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MECHANISMS OF DEVELOPMENT

Functional analysis of the *Volvox carteri* asymmetric division protein GlsA

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ABSTRACT

The Zuotin-family J protein chaperone GlsA is essential for the asymmetric divisions that establish germ and somatic cell initials during embryogenesis in the green alga *Volvox carteri*, but it is not known on what cellular process GlsA acts to carry out this function. Most GlsA protein is nuclear, and GlsA possesses two SANT domains, suggesting that GlsA may function as a transcriptional regulator. On the other hand, close homologs from yeast and mice are ribosome-associated factors that regulate translation fidelity, implying GlsA might also regulate translation. Here we set out to gain additional evidence regarding the function of GlsA, specifically with respect to its possible involvement in transcription and translation. We found that like *zuotin* mutants, *glsA* mutants are ultrasensitive to both cold and to the ribosome-binding aminoglycoside antibiotic paromomycin, so some fraction of GlsA is likely to be ribosome associated. We also found that GlsA co-immunoprecipitates with histones and that this interaction is dependent on the presence of intact SANT domains. Through rescue experiments using transgenes that encode GlsA variants, we determined that the growth and asymmetric division defects of the *glsA* mutant are separable—a GlsA variant that rescued the growth defects did not completely rescue the asymmetric division phenotype. Considered in total, our results suggest that GlsA acts both at the level of translation and transcription, but the function that is essential for tolerance to paromomycin and cold is not sufficient for asymmetric cell division.

1. Introduction

Asymmetric cell divisions give rise to daughters that express different cell fates and are essential for much of the cell-type diversity that is critical for the generation of complex multicellular organisms. Many asymmetric divisions involve asymmetric positioning of the division plane and generate daughters that differ in size, and in these and other cases precise positioning of the division plane is necessary for the proper differentiation of the progeny of the division. Studies of different types of asymmetric divisions, mostly in a relatively small number of animal and plant model systems, have revealed that while some factors that regulate cell division geometry may be conserved within certain lineages, multiple mechanisms have evolved for controlling the positioning of division planes (Gonczy, 2008; Heidstra, 2007;

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Abbreviations: aa, amino acid(s); bp, base pair(s); BSA, bovine serum albumin; °C, degree celsius; Gls, Gonidialess; HA, influenza hemaglutinin; kb, kilobase(s); kDa, kilodalton(s); min, minute

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Knoblich, 2008; Ten Hove and Heidstra, 2008). This realization encourages a broader investigation into the types of molecules that can regulate division plane positioning in eukaryotes.

The green alga *Volvox carteri* is an excellent organism in which to study such asymmetric division factors. Progenitors of its two fully differentiated cell types are set aside during embryogenesis by a stereotyped set of asymmetric divisions, and mutants that are defective for these asymmetric divisions (but not for symmetric divisions) are readily isolated (Kirk, 1998; Kirk et al., 1991). Such mutants make only small cells that differentiate into somatic cells; hence they do not make gonidia, and are termed Gonidialess (Gls). Because they prevent the formation of asexual reproductive cells, *gls* mutations are lethal in the wild type genetic background, but they can be maintained in strains defective for the *somatic regenerator* (*regA*) gene, in which somatic cells dedifferentiate, enlarge, and become gonidia (Huskey and Griffen, 1979; Starr, 1970). Gls mutants therefore provide an excellent opportunity to identify genes that encode factors essential for asymmetric division.

Thus, far one *gls* gene has been cloned, *glsA*, which encodes a 748-amino acid protein (GlsA) that has close homologs in every eukaryotic lineage examined, including fungi, ciliates, animals, and higher plants (Miller and Kirk, 1999). Except for the fungal proteins, all known GlsA orthologs share four conserved domains that in GlsA are called the J, M, SANT1, and SANT2 domains; fungal orthologs of GlsA appear to have lost the SANT domains (Braun and Grotewold, 2001). J domain proteins (J proteins) typically act as co-chaperones that target substrate proteins to specific Hsp70 chaperone partners, which modify them through folding/re-folding reactions (Bukau and Horwich, 1998; Suh et al., 1998; Tsai and Douglas, 1996). The J domain of GlsA interacts with Hsp70A, and this interaction is essential for asymmetric division (Cheng et al., 2005), so GlsA is likely to act like a canonical J protein co-chaperone. It has not been reported whether the M or SANT domains are important for GlsA function in asymmetric division, but in other proteins these domains have been implicated in transcriptional control. For instance, the M domain of the mouse ortholog of GlsA (MIDA1) binds the helix-loop-helix (HLH) protein Id1 (an inhibitor of certain basic HLH transcription factors) to stimulate cell proliferation (Shoji et al., 1995), and many SANT (Swi3, Ada2, NcoR1, and TFβIII) domain proteins bind histone N-terminal tails, recruit histone acetylases or deacetylases that modify histone tails, and/or interact with other chromatin-associated proteins (Badri et al., 2008; Boyer et al., 2004; Guelman et al., 2006; Humphrey et al., 2001; Mo et al., 2005; Shi et al., 2005; Wang et al., 2008).

On the other hand, both fungal and vertebrate orthologs of GlsA have been implicated in ribosome function. The *Saccharomyces cerevisiae* ortholog of GlsA, named Zuotin, is part of a ribosome-associated chaperone complex termed RAC that includes Hsp70 protein Ssz1p (Gautschi et al., 2001, 2002; Yan et al., 1998). RAC is essential for another Hsp70 protein (Ssb1/2p) to associate with nascent polypeptides near the ribosome exit tunnel, which in turn appears to be required for targeting of nascent polypeptides to organelles (Ogle et al., 2003). Defects in either RAC protein or Ssb1/2b cause mild defects in codon-reading accuracy and severe defects in translation termination, as determined by both in vivo and in vitro assays (Rakwalska and Rospert, 2004). Notably, *zuotin* and *ssz1* mutants are cold sensitive for growth and ultrasensitive to the aminoglycoside antibiotic paromomycin, which binds the ribosome and reduces translation accuracy (Luce et al., 1985). The human ortholog of GlsA, MPP11 (M-phase phosphoprotein 11), also associates with ribosomes and can rescue yeast RAC mutants for both cold and paromomycin sensitivity when expressed heterologously in that system (Otto et al., 2005). C-terminal deletion mutants of MPP11 that lack both SANT domains retain partial activity in yeast, indicating that the SANT domains are not essential for the ribosomal function of MPP11. Interestingly, *V. carteri glsA* mutants, like *zuotin* mutants, are cold sensitive (Miller and Kirk, 1999). These facts suggest that, like Zuotin and MPP11, GlsA is likely to be a ribosome-associated chaperone, and raise the possibility that GlsA function in asymmetric division might involve regulation of translation.

Here we describe experiments that provide new insights into the role of GlsA in regulating division symmetry in *V. carteri*. We found that *glsA* mutants are hypersensitive to the ribosome-binding drug paromomycin, meaning that GlsA is very likely part of a ribosome-associated chaperone complex, as is its yeast counterpart Zuotin. Interestingly, while all conserved domains that could be tested were found to be essential for the asymmetric division function of GlsA, a variant that lacks the SANT2 domain rescued the cold growth and paromomycin sensitivities of the *glsA* mutant. These findings demonstrate that while GlsA is required for tolerance to cold and paromomycin, this function is not sufficient for its role in asymmetric division. Finally, we report that full-length GlsA co-immunoprecipitates with histones, while the SANT2 deletion variant does not, indicating that GlsA is likely to function at the level of transcriptional regulation in some capacity, and that it requires an intact SANT2 domain to do so.

2. Results

2.1. *At least three conserved domains of GlsA are essential for asymmetric division*

Previously, it was reported that the J domain is essential for GlsA function in asymmetric division (Miller and Kirk, 1999). As a first step toward determining if any of the other three conserved domains of GlsA are essential for asymmetric division, we made transgenes (driven by *glsA* regulatory sequences) that encode GFP-tagged GlsA variants deleted separately for the M, SANT1, and SANT2 domains (Fig. 1A and data not shown) and transformed them into *glsA* mutant 22gls1. We obtained transformants that expressed the variant deleted for the SANT2 domain (ΔS2-GGG; Fig. 1B), but we could not isolate transformants that expressed the other deletion variants (data not shown). Therefore, we made constructs in which the highly conserved M domain residues SWREF (aa 249–253) were mutated to AVAVA and the highly conserved SANT1 domain residues WSE (aa 481–483) were mutated to AAA. The M domain variant (M*-GGG) was expressed (Fig. 1B), but the SANT1 variant was not (data not shown). A second SANT1 variant that contains two different mutations