Curcumin Inhibits Growth of *Saccharomyces cerevisiae* through Iron Chelation📝📝📝

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Curcumin, a polyphenol derived from turmeric, is an ancient therapeutic used in India for centuries to treat a wide array of ailments. Interest in curcumin has increased recently, with ongoing clinical trials exploring curcumin as an anticancer therapy and as a protectant against neurodegenerative diseases. *In vitro*, curcumin chelates metal ions. However, although diverse physiological effects have been documented for this compound, curcumin’s mechanism of action on mammalian cells remains unclear. This study uses yeast as a model eukaryotic system to dissect the biological activity of curcumin. We found that yeast mutants lacking genes required for iron and copper homeostasis are hypersensitive to curcumin and that iron supplementation rescues this sensitivity. Curcumin penetrates yeast cells, concentrates in the endoplasmic reticulum (ER) membranes, and reduces the intracellular iron pool. Curcumin-treated, iron-starved cultures are enriched in un budded cells, suggesting that the G2 phase of the cell cycle is lengthened. A delay in cell cycle progression could, in part, explain the antitumorigenic properties associated with curcumin. We also demonstrate that curcumin causes a growth lag in cultured human cells that is remediated by the addition of exogenous iron. These findings suggest that curcumin-induced iron starvation is conserved from yeast to humans and underlies curcumin’s medicinal properties.

Curcumin is the major chemical component of turmeric, a dietary spice made from the root of the *Curcuma longa* Linn plant and used extensively in traditional Indian medicine (38). Curcumin is a potent bioactive compound that is used to treat cancer (5, 35), atherosclerosis (33), and neurodegenerative diseases, such as Alzheimer’s (26, 45) and Parkinson’s (44) disease, as well as to promote wound healing (15, 36). Curcumin is particularly appealing as a therapeutic agent because of its extremely low toxicity. Many biological activities have been ascribed to curcumin. For example, curcumin suppresses inflammatory responses in cultured cells and in animals and also exhibits antioxidant properties. Furthermore, curcumin’s ability to inhibit tumorigenesis and proliferation of a wide variety of cancerous cells has been well documented. Curcumin is a polyphenol and complexes readily with a number of different metal ions. In aqueous solutions of neutral pH, curcumin is an effective chelator of Fe(III) (2). Curcumin is also lipophilic and readily crosses membranes (19), so therefore it may also chelate metal ions intracellularly. How these chemical properties contribute to curcumin’s biological activities, however, is not understood.

Identifying relevant *in vivo* targets of small molecules is technically challenging. Recently, several genetic and genomic approaches have been developed that use the simple eukaryote *Saccharomyces cerevisiae*, or budding yeast, to study the mechanism of drug action (17, 27, 31). One such method, termed homozygous profiling, uses a comprehensive collection of 4,700 homozygous diploid deletion yeast strains, each bearing a deletion of a single nonessential gene (39), to examine growth in the presence of a bioactive compound (12). Mutant strains that display increased sensitivity to the compound are identified, and the identity of the genes deleted in these hypersensitive strains is used to infer the biological effects of the compound.

We carried out such a screen to identify yeast mutants whose growth is strongly inhibited by curcumin. The results of this study indicate that curcumin antagonizes yeast growth by chelating iron. Furthermore, iron supplementation alleviates the growth-inhibiting effect of curcumin on both yeast and cultured human cells, suggesting a common mechanism. Previous studies established that curcumin treatment causes mouse cells and tissues to display iron depletion characteristics (20). The findings presented here also indicate that curcumin chelates iron *in vivo* and suggest that iron chelation may underlie many of curcumin’s therapeutic activities.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The yeast growth medium and basic methods were as described in reference 34. Curcumin, bathophenanthroline

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disulfonylic acid (BPS), bacitracin, neomycin (Neom). These bacterial cells were grown on yeast extract-peptone-dextrose (YPD) agar plates, and the growth was visually assessed.

The growth of these bacterial cells was further analyzed by determining their optical density (OD) at 600 nm (OD600) using a microtiter plate reader. The OD600 values were recorded at regular intervals, and the growth curve was plotted.

**Results and Discussion**

The bacterial cells grown under control conditions exhibited a typical exponential growth phase, as evidenced by a sharp increase in the OD600 values. The addition of curcumin to the growth medium resulted in a significant decrease in the growth rate, as indicated by a slower increase in the OD600 values. The inhibition of growth was dose-dependent, with higher concentrations of curcumin leading to a more pronounced effect.

The growth inhibition was further confirmed by colony formation assays. Control cultures grown on YPD agar plates formed numerous colonies, whereas the cultures treated with curcumin did not form visible colonies, indicating a complete inhibition of growth. The results were reproducible across multiple experiments.

**Conclusion**

The study demonstrated that curcumin has a potent inhibitory effect on the growth of the bacterial cells used in this study. The mechanism of this inhibition is currently under investigation, and further studies are needed to elucidate the specific target(s) and the molecular mechanisms involved.

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Strains, delete overlapping open reading frames and thus both compromise Ccc2 function. Remarkably, 8 out of the 17 identified genes encode products that function in the transport and homeostasis of iron and copper ions (Table 1). Specifically, genes whose products mediate iron transport (FTR1 and FET3) and the regulation of gene expression under iron limitation conditions (AFT1) were identified (8, 40). Genes that encode proteins required for copper transport (CTR1) and the regulation of gene expression during copper limitation (MAC1) were also identified (6, 23). Interestingly, these processes are coupled in vivo (7), as an essential component of high-affinity iron uptake, Fet3p, is a copper-dependent ferro-oxidoreductase, and strains with deletions of genes whose products are required for the incorporation of copper ion into Fet3 (CCC2, ATX1, and GEF1) are sensitive to curcumin (13, 18, 42). Other identified genes include those that disrupt vacuolar acidification (VMA3, VMA13, and VMA21), a process required for intracellular iron utilization (9, 37). In addition, several mutants that disrupt the lipid

**FIG. 1.** Yeast sensitivity to curcumin can be rescued by iron supplementation and is similar to yeast sensitivity to BPS, an iron chelator. (A) Yeast cells are sensitive to curcumin. Serial dilutions of WT (BY4743) cells were plated on YPD with or without curcumin at the concentrations indicated and grown for 2 days at 30°C. (B) Deletion of iron and copper homeostasis genes exacerbates yeast sensitivity to curcumin. WT (BY4743) and isogenic homozygous deletion strains (lacking the gene indicated) were plated on YPD without any additions or with 150 μM curcumin, either by itself, with 500 μM CuSO₄, or with 50 μM FeSO₄, and grown for 3 days at 30°C. (C) In parallel to the experiments shown in panel B, the same cultures were plated on YPD with either 100 μM curcumin or 80 μM BPS and grown for 3 days at 30°C.

**TABLE 1.** Deleted genes identified in curcumin-hypersensitive strains and their functions

| Biological process | Gene(s) |
|--------------------|---------|
| Transport or homeostasis of iron or copper ions | FET3, FTR1, AFT1 |
| Copper ion import/homeostasis | CTR1, MAC1 |
| Copper and iron homeostasis | ATX1, GEF1, CCC2 |
| Other function | SAC1, GAL11, ERV14, VMA3, VMA13, VMA21, LEM3, ERG3, YSP1 |

*The ydr269cΔ strain also disrupts ccc2.
composition of membranes and thus have wide-ranging defects in membrane function and trafficking are hypersensitive to curcumin (the sac1Δ, erg3Δ, and lem3Δ strains). Overall, these findings suggest that strains with defects in iron and/or copper homeostasis are hypersensitive to curcumin.

**Differential rescue of curcumin hypersensitivity by copper and iron.** To further define the mechanism by which curcumin inhibits yeast growth, we tested the ability of curcumin-hypersensitive deletion strains to grow when iron or copper supplements were added to the growth medium. Importantly, addition of iron or copper to YPD medium at the concentrations employed had no effect on cell growth in the absence of curcumin (data not shown). Addition of 50 μM iron significantly improved the growth of all curcumin-sensitive strains, whereas addition of a 10-fold-larger amount of copper (500 μM) was able to rescue growth of only a small subset of strains (the fet3Δ, gef1Δ, ccc2Δ, atx1Δ, and mac1Δ strains) (Fig. 1B).

We next compared the curcumin sensitivity profile to those of BPS, a known iron chelator, and BCS, a known copper chelator. The 100 μM curcumin sensitivity profile was strikingly similar to the 80 μM BPS sensitivity profile (Fig. 1C), with the erg3Δ strain as the single exception, showing sensitivity to curcumin and not BPS. Similar experiments comparing growth on 100 μM curcumin to growth on as much as 500 μM BPS identified only three of the candidates, the vma21Δ, vma13Δ, and cup5Δ strains, as BCS sensitive (data not shown). Taken together, these observations suggest that curcumin inhibits yeast growth primarily through the limitation of iron availability.

Competitive growth assays similar to the one described here for curcumin have been performed using 75 to 100 μM BPS (21). This BPS concentration range results in a yeast sensitivity profile similar to that observed for 150 μM curcumin (Fig. 1C and data not shown), which was the curcumin concentration employed in our competitive growth assays. Strikingly, 18 of 42 of our curcumin-sensitive candidates were also identified in the BPS sensitivity screen (21). While the curcumin-hypersensitive strains identified in this study were well represented in the BPS hypersensitivity profile, the BPS competitive growth assays described by Jo et al. (21) identified approximately 3-fold-more sensitive strains. This difference could be due to the fact that cells were exposed to BPS for 15 generations as opposed to the 5 generations used in the curcumin screen. Furthermore, a less stringent fitness cutoff was used when assigning BPS-sensitive strains. Taken together, these data further support the interpretation that curcumin-mediated growth inhibition is due to iron starvation.

**Curcumin accumulates in yeast endoplasmic reticulum membranes.** Chelators can deplete the exogenous pool of metal ions from the growth medium, enter cells and deplete the intracellular reserves of metal ions, or act both intra- and extracellularly to alter metal ion pools. Curcumin is able to bind iron in solution, demonstrating that it is an effective extracellular iron chelator (2). To determine if curcumin can also traverse the yeast cell wall and plasma membrane to act intracellularly, we exploited the natural fluorescent properties of curcuminoids. Curcumin autofluorescence can be excited at ~455 nm and emits at approximately 540 nm (19, 24). We treated cells containing an HDEL-DsRed (red fluorescent protein from Discosoma striata) marker, which uniformly stains both cortical and perinuclear endoplasmic reticulum (ER) membranes (16), with either dimethyl sulfoxide (DMSO) or 150 μM curcumin for 5 h and visualized curcumin fluorescence using a filter set for green fluorescent proteins (GFPs) that overlaps with the curcumin excitation and emission wavelength (Fig. 2). Curcumin fluorescence colocalized with the HDEL-DsRed marker in 69% of cells (n = 103) examined, indicating that curcumin not only enters yeast cells but also accumulates within the ER membrane (Fig. 2A). While there is clear colocalization of curcumin with the ER membrane, curcumin may also localize to additional intracellular membranes, accounting for the background of curcumin fluorescence that does not colocalize with the ER HDEL marker (Fig. 2A). These observations are consistent with demonstrations of curcumin accumulation in intracellular membranes, including the ER, of mammalian cells (19, 24). These findings further suggest that curcumin may impact intracellular iron pools in addition to chelation of extracellular iron.
To test whether addition of iron to the growth medium suppresses curcumin sensitivity by preventing curcumin uptake into cells, cells grown in curcumin and iron were examined by fluorescence microscopy. Curcumin also colocalized with the ER membrane in iron-treated cells, indicating that it still enters cells under conditions of iron supplementation (Fig. 2B). Curcumin fluorescence was reduced in iron-treated cells. However, because iron decreases the fluorescence intensity of curcumin-containing solutions in a dose-dependent manner (data not shown), fluorescence per se cannot be used to compare intracellular curcumin concentrations under these two conditions. Nonetheless, our findings indicate that curcumin permeates yeast cells grown in the presence or absence of supplemental iron and are consistent with the hypothesis that curcumin acts intracellularly to chelate iron and induce iron starvation.

Curcumin treatment results in reduced intracellular iron.

We tested the effect of curcumin on cellular iron levels directly by using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Wild-type cells were treated with curcumin, BPS, or BCS (see Materials and Methods). The majority of elements assessed this way did not change as a result of these treatments (data not shown). As expected, the iron content of cells treated with 100 μM BPS was dramatically reduced (80% less iron than cells grown in YPD). Curcumin also significantly decreased cellular iron levels, with cells grown in the presence of 25 μM and 50 μM curcumin containing 20 and 40% less iron, respectively, than cells grown in YPD (Fig. 3A). Treatment with BCS reduced cellular copper content by 90% from that of cells grown in YPD; however, consistent with our earlier findings, curcumin treatment did not significantly alter copper levels from those of YPD-grown cells (Fig. 3A).

The physiological relevance of this cellular iron depletion was next examined by measuring the transcriptional response induced by curcumin. Under conditions of iron starvation, the Aft1 transcription factor upregulates genes responsible for high-affinity iron transport (41). Two targets of Aft1 transcription regulation are fet3, the high-affinity iron transporter, and fre2, a gene encoding an iron reductase that functions as the rate-limiting step in high-affinity iron import (1, 43). Transcription of fet3 can also be induced by Mac1p, a transcription factor that activates gene expression in response to copper starvation (14); however, regulation of fre2 is independent of copper starvation (28). Following 50 μM curcumin treatment, fet3 and fre2 expression increased 3-fold and 6-fold, respectively (Fig. 3B and C). Because the curcumin-mediated induction levels of fre2 and fet3 are similar, the most parsimonious explanation is that curcumin induces predominantly an iron starvation response. These data suggest that the growth of yeast in the presence of curcumin leads to a decrease in available iron and, as a result, induces the iron starvation transcriptional response.

Curcumin slows yeast cell cycle progression with no accompanying decrease in cell viability.

Next, we examined the kinetics of curcumin-induced yeast cell growth inhibition. Yeast cells were grown to mid-log phase and treated with curcumin or BPS in the presence or absence of additional iron, and the growth rate (i.e., doubling time) was monitored. As expected, addition of curcumin or BPS lowered the yeast growth rate (Fig. 4A), with treatments of either 80 μM BPS or 150 μM curcumin increasing the doubling time approximately 2-fold (Fig. 4B). Addition of iron to the BPS- or curcumin-treated cells rescued this growth inhibition, shortening the doubling times to levels similar to those observed for untreated control cells grown in YPD (Fig. 4A and B)

After ~10 h of treatment with either BPS or curcumin, at which point control cells in YPD had doubled twice, cell viability and cell cycle progression were assessed for the various conditions. Viability of cells grown in the presence of curcumin or BPS was monitored using the vital stain methylene blue (see Materials and Methods) and by assessing the number of CFU of washed cells plated on YPD. At 10 h posttreatment, 100% cell viability was observed for all cultures (data not shown), consistent with the hypothesis that these compounds cause a
delay in cell cycle progression rather than cell death. To assess cell cycle progression, we analyzed the morphologies of yeast cells exposed to curcumin and BPS. In G₁ phase, cells are unbudded, while cells in S, G₂, and M phases of the cell cycle possess buds of characteristic sizes (reviewed in reference 25). During mid-log-phase growth in YPD, 25% of cells were unbudded and 75% of cells were budded (Fig. 4C). Exposure to curcumin or BPS caused an increase in the fraction of unbudded cells, which is commonly observed for treatments that increase the length of G₁ (Fig. 4C). Curcumin exposure caused a dose-dependent increase in the proportion of unbudded cells to 40% and 45% for 100 μM and 150 μM curcumin, respectively. BPS treatment increased the unbudded fraction to 55%. Taken together, these observations demonstrate that curcumin treatment lengthens the time required to complete the cell cycle and suggest that the duration of G₁ is increased.

To determine if iron starvation was the cause of this increased doubling time, iron was added to the medium to counter the effect of curcumin on cellular iron levels. Supplementation with 50 μM iron in cultures with 100 μM or 150 μM curcumin or 80 μM BPS completely alleviated the growth inhibition, resulting in restoration of the cell morphology distributions to those observed for YPD-grown cells (Fig. 4C). Thus, BPS- or curcumin-induced iron starvation increases yeast doubling time, and this is, at least in part, due to a prolonged G₁ phase. Treatment of yeast with other metal chelators has been reported to induce a G₁ growth arrest that can be rescued by the addition of exogenous metal ions to the growth medium (22).

**Curcumin and cultured human cells.** Exposure of certain human cancer cells to curcumin results in a G₁ cell cycle arrest (30), and treatment of human cells with other iron chelators also induces a reversible G₁ cell cycle arrest (3, 29). To extend the observations made in yeast, we further explored the effects of curcumin on cell growth by treating human osteosarcoma U2OS cells with curcumin and monitoring cell density relative to that of a DMSO drug vector control. The density of control cultures increased 3-fold in 24 h, whereas the curcumin-treated cell density did not increase (Fig. 5). Addition of iron to the curcumin-treated cells suppressed this growth defect and allowed for population doubling in 24 h (Fig. 5). These data suggest that curcumin’s effects are conserved and that it inhibits growth of both yeast and human cells by limiting iron availability.

An understanding of curcumin’s mechanism of action on human cells has been elusive, due in part to the myriad effects associated with curcumin treatment (reviewed in reference 15). We used yeast as a model system to explore the mechanism of action of curcumin, and our results strongly suggest that curcumin inhibits eukaryotic cell growth predominantly by chelating intracellular iron. Yeast cells with defects in iron homeostasis are sensitive to curcumin, and curcumin delays cell cycle progression in an iron-dependent manner. These findings suggest that the potent chemotherapeutic effects of curcumin may be explained by iron depletion of cancer cells, a process which inhibits their proliferation. Iron chelation may also explain the diverse physiological effects of curcumin. Many cellular enzymes require iron as a cofactor, and several signaling pathways are also affected by iron availability. Curcumin penetrates both yeast and mammalian cells and accumulates in intracel-
luminal compartments, including the ER. This accumulation in intracellular compartments may selectively limit the availability of essential iron cofactors, impairing specific enzymatic functions and altering cellular signaling to produce the diverse physiological effects associated with curcumin treatment.

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