999; Lehmann, 2001; Liu et al., 2008), and cell cycle regulation (Chen and Suter, 2003; Cameroni et al., 2010). It is composed of the core subcomplex, containing six proteins, p34, p44, p52, p62, TTDA, and XPB, and the CAK subcomplex, comprising the CDK7, Cyclin H, and MAT1 proteins. The XPD subunit forms the structural link between the TFIIH core and CAK subcomplexes (Schultz et al., 2000; Chen and Suter, 2003; Zurita and Merino, 2003) and functions as part of a superfamily of DNA helicases with 5′ to 3′ polarity involved in the opening of the DNA duplex around the sites of DNA damage which allow access to NER machinery. XPB, the largest (89 kD) TFIIH subunit, is a ATPase helicase that separates the DNA chains in 3′ to 5′ direction and opens and remodels the DNA during NER and transcription. The helicase activity of XPB is mainly involved with promoter escape during transcription (Lehmann, 2001). The p44 protein of the core subcomplex was shown to have a E3 ubiquitin ligase domain (Takagi et al., 2005) whereas the p34 and p62 are structural proteins of the core subcomplex, with the former have a strong interaction with p44 whilst the latter is important for stabilizing the TFIIH complex and as well as having interactions with transcription factors and NER factors (Compe and Egly, 2012). Both the TTDA and p52 proteins regulate the ATPase activity of XPB (Coin et al., 2006; Compe and Egly, 2012). As part of the CAK subcomplex, CDK7 is important for phosphorylation and activation of the largest subunit of RNA Pol II and nuclear factors (Keriel et al., 2002; Chymkowitch et al., 2011) and is regulated by the Cyclin H protein subunit (Liu et al., 2007). The MAT1 protein of the CAK subcomplex is a key assembly factor which stabilizes a transient CDK7–Cyclin H complex and thereby enhances the specificity of the binding of the CDK-activating kinase (CAK) subcomplex to TFIIH. Mutations in the genes of three of the TFIIH subunits, namely, XPB, XPD, and TTDA, can lead to the human recessive genetic disorders xeroderma pigmentosum (XP), often in combination with Cockayne's syndrome (CS), and trichothiodystrophy (TTD). These autosomal recessive diseases have a wide spectrum of symptoms which reflect the dual role of these TFIIH subunit genes in both transcription and repair (Lehmann, 2001). Patients who suffer from XP are sensitive to light and exhibit a high frequency of malignant

Abbreviations: CAK, CDK activating kinase; CDKs, cyclin-dependent kinases; NER, nucleotide excision repair; TFs, transcriptional factors; TFIIH, transcriptional factor IIH; XPB, xeroderma pigmentosum B; XPD, xeroderma pigmentosum D.

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skin cancer and the XP-causing mutations in the helicase motifs of the XPD or XPB subunit proteins (Oh et al., 2006; Cameroni et al., 2010). These gene mutations affect the ability of the XPD and XPB proteins to participate in the NER pathway and therefore contribute to the accumulation of DNA alterations. Mutations in XPD and XPB are also responsible for the combined XP and CS phenotypes (XP–CS). XP–CS patients suffer from skin abnormalities as seen in XP, including increased cancer incidence, and also the neurological and developmental dysfunctions associated with CS (Fan et al., 2008; Cameroni et al., 2010). By contrast, TTD patients have sulfur-deficient sparse, dry and brittle hair, with a typical banding and other neuroectodermal symptoms that include mental and growth retardation, proneness to infections, ichthyosis, nail abnormalities, skin photosensitivity, microcephaly, facial dysmorphism, decreased fertility and features of premature ageing, such as osteoporosis, hearing loss, cataracts and dental caries (Stefanini et al., 2010). All TTD-causing mutations result in reduced steady-state levels of the entire TFIIH complex, modifications of its architecture, and impaired TFIIH function during DNA repair and transcription.

Fish, and especially zebrafish (*Danio rerio*), are increasingly being used as model organisms to study human genetic disorders since they have many technical advantages (Bolis et al., 2001; Lieschke and Currie, 2007). Because zebrafish possesses genes orthologous to most human genes due to their relatively recent common ancestry it is becoming an important model system to study human biological processes. Indeed, the physiological processes of zebrafish have many similarities to humans, including those affecting bone metabolism (Lieschke and Currie, 2007) which make its study relevant to human physiology and pathology. Most importantly, the amino acid sequences of functionally relevant protein domains in zebrafish are evolutionarily conserved, with many similarities existing between zebrafish and mammalian sequences (Lieschke and Currie, 2007). The objectives of this study were to characterize the TFIIH subunit genes from several fish species, namely, zebrafish (*D. rerio*), stickleback (*Gasterosteus aculeatus*), Japanese ricefish (medaka; *Oryzias latipes*), torafugu (*Takifugu rubripes*), and spotted green pufferfish (*Tetraodon nigroviridis*) and to determine their chromosomal localization. In addition, we aimed to determine the degree of conservation in primary structures of the critical XPD and XPB helicase proteins. These observations allow us not only to show the suitability of zebrafish as a model organism for the study of TFIIH gene function and regulation but also to confirm the relevance of these studies to human biology and health.

2. Materials and methods

2.1. Sequence collection and experimental strategy

Sequence databases at NCBI GenBank (January, 2013; www.ncbi.nlm.nih.gov) and Ensembl (release v72; www.ensembl.org) were searched for annotated TFIIH subunit sequences including XPD, XPB, p44, Cdk7, Cyclin H, p34, p52, p62, TTDA, and MAT1. A total of 59 sequences representing six species were collected (Fig. S1),

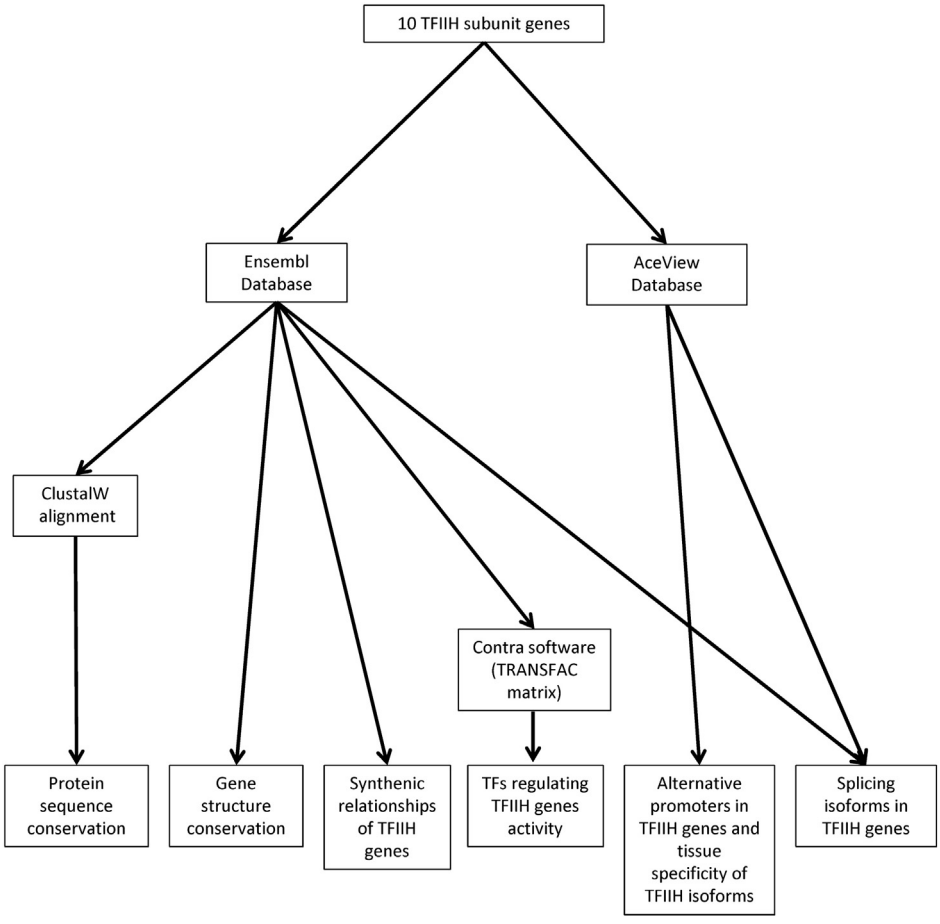

Fig. 1. Workflow and strategies used for database mining and gene identification. Ten TFIIH subunit genes were subjected to six functional database mining tests. (1) Comparison of protein primary sequence between human and five teleost, including zebrafish; (2) comparison of gene structure; (3) identification of neighbour genes to clarify the syntenic relationships of TFIIH genes; (4) identification of alternative promoters and spliced isoforms of TFIIH genes using AceView and Ensembl database; and (5) identification of TF regulating promoters of TFIIH subunit genes (within 1500 bases upstream of the translation start site) retrieved from the NCBI Entrez Gene database.