Gene 567 (2015) 146–153

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Identification and characterisation of synaptonemal complex genes in monotremes

Aaron E. Casey *, Tasman J. Daish, Frank Grutzner

The Robinson Institute, School of Molecular and Biomedical Science, University of Adelaide, Gate 8 Victoria Drive, Adelaide, South Australia 5005, Australia

ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 25 April 2015
Accepted 27 April 2015
Available online 15 May 2015

Keywords:
Synaptonemal complex
Monotremes
Meiosis
SYCP1
SYCP2
SYCP3

ABSTRACT

The platypus and echidna are the only extant species belonging to the clade of monotremata, the most basal mammalian lineage. The platypus is particularly well known for its mix of mammalian and reptilian characteristics and work in recent years has revealed this also extends to the genetic level. Amongst the monotreme specific features is the unique multiple sex chromosome system (5X4Y in the echidna and 5X5Y in the platypus), which forms a chain in meiosis. This raises questions about sex chromosome organisation at meiosis, including whether there has been changes in genes coding for synaptonemal complex proteins which are involved in homologous synapsis. Here we investigate the key structural components of the synaptonemal complex in platypus and echidna, synaptonemal complex proteins 1, 2 and 3 (*SYCP1*, *SYCP2* and *SYCP3*). *SYCP1* and *SYCP2* orthologues are present, conserved and expressed in platypus testis. *SYCP3* in contrast is highly diverged, but key residues required for self-association are conserved, while those required for tetramer stabilisation and DNA binding are missing. We also discovered a second *SYCP3*-like gene (*SYCP3*-like) in the same region. Comparison with the recently published Y-borne SYCP3 amino acid sequences revealed that SYCP3Y is more similar to SYCP3 in other mammals than the monotreme autosomal SYCP3. It is currently unclear if these changes in the *SYCP3* gene repertoire are related to meiotic organisation of the extraordinary monotreme sex chromosome system.

1. Introduction

During prophase I homologous chromosomes align and pair with one another. Pairing is triggered by the homologous repair of DNA double strand breaks (DSBs) and stabilised by the formation of a tripartite structure called the synaptonemal complex (SC) (reviewed by Page and Hawley, 2004). The SC assembles as a three-dimensional ladder-like structure comprising two axial elements (called lateral elements in the fully formed SC) joined by transverse filaments and a central element (Solari, 1970b; Holm and Rasmussen, 1977). In mammals, axial elements include two known proteins; synaptonemal complex protein 2 (SYCP2) (Offenberg et al., 1991; Offenberg et al., 1998) and synaptonemal complex protein 3 (SYCP3) (Heyting et al., 1988). A single protein is known to form the transverse filaments, synaptonemal complex protein 1 (SYCP1) (Offenberg et al., 1991), while the central element has numerous components: synaptonemal complex element 1 (SYCE1) (Costa et al., 2005), synaptonemal complex element 2 (SYCE2) (Costa et al., 2005), synaptonemal complex element 3 (SYCE3) (Schramm et al., 2011) and testis expressed 12 (Tex12) (Hamer et al., 2006). The lateral element SYCP2 protein has been shown to bind SYCP1 providing a demonstrated structural link between the axial elements and transverse filaments (Winkel et al., 2009).

The SC forms and disassembles during prophase I to mediate association of homologous chromosomes. At leptotene, the chromosomes are organised along the axial elements (Heyting et al., 1988; Offenberg et al., 1998) and at this stage DNA DSBs interact with their matching sequence on the homologous chromosome via interaxis bridges, bringing the homologs into close proximity (reviewed by Page and Hawley, 2004). Over the duration of leptotene a proportion of the bridges

Abbreviations: °C, degrees Celcius; aa, amino acid; BLAT, BLAST-like alignment tool; BP1, basic patch 1; BP2, basic patch 2; cDNA, DNA complementary to RNA; *CHPT1*, choline phosphotransferase 1; cm^3, centimetre cubed; COR1, meiotic chromosome core protein 1; Ct6, C-terminal 6 amino acid domain; DNA, deoxyribonucleic acid; DSB, double strand break; g, gravity; *GNPTAB*, N-acetylglucosamine-1-phosphate transferase, gamma subunit; Ka, Number of non-synonymous substitutions per non-synonymous site; Ks, Number of synonymous substitutions per synonymous site; mL, millilitre; *NR1H5*, nuclear receptor subfamily 1, group h, member 5; Nt6, N-terminal 6 amino acid domain; nm, nanometre; PAR, Pseudoautosomal region; *PHACTR3*, phosphatase and actin regulator 3; Phyre2, protein homology/analogy recognition engine v 2.0; *PPP1R3D*, protein phosphatase 1, regulatory subunit 3d; RNA, ribonucleic acid; RT-PCR, reverse transcription polymerase chain reaction; SC, synaptonemal complex; *Sly*, *SYCP3*-like, Y-linked; *Slx*, *SYCP3*-like, X-linked; *Slx2*, *SYCP3*-like, X-linked 2; *SYCE1*, synaptonemal complex element 1; *SYCE2*, synaptonemal complex element 2; *SYCE3*, synaptonemal complex element 3; *SYCP1*, synaptonemal complex protein 1; *SYCP2*, synaptonemal complex protein 2; *SYCP2*-like, synaptonemal complex protein 2-like; *SYCP3*, synaptonemal complex protein 3; *SYCP3*-like, synaptonemal complex protein 3-like; *SYCP3Y*, synaptonemal complex protein 3 y; *Tex12*, testis expressed 12; *TSHB*, thyroid stimulating hormone, beta; *Xlr*, X-linked lymphocyte-regulated; *Xmr*, *Xlr*-relate, meiosis regulate.

* Corresponding author.
E-mail address: aaron.casey@adelaide.edu.au (A.E. Casey).

http://dx.doi.org/10.1016/j.gene.2015.04.089
0378-1119/

mature into axial associations (Rockmill et al., 1995) that become initiation sites for SC formation. As the cell enters zygotene, homologous chromosomes continue to be pulled into close proximity, particularly at telomeric ends which focus to a single pole (Scherthan et al., 1996). As homologous sequences associate, the transverse filaments and corresponding central elements begin to assemble (Heyting et al., 1988; Offenberg et al., 1991). At pachytene the SC is fully formed between autosomal pairs while the sex chromosome SC is restricted to pseudoautosomal regions (PARs) (Solari, 1970b), with only axial elements being present along the entire length of the chromosomes (Solari, 1970b). At diplotene, the central elements and transverse filaments disassemble with chromosomal association maintained by chiasmata, the sites of recombination (Solari, 1970a). By late diplotene, the axial elements are also lost thus completing the role of the SC in meiosis of most species (Solari, 1970a). In metatherian mammals which lack a sex chromosome PAR, the SC proteins SYCP3 and SYCP1 are essential in maintaining sex chromosome association through diplotene via formation of the 'dense plate' (Solari and Bianchi, 1975; Page et al., 2003; Page et al., 2006). This structure tethers the sex chromosomes until anaphase I after which time the dense plate dissolves (Page et al., 2006).

The ultrastructure and mode of assembly of SCs is conserved from yeast to humans (von Wettstein et al., 1984), with recent findings confirming sequence homology between all studied Metazoa, particularly for SYCP1 and SYCP3 (Fraune et al., 2012). Further study of central element proteins revealed SYCE2 and Tex12 arose as early as Eumetazoa (all but the simplest forms of animals); SYCE1 in Bilateria (animals with an anterior/posterior and dorsal/ventral polarity) and SYCE3 much later in Teleostomi (jawed vertebrates excluding cartilaginous fish) (Fraune et al., 2013). Of all the component SC proteins, SYCP3 is the most highly conserved at the amino acid (aa) level and can be split into several functional domains. Two such domains are involved in self association; an N-terminal 6aa (Nt6) and a C-terminal 6aa (Ct6) domain with two other domains being responsible for DNA binding; basic patch 1 (BP1) and basic patch 2 (BP2) (Tarsounas et al., 1997; Baier et al., 2007; Syrjänen et al., 2014). The first conserved self-association motif (Nt6) reveals that 5 of 6 amino acids are conserved between zebrafish and human, with the middle amino acid substituted for a similar polar neutral amino acid. The second conserved self-association motif (Ct6) has 100% similarity at the amino acid level between zebrafish and human. Heterologous expression of mutated SYCP3 (either at the Nt6 or Ct6) in mitotic cell cultures results in failure of the proteins ability to polymerise and form 5–10 nm fibres (that resemble SYCP3 ultrastructures observed in meiotic cells) and thus such mutations are predicted to affect the role of SYCP3 in the meiotic cell (Yuan et al., 1998; Baier et al., 2007; Syrjänen et al., 2014). The first DNA binding motif (BP1, KXXKKR) is conserved from zebrafish to human. The second DNA binding motif (BP2, KRKR) shows 100% conservation at the amino acid level between zebrafish and human. SYCP3 deletion results in impaired axial element assembly leading to defects in synapsis, chiasmata formation, chromosome condensation (Yuan et al., 2000; Liebe et al., 2004) and ultimately meiotic arrest and sterility in males, while in females aneuploidy is observed with a resulting increase in embryonic death (Yuan et al., 2002). Recently SYCP3 has been shown to exist as a tetramer consisting of four-helix bundles and coiled coil motifs with DNA binding N-terminal sequences that protrude from each end of the core (Syrjänen et al., 2014). In conjunction with the ability of these tetramers to interact with one another, SYCP3 is proposed to provide the structural framework for the axial element structure.

The platypus has 21 autosomal pairs and an extraordinary sex chromosome system comprising 5X and 5Y chromosomes that form an alternating chain ($X_1Y_1X_2Y_2X_3Y_3X_4Y_4X_5Y_5$) during meiosis (Grutzner et al., 2004). Previously we have shown this complex forms in a highly ordered stepwise manner during zygotene (Daish et al., 2009). This pairing is mediated by multiple PARs of varying size, with some yet to be identified, specifically between the X_5Y_5 and X_4Y_4 chromosome pairs. This has interesting implications for SYCP3 function given that marsupials, who also lack a detectable PAR, form a dense plate enriched with SYCP3 (Page et al., 2003) and SYCP1 (Page et al., 2006) to maintain X and Y chromosome proximity. Here we identify and characterise key SC component genes and report the expression of three distinct copies of SYCP3 in platypus testis.

2. Materials and methods

2.1. Genome database search

All genome database searches were carried out on the Ensembl Asia Database website (http://asia.ensembl.org/index.html).

2.2. Domain prediction

To obtain domain predictions, amino acid sequences were entered into InterPro (http://www.ebi.ac.uk/interpro/) using default settings.

2.3. Sequence alignments

Sequence alignments were carried out using "Geneious version 6.1.8" (http://www.geneious.com, Kearse et al., 2012) and MAFFT (Katoh et al., 2002). For protein alignments the algorithm was set to "Auto", the scoring matrix set to "BLOSUM62", the gap open penalty set to "1.53" and offset value set to "0.123". For nucleotide alignments the algorithm was set to "Auto", the scoring matrix to "200PAM/k = 2", gap open penalty to "1.53" and offset value to "0.123".

2.4. Phylogenetic tree generation

Trees were created using "Geneious version 6.1.8" (http://www.geneious.com, Kearse et al., 2012) using the MrBayes plugin (Huelsenbeck and Ronquist, 2001) with the rate matrix (fixed): Poisson, rate variation: gamma, outgroup: zebrafish, gamma categories 4, chain length: 1,100,000, subsampling frequency: 200, heated chains: 4, burn-in length: 100,000, heated chain temp: 0.2, random seed: random, branch lengths ~ exponential: 10, and shape parameter ~ exponential: 10

2.5. Immunocytochemistry

Meiotic spreads were performed using a dry down protocol (Peters et al., 1997) with a paraformaldehyde (pH 10) for increased spreading of platypus material. Immunostaining was carried out as previously described (Schoenmakers et al., 2009) with the following antibodies: rabbit anti-mouse SYCP1 (Novus, NB3000-229), mouse anti-human SYCP2 (Abcam, ab67694), mouse anti-hamster SYCP3 (Novus, NB100-2065), rabbit anti-mouse SYCP3 (Abcam, ab15092), guinea pig anti-SYCP3 (serum, a kind gift from Christa Heyting). All primary antibodies were used at a 1:200 dilution. For rabbit primary antibodies, either Goat anti-Rabbit IgG (H + L) Secondary Antibody, Alexa Fluor® 568 conjugate (Life Technologies) or Goat anti-Rabbit IgG (H + L) Secondary Antibody, Alexa Fluor® 488 conjugate (Life Technologies) were used, for mouse primary antibodies, a Goat anti-Mouse IgG (H + L) Secondary Antibody, Alexa Fluor® 568 conjugate (Life Technologies) was used and for the guinea pig primary antibody, a Goat anti-Guinea Pig IgG (H + L) Secondary Antibody, Alexa Fluor® 488 conjugate (Life Technologies) was used. All secondary antibodies were used at a 1:400 dilution. All immunostaining experiments were carried out in mouse in parallel with platypus using the same paraformaldehyde, pH and antibody dilutions.