



OBSERVATION ARTICLE

REVISED Possible identification of CENP-C in fish and the presence of the CENP-C motif in M18BP1 of vertebrates. [version 2; referees: 3 approved]

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Abstract

The centromeric protein CENP-C is a base component of the kinetochore. This protein, along with CENP-A has been shown to adaptively evolve in a number of animal and plant species. In order to determine if CENP-C also evolves in fish species, I attempted to retrieve fish CENP-C sequences from GenBank. No Teleostei CENP-C sequences were found either by name or by BLASTP searches with the vertebrate CENP-C motif sequence. A number of putative Teleostei protein sequences were identified in GenBank that have homology to the C-terminal cupin domain of vertebrate CENP-C. These proteins only have partial homology to the CENP-C motif, but evidence is presented that makes it likely that these fish proteins are orthologs of CENP-C. Interestingly, it was also discovered that the CENP-C motif sequence is also mostly present in M18BP1 proteins of fish and some other vertebrates but not in mammals. This finding may have implications for CENP-C and M18BP1 assembly in centromeric regions of different vertebrate taxa.

Open Peer Review

Referee Status:

	Invited Referees		
	1	2	3
version 2 published 20 Jan 2016	 report	 report	 report
version 1 published 05 Aug 2015	 report		

- 1 **Tatsuo Fukagawa**, Osaka University
Japan
- 2 **Nikolina Sekulic**, University of Oslo
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Competing interests: No competing interests were disclosed.

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REVISED Amendments from Version 1

This update incorporates revisions suggested by the reviewer, includes common name categories of species names utilized in this study in the legend to Figure 1 and makes a clear distinction between lobe-finned fishes in which CENP-C contains the "CENP-C motif" vs. non-lobe-finned fishes (specifically teleost fishes and shark) which lack an obvious "CENP-C motif".

See referee reports

Introduction

The kinetochore is a structure that connects chromosomal centromeric DNA to microtubules during mitosis and meiosis¹. The centromere is epigenetically defined by the deposition of nucleosomes that contain the histone H3 variant CENP-A². Centromeric protein CENP-C is required for both the recruitment of new CENP-A to the centromeric region as well as the initial assembly of the kinetochore. The CENP-C protein is generally considered to be ubiquitous in all eukaryotic taxa since homologs of CENP-C have been identified in yeast³ and *Drosophila*⁴ as well as many plants and vertebrates⁵. While CENP-C evolves so rapidly that very little homology is observed between distantly related taxa, a conserved CENP-C motif has been identified across all lineages studied⁵. This conserved motif should, therefore, be of utility to identify CENP-C orthologs in other species.

CENP-A has been initially shown to evolve adaptively in *Drosophila*⁶, in members of the Brassicaceae family⁷ and more recently in primates⁸ and in percid fishes^{9,10}. CENP-C has also been shown to evolve adaptively in a number of animal and plant species⁵ as well as in primates⁸. In an effort to determine if CENP-C also evolves adaptively in fish species, searches were conducted in GenBank for Teleostei proteins that had been already identified as CENP-C or for genes that had been annotated as coding for CENP-C. No such teleost fish proteins or genes were found. BLASTP searches of just the Teleostei subset of GenBank were performed with the

conserved vertebrate CENP-C motif and these too failed to find identified fish CENP-C proteins or genes. However, a set of orthologous C-terminal cupin domain containing genes have been identified in the elephant shark *Callorhynchus milii* and several teleost fish species that, while lacking most of the conserved CENP-C motif, have features that make these likely to be fish CENP-C orthologs.

Methods

Standard BLASTP searches were performed on the NCBI blast server. The vertebrate CENP-C motif NVRRTKRXRLKPLEYWRGERVBY used in BLASTP searches in this study was obtained from an alignment of 25 species including the lobe-finned fish *Latimeria chalumnae*, amphibians, reptiles, birds and mammals (Supplementary File S1). Retrieved sequences were aligned with the MUSCLE alignment feature in Geneious (version 6.1) sequence analysis software.

Results and discussion

BLASTP searches with the vertebrate CENP-C motif identified CENP-C proteins from a variety of taxa, including plants, but did not identify any CENP-C in non-lobe-finned fish lineages. It is possible that CENP-C may be absent in ray-finned fish, but the ubiquity of this protein in other lineages and the central role of this protein in centromeric function make this unlikely. A C-terminal cupin domain protein encoded by a shark gene annotated in GenBank as CENP-C was used to identify homologs in Teleostei genomes by BLASTP. The retrieved teleost fish homologs were annotated as either calponin homology domain containing protein, neurofilament heavy polypeptide-like protein, or myb-like protein. The cupin domains of these proteins share significant homology to the cupin domain of other vertebrate CENP-C proteins (Figure 1). Within vertebrate CENP-C proteins the RxxRxxxxPLxYWxGERxxY sequence defines identities within the CENP-C motif located within about 100 amino acids upstream of the cupin domain (Figure 2). However, within the shark and teleost fish C-terminal cupin domain-containing protein sequences, only some of these CENP-C motif sequence identities were present (Figure 3) and, therefore, unambiguous identity of these proteins as CENP-C was not obvious.

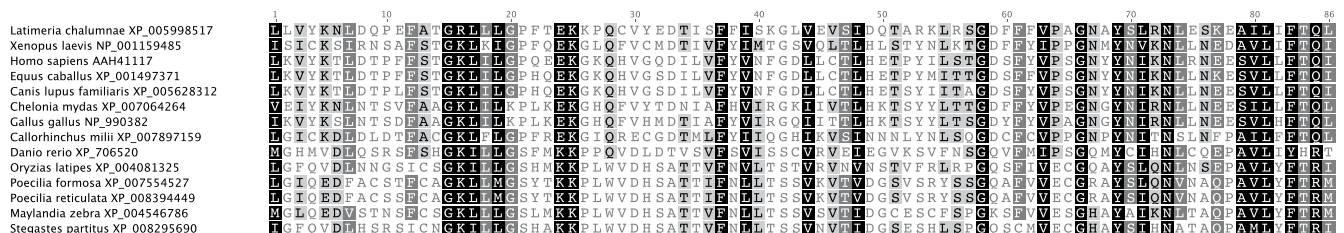


Figure 1. Homology of shark and teleost fish putative CENP-C cupin domains to the cupin domains of other vertebrate CENP-C proteins. Lobe-finned fish: *Latimeria chalumnae*. Amphibian: *Xenopus laevis*. Mammals: *Homo sapiens*, *Equus caballus*, *Canis lupus familiaris*. Reptile: *Chelonia mydas*. Bird: *Gallus gallus*. Shark: *Callorhynchus milii*. Teleost fish: *Danio rerio*, *Oryzias latipes*, *Poecilia formosa*, *Poecilia reticulata*, *Maylandia zebra*, *Stegastes partitus*.

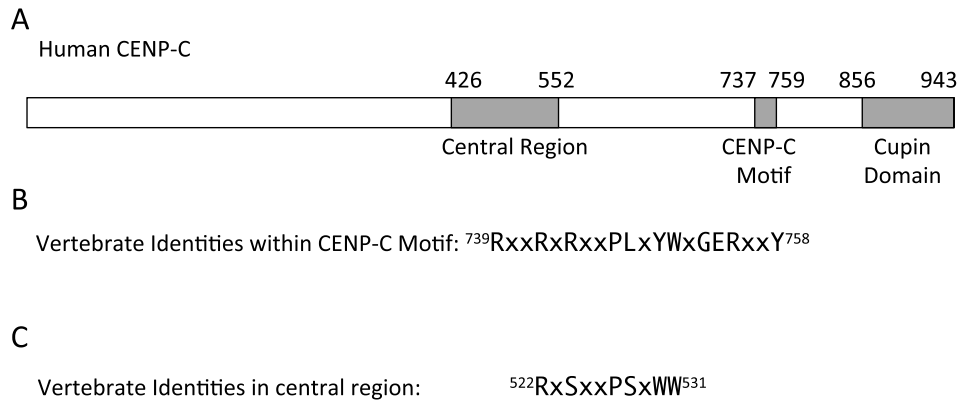


Figure 2. Conserved domains and sequences in vertebrate CENP-C. (A) Diagram of human CENP-C. (B) Amino acids that are identical in the CENP-C motif in vertebrates in which CENP-C has been identified. (C) Conserved sequence in the CENP-C central region that is homologous to part of the CENP-C motif. Amino acid locations within human CENP-C protein of conserved sequences are indicated at the beginning and end of each sequence.

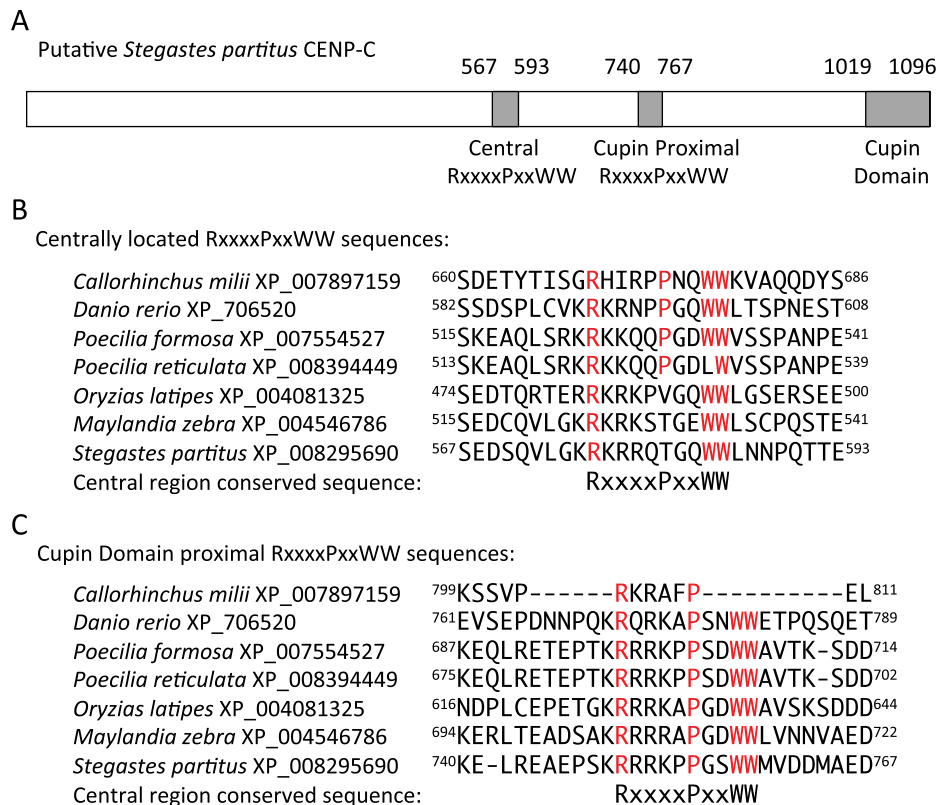


Figure 3. Conserved regions homologous to a portion of the CENP-C motif present in putative non-lobe-finned fish CENP-C. (A) Diagram of putative *Stegastes partitus* CENP-C. (B) Alignment of fish central region sequences that contain the conserved RxxxxPxxWW sequence. (C) Alignment of the fish cupin domain proximal sequences that contain the conserved RxxxxPxxWW sequence. Amino acids matching the conserved sequence identities are highlighted in red. Amino acid locations within each species' protein are indicated at the beginning and end of each sequence.

In a recent study that examined the interaction between CENP-C conserved domains and CENP-A containing nucleosomes (or nucleosomes containing histone H3 modified with a CENP-A C-terminal tail), Kato *et al.*¹¹ identified within the conserved central region of CENP-C a RxxSxxPSxWW consensus sequence (Figure 2) that is similar to the core portion of the CENP-C motif. Mutations of the arginine to alanine or the tryptophans to alanine in this sequence prevented the binding of this central region to the nucleosomes. So, functionally, the RxxxxPxxWW portion of the central region sequence is important to centromeric binding of CENP-C. Furthermore, mutations of the arginine, tyrosine and tryptophan in the core CENP-C motif RxxxxPxxYW also reduce the binding affinity the CENP-C to the nucleosomes¹¹. A mutation of arginine to alanine in this core portion of the CENP-C motif was previously shown to prevent the binding of *Xenopus* CENP-C to centromeres¹².

An alignment of the putative shark and teleost fish CENP-C proteins identified two conserved regions that contained the RxxxxPxxWW sequences (Figure 3). The placement of these sequences corresponds roughly to the locations of the central portion and the CENP-C motif of the vertebrate CENP-C (Figure 2). Therefore, it is likely that the combination of the C-terminal cupin domain and the presence of these centromeric nucleosome binding regions in positions generally corresponding to the locations of the central region and the CENP-C motif identifies these teleost genes as possible CENP-C orthologs. It will be necessary, of course, to verify if this protein is actually found at non-lobe-finned fish centromeres. It should be noted, however, that the distance between the cupin domain and the “CENP-C motif” position is about twice as long in the putative teleost fish CENP-C in comparison to this distance in CENP-C of other vertebrates. It is interesting that the

putative shark “CENP-C motif” location lacks the tryptophans of the RxxxxPxxWW sequence and that *Poecilia reticulata* has a replacement of the first tryptophan in the conserved central region sequence (Figure 3). However, depending on other factors acting in the assembly of the centromere in various taxa, it may be possible that just one of those conserved RxxxxPxxWW sequences may be necessary for centromeric binding of the putative non-lobe-finned fish CENP-C. Indeed, the conserved central region is not present in chicken CENP-C¹³ and no homology to the RxxxxPxxWW portion of the conserved central region is detectable in CENP-C of other birds and reptiles. Yet a deletion mutant of chicken CENP-C in which the central portion had been removed was able to rescue CENP-C deficient chicken cells and also co-localized with CENP-T at centromeres¹³. This demonstrates that, at least in chickens, the central region is not necessary for CENP-A binding and, presumably, the C-terminal region which contains the CENP-C motif is sufficient for that purpose.

Interestingly, BLASTP searches of the Teleostei subset of GenBank retrieved centromeric protein M18BP1 sequences. This protein is recruited to centromeres by CENP-C^{14,15} and along with centromeric proteins Mis18 α and Mis18 β functions in the recruitment of CENP-A to centromeres¹⁶. The M18BP1 protein contains almost the entire vertebrate CENP-C motif in all vertebrates examined except in mammals (Figure 4). It appears that the CENP-C motif sequence is not exclusive to just CENP-C. Since both CENP-C and M18BP1 associate with centromeres and with each other, it is tempting to speculate that what has generally been regarded as a CENP-C motif sequence facilitates the interaction of both of these proteins with centromeric nucleosomes. Furthermore, since mammalian M18BP1 lacks this CENP-C motif, it is possible that mammalian M18BP1

CENP-C <i>Latimeria chalumnae</i> XP_005998517	WRSKRMRVRLPEYWRGERVDYKLSPPSGGLVVEGVLS
CENP-C <i>Xenopus laevis</i> NP_001159485	RRSKRTRVKPLAYWKGervNYKIRPSGGFLVEGVVP
CENP-C <i>Chelonia mydas</i> XP_007064264	RRTKRIRLKPLeYWRGERVNYMMRPSGGFVVGIVS
CENP-C <i>Gallus gallus</i> NP_990382	RRTKRIRLKPLeYWRGERVTYTLKPSGRLLISGIAG
CENP-C <i>Homo sapiens</i> NP_001803	RRTKRTRLKPLeYWRGERIDYQGRPSGGFVISGVLS
CENP-C <i>Canis lupus familiaris</i> XP_005628310	RRTKRTRLKPLeYWRGERIDYHGRPSGGFVIGGILS
CENP-C <i>Equus caballus</i> XP_001497371	RRTKRTRLKPLeYWRGERIDYQGRPSGGFVIGGILS
M18BP1 <i>Latimeria chalumnae</i> XP_005992785	SRSGRPIIPPLEYWRGQRI-----VIDHASD
M18BP1 <i>Xenopus laevis</i> NP_001243215	SRSGRLLIKPVLKYWCGERV-----VTDRNLN
M18BP1 <i>Chelonia mydas</i> XP_007066720	SRSGRRIKPPLQYWCQRE-----FVDRKLN
M18BP1 <i>Gallus gallus</i> XP_421481	TRSGRLVKPPLSFWCGERE-----FVDRELN
M18BP1 <i>Callorhinchus milii</i> XP_007901995	SRSGRCIKAPLeYWRGQRI-----VVDSQLK
M18BP1 <i>Esox lucius</i> XP_010877796	SRSGRLLKPLeYWKGARV-----ILDSDLN
M18BP1 <i>Danio rerio</i> XP_005158813	SRSGRLLIKPLeYWKGGRI-----VMDSEMN
M18BP1 <i>Oryzias latipes</i> XP_004082593	SRSGRVIKPLeYWRGGRV-----LLDAEMN
M18BP1 <i>Stegastes partitus</i> XP_008282233	SRSGRVIKPLeYWKGGRV-----ILDAFMN
M18BP1 <i>Homo sapiens</i> NP_060823	CHSNCQNKPTLR-----FPDDQVN
M18BP1 <i>Equus caballus</i> XP_001915365	CHSNHQNPPLR-----LPDDQIS
M18BP1 <i>Canis lupus familiaris</i> XP_003435173	CHGNCQNKPPGR-----LPDDQIN

Figure 4. Alignment of regions of vertebrate CENP-C and M18BP1 that contain the CENP-C motif sequences. The vertebrate CENP-C motif sequence identities (Figure 2A) are highlighted in red.

may be more dependent on association with CENP-C to localize to the centromere than the M18BP1 of other vertebrate taxa.

Competing interests

No competing interests were disclosed.

Grant information

This study was supported by a Faculty Research Grant funded by the University of West Georgia.

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary material

Supplementary File S1. Alignment of the C-terminal portion of vertebrate CENP-C proteins.

The vertebrate CENP-C motif containing consensus sequence utilized in BLASTP searches spans amino acids 11 to 33 and is highlighted in red.

[Click here to access the data.](#)

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Open Peer Review

Current Referee Status:



Version 2

Referee Report 22 April 2016

doi:10.5256/f1000research.8419.r13524



Inna Lermontova

Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany

The CENP-C protein is required for the assembly and proper function of kinetochores in most eukaryotes. In this paper the author has identified the putative CENP-C protein homologue of Teleostei using BLASTP searches. This protein does not contain a typical CENP-C motif, but a motif with a partial homology to CENP-C. However, presence of the Cupin domain at the C-terminus and two CENP-C-like motifs at the central and proximal regions, respectively, have allowed the author to conclude that the identified protein is a CENP-C ortholog.

Additionally, the author has identified the CENP-C motif in M18BP1 proteins of fishes and some other vertebrates. It is interesting that the CENP-C motif cannot be identified in M18BP1 of mammals. From my point of view, this is very important finding of the current study. Therefore, I recommend to show in the paper the structure of the M18BP1 protein homologues of vertebrates with the SANTA, SANT and CENP-C domains. These domains are present in M18BP1 proteins of most vertebrates, but M18BP1 proteins of mammals have only SANTA and SANT domains and in *Latimeria chalumnae* only SANTA and CENP-C domains are present. I suggest to mention this in the paper and to discuss it.

I think the study is a significant contribution in the field of centromere research and the information presented in the paper is applicable by experimental biologists.

Minor comments:

- I suggest to change the title to: "Identification of the putative CENP-C protein homologue in fish and the CENP-C motif in M18BP1 of vertebrates."
- Abstract: "[...]but evidence is presented that makes it likely[...]" Please write which evidence.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 04 April 2016

doi:10.5256/f1000research.8419.r12934





Nikolina Sekulic^{1,2}

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² Biotechnology Centre, University of Oslo, Oslo, Norway

The paper describes identification of putative CENP-C proteins in fish and points to the presence of CENP-C motif in M18BP1 protein of many vertebrates but not mammals.

CENP-C is an essential protein in building centromeres that interacts directly with CENP-A, epigenetic mark of centromeres, and its underlining DNA. Lack of conservation between CENP-C of different species has been attributed to adaptive evolution of the protein. Great evolutionary variability has made it difficult to identify CENP-C proteins based only on simple sequence homology search. The author has noticed the lack of annotated CENP-C protein in fish and undertook an effort to use targeted database searches to identify putative CENP-C proteins.

The paper fills in the gap of knowledge in the field. It presents interesting and valuable findings but I suggest a couple of changes that would help improve it.

1. The central region of the human CENP-C should be labeled as 426-537 and not 426-552. For references see Carroll CW *et al.*, 2010¹; Kato H *et al.*, 2013²; Falk SJ *et al.*, 2015³; Falk SJ *et al.*, 2016⁴.
2. The author couldn't identify CENP-C motif in the putative proteins in fish but has instead identified a region that is more similar to the central region. Here previous findings on the role of central region and CENP-C motif should be clarified, emphasizing that central region specifically binds CENP-A nucleosomes (which targets it to the centromere) (References: Carroll CW *et al.*, 2010¹; Falk SJ *et al.* 2015³) while CENP-C motif binds both types of nucleosomes non-selectively (H3 and CENP-A) with lower affinity (Kato H *et al.*, 2013²). This can be followed with an exception in the chicken system, as suggested by the previous reviewer, Tatsuo Fukagawa.

In this context, I find the absence of CENP-C motif less surprising, since the major function of CENP-C protein (its association with CENP-A nucleosomes) comes from central region anyway – making identified candidate proteins very likely fish CENP-C homologs. Subsequently, identification of the CENP-C motif in M18BP1 proteins is also less surprising knowing that it might just be a nucleosome binding module.

- In my opinion, region that has similarity to central region should be named “central region-like motif” instead of “cupin-proximal region”. I find this name very confusing.

Minor comments:

1. For the more lay audience, specify that teleost is the most populated branch of fish phylogenetic tree
2. In figures 1 and 4, make a distinction between fish and other species, and indicate amino-acid specifications within each species protein like in figures 2 and 3.

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I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 28 January 2016

doi:[10.5256/f1000research.8419.r11994](https://doi.org/10.5256/f1000research.8419.r11994)



Tatsuo Fukagawa

Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan

I found that author almost addressed my concerns. The quality of paper has been improved and the paper is of indexable quality.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 25 September 2015

doi:[10.5256/f1000research.7336.r10498](https://doi.org/10.5256/f1000research.7336.r10498)



Tatsuo Fukagawa

Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan

The centromere protein CENP-C is well known as an essential component for functional kinetochore assembly. Due to importance of this molecule, CENP-C must be conserved in Fish species. The author performed BLASTP searches with the conserved CENP-C motif sequence, but any CENP-C homologues in Fish lineages were not identified with this sequence. However, as there is a putative CENP-C sequence in shark genome, BLASTP searches were carried out with C-terminal domain sequence of putative shark CENP-C. Then, the author identified CENP-C candidates from various teleost genomes. Although the author does not show that candidate proteins localize to centromeres, these candidates contains related sequences of CENP-C motif, which were a little divergent from the vertebrate consensus sequences.

Interestingly, the authors found that various vertebrate M18BP1s, which are recruited to centromeres by CENP-C, contain the CENP-C motif sequence, but mammalian homologues do not contain the motif sequence.

This is an observation article and finding of the CENP-C motif in the M18BP1 sequence is interesting. However, to improve the quality of the paper, the author should revise the manuscript. My specific concerns are following.

1. As the author recognizes, it is necessary to verify whether CENP-C candidates from teleost genome really localize to Fish centromeres. As the author obtained a candidate from Zebrafish genome, such an experiment is not difficult with the Zebrafish experimental system. If the author added localization data, Figure 2 would be interesting.
2. Related to Figure 2. If the author shows sequence comparison of Cupin domain in teleost sequences, it would be helpful.
3. When the author discuss about central region of human CENP-C, it may be better to cite a recent paper by Nagpal *et al.* (Mol. Biol. Cell, 2015), which says that central region sequence does not exist in chicken CENP-C. Then, the author can emphasize that the central motif is really important for CENP-A binding.
4. The author described some results of the analysis in the Introduction. This is not necessary and it would be better to cut of redundant description.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 12 Jan 2016

Leos Kral, University of West Georgia

I have revised the manuscript to address concerns #2, #3 and #4. Unfortunately, I do not have the resources to carry out the localization experiment (concern #1). My main motivation in publishing these observations is to bring awareness of this issue to individuals who may have the resources and interest to follow up with the relevant experiments.

Competing Interests: No competing interests were disclosed.