CHPA, a Cysteine- and Histidine-Rich-Domain-Containing Protein, Contributes to Maintenance of the Diploid State in Aspergillus nidulans

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The alternation of eukaryotic life cycles between haploid and diploid phases is crucial for maintaining genetic diversity. In some organisms, the growth and development of haploid and diploid phases are nearly identical, and one might suppose that all genes required for one phase are likely to be critical for the other phase. Here, we show that targeted disruption of the chpA (cysteine- and histidine-rich-domain- [CHORD]-containing protein A) gene in haploid Aspergillus nidulans strains gives rise to chpA knockout haploids and heterozygous diploids but no chpA knockout diploids. A. nidulans chpA heterozygous diploids showed impaired conidiophore development and reduced conidiation. Deletion of chpA from diploid A. nidulans resulted in genome instability and reversion to a haploid state. Thus, our data suggest a vital role for chpA in maintenance of the diploid phase in A. nidulans. Furthermore, the human chpA homolog, Chp-1, was able to complement haploinsufficiency in A. nidulans chpA heterozygotes, suggesting that the function of CHORD-containing proteins is highly conserved in eukaryotes.

All meiotic organisms spend some proportion of their life as haploids and as diploids. In many higher organisms, the diploid state is dominant and the haploid phase generally occurs only in gametes (18). On the other hand, several taxa such as green and brown algae have developed a long haploid phase and a short diploid phase. In some cases, such as the filamentous fungus Aspergillus nidulans, vegetative diploid and haploid phases are capable of extensive growth and similar development (1, 7, 29). These isomorphic haploid-diploid organisms provide excellent model systems for studying genes specifically required for diploidy, because differences between the diploid and haploid cells in the vegetative phases are minimal.

A. nidulans is a useful model for studying cellular differentiation processes, including sexual and asexual development (27). In sexual reproduction, foci within the vegetative mycelium begin to coil and fuse, eventually forming a red-pigmented fruiting body called the cleistothecium (6). Large thick-walled multinucleated cells develop by budding at the tips of the specialized hyphae and form a tissue that envelops the young cleistothecium (the procleistothecium). Reproductive asogenous hyphae proliferate within an expanding cleistothecial wall. This tissue type gives rise to large numbers of differentiated ascus mother cells, within which karyogamy occurs to give transient diploid nuclei. Nuclear fusion is immediately followed by meiosis and differentiation of red-pigmented binucleate haploid ascospores (6). Asexual reproduction is characterized by the switch from a highly polarized filamentous growth of the vegetative hyphae to a budding one. In this case, however, the hyphae are differentiated into other types of cells comprising the reproductive structure (conidiophore) and unicellular conidia (28). In contrast to the transient diploid phase in sexual development, somatic diploids formed from haploid nuclear fusion are stable and undergo asexual reproduction cycles similar to those of haploids, producing isomorphic developmental structures (10). However, little is known of the genes that control the formation and maintenance of diploids during the sexual or asexual life cycle. Identification of genes that are required only for the formation and/or maintenance of the diploid state, therefore, should facilitate our understanding of how A. nidulans controls the diploid phase of its life cycle differently from the haploid phase.

The cysteine- and histidine-rich domain (CHORD) is a novel zinc binding domain found in most eukaryotes except Saccharomyces cerevisiae. In Caenorhabditis elegans, a CHORD-containing protein (also designated CHP) has been identified as an essential component in development; silencing the chp gene results in reduction in fecundity and embryo lethality (23, 32). In vertebrates, two closely related CHORD-containing proteins have been identified; the one more homologous to CHP is designated CHP-1, and the more-diverged protein is melusin (5, 23). Melusin was originally isolated as a protein binding to the cytoplasmic domain of β-integrin and specifically expressed in striated muscle tissue, where it localizes at costameres in proximity of the Z line along with β-integrins (5). It has been shown that knocking out the melusin gene in mice does not affect skeletal muscle or heart development during embryogenesis and has no effect on basal cardiac and skeletal muscle function. However, melusin knockout mice develop dilated cardiomyopathy and cardiac failure when subjected to chronic pressure overload. This indicates a crucial role of melusin in transducing signaling in response to mechan-
ical stress in cardiomyocytes (4). Nevertheless, the biochemical function of the vertebrate CHP homologs remains to be eluci-
dated.

In addition to two CHORD domains, vertebrate and C. ele-
gans CHP proteins contain a domain called the CS motif (CHP and SGT1) that is also found in SGT1, a multifunctional pro-
tein originally identified in yeast cells (17, 23, 24). In yeast cells, SGT1 performs diverse functions, including regulation of the cell cycle and the cyclic AMP pathway (9, 17). In plants, SGT1 also serves multiple functions, including regulation of disease resistance and auxin signaling pathways (2, 12). Interestingly, the CS domain of SGT1 binds the CHORD-II domain of RAR1, a plant CHORD-containing protein that is also re-
quired for resistance triggered by a number of resistance genes (2). Both RAR1 and SGT1 specifically interact with heat shock protein 90 (HSP90), which is also essential for disease resis-
tance, suggesting that RAR1 and SGT1 may function as co-
chaperones of HSP90 (26). However, whether vertebrate and C. elegans CHP proteins also function through HSP90 remains to be investigated.

In this study, we identified an A. nidulans CHP homolog and cloned the corresponding gene, chpA. We showed that chpA4 is required for the somatic diploid phase but not for the haploid phase. Furthermore, diploids are sensitive to chpA4 gene dosage, requiring both copies for normal development. Human Chp-1 can complement the haplo-insufficiency seen in A. nidu-
lans chpA4 heterozygotes. These data suggest that chpA plays a crucial conserved role in diploid state maintenance in A. nidu-
lans.

**MATERIALS AND METHODS**

**Strains and gene isolation.** Table 1 lists the genotypes of the A. nidulans strains used in this study. Previously described techniques (13) were employed for growing, crossing, and handling A. nidulans. Heterokaryon generation and propagation were performed as previously described (15). Ascospore yield was determined by clearing cleistothecia of Hule cells and conidia, opening cleistoth-
ecia individually in water, and counting the ascospores in an aliquot obtained from each cleistothecium with a hemocytometer. Ascospore viability was deter-
ned by germinating the ascospores at 37°C and microscopically examining 200 ascospores from each cleistothecium for the presence of germ tubes. A. nidulans chpA4 cDNA (GenBank accession no.AY373584) was amplified from an A. nidulans cDNA library by using PCR primer Apchp1 (5'-ACA ATG GCC ACC AAG TGC GTA CAC-3') based on the expressed sequence tag sequence for chpA (GenBank accession no. AA965935) and an oligo(dT) primer (Promega). The genomic region containing approx. 1.1 kbp of DNA extending 5' and 3' from the chpA gene locus (GenBank accession no.AY375534) was cloned using a Marathon Genome Walker kit (Clontech, Palo Alto, Calif.). The full-length chpA coding sequence was cloned downstream of the A. nidulans chpA4 promoter in a plasmid, pMCB17, which contained the prur selectable marker gene (20). Human Chp-1 (GenBank accession no. AF192466) was cloned by reverse transcription-PCR with primers HsChp1 (5'-GAT CGG TGC GTT GTG TAA ACA TAC CAT GCT TCT CTA G-3') and HsChp2 (5'-GAT CGG TGC GTT GTG TAA ACA TAC CAT GCT TCT CTA G-3') and HsChp2 (5'-GAT CGG TGC GTT GTG TAA ACA TAC CAT GCT TCT CTA G-3') and HsChp2 (5'-GAT CGG TGC GTT GTG TAA ACA TAC CAT GCT TCT CTA G-3'). The respective primers were extensively purified on selection plate and the chpA4 knockouts were confirmed with the same primer sets. Southern blotting was used to further confirm the correct insertions.

**TABLE 1. A. nidulans strains used in this study**

| Strain(s) | Genotype | Reference or source |
|-----------|----------|---------------------|
| **Haploid** | | |
| GR5 | pyrG89; pyroA4; wA2 | 13 |
| P22L, P241, and P27L | pyrG89 + ΔchpA::pyrG4; pyroA4; wA2 | 13 |
| P74 | pyrG89 + pyr4+; pyroA4; wA2 | 13 |
| 20.3.10 | pyrG89; pabaA1; argB2; fwA1 | This study |
| A9 | argB2 + ΔchpA::argB; ppyR89; pabaA1; fwA1 | This study |
| A17 | argB2 + argB; ppyR89; pabaA1; fwA1 | This study |
| **Diploid** | | |
| P74A9 | argB2 + ΔchpA::argB::pyrG4; ppyR89 + pyr4+; pyroA4; pyroA4; wA2;fwA1 | This study |
| P22D, P24D, and P27D | ppyR89 + ΔchpA::pyrG4; pyroA4;pyroA4; wA2;wA2 | This study |
| GR5A9 | argB2 + ΔchpA::argB::pyrG4; pyroA4;pyroA4; pabaA1;pyrG89; ppyR89; wA2;fwA1 | This study |
| Dan2 | argB2 + argB; ppyR89 + pyr4+; pyroA4;pyroA4; pabaA1; pabaA1; wA2;fwA1 | This study |
| Dan3 | argB2 + ΔchpA::argB::pyrG4; ppyR89 + pyr4+::HsChp1; pyroA4;pyroA4; pabaA1; pabaA1; wA2;fwA1 | This study |
| Dan4 | argB2 + ΔchpA::argB::pyrG4; ppyR89/pyr4+; pyroA4;pyroA4; pabaA1; pabaA1; wA2;fwA1 | This study |
SEM. Sporulating *A. nidulans* hyphae were placed on aluminum scanning electron microscopy (SEM) stubs with optimal cutting temperature compound (BDH Laboratory Supplies, Poole, United Kingdom) and then immediately plunged into liquid nitrogen slush at approximately -195°C to cryopreserve the material. The sample was transferred to the cryostage of a CT1500HF cryotransfer system (Oxford Instruments, Oxford, United Kingdom) and observed with an XL30 FEG SEM (Philips Electron Optics, FEI UK Ltd., Cambridge, United Kingdom).

**RESULTS**

**Isolation and cloning of the *A. nidulans* chpA gene.** To elucidate the function of CHORD-containing proteins (CHPs), we chose a simple eukaryote, *A. nidulans*, as a model system (8). We isolated a single-copy gene, designated *chpA*, encoding the CHP homolog in *A. nidulans* (Fig. 1A). Similar to metazoan CHP proteins, *A. nidulans* CHPA can be divided into three discrete domains: CHORD-I, CHORD-II, and the CS motif. Figure 1B shows the alignment of these three domains. The high similarity in CHORD domains from plant, metazoan, and fungal CHP proteins suggests that their basic function might be conserved. Interestingly, the level of sequence similarity was higher for corresponding CHORD motifs from various species. Black, dark grey, and light grey boxes indicate >100%, >70%, and >50% identity through conserved amino acid substitutions, respectively.
tifs in different phyla than between CHORD motifs in a single species. This finding is indicative of distinct conserved functions for CHORD-I and CHORD-II. A high degree of similarity is also evident within CS motifs from *A. nidulans*, humans, *C. elegans*, and *Drosophila* and the SGTL1 proteins from yeast strains, humans, and *Arabidopsis*. This finding may indicate a conserved functional link between CHP and SGTL1 via the CS motif.

**Targeted knockout of chpA gene.** To understand the function of *chpA*, we created *chpA* knockouts in *A. nidulans*. An initial gene deletion experiment with the haploid *A. nidulans* strain GR5 (19) used homologous recombination to replace the endogenous *chpA* gene with the *pyr4* selectable marker (3), which complements a uridine/uracil auxotrophy (Fig. 2A). Three independent site-specific recombinants were identified by PCR screening from a population of 60 transformants. Surprisingly, all three colonies (P22, P24, and P27) showed clear growth sectors on plates, whereas non-site-specific recombinant colonies, represented by P74, gave rise to single light-color (wild-type) colonies (Fig. 2B). The mycelia from the light-colored sector of P24 were designated P24L, and the mycelia from the darker sectors were designated P24D (Fig. 2B). Southern blotting analysis of the DNA from P24D and other darker sectors (P22D and P27D) with the promoter region of *chpA* used as a probe showed a 2.5-kbp band corresponding to the wild-type *chpA* locus and a 3.5-kbp band representing the *chpA* locus correctly replaced by *pyr4* (Δ*chpA::pyr4*) (Fig. 2A and C). In contrast, the DNA from light-color sectors (P22L, P24L, and P27L) contained only the larger band corresponding to Δ*chpA::pyr4*+, indicating that these are indeed *chpA* knockouts. Despite its lack of the *chpA* gene, P24L could complete both asexual and sexual life cycles as a wild type (Fig. 3; Table 2). Sensitivity in P22L, P24L, and P27L to chemicals such as benomyl, hydroxyurea, or the detergent Triton X-100 was not significantly different from that of non-site-specific recombinant control P74 (data not shown).

The presence of both the *chpA* gene and the *pyr4* gene in P24D genomes arising from uninucleate spores suggested that P24D could be diploid. Indeed, flow cytometric analysis of dormant spores showed that strain P24D is diploid while P24L is haploid (Fig. 2D). Thus, removing *chpA* from the haploid *A. nidulans* genome seems to induce high rates of nuclear fusion within the heterokaryon mycelia, giving rise to stable heterozygous diploids. However, we never recovered any diploid *chpA* double knockouts from these experiments.

*A. nidulans* *chpA* heterozygotes show impaired conidiophore development and reduced conidiation. To understand the cellular basis of the sectoring phenotype, we compared the morphology of the *chpA* heterozygote strain P24D (Δ*chpA::chpA*: *pyr4*+) to those of wild-type controls and the haploid *chpA* knockout strain P24L (Δ*chpA::pyr4*) by SEM (Fig. 3A). In both diploid and haploid wild-type strains, developing conidiophores are highly symmetrical and consist of a stalk ending in a vesicle crowned by tiers of specialized metulae and phialide cells. These phialide cells continue to bud off into uninucleate spores by repeated budding coupled to asymmetric mitotic divisions. In strain P24D, hyphal growth morphology is indistinguishable from that of wild-type diploid strain P74A17 (Δ*chpA::chpA*) and P24L until the metula stage of conidiophore development. At this stage, the number of metula cells are
Conidiophore formation in A9 (fawn 20.3.10 background (Fig. 2A). The hyphal growth and which the strain (31). For this purpose, we created a haploid strain A9 in nuclear fusion of the white GR5 strain and the fawn 20.3.10 heterokaryons by green spore coloration resulting from the this experiment, diploid colonies can be distinguished from 
diploid from two haploid gene can form functional diploids, we attempted to form a

FIG. 3. Conidium formation defects in the chp4 heterozygous diploid strains. (A) SEMs of conidiophore from wild-type diploid P74A17 (chpA/chpA), heterozygous diploid mutants P24D (chpA/ΔchpA::pyr4+) and P74A9 (chpA/ΔchpA::argB), haploid wild-type GR5, and haploid chpA knockouts P24L (ΔchpA::pyr4+) and A9 (ΔchpA::argB). Abbreviations: C, conidia; P, phialides; M, metulae. Scale bars, 10 μm. (B) Quantification of conidium production. Diploid and haploid strains were grown on potato dextrose agar medium, and the numbers of conidia were counted with a hemocytometer. The results shown are means ± standard deviations for triplicate cultures.

dramatically reduced, and malformed phialides are observed in P24D but not in P74A17 or P24L (Fig. 3A). In addition, there is a loss of symmetrical development of the metulae in the strain. The basis of the segregating phenotype, therefore, is due to impaired sporulation in chpA heterozygotes (Fig. 3B). These data suggest that the absolute level of chpA is crucial for conidiophore development in the diploid state.

Haploid chpA deletion mutants are unable to form viable diploids. To investigate whether A. nidulans lacking the chpA gene can form functional diploids, we attempted to form a diploid from two haploid chpA knockouts in different genetic backgrounds that contain distinctive spore color markers. In this experiment, diploid colonies can be distinguished from heterokaryons by green spore coloration resulting from the nuclear fusion of the white GR5 strain and the fawn 20.3.10 strain (31). For this purpose, we created a haploid strain A9 in which the chpA gene was replaced by the argB gene (30) in the fawn 20.3.10 background (Fig. 2A). The hyphal growth and conidiophore formation in A9 (ΔchpA::argB) are comparable to that of P24L and the wild type (Fig. 3A). Spores from P24L and A9 were mixed together, allowed to germinate and un-dergo cellular fusion, and plated out onto double-selection medium that would allow growth only of heterokaryon or diploid colonies carrying the full complement of markers from both haploid strains (Fig. 4A). The only viable colonies recovered from the mixture were white and fawn heterokaryons, as indicated by the mixed spore colors, and no green diploid colonies were found. This result is in contrast to the control combination of wild-type haploids P74 and A17, which produced green spores at the expected frequency. Other control strains showed efficient diploid formation with P74 and A9, as well as with P24L and fawn haploid strain A17 (chpA), resulting in green chpA heterozygous diploid spores. The phenotype of the P74A9 strain (chpA/ΔchpA::argB) (Fig. 3A) was identical to that of P24D (chpA/ΔchpA::pyr4+) as its conidiophore formation was severely affected, thus confirming that this phenotype was not due to the marker genes. In summary, the combination of P24L and A9 could produce heterokaryons but not diploids. This evidence argues that the effect of chpA is not restricted to conidiophore development. These results are consistent with the idea that chpA is required for the efficient formation of stable diploids in A. nidulans.

Attempts to disrupt the remaining chpA gene copy in chpA heterozygotes lead to unstable aneuploids. To test if chpA is required for the maintenance of diploid status rather than for the actual nuclear fusion process, we used the heterokaryon rescue method (21) to remove the chpA gene in a heterozygote diploid. For this experiment, we created a chpA heterozygote by fusing the nuclei from wild-type strain GR5 and haploid chpA knockout A9. The resulting green diploid strain, GR5A9 (chpA/ΔchpA::argB) had the developmentally defective characteristics of the previously generated chpA heterozygote, P24D. In addition, this strain carried the pyrG89 mutation, which allowed us to use the pyr4 selectable marker to disrupt the remaining chpA allele. After screening 200 transformants by PCR, we found two site-specific recombinant strains, G114 and G65, in which the chpA gene was correctly replaced by the pyr4 gene. Since such primary transformants normally contain a mixture of the parental GR5A9 (untransformed) nuclei with an intact chpA gene and nuclei containing the chpA deletion, they were purified on selection plates to remove any parental strains or heterokaryons. We analyzed DNA from these spore-purified strains by Southern blotting. Surprisingly, we detected an extra band corresponding to wild-type chpA in addition to bands which represent ΔchpA::pyr4+ and ΔchpA::argB (Fig. 4B). The presence of all three genes (chpA, pyr4, and argB) at the chpA locus in G114 and G65 suggested that the strains could be persistent diploid heterokaryons, tetraploids, or aneuploids. These states are all likely to be unstable to a greater or lesser degree on nonselective medium (16). Indeed, strain

| Strain  | Yield a | Viability b |
|---------|---------|-------------|
| GR5     | 3.1 × 10^4 ± 0.5 × 10^4 | 71 ± 5.5 |
| P24L    | 2.8 × 10^3 ± 0.2 × 10^3 | 73 ± 1.5 |
| P74     | 2.5 × 10^3 ± 0.2 × 10^3 | 75 ± 1.5 |

a Values are the average number of ascospores per cleistothecium (from three experiments).
b Values are the average number of ascospores with a germ tube after 8 h of incubation at 37°C (from three experiments).

TABLE 2. Sexual sporulation of the chpA mutant
G114 showed a severe sectoring phenotype when grown on nonselective medium, whereas the non-site-specific control strain G88 showed no such sectoring, indicating that the G114 genome is unstable (Fig. 4C). Flow cytometric analysis demonstrated that the DNA content of G114 was greater than that of the diploid and that strains isolated from any of the sectors (i.e., 114S1) that had lost the chpA gene had reverted to the haploid state (Fig. 4D). These data strongly suggest that A. nidulans cannot exist as a diploid without the chpA gene and that the original G114 strain is either a diploid aneuploid addition line, a triploid, or possibly a tetraploid.

**Human Chp-1 can functionally replace A. nidulans chpA.** If the function of metazoan and fungal chpA proteins is conserved, we reasoned that human Chp-1 should complement the developmental defect in the A. nidulans chpA heterozygote strains. We transformed the chpA heterozygote strain GR5A9 with a human Chp-1 cDNA (23) driven by the A. nidulans chpA promoter. The conidiation defect in GR5A9 is reflected in the pale green coloration of the colony, resulting from fewer conidia, when compared to a green wild-type diploid, Dan1 (Fig. 5). The reduced-conidiation phenotype in GR5A9 was fully complemented by the introduction of the genomic fragment of chpA (Dan3) as a control (Fig. 5B). GR5A9 colonies transformed with the vector alone (Dan4) showed no complementation of the conidiation defect (Fig. 5). The chpA heterozygote colonies carrying an extra human Chp-1 copy (Dan2), however, have a deeper green coloration resembling that of the wild-type chpA diploid (Fig. 5A) and increased conidium production (Fig. 5B). Thus, human Chp-1 is able to rescue the haploinsufficiency phenotype seen in the chpA heterozygotes, indicating that the function of CHORD-containing protein is highly conserved among eukaryotes.

**DISCUSSION**

Since the life cycle is one of the most fundamental attributes of an organism, understanding the variation in haploid and diploid phases in the life cycle among meiotic organisms is an important problem in evolutionary biology. In this study, we provide molecular and genetic data suggesting that chpA is a crucial determinant of the diploid state in the asexual phase of the life cycle of A. nidulans.

Attempts to delete chpA from haploid A. nidulans conidia result in the formation of two types of spores, a chpA null haploid and a chpA/ΔchpA heterozygote. chpA/ΔchpA isolates show a clear sporulation defect in asexual conidiation, while chpA-null haploids are entirely unaffected during asexual or sexual reproduction. Thus, asexual conidiophore development in diploids and haploids is differently controlled; the diploid phase requires proper dosage of chpA, but the haploid phase does not require chpA at all. Furthermore, no diploid chpA-null spores were isolated, indicating that chpA may be essential for somatic diploids of A. nidulans.
To determine whether chpA was required for A. nidulans diploid viability, we carried out two independent experiments to isolate somatic diploid chpA gene knockouts. First, haploid spores from two different chpA deletion mutants were isolated, mixed together, and plated onto medium favoring only colonies that contain both selectable markers. From this experiment, we isolated only heterokaryon mycelia containing nuclei from both haploid chpA deletion mutants. All the control spore mixtures having at least one wild-type copy of chpA produced diploids, indicating that the double selection pressure was efficient to ensure nuclear fusion events and that nuclei from two haploid chpA deletion mutants must have fused to form diploids. Therefore, these ΔchpA/ΔchpA diploids do not form viable colonies after nuclear fusion. Second, we tried to delete the second copy of chpA from a chpA/ΔchpA heterozygote. This experiment was intended to directly address the question of whether chpA is required for diploid state maintenance. Attempts to delete the extra copy of chpA resulted in the formation of aneuploids with genomes that showed great instability on nonselective medium. Segregating sectors that did not contain chpA were all haploid in nature as determined by flow cytometry. All diploid sectors isolated had at least one copy of wild-type chpA. These data suggest that A. nidulans lacking chpA cannot form viable diploid colonies.

Since haploid chpA deletion mutants undergo normal sexual life cycle, it is suggestive that chpA is not required for the formation of the unstable diploid formed from dikaryotic hyphal cells prior to meiosis.

In the sexual cycle of A. nidulans, dikaryotic hypha formation is achieved by fusion of ascogonium- and antheridium-like structures (9). Nuclei within the dikaryotic cell divide synchronously, which allows for the branching growth of the ascogenous hyphae. Karyogamy occurs in the penultimate crozier cells of hyphal branches to form diploid nuclei that immediately undergo reductive meiosis to produce haploid gametes. Since the diploid formed during sexual reproduction is unstable, it is feasible that the diploid mother cells do not require chpA function because they do not need a stable diploid state. This would be consistent with our data suggesting that chpA is probably required for diploid state maintenance. Our data, then, provide molecular evidence in A. nidulans for a genetic mechanism specifically controlling the diploid state. Since the human chpA homolog, Chp1-1, is able to rescue the haploinsufficiency seen in ΔchpA/ΔchpA heterozygotes, this indicates that the diploid state maintenance mechanism could be a conserved genetic pathway occurring across phyla.

Since this study reveals a novel genetic requirement, i.e., chpA for the diploid state in A. nidulans, it would be interesting to determine how chpA stabilizes the diploid A. nidulans nuclei and prevents genomic instability. Experiments with an ectopic inducible chpA construct in a ΔchpA/ΔchpA nuclei would allow us to understand how chpA achieves genomic stability in diploids and would also provide evidence for the mechanism of diploid genome instability when chpA is systematically removed under noninducing conditions. A. nidulans CHPA proteins contain the highly conserved CS motif also found in the yeast protein SGT1, which is essential for the kinetochore complex (17), suggesting that CHPA may operate at the point of homologous chromosome segregation through the kinetochore.

In plants, the RAR1 protein, which contains CHORD domains but not the CS motif, binds the CS motif of SGT1, a domain structurally similar to p23, a cochaperone of HSP90 (2, 9). In addition, the CHORD-1 domain of RAR1 specifically binds to HSP90 (26). Thus, an alternative scenario is that chpA may be involved in chaperone activity, regulating key components essential for the maintenance of diploidy. These data will provide further evidence for the role of a novel genetic mechanism of which chpA is a part, which controls ploidy levels in A. nidulans. Such experiments are beyond the scope of this particular study but will reveal how the diploid state is maintained differently from that of haploid.

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