Biological Sciences: Genetics

Atr1 and Chk1 are essential for fruiting body formation in the ink cap mushroom *Coprinopsis cinerea*

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ABSTRACT

The fruiting body or mushroom in the Agaricomycetes is a relatively large multicellular structure essential for sexual reproduction. The pileus bears the hymenium, a layer of cells that includes the specialised basidia in which nuclear fusion, meiosis and sporulation occur. *Coprinopsis cinerea* is a well-known model fungus used to study developmental processes associated with the formation of the fruiting body. Here we describe that knocking down the expression of Atr1 and Chk1, two kinases involved in the response to DNA damage in a number of eukaryotic organisms, dramatically impairs the ability to develop fruiting bodies in *C. cinerea*, as well as other developmental decisions such as sclerotia formation. These defects correlate with the inability to sustain an appropriated dikaryotic cell cycle, suggesting a connection between the DNA damage response cascade, cell cycle regulation and developmental processes in this class of fungi.

INTRODUCTION

Development of any biological structure involves individual cells undergoing different sorts of specialization to carry out different functions in the final structure. Progressing in differentiation steadily reduces the options a cell can follow, so once it becomes committed to a pathway the ability to revert back to a "less-specialized" state is dramatically reduced. However, this principle that applies for a large number of eukaryotic organisms, cannot be easily applied for multicellular fungi: no matter how differentiated cells become to particular functions in the final structure, most are able to revert to the original undifferentiated state. An example of it is the fungal mushroom, a relatively large, highly differentiated structure, composed by cells that when removed from the multicellular structure and put onto some nutritive artificial medium are able to resume growth and all developmental choices of mycelium cells. The only exception are the meiocytes that are committed to their morphogenetic pathway [1].

The molecular basis of this cellular plasticity is not well understood [2]. *Coprinopsis cinerea* is a well-known model organism to study developmental processes associated with the formation of the fruiting body or mushroom [3,4]. On a fertile dikaryotic mycelium, the fruiting body is initiated as a loose hyphal aggregation (primary hyphal knot). Primary hyphal knots are not specific to fruiting body formation but also serve as precursors in the dark-dependent development of sclerotia (small multicellular globose structures serving long-term survival) [5,6]. The formation of compact fruiting initials (secondary hyphal knots) is the first fruiting body-specific stage. Tissue differentiation leads to

fruiting body primordia composed of the two major organs of the fruiting body: the pileus or cap with the gills and the stipe, which supports the cap. In the cap on the gill surfaces, further development produces the hymenium, a layer of cells carrying the basidia in which synchronously karyogamy takes place. Meiosis follows directly to produce haploid basidiospores, which disperse when the pileus expands [5].

Fruiting body formation is part of the sexual cycle in *C. cinerea,* and it is initiated after mating of two compatible sterile monokaryotic mycelia to give the fertile dikaryon with two distinct haploid nuclei in the hyphal segments. Therefore, compatibility in the mating type genes is required to trigger the process. *C. cinerea* is a tetrapolar species with two multiallelic mating type loci, *A* and *B*, which must be different to establish a dikaryon and induce the formation of the fruiting body. The *A* mating type locus encodes two distinct protein subunits of a heterodimeric homeodomain transcription factor and the *B* locus encodes pheromones and pheromone receptors [7,8,9]. Interestingly, the roles of mating type genes go further than the mating process and actually they are essential regulators during defined steps in the process of fruiting body formation [10].

The fruiting body is composed of dikaryotic cells. In these cells, two nuclei, each coming from a different parental monokaryotic mycelium, remain discrete during somatic cell divisions. Dikaryotic maintenance involves an intricate cell division that includes the formation of a structure known as the clamp connection or clamp cell, as well as the sorting of one of the nuclei to this structure [11,12]. Prior to nuclear division, a clamp cell develops on the side of the apical cell and one nucleus migrates into this clamp while the other remains in the main cell. Nuclear divisions are synchronic and septa are laid down across the mitotic

spindles planes: one septum between the clamp cell and the apical cell trapping a single nucleus in the clamp and one septum within the cell hyphal subapical to the clamp separating one nucleus in the novel subapical cell from two distinct haploid nuclei in the apical cell [13]. Subsequently the clamp cell fuses with the subapical cell and its nucleus migrates into this cell. This way nuclear division occurs in a synchronous and independent way in two distinct subcellular compartments. Clamp connections therefore guarantee a correct nuclear distribution that keeps the presence of two distinct nuclei per cell. Mutants unable to produce clamps such as *clp1-1* mutants showed severe defects in sexual development [14]. Mating type genes govern the formation of the dikaryon starting with the nuclear pairing and ending with the clamp cell fusion [15], suggesting a connection between these genes and the control of the dikaryotic cell cycle, although the details behind this control are largely unknown. How important is the correctness in dikaryotic cell division for the appropriated development of the fruiting body was recently suggested by the discovery that a mutant strain carrying a thermosensitive allele of the gene *smc1*, encoding a component of cohesion complex, showed an impairment in the dikaryotic cell cycle as well as an arrest of hyphal knot development at an early fruiting stage [16].

Recently we have proposed a role for the kinases Atr1 and Chk1 in the regulation of the dikaryotic cell cycle in the phytopathogenic basidiomycete *Ustilago maydis* [17,18,19]. These kinases are broadly conserved regulators involved in the response to DNA damage [20,21]. Activation of Atr1-Chk1 axis in *U. maydis* was under the control of *b* proteins, the orthologs of *A* mating-type proteins from *C. cinerea* [22]. We proposed that mating-type dependent timely

activation of the Atr1-Chk1 axis in *U. maydis* enables the nuclear synchronization of the dikaryotic cell cycle as well as the appropriated formation of clamp connections. In this work we show that Atr1 and Chk1, proteins involved in the DNA-damage response (DDR) in *C. cinerea* are also essential for a correct mitosis of the dikaryotic cell and that their absence prevent the mature fruiting body formation.

RESULTS

The kinases Atr1 and Chk1 are required for the DNA damage response in *C. cinerea*.

The DNA damage response (DDR) pathway detects damaged DNA and co-ordinates its repair with the cell cycle progression [20,23]. Central to this cascade are the phosphatidylinositol 3-kinase related kinases (PIKKs) ATM and ATR, which share a significant sequence homology between them and canalize all signaling through this cascade [24]. Downstream of these kinases, there is a group of serine-threonine kinases that belongs to two different families: Chk1 and Chk2/Rad53. These are phosphorylated in an ATR/ATM-dependent manner and their activation produces, among other effects, a cell cycle delay enabling the DNA repair before mitosis is complete [25,26].

Using the BLAST program and the sequence of DDR kinases from different fungal species as well as human as queries, we found Atr1 (CC1G_08126.3), Atm1 (CC1G_00839.3), Chk1 (CC1G_02812.3), and Chk2 (CC1G_09319.3) homologues in the *C. cinerea* genome database (http://www.broadinstitute.org/annotation/genome/coprinus_cinereus/MultiHome.html) (Fig. S1).

Based in our previous results in *U. maydis*, we played with the hypothesis that Atr1 and Chk1 might have some role during the dikaryotic cycle in *C. cinerea*. To address this hypothesis we tried to impair the function of these regulators during the dikaryotic cell cycle using dsRNA-mediated gene silencing [27,28]. Antisense constructs were designed from different *chk1* and *atr1* exons (RNAi plasmids, see Material and Methods in Supporting Information for a description). As receptor strain for these silencing plasmids we used the self-compatible homokaryon AmutBmut. This particular strain carries specific mutations in both mating-type loci (*A43mut* and *B43mut*) that enable the formation of fruiting bodies without prior mating to another strain [29,30]. It shows typical characteristics of the dikaryon such as the formation of fused clamp cells at hyphal septa and it has been used to screen for mutations affecting dikaryotic phenotypes, such as clamp connection formation [14], fruiting body development [16,31,32] and meiosis [33].

We transformed AmutBmut with *chk1* and *atr1* RNAi plasmids as well as the empty plasmid used for the silencing as a control (RNAi control). As selective marker *pab1* gene was used [34]. Two independent silenced clones for each gene -showing a different silencing degree- as well as one clone carrying the control construct were chosen for further analysis. Strain RNAi *chk1*#1 showed around 70% of expression of *chk1* relative to the control strain while strain RNAi *chk1*#2 showed 50% of mRNA levels (Fig.1A). We were unable to find clones showing a much higher level of silencing for this gene. In the *atr1* clones, RNAi *atr1*#1 and RNAi *atr1*#2 strains showed 70% and 20% mRNA levels in comparison to the control strain, respectively (Fig. 1B). The gene silencing process was stable in all strains with the exception of RNAi *chk1*#2 strain, which

frequently raised colony sectors with cells showing low levels of silencing (they expressed the *chk1* gene at levels as high as 90% of wild-type expression, Fig. S2) when plated in conditions were gene silencing impaired growth (see below). Silenced strains were affected at various degrees in their ability to grow. Control strains (AmutBmut and RNAi control) took around 6 days at 37°C to fully cover the plate, while RNAi *chk1*#1 and RNAi *chk1*#2 needed 7 days to grow at the same extent. RNAi *atr1*#1 and RNAi *atr1*#2 needed 8 days and 10 days, respectively.

Atr1 and Chk1 are main players of the DDR pathway in eukaryotic organisms and its lack of function produces a high sensitivity to genotoxic agents in the cells in particular to agents that cause DNA replication stress such as methylmethane sulfonate (MMS), which induces DNA-alkylation [35] and hydroxyurea (HU), which inhibits ribonucleotide reductase and therefore affects replication by depletion of dNTPs [36]. Therefore, the sensitivity of the selected silenced clones against these genotoxic agents was tested. Strains were grown on rich media supplemented with HU or MMS. As expected, when the *atr1* or *chk1* gene expression was silenced the cells were more sensitive to these genotoxic stresses (Fig.1C). We found a good grade of correlation with the level of silencing in these strains and the level of sensitivity to HU or MMS. It is worth noting that for *chk1*, a dramatic difference with respect HU and MMS sensitivity was observed between RNAi *chk1*#1 (70% of wild-type expression) and RNAi *chk1*#2 (50% of wild-type expression). Moreover, plating RNAi *chk1*#2 cells in stress conditions often resulted in the appearance of fast-growing sectors (Fig. S2A). Further analysis of these fast-growing cells revealed that they reduced the level of silencing allowing almost wild-type expression levels for *chk1* (while

retained the RNAi transgene) (Fig. S2B) and regained the ability to survive in the presence of genotoxic stress (Fig. S2C). One of these silencing revertants, named RNAi *chk1*#2 Rev, was used as additional control in the characterization of the roles of these kinases in the dikaryotic cell cycle (see below).

Atr1 and Chk1 kinases are required for appropriated mitosis of the dikaryon in *C. cinerea*.

We analyzed the consequence of knocking-down *atr1* or *chk1* for the dikaryotic cell cycle in *C. cinerea*. For this, we performed microscopic analysis of the *atr1* and *chk1* silenced strains as well as the controls (AmutBmut, RNAi control and a *chk1*-revertant obtained from a fast-growing sector of RNAi *chk1*#2). The different strains were grown on a thin agar layer upon a microscope glass slide for two days. Then, they were co-stained with Hoechst and Calcofluor White, to visualize nuclei and septa, respectively. Hyphae from control strains showed cell compartments flanked by septa associated to clamp connections and carrying two nuclei each (Fig. 2A). In contrast, *chk1* and *atr1* silenced strains cells showed a range of abnormalities such as more than 2 nuclei per cell compartment, septa without associated clamp connection or clamp connections with “trapped” nuclei (Fig. 2A and B). We quantified the abnormalities observed in the *chk1* and *atr1* silenced strains. For this, the number of nuclei per cellular compartment as well as the presence of normal or aberrant clamps in each of these cells was counted. In Figure 2C is shown the resulting graphic, where “wt mitosis” gathers all the cells where the presence of nuclei and clamps was normal, “aberrant mitosis” groups those cells where either the nuclear distribution or the clamp formation was aberrant and “trapped nuclei” shows the

nuclei that were observed “locked” inside the clamps. In the control strains the number of aberrant phenotypes was very low and similar to previously reported results [37]. In contrast, in the silenced strains the number of aberrant mitosis was dramatically increased (Fig. 2C). As we noted with the sensitivity to genotoxic stress, we observed a nice correlation between the degree of silencing and the severity of the phenotype.

Atr1 and Chk1 are necessary for mature fruiting body formation in *C. cinerea*.

C. cinerea dikaryotic cells have a wide-ranging developmental potential. This potential ranges from the fruiting body, with the meiotic basidiospores, to mitotic aerial spores (oidia) and mitotic submerged spores (chlamydospores) as well as a plethora of multicellular structures such as sclerotia, mycelial cords, pseudorhizas and rockeries [5]. Fruiting-body formation is the most complex developmental process in the life cycle of *C. cinerea* that starting from a mesh of free undifferentiated hyphae ends up in a compact structure with differentiated tissues inside. The production of fruiting bodies depends on environmental conditions which include temperature, humidity, light and nutrients [38]. Oidia formation on the dikaryon as on mycelium of AmutBmut is dependent on light conditions [39] while sclerotia arise when the fungus has been kept for a long period in the dark [6]. We wondered about the consequences that the impaired dikaryosis observed in Atr1-Chk1 defective cells might have in the developmental choices of *C. cinerea*. For this, we evaluated in the silenced strains the formation of mushrooms under nutrient-controlled conditions as well as the ability to produce oidia and sclerotia.

To induce fruiting body formation, each strain was grown on 15 YMG/T plates until the mycelium filled up all the plate surface and just before the hyphae reached the border, the growing conditions of these plates were changed from 37ºC and 24 hours dark to 25ºC, >90% humidity and a 12 hours light/12 hours dark regime. This experiment was done three times with similar results. In the control strains, all the different developmental stages that had been previously described [29] were observed: Hyphal knots that became primordia and finally gave rise to mature fruiting bodies were developed normally. In contrast, in the silenced strains no fully developed mushrooms were observed (Fig. 3). Both *atr1* and *chk1*-silenced strains could only form hyphal knots. Interestingly, for the RNAi *chk1*#2 Rev strain around 70% of the cultures showed early stage primordia that aborted before becoming a mature primordia, indicating that even a small reduction in *chk1* expression levels affected the ability to produce a full life cycle in this fungus.

With respect to oidia and sclerotia production we observed a distinct response in silenced strains. While we observed no differences between control and silenced strains in oidia production (Fig. 4A, B), we found that sclerotia formation was dramatically impaired in strains carrying the silenced genes (Fig. 4C, D).

In summary, alteration of expression levels of the DDR kinases Atr1 and Chk1 resulted in inability to carry out some developmental choices such as fruiting body formation or sclerotia formation, while other processes such as oidia formation were unaffected.

DISCUSSION

The main conclusion of this study is that the Atr1 and the Chk1 kinases, required for the response to DNA damage in a number of eukaryotic organisms, have in the fungus *C. cinerea* roles during the control of dikaryotic cell cycle as well as in the developmental choices of this fungus.

During the dikaryotic cell cycle, nuclear division and septation have to be coordinated in such a way that each daughter cell inherits a balance of each parental genome. Although in some fungi the formation of clamp connections is not a requisite for this intricate process, in *C. cinerea* clamp connections are necessary for appropriated cell division [13]. We found that down-regulation of the levels of Chk1 and Atr1 seems to result in a high frequency of abnormal mitosis. The outcome of this abnormal mitosis is the presence of an excess of nuclei per hyphal cell, nuclei trapped in the clamp cell or septa with no associated clamp structure. How Atr1 and Chk1 deficiency is connected at molecular level with these abnormalities is not addressed in our study. We envisioned several possible explanations. One is a plausible connection between Atr1/Chk1 axis with the formation of the clamp structure. In *Saccharomyces cerevisiae* it has been reported that DDR checkpoint kinase Rad53 is able to control morphogenetic events during bud formation via its interaction with septins [40,41]. Although no study addressed so far whether septins are involved in clamp formation in *C. cinerea*, most likely it will be the case, as it was reported recently in dikaryons from *Cryptoccocus neoformans*, another basidiomycete [42]. Clamp connection formation is coupled somehow to nuclear position as well as cell cycle stage although the details of such a coupling are not well understood [13]. It could well be that Atr1 and Chk1 are elements involved in this connection. When the clamp formation is impaired or

disconnected from nuclear position and/or division, then abnormal mitosis are likely to arise.

We also entertained a second explanation more related with the “canonical” role of the Atr1-Chk1 cascade during the cell cycle (to delay or arrest the G2/M transition). An important feature during nuclear division in dikaryotic cells is that even when nuclei are located in different cell compartments -one in the main hypha, the other in the clamp cell-, the two nuclei have to divide synchronously [13]. Activation of S phase in both nuclei is likely to occur at the same time. However, some mechanism has to be in charge to assure that in the case the replication of one of the nuclei is delayed with respect to the other one, both will enter mitosis at the same time. We proposed that the Atr1/Chk1 pathway could be the surveillance system that avoids the entry into mitosis until both nuclei finished their respective S phases. In the absence of Atr1 or Chk1 this coupling is lost and then different rates of replication in the distinct nuclei will result in a differential entry into mitosis, which may affect the ability to produce or to resolve an appropriated clamp connection, and as a result an abnormal division. The importance of the connection between mitosis and clamp formation was uncovered upon the study of the consequences in dikaryotic cell cycle of several α and β tubulin mutants [13].

In addition to the observed cell cycle defects, a striking result we found upon down-regulation of Atr1 and Chk1 was the inability to assume some developmental choices, such as the fruiting body formation or the induction of sclerotia. Although we cannot establish a cause-effect relationship between abnormal cell divisions and these developmental defects, we believe this is the most obvious explanation. Alternative explanations such as a direct role of Atr1-

Chk1 cascade in the developmental choices in *C. cinerea* cannot be discarded although we consider it unlikely. Recently, a report described a mutation in one of the components of cohesion complex in *C. cinerea*, and as we described here, the outcome was an impaired cell cycle as well as the inability to produce fruiting bodies [16]. Although in this case the authors also consider alternative explanations, such as a direct role of Smc1 in gene regulation, as it was described in humans [43], we are tempting to assume that in both cases, the *smc1* mutant and the down-regulation of Atr1-Chk1 pathway, the observed developmental defects were a direct consequence of a defective cell cycle.

The *A* mating type proteins are required for fruiting body initiation in *C. cinerea* [44,45] while the *B* mating type proteins optimize the frequency of fruiting [46]. There is thus likely a link to mating type-control based communication in synchronization of the cell cycle of the two nuclei. Further to regulation of fruiting body initiation, sclerotia production is much enhanced by the *A* mating type proteins compared to the monokaryotic situation [44] and disruption in nuclear communication/synchronization should restore the monokaryotic situation as found in the study. Why is then oidiophore and oidia production not affected in the silencing mutants? In contrast to fruiting body development and the sclerotium formation on the dikaryon, oidiation is a process that involves also on the dikaryon only a single nucleus that undergoes a pathway of specific development of uninuclear cells as defined for the monokaryon [37,46].

Extensive studies have led to a thorough understanding of the core mechanisms that drive the eukaryotic cell cycle. It has also become increasingly clear that these core mechanisms are modulated during development. But also, recent findings demonstrate that components of the cell cycle machinery can in

turn regulate development [47]. We believe that studies dedicated to analyze the connections between fruiting body induction and dikaryotic cell cycle regulation in *C. cinerea* will help to increase our knowledge in these important connections.

MATERIAL AND METHODS

Strains and growth conditions

Coprinopsis cinerea strains are derived from AmutBmut genetic background (*A43mut B43mut pab1-1*) [30]. Strains were grown on YMG/T (0.4% yeast extract, 1% malt extract, 0.4% glucose, 100 mg/l tryptophan and 1% agar) and MM (0.1% KH_2PO_4, 0.225% Na_2HPO_4, 0.029% Na_2SO_4, 0.05% di-ammonium tartrate, 0.025% $MgSO_4x7H_2O$, $4x10^{-5}$% thiamine, 0-.005% adenine sulphate, 0.2% asparagine, 1% glucose and 1% agar) media at 37ºC [32,48]. To check the sensitivity of the strains to different genotoxic stresses, a cylindrical piece of mycelium with a diameter of 4 mm was inoculated on YMG/T plates supplemented with 2.5 mM HU or 0.015% MMS. Plates were grown until the plate was fully covered of mycelium (around 6 days for control strains). Induction of fruiting body, oidia and sclerotia formation were done as described [32,39,44].

Microscopic observations

Samples were visualized in a Nikon Eclipse 90i microscope equipped with a Hamamatsu ORCA-ER CCD camera. Pictures were taken using the appropriate filter set, Nikon Plan Apo *VC* 100X NA 1.40 and Plan Apo *VC* 60X NA 1.40 lenses with Nikon Immersion Oil type A nd=1.5151. The software used with the microscope was Metamorph 6.1 and the pictures were further processed with

Adobe Photoshop CS5. To perform septa and nuclei staining, *C. cinerea* was grown for two days at 37ºC on a thin agar layer over a glass microscope slide with a small window in the middle of the size of the cover slip. After this time, 70% EtOH was added to dry out the hyphae and then the hyphae were stained with 140 nM Hoechst 33258 and 273 nM Calcofluor on a 0.5% DMSO solution, for 20 minutes before observing [49].

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LEGENDS TO FIGURES

Fig 1. Silencing *chk1* and *atr1* expression in *Coprinopsis cinerea* resulted in sensitivity to DNA damage agents. (*A*) (*B*) Relative gene expression values, measured by RT-qPCR, of *chk1* and *atr1* in control and silenced strains, respectively. The data show the average and SE of three biological replicates ($p<0.01$). Expression rates are normalized with respect transcript abundance of *benA* (control), a housekeeping gene. (*C*) Sensitivity of control and silenced strains to the DNA-damage agents, hydroxyurea (HU) and methylmethane sulfonate (MMS). Plates were incubated for 8 days at 37ºC.

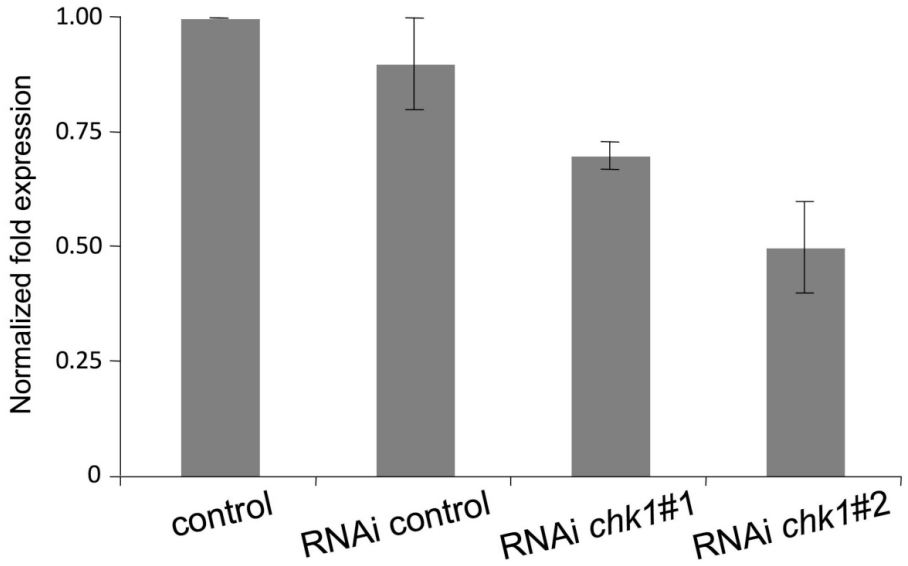
Fig. 2. Down-regulation of *atr1* and *chk1* resulted in an increase in abnormal mitosis in the dikaryon. (*A*) Cell images from the indicated strains. Septa with associated clamp connections are marked with arrowheads, while septa without clamp connections are marked with arrows. Nuclei are marked with asterisks. Dots marked the presence of nuclei trapped by a clamp connection. Bar: 20 μm. (*B*) Examples of cells resulting from abnormal mitosis. In B1: a cell compartment flanked by a septum with no associated clamp connection (arrow) and five nuclei (asterisk). In B2: nuclei trapped inside the clamp connection (asterisk). (*C*) Quantification of the number of aberrant mitosis (cell compartments with clamp-free septa or/and more than 2 nuclei) as well as clamps with trapped nuclei inside in hyphae from the indicated strains. The graph shows the result from 3 independent experiments, counting more than 100 cell compartments each. Means and standard deviations are shown.

Fig. 3. Fruiting defects in *chk1* and *atr1* knock-down strains. The upper panel

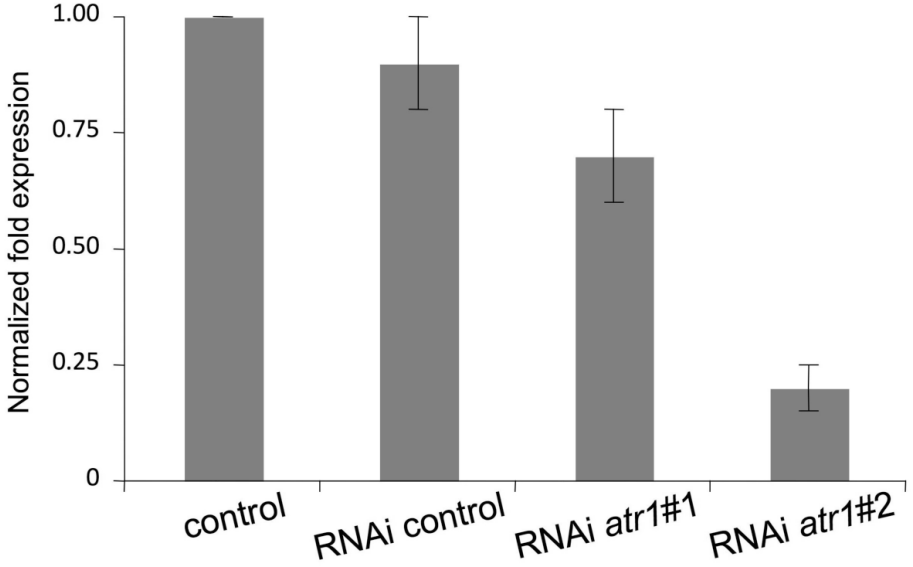
shows the approximate time course of fruiting and the times when photographs (lower panels) were taken. Note that knock-down mutants never develop into fruiting body primordia. Only the reverted strain RNAi *chk1#2* Rev was able to develop into fruiting body primordia, although it stopped at this stage.

Fig. 4. Silencing of *atr1* and *chk1* affects other developmental decisions. (*A*) Images of oidiophores and oidia in the indicated strains. The ability to form oidiophores after light induction (Polak et al. 1997) was not affected in silenced strains. (*B*) Quantification of the ability to produce oidia in the silenced strains. A similar number of oidia was produced by silenced strains in comparison with controls, both in light and dark conditions. (*C*) (*D*) Sclerotia formation was severely inhibited in silenced strains. Sclerotia can be observed as black dots in the surface of the aerial mycelium. Note the scarce number of sclerotia per mycelial camp in silenced strains.

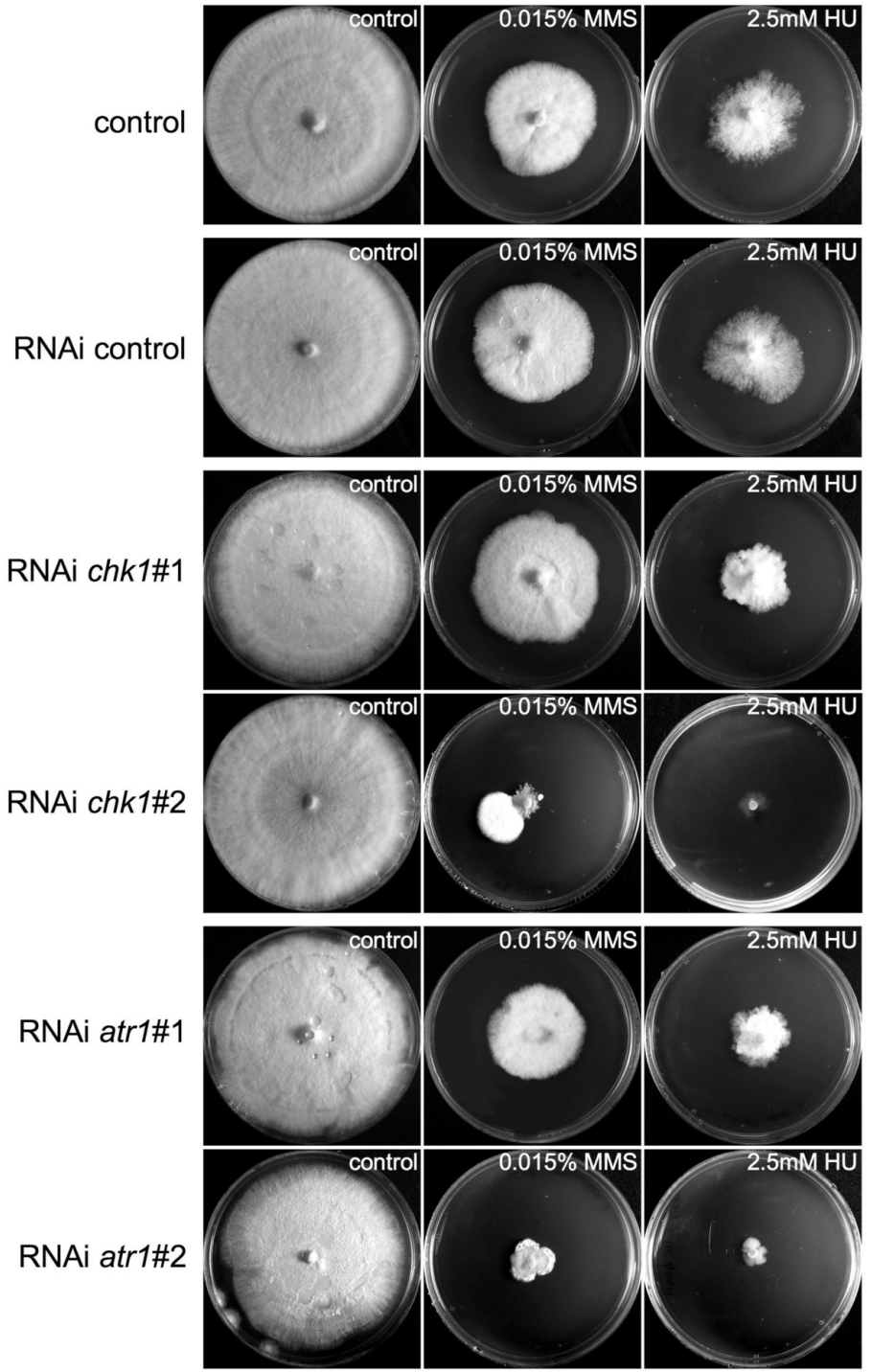
A

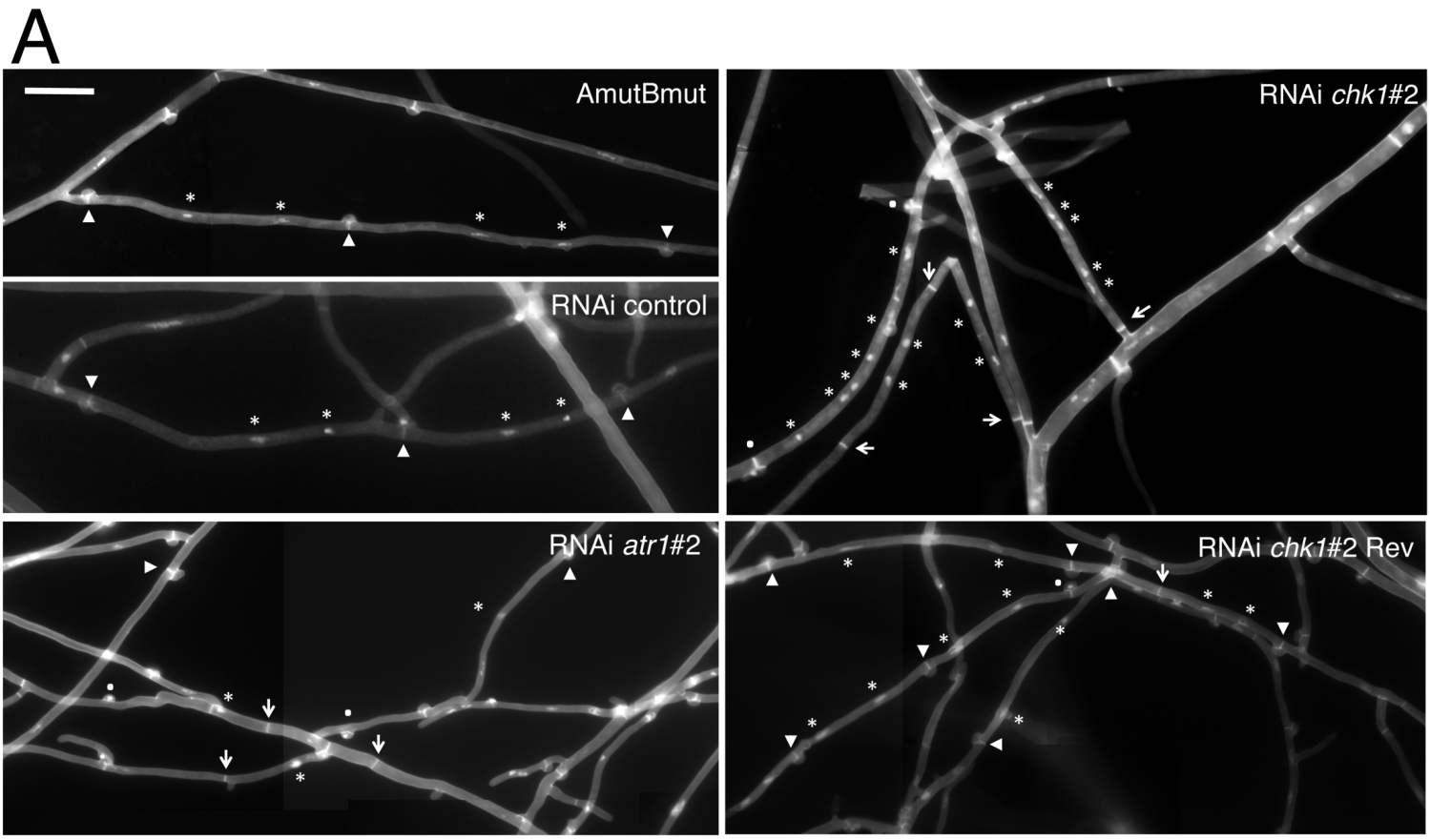

chk1 gene expression
Normalized fold expression
1.00
0.75
0.50
0.25
0
control
RNAi control
RNAi chk1#1
RNAi chk1#2

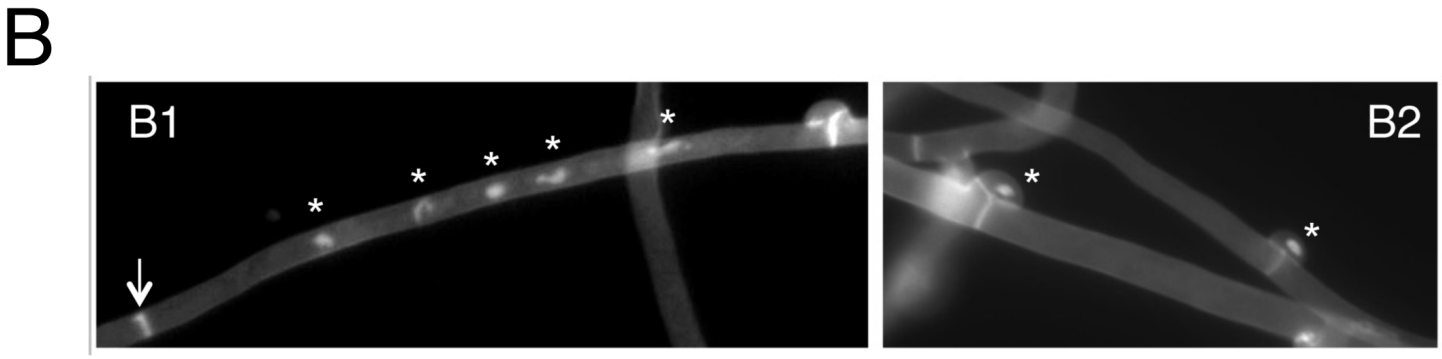
B

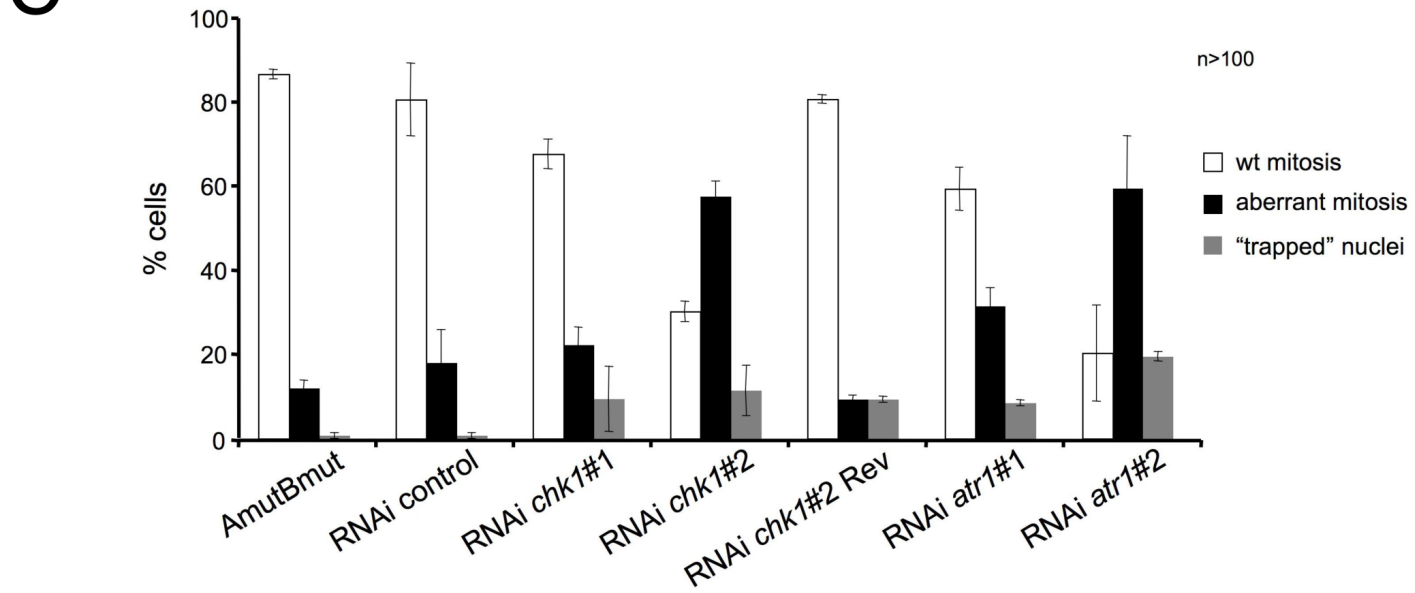

atr1 gene expression
Normalized fold expression
1.00
0.75
0.50
0.25
0
control
RNAi control
RNAi atr1#1
RNAi atr1#2

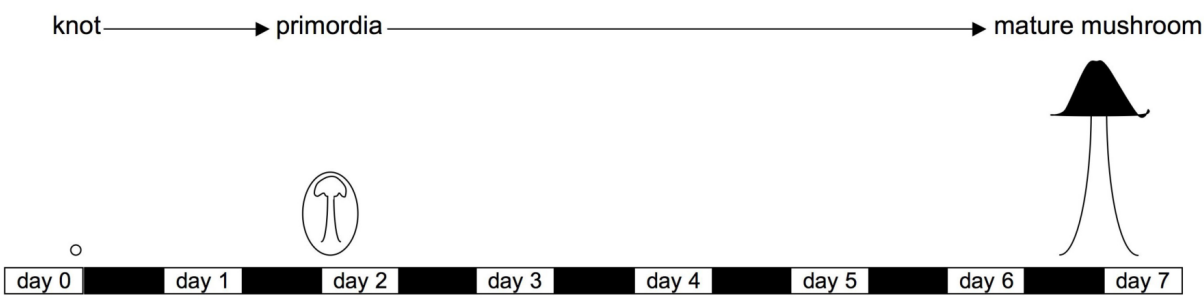
C

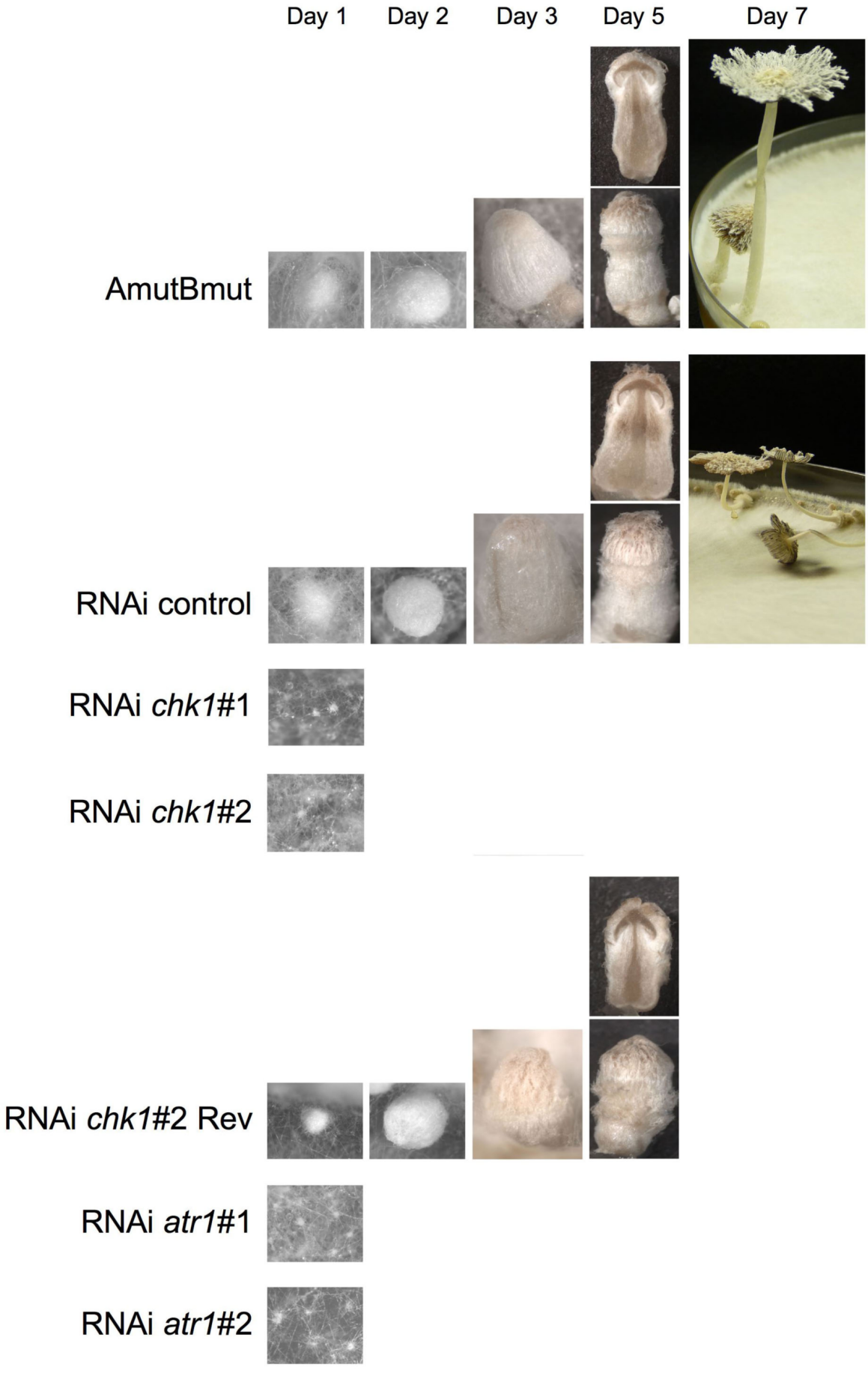

control
RNAi control
RNAi chk1#1
RNAi chk1#2
RNAi atr1#1
RNAi atr1#2
control
0.015% MMS
2.5mM HU

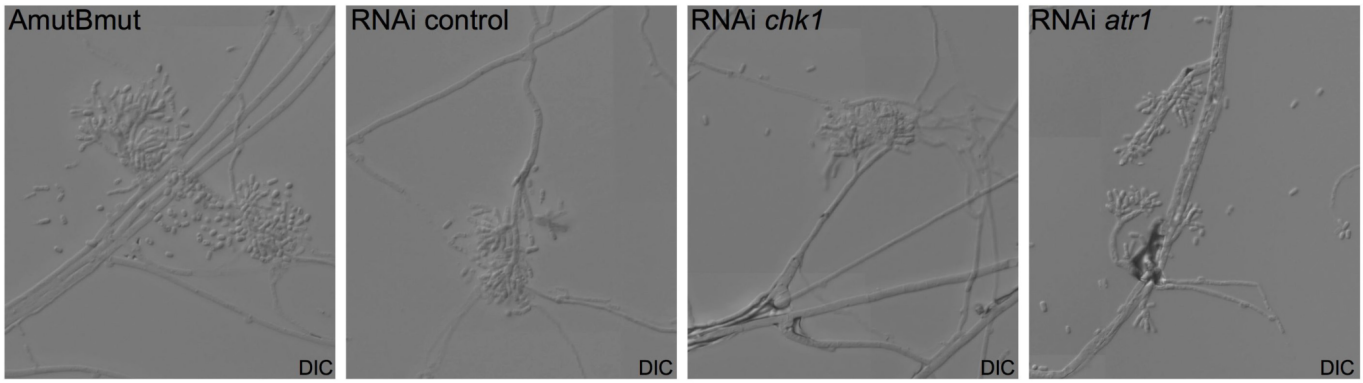

A
AmutBmut
RNAi control
RNAi atr1#2
RNAi chk1#2
RNAi chk1#2 Rev

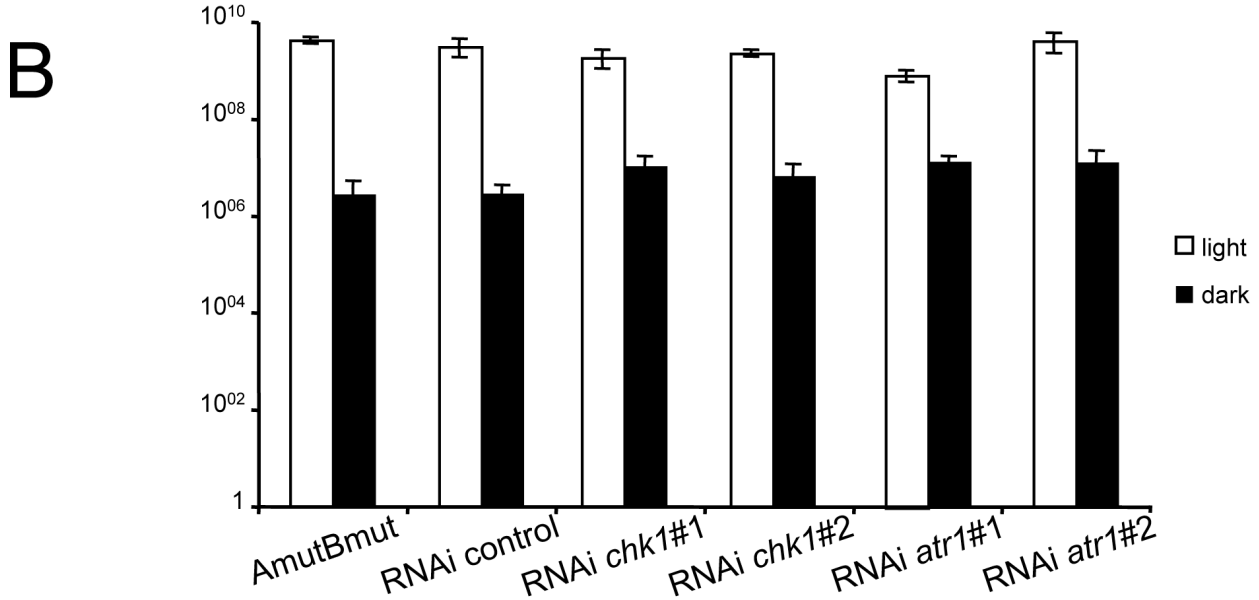

B
B1
B2

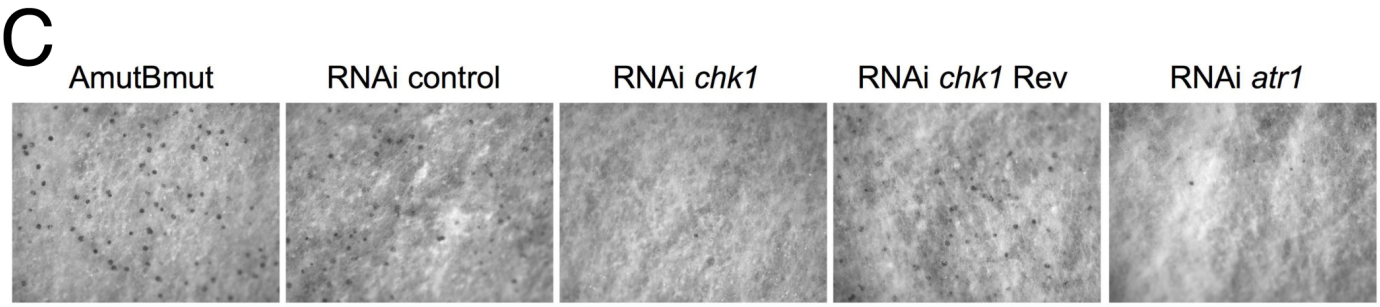

C
100
80
60
40
20
0
% cells
n>100
wt mitosis
aberrant mitosis
"trapped" nuclei
AmutBmut
RNAi control
RNAi chk1#1
RNAi chk1#2
RNAi chk1#2 Rev
RNAi atr1#1
RNAi atr1#2

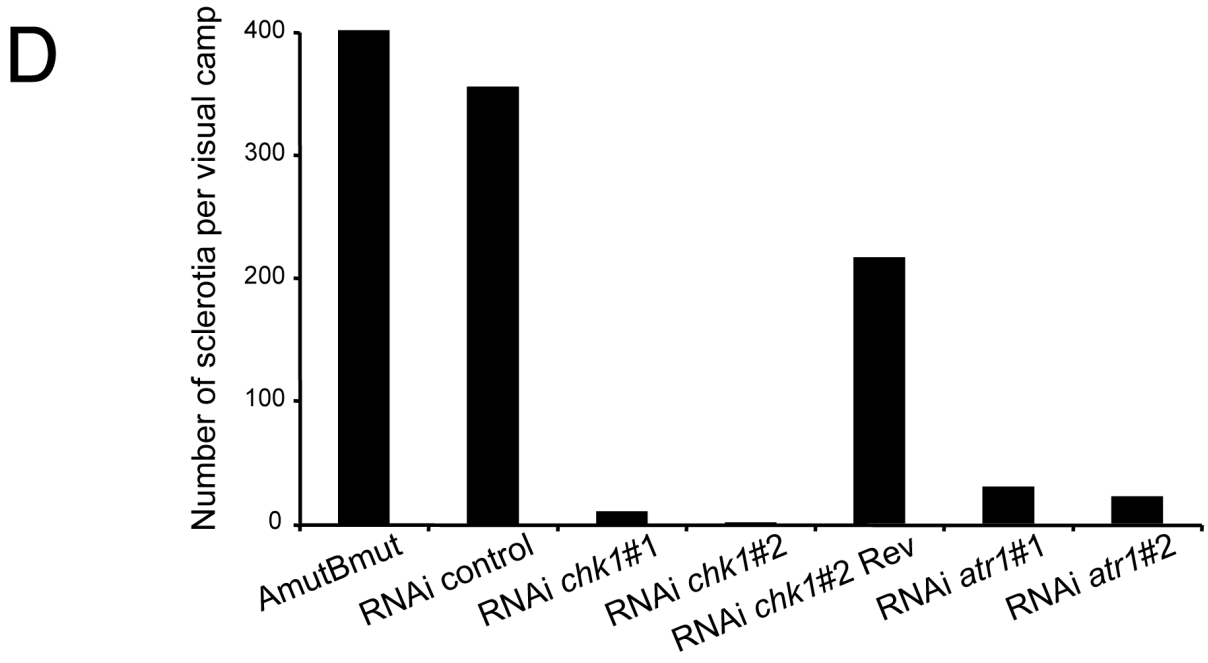

knot
primordia
mature mushroom
day 0
day 1
day 2
day 3
day 4
day 5
day 6
day 7


Day 1
Day 2
Day 3
Day 5
Day 7
AmutBmut
RNAi control
RNAi chk1#1
RNAi chk1#2
RNAi chk1#2 Rev
RNAi atr1#1
RNAi atr1#2


A
AmutBmut
RNAi control
RNAi chk1
RNAi atr1
DIC
DIC
DIC
DIC


B
10^10
10^08
10^06
10^04
10^02
1
light
dark
AmutBmut
RNAi control
RNAi chk1#1
RNAi chk1#2
RNAi atr1#1
RNAi atr1#2


C
AmutBmut
RNAi control
RNAi chk1
RNAi chk1 Rev
RNAi atr1


D
Number of sclerotia per visual camp
400
300
200
100
0
AmutBmut
RNAi control
RNAi chk1#1
RNAi chk1#2
RNAi chk1#2 Rev
RNAi atr1#1
RNAi atr1#2