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Meiotic shugoshins differ from mitotic ones by arginine-reach C-terminal motif in yeast, plant, animals, and human

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Background. Shugoshins (SGOs) are proteins that protect cohesins located at the centromeres of sister chromatids from their early cleavage during mitosis and meiosis in plants, fungi, and animals. Their function is to prevent premature sister-chromatid disjunction and segregation. Meiotic SGOs prevent segregation of sister chromatids in meiosis I, thus permitting homologous chromosomes to segregate and reduce chromosome number to haploid set. The study focused on the structural differences among shugoshins acting during mitosis and meiosis that cause differences in chromosome behavior in these two types of cell division in different organisms.

Methods. A bioinformatics analysis of protein domains, conserved amino acid motifs, and physicochemical properties of 32 proteins from 25 species of plants, fungi, and animals was performed.

Results. We identified a C-terminal arginine-reach amino acid motif that is highly evolutionarily conserved among the shugoshins protecting centromere cohesion of sister chromatids in meiotic anaphase I, but not among mitotic shugoshins. The motif looks like “arginine comb” capable of interaction by hydrogen bonds with guanine bases in the small groove of DNA helix. Shugoshins in different eukaryotic kingdoms differ also in the sets and location of amino acid motifs and the number of α-helical regions in the protein molecule.

Discussion. Meiosis-specific arginine-reach motif may be responsible for formation of SGO-DNA nucleoprotein complex, thus protecting meiotic shugoshins from degradation during meiotic metaphase I and anaphase I, while mitotic SGOs have a motif with less number of arginine residues. This structural difference between meiotic and mitotic shugoshins, probably, could be a key molecular element of the prolonged shugoshin resistance to degradation during meiotic metaphase I and anaphase I and be one of the molecular elements causing the difference in chromosome behavior in meiosis and mitosis. The finding of differences in SGO structure in plant, fungi and animals supports idea of independent evolution of meiosis in different lineages of multicellular organisms.

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Abstract

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Introduction

After DNA replication in the S-phase of the cell cycle, the sister DNA molecules, chromatids, are held together until their disjunction occurs in anaphase of cell division. The phenomenon of holding chromatids together, called cohesion, depends on the complex of a few proteins named cohesins. They play a main role in cohesion, but the process depends on more than ten other proteins (Peters *et al*., 2008). Shugoshin (SGO) is one of them, and SGOs of different organisms have been state to make a family of more or less conserved proteins.

During cell division SGOs protect cohesion of centromere regions of sister chromatids up to the beginning of anaphase, while cohesion in chromosome arms is already lost as early as prophase. This order of events i**s** true for mitosis and the second division of meiosis (meiosis II), but not for the first meiotic division (meiosis I). Cohesion of sister centromeres is protected during metaphase and anaphase of meiosis I by some kind of meiosis-specific shugoshin. As the result, sister chromatids are incapable to separate, and homologous chromosomes, each consisting of two sister chromatids, move in their stead to the cell poles in meiotic anaphase I. The chromosome number is thereby reduced to a haploid set. Thus, the usual shugoshin function is essential for somatic cell divisions through all ontogenesis, but not for meiosis I. Proper segregation of homologous chromosomes in meiosis I depends on expression of a meiosis-specific shugoshin form (Gutiérrez-Caballero *et al*., 2012). Specific shugoshin function is active during only one division cycle, while the somatic function is restored in meiosis II. What is the difference between somatic (mitotic) and specific meiotic shugoshin forms? Some structural differences have been reported for particular proteins in particular biological species, while general rules have not been found yet. We aimed on

comparative analysis within a large pool of different SGOs, trying to find key structural differences between somatic and meiotic shugoshins.

The protein that protects pericentric cohesion of sister chromatids in meiosis I has been discovered experimentally by two independent research groups (Kitajima *et al*., 2004; Rabitsch *et al*., 2004) when studying meiosis in the yeast *Schizosaccharomyces pombe* and termed shugoshin (Sgo1). A direct BLAST search for its orthologs in proteomes of other eukaryotes has revealed related proteins only in two fungi species, *Saccharomyces cerevisiae* and *Neurospora crassa.* In addition, Sgo2 has been identified as a Sgo1 paralog (a form that occurs in mitosis) in *S. pombe* (Kitajima *et al*., 2004; Rabitsch *et al*., 2004). A comparison of Sgo1 and Sgo2 has shown similarity for two protein regions, a conserved C-terminal basic region and a less conserved N-terminal coiled coil (Kitajima *et al*., 2004; Rabitsch *et al*., 2004). These two domains were identified earlier in MEI-S332 protein (shugoshin) of Drosophila. The C-terminal domain was shown to be crucial for centromere localization of MEI-S332 protein (Kerrebrock *et al*, 1995; Tang *et al*, 1998).

Another bioinformatics method has been employed to further search for orthologs, considering the domain protein structure. The search has revealed related proteins in conventional genetic model species: *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the plant *Arabidopsis thaliana*, and mouse, as well as in humans (Kitajima *et al*., 2004; Rabitsch *et al*., 2004). Similar proteins have been found in 15 other eukaryotes, including fungi, animals, and plants (Watanabe, 2005; Hamant *et al*., 2005; Gomez *et al*., 2007; Wang *et al*., 2011; Gutiérrez-Caballero *et al*., 2012; Zamariola *et al*., 2014). The proteins all have only short similar motifs at the C ends of their molecules, and their N-terminal regions show even lower conservation apart from a coiled-coil structure. There are six conserved amino acid residues in the C-terminal region and only two in the N-terminal domain in these proteins. Therefore, this limited homology is functional (Kitajima *et al*., 2004).

Single shugoshin form, SGO1, occurs in some organisms (*S. cerevisiae, N. crassa,* and *Zea mays*), while two forms persist in some others, acting differently in mitosis and meiosis I. The meiotic form is SGO1 in the plant *A. thaliana* and yeast *S. pombe*, while SGOL2 (SGO-like 2) plays the same role in vertebrates, including *Homo sapiens*. SGO1 and SGO2 differ in size and the role during mitosis and meiosis.

Shugoshins work similarly, but interact with different partner proteins in mitosis and meiosis. In higher eukaryotic mitosis, shugoshin SGO1 is phosphorylated by kinases PLK1 (Polo-like kinase) and AuroraB. Phosphorylated SGO1 acts as a homodimer to bind with one serine/threonine protein phosphatase PP2A-B' molecule and is then directed to pericentromeric heterochromatin (Xu *et al*., 2009; Kateneva, Higgins, 2009; Tanno *et al*., 2010). Its binding to chromatin requires kinase Bub1 and proteins of the MCAK (**m**itotic **c**entromeric-**a**ssociated **k**inesin) complex. Shugoshin-associated PP2A dephosphorylates one of the cohesin complex subunits, stromalin SA2/STAG, and thus protects cohesin from ESL1/Separase cleavage (Sakuno, Watanabe, 2009; Yin *et al*., 2013). Shugoshin has additionally been identified as a conserved centromeric adaptor of the CPC (chromosomal passenger complex) (Tsukahara *et al*., 2010). The CPC is needed for proper chromosome segregation in mitosis (Gutiérrez-Caballero *et al*., 2012).

In meiosis, SGO2 is similarly phosphorylated by kinase AuroraB and similarly binds as a homodimer with phosphatase PP2A and MCAK complex. The complex dephosphorylates kleisin REC8 which is another subunit of the cohesin complex, to protect it from separase (cysteine protease) (Xu *et al*., 2009; Macy *et al*., 2009; Tanno *et al*., 2010; Klift, Marston, 2011). The association with pericentromeric heterochromatin requires the specific HP1 protein (Swi6 in the yeast *S. pombe*) and histone H2A phosphorylation at one amino acid residue by kinase Bub1 (Sakuno, Watanabe, 2009; Macy *et al*., 2009).

It was believed for a long time that shugoshins are a conserved protein family whose members have approximately the same function, localization, and protein partners, but show a moderate similarity only within the N- and C-terminal regions when compared among different plants and animals (Kitajima *et al*., 2004; Rabitsch *et al*., 2004; Watanabe, 2005; Hamant *et al*., 2005; Gomez *et al*., 2007; Wang *et al*., 2011). However, recent studies have revealed substantial differences in both amino acid sequence and certain accessory functions among shugoshins from different species (Gutiérrez-Caballero *et al*., 2012; Zamariola *et al*., 2013, 2014).

Shugoshins are only conventionally classified as meiotic and mitotic. In the yeast *S. pombe*, Sgo1 acts only in meiosis I indeed to protect centromeric cohesion, while Sgo2 occurs in both mitosis and meiosis, but performs other functions rather than protecting cohesion (Rabitsch *et al*., 2004; Watanabe and Kitajima, 2005; Sakuno and Watanabe, 2009). In *A. thaliana*, both of the shugoshin forms occur in meiosis, but only SGO1 protects cohesion. The mitotic function of SGO2 is still unclear (Zamariola *et al*., 2013; Cromer *et al*., 2013). In vertebrates, the two shugoshin forms occur in both mitosis and meiosis (Sakuno and Watanabe, 2009), SGOL2 protecting cohesion in meiosis I and playing many other roles (Gregan *et al*., 2008; Lee *et al*., 2008; Llano and Sherman, 2008; Sakuno and Watanabe, 2009; Klift and Marston, 2011; Gomez *et al*, 2014). There is no consensus as to the mitotic function of SGOL2. The other form, SGOL1, is similarly found in all cells in mice (Gregan *et al*, 2008). SGOL1 protects centromeric cohesion of chromatids in mitosis (Watanabe, 2005; Kitajima *et al*., 2006; McGuinness *et al*., 2005; Gutiérrez-Caballero *et al*., 2012) and possibly has additional functions (cited from Gutiérrez-Caballero *et al*., 2012) in many vertebrates. In particular, mammalian SGOL1 is involved in maintaining centriole cohesion (Macy *et al*., 2009).

In budding yeasts, the only shugoshin Sgo1 plays a minor role in segregation of homologous chromosomes during meiosis I, but is important for the sister kinetochore bias toward a biorientation (Kiburz *et al.*, 2008). According to Kitajima *et al*. (2004), Sgo1 plays an important role in mitosis as well. It is necessary for proper chromatids segregation, but does not act by protecting centromere cohesion in mitosis.

In *D. melanogaster* MEI-S332 has been described as a meiotic shugoshin. Its role in mitosis is a matter of discussion. Shugoshin mutants in *D. melanogaster* do not show any mitotic defects, and this protein does not protect mitotic centromere cohesion (Kerrebrock *et al*., 1995). Therefore, MEI-S332 is not essential for mitosis. This does not exclude the possibility that it contributes to congression, kinetochore biorientation, or spindle assembly in a nonessential manner (Nogueira *et al*., 2014). SGO1 of *O. sativa* (Wang *et al*., 2011) and *Z. mays* (Hamant *et al*., 2005) are dispensable for mitosis.

Thus, meiotic and mitotic shugoshin forms are recognized only with respect to their main function of protecting centromeric cohesion and only in certain organisms. We still tried to identify the structural features that would allow pooling meiotic shugoshins in one functional group.

A problem of the origin and evolution of meiosis is discussed, including the variation and evolution of several specific meiotic proteins like recombination proteins, proteins of synaptonemal complex, etc. (Marcon and Moens, 2005; Egel and Penny, 2007; Bogdanov *et al*., 2007; Grishaeva and Bogdanov, 2014). The objective of our work was accordingly to analyze the structural features of meiotic shugoshins by a set of bioinformatics methods, such as COBALT, CDART, MEME, COILS program, Mobile portal – charge, and Compute pI/Mw tool. In particular, we compared the extent of conservation among eukaryotic taxa for different shugoshin forms, classifying them by structure (SGO1 and SGO2) and by their function (meiotic and mitotic).

Materials and Methods

Shugoshin amino acid sequences were sought in the NCBI (http://www.ncbi.nlm.nih.gov/) and UniProtKB/TrEMBL (http://www.uniprot.org/uniprot/) databases. The search was performed by protein identifiers (IDs) reported for shugoshins or by key words. Because data on several proteins (with different IDs) were available from experimental articles and the databases for each eukaryotic species, an essential step was comparing the proteins retrieved for each species and choosing one for further analysis. A multiple sequence alignment was made for each of the shugoshin forms from one organism with the COBALT program (Cobalt Constraint-based Multiple Protein Alignment Tool, http://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi?CMD=Web).

In total, 32 shugoshins from the proteomes of 25 eukaryotic species were analyzed by bioinformatics methods. The species included 3 plants, 12 fungi, 5 invertebrates, and 5 vertebrates. Among more than 120 candidate proteins, we chose those that had been identified experimentally, had been recommended for shugoshins of the given species, or were the closest to the full size (Table 1).

Conserved functional domains of shugoshins were identified using the CDART program (Conserved Domain Architecture Retrieval Tool, http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?).

The set and order of conserved motifs in shugoshin molecules were determined using the MEME program (Multiple Em for Motif Elicitation, http://meme.nbcr.net/meme/tools/meme) with the following parameters: maximal number of motifs, 100; motif distribution in sequences, any number of repetitions; motif width, 6 to 300 amino acid residues. Default values were used for other parameters. Figures summarizing the MEME results are schematic and only approximately show the actual motif sizes because of their great variation.

The secondary structure of the proteins under study (the probability that an α-helical structure is formed) was identified using the COILS program (Prediction of Coiled Coil Regions in Proteins, http://www.ch.embnet.org/software/COILS_form.html) with a window width of 28 and default other parameters.

The static electrical charge distribution along a shugoshin molecule was studied using the "Mobile portal – charge" program of the Mobile Pasteur package (http://mobyle.pasteur.fr/cgi-bin/portal.py?#forms::charge) with the following parameters: window width, 25; data plotting, yes; and image format, png. Default values were used for other parameters.

The isoelectric point (pI) was determined using the Compute pI/Mw tool, which is available from the SIB Bioinformatics Resource Portal, ExPASy (http://web.expasy.org/compute_pi/).

Results

Functional domains of shugoshins

Knowing from the literature that the N- and C-terminal functional domains are moderately conserved among shugoshins, we tried to apply an apparently formal procedure to identifying the domains in the selected proteins (Table 1). However, CDART did not identify the domains in almost half of the proteins, including both predicted ones and proteins examined experimentally. The N-terminal functional domain was not detected in the SGO1 proteins of *A. thaliana*, *O. sativa*, and several invertebrate and vertebrate species (Table 2). The C-terminal domain was not found in the SGO1 proteins of Drosophila, certain fungi, and the snake *O. hannah* (in the last case, the failure was likely explained by the fact that only a truncated protein variant was available from the databases). Both of the domains were not detected in human SGOL2 by CDART, although the protein has been annotated as having an

N-terminal coiled-coil shugoshin domain in the NCBI database. The two domains were similarly not detected in chordate animal, ascidian *Ciona* SGO1. The ascidian protein has been predicted by bioinformatics methods, but has not been characterized in the NCBI annotation. Hence, to clear up the situation we analyzed other parameters reflecting the properties of the proteins.

Physicochemical properties of shugoshins

A study of the secondary structure and, in particular, the propensity to form an α-helix showed that the α-helix colocalizes with the N-terminal functional domain in the proteins that we found to have one (Table 2, Figure 1). The only exception is *Zea mays* shugoshin SGO1 where α-helix is located downstream the domain. We assumed on this ground that other α-helix-forming shugoshins similarly possess an N-terminal functional domain. Thus, all of the proteins examined were assumed to be shugoshins in fact and to possess an important N-terminal domain. The assumption pertains to both meiotic forms and all other shugoshins (Figures 2, 3).

Another important feature was revealed by studying the electrostatic charge distribution along the protein molecule in our protein set. An N-terminal positive charge peak preceded the α-helical region in almost all of the proteins (Figures 1-3, Table 2). Exceptions to this rule are observed when a protein molecule begins immediately with the domain and/or the α-helix. A positive charge peak either colocalized with the α-helix (in both of the *O. hannah* shugoshins) or was absent (in Drosophila and several fungal proteins) in this case. Meiotic shugoshins did not differ in this feature from other shugoshins. A positive charge peak showed a strong colocalization with the C-terminal domain in the proteins wherein the domain was identified by CDART (Figures 1, 2). In the proteins wherein CDART failed to detect a C-terminal domain, the domain was impossible to predict by charge distribution because many positive charge peaks were observed along a shugoshin molecule (Figure 3).

The isoelectric point (pI) was another parameter used in the analysis. The parameter varied greatly, from 6.55 to 9.25, in meiotic shugoshins (*S. pombe* and *A. thaliana* SGO1 and vertebrate SGOL2 proteins, Table 2). Still, their pI values were within the variation range observed for other shugoshins (from 5.15 to 9.87).

Conserved amino acid motifs in shugoshin molecules

In addition, the sets of conserved amino acid motifs were analyzed using MEME. Only a minor similarity was observed a) for meiotic shugoshins from the organisms that possess two shugoshin forms, and b) for shugoshins of *O. sativa*, *Z. mays*, and *D. melanogaster* that were shown to be truly meiotic. A C-terminal motif was the only motif traceable in all meiotic shugoshins (Figure 4, the motif is asterisked). Common N-terminal motifs, which coincided in location with functional domains (as observed in our separate study), were found only in vertebrates (Figure 4, the motifs are indicated with arrows).Even a lower similarity was found for mitotic shugoshins from the organisms that possess two shugoshin forms (Figure 5). The common C-terminal motif was not detected, while the N-terminal motif was observed only in vertebrates, except *D. rerio* (arrows).

The localization of the C-terminal functional domain (CDART program) and the conserved amino acid sequence motif (MEME program, Figure 4) were compared among meiotic shugoshins. Nearly coincident coordinates were observed for the two structural elements. The greatest deviation was one or two amino acid residues. The consensus sequence of the C-terminal motif was identified as RYRRRRACKPVSYKEPSLRCKMRR, being rich in arginine (R). We performed an analogous study of mitotic shugoshins in seven species having two shugoshin forms (Figure 5). The common C-terminal motif was detected only in vertebrates (SGOL1 Mm, Hs, Xl, and Dr; asterisked), and its consensus was identified as KRRCTAAVNYKEPTLASKLRRGDPFTDLCFLNSPIFKQ, having less arginine residues.

Two features were noted when comparing the two, meiotic and mitotic, shugoshin forms (Figures 4, 5, Table 2). First, fungi and plants stood quite apart because even the C-terminal motifs of their proteins slightly differed from those in vertebrates. The N-terminal motifs were also different. Taking into account other parameters of proteins (Table 2), one can note a far greater similarity within the SGO2 group and especially within the SGO1 group. As it is seen, pI values were high in the majority of the SGO1 proteins, amounting to 9 or more, either to 8 in fewer cases (Table 2, shadowed gray). The SGO2 proteins had lower pI values. On the other hand, additional α-helical structural fragments occurring in the central part of the molecule were more common in the SGO2 group (Table 2, shadowed gray). The N-terminal functional domain was more often undetectable by CDART in the SGO2 group.

Discussion

To study the variation of shugoshins in a broad evolutionary range of organisms, we employed a set of bioinformatics methods to analyze both structural and physicochemical features of the proteins. A diagnostic signature of all shugoshins was identified; namely, a positively charged region precedes an α-helix at the N end of the molecule. The signature was the most conserved among the shugoshins from the 25 plant, fungal, and animal species examined in our work. We did not observe the signature only in the shugoshins that had the α-helix at the very terminus of the molecule that was in *Drosophila melanogaster*, *Candida glabrata* and *Villosiclava virens* (Table 2).

Starting our *in silico* study of shugoshins, we observed that their N-terminal domains show an extremely low similarity even within a taxon. CDART failed to identify the N-terminal domain in 11 out of the 32 proteins in our set (Table 2). The observation is in line with the slight similarity reported for the N-terminal shugoshin domains in fungi in one of the earliest works on the shugoshin family (Rabitsch *et al*., 2004). The conventionally conserved shugoshin domain seems to vary greatly in primary structure among different eukaryotic kingdoms. Such a situation is not seldom with structural chromosomal meiotic proteins. For instance, proteins of the Scc1/RAD21/REC8 cohesin family differ in the set of conserved amino acid motifs even within the functional cohesin domain (Bogdanov *et al*., 2007; Grishaeva *et al*., 2007). The Scc3/SA/STAG stromalins, which belong to another cohesin family, are conserved only among vertebrates and show an extremely low similarity to their analogs found in early eukaryotes (Grishaeva *et al*., 2010).

While the primary structure, i.e. amino acid sequence, is low conserved among shugoshins, their secondary structure has features that are more typical. All members of the family have a distinct α-helix at the N end (Figures 1-3, Table 2). Two α-helical regions occur in tandem at the N end in the vertebrate SGOL2 proteins. In addition, α-helical regions are found in the central region of the shugoshin molecule in vertebrates, the rice *O. sativa*, and certain fungi (Table 2). Thus, the secondary structure is conserved indeed in the shugoshin family, but the structural pattern is equally characteristic of both meiotic and mitotic shugoshins.

Our analysis of the set of conserved amino acid motifs in shugoshins, the charge distribution along the protein molecule, and pI values allowed us to conclude that the functional classification of shugoshins into meiotic and mitotic lacks a structural basis apart from the fact that the meiotic proteins always have a small, highly conserved domain (or a motif when the domain is undetectable) at the C end (Figure 4, Table 2). The C-terminal domain/motif is short, approximately 30 amino acid residues, but it is necessary for the exact shugoshin localization in the centromeric region of chromosomes (Tang *et al*., 1998).

A greater similarity in several parameters is observed within shugoshin groups, SGO2 and especially SGO1. The most interesting features are shadowed gray in Table 2 and described in Results. Fungi and plants stay apart because their shugoshins display only a low

primary structure similarity to vertebrate shugoshins. Shugoshins apparently differ between different eukaryotic kingdoms.

As already mentioned in Introduction, partitioning of shugoshins into meiotic and mitotic groups is conventional, and shugoshins are recognized as meiotic and mitotic only by their main function of protecting centromeric cohesion and only in some organisms. It seems that protecting cohesion is not the most important function in the case of SGOL2, and that other functions are of greater significance, being acquired during evolution by Sgo2 of primitive eukaryotes, such as *S. pombe*. New functions of this shugoshin developed with genome complication. For instance, human SGOL2 recruits kinesin MCAK to the centromere, where MCAK depolymerizes spindle microtubules attached in an improper manner. In *X. laevis*, the same shugoshin regulates CPC-dependent spindle assembly (Gutiérrez-Caballero *et al*., 2012).

In contrast, SGOL1 preserved the function of protecting cohesion, but only in mitosis. The function is of importance indeed, given that meiotic cohesion is dissolved via two steps in higher eukaryotes, first in chromosome arms (the so-called prophase pathway) and then in the centromere. Yet the function was preserved by SGO1 and was not transferred to SGO2, as is evident from our findings. Gutiérrez-Caballero *et al*. (2012) have speculated that the original shugoshin function was protecting centromeric cohesion in meiosis and that the capability of protecting cohesion in mitosis was acquired by SGOL1 in vertebrates.

Conclusions

Historically, shugoshins were considered to be orthologs and to belong to a conserved family. However, recent studies showed that shugoshins have a low amino acid sequence homology and display functional differences. Their functions should therefore be considered individually for yeasts, flies, and vertebrates. In spite of their common name, shugoshins lack direct orthology and are highly diverse in amino acid sequence and functions (Gutiérrez-Caballero *et al*., 2012).

Thus, any information obtained by comparing the shugoshin structure for different organisms is of importance for understanding the actual functions and mechanisms of action of shugoshins. The conserved motif found by us in the C-terminal region of shugoshins is of particular interest in this respect, being conserved to the greatest extent in meiotic shugoshins, but not among mitotic shugoshins. This structural difference in meiotic and mitotic shugoshins, probably, can be responsible for resistance of shugoshin against degradation during meiotic metaphase I and anaphase I, providing differences in sister-chromatids behavior in meiosis I and mitosis.

Two directions of further investigation could be proposed. One is to test capability of meiotic shugoshins to interact with other accessory proteins that could protect shugoshins from degradation during meiosis I. Another way is to pay attention to possible association of meiosis-specific arginine-reach motif of shugoshins (found in our study) with centromere DNA during meiosis I. Seeman *et al*. (1976) hypothesized that, in the DNA major groove, an arginine side group can form hydrogen bonds with a guanine base. Thus, several clustered arginine residues can make “arginine comb”. Indeed, there is qualitative observation that arginine readily has high affinity to DNA. Recent thorough analysis (Suvorova *et al.*, 2015) confirms this conclusion. In this case, the arginine-guanine association could be involved in protection of meiotic shugoshin from degradation in meiosis I, while shugoshins of mitotic chromosomes, which have a motif with less number of arginine residues, lakes such kind of association.

We conclude that meiotic shugoshins are combined in one family by their function rather than by parameters characterizing their structure. Our results additionally indicate that either SGO1 or SGO2 evolved to act as a main meiotic form, the choice being made

independently in different multicellular lineages, designated by Cock *et al*. (2011), namely, red and brown algae, green algae/plants, fungi, and animals, and being determined by a yet unclear factor: capability of meiotic shugoshin to interact with another, accessory potein, or with DNA at centromere region.

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Table 1. Shugoshins chosen for further analysis, their names used in this work, sizes (amino acid residues, a.a.), and NCBI IDs

Protein	NCBI ID	Size, a.a.	Protein	NCBI ID	Size, a.a.
Sgo1 Sp[a]	Q9P7A0.1	319	SGO1 Dm (MEI-S332)	Q24141.1	401
Sgo2 Sp	O13734	647	SGO1 Ce	Q18412.2	307
Sgo1 Sc	Q08490.1	590	SGO1 Cb	Q60ZS1.1	306
Sgo1 Nc	Q872U8.1	774	SGO1 Bm	CDP98524.1	1107[b]
Sgo1 Ag	NP_984314.2	648	SGO1 Ci	XP_002129751.1	426[b]
Sgo1 Mg	EAA54538.1	552[b]	SGOL1 Xl (SGO-like)	NP_001090071.1	663
Sgo1 Mo	XP_003709333.1	544[b]	SGOL2 Xl	NP_001243696.1	1029
Sgo1 Vv	KDB14582.1	621	SGOL1 Oh, partial	ETE65485.1	553
Sgo1 Tv	EHK16025.1	636[b]	SGOL2 Oh, partial	ETE62590.1	874
Sgo1 Yl	CAG81849.1	823[b]	SGOL1 Dr	NP_001074089.	618
Sgo1 Tm	KFX44805.1	659	SGOL2 Dr	NP_001116771.1	847
Sgo1 An	Q5BDI1.1	479	SGOL1 Mm	Q9CXH7.1	517
Sgo1 Cg	Q6FMT2.1	603	SGOL2 Mm	Q7TSY8.1	1164
SGO1 At	NP_187655.2	572	SGOL1 Hs	Q5FBB7.1	561
SGO2 At	NP_196052.2	419	SGOL2 Hs	Q562F6.2	1265
SGO1 Os	ADO32586.1	486			
SGO1 Zm	Q4QSC8.1	474			

[a] Fungi: Sp, *Schizosaccharomyces pombe*; Sc, *Saccharomyces cerevisiae*; Nc, *Neurospora crassa*; Ag, *Ashbya gossipii*; Mg, *Magnaporthe grisea*; Mo, *Magnaporthe orizae*; Vv, *Villosiclava virens*; Tv, *Trichoderma virens*; Yl, *Yarrowia lipolytica*; Tm, *Talaromyces marneffei*; An, *Aspergillus nidulan*; and Cg, *Candida glabrata*; plants: At, *Arabidopsis thaliana*; Os, *Oryza sativa*; and Zm, *Zea mays*; an insect: Dm, *Drosophila melanogaster;* nematodes: Ce, *Caenorhabditis elegans*; Cb, *Caenorhabditis briggsae*; and Bm, *Brugia malayi*; an ascidian: Ci, *Ciona intestinalis*; vertebrates: Xl, *Xenopus laevis*; Oh, *Ophiophagus Hannah*; Dr, *Danio rerio*; Mm, *Mus musculus*; and Hs, *Homo sapiens.*

[b] The protein has been annotated as predicted or hypothetical or otherwise. In all other cases, the protein is a conventional shugoshin.

Table 2. Comparison of shugoshins by four parameters (colocalization of the α-helix and the N-terminal domain, the presence of a positive charge peak ahead them, colocalization of the positive charge peak and the C-terminal domain, and the isoelectric point, pI)

Shugoshin, eukaryotic species, protein function	N-terminal region			C-terminal region			pI
	Functional domain	α-Helix	Positive charge peak	Functional domain	α-Helix	Positive charge peak	
SGO1, Dm	+ [b]	+	–	–	–		8,80
SGO1, Ce	+	+	+/–	+	–	+	9,83
SGO1, Cb	+	+	+/–	+	–	+ [c]	9,69
SGO1, Bm	– [d]	+	+	+ [a]	–	+	7,97
SGO1, Ci	–	+	+	–	–		9,17
SGO1, Os	– [d]	+/–	+	+	+/– [h]	+	9,40
SGO1, Zm	+	+ [f]	+	+	–	+	9,39
Sgo1, Sc (Saccharomycetes)	+	+	+	+ [a]	–	+	9,26
Sgo1, Ag (Saccharomycetes)	+	+	+	+ [a e]	–	+	6,42
Sgo1, Yl (Saccharomycetes)	+	+	+/–	– [e]	+ [h]		9,13
Sgo1, An (Saccharomycetes)	+	+	+	–	–		6,18
Sgo1, Tm (Eurotiomycetes)	+	+	+	+ [a]	–	+	6,52
Sgo1, Cg (Eurotiomycetes)	+ [b]	+	–	+ [a]	+/– [h]	+	9,22
Sgo1, Nc (Sordariomycetes)	+	+	+	+ [a]	–	+	8,09
Sgo1, Mg (Sordariomycetes)	+	+	+	+	–	+	9,93
Sgo1, Mo (Sordariomycetes)	+	+	+	+	–	+	9,80
Sgo1, Tv (Sordariomycetes)	+	+	+	+	–	+	9,40
Sgo1, Vv (Sordariomycetes)	+ [b]	+	–	– [e]	–		9,74
Sgo1, Sp, meiotic	+	+	+	+	–	+	8,91
SGO1, At, meiotic	–	+	+	+	–	+	9,25
SGOL1, Xl, mitotic	+	+/- (2)	+	+	+/– [h]	+	9,55
SGOL1, Oh, mitotic (partial)	–	+ (2) [b]	+ [g]	–	–		6,26
SGOL1, Dr, mitotic	–	+ (2)	+	+	–	+	9,87
SGOL1, Mm, mitotic	+	+ (2)	+	+	+ [h]	+	9,65

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SGOL1, Hs, mitotic	+	+ (2)	+	+	+[h]	+	9,27
Sgo2, Sp, mitotic	+	+	+	+	+/-[h]	+	5,15
SGO2, At, mitotic	–	+	+	+	–	+	9,56
SGOL2, Xl, meiotic	–	+	+	+	+ [h]	+	7,35
SGOL2, Oh, meiotic	–	+ [b]	+/– [g]	+	+[h]	+	9,12
SGOL2, Dr, meiotic	–	+	+	+	-	+	6,55
SGOL2, Mm, meiotic	+	+	+	+	+/- [h]	+	8,97
SGOL2, Hs, meiotic	–	+	+	–	+/-[h]		8,09

Shadowed are the most important protein characteristics. For details, see the text
+, distinct peak.
+/–, small peak.
(2), double peak.
[a] The shugoshin domain is displaced to the central region of the molecule.
[b] The shugoshin domain or α-helix occur at the start of the molecule.
[c] Peaks of positive charge are placed before and after the shugoshin domain.
[d] A domain other than the shugoshin domain colocalizes with the α-helix.
[e] There is an additional domain(s) other than shugoshin domain.
[f] An α-helix is located after the shugoshin domain.
[g] A positive charge peak colocalizes with α-helix rather than precedes it.
[h] There are additional α-helices in the central region of the molecule.
The organisms are designated as in Table 1.

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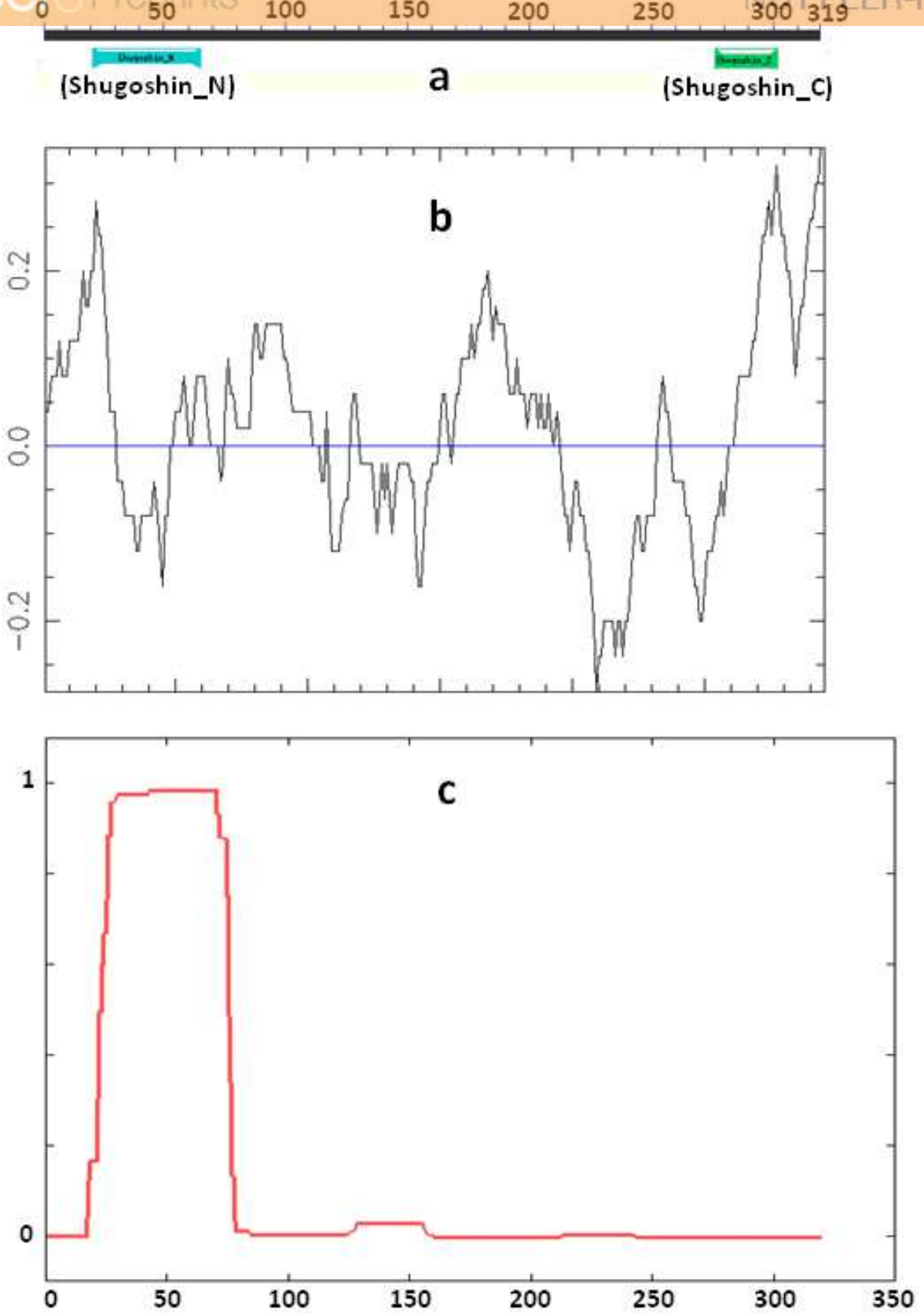

Figure 1. Three parameters of the *S. pombe* Sgo1 meiotic shugoshin
(a) The N- and C-terminal functional domains, (b) the electrostatic charge distribution along the protein molecule, and (c) the probability for an α-helical structure to be formed. Abscissa, amino acid sequence. Ordinate, charge (b) or probability (c).

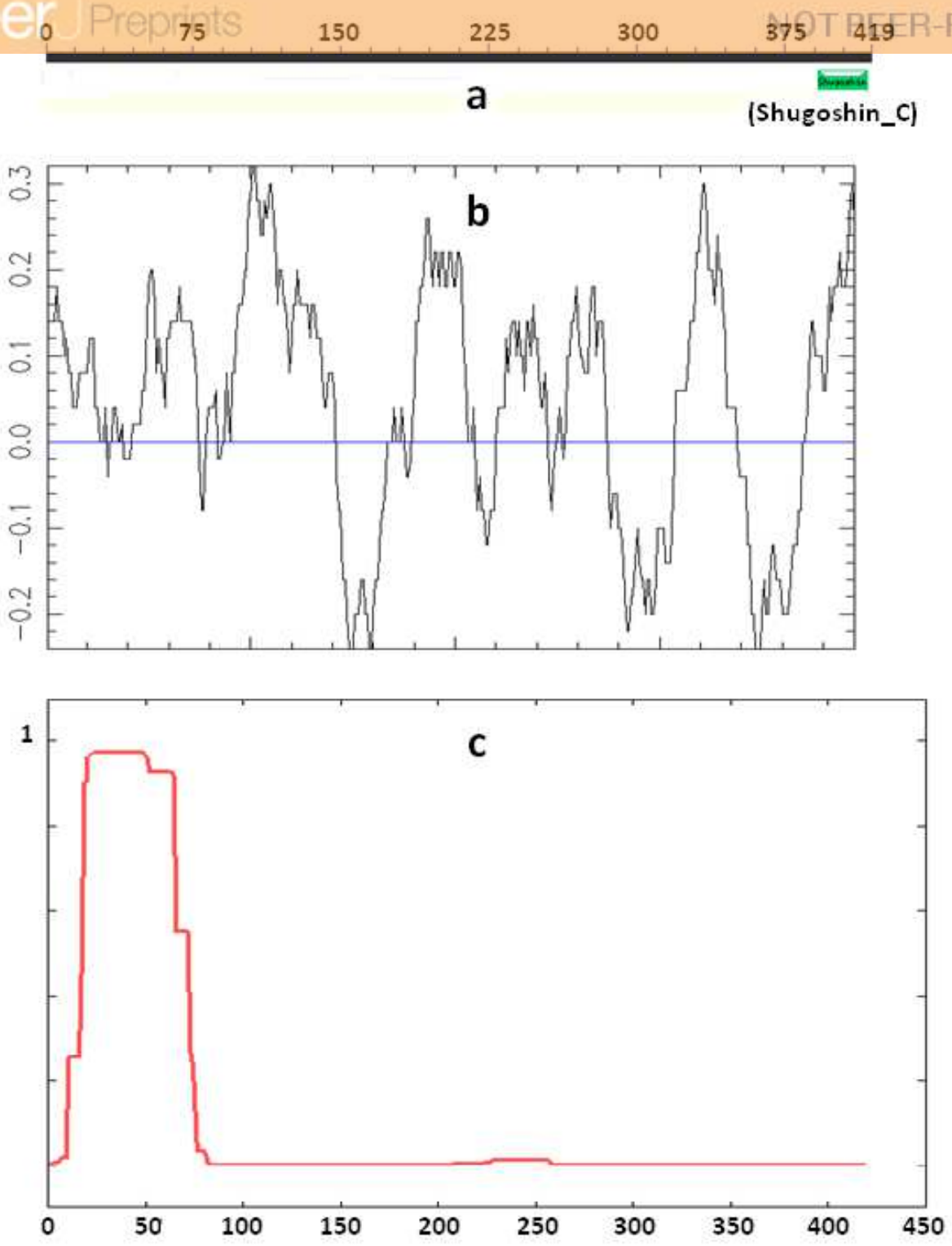

Figure 2. Three parameters of the *A. thaliana* SGO2 mitotic shugoshin
The N-terminal functional domain is absent from the protein. Designations are as in Figure 1.

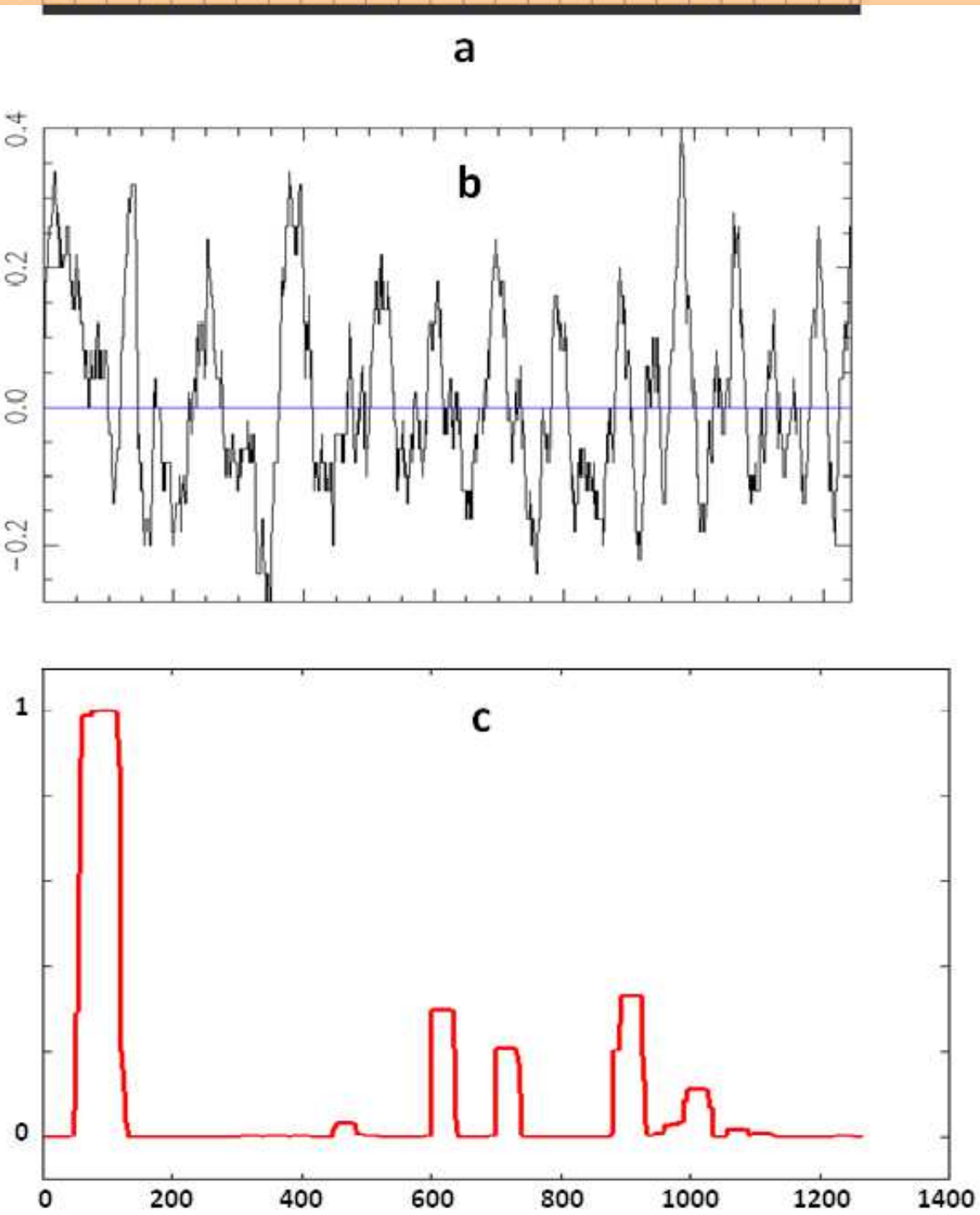

Figure 3. Three parameters of the *H. sapiens* SGOL2 meiotic shugoshin
Designations are as in Figure 1. Both N- and C-terminal functional domains are absent. The formation of α-helices is observed not only in the N-terminal, but also in the central region of the molecule.

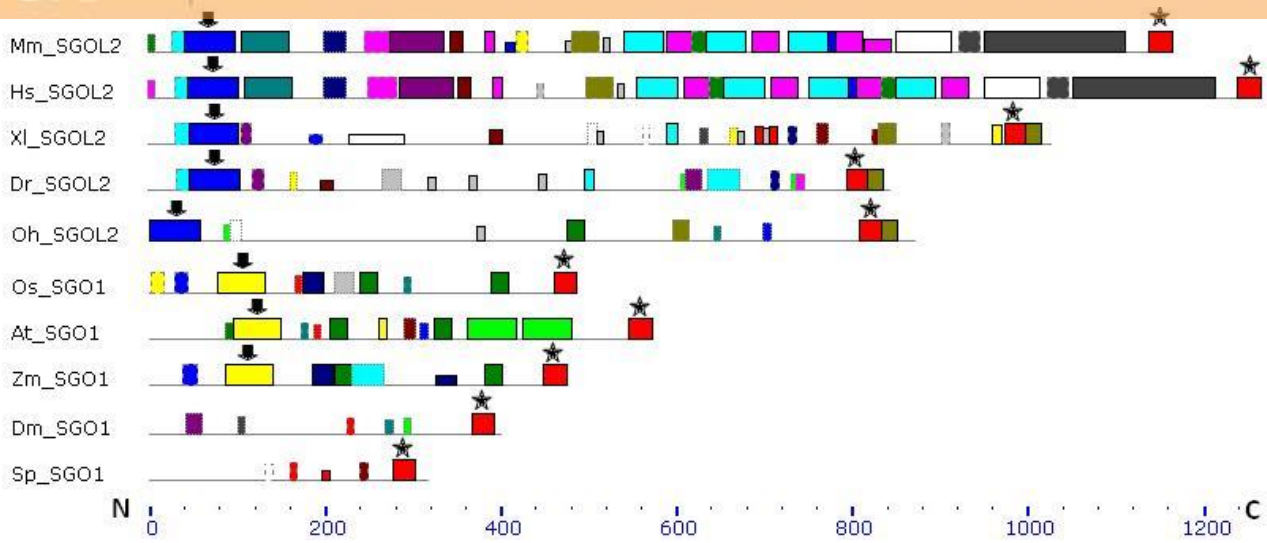

Figure 4. Order of conserved amino acid motifs in meiotic shugoshins including those of *O. sativa*, *Z. mays*, and *D. melanogaster*

Species are indicated as in Table 1. A scale shows the amino acid sequence of a protein from the N toward the C end. Similar motifs are shown with bars of the same color and size. See text for more details.

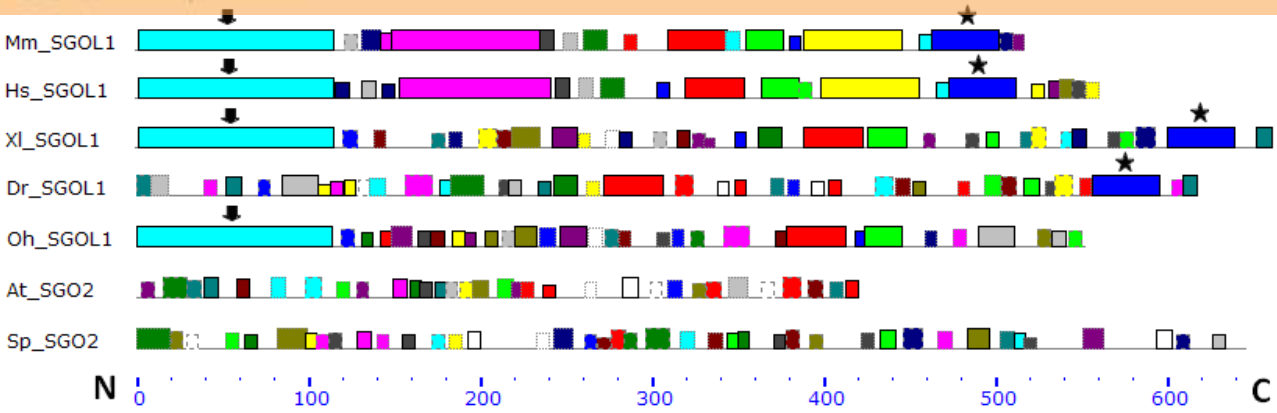

Figure 5. Order of conserved amino acid motifs in mitotic shugoshins from species having two shugoshin forms

Species are indicated as in Table 1. A scale shows the amino acid sequence of a protein from the N toward the C end. Similar motifs are shown with bars of the same color and size. See text for more details.