Curcumin targets a circumferential band at the cleavage furrow of dividing fission yeast cells

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Abstract

Curcumin is the active ingredient of one of the most widely used spices in the world for millennia. It is also highly valued as a traditional health supplement in many South Asian countries. It is not surprising that this yellow turmeric has attracted strong attention for its therapeutic potential in recent years. Nevertheless, the molecular and cellular targets of curcumin remain unknown. Here, we took a novel imaging-based approach to determine the intracellular localization of curcumin using the model organism fission yeast *Schizosacchromyces pombe*. We took advantage of the intrinsic fluorescence of curcumin to track its whereabout in yeast cells. Live fluorescence microscopy revealed for the first time that curcumin, at a concentration as low as one micromolar, formed a narrow circumferential band around the equatorial plane of dividing cells within 10 minutes of addition. The intensity of this band increased proportionally to the concentration of curcumin and gradually over time. Inhibition of cell division by the *cdc25* temperature-sensitive mutation inhibited the curcumin band. During cytokinesis, curcumin co-localized with the integrated plasma membrane protein Bgs1 at the cleavage furrow, not with either the contractile ring or the septum markers. Besides fission yeast, such equatorial curcumin band was found in the dividing cells of two other yeasts, *Saccharomyces cerevisiae* and *Candida albicans*. Our study thus discovered that this widely used yellow turmeric targets the cleavage furrow of dividing yeast cells, suggesting that curcumin may have anti-fungal therapeutic potential (223 words).

Introduction

Curcumin is the main active compound of a widely used spice around the world. This polyphenol molecule ($C_{21}H_{20}O_6$, Diferuloylmethane) can be isolated from the root of the plant *Curcuma longa*. Originated in India, this yellow turmeric has been used in cooking in South Aisa for millennia. Over the last few centuries, curcumin has gained even more popularity, adopted by many other countries around the world as a spice.

Besides being used as a spice, curcumin has a long history of being used as either dye, or herbal medicine, or cosmetics. Over the last decade, curcumin has especially attracted strong interests in biomedical research for its therapeutical potential as an anti-tumor, anti-oxidant or anti-inflammatory supplement (Gupta et al., 2013; Stepien et al., 2020; Zhou et al., 2011). The heightened interest in curcumin can be gleaned from the large number of scientific studies focused on the compound. For example, in 2024 alone, more than 2,400 papers can be found by searching PubMed using "Curcumin" as the keyword. Despite such strong interest in the potential of curcumin for human health, the cellular and molecular targets of this small molecule remain largely unknown.

Yeasts have long been instrumental in elucidating the cellular mechanisms of various bioactive compounds due to their genetic tractability and conserved eukaryotic pathways (Botstein and Fink, 2011). The budding yeast *Saccharomyces cerevisiae* has been used as a model organism to identify the potential molecular targets of curcumin with some successes. At high concentrations of more than 100 micromolar, curcumin accumulates intracellularly in the endoplasmic reticulum (ER) (Minear et al., 2011). It can chelate intracellular iron, potentially disrupting the iron homeostasis (Azad et al., 2014; Minear et al., 2011). However, several reports also documented the pleiotropic effects of curcumin in *S. cerevisiae*. Curcumin can activate the MAPK kinase cascade (Azad et al., 2014). It also delays the cell cycle progression (Minear et al., 2011). Finally, curcumin may also extend the chronological lifespan

of yeast (Stepien et al., 2020). Overall, it remains unclear what the main cellular and molecular targets of curcumin are in budding yeast.

Fission yeast *Schizosacchromyces pombe* has also been widely used as a unicellular eukaryotic model organism (Hoffman et al., 2015). The cylindrical shaped fission yeast cells grow through tip extension. They enter mitosis when the cell length reaches ~14 μm. Like animal cells, fission yeast cells start cytokinesis through the assembly of an actomyosin contractile ring at the equatorial division plane. This is followed by the cleavage furrow ingression and the septation. Unlike *S. cerevisiae*, dividing fission yeast cells split into two equal sized daughters. Despite some similarities, *S. pombe* and *S. cerevisiae* differ significantly from each other in many cellular processes. This is not surprising, considering that they have diverged from each other more than 300 million years ago. To our knowledge, few have studied the cellular effects of curcumin in fission yeast.

Here, we employed a novel imaging-based approach to track the intracellular localization of curcumin in fission yeast cells through live fluorescence microscopy. To our surprise, we discovered a circumferential band of curcumin at the equatorial division plane even at a concentration of one micromolar. Curcumin specifically targeted the plasma membrane at the division plane, based on its co-localization with the integrated membrane protein Bgs1 during cytokinesis. We found similar curcumin band at the equatorial division plane of two other yeasts, *S. cerevisiae* and *C. candida* cells. The discovery of this curcumin band in dividing yeast cells demonstrates both the therapeutic potential of curcumin and the promising future of using fission yeast as a model organism to study this popular yellow turmeric.

Results and discussion

One unique property of curcumin is its intrinsic fluorescence, which allows for the direct visualization of this small molecule (Fig. 1A). This yellow turmeric can be excited at 488nm

with a peak emitting fluorescence at 520nm (Ferreira et al., 2023; Priyadarsini, 2009; Zsila et al., 2003). We hypothesized that the intracellular localization of curcumin can be determined precisely through live microscopy. We inoculated the exponentially growing fission yeast cells in the rich YE media supplemented with 0.5, 1, 2, 4 and 8 μM of curcumin respectively for 10 minutes (Fig. 1B). We then imaged this curcumin labeled cells using a confocal fluorescence microscope. Little or no fluorescence was detected in the cells treated with either the vehicle DMSO or 0.5μM curcumin (Fig. 1B). In comparison, at 1μM, a bright fluorescent band formed at the equatorial plane of dividing cells. No other intracellular structures were fluorescent (Fig. 1B). The fluorescence intensity of these bands increased gradually as the curcumin concentration increased (Fig. 1B). At 8μM, in addition to the band, the diffuse fluorescence of curcumin was visible throughout the cytoplasm of some interphase cells (Fig. 1B). At these concentrations, curcumin had no observable effect on the morphology of these fission yeast cells (Fig. 1B). Fluorescence intensity of the curcumin band increased gradually in the presence of this small molecule (Fig. 1C). To confirm that curcumin specifically targets the dividing cells, we prevented the cells from entering mitosis by using the *cdc25-22* temperature-sensitive mutant (Gould et al., 1990). At the restrictive temperature of 36°C, these mutant cells became much longer than the wild-type cells due to their failure to divide (Fig. 1D). As expected, no curcumin band was detected in these mutant cells (Fig. 1D). However, the intensity of the curcumin band in the wild type cells also weakened significantly at 36°C. Therefore, the curcumin band is likely temperature dependent. We concluded that curcumin targets the dividing fission yeast cells with high affinity by forming a band at the equatorial plane.

At the division plane, the curcumin band may represent the actomyosin contractile ring, or the cleavage furrow or the septum. To differentiate such scenarios, we characterized the curcumin band in the cells expressing the fluorescence protein markers for each of these three subcellular structures separately. First, we examined the cells expressing the contractile ring

marker Rlc1 (Le Goff et al., 2000), the myosin regulatory light chain, tagged with the fluorescence protein tdTomato. The curcumin band and the contractile ring only partially overlapped at the equatorial division plane (Fig. 2A). Although both appeared at the beginning of cytokinesis (Fig. 2A), the curcumin band did not constrict as the contractile ring did. Therefore, the curcumin band is distinct from the contractile ring. Secondly, we examined the cells expressing an integrated membrane protein, the glucan synthase Bgs1 (Cortes et al., 2002), tagged with tdTomato at the cleavage furrow. The localization of Bgs1-tdTomato at the division plane largely overlapped with the curcumin band (Fig. 2B). To confirm this, we carried out time-lapse fluorescence microscopy of these Bgs1-tdTomato expressing cells in the YE media supplemented with 2μM curcumin. In the dividing cells, the curcumin band overlapped with that of Bgs1-tdTomato during cytokinesis (Fig. 2C). After the separation of daughter cells, the curcumin band split into two, one remaining in each daughter cell. In contrast, Bgs1-tdTomato diffused on the plasma membrane of the cell tips (Fig. 2C). Lastly, we co-localized the curcumin band with the septum. The cells were incubated in the presence of both curcumin (2μM) and calcofluor (1μM) which stained the septum (Ribas and Cortés, 2016). The curcumin band formed in many cells whose septum had not yet been assembled (Fig. 2D). Even among the cells with septum, the head-on view of the cleavage furrow, by 3D reconstruction, revealed that the curcumin band did not overlap with the disc-shaped septum (Fig. 2E). We concluded that curcumin accumulates in a band on the plasma membrane of the cleavage furrow during cytokinesis.

Lastly, we determined whether a similar curcumin band assembles in other yeasts cells. As in the fission yeast cells, a curcumin band formed at the bud neck of dividing budding yeast *S. cerevisiae* cells (Fig. 3A). However, compared to those in fission yeast, the fluorescence intensity of these curcumin bands was significantly lower. Even at 2μM, the cytoplasmic curcumin fluorescence was higher. Similarly, the curcumin bands decorated the bud neck of

the dividing pathogenic yeast *C. albicans* cells (Fig. 3B). We concluded that the curcumin band targets the equatorial division plane of two other yeasts.

Compared to past studies, ours revealed the existence of a unique equatorial curcumin band in yeast cells by using much lower concentrations of this molecule. The past studies typically examined the effects of curcumin at concentrations exceeding 100μM, sometime as high as several mM (Minear et al., 2011). In comparison, we carried out most of our experiments using just 2μM curcumin supplemented in the media. At this concentration, curcumin likely binds those high-affinity intracellular targets first. With increased concentrations of curcumin, it may have pleiotropic effect through binding many other molecules nonspecifically. Consistent with the hypothesis, we observed that curcumin started to stain other intracellular structures in addition to the equatorial band at 8μM. The unspecific binding of other intracellular targets by curcumin may have prevented the observation of the curcumin band in those earlier studies.

This other critical factor that may have worked to our favor is that we chase-labeled the yeast cells with curcumin before determining its localization with microscopy. In comparison, the other studies typically wait for hours after addition of curcumin before imaging these yeast cells (Minear et al., 2011). This may have allowed curcumin to be trafficked through the intracellular membrane pathway. We similarly found that the curcumin band disappeared after incubating yeast cells with this small molecular for more than 8 hours. Therefore, our study suggests that curcumin binds the plasma membrane at the equatorial division plane first.

Our study strongly suggests that curcumin binds to the lipids in the plasma membrane of the cleavage furrow in both budding and fission yeast. Curcumin may permeate the yeast cell wall due to its lipophilic character (Tonnesen et al., 2002). It does not appear to permeate the plasma membrane completely. Curcumin co-localizes with the integrated membrane protein Bgs1 at the cleavage furrow. Our conclusion is consistent with the previous study using

budding yeast cells to identify the molecular targets of curcumin. The genetic screen for hypersensitivity to curcumin using the budding yeast identified two groups of genes, one of which regulates the lipid biosynthesis. These include two genes encoding the synthesis of ergosterol and phospholipids respectively. Of these two, ergosterol which is specific to yeast, is the most likely target.

Our study explains a few observations made of curcumin in fission yeast. A genome-wide study of the effect of hydrazinocurcumin found that this derivative form of curcumin may target the septum and the septation pathway (Baek et al., 2008). A fission yeast mutant of one of the septin genes is hyper-sensitive to hydrozinocurcumin (Baek et al., 2008). (Iurciuc-Tincu et al., 2020; Luo et al., 2023).

Taken together, our study revealed a previously unknown ability of curcumin to target dividing fission yeast cells even at a relatively low concentration. Curcumin can form a band on the plasma membrane at the cleavage furrow of dividing fission yeast cells quickly and continue to accumulate there. In the future, curcumin may be explored for its ability for its antifungal potential. This is particularly promising considering that curcumin is well-tolerated in human bodies as a widely used spice.

Materials and Methods

Yeast Strains and growth conditions: We followed the standard procedures for yeast cell culture (Moreno et al., 1991). The *S. pombe* yeast cells were inoculated in liquid YE5s media at 25°C in an orbital shaker (Innova, New Brunswick, USA). For both *S. cerevisiae* cells and *C. albicans* cells, we inoculated them in the YPD media at 30°C.

For the temperature-sensitive mutants, we grew the exponentially growing yeast cell at 36°C 4 hrs before imaging them with curcumin.

Fluorescence microscopy: For microscopy of live yeast cells, we used a previously described method to image them in a lectin-coated glass-bottomed petri dish (Poddar et al., 2021). Briefly, to prepare the dish (Cellvis, USA) for confocal microscopy, we added 50ul of 50µg/ml of lectin (Sigma, L2380) to the center of the coverslip and allowed it to dry out at 30°C for 3-4 hours. We deposited 20ul of the exponentially growing yeast culture to the cover slip and left it to stand for 10 mins. Afterwards, we added 2 ml of YE5S media to the petri dish and proceeded to live microscopy.

To perform time-lapse microscopy, we used a spinning disk confocal system managed by iQ3 software (Andor). It consists of an Olympus IX71 microscope body and a CSU-X1 spinning disk unit (Yokogawa, Japan). It is equipped with a motorized stage (ASI, USA) and a Piezo Z Top plate. Images were captured on an EMCCD camera (IXON-897, Andor). The microscope employed solid-state lasers with wavelengths of 488 nm and 561 nm. Unless otherwise noted, a 60x objective lens (Olympus, Plan Apochromat, NA = 1.40) was used. Typically, a Z-series of 8 slices at 1µm intervals was acquired for each time point. For the temperature-sensitive mutants, we imaged the cells in a temperature-controlled chamber maintained at 36°C (Okolab, Italy).

Chemicals: Curcumin is commercially available from Millipore Sigma (C1386). A 10mM stock solution of curcumin was prepared in DMSO and stored at -20°C for long-term usage. The curcumin supplemented yeast media was freshly prepared by diluting the stock solution into YE5s on the day of experiment. Calcofluor White (1mg/ml) was commercially available from Millipore Sigma (18909). Lectin (Sigma, L2380) was prepared in water for 1mg/ml stock solution.

Image analysis: All the micrographs were analyzed using NIH Image J.

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Author Contributions

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Varmila Kulasegaram: Investigation, Formal analysis, Methodology, Writing - review & editing

Anna Okorokova-Façanha: Writing - review & editing

Qian Chen: Investigation, Formal analysis, Conceptualization, Funding acquisition, Project administration, Writing - original draft, Writing - review & editing

Table 1: List of fission yeast strains used in this study

Name	Genotype	Source
FY527	*h- leu1-32 ura4-D18 his3-D1 ade6-M216*	Lab stock
QCY795	*h- leu1-32 ura4-delta18 his3-delta1 bgs1::ura4+ Pbgs1+::tdTomato-bgs1+:leu1+*	Lab stock
JW1341	*h- rlc1-tdTomato-natMX6 ade6-M210 leu1-32 ura4-D18*	Lab stock
JW1009	*h- Cdc25-22 ade6-M210 ura4-D18*	Lab stock

Figure 1

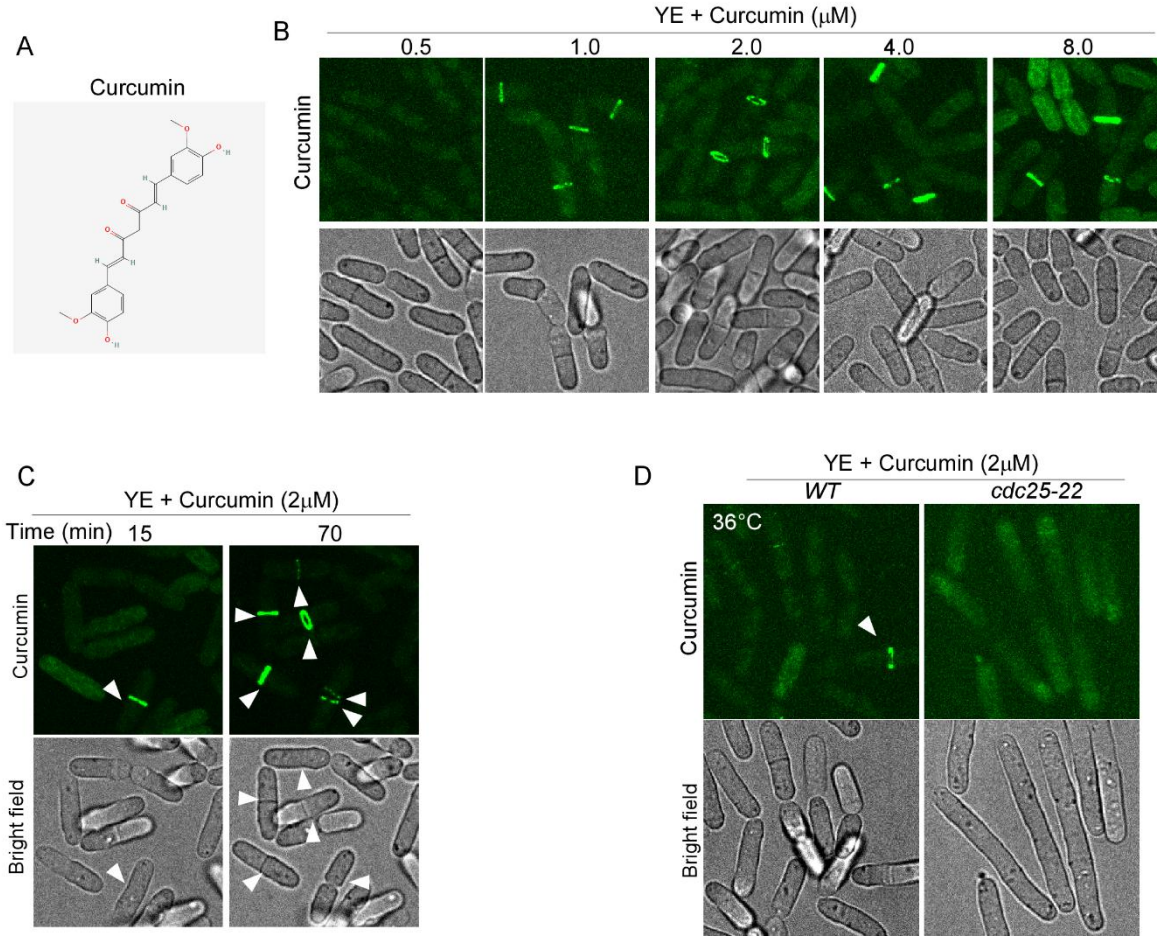

Figure 1. Curcumin stains dividing fission yeast cells. (A) Chemical structure of curcumin **(B)** Micrographs of fission yeast cells in the presence of various concentrations of curcumin for 10 mins. **(C)** Micrographs of fission yeast cells in the presence of 2μM curcumin. Number indicates minutes after the addition of curcumin in the media. **(D)** Micrographs of the wild type (left) and *cdc25-22* mutant (right) cells in the presence of curcumin at the restrictive temperature of 36°C.

Figure 2

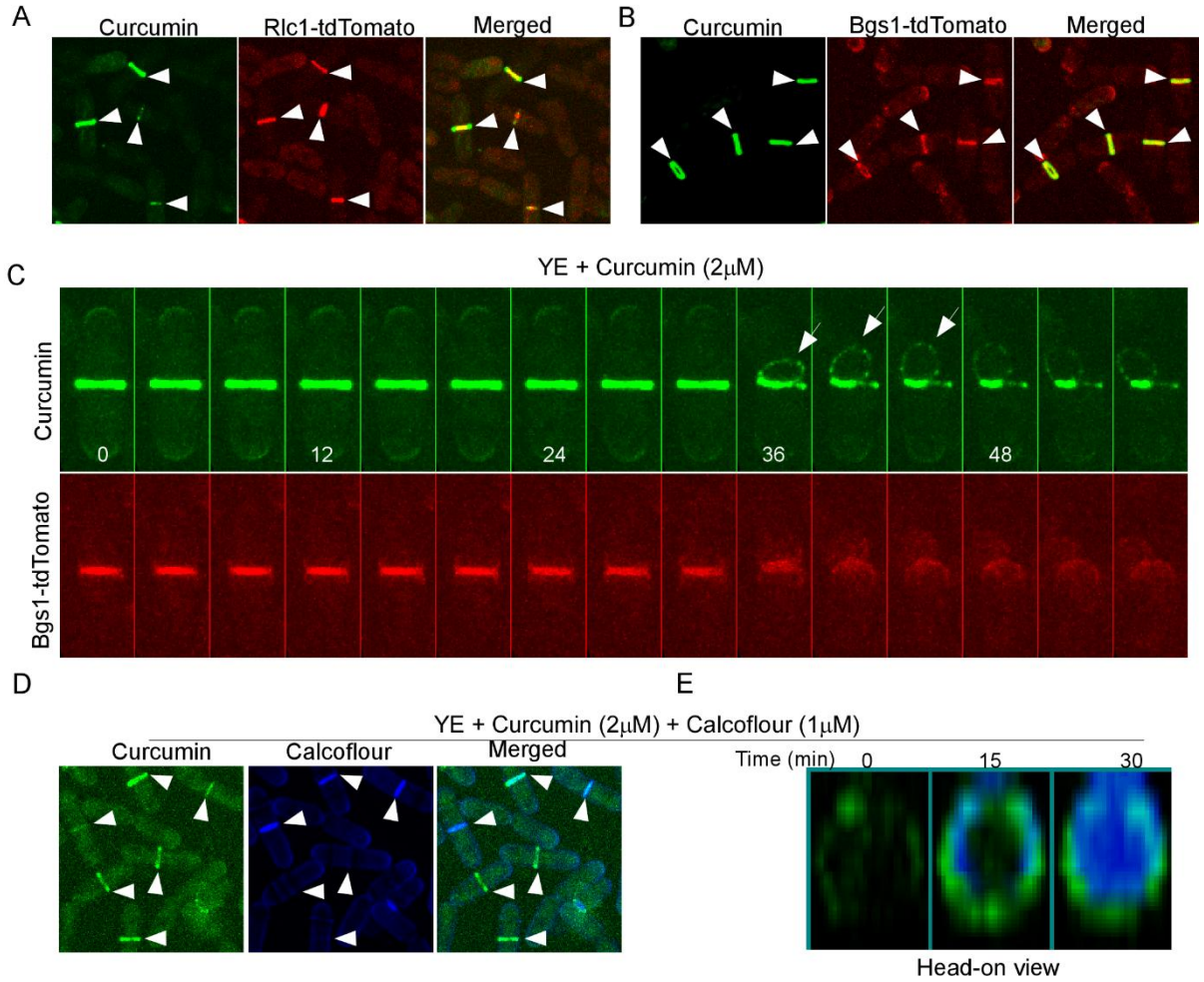

Figure 2. The curcumin band is on the plasma membrane at the equatorial division plane. (A-B) Micrographs of Rlc1-tdTomato (top, red) expressing or Bgs1-tdTomato (bottom, red) expressing cells in the presence of 2μM curcumin (green). Arrowhead: curcumin band.

(C) Time-lapse micrographs of a cell expressing Bgs1-tdTomato (red) in the presence of curcumin. Arrows: splitting of the curcumin band into two after the daughter cell separation.

(D) Micrograph of the wild-type cells in the presence of both curcumin and calcofluor.

(E) 3D reconstructed micrographs of the cleavage furrow (head-on view) the wild type cells in the presence of both curcumin (green) and calcofluor (blue). Number: time from the start of the time-lapse series.

Figure 3

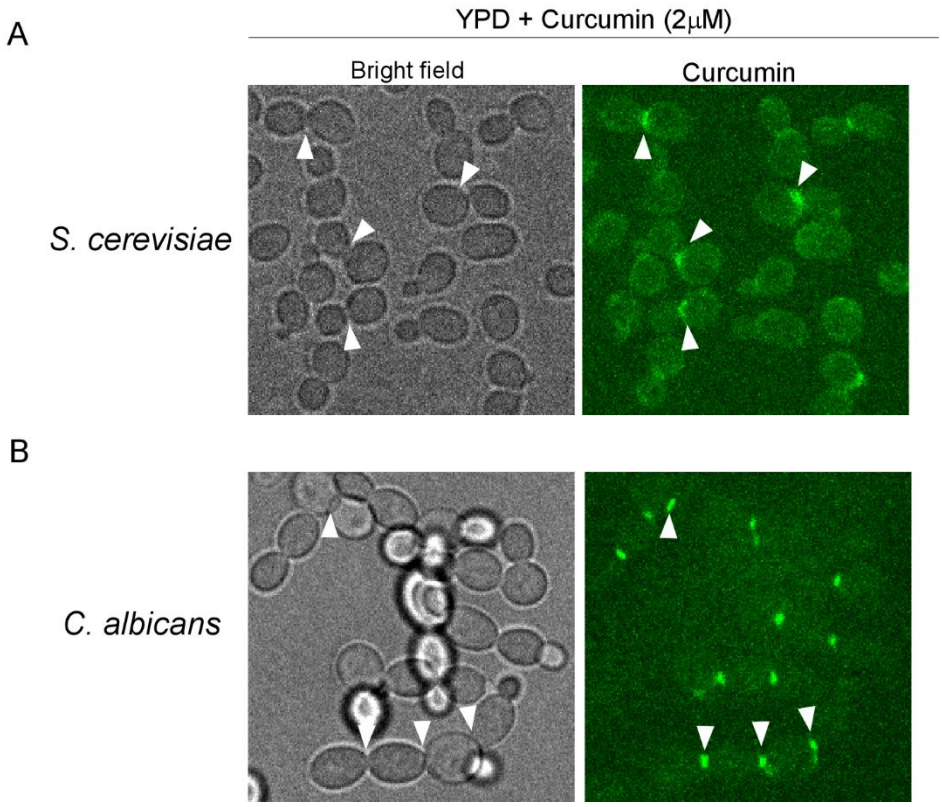

Figure 3. The curcumin band forms at the division planes of two other yeasts, *S. cerevisiae* and *C. albicans*. (A-B) Micrographs of the wild-type *S. cerevisiae* or *C. albicans* cells in the presence of 2μM curcumin. Arrowhead: the curcumin band at the bud neck.

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Figure 1

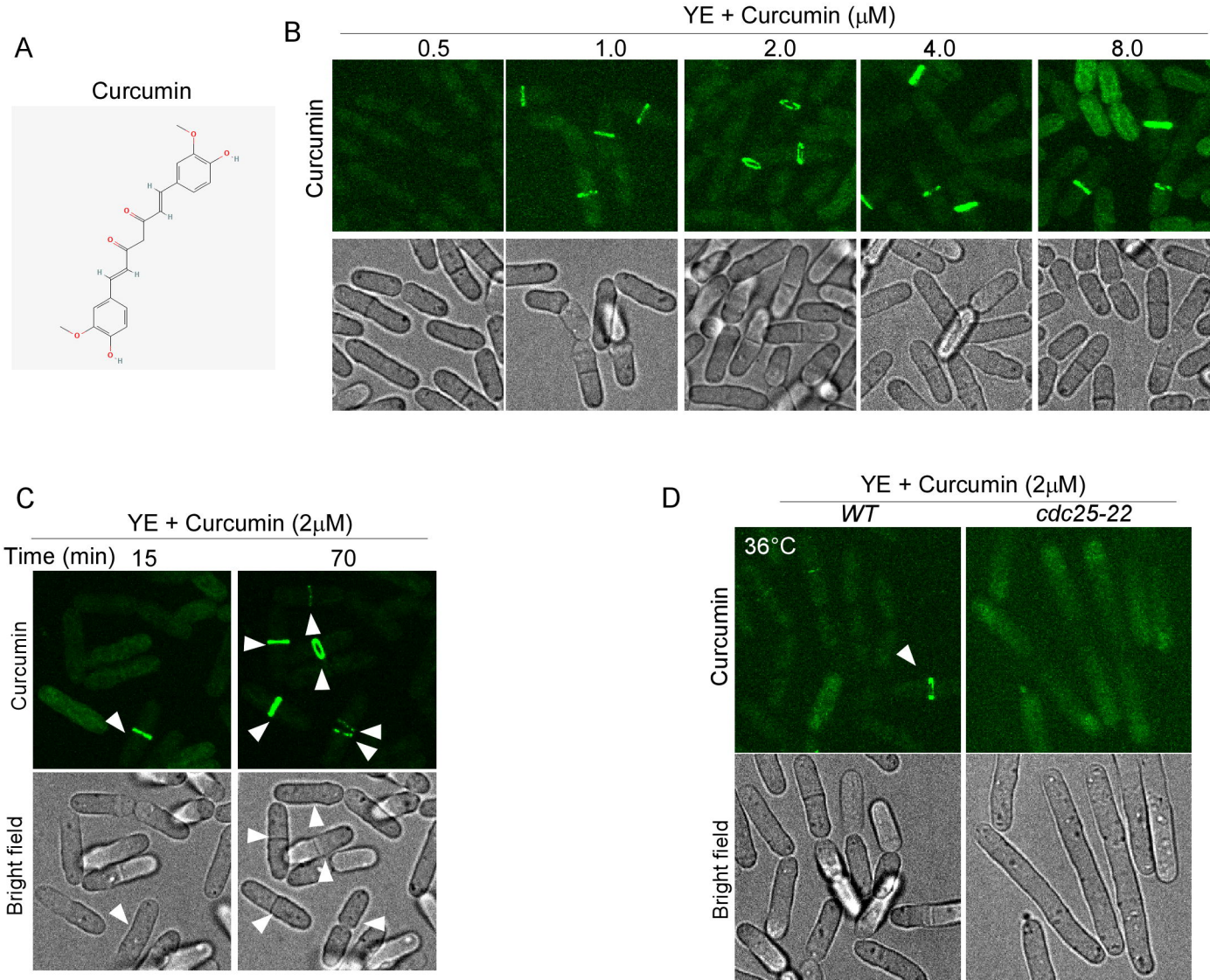

A
Curcumin
B
YE + Curcumin (μM)
0.5
1.0
2.0
4.0
8.0
Curcumin
C
YE + Curcumin (2μM)
Time (min)
15
70
Curcumin
Bright field
D
YE + Curcumin (2μM)
WT
cdc25-22
36°C
Curcumin
Bright field

Figure 2

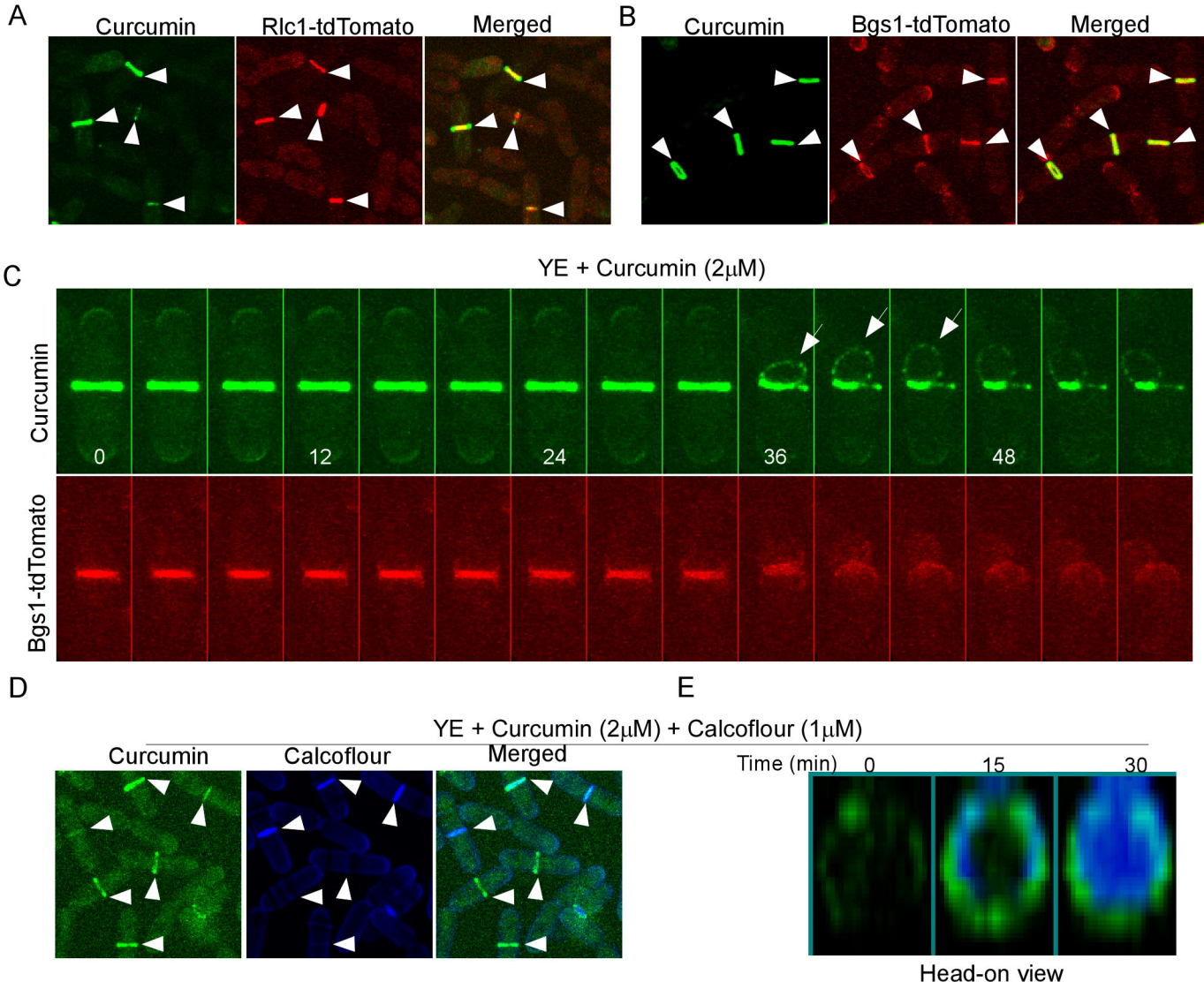
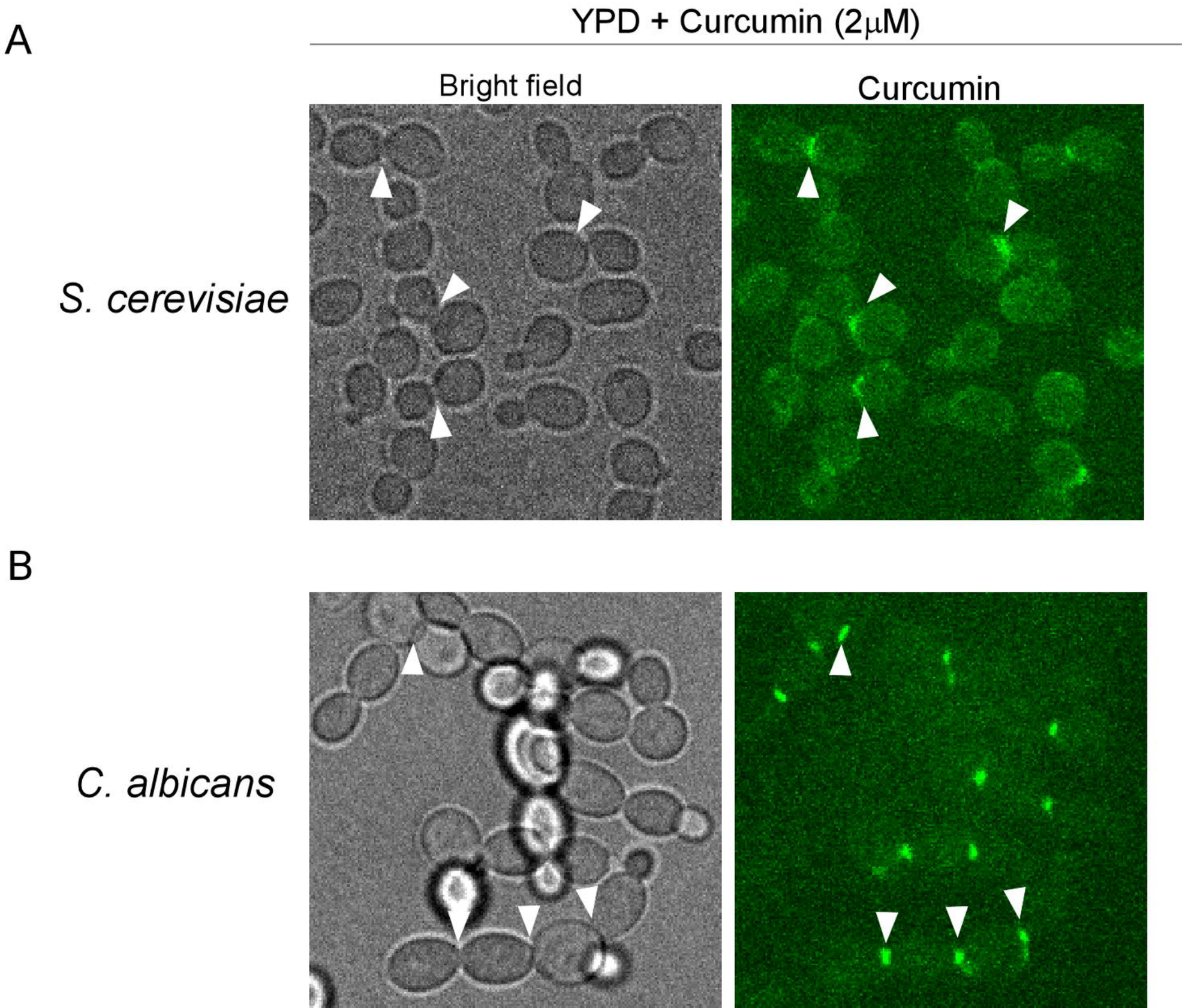

A
Curcumin
Rlc1-tdTomato
Merged
B
Curcumin
Bgs1-tdTomato
Merged
C
YE + Curcumin (2μM)
Curcumin
Bgs1-tdTomato
0
12
24
36
48
D
Curcumin
Calcoflour
Merged
E
YE + Curcumin (2μM) + Calcoflour (1μM)
Time (min)
0
15
30
Head-on view

Figure 3


A
YPD + Curcumin (2μM)
Bright field
Curcumin
S. cerevisiae
B
C. albicans