Does *Candida albicans* Als5p Amyloid Play a Role in Commensalism in *Caenorhabditis elegans*?

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**Candida albicans**, a dimorphic fungus and an opportunistic pathogen, possesses a myriad of adherence factors, including members of the agglutinin-like sequence (Als) family of mannoproteins. The adhesin Als5p mediates adhesion to many substrates and is upregulated during commensal interactions but is downregulated during active *C. albicans* infections. An amyloid-forming core sequence at residues 325 to 331 is important for Als5p function, because a single-amino-acid substitution at position 326 (V326N) greatly reduces Als5p-mediated adherence. We evaluated the role of Als5p in host-microbe interactions by using *Caenorhabditis elegans* nematodes as a host model and feeding them *Saccharomyces cerevisiae* expressing Als5p on the surface. Als5p-expressing yeast had 8.5- and 3.5-fold-increased intestinal accumulation rates compared to Als5p-nonexpressing *S. cerevisiae* or yeast expressing amyloid-deficient Als5pV326N, respectively. Surprisingly, this accumulation delayed *S. cerevisiae*-induced killing of *C. elegans*. The median survival time was nearly twice as long as that of nematodes fed nonexpressing or non-amyloid-forming Als5pV326N-expressing *S. cerevisiae*. Treatment with the amyloid-inhibiting dye Congo red or repression of Als5p expression abrogated the protective effect of Als5p. Furthermore, Als5p had no effect on oocyte quantity or quality, since nematodes fed either empty vector (EV)- or Als5pV326N-expressing *S. cerevisiae* had similar egg-laying and egg-hatching rates. This study is the first, to our knowledge, to show that expression of an amyloid-forming protein can attenuate pathogenicity in *C. elegans*.

*Candida albicans* is a dimorphic commensal yeast that normally colonizes human nasopharyngeal and urogenital tracts. Although *C. albicans* is one of the most commonly isolated causes of nosocomial fungal infections in immunocompromised patients, it is generally harmless to the healthy population. In the healthy population, the host has mechanisms such as the microbial flora, epithelial barriers, and the innate immune system that control its presence (1).

*C. albicans* adheres to and colonizes the host with the help of a myriad of adherence proteins which are important in both commensalism and pathogenesis. The agglutinin-like sequence (Als) family of proteins constitutes one prominent class of *Candida* adhesins. There are eight ALS genes in the *Candida albicans* genome, and their products are glycosylphosphatidylinositol (GPI)-anchored glycoproteins homologous to the α-agglutinin protein of *Saccharomyces cerevisiae* (2). Each Als protein has a similar domain structure that includes conserved N-terminal Ig-like invasin domains that determine substrate specificity, a T-domain that allows for amyloid formation, a region with a variable number of 36-amino-acid tandem repeats (TR domains), and a glycosylated C-terminal serine-threonine-rich stalk domain that links to a modified GPI anchor that covalently attaches Als proteins to the cell wall of *C. albicans* (3, 4). When transgenically expressed in *Saccharomyces cerevisiae*, Als5p mediates fungal aggregation and also causes adherence to laminin, gelatin, fibronectin, and epithelial cells, as well as endothelial cells in *vitro* (5, 6).

The amyloid-forming region in Als5p and other adhesins is important for robust adherence and yeast aggregation onto a variety of surfaces (7–10). This region mediates clustering of adhesin molecules on the surface to form amyloid-like nanodomains that have high avidity for ligands (11, 12). Consequently, a single-amino-acid substitution, V326N, in the amyloid-forming region of Als5p abolishes its ability to form amyloids, cell surface nanodomains, cell aggregates, and biofilms on polystyrene (11–13). As a natural extension, we sought to assay the role of Als5p and its *in vivo* interactions with the host, as these functions are thought to play a role in both pathogenesis and commensalism. *Caenorhabditis elegans* is a free-living microbivore nematode whose use as a model organism across various fields of biology has been expanding in recent years. *C. elegans* is an emerging model for the study of innate immunity and host-pathogen interactions (14–24). A variety of virulence factors that are important in mediating bacterial and fungal pathogenesis in humans also cause disease in nematodes. To date, however, *C. elegans* has never been used to study commensal-associated proteins that may be important in this dynamic interplay between host and microorganism. Our study represents the very first attempt, to our knowledge, to assay and demonstrate the role of the cell wall adhesin Als5p and its functional amyloid in commensalism and adherence *in vivo*.

**MATERIALS AND METHODS**

**Strains and media.** Fluorescent *Saccharomyces cerevisiae* strains containing pJL1-Als5, pJL1-EV, and pJL1-Als5V326N (11) were generated by transformation with green fluorescent protein (GFP) and red fluorescent protein (RFP) expression plasmids pADH1-GFP and yEPGAP-Cherry, respectively, which were kindly provided by Neta Dean’s laboratory (Stony Brook University, New York, NY) (25). The resulting strains were grown in complete synthetic medium lacking uracil and tryptophan (CSM–Ura–Trp) at 24°C to induce expression. Galactose (2%) was the carbon source unless otherwise stated. *Candida albicans* strain BWP17
expressing ADH1pyEmRFP (referred to as BWP17 throughout the study) was also gifted to us by the Dean laboratory and was grown in yeast extract-pectone-dextrose (YPD) broth at 24°C. Nematodes were propagated on modified nematode growth medium (NGM) (40 mg/liter adenine, 3g/liter NaCl, 17g/liter agar, 2.5g/liter peptone, 20g/liter galactose, 10g/liter yeast extract, 1 ml 1 M CaCl2, 1 ml 1 M MgSO4, 25 ml 1 M KPO4, and 1 ml of 5 mg/ml cholesterol in 95% ethyl alcohol [EtOH]) and fed Esherichia coli OP50. C. elegans nematodes were maintained at 18°C. E. coli OP50 was grown overnight in LB broth at 37°C. In Congo red (CR) experiments, plates were made as described above, with the addition of Congo red at a final concentration of 30 μM. All C. elegans N2 strains and E. coli OP50 were provided by the Caenorhabditis Genetics Center (CGC), which is funded by the NIH Office of Research Infrastructure Programs (under NIH grant no. P40 OD010440).

Intestinal accumulation assay. C. elegans nematodes grown on modified NGM plates were inoculated with E. coli OP50 as previously described (26). For experiments with S. cerevisiae, the nematodes were fed on suspensions of S. cerevisiae transformed with pLP1, pLP1Ak5V326N, or pLP1EV (denoted “Als5p-expressing,” “Als5pV326N-expressing,” and “EV” strains, respectively, below) (11). The agar plates were modified NGM plates spotted with 100 μl of a suspension of Als5p-expressing, Als5pV326N-expressing, or EV S. cerevisiae cells (optical density at 600 nm [OD600] = 2) or Candida albicans BWP17 (OD600 = 2). For mixing experiments, 100 μl of a 1:1 mixture of Als5p-expressing (OD600 = 2) and EV cells was spotted onto NGM plates. After 24 h, the plates were washed twice in 3 ml M9 buffer (5.8 g/liter NaH2PO4, 3.0 g/liter KH2PO4, 0.5 g/liter NaCl, 1.0 g/liter NH4Cl) to rinse off nematodes, which were collected into a 15-ml conical tube (~5.5 ml total). The pooled suspensions were centrifuged for 1 min at 500 rpm, after which the supernatant was slowly decanted. This washing procedure was performed five times in order to ensure that residual yeast was removed. The nematode pellet was then resuspended in 500 μl of M9 buffer. To enumerate the number of nematodes in the pellet, we visually inspected ~100 μl aliquots under a stereoscope (in triplicate). One hundred microliters of worm suspension was then transferred into a tube containing 900 μl 6 M urea and five glass beads (5 mm in diameter), vortex mixed at maximum speed for 5 min, and immediately diluted 1:10, 1:100, and 1:1,000 into M9 buffer. The yeast suspension (100-μl aliquots) were then plated onto appropriate selective media (CM-S-S, -Ura-Trp+2% galactose and 40 mg/liter adenine for EV/Als5p/Als5pV326N and YPD for BWP17) and incubated at 30°C for 48 h.

C. elegans survival assays. In preparation for survival assays, eggs were obtained by standard bleaching techniques according to methods described previously by Sternagel (26). Briefly, C. elegans stock plates that had many gravid hermaphrodites were washed with sterile H2O. The suspension was collected into a sterile 15-ml conical centrifuge tube with a cap. Sterile H2O was added to a total of 3.5 ml. A total of 0.5 ml of 5 M NaOH was premixed with a 5% household bleach and added to this 3.5-ml solution. The solution was then vortex mixed and centrifuged, and the pellet was washed twice. The pellet was resuspended in 3 ml sterile M9 buffer. Eggs were placed onto lawns of E. coli OP50 at 24°C until they were L4 larvae to early adult nematodes. Modified NGM plates, supplemented with 2% galactose (unless otherwise stated), kanamycin, and ampicillin (to inhibit the growth of E. coli OP50), were spotted with yeast strains (OD600 = 2) and approximately 50 L4 larvae to early adult nematodes unless otherwise indicated. All survival assay plates were incubated at 18°C for 96 h. During the reproductive period, adults were transferred daily onto fresh plates. Worm mortality was scored over time with a worm being considered dead when it failed to respond to touch. Nematodes that were not recovered from the plates were censored from the study. The percentage of C. elegans nematodes alive for each given day was calculated by using the following formula:

\[
\text{% Alive} = 1 - \left( \frac{\text{worms dead}}{\text{worms alive}} \right) \times (\text{% alive previous day}) \times 100
\]

Microscopic analysis. For visualization of yeast in the nematode intestines, nematodes were fed the yeast strains at 18°C, after which they were picked from experimental plates and mounted onto 2% agar pads and immobilized in 7 to 10 μl of 15 mM sodium azide. Slides were viewed under an Olympus BX51 microscope using an Olympus DP71 camera. Overlays of fluorescent and bright-field images were inspected by an evaluator blinded to the identity of the yeast in each experiment. Each sample was scored on a semiquantitative log-based scale: <10 cells, 1 unit; 11 to 20 cells, 2 units; 21 to 40 cells, 3 units; 41 to 80 cells, 4 units; and >80 cells, 5 units.

Oocyte quality analysis. In preparation for the oocyte quality analysis, early L4 larvae were obtained by techniques used for the survival assay described above. Modified NGM plates, supplemented with kanamycin and ampicillin, were spotted with Als5p-expressing, Als5pV326N-expressing, or EV S. cerevisiae cells as well as E. coli OP50, and a single nematode was placed onto each plate. Plates were then incubated at 18°C. Each independent assay was carried out four times, with a total population size of 5 to 7 nematodes for each microorganism strain, per experiment. During the reproductive period, adults were transferred daily onto fresh plates. Eggs laid and those which hatched were scored over time at 24-h intervals. The percentage of viable C. elegans eggs for each given day was calculated by using the following formula:

\[
\text{% Viability} = \left( \frac{\# \text{ eggs laid}}{\# \text{ eggs hatched}} \right) \times 100
\]

RESULTS

Als5p expression increases yeast occupancy within the C. elegans intestine. Accumulation of intact microbial cells in the intestine of C. elegans has been proposed to be an indicator of infection (20, 24, 27). We investigated whether Als5p mediates intestinal residence in C. elegans by using yeast cells expressing cytoplasmic fluorescent proteins. Candida albicans strain BWP17 accumulated within the nematode intestine in large numbers, with pronounced intestinal distention (Fig. 1A and 2A). We therefore hypothesized that if Als5p mediates binding to C. elegans, protein expression on the cell surface of S. cerevisiae would render this yeast pathogenic and cause yeast to accumulate in the nematode intestine. As expected, we observed Als5p-expressing yeast cells throughout the intestine of the nematode (Fig. 1B). In contrast, there were many fewer cells in worms harboring an empty vector (EV cells), with a distinct diffuse fluorescence throughout the intestine (Fig. 1D). Therefore, expression of Als5p in S. cerevisiae resulted in much greater fungal retention than in nonexpressing cells (Fig. 1, A and D).

To support these results, we counted fungi in the intestine and represented the numbers using a log scale, as described in Materials and Methods. The counter was blinded to the identity of the yeast in each experiment. Each sample was scored on a semiquantitative log2-based scale: <10 cells, 1 unit; 11 to 20 cells, 2 units; 21 to 40 cells, 3 units; 41 to 80 cells, 4 units; and >80 cells, 5 units.
strains (Fig. 2B), similar to what was observed microscopically (Fig. 1 and 2A).

The Als5p amyloid sequence is critical for intestinal accumulation. The amyloid-forming region of Als5p potentiates adherence in the S. cerevisiae expression model, and a V326N mutation is sufficient to abolish amyloid formation and severely inhibit adhesion in vitro (11). Therefore, we assayed the effect of the amyloid sequence in the C. elegans infection model. Intestinal accumulation of cells expressing Als5pV326N was less than that of Als5p-expressing cells and similar to or slightly more than that of EV cells (Fig. 1C versus B and D). Intact Als5p-expressing S. cerevisiae accumulated in the intestinal tract of C. elegans in larger numbers than Als5pV326N-expressing cells (Fig. 2A). CFU counts showed 3.5-fold fewer mutant Als5pV326N-expressing cells isolated from the C. elegans intestine than Als5p-expressing cells (Fig. 2B). These results suggested that Als5p amyloid function mediated adherence in C. elegans and led to decreased mortality of the nematodes.

Als5p decreases S. cerevisiae-induced mortality in C. elegans. We assayed survival times by comparing nematodes fed C. albicans or S. cerevisiae expressing Als5p or Als5pV326N with those fed EV cells or E. coli OP50 to determine whether increased intestinal occupation of viable Als5p-expressing yeast cells was lethal to the host (Fig. 3A). Candida albicans BWP17 killed C. elegans more rapidly than did S. cerevisiae, killing most nematodes by day 2 and all of them by day 4 (Fig. 3A). Feeding on S. cerevisiae killed the nematodes, but it was less lethal than feeding on C. albicans. Interestingly, the worms fed S. cerevisiae Als5p survived longer than nematodes fed EV control yeast. Specifically, at 48 h postexposure, there were 20% more C. elegans worms alive on Als5p-expressing yeast cells (P < 0.005) than nematodes fed EV control yeast cells (Fig. 3A). This delayed lethality continued through days 3 (P < 0.005) and 4 (P < 0.05), with 20% and 14% more nematodes alive on Als5p-expressing cells than on EV control cells, respectively (Fig. 3A). Nematodes fed Als5p-expressing cells had a time to 50%
death (TD₅₀) value of 84.3 h, 35.6 h more (P < 0.005) than nematodes fed EV control cells (Fig. 3B). This mortality was similar to that observed for nematodes fed \textit{E. coli} OP50 (Fig. 3C). Expression of fluorescent proteins had no effect on the mortality rates of \textit{C. elegans}, since nonfluorescent strains exhibited survival rates similar to those of their fluorescent counterparts (data not shown). Together, these results indicated that the \textit{C. albicans} adhesin Als5p increased colonization in the intestine of the nematodes and reduced the lethality of \textit{S. cerevisiae}.

We hypothesized that if Als5p contributed to the decreased mortality, then feeding nematodes on yeast grown on glucose would abrogate the observed reduction in mortality, because Als5p was expressed under a \textit{GAL1} promoter. Indeed, \textit{S. cerevisiae} cells harboring pPL1 and pPL1-EV killed nematodes at rates similar to each other and to EV yeasts in galactose-containing medium (see Fig. S1 in the supplemental material). Thus, repression of Als5p expression led to more rapid death of the nematodes. These data support that Als5p is necessary for prevention of rapid \textit{S. cerevisiae}-induced mortality.

\begin{figure}
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\caption{Effect of functional Als5p amyloid on intestinal occupation in \textit{C. elegans}. (A) Accumulation of intact yeast cells counted in \textit{C. elegans} alimentary tracts after 24 h (mean \pm standard error of the mean for n = 10 worms per food source). See Materials and Methods for details. (B) Live fungi recovered from \textit{C. elegans} after 24 h of feeding. Shown are means \pm standard errors of the means from two independent experiments where more than 50 animals were used in each trial. CFU are per nematode. * indicates a Student t test P value of <0.05; ** indicates a Student t test P value of <0.005.}
\end{figure}

\begin{figure}
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\caption{Survival of \textit{C. elegans} challenged with \textit{Saccharomyces cerevisiae} strains. (A) Percentage of \textit{C. elegans} nematodes alive, starting with young adult animals fed on Als5p-expressing, Als5pV326N-expressing, or EV \textit{S. cerevisiae} cells or \textit{C. albicans} BWP17 over 4 days. Data are from eight independent experiments; more than 50 animals were used in each case. (B) Hours postexposure at which 50% of nematodes fed the indicated yeast strains died. Data are from eight independent experiments; more than 50 animals were used in each case. * indicates a Student t test for difference from Als5p with a P value of <0.05; ** indicates a Student t test with a P value of <0.005. (C) Percentage of \textit{C. elegans} nematodes alive, starting with young adult animals fed on Als5p-expressing \textit{S. cerevisiae} cells or \textit{E. coli} OP50 over 4 days. Data are from three independent experiments; more than 50 animals were used in each case.}
\end{figure}
The Als5p amyloid sequence is necessary for mortality reduction in *C. elegans*. Because amyloid-forming Als5p increased both fungal burden and survival of the worms, we determined whether the amyloid sequence was responsible for the increased survival. We fed nematodes yeast cells expressing the non-amyloid-forming adhesin Als5pV326N. The amyloid-deficient mutant protein did not prolong nematode survival (Fig. 3A). More specifically, nematodes fed Als5pV326N-expressing cells had a TD50 value of 57.3 h, 27 h less (*P* < 0.005) than that of nematodes fed Als5p-expressing cells (Fig. 3B) and similar to that of control EV yeasts. On day 2, there were 17% more nematodes alive on Als5p- than on Als5pV326N-expressing yeast cells, with that number going to 20% and 14% on days 3 and 4, respectively (Fig. 3A). The statistical significance of the difference between Als5p and Als5pV326N was a *P* value of <0.05 throughout the study. The viabilities of *C. elegans* fed Als5p and Als5pV326N-expressing *S. cerevisiae* and EV negative-control cells were similar for days 1 through 4, strongly suggesting that Als5pV326N mutants behaved more like EV cells than Als5p-expressing yeast cells.

*C. elegans* nematodes fed Als5p-expressing *S. cerevisiae* cells exhibit normal physiological processes. In order to determine if any of the nematodes fed Saccharomyces strains exhibited signs of infection, we assayed muscle function and movement in defection (28, 29). Defecation is a highly regulated process that is vital to the infecting, we hypothesized that performing our survival assays on plates containing CR would prevent amyloid formation and negate the Als5p-mediated protective effect. In fact, the presence of 30 μM CR inhibited Als5p amyloid-mediated life extension (Fig. 6A). In detail, addition of 30 μM CR to the agar plates led to death of the nematodes fed Als5p-expressing yeast at rates similar to those of worms fed EV cells (Fig. 6B versus C). The mortality of nematodes fed Als5p strains in the presence of CR increased at statistically significant amounts at 48 h and 72 h post-exposure compared to those in the absence of CR. Specifically, there were about 20% more nematodes alive that were fed Als5p-expressing *S. cerevisiae* in the absence of CR than in its presence during this time (*P* = 0.05). CR did not affect the viability of the yeast cells or nematodes (data not shown). The addition of CR did not affect the survival of worms fed amyloid mutant protein-expressing cells or EV nonexpressing cells (Fig. 6B and C). Together, these data strongly suggest that the diminished lethality observed for nematodes fed Als5p-expressing yeast cells was not only dependent on Als5p but also specifically mediated by the amyloid-forming ability of this protein.

Oogenesis is a sensitive marker of reproductive changes resulting from starvation. Nematodes undergo an oogenic germ line starvation response, which results in reduced embryo viability and increased life span (31). Starvation can lead ultimately to matricide by eggs hatching within the hermaphrodite’s uterus. To determine if nematodes were being starved in the presence of Als5p-expressing, Als5pV326N-expressing, and *E. coli* cells, we fed nematodes the above-mentioned fungal cells and counted the number of eggs laid and the number of eggs hatched over time. Nematodes fed Als5p-expressing cells laid a similar number of eggs as nematodes fed either Als5pV326N-expressing or EV cells at 24 to 72 h postfeeding (Fig. 5). The number of eggs laid was similar to those laid by worms fed *E. coli* OP50 for the first 24 h but was decreased at 48 and 72 h. In contrast, the fraction of eggs that hatched was not significantly different between nematodes fed any *S. cerevisiae* strains or OP50. These results, together with the differences in mortality rates of nematodes fed Als5p-expressing, Als5pV326N-expressing, or EV cells, suggest that nematodes are not undergoing starvation and that the decreased mortality rate observed in this study is Als5p dependent.

**Amyloid binding dye Congo red negates Als5p effects.** Amyloid binding dyes are used for visualization of these insoluble fibrils, but at higher concentrations, their presence can also prevent amyloid formation (32). Accordingly, Congo red (CR) decreases aggregation of Als5p-expressing *S. cerevisiae* cells to each other (11). Therefore, we hypothesized that performing our survival assays on plates containing CR would prevent amyloid formation and negate the Als5p-mediated protective effect. In fact, the presence of 30 μM CR inhibited Als5p amyloid-mediated life extension (Fig. 6A). In detail, addition of 30 μM CR to the agar plates led to death of the nematodes fed Als5p-expressing yeast at rates similar to those of worms fed EV cells (Fig. 6B versus C). The mortality of nematodes fed Als5p strains in the presence of CR increased at statistically significant amounts at 48 h and 72 h post-exposure compared to those in the absence of CR. Specifically, there were about 20% more nematodes alive that were fed Als5p-expressing *S. cerevisiae* in the absence of CR than in its presence during this time (*P* = 0.05). CR did not affect the viability of the yeast cells or nematodes (data not shown). The addition of CR did not affect the survival of worms fed amyloid mutant protein-expressing cells or EV nonexpressing cells (Fig. 6B and C). Together, these data strongly suggest that the diminished lethality observed for nematodes fed Als5p-expressing yeast cells was not only dependent on Als5p but also specifically mediated by the amyloid-forming ability of this protein.

**Als5p can attenuate mortality due to *S. cerevisiae*.** In order to evaluate if Als5p-expressing and -nonexpressing *S. cerevisiae* strains can cohabit the intestine of the *C. elegans* host, we fed nematodes a 1:1 mixture of Als5p-expressing and EV cells. We found that many nematodes retained a large number of EV cells along with a similar number of Als5p-expressing cells (Fig. 7C and D). Similarly, in nematodes whose intestines retained few Als5p-expressing cells, there were few EV cells present (data not shown). We also evaluated the mortality rates of these nematodes. Nematodes fed a mixture of Als5p-expressing and EV cells had mortality rates similar to those fed only Als5p-expressing cells (Fig. 7A). Both of these experimental groups had decreased mortality rates compared to those of nematodes fed solely EV control cells.

![Graph](attachment:image.png)
Our data suggest that Als5p is not critical for pathogenesis but rather that it can move the relationship toward a commensal-like state. The expression of Als5p moved the yeast-host interaction toward each of the hallmarks of commensalism: (i) increased occupancy of the host nematode by Als5p-expressing cells, (ii) maintenance of a nondiseased physiological state in the host, and (iii) possible fungal evasion of the nematode innate immune response to allow survival of the microbe. For each of these criteria, the amyloid-forming sequence in Als5p was necessary for activity.

**Als5p mediates a commensal-like state.** Als5p was sufficient to enable *S. cerevisiae* cells to accumulate within the intestine of the nematode host. Furthermore, the yeast in the alimentary tract remained viable. In CFU assays, 8-fold more Als5p-expressing yeast cells were isolated from nematodes than EV yeast cells (Fig. 2). Thus, expression of Als5p led to fulfillment of the first two criteria of commensalism: increased occupancy and viability of the yeast. The expression of Als5p on the yeast surface also led to a nondiseased state in the host, the third condition. The defecation rates were indistinguishable from those of worms fed *E. coli*, whereas worms fed EV yeast had a significant decrease in the rate (Fig. 4), a sign of disease (28, 29). In addition, feeding on *C. elegans* on *S. cerevisiae* did not affect egg viability. The fraction of eggs hatched was constant and similar to that with *E. coli* for the yeast strains tested. Most remarkably, there was increased survival of the nematodes, even in the presence of increased fungal occupancy. The viability of worms feeding on Als5p-expressing yeast was in fact similar to that of worms fed *E. coli* (Fig. 3C), whereas nematodes fed EV yeast died significantly faster (Fig. 3A). The increased survival was dependent on expression of the Als5p protein (see Fig. S1 in the supplemental material). Therefore, by measures or nematode viability, defecation rates, and egg viability, the host organism was in a nondisease state after feeding on Als5p-expressing yeast (the third criterion of commensalism).

**Is the amyloid sequence in Als5p a commensal-associated molecular pattern?** The amyloid-forming sequence in Als5p was necessary for each of the changes in the yeast-host response. The amyloid-forming sequence was key in both gut occupancy and viability: numbers of Als5p-expressing yeast cells were visually greatly increased in the gut over yeast cells expressing the nonamyloid mutant form of the protein, Als5pV326N (Fig. 1), with a 3-fold increase in the number of viable yeast cells (Fig. 2). Similarly, yeast cells expressing Als5pV326N, a sequence that does not form amyloid, showed decreased defecation rates (Fig. 4), a symptom of a diseased state (29).

To us, the most remarkable result was that feeding with yeast expressing the amyloid-forming wild-type version of Als5p led to longer nematode survival than did feeding with yeast expressing the Als5pV326N protein, whose sequence was mutated to inhibit amyloid formation (Fig. 2). Indeed, the average life span of the worms fed yeast with the nonamyloid Als5pV326N protein was statistically similar to that of the worms feeding on EV yeast, which did not express Als5p at all.

Therefore, the amyloid-forming sequence in Als5p was necessary for accumulation of viable yeasts in the nematode gut and for increased survival of the worms. These results support the hypothesis that Als5p can function in modulating innate immunity to promote a more commensal-like state in the relationship between nematodes and *S. cerevisiae*. The Als5p amyloid-forming sequence
is necessary for this activity. In this model, the Als5p amyloid sequence functions as a commensal-associated molecular pattern (CAMP), analogous to a pathogen-associated molecular pattern (PAMP) in pathogenesis (33). This concept helps to define the differences and similarities between commensalism and pathogenicity, with both states being outcomes of host-microbe interactions linked to characteristics of both the host and microbe (34).
Indeed, our results identify a particular protein, region, and indeed a single-amino-acid residue that can shift the host-microbe relationship between pathogenicity and commensalism.

Our mixing experiments support this hypothesis. Als5p-expressing cells can enhance the retention of otherwise nonretained EV-expressing *S. cerevisiae* cells within the intestine of *C. elegans*. Furthermore, this retention of EV control cells was not accompanied by an increase in the mortality rate. On the contrary, nematodes fed the mixture of Als5p-expressing and EV *S. cerevisiae* cells succumbed at rates similar to those of nematodes fed only Als5p-expressing cells. This observation is directly in line with our hypothesis that Als5p may possess CAMP-like properties, perhaps downregulating innate immune functions in the nematode to promote or enhance survival of both yeast and host. This proposed ability may help explain why EV cells are found in larger numbers within the intestine of the nematodes fed the *S. cerevisiae* mixture. We hypothesize that Als5p prevents robust activation of host immune functions, which allows for the microbe’s survival in this host niche.

The results of our study do not rule out other explanations. For instance, amyloid-forming Als5p might function to sequester *S. cerevisiae* toxins, thereby promoting tolerance. Alternately, surface amyloids may strengthen the yeast cell wall, preventing disruption by the grinder and allowing persistence in the gut with disease (for *C. albicans*) or without disease (*S. cerevisiae*). Nevertheless, the commensalism model appears to be supported by previous work including the finding that Als5p is upregulated in non-pathogenic states (1).

**Amyloids in clinically important interactions.** Our study adds to the clinical relevance of amyloids since they are naturally present in any host, including human. There are many examples of host interactions with amyloids on microorganisms that can affect the balance within the dynamic interplay between host and microbe (35). For instance, amyloid fibrils formed by prostatic acid phosphatase augment HIV infection and thus tip the balance toward HIV survival and replication (36). Amyloids are present in human native hemostasis components that represent crucial regulatory elements in blood coagulation and clot clearance (hemostatic system). This hemostatic system can be exploited with the help of amyloids produced by microorganisms such as *E. coli* (a commensal bacterium) and *Salmonella* spp. (pathogenic bacteria), thus increasing their chances of colonizing their host (37). Hemostasis, amyloid, and microbial pathogenesis are closely related, and interactions between host and bacterial amyloids play a critical role (37).

There are several additional lines of evidence that Als amyloid interactions shift host-microbe interactions in *C. albicans*. Als5p expression does not correlate with either acute-stage or convalescent-stage candidiasis, and many clinical *C. albicans* strains have natural ALS5 deletions (34, 38). In contrast, ALS5 expression is obvious in asymptomatic, nonpregnant women, suggesting that it is transcribed under commensal conditions (39). Also, we have recently shown that surface amyloids are present on the surface of *C. albicans in situ* in autopsy sections of abscesses from candidiasis victims (40). In humans, these surface amyloids can affect the host response by binding serum amyloid P component (SAP), an innate immune system pattern recognition receptor (PRR) that suppresses the inflammatory response (40, 41).

Clearly, the outcome of the interaction between microbial amyloids and the host can determine the benefit or harm to the host. Our data and those of others support the paradigm that microbial surface amyloids change host-microbe interactions and can promote either infection or commensalism. This study only scratches the surface of the complexity of host-microbe interactions and gives novel insights into the molecular basis for how a successful commensal like *C. albicans* can inhabit its host.

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