Original Research Article

Mechanistic Insights Into HORMAD1-mediated Meiotic Regulation Using AlphaFold

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ABSTRACT

Meiosis is an essential process for gametogenesis and fertility, requiring a network of proteins that coordinate homologous chromosome pairing, synapsis, and recombination. The HORMA domain-containing protein HORMAD1 plays a key role in these events by localizing to unsynapsed chromosomal axes, promoting synaptonemal complex formation, and activating meiotic checkpoints. Disruptions in HORMAD1 function have been linked to infertility, yet the molecular basis of its interactions and regulation remains incompletely understood. In this study, AlphaFold was used to investigate how HORMAD1 recognizes its own closure motif and interacts with known meiotic partners. Validation with canonical and mutant peptides confirmed that AlphaFold accurately predicts established binding behaviors. Screening of potential closure motif–like sequences revealed novel candidate interactions with MCM9, SYNP2, and ATR, while IHO1 and BRCA1 showed minimal binding confidence. Additionally, modeling of phosphorylated peptides suggested that posttranslational modification can disrupt HORMAD1's autoinhibitory conformation and regulate its interactions with other meiotic factors, potentially regulating its activity during meiosis. These findings demonstrate that AlphaFold can recapitulate known biochemical results and predict new interaction candidates, providing a structural framework for future experimental validation. Understanding HORMAD1's binding specificity and regulation advances insight into the molecular mechanisms underlying meiotic control and fertility disorders.

Keywords: HORMAD1; meiosis; AlphaFold; fertility; posttranslational modifications

INTRODUCTION

Meiosis is a fundamental biological process that ensures the accurate segregation of chromosomes and the generation of haploid gametes, making it necessary for sexual reproduction (1). This process depends on a complex network of proteins that coordinate homologous chromosome pairing, synapsis, and recombination. Among these proteins, HORMA domain-containing protein 1 (HORMAD1) has emerged as a crucial regulator of meiotic progression. HORMAD1 localizes to unsynapsed chromosomal regions, facilitates the formation of the synaptonemal complex (SC), and supports the activation of meiotic checkpoints. Disruptions in its function can have consequences for gametogenesis and fertility (2, 3).

Recent studies suggest that mutations in HORMAD1 or alterations through post-translational modifications may impair its ability to regulate meiotic checkpoints and chromosome synapsis (4). Such defects can

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contribute to infertility by promoting aneuploidy, meiotic arrest, or defective gamete formation (5). Despite these implications the precise molecular mechanisms by which HORMAD1 orchestrates key meiotic events, and how its dysregulation leads to fertility disorders, remains poorly understood.

Computational modeling offers a powerful approach to address these gaps. While biochemical experiments have illustrated many aspects of HORMAD1 function, including the identification of interaction partners, they do not provide the direct mechanism for how HORMAD1 associates and functions through these interactions (5, 6). Standard experimental techniques to elucidate the structural basis for protein-protein interactions require labor intensive processes and optimization. Further, they can be limited in capturing dynamic interactions (7).

This paper explores the use of structural prediction tools to recapitulate biochemical experiments involving mutations to residues involved in protein-protein interactions. By using Alphafold, this study predicts the mechanism of HORMAD1 interactions with its known meiotic factors by evaluating potential closure-motif recognition and the effect of posttranslational modifications on these interactions (8). This provides a rapid method to generate hypotheses about regulatory mechanisms that can then be tested experimentally. This approach allows researchers to evaluate aspects of HORMAD1 regulation that remain inaccessible through traditional methods, providing a complementary framework to understand the molecular basis of infertility. By understanding these mechanisms, we can better determine the molecular basis of certain infertility disorders and open pathways for potential diagnostic or therapeutic advances.

LITERATURE REVIEW

Overview of Meiosis and Gametogenesis

Meiosis is a specialized form of cell division that reduces the chromosome number by half, producing haploid gametes essential for sexual reproduction (1). Proper meiotic progression is critical for maintaining genomic integrity and generating genetic diversity, both of which are fundamental to evolutionary fitness. This process involves homologous chromosome pairing, synapsis, recombination, and activation of meiotic checkpoints, all of which are tightly regulated to prevent chromosomal abnormalities and infertility (9, 10). Errors in meiosis can result in gametes with incorrect chromosome numbers, a major cause of miscarriages and congenital disorders in humans (10).

The orchestration of meiosis requires the coordinated action of numerous proteins that regulate chromosomal behavior and ensure successful gametogenesis. Among these, HORMA domain-containing protein 1 (HORMAD1) has emerged as a central regulator of meiotic progression, particularly during prophase I (10, 11). HORMAD1 plays key roles in chromosome synapsis, recombination, and meiotic checkpoint activation, and its localization to unsynapsed chromosome axes is essential for maintaining the integrity of meiotic processes (7). By regulating these critical steps, HORMAD1 ensures accurate segregation of homologous chromosomes and prevents the formation of defective gametes (4, 8).

HORMAD1 Function in Chromosome Synapsis

HORMAD1 plays a pivotal role in the assembly and stabilization of the synaptonemal complex (SC), a protein scaffold that physically links homologous chromosomes during prophase I (5). Proper formation of the SC is crucial for recombination and the precise segregation of chromosomes. In both mouse and human studies, HORMAD1 has been shown to interact with SC proteins such as HORMAD2 and SYCP2, which together stabilize the SC and ensure homologous chromosomes remain aligned until recombination is complete (6, 7). Without HORMAD1, chromosomes fail to synapse properly, resulting in meiotic arrest or the production of aneuploid gametes (4, 9).

Mechanistically, HORMAD1 contains a HORMA domain that functions like a “safety belt,” enabling it to encircle and bind chromosomal axes and other meiotic proteins through its closure motif (10). This structural configuration allows HORMAD1 to recruit and stabilize key meiotic factors at unsynapsed regions, including checkpoint proteins that monitor synapsis and recombination progression (11, 12). By dynamically localizing to sites of incomplete synapsis, HORMAD1 not only reinforces the structural integrity of the SC but also signals the presence of unsynapsed regions, coordinating the activation of meiotic checkpoints and ensuring proper recombination and genomic integrity (7, 11). Further, HORMAD1 contains its own closure motif at its C-terminus, which can interact with the safety belt in an autoinhibited state. This state enables further regulation and can be perturbed by post-translational modifications (10, 13).

Beyond its structural role, HORMAD1 is instrumental in meiotic checkpoint activation. It recruits DNA damage response proteins, including ATR, BRCA1, and RAD51,

to unsynapsed chromosome regions, initiating signaling pathways that monitor and respond to errors in synapsis or recombination (7, 12, 14). This surveillance function is critical; in its absence, meiotic checkpoints fail, allowing defective gametes to form, which can lead to infertility or developmental abnormalities (4, 8). In mouse models, the lack of HORMAD1 results in premature disassembly of the SC, defective recombination, and meiotic arrest at the pachytene stage, highlighting its central role in maintaining meiotic fidelity (4, 9).

Post-Translational Regulation of HORMAD1

HORMAD1 activity is finely tuned by post-translational modifications, which regulate its function during various stages of meiosis. Phosphorylation of HORMAD1 is a key mechanism for modulating its interactions with other meiotic proteins and its binding to chromosome axes (6, 13). Specific phosphorylation events can enhance or inhibit HORMAD1's ability to support synapsis and checkpoint signaling, demonstrating the complexity of its regulation (14). One key example includes phosphorylation of HORMAD1's C-terminal closure motif (13).

In addition to phosphorylation, SUMOylation has been shown to stabilize HORMAD1 on unsynapsed chromosomes during late prophase I (10). This modification ensures the protein continues to support checkpoint activity until synapsis is complete. Dysregulation of post-translational modifications, whether through mutation or environmental factors, can compromise HORMAD1 localization and function, resulting in meiotic errors (9, 14). Recent studies also suggest that ubiquitination may contribute to the temporal turnover of HORMAD1, allowing the cell to remove the protein from chromosomes once its role in checkpoint activation and synapsis has been fulfilled (12).

Genetic Mutations and Meiotic Defects

Genetic mutations affecting HORMAD1 function have been linked to reproductive disorders in both humans and animal models. Biallelic loss-of-function mutations in HORMAD1 are associated with spermatogenic arrest in men, underlining its essential role in male fertility (11). In mice, Hormad1 knockout models show meiotic arrest at the pachytene stage, resulting in infertility in both sexes (12). These findings demonstrate that HORMAD1 is indispensable for both male and female gametogenesis (4, 8).

Mutations in HORMAD1 disrupt both recombination and checkpoint function, leading to the production of aneuploid gametes or meiotic arrest (9, 12). In females, dysregulated HORMAD1 expression in oocytes has been linked to impaired homologous recombination and synapsis, potentially reducing oocyte quality and contributing to infertility (5, 7). Such defects underscore the importance of HORMAD1 for maintaining genome integrity and ensuring successful gametogenesis.

Species Comparisons: Humans and Mice

Mouse models have been invaluable in studying HORMAD1, as gene manipulation allows researchers to observe meiotic consequences *in vivo* (4, 9). Many HORMAD1 functions are conserved between humans and mice; however, human studies reveal additional complexity. Variations in HORMAD1 expression or partial loss-of-function mutations may have sex-specific effects, with differences in checkpoint sensitivity and SC dynamics between males and females (7, 11). These species differences must be considered when applying findings from animal models to human infertility research.

Knowledge Gaps and Study Objectives

Despite considerable progress, significant gaps remain in understanding HORMAD1 function. The precise molecular mechanisms coordinating SC assembly, checkpoint signaling, and recombination remain incompletely defined (6, 12). Specifically, the molecular basis of HORMAD1 protein-protein interactions that govern its role in synapsis, checkpoint activation, and regulation by post-translational modifications is poorly understood due to lack of structural information (10, 13). Several interaction partners such as HORMAD2 and MCM9 have annotated closure-motif-like sequences. BRCA1, ATR, SYCP2, and IHO1 have been characterized as direct interaction partners of HORMAD1, but the structural basis for their interactions remains unknown (12, 14). It is unknown whether subtle variation in closure motif sequences can be tolerated by HORMAD1 and may be present in these meiotic factors. Further, it is not clear whether these sites may be regulated by post-translational modifications analogous to HORMAD1's own closure-motif to regulate the protein-protein interactions required for meiosis progression (13).

Additionally, the consequences of complete HORMAD1 loss are well documented. It is less understood how partial loss-of-function mutations or subtle dysregulations of post-translational modifications mechanistically affect gametogenesis (12). The molecular basis of HORMAD1-associated infertility in humans

remains to be fully elucidated (14). Future research should focus on the dynamic interactions of HORMAD1 with other meiotic proteins, the role of specific post-translational modifications, and sex-specific differences in function. Addressing these gaps could lead to improved diagnostic approaches for infertility and inform potential therapeutic strategies aimed at correcting meiotic defects (10, 12).

Computational modeling, particularly with AlphaFold, provides an opportunity to address these gaps by predicting the structural interactions between HORMAD1 and its potential binding partners (15). By evaluating canonical and candidate closure motifs in silico, AlphaFold can reveal which residues contribute to binding specificity, highlight potential regulatory mechanisms such as phosphorylation-induced conformational changes, and suggest novel interaction candidates for experimental follow-up. This approach allows mechanistic insight beyond what has been achieved in prior biochemical studies and complements existing in vivo and in vitro research (15).

This paper addresses the unknown mechanism for how HORMAD1 interacts with its known interaction partners. Using knowledge of canonical closure-motif sequences, potential interaction peptides among known HORMAD1 interactors were predicted. AlphaFold was used to determine whether HORMAD1 could recognize these peptide sequences and was able to identify several novel peptides as potential HORMAD1 interaction candidates that should be tested experimentally (16).

METHODS AND MATERIALS

Research Approach and Design

This study employs a computational and literature-based methodology to investigate the role of HORMAD1 in meiosis, its functional interactions, and how structural alterations may contribute to fertility disorders. By combining protein structural prediction, sequence analysis, and literature synthesis, this approach provides detailed mechanistic insights without requiring experimental manipulation of human gametes. The study focuses on understanding HORMAD1's domain organization, potential regulatory interactions, and the structural consequences of post-translational modifications or mutations (9).

Sources of Data

UniProt: Protein sequences and functional annotations for HORMAD1 and known partner proteins (BRCA1, ATR, SYCP2, IHO1, MCM9) were retrieved. Known interaction motifs and post-translational modifications were identified from literature and UniProt annotations.

AlphaFold (ColabFold implementation): Structural models of HORMAD1 and predicted complexes with short peptides corresponding to closure motifs from partner proteins were generated using AlphaFold (14) through the ColabFold implementation. Models were evaluated using three confidence metrics:

ChimeraX (v1.5): Structural models were visualized using UCSF ChimeraX to assess spatial orientation of closure motifs, interaction interfaces, and domain organization. Distance measurements and residue accessibility were analyzed for key binding regions.

Literature Review: Peer-reviewed studies were used to identify known HORMAD1 interaction partners, validated closure motif sequences, and to contextualize AlphaFold predictions (9).

Motif Identification and Structural Analysis

Candidate closure motifs within partner proteins were identified by aligning sequences to canonical motifs, specifically looking for serine residues followed by glutamic acids in HORMAD1 interacting proteins that were previously reported in the literature. Short peptide sequences corresponding to these motifs were used in AlphaFold to predict binding to HORMAD1. Models were scored and interpreted based on pLDDT, pTM, and ipTM values to evaluate both structural reliability and interaction confidence (Table 1). Peptide sequences, model scores, and predicted binding outcomes are summarized in Table 2.

RESULTS

Structural predictions of HORMAD1 bound to its own closure motif

To validate the ability of AlphaFold to predict HORMAD1 closure motif interactions, several controls were first performed. Full-length HORMAD1 was modeled to verify that the protein adopted its expected self-closed conformation, with the C-terminal closure motif engaged by the HORMA domain safety belt, recapitulating the experimentally determined X-ray crystal structure (13) (Figure 1).

When residues 1–235 of HORMAD1 were modeled in isolation, the domain structure was maintained but the autoinhibitory interaction was absent, as expected. Inclusion of the canonical closure motif peptide sequence (VPKRRKFSEPKEHI) with HORMAD1 residues 1–235

Table 1. Predicted structural and interaction confidence metrics for HORMAD1 by AlphaFold-Multimer, including pLDDT and ipTM scores to assess model reliability and potential binding interfaces.

Metric	Description	Interpretation/Typical Range
pLDDT	evaluates the accuracy of local residue-level structure	>80: high confidence (dark blue) 50–70: low confidence (yellow) <50: very low confidence (orange/red)
pTM	measure of confidence in the relative domain arrangement within a single protein chain	Higher values indicate more reliable domain placement
ipTM	onfidence in predicted inter-chain interactions, such as protein–protein or protein–peptide binding	>0.7: high confidence 0.5-0.7: moderate confidence <0.5: low confidence; unlikely to bind

Table 2. Predicted structural and interaction confidence metrics for HORMAD1 and its putative meiotic protein partners as generated by AlphaFold-Multimer. Peptide sequences represent candidate closure motifs or motif mutants used to evaluate HORMAD1's predicted binding specificity. Sequences were chosen based on known binders (canonical, MCM9), putative motifs, or engineered mutations.

HORMAD1 Residues	Peptide Sequence	Peptide Type	ipTM	pTM	Purpose of Test	Interpretation
1–235	ESKTRS	Canonical closure motif (WT)	0.73	0.81	Positive control to test AlphaFold's ability to predict known binding	Strong prediction of binding to canonical motif
1–235	RKFSEPK	S26K safety-belt mutant	0.61	0.79	Test whether AF2 detects decreased binding caused by mutation	Predicted interaction reduced compared to WT
1–235	AAPKEH	SE→AA mutant (negative control)	0.39	0.80	Test whether complete motif disruption prevents binding	Low ipTM indicates little/no predicted binding
1–235	EKSEPG	MCM9 verified motif	0.61	0.81	Test if HORMAD1 recognizes known MCM9 binder	Moderate ipTM suggests possible binding
1–235	SESKSK	MCM9 putative motif	0.40	0.80	Test if HORMAD1 binds weaker predicted MCM9 motif	Minimal predicted interface
1–235	SLLQNSEGKRGTGAGG	SYNP2 motif 1	0.41	0.81	Test if HORMAD1 interacts with SYNP2	No strong predicted interaction
1–235	LRRSES LSEKQVK	SYNP2 motif 2	0.68	0.82	Test second SYNP2 motif	Possible predicted interaction
1–235	CMLPKSAALSEK LKFREFLLPR	ATR motif	0.65	0.81	Test whether HORMAD1 binds ATR closure-like motif	Moderate ipTM suggests potential interaction

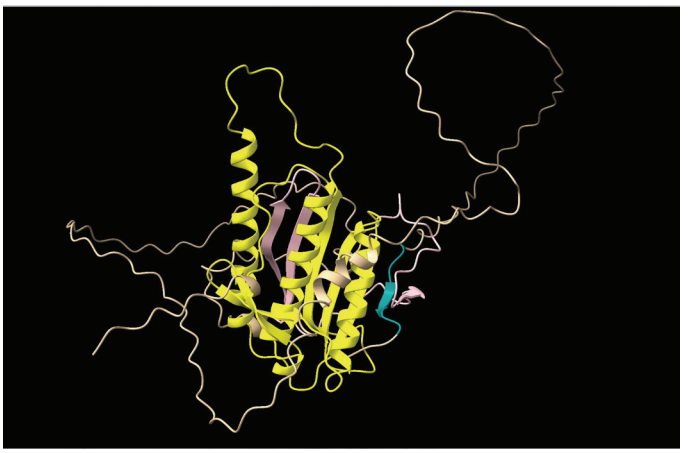

Figure 1. ChimeraX Prediction of HORMAD1 bound to canonical closure motif peptide. The ChimeraX prediction of HORMAD1 residues 1-391(chain A). The yellow colored structure (residues 12-179) are the HORMA domain. The pink structure (residues 180-229) is the safety belt. The teal structure (residues 385-391) is the closure motif.

resulted in high inter-chain confidence (ipTM = 0.73, pTM = 0.81), consistent with binding (Figure 2). In contrast, pairing the canonical sequence with the HORMAD1 S26K mutant produced a moderately decreased ipTM (0.61), suggesting reduced binding capacity, consistent with previous biochemistry.

Further, a mutated peptide in which the SE residues were replaced by alanines (SE→AA) (SSQESVPKRRKFAAPKEHI) yielded a very low ipTM (0.39), indicating that AlphaFold did not predict a confident interaction, consistent with the loss of binding observed in biochemical experiments. Further, when comparing the pLDDT scores for the canonical closure motif versus the SEtoAA peptide, only the canonical closure motif has a high confidence score. The expected position error of the canonical closure motif is low with respect to the HORMAD1 residues whereas the SEtoAA peptide has a high expected position error further supporting that the canonical motif interacts with HORMAD1 whereas the SEtoAA peptide does not (Figure 3). These results confirmed that AlphaFold

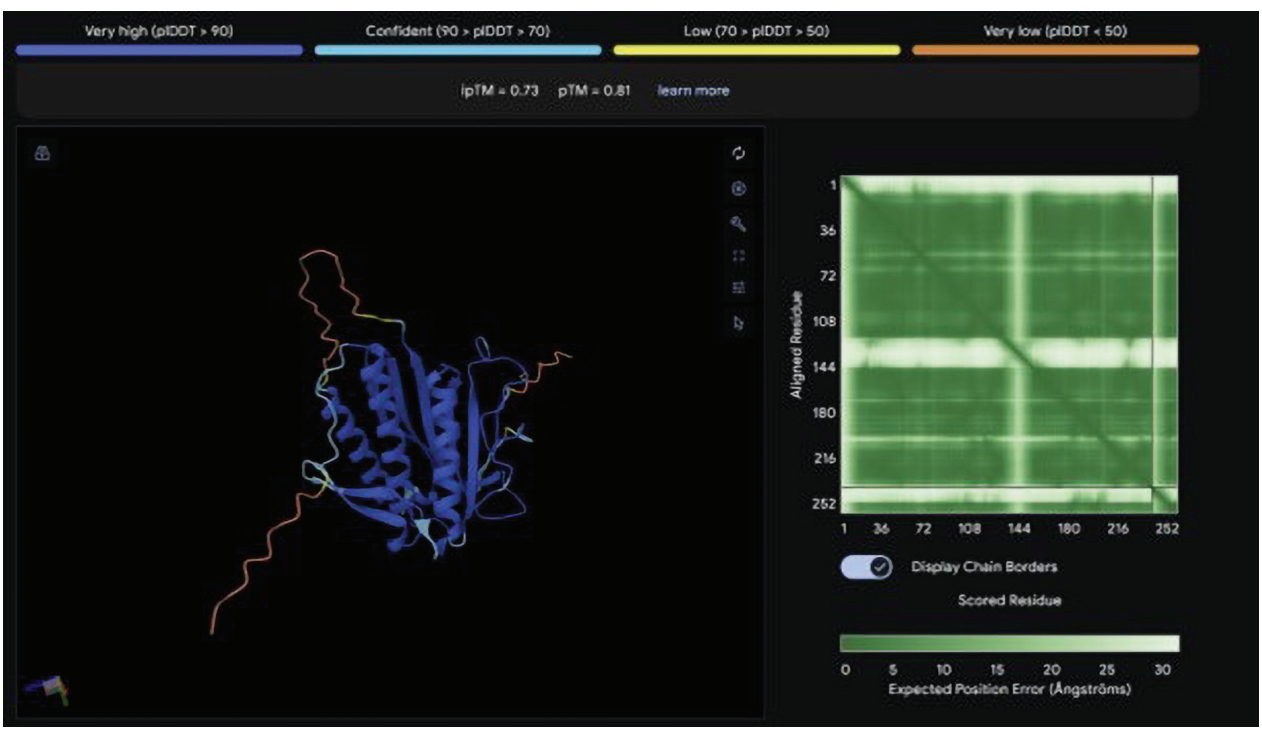

Figure 2. AlphaFold Prediction of HORMAD1 bound to canonical closure motif peptide. The AlphaFold prediction of HORMAD1 residues 1-235 (chain A) and closure motif sequence ESKTRS (chain B) have an ipTM score of 0.73 and pTM score of 0.81. The predicted structure is colored by pIDDT score where Hormad1 residues in the HORMAD domain and safety belt have pIDDT scores greater than 90 or high confident range and the closure motif has pIDDT scores greater than 70 or in the confident range. The expected position error of the closure motif is low.

could recapitulate known HORMAD1 binding behaviors observed in *in vitro* biochemical experiments.

Structural predictions of HORMAD1 bound to MCM9 closure motif

AlphaFold predictions using HORMAD1 residues 1-235 and the confirmed MCM9 motif (Figure 4) produced an ipTM of 0.61 and moderate confidence in the interaction, supporting its role as a bona fide HORMAD1 interactor (Figure 4). By comparison, the canonical binding sequence yielded a higher ipTM of 0.73 (high confidence, blue), whereas a nonbinding peptide sequence scoring 0.39 (low confidence, yellow), suggesting it does not form a stable interface (Figure 3).

Having established confidence in the predictive approach, candidate partner proteins were next examined for potential closure-motif–like sequences that were not previously described. A second putative MCM9 motif (STSQGKEKSEPGQRSKVDI) harboring the SE feature of the canonical closure motif produced a much lower ipTM of 0.40 (low confidence, yellow), suggesting it does not form a stable interface (Figure 5).

Structural predictions of HORMAD1 bound to SYNP2, IHO1, BRCA, and ATR

Several closure-like motif sequences were identified among the meiotic factors SYNP2, IHO1, BRCA, and ATR of which had previously been reported as direct interactors of HORMAD1, but through unknown mechanisms. The SYNP2 and ATR-derived motif produced a moderate confidence (Figure 6 and 7). In contrast, predicted interactions with motifs from IHO1 and BRCA1 were weak, suggesting no meaningful binding (Table 1**)**. Together, these results indicate that while most candidate motifs are not predicted to bind HORMAD1, a subset, including SYNP2, ATR, and MCM9, may represent novel closure motif sequences worth experimental validation.

Structural predictions of HORMAD1 bound to phosphorylated closure motif

In order to test how posttranslational modifications may impact HORMAD1 regulation, an AlphaFold prediction was performed for HORMAD1 residues 1-235 and the canonical closure motif sequence harboring

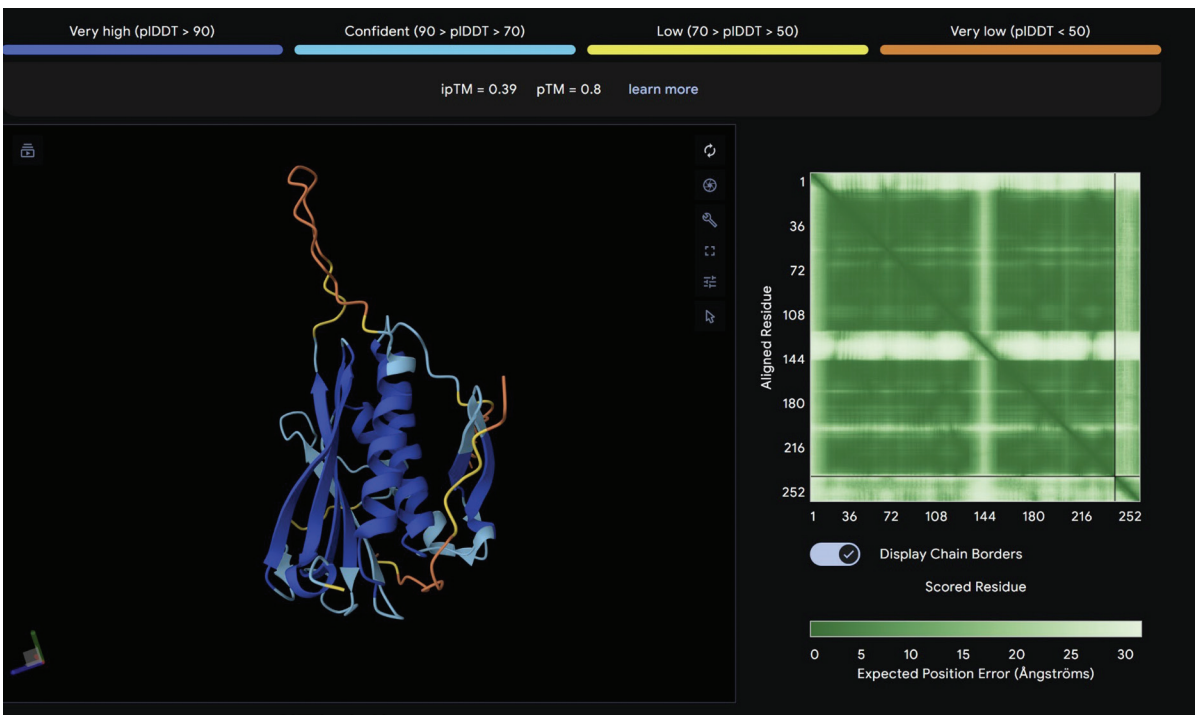

Figure 3. AlphaFold Prediction of HORMAD1 bound to canonical closure motif peptide SE changed to AA. The AlphaFold prediction of HORMAD1 residues 1-235 (chain A) and mutated closure motif sequence ESKTRS (chain B) have an ipTM score of 0.39 and pTM score of 0.8. The predicted structure is colored by pIDDT score where HORMAD1 residues in the HORMAD domain and safety belt have low pIDDT scores due to their yellow and orange colors. Shows high expected position error showing that the SE to AA peptide does not interact well with HORMAD1.

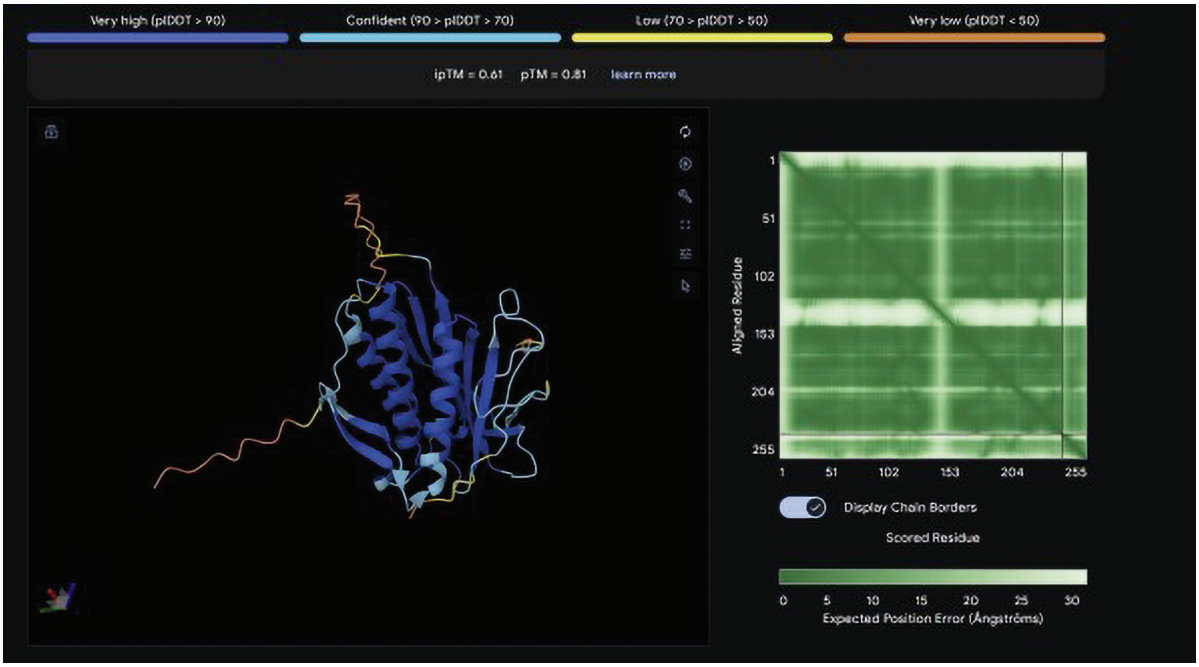

Figure 4. AlphaFold Prediction of HORMAD1 bound to MCM9 closure motif peptide. The AlphaFold prediction of HORMAD1 residues 1-235 (chain A) and closure motif sequence EKSEPG (chain B) have an ipTM score of 0.61 and pTM score of 0.81. The predicted structure is colored by pIDDT score where Hormad1 residues in the HORMAD domain and safety belt have pIDDT scores greater than 90 or high confident range and the closure motif has pIDDT scores greater than 70 or in the confident range. The iPTM is reduced compared to the canonical motif but it still is predicted to interact with moderate confidence.

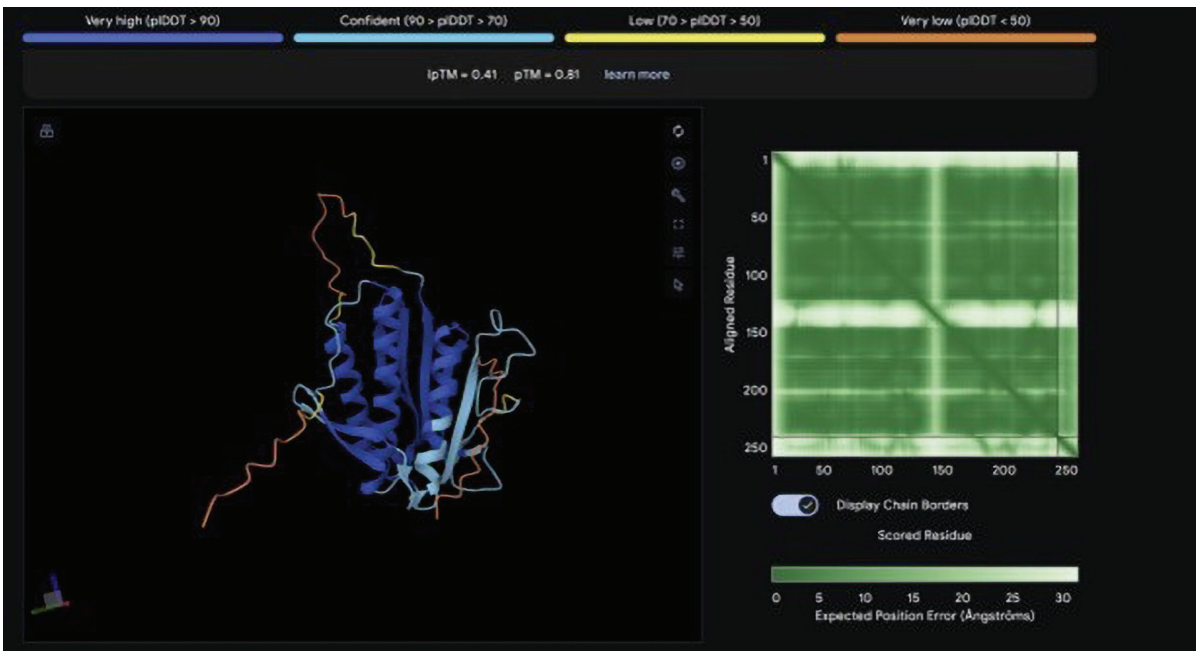

Figure 5. AlphaFold Prediction of HORMAD1 bound to MCM9 second motif peptide. The AlphaFold prediction of HORMAD1 residues 1-235 (chain A) and closure motif sequence ESKTRS (chain B) have an ipTM score of 0.4 and pTM score of 0.8. The predicted structure is colored by pIDDT score where Hormad1 residues in the HORMAD domain and safety belt have low pIDDT scores. There is no interaction between MCM9 and motif 1.

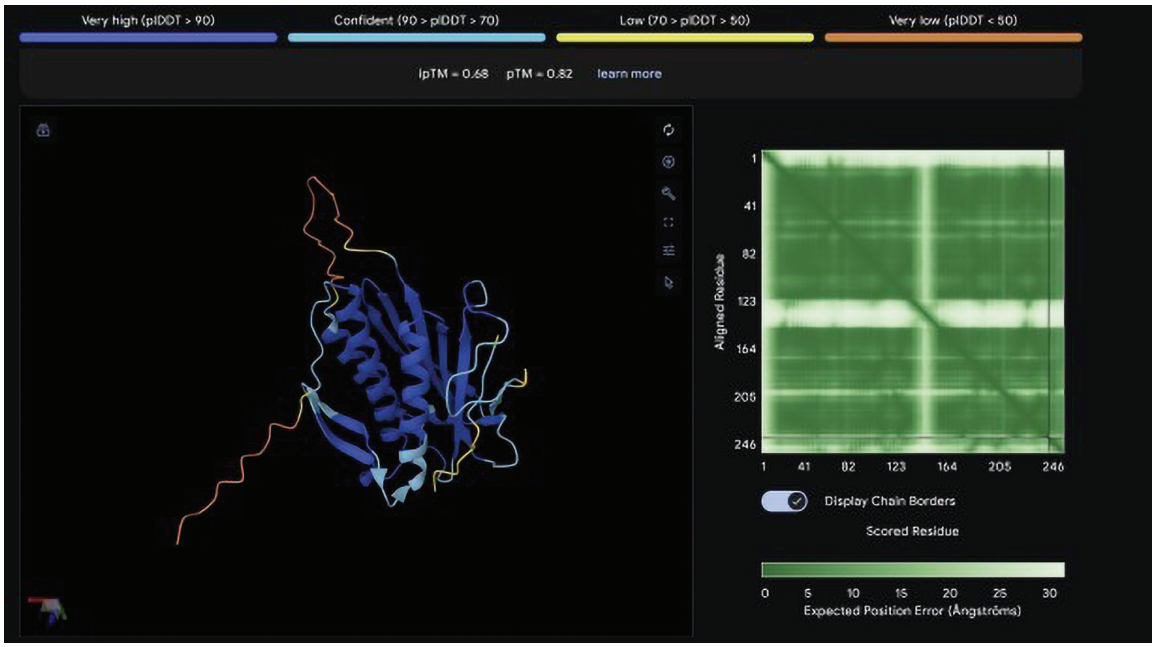

Figure 6. AlphaFold Prediction of HORMAD1 bound to SYNP2 closure peptide. The AlphaFold prediction of HORMAD1 residues 1-235 (chain A) and closure motif sequence ESKTRS (chain B) have an ipTM score of 0.68 and pTM score of 0.82. The predicted structure is colored by pIDDT score where Hormad1 residues in the HORMAD domain and safety belt have pIDDT scores greater than 90 or high confident range and the closure motif has pIDDT scores greater than 70 or in the confident range. The expected position error of the closure motif is low and there is possible interaction between SYNP2 and HORMAD1.

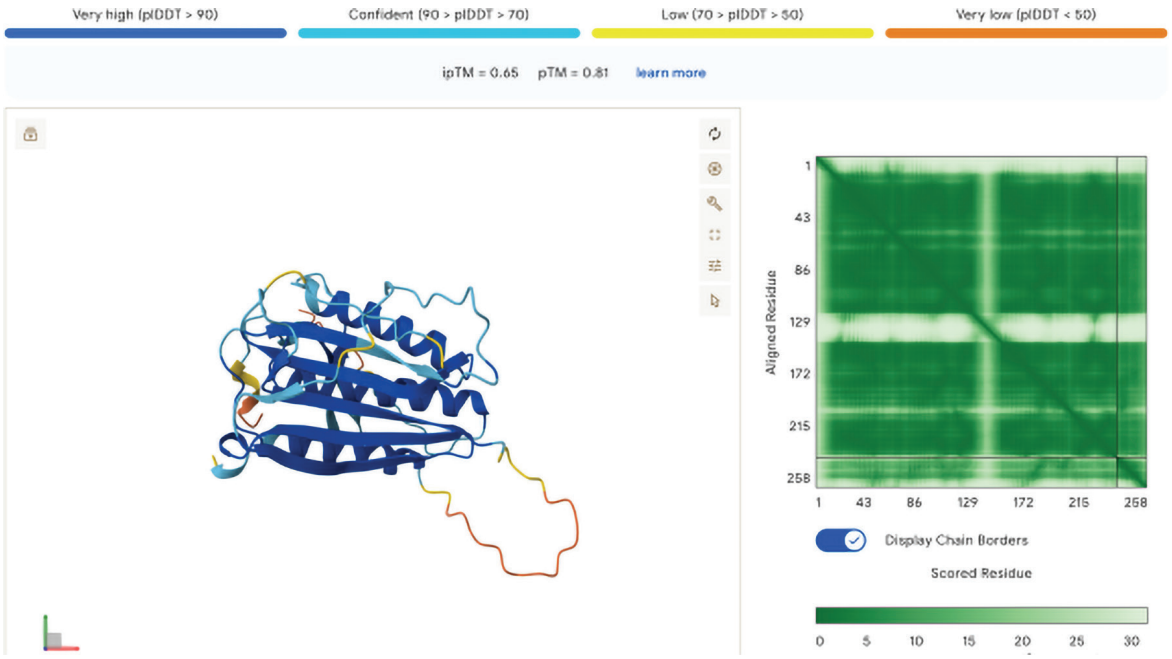

Figure 7. AlphaFold Prediction of HORMAD1 bound to ATR closure peptide. The AlphaFold prediction of HORMAD1 residues 1-235 (chain A) and closure motif sequence ESKTRS (chain B) have an ipTM score of 0.65 and pTM score of 0.81. The predicted structure is colored by pIDDT score where Hormad1 residues in the HORMAD domain and safety belt have pIDDT scores greater than 90 or high confident range and the closure motif has pIDDT scores greater than 70 or in the confident range. There is possibly an interaction between ATR and HORMAD1.

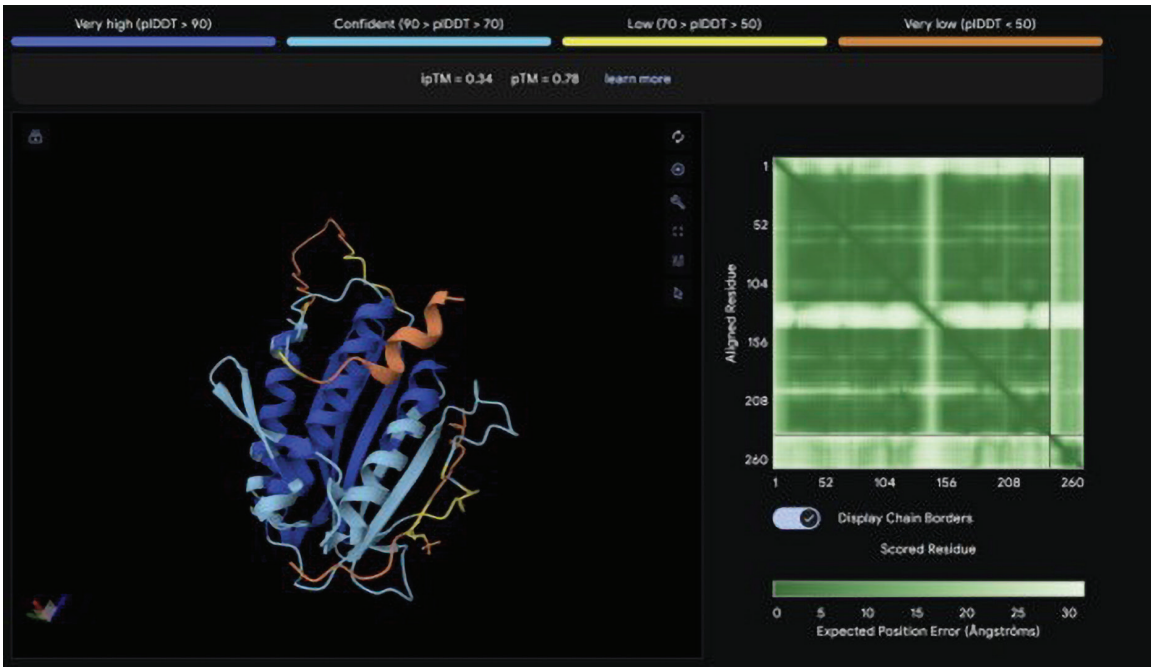

Figure 8. AlphaFold Prediction of HORMAD1 bound to phosphorylated peptide. The AlphaFold prediction of HORMAD1 residues 1-235 (chain A) and phosphorylated closure motif sequence EPKTRS (chain B) have an ipTM score of 0.34 and pTM score of 0.78. The predicted structure is colored by pIDDT score where Hormad1 residues in the HORMAD domain and safety belt have pIDDT scores greater than 90 or high confident range and the closure motif has pIDDT scores lower than 50, in the very low range. The expected position error of the closure motif is high.

a phosphorylation posttranslational modification on residue S387. This predicted an a low ipTM score and low pLDDT scores for the peptide sequence position in the predicted structure (Figure 8). This suggests that phosphorylation may disrupt HORMAD1 recognition of its own closure motif or its interaction with partner proteins.

DISCUSSION

The results demonstrate that AlphaFold can reproduce the known structural behavior of HORMAD1 closure motif binding, including canonical interactions and mutations that disrupt complex formation (13, 14). The ability to recover these features provides confidence in using this approach to screen for potential new HORMAD1 interactors. Importantly, AlphaFold distinguished between positive and negative controls, with high ipTM for the canonical motif, reduced ipTM for the S26K mutant, and near-background values for the SE→AA peptide. This trend closely mirrors biochemical pull-down assays reported in the literature, which showed that safety belt and closure motif mutations abolish binding (4, 6).

When applied to candidate motifs from meiotic and DNA repair proteins, the predictions were more nuanced. MCM9, a known interactor, was recovered with intermediate ipTM values, consistent with its established role in binding HORMAD1 (12). Interestingly, AlphaFold suggested possible binding between HORMAD1 and motifs from SYNP2 and ATR, while sequences from IHO1 and BRCA1 did not produce confident predictions. A common feature of the predicted binders is the presence of hydrophobic residues adjacent to acidic side chains, resembling the canonical consensus (13, 15). While these observations point to potential sequence features important for recognition, AlphaFold cannot provide quantitative binding affinities and may overestimate transient interactions (16).

The predicted interactions between HORMAD1 and proteins such as ATR and SYNP2 may have important biological implications for meiotic regulation. HORMAD1 is a key mediator of the meiotic recombination checkpoint, ensuring that synapsis and double-strand break repair are properly coordinated. The potential ATR interaction suggests a direct structural basis for ATR

recruitment to unsynapsed chromosome regions, where it initiates checkpoint signaling and maintains meiotic arrest until repair is complete. Likewise, the possible association with SYNP2 points to a role for HORMAD1 in early synaptonemal complex assembly, potentially acting as a scaffold that links chromosome axis proteins with developing SC components. Together, these findings raise the possibility that closure-motif mimicry enables HORMAD1 to toggle between checkpoint activation and synapsis promotion depending on its binding partners, offering a new framework for understanding how its regulation orchestrates meiotic progression (10, 11).

Another possible layer of regulation is phosphorylation. Many of the closure-motif–like sequences examined contain serine or threonine residues, which are frequent targets of cell-cycle–dependent kinases (11). Phosphorylation at these positions could alter binding to HORMAD1 by either strengthening or weakening the interaction, providing a way to turn motif recognition on or off at specific stages of meiosis. Interestingly, when phosphorylation of S387 in the canonical closure motif, a known site of phosphorylation, the interaction between HORMAD1 and itself is reduced (12). This suggests that post-translational modification may play an important role in controlling when and how HORMAD1 engages with its partners.

A key limitation of this approach is that AlphaFold predictions are based on static structures and cannot model the conformational dynamics that may regulate HORMAD1 interactions in vivo. In addition, the ipTM metric provides only a heuristic measure of interface confidence rather than experimental binding strength (16). Nevertheless, the predictions highlight potential interaction partners that could be prioritized for biochemical testing, especially SYNP2 and ATR, which have not yet been confirmed experimentally. Further, several interaction partners of HORMAD1 may not interact through a closure-like motif and their mechanism of interaction should be further explored (9). Given the lack of structural information available for HORMAD1 and its essential role in meiotic chromosome axis assembly, these computational predictions provide a valuable starting point for guiding future experiments aimed at defining how HORMAD1 regulates meiosis through closure motif mimicry.

CONCLUSION

This study aimed to investigate the structural and functional interactions of HORMAD1 in meiosis using computational modeling and literature-based analysis. By applying AlphaFold to predict HORMAD1's structure and its interactions with partner proteins, the study identified potential closure motif–like binding between HORMAD1 and proteins such as MCM9, SYCP2, and ATR. These predictions provide new insight into how HORMAD1 may regulate meiotic progression and how post-translational modifications could modulate its activity. Although the findings are based on computational models and require experimental validation, they establish a valuable framework for future studies examining HORMAD1's role in fertility. Overall, this research demonstrates how structural prediction tools can advance understanding of protein interaction critical to meiotic control and reproductive health.

While AlphaFold offers powerful structural prediction capability, its models are inherently static and do not capture the dynamic conformational changes or regulatory mechanisms occurring during meiosis. Confidence metrics such as pLDDT, pTM, and ipTM reflect prediction reliability rather than actual binding affinity or temporal behavior. Therefore, these findings should be regarded as hypotheses pending empirical confirmation.

Future work should focus on biochemical validation through peptide pull-down and co-immunoprecipitation assays to confirm direct interactions between HORMAD1 and its predicted partners. Mutagenesis of candidate closure motifs, followed by binding studies, will further clarify sequence features critical for recognition. Such experimental validation may strengthen the structural interpretations proposed here and advance understanding of meiotic regulation and reproductive health.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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