Our data that the polypeptide with expanded polyQ forms inclusions only in the presence of the [RNQ⁺] prion are consistent with the latest report showing that aggregation of the MJD protein with polyQ expansion is facilitated in so-called [PIN⁺] cells (Osherovich and Weissman, 2001). [PIN⁺] is a non-Mendelian genetic trait promoting emergence of [PSI⁺] (Derkatch et al., 1997; Osherovich and Weissman, 2001). It was shown that [PIN⁺] may be caused by a number of potential prion-forming proteins, for example, Rnq1 and New1 (Derkatch et al., 2001). Interestingly, deletion of the NEW1 gene did not significantly affect aggregation of 103Q polypeptide, suggesting the major role of [RNQ⁺] prion in this process. Whether aggregates of prion form of Rnq1, which has a QN-rich domain, could directly serve as a nucleation site for polyQ, or Rnq1 acts on the polyQ aggregation indirectly is yet to be established.

Huntingtin toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1

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The cause of Huntington's disease is expansion of polyglutamine (polyQ) domain in huntingtin, which makes this protein both neurotoxic and aggregation prone. Here we developed the first yeast model, which establishes a direct link between aggregation of expanded polyQ domain and its cytotoxicity. Our data indicated that deficiencies in molecular chaperones Sis1 and Hsp104 inhibited seeding of polyQ aggregates, whereas ssa1, ssa2, and ydj1–151 mutations inhibited expansion of aggregates. The latter three mutants strongly suppressed the polyQ toxicity. Spontaneous mutants with suppressed aggregation appeared with high frequency, and in all of them the toxicity was relieved. Aggregation defects in these mutants and in sis1–85 were not complemented in the cross to the hsp104 mutant, demonstrating an unusual type of inheritance. Since Hsp104 is required for prion maintenance in yeast, this suggested a role for prions in polyQ aggregation and toxicity. We screened a set of deletions of nonessential genes coding for known prions and related proteins and found that deletion of the RNQ1 gene specifically suppressed aggregation and toxicity of polyQ. Curing of the prion form of Rnq1 from wild-type cells dramatically suppressed both aggregation and toxicity of polyQ. We concluded that aggregation of polyQ is critical for its toxicity and that Rnq1 in its prion conformation plays an essential role in polyQ aggregation leading to the toxicity.

Introduction

Expansion of polyglutamine (polyQ) domains in certain proteins causes several fatal neurodegenerative diseases, for example, Huntington’s disease. All of these diseases are inherited as a dominant trait, and their onset and severity closely correlate with the length of abnormal polyQ sequences (Gusella and Macdonald, 1998; Cummings et al., 1999a). These and other observations led to a conclusion that polyQ expansion is a gain-of-function mutation, causing polypeptides with expanded polyQ (for example, huntingtin) to acquire an unusual conformation, which facilitates their aggregation into intracellular inclusion bodies (IBs)* and causes cell toxicity (Difiglia et al., 1997; Scherzinger et al., 1997; Bates et al., 1998).

It was initially assumed that denatured or abnormal proteins aggregate and form IBs simply due to their intrinsic tendency to associate with each other until the aggregates eventually become insoluble. However, recent reports demonstrated that formation of cytoplasmic IBs in mammalian cells may be a complex process involving transport of small protein aggregates from the cell’s periphery to the centrosome along microtubules. These IBs contain Hsps, ubiquitin, ubiquitin-conjugating enzymes, and 26S proteasomes forming a large structure termed aggresome (Vidair et al., 1996; Wojcik et al., 1996; Johnston et al., 1998; Anton et al., 1999; Garcia-Mata et al., 1999; Wigley et al., 1999; Fabunmi et al., 2000). Recent findings from our and other groups indicate that IBs formed by polyQ-containing polypeptides have similarities with the aggresome (Steenoien et al., 1999; Wyttenbach et al., 2000; Meriin et al., 2001; Waelter et al., 2001). Furthermore, we have found recently that a stress-activated protein kinase MEKK1 stimulates early stages of IB formation (Meriin et al., 2001), suggesting that various steps of IB formation are tightly controlled by the cell.

*Abbreviations used in this paper: IB, inclusion body; GFP, green fluorescent protein; GuHCl, guanidine hydrochloride; polyQ, polyglutamine.

Key words: aggregation; polyglutamine; toxicity; prions; yeast
Close correlation between formation of the huntingtin-containing IBs and death of the affected neurons suggests that IB may be involved in neurodegeneration (Davies et al., 1998). However, there is evidence that IB formation may not be necessary for the neuronal death, since artificial inhibition of aggregation of polyQ-containing polypeptides in certain animal and cellular models did not inhibit and even enhanced neuronal apoptosis (Klement et al., 1998; Saudou et al., 1998; Cummings et al., 1999b). Therefore, IB formation may be irrelevant to the neurodegeneration process or may even serve a protective role by capturing toxic soluble polyQ molecules (for review see Sherman and Goldberg, 2001). The question whether soluble abnormal proteins or IBs cause toxicity and neurodegeneration is the focus of ongoing discussion in the field. Because of the complexity of polyQ-induced neuronal death and IB formation, development of adequate cellular and animal models is critical to dissect cellular mechanisms of these processes. The practical application of this research would be identification of proteins involved in neurodegeneration and IB formation in order to use them as targets for drug design.

In mice, Drosophila and Caenorhabditis elegans expression of extended polyQ polypeptides caused toxicity and neurodegeneration, and IBs in neurons were detectable, whereas polypeptides with polyQ of normal length were not toxic and did not form IBs (Davies et al., 1997; Scherzinger et al., 1997; Kazemi-Esfarjani and Benzer, 2000; Satyal et al., 2000). Notably, in mice model expression of exon 1 of huntingtin, a small NH₂-terminal fragment with extended polyQ domain, was sufficient to cause both neurodegeneration and IB formation (Davies et al., 1997). Yeast Saccharomyces cerevisiae has also been used as a model, since it provides a useful tool for screening of genes involved in IB formation and potential polyQ-induced toxicity and for screening of chemical compounds, which inhibit these processes. Short polyQ was shown to be soluble in yeast, whereas long polyQ polypeptide–formed IBs; however, no toxicity of polyQ polypeptides in yeast has been reported so far (Krobitsch and Lindquist, 2000; Muchowski et al., 2000). In these yeast models, the molecular chaperones Hsp104 was reported to be essential for the aggregation of polyQ (Krobitsch and Lindquist, 2000). Overexpression of other molecular chaperones Hsp70 and Hdj1 also affected polyQ aggregation suppressing formation of fibrous aggregates and promoting instead formation of amorphous structures (Muchowski et al., 2000).

The molecular chaperones Hsp104, Hsp70, and Sis1 were implicated in emergence and maintenance of prion conformation of certain yeast proteins, for example, Sup35, Rnq1, Ure2 (Chernoff et al., 1995, 1999; Derkatch et al., 1997; Newnam et al., 1999; Jung et al., 2000; Moriyama et al., 2000; Sondheimer and Lindquist, 2000; Sondheimer et al., 2001; Wegrzyn et al., 2001). These polypeptides in prion conformation aggregate and convert normal polypeptides of the same type into prion conformation, thus recruiting them into IBs. The phenotypic traits resulting from such aggregation are inherited in a non-Mendelian fashion (Wickner et al., 1999; Serio and Lindquist, 2000). These mechanisms closely resemble aggregation of mammalian prion PrP, a cause of a group of neurodegenerative disorders (Prusiner, 2001).

In these yeast models, the molecular chaperone Hsp104 was reported to be essential for the aggregation of polyQ (Krobitsch and Lindquist, 2000; Muchowski et al., 2000). In contrast to 25Q, expression of 103Q is toxic to yeast cells, since colonies expressing 103Q ceased to grow within 1–2 d after induction on galactose medium (Fig. 1 A, top). No difference in colony size was seen on glucose-containing medium without polyQ expression (Fig. 1 A, bottom), indicating that accumulation of 103Q was responsible for the growth defect.

Notably, the cellular levels of accumulated 103Q were much lower than the level of 25Q as seen by a decrease in in-
tensity of fluorescence of the cells expressing 103Q (Fig. 1B). This difference in accumulation of the polyQ polypeptides evidently was not related to 103Q degradation, since both 25Q and 103Q polypeptides were relatively stable in yeast (unpublished data). The difference in expression may be related to reduction of the plasmid copy number in cells expressing 103Q due to plasmid instability. Accordingly, in spite of constantly maintained selection for the plasmid’s URA marker cells expressing 103Q were losing this plasmid rapidly. After 3 d on selective galactose plate, only 0.1% of cells expressing 103Q retained the plasmid with the 103Q gene, whereas as much as 25% of cells expressing 25Q still maintained the plasmid (unpublished data). Therefore, the 103Q-induced toxicity appears to be so severe that cells which lost the plasmid have an advantage over 103Q-expressing cells even on the selective medium.

To study whether expression of expanded polyQ inhibits growth by affecting the cell cycle progression, we compared cellular DNA contents in the cultures expressing 25Q and 103Q. Cells induced in selective galactose medium for 20 h were stained with propidium iodide and then analyzed by FACS®. Expression of 103Q in strain W303 caused a slight increase of G2 phase cells in comparison with cells expressing 25Q (unpublished data), whereas in 103Q-expressing JN54 cells no changes in the cell cycle were observed (Fig. 1C) in spite of significantly decreased growth. This indicates that there is no substantial cell cycle delay, which could account for the growth inhibition upon accumulation of 103Q.

**Molecular chaperones are essential for polyQ aggregation and toxicity**

Microscopic observation showed that 25Q was diffusely distributed in yeast cells, whereas every cell expressing 103Q formed multiple aggregates of two types (Fig. 2A): appearing either as large disordered lumps or as small grains (or flakes), some of which were swarming in a cell. A fraction of cells with small aggregates increased in expense of cells with large aggregates upon prolonged 103Q accumulation, suggesting that aggregates are dynamic formations capable of further evolution. Fractionation of cell homogenates in the presence of 1% Triton X-100 provided independent evidence that the majority of 25Q (>75%) were soluble in the cells (Fig. 2B).

Notably, a fraction of the 25Q was found in 800 g pellets, indicating that although visible aggregates were not seen with short polyQ, some of the molecules associated with large detergent-insoluble cellular structures. With 103Q, from 50 up to 95% of the polypeptide was found in pellets, depending on duration of induction and yeast strain.

Because polyQ expression in yeast shows a length-dependent toxicity, this system offers a simple method to study the mechanisms of polyQ aggregation and toxicity genetically. In search of cellular elements controlling the process of aggregation, we screened a set of mutants of chaperones and related proteins, including _hsp104_, _ssa1_ _ssa2_, _ssa1_ _ssa3_, _ssa1_ _ssa4_, _ssb1_ _ssb2_, _sei1_, _sei2_, _pdr_, _ydj1–151_, _sis1–85_, _xdj1_, _zuo1_, _ypr061c_, _ynl227c_, _ypr041c_, _ynl077w_, _sti1_, and _sba1_. Among these mutants, _hsp104_ deletion, _sis1–85_ mutation, _ssa1_ _ssa2_ double deletion (Fig. 2C) and _ydj1–151_ mutation (unpublished data) significantly suppressed 103Q aggregation as assayed by fractionation. Two distinct types of the aggregation patterns were seen with these chaperone mutants. In _ssa1_ _ssa2_ (Fig. 2D) and _ydj1–151_ (unpublished data) mutants, 103Q formed a multitude of very small grain-like aggregates against a bright diffused background representing soluble 103Q. (Of note, there was no compensatory induction of other chaperones in this particular strain of _ssa1_ _ssa2_ mutant.) In _hsp104_ and _sis1–85_ strains, the majority of cells did not form any visible aggregates, and the rest of the cells formed one or few large condensed aggregates (Fig. 2D). These large aggregates resembled a compressed bundle of filaments, whose appearance was completely different from the IBs seen in the wild-type strain (compare Fig. 2A).

The differences in 103Q aggregation patterns in the chaperone mutants may reflect different roles of the chaperones in polyQ aggregation. We followed the process of aggregation in wild-type and mutant cells in real time by taking pictures every 2 min of several selected cells expressing 103Q. In the wild-type, initially all fluorescent cells contained small aggregates. With time, intensity of fluorescence in aggregates and aggregate size grew, whereas the soluble (diffusely distributed) fluorescent material gradually disappeared (Fig. 3). However, even after 60 min of observation a certain pool of

![Figure 2](image-url)
103Q still remained soluble. Unlike the wild-type cells, in ssa1 ssa2 cells no apparent redistribution of soluble 103Q into numerous aggregates was seen within 60 min (unpublished data), indicating that although the nucleation of aggregates is unimpeded in this mutant, further growth of the aggregates is inhibited. We monitored rare events of IB formation in the hsp104 mutant and found a remarkable difference with polyQ aggregation compared with the wild-type cells (Fig. 3). Once started, the aggregation of 103Q in hsp104 mutant proceeded rapidly, so 6–12 min after the first appearance of visible seeds almost all soluble polyQ in the cell collapsed into them, forming large IBs (Fig. 3). Therefore, the rate-limiting step in the hsp104 mutant is not growth of prenucleated aggregates but initiation of the aggregation process. In a small number of cells where aggregates are seeded despite the lack of Hsp104, the consequent growth of the IB proceeds rapidly. Therefore, Hsp104 appears to be involved (possibly indirectly as discussed below) in nucleation of polyQ aggregates, whereas members of the Ssa family appear to be involved in expansion of prenucleated aggregates, which defines the difference in aggregation phenotypes in the chaperone mutants.

Most importantly, suppression of aggregation in the hsp104, ssa1 ssa2, and ydj1–151 chaperone mutants, independently on the mechanism, correlated with suppression of polyQ toxicity. The ssa1 ssa2 and ydj1–151 mutants grew slower than the parental strain as seen with control cells expressing 25Q. By contrast, expression of 103Q, although dramatically reducing growth of wild-type colonies, did not significantly affect growth of these mutants (Fig. 4, bottom). It was difficult to make a conclusion about the effect of sis1–85 mutation on polyQ toxicity, since SIS1 is an essential gene, and this mutation by itself dramatically slowed growth of yeast. The hsp104 deletion, which did not affect yeast growth (unpublished data), relieved 103Q toxicity (Fig. 4, top), so colonies of cells lacking Hsp104 transformed with either 103Q or 25Q were large and did not differ in size on galactose plate (unpublished data). We studied whether relief of polyQ toxicity in the hsp104 mutant was caused by suppression of aggregation. Since 103Q aggregated in a fraction of hsp104 cells, we investigated whether in these cells aggregation was toxic. Accordingly, individual cells of the hsp104 strain induced in the galactose medium for 16 h were placed on a grid on galactose-selective plates using a micro-manipulator. Cells containing IBs were identified, and emergence of colonies was monitored. Although 14 out of 18 cells without aggregates gave rise to colonies, only 1 out of 9 cells with aggregates formed a colony, suggesting that even for hsp104 mutant cells IBs are toxic. These data indicate that the growth defect in yeast cells expressing expanded polyQ is caused by its aggregation.

**Yeast prions are required for 103Q aggregation**

To find additional cellular components involved in polyQ aggregation and toxicity, we selected spontaneous mutants in which aggregation of 103Q was suppressed. We expected that 103Q would not be toxic in the cells defective in aggregation; hence, the block on the polyQ expression (assayed as cell fluorescence) would be relieved. Accordingly, using a cell sorter we isolated a fraction of cells with the highest fluorescence, which was expected to be enriched with the aggregation mutants. Two consequent cycles of cell sorting yielded >85% of cells without visible aggregates, indicating that the enhanced fluorescence indeed was associated with the reduced aggregation. The calculated frequency of the spontaneous mutations was unexpectedly high (close to
Mating of all 25 spontaneous mutants and *hsp104* and *sis1–85* mutants with the wild-type haploid strain (BY4742) demonstrated that the aggregation defects were recessive (unpublished data). Surprisingly, all diploids obtained by mating the spontaneous mutants with the *hsp104* deletion strain displayed defects in aggregation similar to that of parental mutants (unpublished data), indicating the lack of complementation. Since a frequency of spontaneous mutations of $10^{-3}$ in the *hsp104* gene is improbable, these data implied an unusual type of inheritance. Crossing of the *hsp104* deletion mutant with *sis1–85* also yielded diploid cells with defects in aggregation similar to that of parental mutants (unpublished data), indicating that defects in polyQ aggregation were inherited in a non-Mendelian manner.

The conclusive proof of such type of inheritance came from sporulation/dissection of diploids resulting from mating of the mutants with the wild-type strain. All four spores in a tetrad usually displayed wild-type–like aggregation and toxicity of 103Q, indicating a non-Mendelian mode of inheritance. In contrast, sporulation and dissection of diploids originating from a mating of wild-type strain and *hsp104* mutant resulted in 2:2 segregation of aggregation/toxicity pattern. In these tetrads, suppressed aggregation was always associated with the *hsp104* deletion. The non-Mendelian mode of inheritance of polyQ and toxicity was reminiscent of inheritance of prion elements in yeast (Wickner et al., 1999; Sondheimer and Lindquist, 2000).

To test this possibility, we screened a set of deletion mutations in nonessential prion genes and in genes involved in aggregation of prion proteins (*i.e.*, *mks1*, *new1*, *sla1*, *rnq1*, *ure2*, and *ybr016w*) for their effects on polyQ aggregation. Of all the mutants tested, only cells with the *rnq1* deletion displayed an aggregation defect. The 103Q aggregation pattern in these cells was similar to that of *hsp104* and *sis1–85* mutants (Fig. 5 A, middle). No 103Q-related toxicity was detected in *rnq1* mutant cells (Fig. 5 B) despite the fact that 103Q was accumulated to higher levels than in the wild-type strain (Fig. 5 C). Mating of the *rnq1* mutant with the isogenic wild-type strain followed by sporulation and tetrad dissection resulted in a 2:2 segregation of suppressed aggregation to toxicity as was shown before with the *hsp104* mutant. Suppressed aggregation was always associated with *rnq1* deletion. These data strongly indicate that Rnq1 is essential for aggregation and toxicity of polyQ polypeptides.

To investigate whether prion conformation of Rnq1 is critical for polyQ aggregation, we employed a method of curing yeast cells of prions by growing them in the presence of guanidine hydrochloride (GuHCl), which prevents propagation of prion conformation (for review see Chernoff, 2001). Accordingly, yeast cells were grown for three passages on glucose-containing medium (to block 103Q induction)
in the presence of 5 mM GuHCl. Expression of 103Q was induced in 22 randomly chosen individual clones originating from this procedure, and 21 of them displayed the aggregation phenotype similar to that of the spontaneous mutants described above (Fig. 5 A, bottom). Moreover, none of the 21 clones demonstrated a growth defect in response to accumulation of 103Q (Fig. 5 B) despite 103Q levels being significantly higher than the levels in the original cells (Fig. 5 C), which underscores the importance of polyQ aggregation for its toxicity. In two randomly chosen clones with no polyQ aggregation, all Rnq1 was found in the 100,000 g supernatant fraction in contrast to the original strain where most of Rnq1 was found in the insoluble fraction (Fig. 5 D). This confirms that the GuHCl treatment indeed cured cells of prion form of Rnq1 ([RNQ⁺]). These data strongly suggest that prion conformation of Rnq1 is essential for aggregation and toxicity of polyQ.

Since hsp104 and sis1 mutations are known to cure cells of [RNQ⁺] (Derkatch et al., 1997; Sondheimer et al., 2001), it appears that suppression of polyQ aggregation and toxicity by these mutations is caused primarily by the loss of Rnq1 prion. On the other hand, the ssa1saa2 and ydj1–151 mutations, which also relieved polyQ-related toxicity (Fig. 4), did not affect prion state of Rnq1, since all Rnq1 remain associated with the insoluble fraction in the ssa1saa2 or ydj1–151 cells homogenates (Fig. 5 D). Therefore, effects of ssa1saa2 and ydj1–151 mutations appear to be unrelated to [RNQ⁺]. These data support the notion that Ssa and Ydj1 apparently affect a step in IB formation different from one controlled by Hsp104 and Sis1.

Discussion

Here, we describe the first yeast model of polyQ expansion diseases in which accumulation of the polyQ domain was cytotoxic. The fact that 103Q is toxic for yeast cells may imply that the deleterious effects of polyQ extension are more general than they appeared before and that the mechanisms of toxicity could be conserved through evolution. The novel yeast model sheds light on the debated connection between aggregation of polyQ and its toxicity. We present several lines of evidence that the toxicity strongly associates with polyQ aggregation: (a) short polyglutamine, which did not form aggregates, was not toxic for the cells in spite of being accumulated at higher levels, whereas long polyglutamine aggregated and was toxic; (b) impairment of aggregation at apparently two distinct steps in chaperone mutants hsp104 and ssa1saa2 or ydj1–151 led to suppression of 103Q toxicity, whereas in the rare hsp104 mutant cells, which formed IBs, 103Q remained toxic; (c) deletion of the RNQ1 gene encoding a prion protein suppressed both aggregation and toxicity; and (d) curing the wild-type cells of prions suppressed 103Q aggregation and was sufficient to relieve the cytotoxicity in the course of subsequent 103Q induction. The most plausible conclusion from these data is that 103Q aggregation is essential for its toxicity.

Our data indicate that in addition to genetic factors the presence of prions determines polyQ aggregation and consequently cytotoxicity in yeast cells. It is possible that the lack of polyQ toxicity in previously reported yeast models may be related to the lack of [RNQ⁺] prion in the strains used in those experiments (Krobitsch and Lindquist, 2000; Hughes et al., 2001). This suggestion is supported by the morphology of aggregates in these reports, which resembled that in our clones cured of [RNQ⁺] prion. Another critical factor in toxicity appears to be the intracellular level of expanded polyQ (unpublished data).

Our data demonstrate that, counterintuitively and in contrast to in vitro results, aggregation of polyQ in vivo is strongly dependent on cellular factors. We found that among chaperones, in addition to Hsp104 (Krobitsch and Lindquist, 2000), Sis1, Ssa1/2, and Ydj1 also play an essential role in formation of the IBs. The majority of cells mutated in either hsp104 or sis1 genes were unable to form any visible 103Q aggregates. In rare hsp104 mutant cells in which nucleation of aggregates occurred, IBs grew efficiently. These data suggest that Hsp104 (and probably Sis1) is not essential for expansion of aggregates but rather for seeding of IBs. Likely, these chaperones control seeding of aggregates via maintenance of the [RNQ⁺] prion (Derkatch et al., 1997; Sondheimer et al., 2001; Wegrzyn et al., 2001). In addition, Hsp104 could be directly involved in shearing of polyQ aggregates. Such a role for Hsp104 would be consistent with its involvement in shearing of Sup35 aggregates (Patino et al., 1996; Paushkin et al., 1996), which is critical for maintenance of prion form of Sup35 ([PSI⁺]) (for review see Chernoff, 2001; Wegrzyn et al., 2001).

Ssa1/Ssa2 and Ydj1 were also essential for formation of IBs but at a different step. Real-time observation of this process indicated that Ssa1/Ssa2 (possibly with the help of Ydj1) might be involved in expansion of preformed seeds. This agrees with the previously proposed role of Ssa in the growth of prion aggregates in yeast (for review see Chernoff, 2001). In cells with decreased levels of Ssa (or deprived of Ydj1), growth of aggregates might become a limiting step, and some 103Q molecules unable to join IBs would remain soluble, which in turn would allow more nucleation centers to emerge in a cell and a much higher number of small aggregates to be formed. Together these data indicate a novel and unexpected role of molecular chaperones in promoting formation of protein aggregates. Furthermore, different chaperones facilitate two distinct steps in the aggregation process. Interestingly, although physiological levels of chaperones are necessary for the aggregation, overexpression of Hsp104, Hsp70, and Hsp40 was reported to keep polyQ polypeptides in a soluble form (Chan et al., 2000; Satyal et al., 2000). However, it should be noted that it is not clear whether strains used in these studies contained the [RNQ⁺] prion.

Our data that the polypeptide with expanded polyQ forms inclusions only in the presence of the [RNQ⁺] prion are consistent with the latest report showing that aggregation of the MJD protein with polyQ expansion is facilitated in so-called [PIN⁺] cells. [PIN⁺] is a non-Mendelian genetic trait promoting emergence of [PSI⁺] (Derkatch et al., 1997). It was shown that [PIN⁺] may be caused by a number of potential prion-forming proteins, for example, Rnq1 and New1 (Derkatch et al., 2001). Interestingly, deletion of the NEW1 gene did not significantly affect aggregation of 103Q polypeptide, suggesting the major role of [RNQ⁺] prion in this process. Whether aggregates of prion form of
Rnq1, which has a QN-rich domain, could directly serve as a nucleation site for polyQ, or Rnq1 acts on the polyQ aggregation indirectly is yet to be established.

Our data show that the toxicity of expanded polyQ polypeptide in yeast cells depends on the presence of prion in a cell. This fact suggests that in mammalian tissues some (possibly other than PrP) prion-like proteins may be required for aggregation and neuronal pathology of mutant polypeptides with expansion of the polyglutamine tract. This consideration establishes a link between the two seemingly distant groups of neurodegenerative diseases.

Presented here is a yeast model of cytotoxicity caused by polyQ aggregation, which can be further used in a search for yeast genetic modifiers of aggregation and viability. The identification of such cellular factors will ultimately facilitate the quest for genetic modifiers in humans and be crucial for understanding the mechanisms of the development of polyQ expansion-related pathology.

Materials and methods

Strains and plasmids

Deletion mutants of the S. cerevisiae wild-type strains BY4739 (MATa leu2Δ lys2Δ ura3Δ) and BY4742 (MATa his3Δ leu2Δ lys2Δ ura3Δ) were obtained from the deletion library (ResGen; Invitrogen) of yeast nonessential genes (Winzeler et al., 1999). Table I lists all other strains used in this work.

Vectors for expression of polyQ constructs under control of Gal1 promoter were a gift from A. Kazantsev (Massachusetts Institute of Technology). DNA constructs for mammalian expression of huntingtin with polyQ expansion-related pathology (Winzeler et al., 1999). Table I lists all other strains used in this work.

Observation of yeast growth and induction

Cells were routinely grown at 30°C on selective SD medium and then transferred into SG liquid or solid medium for induction of 25Q or 103Q expression.

To stain cells with propidium iodide for flow cytometry, 0.5–5 ml of cells grown in SG medium overnight were harvested, washed with 50 mM Tris-HCl (pH 7.5), and treated as follows. Cells were fixed in 70% ethanol for 1 h, washed, and treated with 1 mg/ml RNase A in 50 mM Tris-HCl (pH 7.5) for 1 h, incubated with 0.05 mg/ml propidium iodide in 7.5) for 1 h with rotation. Then, cells were treated with 0.5% pepsin in 55 mM HCl for 5 min and incubated with 0.05 mg/ml proteinase K in 180 mM Tris-HCl, pH 7.5, 200 mM NaCl, and 7 mM MgCl2 overnight at 4°C with rotation.

Microscopy

Confocal microscopy was performed with laser scanning system Radiance 2000 (Bio-Rad Laboratories). To follow a process of aggregation in real time, 103Q was induced for 3 h, cells were harvested, mounted on glass slides covered with solid (1.5% agarose) galactose medium, and observed under the deconvolution epifluorescent microscope (Delvosight; Applied Precision, Inc.). Every 2 min, 10–15 serial sections (0.25 mm/section, 0.5 s exposure/snow) were taken to cover the whole thickness of the cell for a total of 1 h.

Analysis of solubility of polyQ polypeptides and Rnq1 protein in cell lysates

Collected cells were disrupted by vortexing with 425–600 µm acid-washed glass beads in 40 mM Hepes, pH 7.5, 50 mM KCl, 1% Triton X-100, 2 mM DTT, 1 mM Na2VO4, 50 mM β-glycerophosphate, 50 mM NaF, 5 mM EDTA, 5 mM EGTA, 1 mM PMSF, 1 mM benzamidine, and 5 µg/ml each of leupeptin, pepstatin A, and aprotinin. Disintegration by lyticase treatment provided similar results (unpublished data). After disintegration, cell lysates were left in narrow tubes for 1 h to allow ~95% of unbroken cells to sediment by the gravity force, whereas almost all of the aggregates released from the broken cells stayed in the solution (unpublished data).

The upper portion of supernatant was carefully removed and used for fractionation. Samples were normalized by the amount of total protein. Lysates were subjected to centrifugation at 800 g for 10 min. The pellets were washed once with the lysis buffer and in a volume of water equal to the volume of the supernatant. To assess intracellular aggregation of Rnq1, cells were disrupted by vortexing with glass beads in the buffer containing 50 mM Tris-HCl, pH 8.0, 10 mM KCl, 100 mM EDTA, 1 mM DTT, 1.0% Triton X-100, and 0.2% SDS. Then cell homogenate was cleared from debris by 10 min centrifugation at 3,000 g and subjected to centrifugation at 100,000 g to separate soluble and insoluble forms of the protein. All samples were supplemented with loading SDS-PAGE buffer containing 2% SDS and boiled for 3 min before being subjected to immunoblotting. GFP-tagged polyQ polypeptides were visualized with anti-GFP antibody (polyclonal) (CLONTECH Laboratories, Inc.). Rnq1 antibody was a gift from S. Lindquist (Whitehead Institute, Cambridge, MA).

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References


Chernoff, Y.O., G.P. Newnam, J. Kumar, K. Allen, and A.D. Zink. 1999. Evi-

Table I. Strains used

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<th>Strain</th>
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