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The F658G substitution in *Saccharomyces cerevisiae* cohesin Irr1/Scc3 is semi-dominant in the diploid and disturbs mitosis, meiosis and the cell cycle

Agata Cena[a], Ewa Kozłowska[b], Danuta Płochocka[a], Marcin Grynberg[a], Takao Ishikawa[c], Jan Fronk[c], Anna Kurlandzka[a,*]

[a]*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland*
[b]*Department of Immunology, Institute of Zoology, Faculty of Biology, Warsaw University, Warsaw, Poland*
[c]*Institute of Biochemistry, Faculty of Biology, Warsaw University, Warsaw, Poland*

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Abstract

The sister chromatid cohesion complex of *Saccharomyces cerevisiae* includes chromosomal ATPases Smc1p and Smc3p, the kleisin Mcd1p/Scc1p, and Irr1p/Scc3p, the least studied component. We have created an *irr1-1* mutation (F658G substitution) which is lethal in the haploid and semi-dominant in the heterozygous diploid *irr1-1*/*IRR1*. The mutated Irr1-1 protein is present in the nucleus, its level is similar to that of wild-type Irr1p/Scc3p and it is able to interact with chromosomes. The *irr1-1*/*IRR1* diploid exhibits mitotic and meiotic chromosome segregation defects, irregularities in mitotic divisions and is severely affected in meiosis. These defects are gene-dosage dependent, and experiments with synchronous cultures suggest that they may result from the malfunctioning of the spindle assembly checkpoint. The partial structure of Irr1p/Scc3p was predicted and the F658G substitution was found to induce marked changes in the general shape of the predicted protein. Nevertheless, the mutant protein retains its ability to interact with Scc1p, another component of the cohesin complex, as shown by coimmunoprecipitation.

Keywords: *Saccharomyces cerevisiae*; Sister chromatid cohesion; Mitosis; Meiosis

Introduction

The maintenance of proper ploidy during cell division is crucial for the cell functioning. It requires both an accurate replication of chromosomes and their faithful segregation during mitosis and meiosis. In *Saccharomyces cerevisiae* the majority of cell cycle events are similar to the processes in other eukaryotes. The major differences are that in yeast, unlike in higher eukaryotes, DNA replication and spindle assembly are initiated simultaneously and the nuclear envelope remains intact throughout mitosis.

It is now generally accepted that the proper physical association of sister chromatids is maintained by the sister chromatid cohesion complex (SCC), and the unperturbed functioning of this complex is critical for genome stability (Hirano, 2000; Nasmyth, 2001, 2005; Uhlmann, 2004). The SCC, conserved in structure and function from yeast to mammals, is called cohesin in *S. cerevisiae*. It is proposed that cohesins form a ring around sister chromatids (for a review, see Nasmyth and

*Corresponding author. Tel.: +48 2 2592 1318; fax: +48 2 2658 4636.
E-mail address: ania218@poczta.ibb.waw.pl (A. Kurlandzka).

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Haering, 2005). The cohesin complex is composed of a heterodimer of SMC (structural maintenance of chromosomes) proteins and of two non-SMC subunits: Mcd1/Scc1 (YDL003W) and Irr1/Scc3 (YIL026C). All genes encoding cohesins are essential – their deletion causes cell death. Mutations in genes encoding the proteins forming cohesin are either lethal or cause severe defects in chromosome segregation (Michaelis et al., 1997; Guacci et al., 1997; Klein et al., 1999; Kurlandzka et al., 1995; Uhlmann, 2003). The dissolution of the cohesion complex is precisely regulated by mechanisms that remain only partially known.

In mitosis, the cohesin complex holds sister chromatids together and, simultaneously, sister kinetochores are pulled to opposite cell poles by microtubules of the elongating mitotic spindle. This is probably the basis of the tension which signals proper bipolar attachment of kinetochores. This bipolar attachment is required for the proper assembly and functioning of the proteins engaged in the spindle assembly checkpoint (SAC). The correct functioning of this checkpoint is indispensable for mitosis exit (for reviews, see Lew and Burke, 2003; Pinsky and Biggins, 2005). Once all chromatids are aligned, the inhibitory signal from SAC that monitors bi-orientation is relieved. The anaphase-promoting complex (APC) then targets Pds1/securin for ubiquitin-mediated degradation. Pds1 proteolysis allows Esp1/separin to cleave the Mcd1/Scc1 cohesin subunit, triggering chromatid separation (Cohen-Fix et al., 1996; Ciosk et al., 1998).

When mitotically dividing yeast cells are induced to enter meiosis, cells exit the mitotic cell cycle at the G1 phase (Kupiec et al., 1997; Neiman, 2005). The switch from mitosis to meiosis has been thoroughly investigated at the transcriptional level (Kassir et al., 2003; Pnueli et al., 2004), and the mechanisms of chromosome segregation during meiosis are quite well known (Nasmyth, 2005). It has been established that accurate meiotic segregation requires replacement of the mitotic cohesin subunit Scc1 by the meiosis-specific kleisin Rec8. At the first meiotic division (meiosis I), Rec8 is cleaved along chromosome arms but is protected at the centromeres, where it is only cleaved during the second division (meiosis II) (Lee and Orr-Weaver, 2001; Watanabe, 2005).

The yeast Irr1/Scc3 protein is the least studied element of the cohesion complex, although homologues of this protein have been identified in other eukaryotes from fungi to plants and mammals (Wang et al., 2003; Valdeolmillos et al., 2004; Chelysheva et al., 2005; Hauf et al., 2005). In mammals, three homologues, called STAG1-3 or stromalins, were described, two of them being involved in mitotic cohesion and one being specific to meiosis (Carramolino et al., 1997; Prieto et al., 2001). The aminoterminal part of Irr1 (first 496 amino acids), conserved among eukaryotes, is 18–25% identical to members of the stromal antigen protein family (Toth et al., 1999), whereas the C-terminal part is variable.

The proposed models of *S. cerevisiae* cohesin do not specify the position of Irr1p in the complex, although it is assumed that the primary role of Irr1p consists in closing the cohesin ring, and this protein is usually depicted as an element attached to the Mcd1/Scc1 kleisin subunit (Haering and Nasmyth, 2003; Nasmyth, 2005). Recent data obtained from proteome-wide purification of yeast protein complexes (Krogan et al., 2006) confirmed the previously identified interactions among Irr1p, Mcd1p and Smc3p, but also detected new Irr1p interactions with Hta2p (histone H2A subtype) and Yra1p (a nuclear protein required for export of mRNA from the nucleus).

The role of Irr1p/Scc3p in meiosis is poorly known. A meiosis-specific variant of Irr1p – STAG3 – has been described in mammalian cells (Pezzi et al., 2000). In *Caenorhabditis elegans*, the localization of a Rec8 homologue to chromosomes has been shown to depend on the presence of an Irr1p homologue – SCC-3 (Wang et al., 2003; Pasierbek et al., 2003). It has also been shown that *Arabidopsis* cohesins, AtREC8 (homologue of Rec8p) and AtSCC3 (homologue of Irr1p), are necessary for the monopolar orientation of kinetochores at meiosis I and for the maintenance of centromeric cohesion at anaphase I (Chelysheva et al., 2005).

Here we present data on an *irr1-1* mutation (F658G substitution in the Irr1p/Scc3p cohesin) of *S. cerevisiae* which is lethal in the haploid and semi-dominant in diploid yeast cells. In our recent paper (Cena et al., 2007), we described a rather unexpected influence of the presence of this mutated copy of *IRR1* on the cell wall integrity. Here we show that the heterozygous diploid *irr1-1*/*IRR1* exhibits significant irregularities in mitotic divisions: chromosome segregation errors, disturbances in segregation of nuclei and in cytokinesis. Moreover, this diploid is severely affected in meiosis. Our data suggest that these irregularities could result from malfunctioning of the spindle assembly checkpoint. These defects exhibit incomplete penetrance, in which they resemble phenotypes observed in pre-cancerous mammalian cells. Thus, our yeast *irr1-1*/*IRR1* diploid may serve as a model to investigate the general aspects of genome integrity maintenance.

Materials and methods

Strains and media

Yeast strains used in the present study, isogenic with the strain W303, are listed in Table 1. *Escherichia coli* XL1-Blue MRF′ (Stratagene, Saint Quentin en Yvelines, France) was used for molecular manipulations.