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Molecules in focus

Zinc fingers of the cerebellum (Zic): Transcription factors and co-factors

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ABSTRACT

The *Zic* genes encode zinc finger containing proteins that can bind proteins and DNA. The understanding of Zic molecular networks has been hampered by functional redundancy amongst family members, and because their loss-of-function phenotypes are indicative of a role in many signalling pathways. Recently molecular evidence has emerged confirming the pleiotropic nature of these proteins: they act both as classical transcription factors and as co-factors to directly and indirectly influence gene expression. It has long been known that germ-line mutation of the *Zic* genes in human and mouse causes a range of congenital disorders. Recently connections between Zic proteins and stem cell function have also emerged suggesting a role in adult onset diseases. The immediate challenge is to determine when and where these proteins act as transcription factors/co-factors during development and disease and how the switch between these roles is controlled.

1. Introduction

Members of the *Zic* (*z*inc finger of the *c*erebellum) gene family have been identified in a range of vertebrate and invertebrate species. The founding member, Drosophila odd-paired (*opa*), was isolated during the landmark Drosophila genetic screen (Nusslein-Volhard and Wieschaus, 1980) because *opa* mutants fail to generate alternate segments during embryonic patterning. Accordingly, *opa* was classed as a member of the pair-rule genes which, in addition to the presence of zinc fingers, suggested that *opa* may encode a transcription factor (Benedyk et al., 1994). The first vertebrate orthologue of *opa* was isolated from the cerebellum of the mouse and was named *z*inc finger of the *c*erebellum (*Zic*) but upon identification of other mouse *Zic* genes was renamed *Zic1* (Aruga et al., 1996). The number of *Zic* genes within a species varies from the single Drosophila orthologue to the seven in zebrafish. This review focuses on the mammalian Zics, and in humans and mouse there are five genes numbered *ZIC1–ZIC5* and *Zic1–Zic5*, respectively (reviewed by Merzdorf, 2007). Despite three decades elapsing since their discovery much remains unknown about this family and little is known about expression control of and by the Zic genes/proteins. Moreover, the long-running uncertainty regarding whether Zic proteins act as *bone fide* transcription factors or as co-factors is only now beginning to be clarified with evidence that they can act as either class of molecule, presumably in a context dependent manner.

2. Structure

The five *Zic* genes in humans and mice reside at three genomic locations. *Zic1* and *Zic4* exist as a divergently transcribed tandem gene pair (on human Chr3, MMu Chr9) as do *Zic2* and *Zic5* (on human Chr13, MMu Chr14) whilst *Zic3* is an X-linked singleton in each species (Fig. 1A). The defining feature of the Zic proteins is the highly conserved zinc finger domain that consists of five tandem C2H2 zinc fingers (reviewed by Merzdorf, 2007) (Fig. 1B). The first zinc finger is well conserved amongst Zic1–3, but is more divergent in Zic4 and Zic5, which suggests that the division of Zic proteins into two subclasses may be warranted. This is supported by the fact that mammalian Zic1–3 and opa have a Zic opa conserved motif (ZOC) required for transcriptional activation of target genes in vitro and for binding of the myogenic repressor protein, I-mfa. Zic proteins also possess a zinc finger N-flanking conserved region (ZF-NC) proximal to the first zinc finger domain; the function of which remains unknown (Aruga et al., 2006 and references therein) (Fig. 1B). All Zic proteins lack a canonical nuclear localisation signal (NLS) but an interspersed type of NLS within the zinc fingers controls nuclear import of ZIC3 via the importin pathway (Bedard et al., 2007; Hatayama et al., 2008). With the exception of Zic4 (Ishiguro et al., 2004; Aruga et al., 1996), outside the zinc finger domain and ZF-NC, each of the Zics contain low complexity regions, most notably ZIC2 which possesses a polyalanine tract; the expansion of which is linked to a human disorder (Brown et al., 2005) (Fig. 1B). The genomic arrangement and protein sequence conservation patterns suggest that the ancestral *Zic* gene first underwent tandem duplication followed by sequential duplication of the gene pair.

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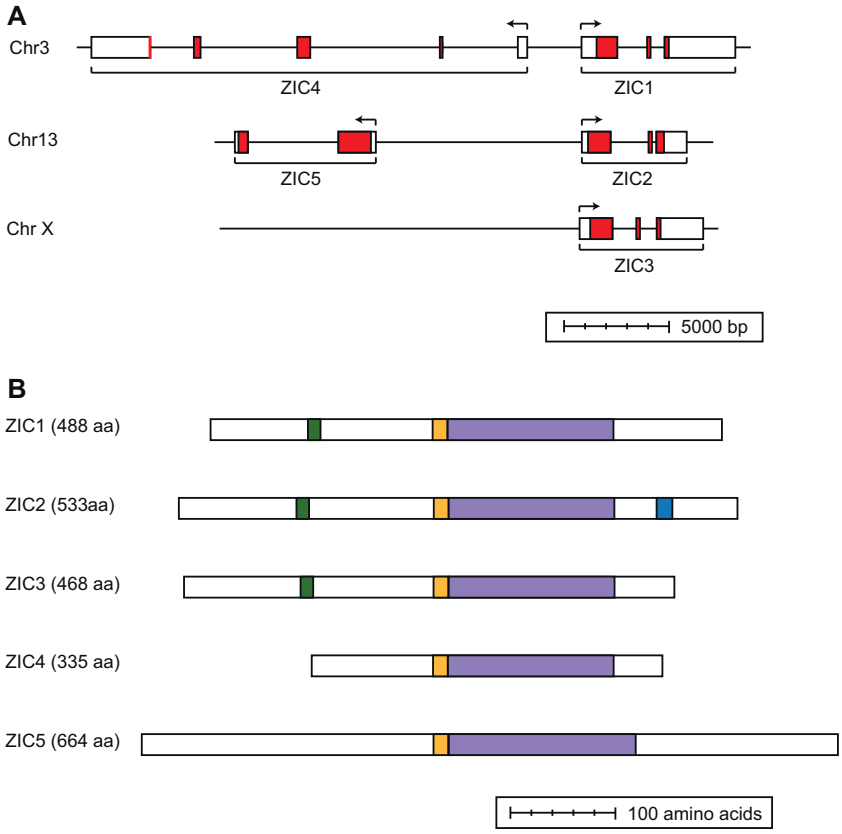

Fig. 1. ZIC genomic organization and protein structure. (A) Representation of human *ZIC* genomic organisation. *ZIC1* and *ZIC4* are located on human Chr3 whilst *ZIC2* and *ZIC5* reside on human Chr13. The arrows indicate divergent transcription of the tandem gene pairs. *ZIC3* is an X-linked singleton. *ZIC1*, *ZIC2* and *ZIC3* are spread across three exons (red and white boxes), whereas *ZIC4* and *ZIC5* are coded by five and two exons, respectively. The red boxes demarcate the portion of the exon that contributes to the coding regions whilst the white boxes define untranslated (UTR) regions of the *ZIC* mRNA. (B) Representation of ZIC protein structure. The N-terminally located ZOC domain is conserved in ZIC1–3 (green box), whilst the ZF-NC (yellow box) and the zinc finger domain (purple box) are completely conserved amongst the ZICs. Only ZIC2 possesses a poly-alanine tract at the C-terminus (blue box).

3. Expression, activation and regulation

The retention of tandem gene pairs within the genome is often associated with co-expression and there is evidence that this is the case for the *Zic* genes. *Zic2* and *Zic5*, along with *Zic3* share largely overlapping domains of expression during early murine embryogenesis. *Zic2* transcription is first detected in the blastocyst (Brown and Brown, 2009) and *Zic2*, *Zic3* and *Zic5* are each expressed in the post-implantation egg cylinder and in the ectoderm and mesoderm of the gastrula (Elms et al., 2004; Furushima et al., 2000). As organogenesis proceeds transcripts from each of these genes are found in specific regions of the neurectoderm of the developing central nervous system (CNS) and precursors of the musculo-skeletal system including the somites and limb buds. This is accompanied at early organogenesis by the initiation of *Zic1* and *Zic4* transcription, also within specific regions of the neurectoderm and in the somites (Elms et al., 2004; Gaston-Massuet et al., 2005). *Zic* gene expression at later stages of embryogenesis and in the adult has not been well characterized. The available data however suggest ongoing expression within the nervous and skeletal system (Brown and Brown, 2009; Kalogeropoulos et al., 2010). There is little known regarding the control of mammalian *Zic* gene expression with no in vivo confirmed regulatory proteins identified although information from ES cells suggests that *Zic3* expression is promoted by the Nanog transcription factor (Lim et al., 2007). Extensive experiments in other organisms indicate that multiple signalling pathways and transcription factors regulate *Zic* gene expression (reviewed by Merzdorf, 2007).

Biochemical analysis of the mutant Zic proteins found associated with human congenital defects suggests the control of Zic nuclear accumulation is critical for function (Brown et al., 2005; Ware et al., 2004). ZIC3 nuclear localisation can be controlled by the importin pathway via an interaction with the Kpna1 and Kpna6 import receptor proteins (Bedard et al., 2007; Hatayama et al., 2008) and it is possible that the other Zics also interact with this pathway. Drosophila genetics and protein interaction studies suggest other protein partners also regulate this process. The Drosophila pair rule gene *ten-m* is thought to regulate opa activity and Zic1 can physically interact with the cleaved intracellular domain of the mammalian Ten-m orthologous Teneurin 2 protein; this interaction influences Zic1 transcriptional activity and sub-cellular localization of the two proteins in cultured cells (Baumgartner et al., 1994; Bagutti et al., 2003). The Gli proteins and Zic1–3 can associate in Xenopus and mammalian cells to influence the nuclear localization and transcriptional activity of both proteins in a context specific manner (reviewed by Merzdorf, 2007). Similarly, Zic1–3 interaction with the I-mfa protein prevents nuclear import and decreases transcriptional activity of the Zic protein (reviewed by Merzdorf, 2007). More regulators (and regulatory mechanisms) of Zic expression and activity undoubtedly remain to be identified and it will be fundamental to understand when and where these mechanisms operate in the in vivo context.

4. Biological function

Human genetics has shown that loss-of-function mutations in *Zic* genes result in a range of congenital defects (reviewed