Propagation of a Novel Cytoplasmic, Infectious and Deleterious Determinant Is Controlled by Translational Accuracy in *Podospora anserina*

Philippe Silar, Vicki Haedens, Michèle Rossignol and Hervé Lalucque

Institut de Génétique et Microbiologie, Université de Paris Sud, 91405 Orsay Cedex, France

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ABSTRACT

Some mutant strains of the filamentous fungus *Podospora anserina* spontaneously present a growth impairment, which has been called Crippled Growth (CG). CG is caused by a cytoplasmic and infectious factor, *C*. *C* is efficiently transmitted during mitosis but is not transmitted to the progeny after sexual crosses. *C* is induced by stationary phase and cured by various means, most of which stress the cells. Translational accuracy is shown to tightly regulate the propagation of *C* during the active growth period, because its propagation in dividing hyphae is restricted to cells that display an increased translational accuracy. However, induction of *C* in stationary phase proceeds independently from the translational accuracy status of the strain. CG does not seem to be accompanied by mitochondrial DNA modifications, although *C* activates the action of the Determinant of Senescence, another cytoplasmic and infectious element, which causes a disorganization of the mitochondrial genome. In addition, presence of *C* drastically modifies the spectrum of the mitochondrial DNA rearrangements in *AS6-5 mat*− cultures during Senescence. *C* seems to belong to the growing list of unconventional genetic elements. The biological significance of such elements is discussed.

AGING, in metazoa, is a complex phenomenon, which likely results from several causes (Finch 1990; Holliday 1995). Recent data show that the genome exerts an important control over it (Johnson 1997). However, to date, no clear picture emerges of how these genes act on senescence. Fungi may help in deciphering some of the issues related to aging because they are easily amenable to molecular genetic analysis. In these organisms, declines of vegetative and/or reproductive potentialities, usually due to degenerations at the cellular level, are considered as aging processes (Griffiths 1992). In addition to the yeast *Saccharomyces cerevisiae* (Mortimer and Johnston 1959; see Sinclair *et al.* 1998 for a review), the filamentous fungus *Podospora anserina* stands as a valuable model. This fungus presents an unavoidable arrest of vegetative growth, called Senescence, that culminates with cellular death (Rizet 1953). This phenomenon of clonal aging is caused by a cytoplasmic and infectious element called the Determinant of Senescence (Marcou 1961) and is correlated with massive mitochondrial DNA (mtDNA) alterations (Dujon and Belcour 1989). However, the debate as to whether the mtDNA modifications are causal to or are a consequence of Senescence remains open (Silar *et al.* 1997a).

Other cytoplasmic and infectious determinants have been described in many filamentous fungi, in which they promote various degenerative or differentiation processes (Daboussi-Bareyre 1979). Although mtDNA alterations have been implicated in some of these processes (Griffiths 1992), the nature of the determinants for many of them has not yet been elucidated because no mtDNA alterations or extrachromosomal elements have been observed in the modified hyphae. Here, we report on the discovery in some mutant strains of *P. anserina* of a novel clonal cellular degenerative process that does not culminate in death. This alteration is caused by a novel cytoplasmic and infectious element, which is induced by stationary phase, cured by stresses, and controlled by translational accuracy. This element is not associated with mtDNA modifications but does interact in some way with the Determinant of Senescence. To our knowledge, these properties are unique to this endogenous and deleterious element.

Corresponding author: Philippe Silar, Institut de Génétique et Microbiologie, URA 2225, Université de Paris Sud, 91405 Orsay cedex, France. E-mail: silar@igmors.u-psud.fr

MATERIALS AND METHODS

Strains and growth conditions: All strains are derived from the S strain. All strains are thus isogenic except for the mating type region and the indicated mutations. Descriptions of suppressor (that decrease translational accuracy) and antisuppressor (that increase translational accuracy) mutations can be found elsewhere (Dequard-Chablat *et al.* 1986; Coppin-Raynal *et al.* 1988). Culture conditions and genetic methods for *P. anserina* have been described (Esser 1974). M2 medium is 0.25 g/liter KH_2PO_4, 0.3 g/liter K_2HPO_4, 0.25 g/liter $MgSO_4$, 0.5 g/liter urea, 0.2 mg/liter biotin, 0.2 mg/liter thiamine, oligo-elements, 5 g/liter dextrin, and 12 g/liter agar. G medium is 4.4 g/liter of CH_3COONH_4, 1.5% Bacto-peptone, and 1.5% agar. Longevity was measured as described by Belcour and Begel (1980).

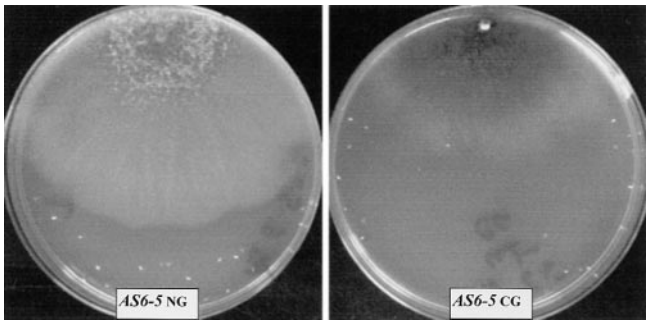

Figure 1.—Morphology of normal growth (NG) and Crippled Growth (CG) in cultures of an *AS6-5* strain. Cultures were grown for 5 days on M2 at 27°.

Strain construction: The double mutant strains were constructed by crossing the parental stains containing the single mutations. In each case, the putative double mutant was crossed with wild type to check for the segregation of both mutations and hence confirm the genotype of the double mutant.

Suppression level analysis: Suppression level measures were performed as described (Picard 1973; Coppin-Raynal 1981), with the *leu1-1* and/or *193* UGA nonsense mutations that affect leucine biosynthesis and spore color, respectively.

mtDNA analysis of the *AS6-5* strain: Fresh *AS6-5 mat*+ and *AS6-5 mat*− strains were recovered from crosses between wild type and the *AS6-5* strain of our collection. Immediately after germination, normal and CG cultures were set up and mtDNA was analyzed. The cultures were subsequently grown until Senescence appeared and mtDNA was again analyzed. mtDNA was extracted with the rapid extraction procedure (Lecellier and Silar 1994). Subsequent analysis is done using standard methods (Ausubel *et al.* 1987). Probes used are described in Jamet-Vierny *et al.* (1980) and Vierny-Jamet (1988).

RESULTS

AS6-5 cultures can be obtained in two different states despite being grown in the same conditions: While analyzing the *AS6-5* strain of *Podospora anserina* (Silar *et al.* 1997a), we uncovered a surprising phenomenon. During growth at 37° on M2 medium, some cultures of this strain differentiate mycelial sectors with diminished growth rate, increased pigmentation, irregular hyphal branching, and twisted hyphae. In our standard conditions (*i.e.*, in race tubes containing M2 medium at 27°), a sector appears on average in 1 out of 12 cultures. This growth alteration is referred to as Crippled Growth (CG) with respect to normal growth (NG). Appearance of these sectors is a rare event that seems to occur randomly. Upon subculturing with large agar plugs (at least 1 mm^3), cultures of either morphology (Crippled or normal) can be maintained and, hence, the cause of this growth alteration is efficiently transmitted through mitosis (Figure 1).

It is possible to render normal Crippled cultures and vice versa by various means: Apart from their spontaneous appearance, we observe that Crippled sectors that start from the inoculum arise at high frequency in *AS6-5* cultures issued from mycelia that have been incubated at 27° on M2 medium for at least 2 days (Figure 2). Prolonged incubation in the same conditions for at least 4 days yields only pure Crippled cultures. This "stationary phase" effect occurs, albeit with varying speed, on all the media that we test and at temperatures ranging from 11° to 37°.

The recovery of normal cultures from Crippled ones can be achieved by at least five means:

1. By subculturing Crippled cultures at 37° on M2 medium.
2. By subculturing them at 27° on M2 medium supplemented with 200 g/liter of saccharose, 500 mg/liter of paromomycin, or 1 g/liter of kasugamycin. Subculturing on our ascospore germination medium, which contains a low level of nutrient, is also very potent

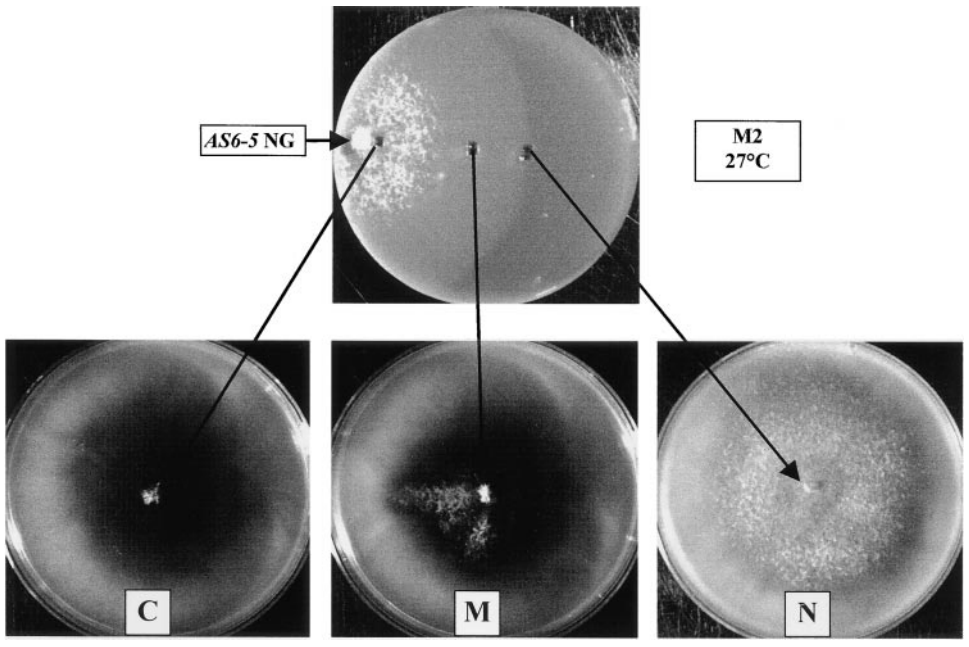

Figure 2.—Installation of CG after passage in stationary phase. A Petri plate of M2 medium was inoculated with a normal growing explant of an *AS6-5* culture and incubated for 6 days at 27°. Explants were then taken at the edge of the culture, at 1.5 cm away from the edge (corresponding to 2 days in stationary phase) or at 4 cm away from it (corresponding to 6 days in stationary phase) and inoculated on new M2 plates. Clearly, the explant taken at the edge generates a normal culture, whereas the one taken at 4 cm from the edge generates Crippled culture, showing that CG is promoted by prolonged passage in stationary phase. The explant taken at 1.5 cm generates a mosaic culture constituted of normal and Crippled sectors.

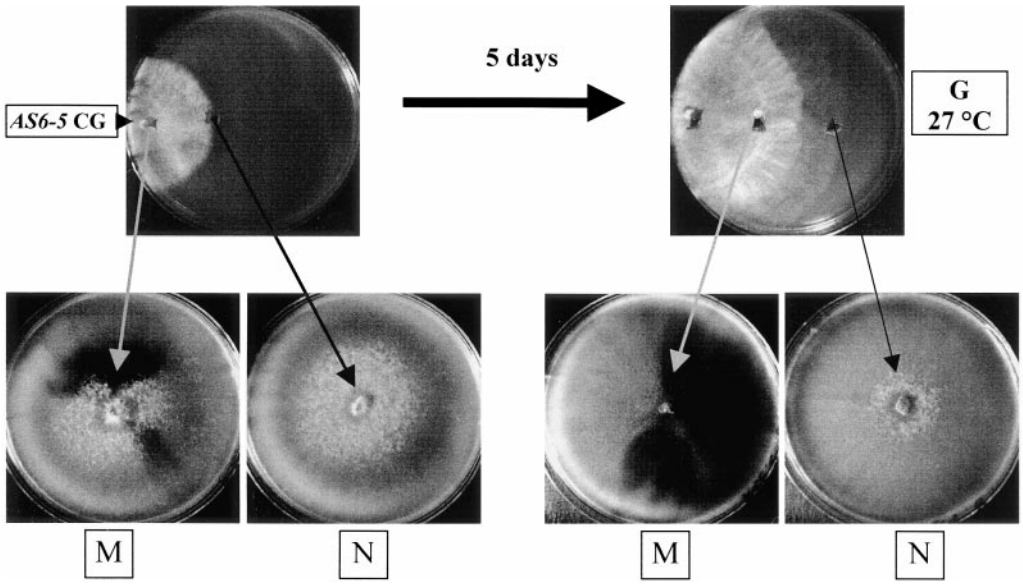

Figure 3.—Reversion of CG by growth on ascospore germination medium. A Petri plate of ascospore germination medium was inoculated with a Crippled explant of an *AS6-5* culture and incubated for 4 days at 27°. Explants were then taken at the edge of the culture, or near the explant. When inoculated on new M2 plates, the explant taken at the edge generates a normal culture showing that growth has been reversed to normal. The explant taken near the inoculum generates a mosaic culture. The initial plate was then further incubated for 5 days and explants were taken at the edge or beside the one that was located 5 days before at the edge of the culture. The first one generates a normal growing culture whereas the latter regenerates a mosaic culture showing that reversion is temporary.

in reversing growth to normal and is used routinely (Figure 3). Explants taken from the growing edge of the culture, done in conditions (1) or (2), give birth to normal growing cultures. However, as stated in the previous paragraph, after several days of incubation, similar explants taken in the same area give birth to Crippled cultures, showing that curing is temporary (Figure 3).

3. By storing Crippled cultures at 4° for at least 3 months; mycelium explants from these stored Crippled cultures yield mosaic cultures consisting of Crippled and normal sectors.
4. By starting new cultures with minute explants of a few articles; the resulting mycelia always have a normal morphology. By this procedure we have not observed a reproducible diminished regeneration frequency of the Crippled articles when compared to normal ones, suggesting that curing occurred through elimination of the alteration rather than selection of healthy hyphae.
5. By irradiating Crippled mycelia with UV light (514 nm) with at least 100 J/m^2; cells from the growing edge recover NG when further grown.

Appearance of Crippled Growth is controlled by translational fidelity: The *AS6-5* strain contains a mutation, located in a ribosomal protein, that restricts the readthrough brought about by suppressors during the translational decoding of UGA stop-codon and hence increases translational accuracy (Coppin-Raynal *et al.* 1988). To test if translational accuracy level is involved in the expression of CG, we screen for the presence of CG in the various suppressor (with increased readthrough level) and antisuppressor (with decreased readthrough level) strains of our collection. To this end, cultures are incubated at 27° on M2 medium for several days and then large explants are reinoculated on fresh medium. Cultures issued from strains carrying any of the 26 antisuppressor alleles, located in 7 different genes (*AS2*, *AS3*, *AS4*, *AS6*, *AS7*, *AS9*, and *su12*), present sectors of altered growth that resemble CG. Seemingly, antisuppressor alleles of the *AS1* gene also present CG (Contamine *et al.* 1996; M. Dequard-Chablat, personal communication). On the contrary, wild type and strains carrying any of the 15 suppressor alleles, located in 9 different genes (*su1*, *su2*, *su3*, *su4*, *su7*, *su8*, *su11*, *su12*, and *su15*), never present them. This is particularly striking in the case of the strains carrying a mutation in the *su2*/*AS2* or *su12* genes (Dequard-Chablat 1986; Silar *et al.* 1997a; Gagny and Silar 1998). Indeed, strains carrying the suppressor alleles of these genes (*su2-1*, *su2-5*, *su12-1C1*, *su12-1C5*, and *su12-2*) never present altered sectors, whereas the strains carrying antisuppressor alleles (*AS2-1*, *AS2-2*, *su12-2PR5*, *su12-2PR8*, and *su12-2PR27*) do. In many of the antisuppressor strains, the time spent in stationary phase that is necessary to obtain the altered sectors was much longer than that for *AS6-5* and the growth alteration may not be stable upon subculture.

The *AS4-44* strain, which carried a mutation in the elongation factor eEF1A (Silar and Picard 1994), is investigated in more detail. This strain has to date never presented altered sectors spontaneously, and CG cul-

tures are obtained after passage in stationary phase (wild-type and suppressor strains never present CG even after passage in stationary phase). CG cultures revert to the normal state, albeit slowly. In this strain, a strongly diminished female fertility is observed when growth is altered. Apart from this, the modalities of induction and curing of the morphological alteration are the same as the ones observed for the *AS6-5* strain, suggesting that it is due to the same cause (proof of this is given in the next section). This growth defect thus is also referred to as CG. In this strain, as well as in other antisuppressor strains, CG is enhanced and stabilized by addition to M2 medium of 8.8 g/liter of ammonium acetate. Note that this addition does not promote the appearance of CG in wild-type or in suppressor strains.

The role of antisuppressor mutations in promoting CG was confirmed by introducing a wild-type ectopic copy of *AS4* into the *ASA-27* mutant strain. Analysis reveals that CG could not be induced after passage in stationary phase in the resulting strain, whereas in the same conditions it was induced in the *ASA-27* strain. The possibility for a strain to present Crippled is thus a recessive phenotype.

To further establish the relationship between accuracy and CG, double mutant strains were constructed and analyzed as previously. Results lead to three conclusions: (1) Increasing antisuppression enhances CG because the *su12-2PR8 AS6-2* double antisuppressor mutant presents a very stable CG alteration very much like the one in *AS6-5*, whereas the *su12-2PR8* and *AS6-2* strains have unstable Crippled sectors. The double mutant strain needs to be incubated in stationary phase only 2 days, in order to obtain 100% explants (24 explants tested) that regenerate completely Crippled cultures. At least 6 days are necessary for the single mutants to obtain 100% of mosaics cultures (12 explants tested for each strain). (2) Decreasing global fidelity diminishes CG. Indeed, explants taken from the *su12-1C1 AS6-5*, *su12-1C1 AS3-1* strains, which carry an omnipotent suppressor mutation associated with an antisuppressor, never produce CG cultures after passage in stationary phase even if incubated 10 days (12 explants tested for each strain). The *su1-1 AS6-5* and *su1-1 AS4-44* double mutants present an alteration that is less pronounced than that of the strain carrying the antisuppressor mutation alone, but do so with the same kinetics. (3) Increasing UGA readthrough has no effect on CG because the *su4-1 AS6-5*, *su8-1 AS6-5*, *su4-1 AS4-44*, and *su8-1 AS4-44* double mutants, which carry the *su4-1* or *su8-1* $tRNA_{UGA}$ suppressor (Debuchy and Brygoo 1985) associated with an antisuppressor mutation, still display CG. Moreover, the alteration has the same intensity and appears with the same kinetics as in the strain carrying the antisuppressor mutation alone. Notably, in these double mutants, despite the presence of an antisuppressor mutation, suppression of the *leu1-1* mutation is readily detected, showing that UGA readthrough is strongly increased when compared to wild type.

TABLE 1

Transmission of CG from various donor strains to *AS6-5* NG recipient

Donor	Phase	Success/attempt
AS6-5 CG	Growth	7/12
	Stationary	9/12
AS6-5 NG	Growth	0/21
	Stationary	6/12
AS4-44 CG	Growth	7/12
	Stationary	5/12
AS4-44 NG	Growth	0/12
	Stationary	7/12
su12-1C1 NG	Growth	0/15
	Stationary	4/12
WT NG	Growth	0/30
	Stationary	12/30

The table gives the number of crippled sectors obtained *vs.* the number of attempts when *AS6-5* NG cultures were contaminated with explants from various donor strains grown normal or Crippled and taken either in the growth period or in the stationary phase.

CG is caused by a cytoplasmic and infectious element and is not transmitted through meiosis: The modalities of appearance and disappearance of CG suggest that a nuclear mutation is not involved in the generation of the growth alteration. This is confirmed by "contamination" experiments. In these experiments, a small explant of Crippled donor mycelium is put 1 mm away from the growing edge of a normal recipient mycelium. Local anastomoses occur between the two mycelia. After several days of incubation, the resulting plates are observed. When some donor strains are used on *AS6-5* NG recipient cultures (Table 1), CG sectors may be obtained on some plates downstream of the inoculi. A judicious choice in the mating types of the donor and recipient mycelia allows one to follow the nuclei after the anastomoses. Mating type analysis of the altered sectors shows that they contain only recipient nuclei, demonstrating that the element responsible for CG is cytoplasmic and infectious. We have called this element *C*. As proposed for other cytoplasmic traits, cultures that carry *C* are now referred to as $[C^+]$ and those that do not as $[C^-]$. Data of such experiments (Table 1) show that growing NG mycelia never transmit the determinant, but such mycelia do so with the same efficiency as CG mycelia when they are in stationary phase, confirming that *C* is present during this phase. *AS4-44* $[C^+]$ mycelia induce sectors in the *AS6-5* $[C^-]$ recipient, demonstrating that the growth alteration, in these mutants, is indeed caused by the same element. Interestingly, wild-type and suppressor mycelia that are in stationary phase can also transmit CG, showing that all strains are $[C^+]$ during

TABLE 2

Transmission of CG to various recipient strains

Recipient	Donor	
	AS6-5 [C^+]	*AS4-44* [C^+]
AS4-44	1/12	5/12
AS7-2	2/24	NT[a]
su12-2PR8	0/24	6/24
su12-1C1	0/24	NT
WT	0/33	0/24

The table gives the number of crippled sectors obtained *vs.* the number of attempts when various recipient strains were inoculated with either *AS6-5* [C^+] or *AS4-44* [C^+] cultures.
[a]Not tested.

this phase. The difference between antisuppressor and wild-type/suppressor strains is thus the inability of the former to prevent the propagation of *C* in dividing hyphae. Indeed, *C* is never transmitted to wild-type and suppressor strains (0 successes in 81 attempts), while it is readily transmitted to antisuppressor strains (14 successes in 96 attempts; Table 2). As seen in Table 2, efficiency of contamination seems to depend on the donor/receptor couple.

Meiotic transmission was analyzed with the following crosses: ♀ *AS7-2* [C^+] × ♂ *AS6-2* [C^+], ♀ *AS6-2* [C^+] × ♂ *AS7-2* [C^+], ♀ *AS4-44* [C^+] × ♂ *AS7-2* [C^+], ♀ *AS7-2* [C^+] × ♂ *AS4-44* [C^+] (all the cultures had grown Crippled). Because germination occurs on a special medium that cures *C* (see above), care was taken to transfer germinating ascospores very rapidly with the smallest bit of agar possible on M2 medium. Nevertheless, all 32 ascospores obtained from these crosses and carrying an antisuppressor mutation yield mycelia that are NG and thus [C^-], showing that *C* is not efficiently transmitted through meiosis. In addition, the numerous crosses made over the years between antisuppressor strains or between antisuppressor strains and wild type have never given ascospores yielding directly Crippled mycelia, though *C* is present in the hyphae surrounding the cells that undergo the sexual process (crosses in *P. anserina* require about 1 wk to proceed in our standard conditions at which time cells are [C^+]). However, all the ascospores that are recovered from these crosses can readily express *C* after passage in stationary phase.

***C* is different from the Determinant of Senescence:** Because *P. anserina* presents an unavoidable growth arrest caused by a cytoplasmic and infectious determinant (Senescence), we have investigated the relation between *C* and the Determinant of Senescence. The *AS6-5* strain was chosen for these investigations. From young or senescent cultures of this strain, we could obtain subcultures in either the normal or Crippled morphologies, showing that *C* is different from the Determinant of Senescence. Whereas Senescence is correlated with

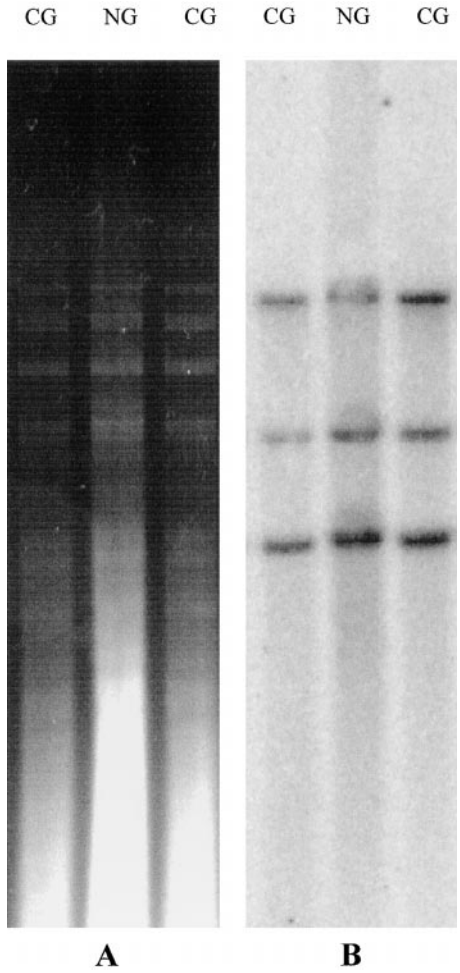

Figure 4.—(A) mtDNA structure of young *AS6-5 mat*− cultures grown normal (NG, center lane) or Crippled (CG, other lanes). DNA was extracted from the cultures grown in either morphological form, digested with *Hae*III, and separated by electrophoresis on an agarose gel. (B) Autoradiogram for the same gel obtained with a probe for the β region. This region is not rearranged as observed during Senescence because the three detected bands correspond to unmodified mtDNA.

mtDNA modifications, no obvious differences are observed in the mtDNA between CG or NG young *AS6-5* cultures (Figure 4). This suggests that Crippled Growth is likely not due to some mtDNA mutation. However, the *AS6-5* CG cultures have a drastically diminished lifespan when compared with NG cultures because they have a longevity of 13.5 ± 2.0 cm whereas NG cultures have a longevity greater than 30 cm (Silar *et al.* 1997a). In addition, the spectrum of rearrangements is influenced by the presence of CG. We previously reported (Silar *et al.* 1997a) that, as wild-type cultures, *AS6-5 mat*+ senescent cultures amplify senDNAα, whereas *AS6-5 mat*− cultures do not. Further analysis of the senescent *AS6-5* normal or Crippled cultures (Figure 5) reveals that (1) *AS6-5 mat*+ cultures do amplify the senDNAα independently of their growth history, and (2) *AS6-5 mat*− cultures do not amplify senDNAα

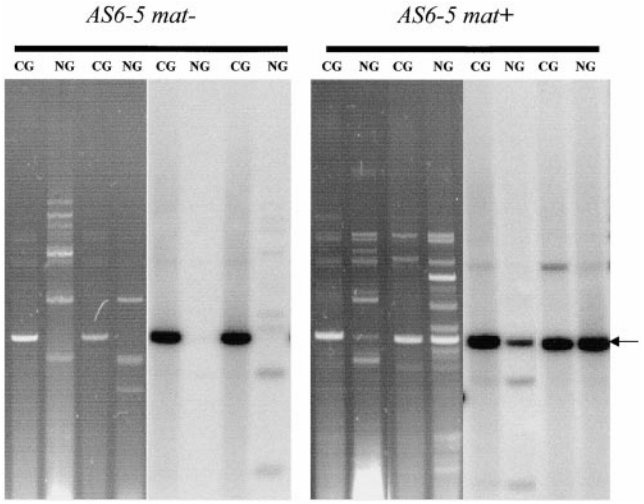

Figure 5.—mtDNA structure of senescent *AS6-5 mat−* or *AS6-5 mat+* cultures grown either normal (NG) or Crippled (CG). DNA was analyzed as in Figure 4 but the probe used is specific for the α intron that is amplified as senDNAα during Senescence in wild type. The arrow points toward the senDNAα monomer.

when grown normal but do amplify a large amount of senDNAα when grown Crippled.

DISCUSSION

We have discovered a novel growth alteration that occurs in some strains of *P. anserina*. This Crippled Growth is due to some cellular malfunction, which results in slower mycelial growth speed, abnormal hyphal morphology, accumulation of pigment, shorter longevity, and reduced female fertility. It is caused by a novel cytoplasmic and infectious element, which we have called *C*, that is clearly different from the one that causes Senescence. However, *C* interacts in some way with the Determinant of Senescence because *AS6-5* CG cultures have a drastically reduced longevity and a modified spectrum of mtDNA rearrangements during Senescence. *C* seems to belong to a large group of cytoplasmic and infectious determinants described in filamentous fungi, which cause morphological and/or fertility modifications (Daboussi-Bareyre 1979). Because of its deleterious effects, *C* can be considered as promoting an aging phenomenon of the cultures that express it during their growth phase, as proposed for other similar phenomena (Griffiths 1992).

Classically, two broad groups of hypotheses are put forward to explain determinants such as *C*. The first group relies solely on nucleic acid modifications (genetic explanation), whereas the second one postulates inheritable regulatory changes (epigenetic explanation). According to the first group of hypotheses, for example, either mutations located in the mtDNA that promote some kind of replicative advantage to the molecules that carry them, or activation of cryptic virus-like elements can result in cytoplasmic and infectious phenomena. This is the case, for example, in the "kalilo-based" senescence in Neurospora (Griffiths 1992). Three observations argue against such being the case for *C*: (1) the ease with which *C* is either induced or cured within the same mycelium; (2) the fact that *C* appears in all the strains of our collection that we have so far tested; and (3) the absence of meiotic transmission of *C*, coupled with the fact that all strains that have freshly been recovered from meiosis can express *C* in stationary phase. In addition, we have no evidence of mtDNA modifications, and virus-like elements have never been detected in the strains we use (Delay 1963).

We thus favor the second group of hypotheses, which relies on epigenetic explanations. This group includes, but is not limited to, the following hypotheses:

1. A self-activated structural modification of nucleic acid or an epigenetic modification *sensus stricto* (see Selker 1997 for a review of filamentous fungi epigenetic phenomena).
2. A self-propagating alternative structure of a macromolecule, also now called prion. Such events have been demonstrated in the cases of the yeast Ψ and URE3 elements (see Tuite and Lindquist 1996; Lansbury 1997 for reviews). It is likely to be the case for the s* → s transition, another cytoplasmic and infectious determinant of *P. anserina* that is involved in the control of vegetative incompatibility (Coustou *et al.* 1997).
3. A self-positively regulated biosynthetic pathway as originally described for the lactose regulation in *Escherichia coli* (Novick and Weiner 1957).
4. A self-positively regulated decision pathway like the lytic/lysogenic choice in lambda bacteriophage (Ptashne 1992).

As is obvious from the above list, the main point of these hypotheses is the activation of a self-fueled phenomenon. It was pointed out a long time ago (Nanney 1958) that a product, whose production is self-activated, can be transmitted through cellular continuity and that both cellular states (with or without the product) are thus inheritable. As already discussed, such processes likely participate in cell differentiation (Beisson 1977). Installation of CG in a NG culture seems thus to be mechanistically akin to a deleterious differentiation process.

CG displays five main characteristics that may or may not be encountered in the other described cases of nonconventional genetic elements.

First, *C* is inheritable during mitosis but is not efficiently transmitted during meiosis. This has already been observed for other elements like the "Secteurs" and "Anneaux" in the ascomycete *Nectria haematococca* (Daboussi-Bareyre 1979). Several hypotheses can be proposed to explain this. For example, either the condi-

tions required to accomplish the sexual cycle (especially translational accuracy) are incompatible with the propagation of *C* or a protein necessary for the production of *C* may not be expressed during reproduction. Alternatively, hyphae containing too high a level of *C* may not be able to engage in the differentiation of the female organs.

Second, propagation of *C* seems to depend on translational accuracy. *C* is the third element to be discovered that is connected with translational accuracy and that has a nonconventional genetic transmission (Cox 1965; Liebman and All-Robyn 1984). It was recently suggested (Wickner 1994) and shown (Glover *et al.* 1997; Paushkin *et al.* 1997) that one of them, Ψ, behaves as a prion. So far no explanation has been given for the other. It is noteworthy that the two cellular degeneration syndromes of *P. anserina* are tightly regulated by translation, emphasizing the need for further study on the role of translation in the cell homeostasis. However, translation acts differently upon them because accuracy level *per se* does not seem to be involved in control of Senescence (Belcour *et al.* 1991; Silar *et al.* 1997b), whereas it is in the control of CG.

Third, *C* is induced by stationary phase in all strains. Several studies have shown that the regulatory network of the cell responds to growth-arrested conditions by inducing or repressing several genes (Werner-Washburne *et al.* 1993; Nystrom 1998). These modifications may promote the induction of *C* by enhancing the probability that the first infectious particles will be formed. Indeed, all the material necessary for its formation may be present in the cytoplasm during active growth, because *C* may spontaneously appear in dividing hyphae as seen in the *AS6-5* strain. However, *C* is allowed to efficiently propagate during the active growth phase only in antisuppressor strains. To account for this fact, we propose two hypotheses.

1. During stationary phase, translational fidelity could be increased in all strains. This allows for the establishment of *C*. Upon growth renewal of wild type and suppressor strains, decrease of accuracy would impair production of *C*, which would then be diluted, allowing for normal growth. On the contrary, error level would be too low during the active growth phase in antisuppressor strains to prevent stoppage of *C* production. Antisuppressor mutations would thus mimic stationary phase conditions. We have as yet no proposition to explain how increased accuracy could generate a self-fueled process. Note that this hypothesis is in direct opposition to the one formulated by Orgel (1963) to explain aging, which stated that more error during translation would lead to an error catastrophe. Modifications of translation accuracy during stationary phase have been detected in *E. coli*, since during this phase translational frameshifting is increased (Barak *et al.* 1996; Wenthzel *et al.* 1998) and readthrough is decreased (Wenthzel *et al.* 1998).
2. Alternatively, antisuppressor strains may lack a protein factor, which would prevent propagation of *C* during growth. This factor would be produced thanks to a translational error, because appearance of CG in a strain is negatively correlated with translational accuracy. Note that, despite having an increased UGA readthrough level, the double mutant strains carrying an antisuppressor mutation and a UGA suppressor tRNA still display CG, suggesting that a UGA readthrough should not be involved in the elimination of *C*.

Fourth, curing of *C* can be effected in numerous ways, most of which clearly stress the cells (high temperature, low temperature, high osmotic pressure, starvation, or UV-irradiation). This suggests that HSPs may be involved in the formation and/or maintenance of *C*. This has already been observed for the Ψ prion (Chernoff *et al.* 1995). Antibiotics that interfere with translational accuracy, namely paromomycin (that increases translational errors *in vivo*; Singh *et al.* 1979; Coppin-Raynal 1981) and kasugamycin (that may decrease errors in *P. anserina*; Kieu-Ngoc and Coppin-Raynal 1988), result in curing of *C*. It is possible that this is a translational effect. Alternatively, it may be due to the stress caused by these antibiotics to the cells because both exert their effect only when they are present at doses high enough to entail growth impairment. Additionally, paromomycin has been shown to induce the heat-shock response in yeast (Grant *et al.* 1989).

Fifth, *C* influences Senescence, suggesting that both *C* and the Determinant of Senescence may act in similar pathways. We have recently proposed that the Determinant of Senescence acts by modifying, possibly at the translational level, the spectrum of the protein produced in the cytosol (Silar *et al.* 1997a). It is tempting to suggest that *C* may do the same.

The last question that we speculate upon is the relevance of CG and similar cytoplasmic and infectious phenomena to aging. As pointed out above, the establishment during evolution of such processes can be effected by the closure with a positive loop of a biosynthetic or regulatory pathway, through some mutational event in one of the actors of the cellular network. Some of these positive regulatory loops may surely be beneficial for survival and be selected by evolution. They would thus appear as differentiation processes. However, some, like *C*, may not always be advantageous. Their control is then crucial for the survival of the organisms. Uncontrolled loops may self-activate during the lifetime of the organism, spontaneously or in response to some environmental cue, and participate in the breakdown of the cellular homeostasis observed in old organisms. Activation of such loops would thus appear as an aging phenomenon. Through a complete change of the regulatory network,

sexual reproduction could be an efficient way to eradicate the activated loop as seen in *P. anserina*. Sex may thus participate not only in clearing deleterious mutations but also in resetting such loops to the inactive state.

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