Curr Genet (1993) 24:268–270

Current Genetics

Short communications

Multicopy *SUP35* gene induces de-novo appearance of *psi*-like factors in the yeast *Saccharomyces cerevisiae*

Yury O. Chernoff*, Irina L. Derkach, Sergey G. Inge-Vechtomov

Department of Genetics, Saint-Petersburg University, Universitetskaya 7/9, 199034 Saint-Petersburg, Russia

Received: 28 December 1992 / Accepted: 25 February 1993

Abstract. Previously, we have shown that plasmid-mediated multiplication of *Saccharomyces cerevisiae* wild-type *SUP35* gene leads to omnipotent suppression and is incompatible with *psi*-factor, which is an endogenous extrachromosomal suppressor. Here, we describe a frequent de-novo appearance of *psi*-like factors in mitotic progeny of yeast transformants containing multicopy *SUP35* gene.

Key words: Mistranslation – Omnipotent suppression – Plasmid – Extrachromosomal element

The *psi*-factor has been shown to be an extrachromosomal element acting as an allosuppressor and omnipotent suppressor in *Saccharomyces cerevisiae* (see Cox et al. 1988 for review). Some other extrachromosomal *psi*-like elements (*eta*-factor, for example) affecting fidelity of translation have also been identified (Liebman and All-Robyn 1984; All-Robyn et al. 1990). Both *psi* and *psi*-like elements are inherited in non-Mendelian fashion and can be eliminated by some mutagens or by so-called "stress-inducing" agents; for example, guanidine-HCl (Tuite et al. 1981; Liebman and All-Robyn 1984; Cox et al. 1988). Some, but not all, *psi*-strains can give rise to strains that are psi^+ (Cox et al. 1988). However, psi^- strains cured of the factor by guanidine-HCl treatment are usually stable (*Ibid*). De-novo appearance of the *psi*-factor in such *psi*-derivatives has been reported only for some strains of the Peterhoff Breeding Collection (Tikhodeyev et al. 1990).

The molecular basis of *psi* is unknown. It does not reside on mitochondrial DNA, two-micron or killer plasmids (see Cox et al. 1988). The frequency of *psi* de-novo appearance was shown to be elevated by transformation with extrachromosomal rDNA (so-called "three-micron DNA"); this was explained by a hypothetic relationship between *psi* and rDNA (Dai et al. 1986). However, this explanation is not in an agreement with later observations (see Cox et al. 1988).

It is known that *psi* and *psi*-like factors are incompatible with some other suppressors (Liebman and All-Robyn 1984; Cox et al. 1988; All-Robyn et al. 1990). Previously, we have found that multiple copies of the wild-type *SUP35* gene, which acts as omnipotent suppressor, are also incompatible with *psi* (Chernoff et al. 1988, 1992). As a result, *SUP35*-containing episomal plasmids are extremely unstable in psi^+ strains. The apparent reason for such incompatibility is a high level of mistranslation in yeast cells bearing both the *psi*-factor and multiple copies of the *SUP35* gene.

Here we describe an unusual phenomenon which was observed in the mitotic progeny of psi^- extra-*SUP35*-containing transformants and explained as the de-novo induction of the *psi* (or *psi*-like) factor by multicopy *SUP35*.

The experimental strategy is as follows. The psi^- strain 33G-D373 (*MATα ade2-144,717 his7-1*$_{UAA}$ *leu2-3,112 lys9-A21*$_{UAA}$ *pheA10 trp1-289*$_{UAG}$ *ura3-52*) (Chernoff et al. 1988, 1992) or else a guanidine-HCl *psi*-cured derivative of the psi^+ strain L28-2V-P3982 (*MATa ade1-14*$_{UGA}$ *his7-1*$_{UAA}$ *leu2-01 lys2-87*$_{UGA}$ *thr4-B15*$_{UGA}$) were transformed with the original episomal *LEU2* vector YEp13 (Broach and Hicks 1980) and its derivatives bearing either an intact *SUP35* gene (pYST2 and pSTR7) or non-functional subfragments of *SUP35* (pSTR1 and pSTR2). Construction of these plasmids (Telckov et al. 1986) and the sequence of the *SUP35* cloned segment (Kushnirov et al. 1988) have been published previously. In contrast with the other plasmids, pYST2 and pSTR7 transformants were Sup^+, indicating that nonsense-mutations (with the exception of *thr4-B15*) were suppressed

* *Present address:* Laboratory for Molecular Biology (M/C 067), Department of Biological Sciences, University of Illinois at Chicago, PO Box 4348, Chicago, IL 60680, USA

Correspondence to: Y. O. Chernoff

Table 1. The appearance of *psi*-like factors (Psu^+) in mitotic progeny of yeast transformants

Plasmid	Presence of *SUP35*	Suppression	Strain	Number of transformants subcloned	Number of Leu^- subclones tested	Number (%) of Leu^- Psu^+ subclones
YEp13	–	–	*psi*⁻ L28-2V-P3892	15	223	0
			33G-D373	27	345	1 (0.3%)[a]
pSTR7	+	+	*psi*⁻ L28-2V-P3892	16	239	51 (21.4%)
			33G-D373	25	427	64 (12.6%)
pYST2	+	+	33G-D373	8	170	26 (15.3%)
pSTR1	3′-part	–	*psi*⁻ L28-2V-P3892	14	226	0
			33G-D373	1	11	0
pSTR2	5′-part	–	*psi*⁻ L28-2V-P3892	16	229	0
			33G-D373	11	163	1 (0.6%)

[a] No Psu^+ have been found among 114 Leu^+ subclones tested

in the presence of multicopy *SUP35*, as previously described (Chernoff et al. 1992). Transformants were subcloned in non-selective conditions and tested for both the presence of plasmid and suppression. The Sup^+ phenotype has usually been co-retained or co-lost with the plasmid *LEU2* marker, as described in our previous studies. However, suppressed Leu^- subclones were exceptionally, and surprisingly found. These subclones, designated as Psu^+ ("Phenotypic Suppression"), were more clearly detectable on non-fermentable media containing ethanol instead of glucose, in strong contrast with multicopy *SUP35*-mediated suppression, which was efficiently expressed on glucose-containing media. Suppression spectra of Psu^+ derivatives were also clearly distinguishable from the spectra of the original Sup^+ transformants. In the strain 33G-D373, for example, *his7-1*$_{UAA}$ was more efficiently suppressed by multicopy *SUP35* than *lys9-A21*$_{UAA}$ (see Chernoff et al. 1992). However, the suppression of *lys9-A21* was more efficient than *his7-1* in 33G-D373 Psu^+ derivatives. Carbon source-dependent suppression was previously described for some weak suppressors (Inge-Vechtomov and Karpova 1984; Inge-Vechtomov 1986), including *psi*-like factors (Inge-Vechtomov et al. 1988; Tikhodeyev et al. 1990). Re-transforming Psu^+ subclones with the *SUP35*-containing plasmid pSTR7, we have detected both an extreme instability of transformants bearing autonomous plasmids and a high frequency of integrants (data not shown). These effects are characteristic for *SUP35*-containing plasmids in *psi*$^+$ strains (Chernoff et al. 1992). In all Psu^+ derivatives tested, the Psu^+ phenotype was mitotically unstable in the presence of 5 mM of guanidine-HCl, suggesting its extrachromosomal determination. We conclude that Psu^+ subclones have resulted from the de-novo appearance of a *psi*-like extrachromosomal factor which increases translational ambiguity.

No Psu^+ subclones have been detected in the mitotic progeny of L28-3B-P3982 transformants bearing the original YEp13 plasmid. The frequency of Psu^+ subclones was about 0.3% in the mitotic progeny of YEp13 transformants of 33G-D373 (Table 1). This result is in an agreement with our observation that Psu^+ subclones can appear de novo in 33G-D373 with approximately the same frequency in the absence of any vector plasmid (data not shown). However, the frequency of Psu^+ among plasmid-less subclones, recovered from multicopy *SUP35*-bearing transformants, was 12.6–21.4% depending on the strain (Table 1). The frequency of mitotic plasmid loss for pSTR7 and pYST2 was 30–50%, depending on the transformant tested. So, the minimal frequency of Psu^+ among all subclones, derived from multicopy *SUP35* transformants, can be calculated as $0.126 \times 0.3 = 0.038$ (3.8%). This value is at least one order higher than the frequency of Psu^+ mitotic segregants in the progeny of YEp13-containing transformants, even for 33G-D373. The differences in the frequency of Psu^+-derivatives between YEp13-containing and multicopy *SUP35*-containing transformants are statistically significant. This result appears not to be strain-specific, because it was detected for both *psi*⁻ L28-2V-P3982 and 33G-D373. Hence, we conclude that the de-novo appearance of a *psi*-like factor is induced by plasmids bearing the *SUP35* gene. The resulting *psi*$^+$ cells either rapidly lose the plasmid or die, because of the incompatibility between multicopy *SUP35* and *psi*, as described above.

The patterns of transformants bearing subfragments of *SUP35* were the same as of YEp13: no Psu^+ have been induced in *psi*⁻ L28-2V-P3982 by either pSTR1 or pSTR2 or in 33G-D373 by pSTR1 (Table 1). One Psu^+ subclone has been recovered among 163 Leu^+ mitotic segregants derived from pSTR2 transformants of 33G-D373. This frequency (0.6%) is not significantly different from the case of YEp13. pSTR2 includes about 100 bp of the 5′-part of the *SUP35* coding sequence accompanied by the adjacent 5′-flanking region, while pSTR1 includes most of the residual 3′-part of the coding sequence accompanied by the 3′-adjacent region. Together, these plasmids overlap almost the whole *SUP35*-containing fragment present in pSTR7, except for approximately 300-bp of protein-coding sequence localized between to internal *Hin*dIII sites of *SUP35* (Telckov et al. 1986; Kushnirov et al. 1988). This means that Psu^+ induction depends on the presence of a functional *SUP35* gene but not on any adjacent sequence.

As a result, we have developed a simple and highly-efficient method for produce isogenic *psi*$^+$ strains from the

original *psi*$^-$. The molecular basis of this phenomenon is not clear. It seems improbable that the *SUP35* gene itself is physically involved in the control of the Psu$^+$ phenotype. Using Southern hybridization analysis, we have not detected any *SUP35*-homologous extrachromosomal sequences in Psu$^+$ clones (M.V. Ptyushkina and Y.O. Chernoff, unpublished). However, an indirect role of *SUP35* in the determination of *psi* can not be excluded. Alternatively, the appearance of *psi*-like factors may be a type of compensatory response to an imbalance of the components of the translational machinery resulting from the overproduction of *SUP35*. On the basis of our data, induction of *psi* by extrachromosomal rDNA (Dai et al. 1986) could also be explained as a response the the extradosage of rRNA genes, caused by transformation with extrachromosomal rDNA circles. Further experiments focused on other genes involved in translational fidelity control, as well as on deletional variants of the *SUP35* gene, will help us to confirm or reject these hypotheses.

Acknowledgements. We thank Dr. M. V. Ptyushkina, G. Richter, O. N. Semichayevskaya and O. V. Tarunina for help in some experiments and discussion of the data. We also thank Dr. M. D. Ter-Avanesyan for kindly providing us the *SUP35*-containing plasmids. This study was supported in part by grant from the Russian National Program „Frontiers in Genetics".

References

All-Robyn JA, Kelley-Geraghty D, Griffin E, Brown N, Liebman SW (1990) Genetics 124:505–514

Broach JR, Hicks JB (1980) Cell 21:501–508

Chernoff YO, Derkach IL, Dagkesamanskaya AR, Tikhomirova VL, Ter-Avanesyan MD, Inge-Vechtomov SG (1988) Dokl Akad nauk SSSR (Biol Sci) (Russian) 301:1227–1229

Chernoff YO, Inge-Vechtomov SG, Derkach IL, Ptyushkina MV, Tarunina OV, Dagkesamanskaya AR, Ter-Avanesyan MD (1992) Yeast 8:489–499

Cox BS, Tuite MF, McLaughlin CS (1988) Yeast 4:159–178

Dai H, Tsay S-H, Lund P, Cox BS (1986) Curr Genet 11:79–82

Inge-Vechtomov SG (1986) Issled Genet (Russian) 10:60–65

Inge-Vechtomov SG, Karpova TS (1984) Genetika (Russian) 10:1620–1627

Inge-Vechtomov SG, Tikhodeyev ON, Tikhomirova VL (1988) Genetika (Russian) 24:2110–2119

Kushnirov VV, Ter-Avanesyan MD, Telckov MV, Surguchov AP, Smirnov VN, Inge-Vechtomov SG (1988) Gene 66:45–54

Liebman SW, All-Robyn JA (1984) Curr Genet 8:567–573

Telckov MV, Surguchov AP, Dagkesamanskaya AR, Ter-Avanesyan MD (1986) Genetika (Russian) 22:17–25

Tikhodeyev ON, Getmanova E, Tikhomirova VL, Inge-Vechtomov SG (1990) In: Molecular mechanisms of genetic processes (Russian). Nauka, Moscow, pp 218–228

Tuite MF, Mundy CR, Cox BS (1981) Genetics 98:691–711

Communicated by B.S. Cox