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Additional copies of the *NOG2* and *IST2* genes suppress the deficiency of cohesin Irr1p/Scc3p in *Saccharomyces cerevisiae*✪

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The protein encoded by the *IRR1/SCC3* gene is an element of the cohesin complex of *Saccharomyces cerevisiae*, responsible for establishing and maintaining sister chromatid cohesion during mitotic cell division. We noticed previously that lowering the level of expression of *IRR1/SCC3* affects colony formation on solid support. Here we describe two dosage suppressors (*IST2*, *NOG2*) overcoming the inability to form colonies of an Irr1p-deficient strain. Ist2 is probably involved in osmotolerance, Nog2p is a putative GTPase required for 60S ribosomal subunit maturation, but may also participate in mRNA splicing.

The accuracy of mitotic cell divisions may be influenced by a spectrum of intracellular and extracellular factors. It mostly depends on proper functioning of the DNA replication machinery, mitotic spindle, actin cytoskeleton, and of numerous controlling elements. However, cell divisions can also be influenced by non-mutagenic environmental factors like osmotic and temperature stresses and the presence of many toxic chemicals (Yenush *et al.*, 2002; Goossens *et al.*, 2001; Humphrey & Enoch, 1998).

Proteins responsible for maintaining sister chromatid cohesion during mitosis are organized in a complex named cohesin. In *Saccharomyces cerevisiae* this complex comprises four subunits: Smc1p, Smc3p, Scc1p and Irr1p/Scc3p. These proteins are necessary for the cell life and they have homologues in other organisms (for a recent review see: Nasmyth, 2001). We noticed previously that lowering the level of expression of a gene encoding an element of cohesin (after fusion of *IRR1* with the regulatory *CTA1* gene promoter) —

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Irr1p/Scc3p – affects colony formation on solid support (Kurlandzka *et al.,* 1999). Cells expressing Irr1p at a very low level are capable of only slightly reduced growth in liquid media (2.5-fold lower than observed for the wild-type), but will not grow on solid support when forming a very thin layer or when plated individually. Under such conditions less than 1% of the plated cells were able to form colonies. Thus, this phenotype seemed convenient for a search for suppressors overcoming this defect. Such suppressors may provide an information on a link between two processes: colony formation and chromosome segregation.

MATERIALS AND METHODS

Strains, media and general procedures. The *S. cerevisiae* strain used in this study, AKD14/1C (MATα *ade2-1 his3*Δ *leu2-3,112 ura3-1 irr1*Δ*::kanMX4 TRP1::*P_{CTA1}*-IRR1*) is a derivative of W303. *Escherichia coli* strain XL1-Blue MRF′: Δ*(mcrA)183* Δ*(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [*F′ proAB lacI*^{q} *Z*Δ*M15 Tn10 (Tet*^{R}*)*] is from Stratagene.

Yeast were grown in complete (YP) or minimal (ω_o) media supplemented with required amino acids and nucleotides. Media contained 2% ethanol (YPE) or 10% glucose (YPD10%) as carbon sources. Solid media were prepared by adding agar to 2%. Standard procedures for sporulation and spore dissection were used (Rose *et al.,* 1990).

Suppressor isolation and cloning procedure. The Irr1p deficient recipient strain AKD14/1C was grown overnight in YPD10 liquid medium to a density of 2–4 × 10^7cells/ml, spun down and rinsed with 10% glucose. This strain has the original *IRR1* gene deleted and P_{CTA1}*-IRR1* fusion integrated in *TRP1*. It does not grow on solid media. Yeast high fidelity one-step transformation was performed by the improved lithium acetate procedure of Agatep *et al.* (1998) using a genomic DNA library. After transformation cells were incubated for 1 h in liquid minimal medium supplemented with required amino acids and 10% glucose and then plated on the same solid medium at about 1000 cells per plate. After 4 days of incubation at 30^o the well growing colonies were collected and subcloned. Plasmid DNA was isolated from yeast (Robzyk & Kassir, 1992) and used to transform *E. coli*. Plasmids usually contained one or two open reading frames (ORFs) and each ORF was subcloned into pRS316 (Sikorski & Hieter, 1989) and subsequently transformed into AKD14/1C. The number of yeast transformants per 1 μg of DNA was estimated. Finally, six independent clones were isolated and two of them, pAK051/1 and pAK086/1, giving the highest yield of transformants, were subjected to further analysis.

DNA manipulations and sequencing. Standard procedures were used for DNA manipulations (Sambrook *et al.*, 1989). Genomic DNA for library construction was isolated from the W303 strain (Thomas & Rothstein, 1989) and partially digested with *Sau*3AI. Fragments in the range of 6–10 kb were cloned into the pRS316 centromeric vector (Sikorski & Hieter, 1989).

Northern analysis. Total RNA was isolated with TRI reagent (Molecular Research Center, Inc.) according to the manufacturer's protocol. For hybridization, total RNA after agarose electrophoresis was transferred onto Hybond-N (Amersham) membrane and probed with [α-^{32}P]dCTP-labelled *Eco*RI–*Eco*RI 875bp fragment derived from the middle of *IRR1,* as described previously (Kurlandzka *et al.*, 1995).

RESULTS AND DISCUSSION

As it was already mentioned above, lowering the level of expression of *IRR1* affects colony formation on solid media. Simultaneously with our publication it was shown (Toth *et al.,*

1999) that Irr1p/Scc3p is an element of the multiprotein cohesin complex. It is well known that many of the events critical for genomic stability depend on the proper stoichiometry of the components involved. Overproduction of a normal gene product can affect cellular functions. For multisubunit complexes, an excess of one subunit can interfere with the formation of an active stoichiometric complex. Sometimes the phenotype produced by overexpression of a gene is similar to that of the loss of function of the same gene. Numerous examples of this mechanism have been documented, including genes involved in chromosome segregation (Brown *et al.,* 1993; Ouspenski *et al.,* 1999; Kolodrubetz *et al.,* 2001).

Thus, to avoid strong imbalance effects among individual complex constituents, we performed a dosage suppressor screen using the *S. cerevisiae* genomic library on a centromeric vector. We looked for genes coding for proteins overcoming the deficiency of Irr1p by transforming the recipient AKD14/1C strain in which the original *IRR1* gene was replaced by the P_{CTA1}-*IRR1* fusion.

Ist2p and Nog2p suppress Irr1p deficiency

Screening of approximately 10^5 transformants resulted in the identification of four clones showing plasmid-dependent ability to grow on solid medium. The corresponding plasmids recovered from yeast carried inserts which were identified by partial sequencing. In this manner, four genes were identified as dosage suppressors: *IST2* (*YBR086c*), *NOG2* (*YNR053c*), *YNR054c,* and *OSH2* (*YDL019c*). Two of them, *IST2* and *NOG2,* were subjected to further analysis since they produced the strongest suppressor effect. Both cloned genes were sequenced in full to exclude spontaneous mutations. Wild type copies of these genes on centromeric and multicopy plasmids were subsequently introduced into the AKD14/1C strain. The restored ability to grow on solid medium does not depend on the plasmid copy number. Figure 1 shows the effects of suppression of Irr1p deficiency caused by introducing extra copies of *NOG2*; the effects caused by extra copies of *IST2* (not shown) looked exactly the same. To check whether Nog2p may substitute for Irr1p we introduced *NOG2* on centromeric and multicopy plasmids to the Δ*irr1/IRR1* diploid strain and dissected tetrads. However, the increased copy number of *NOG2* did not restore the Δ*irr1* strain viability.

The presence of extra copies of *IST2* and *NOG2* does not influence *CTA1-IRR1* transcription

In Fig. 2 we present results of Northern blot analysis of RNA isolated from strains bearing plasmids with the *NOG2* and *IST2* genes, grown in glucose repression conditions. In both strains we did not detect the signal of

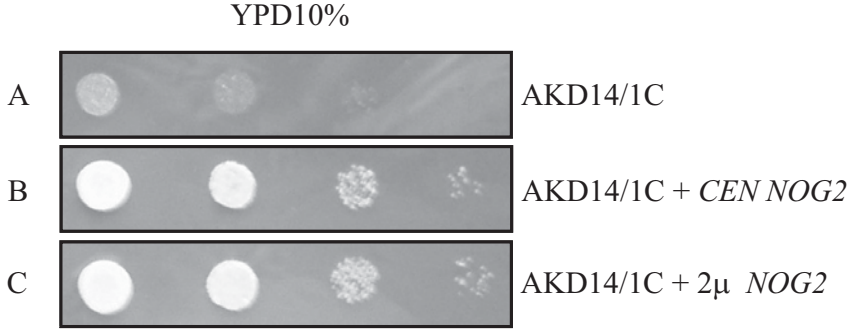

Figure 1. Suppression of Irr1p deficiency caused by extra copies of wild-type *NOG2* gene.

(A) Strain AKD14/1C with chromosomal deletion of *IRR1* and P_{CTA1}-*IRR1* fusion integrated into *TRP1,* (B) the same strain transformed with the centromeric plasmid pRS316 bearing the *NOG2* gene, (C) same as B, but *NOG2* was introduced on the multicopy plasmid YEplac195. The growth of the strains was verified by drop-test on solid medium, under *CTA1* repression conditions (YPD10%).

IRR1 transcript and we concluded that the addition of extra copies of *NOG2* and *IST2* did not disrupt the transcriptional repression of *IRR1*. This result confirmed that we achieved an effect of extragenic overexpression bypass of a deficiency of an essential *IRR1* gene.

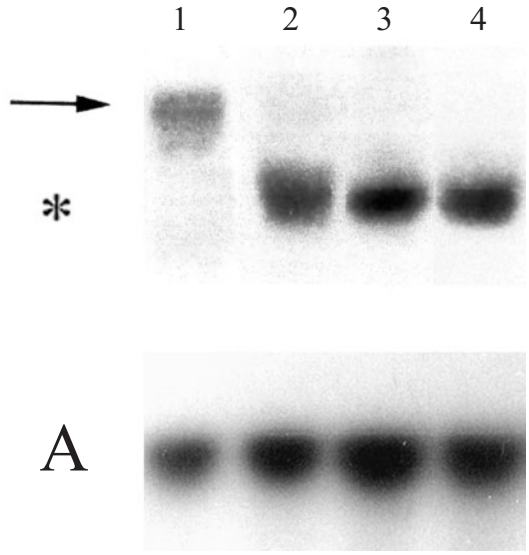

Figure 2. Suppression of Irr1p deficiency is not caused by increased level of *IRR1* transcript.

Total RNA was isolated from the recipient AKD14/1C strain grown under derepression (YPE medium, lane 1), glucose repression (YPD10%, lane 2), and from suppressors grown in YPD10%: lane 3 – suppressor bearing additional copy of *NOG2*, lane 4 – suppressor bearing additional copy of *IST2*. Arrow indicates the localization of the *IRR1* transcript, asterisk indicates non-specific transcript serving as an additional internal control, A indicates control hybridization with actin probe.

The cellular role of the Ist1 and Nog2 proteins

A global functional analysis of 150 ORFs (Entian *et al.,* 1999) revealed that *IST2* (*YBR086c*), that we found to suppress Irr1p deficiency, may be a putative ion channel but its role in conferring sensitivity to NaCl was not defined. A further study (Takizawa *et al.,* 2000) confirmed that Ist2p is a cell membrane protein. It was shown that it has an asymmetric distribution in the cell and changes its localization throughout the cell cycle. It is localized to the mother cell in small-budded cells, but localizes to the bud in medium- and large-budded cells.

There are only a few literature indications of possible connections between salt stress tolerance and mitotic divisions. Schoch *et al.* (1997) observed that a mutation in *KAR3*, encoding a motor-related protein, resulted in an increased sensitivity to high-NaCl stress. They could not explain this observation but conjectured that Kar3p may have multiple functions in addition to its roles in karyogamy and mitosis. However, a large-scale transposon mutagenesis, carried out in a search for genes required for salt-tolerance, revealed that in several cases salt-sensitivity resulted from mutations in motor and cytoskeletal proteins (de Jesus Ferreira *et al.,* 2001). Thus, it cannot be excluded that the compensation of Irr1p deficiency by additional copies of *IST2* is indirect and the increased level of Ist2p may change the intracellular salt concentration. Whether this change influences the functioning of the mitotic spindle remains an open question.

The second dosage suppression of Irr1p deficiency was caused by the presence of an extra copy of *NOG2*. The precise molecular role of this protein has not been established. The gene *NOG2* is essential and it encodes a nuclear protein associated with the nuclear pore complex (Rout *et al.,* 2000). Recent results obtained by Saveanu *et al.* (2001) indicate clearly that it is a putative GTPase associated with pre-60S ribosomal subunit and is required for 60S maturation. However, in the course of exhaustive two-hybrid screens (Formont-Racine *et al.,* 2000) it was found that Nog2p interacts with proteins involved in mRNA processing/splicing: Prp8 Prp9, Prp11, Prp21, Smb1 and Lsm8 (Camasses *et al.,* 1998; Pannone *et al.,* 2001; van Nues & Beggs, 2001; Wiest *et al.,* 1996). Thus, it is very likely that this protein may be involved in more than one function.

Since both the *IRR1* and *NOG2* genes are essential (their deletions are lethal) there was no possibility to construct double deletion mutants to check the putative genetic interac-

tions between them. However, using protein fusions we checked in the two-hybrid system that there is no direct interaction between Nog2p and Irr1p. In an additional experiment (not described), we transformed a ts mutant in the *IRR1/SCC3* gene (*scc3-1*, strain K7518 from F. Uhlmann) selected by Toth *et al.* (1999), with a centromeric plasmid bearing *NOG2*. However, *NOG2* did not suppress the ts phenotype of *scc3-1*. The mutation in *SCC3* causing the ts phenotype has not been described in detail but we assume that an Scc3 mutated protein is present in the *scc3-1* strain. Thus, we suppose that the effect of *NOG2* suppression requires the presence of a low level of intact Irr1p/Scc3p and may, for instance, increase the stability of the cohesin complex.

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