A prion of yeast metacaspase homolog (Mca1p) detected by a genetic screen

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Saccharomyces cerevisiae can be infected with four amyloid-based prions: [URE3], [PSI⁺], [PIN⁺], and [SWI⁺], due to self-propagating aggregation of Ure2p, Sup35p, Rnq1p and Swi1p, respectively. We searched for new prions of yeast by fusing random segments of yeast DNA to SUP35MC, encoding the Sup35 protein lacking its own prion domain, selecting clones in which Sup35MC function was impaired. Three different clones contained parts of the Q/N-rich amino-terminal domain of Mca1p/Yca1p with the Sup35 part of the fusion protein partially inactive. This inactivity was dominant, segregated 4:0 in meiosis, and was efficiently transferred by cytoplasmic mixing. The inactivity was cured by overexpression of Hsp104, but the prion could arise again in the cured strain (reversible curing). Overproduction of the Mca1 N-terminal domain induced the de novo appearance of the prion form of the fusion. The prion state, which we name [MCA], was transmitted to the chromosomally encoded Mca1p based on genetic, cytological and biochemical tests.

cytoduction | metacaspase | prion

The first three amyloid-based prions of *Saccharomyces cerevisiae*, [URE3], [PSI⁺] and [PIN⁺], and the [Het-s] prion of *Podospora* were discovered as non-chromosomal genetic elements (1–4) and later shown to be prions of Ure2p, Sup35p, Rnq1p and HET-s, respectively (5–7). The prion domains of the yeast prion proteins are rich in Q and N residues (8–11), although that of HET-s is not (12). Fusion of Q/N rich domains of various yeast proteins to the non-prion, functional part of Sup35p, Sup35C, showed that the N-terminal 153 residues of Ypl226w/New1p could promote aggregation and inactivation of the Sup35C part of the fusion (13). Finally, random shuffling of the prion domains of either Ure2p or Sup35p produced ten sequences each of which could be (artificial) prion domains (14, 15).

[PIN⁺] increases the frequency with which [PSI⁺] arises *de novo* from undetectable to rare (3), and significantly stimulates [URE3] generation (16). Indeed, overproduction (even without prion formation) of many Q/N-rich proteins can do the same (7). Swi1p was one of the proteins capable of showing the Pin⁺ ([PSI⁺]-inducibility) phenotype (7), and further study showed that it in fact could form a prion, called [SWI⁺] (17).

We approached the search for new prions both by examining those with Q/N rich domains and by an unbiased search using random fusions of yeast DNA with the functional part of Sup35, lacking the Sup35 prion domain. Here, we describe isolation of fragments of the N-terminal domain of Mca1p that can serve as a prion domain. Mca1p is a yeast homolog of metacaspases, proteases that are involved in apoptosis in mammals.

Results

Bank Design and Construction. To find prion domains, we made a bank of fusions to the functional part of Sup35p, called Sup35MC (see methods) using the moderately strong *ADHI* promoter. Using plasmid shuffling, the *LEU2* bank plasmids replaced a *URA3* plasmid encoding Sup35C in a *sup35::kanMX* host, strain 4827. Sup35p is a subunit of the translation termination factor, and its inactivation by aggregation results in elevated readthrough

of nonsense codons by a weak suppressor tRNA, such as the *ade2-1 SUQ5* used by Brian Cox in discovering [PSI⁺] (2).

Screening of Prion Domain Candidates. We selected Ade⁺ clones indicative of partial inactivation of Sup35p. Candidates were then mated with the *sup35::kanMX* strain 4830 of opposite mating type carrying the *URA3* plasmid encoding Sup35C and loss of this *URA3* from the diploids was selected on plates with 5-fluoroorotic acid. The fusion plasmid was then isolated from those diploids showing dominance of the Ade⁺ phenotype and sequenced.

Isolation of N-Terminal Domains of Mca1p. Three independent Ade⁺ clones (p14MCA, p20MCA and pG8MCA) yielded fusions of parts of MCA1 to SUP35MC. We present characterization of p20MCA (Fig. 1). Plasmid p20MCA contains a 109 AA portion of MCA1 fused to the SUP35MC domains. Reintroduction of the p20MCA into strain 4827 or strain 4563 in place of the Sup35C plasmid resulted in mostly red Ade⁻ colonies, showing that the Mca1-Sup35MC fusion protein produced was active and was expressed in amounts adequate for translation termination.

The Ade⁺ Phenotype Is Due to a Nonchromosomal Genetic Element.

We first tested whether the Ade⁺ phenotype showed non-chromosomal inheritance. We crossed the Ade⁺ p20MCA-containing cells with the Ade⁻ strain 4830, eliminated the *URA3-SUP35C* plasmid on plates with 5-fluoroorotic acid, and sporulated the resulting diploids. Ade⁺ segregated uniformly 4Ade⁺:0 in 18 complete tetrads, showing non-chromosomal inheritance (Fig. 2). Spore survival in the cross was only 78%, presumably due to the plasmid not segregating into all spores.

Cytoduction (transfer of cytoplasm from one cell to another by cell fusion without nuclear fusion) was carried out between the original Ade⁺ isolate with p20MCA in strain 4827 as donor and the Ade⁻ strain 4563 with the same plasmid as recipient. The original isolate of strain 4827 containing p20MCA received the K⁺ phenotype via cytoduction with strain 3164. Transfer of the killer dsRNA (18) was used as a marker of transfer of cytoplasm. Colonies with the recipient nuclear genotype were tested for receipt of cytoplasm by streaking on a lawn of the diploid killer-sensitive strain 5 × 47. Twenty-one of the twenty-three K⁺ cytoductants with the nuclear genotype of the recipient (4563) had the Ade⁺ phenotype. This confirms that the Ade⁺ phenotype is determined by a non-chromosomal genetic element, which we denote [MCA].

The Ade⁺ phenotypes are due to prions of the Mca1-Sup35MC fusion.

The genetic criteria for a yeast prion (5) were examined. The first genetic criterion is reversible curability. The other known amy-

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Fig. 1. An Ade⁺ clone containing a portion of the MCA1 sequence. A) Ade⁺ cells with p20MCA, containing the indicated MCA1 sequence fused to SUP35MC and the parent Ade⁻ strain are shown. Cells are Ade⁺ and white or light pink on 1/2 YPD. B) The 109 residue Mca1 N-terminal sequence fused to the SupMC domains expressed by plasmid p20MCA. Note that we have confirmed by Western blot that Mca1p begins at the indicated methionine and not that 21 codons further upstream (data not shown).

loid-based yeast prions, [URE3], [PSI⁺], [PIN⁺] and [SWI⁺], are cured by growth in the presence of the Hsp104 inhibitor, 3 mM guanidine HCl (5, 17, 19, 20). We found no curing by 3 mM or 5 mM guanidine of the Ade⁺ phenotype with plasmids 14MCA or 20MCA. Unlike [URE3] (21, 22), the Ade⁺ phenotype was also not cured by overproduction of Ydj1p or Sse1p (data not shown).

Overproduction of Hsp104 cures [PSI⁺], but not the other yeast prions (17, 20, 21, 23). Transformation of the Ade⁺ p20MCA strain with the high-copy pH221 overproducing Hsp104 (21) resulted in efficient curing of the Ade⁺ phenotype. Allowing loss of the Hsp104-overproducing plasmid from each of 10 transformants left the cells still Ade⁻ and red on 1/2 YPD, indicating that the phenotype was not merely suppressed, but that the [MCA] non-chromosomal gene had been cured. Curing of a prion is expected to be reversible because the protein capable of becoming a prion is still being produced in the cured

Fig. 2. The Ade⁺ phenotype is due to a non-chromosomal genetic element. The Ade⁺ p20MCA-containing isolate was crossed with the Ade⁻ strain 4830 (pURA3-SUP35C). Loss of the URA3-SUP35C plasmid was selected by plating with 5-fluoroorotic acid and resulting diploids were sporulated. Ade⁺ segregated uniformly 4Ade⁺:0 in 18 tetrads, while other markers segregated 2+:2-. A typical tetrad is shown.

Table 1. Overproduction of Mca1Np results in an increase in the frequency with which Ade⁺ clones arise

Plasmid	Carbon source	Ade ⁺ /10 ⁶ cells	Curable/tested
P _{GAL1} -Mca1 ¹⁻¹⁰⁹	Dextrose	8	7/8
P _{GAL1} -Mca1 ¹⁻¹⁰⁹	Galactose	98,000	8/8
Vector	Dextrose	4	0/3
Vector	Galactose	462	3/8

Parental strain 4827 containing p20MCA, lacking p1216, was transformed with either plasmid pJN12 (P_{GAL1}-Mca1¹⁻¹⁰⁹) or vector control (pH772). Induction was performed on galactose-containing medium for 4 days. Viable cells were determined by plating on YPAD and Ade⁺ cells by plating on -Ade medium for 4 days. Ade⁺ colonies were transformed with pH221 to determine whether the Ade⁺ phenotype was curable by overexpression of Hsp104.

strain (5). Cured strains were then plated on -ade medium to determine if the Ade⁻ phenotype is reversible. We found that the cured (Ade⁻) strain carrying p20MCA gave rise to 1080 Ade⁺ clones per 10⁶ cells plated. Thus, [MCA] is reversibly curable, indicating it is due to a prion.

A second criteria for yeast prions is that overproduction of the corresponding protein should increase the frequency with which the prion form arises (5). This is in fact true of the [URE3], [PSI⁺] and [PIN⁺] prions (5, 7, 24). The parental strain 4827 containing p20MCA, and lacking p1216 (URA3 SUP35C), was transformed with pJN12 expressing the 109 residue Mca1 N-terminal sequence found in p20MCA under the control of a galactose inducible promoter. We found that overproduction of the Mca1N protein results in >200 fold increase in the frequency with which Ade⁺ clones arise. Several of these transformants were tested and are cured by overexpression of Hsp104 (Table 1). These results also argue that [MCA] is a prion.

The third genetic criterion for a prion is the unexpected similarity of the phenotype of cells with the prion to that of cells with a mutation in the gene encoding the prion protein. For example, like ure2Δ strains, [URE3] strains show derepressed nitrogen regulation. However, since mca1Δ cells do not have a clear phenotype, this point could not be tested. We show below that propagation of [MCA] depends on Mca1p.

Aggregation of Mca1p in Prion-Containing Cells. Plasmid pTN180, expressing Mca1¹⁻¹⁴⁷-GFP, was introduced into Ade⁺ [MCA]

Fig. 3. Mca1p aggregates in prion-containing cells. The original Ade⁺ [MCA] p20MCA isolate (4827 background) transformed with pTN180 (Mca1N-GFP) shows aggregates (top), while the parent strain 4827 transformed with p20MCA and allowed to lose p1216 (SUP35C) is Ade⁻ [mca-o], and such cells transformed with pTN180 show a diffuse GFP signal (middle). The original isolate cured of [MCA] by overexpression of Hsp104 and transformed with pTN180 also shows a diffuse GFP signal (bottom).

Fig. 4. Ade+ [MCA] cells possess HA-tagged Mca1p primarily in the pellet. HA-tagged Mca1 is visible in a Western blot primarily in the pellet of cell lysates derived from an Ade+ [MCA] derivative of strain TN174. This strain contains an integrated MCA1 with a 3xHA tag as well as an integrated MCA-N-3xHA-URE2C construct. The two HA-tagged proteins are expected to be essentially the same size. In Ade- [mca-o] transformants of this strain, the HA-tagged MCA1 resides equally in the supernatant and pellet. The *mca1* lane shows extract of a strain lacking either tagged protein as a control.

p20MCA cells and into Ade- [mca-o] p20MCA cells, the latter produced by replacing pSUP35C with p20MCA in strain 4827 (Fig. 3). While GFP fluorescence was evenly distributed in [mca-o] cells, it displayed a punctate appearance in [MCA] cells. Cells which had been cured of the putative prion by overexpression of Hsp104 displayed a diffuse GFP pattern similar to that of the [mca-o] strains.

The [MCA] Prion Also Inactivates a Mca-N-Ure2 Fusion Protein. Strain TN174 contains *MCA1* with a 3xHA tag at the normal *MCA1* location, as well as *MCA-N-3xHA-URE2C* (replacing the *URE2* gene), with the prion domain of Mca1p and the nitrogen regulation domain of Ure2p without its own prion domain. Ade+ [MCA] derivatives (here indicating inactivity of Ure2p) were isolated and the aggregation of Mca1p in cells was analyzed by centrifugation. Ade+ transformants possessed HA-tagged Mca1 visible in a Western blot primarily in the pellet, as opposed to Ade- transformants in which the HA-tagged Mca1 resided primarily in the supernatant (Fig. 4). Note that the Mca1-3xHA

Fig. 5. A Tinker to Evers to Chance cytoduction experiment shows that either Mca1p or Mca1N-Sup35MC is sufficient to propagate [MCA]. A) An [MCA] strain containing an intact chromosomal *MCA1* and the *MCA1N-SUP35MC* fusion on p20MCA is used as the donor in a cytoduction in which the intermediate recipient expresses only Mca1p from the chromosome, only the Mca1N-Sup35C fusion protein from p20MCA or neither. The resulting cytoductants were then used as the donor in a cytoduction into a [mca-o] strain. B) An [MCA] strain containing an intact chromosomal copy of *MCA1* and the *MCA1N-Sup35MC* fusion was allowed to lose the fusion plasmid (exchanged for p1216). The *MCA1* chromosomal copy was knocked out (below) in this strain or left intact (above). The fusion plasmid was then reintroduced into the strain, and the phenotype of these transformants is shown.

Fig. 6. MCA1p propagates [MCA]. (Upper) The original 20MCA isolate in which p20MCA has been replaced with pSup35C (p1216) and then transformed with pTN180 (Mca1¹⁻¹⁴⁷-GFP). (Lower) *mca1::kanMX* disruptant in which p20MCA has been replaced with pSup35C and then transformed with pTN180.

and Mca1N-3x-Ure2C are essentially the same size. This experiment shows that [MCA] inactivates an Mca-Ure2 fusion protein, and that, as above, [MCA] is accompanied by aggregation of full length Mca1p.

Evidence That Full-Length Mca1p Can Be a Prion. Several lines of evidence show that the chromosomally encoded full-length Mca1p is recruited into Mca1N-Sup35MC aggregates and can in fact propagate the [MCA] prion in the absence of the fusion protein. First, replacing p20MCA from the original Ade+ isolate with a plasmid expressing just Sup35C resulted in the strain becoming Ade-. However, when p20MCA was reintroduced replacing pSUP35C, cells became Ade+ again (Fig. 5B), suggesting that something was maintaining the [MCA] genetic element in the absence of the Mca1-Sup35MC fusion, presumably the chromosomal Mca1 protein. To test this, the chromosomal *MCA1* gene in the original isolate was replaced with *hph* (hygromycin B resistance). When p20MCA was replaced with pSUP35C, and then p20MCA again replaced pSUP35C, the cells were Ade- (Fig. 5B). This shows that the chromosomal *MCA1* gene is necessary to maintain [MCA]. This indicates that the chromosomally encoded full length Mca1p is propagating the prion.

A second line of evidence showing that full-length Mca1p was propagating the [MCA] prion was the aggregation state of the protein. When p20MCA was replaced with pSUP35C in the original isolate and then pMCA1N-GFP was introduced, green fluorescence of the Mca1N-GFP fusion protein was seen to be in aggregates (Fig. 6). However, if *MCA1* was first disrupted with a hygromycin cassette, then p20MCA was replaced with pSUP35C and pMCA1N-GFP introduced, the fluorescence was diffuse. This shows that the chromosomally encoded full-length Mca1p is aggregated specifically in [MCA] cells and that the Mca1N-GFP fusion protein is recruited into these aggregates.

A third line of evidence involved a 'Tinker to Evers to Chance' cytoduction experiment (8, 25) (Fig. 5). Cytoplasm from an Ade+ [MCA] p20MCA *MCA1* strain was cytoducted into strains expressing either only Mca1p (from the chromosome), only the Mca1N-Sup35MC fusion protein from p20MCA or neither. The cytoductants were then used to transfer cytoplasm to an indicator strain that was [mca-o] Ade- and expressed both Mca1p and Mca1N-Sup35MC. The results showed that either Mca1p or Mca1N-Sup35MC is sufficient to propagate [MCA], but at least one of these is necessary. Moreover,

*Tinker to Evers to Chance refers to a sequential cytoduction experiment, named after the famed double play combination of the Chicago Cubs, shortstop Joe Tinker to second baseman Johnny Evers to first baseman Frank Chance.

Fig. 7. *Mca1p* or *Mca1N-Sup35MC* is sufficient to propagate [MCA]. The intermediate cytoductant in Fig. 5 carrying [MCA] and expressing full length *Mca1p* (but not the *Mca1N-Sup35MC* fusion) was then transformed with pTN180 expressing the *Mca1N-GFP* fusion protein. Fluorescence microscopy of these transformants showed aggregation of the *Mca1N-GFP* protein (top). The control was the intermediate cytoductant from Fig. 5 expressing no *MCA1* sequences and lacking [MCA]. The strain was then transformed with the same plasmid expressing the *Mca1N-GFP* fusion protein and no aggregation is seen by fluorescence microscopy (bottom).

introduction of p*Mca1N-GFP* into the intermediate cytoductants showed that *Mca1p* was aggregated in [MCA] cells expressing *Mca1p* from the chromosome and lacking the plasmid expressing the *Mca1N-Sup35C* fusion protein (Fig. 7).

Thus, the chromosomally encoded full length *Mca1p* can propagate the [MCA] prion and is aggregated when doing so.

Discussion

[MCA] is thus a non-chromosomal genetic element which satisfies two of the genetic criteria for a prion (the third cannot be tested). *Mca1p* or *Mca1N-Sup35MC* are aggregated specifically in [MCA] cells, and the *Mca1N* region that is sufficient to propagate [MCA] is Q/N rich, so it is likely that [MCA] is based on amyloid of *Mca1p*, but this will require further investigation. The QQYG and PPP repeats may play a role in prion formation, but repeats in *Sup35N* are clearly not needed for [PSI⁺] formation (15). *Mca1p* homologs are found in *Candida albicans*, *Kluyveromyces lactis*, *Candida glabrata*, *Ashbya gossypii* and other yeasts and fungi with rapid divergence in residues 1–128 and close similarity thereafter. Unlike the other amyloid-based yeast prions, [MCA] is not cured by growth on millimolar guanidine, a specific inhibitor of Hsp104. Whether this is due to only partial inactivation of Hsp104 by guanidine will require further study. Like [PSI⁺], [MCA] is cured by overexpression of Hsp104, but the mechanism of this curing is unclear, even in the case of [PSI⁺].

Caspases are cysteine-dependent aspartate specific proteases, many of which are involved in mammalian apoptosis. *Mca1p* is a metacaspase, a member of a family of caspase-related proteins, which has been proposed to be involved in one or more yeast programmed cell death processes (reviewed in (26)), and pos-

sibly in cell cycle dynamics (27). Conflicting claims and marginal results make the role of *Mca1p* in apoptosis, and even the existence of a true yeast programmed (purposeful) cell death a subject of ongoing controversy (reviewed in (28)). Our own efforts to reproduce what is described as “One of the most prominent effects of *MCA1* deletion,” namely, resistance of *mca1Δ* strains to valproic acid (29), were unsuccessful (data not shown). Thus we have not yet been able to investigate the physiological consequences of the [MCA] prion.

This is the first prion found by a general molecular cloning search. Experiments of this type should detect protein segments from any organism that can be a prion domain in yeast. With six prions known in *S. cerevisiae*, it is likely that there are many to be discovered in other organisms.

Methods

Construction of Prion Domain Cloning Vector, p1166, and DNA Bank. *SUP35MC* was amplified using oligos 397 (5' gctatcaacgacatacaatcaatcaagct Ggatccaa-caatgtcgCccgggatgtcttgaacgactttcaaaa 3') and 401 (5' ggagacttgaccaactctg-gcgaagaagtcca CTCGAGTTTACTCGGCAATTTTAAATTTTACCAATT 3'), cut with BamHI and XhoI and inserted into pH124 (gift of Herman Edskes, National Institutes of Health, Bethesda) cut with the same enzymes. This CEN *LEU2* plasmid (p1166) has the structure: *ADH1* promoter-BamHI-ATG-SmaI-*SUP35MC*-ter-XhoI-*ADH1* transcription terminator. DNA of *S. cerevisiae* S288C was sonicated to an average size of ~400 bp, and a bank in p1166 was prepared by insertion into the SmaI site by Marligen Biosciences (Ijamsville, MD).

Construction of Other Strains and Plasmids. Strain 4472 (*MATα ade2-1 SUP5 trp1 kar1-1 his3 leu2 ura3 sup35::kanMX pRS316-SUP35* (kind gift of Dan Masison) was made [PIN⁺] by cytoduction from 4212 (*MATα ura2 leu2 his- [PIN⁺]*, Table 2). The pRS316-SUP35 plasmid was replaced first with p1166 (pRS315 backbone: CEN *LEU2 SUP35* promoter-*SUP35C-ADH1* transcription terminator) and then with p1216 (pRS316 backbone CEN *URA3 SUP35* promoter-*SUP35C-ADH1* transcription terminator) forming strain 4827. This strain lacks *SUP35N*, encoding the prion domain of *Sup35p*, and was transformed with the bank in p1166. Strain 4830 (*MATα kar1 leu2 ura3 his3 lys2 sup35::kanMX ade2-1 SUP5 p1216 (URA3 SUP35C)*) was constructed similarly from strain 4563 carrying pRS316-SUP35.

Strain TN174 is a derivative of strain BY319 (*P_{DALS} ADE2 leu2 trp1 ura3 P_{DALS} CAN1 kar1*). The 3 x HA sequence was introduced into BY319 by integrating a pRS406 derivative harboring the full length *MCA1* gene with terminator sequence and tagged with 3x HA (pTN199). Cells with the integrated plasmid were grown on 5-FOA selecting for loss of the plasmid sequence and clones were screened for the 3x HA tag by western-blotting analysis. A MCAN-3xHA-URE2C construct was also integrated into the genome via a similar integration with plasmid pTN160.

Strains 3164 (*MATα kar1-1 arg1 leu1 thr(1,x) L-A-HN K1+*) and 5 × 47 (diploid sensitive to the killer toxin) were used to introduce the killer M₁ dsRNA by cytoduction and to assay strains for its presence.

p20MCA is the plasmid isolated from the Ade⁺ clone indicative of partial inactivation of *Sup35* in the initial bank screen. It contains a portion of *MCA1* encoding residues 1–109 fused to the *SUP35MC* domains within p1166. pJN12 was constructed by PCR amplification of the same part of *MCA1* from p20MCA using oligos BamHI-20MCAF (5' gggggatccaacaatgtcgccctttcaaaat 3') and PstI-SupR (5' gggctgcaggagacttgaccaactctg 3') and inserting it between the BamHI and PstI sites of pH772. pH772 has a multiple cloning site from pVT103 and a galactose promoter inserted into the CEN plasmid pRS314.

pTN180, used for visualization of *Mca1p* aggregation, was constructed by inserting a PCR generated NotI-EcoRI fragment carrying the *ADH1* promoter

Table 2. Strains of *S. cerevisiae*

Strain	Genotype	Source
4472	<i>MATα ade2-1 SUP5 trp1 kar1-1 his3 leu2 ura3 sup35::kanMX pRS316-SUP35</i>	Dan Masison
4827	<i>MATα ade21- SUP5 trp1 kar1-1 his3 leu2 ura3 sup35::kanMX p1216 (CEN URA3 SUP35C)</i>	This work
4563	<i>MATα kar1 leu2 ura3 his3 lys2 sup35::kanMX ade2-1 SUP5 pRS316-SUP35</i>	This work
4830	<i>MATα kar1 leu2 ura3 his3 lys2 sup35::kanMX ade2-1 SUP5 p1216 (CEN URA3 SUP35C)</i>	This work
TN174	<i>MATα P_{DALS} ADE2 leu2 trp1 ura3 P_{DALS} CAN1 kar1 MCA-3xHA ure2::MCAN-3xHA-URE2C</i>	This work
3164	<i>MATα kar1-1 arg1 leu1 thr(1,x) L-A-HN K1+</i>	See ref. 25
5 × 47	Diploid sensitive to the killer toxin	See ref. 25

driving expression of Mca1 residues 1 to 147 fused to GFP into plasmid pRS316 cut with the same enzymes.

Curing. Ade⁺ strains were transformed with plasmid pH221 (high copy Hsp104, (21)), pYdj1 (21), or pSse1 (22) and plated on selective media. Strains were then plated on YPAD and confirmed to have lost the curing plasmid. Strains were then analyzed on -Ade and ½ YPD for color.

Aggregation of MCA Fusion Proteins. Cells carrying pTN180 (Mca1¹⁻¹⁴⁷-GFP) were analyzed with a fluorescence microscope (Zeiss Axioskop II) using a standard FITC filter set (Zeiss, Oberkochen, Germany). Aggregation as reflected in sedimentation was examined in protein extracts of derivatives of strain TN174. Cell lysates were prepared by harvesting cells grown to an OD₆₀₀ of 1.5 and lysing cells using glass beads in buffer A as previously described (30). Supernatant and pellet fractions were separated by spinning for 30 min at 15,000 g. Western blotting was performed with the rat anti-HA antibody 3F10 (Roche) and anti-rat IgG AP conjugate (Promega).

Induction. Strain 4827 containing p20MCA, lacking p1216, was transformed with either pJN12 or pH772 (vector). Transformants were selected on -Trp media. After confirmation that strains were Ura⁻, induction was performed on plates containing galactose for four days. Colonies were enumerated on YPAD and SD-ade. Ade⁺ colonies were transformed with pH221 (high copy, Hsp104) to determine if strains were curable by overexpression of Hsp104.

Cytoduction. Transfer of [MCA] by cytoplasmic mixing was carried out as previously described using kar1 mutants that fail to undergo karyogamy (31). Donor and recipient cells were mixed and grown on YPAD agar at 30 °C for 7 h. The incubated mixture was then streaked on media selecting against the donor. Cytoduction was verified by transfer of the killer phenotype to cells with the recipient nuclear genotype (25).

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