The Drug Diazaborine Blocks Ribosome Biogenesis by Inhibiting the AAA-ATPase Drg1*

Received for publication, November 18, 2013, and in revised form, December 17, 2013. Published, JBC Papers in Press, December 26, 2013, DOI 10.1074/jbc.M113.536110

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Background: Diazaborine is the only known inhibitor of eukaryotic ribosome biogenesis, but its target is unknown.

Results: Diazaborine binds the AAA-ATPase Drg1 and inhibits its ATP hydrolysis, thereby blocking release of Rlp24 from pre-60S particles.

Conclusion: Diazaborine blocks ribosome biogenesis by inhibiting the physiological activity of Drg1.

Significance: Our results highlight the potential of the ribosome biogenesis pathway as target for novel inhibitors.

The drug diazaborine is the only known inhibitor of ribosome biogenesis and specifically blocks large subunit formation in eukaryotic cells. However, the target of this drug and the mechanism of inhibition were unknown. Here we identify the AAA-ATPase Drg1 as a target of diazaborine. Inhibitor binding into the second AAA domain of Drg1 requires ATP loading and results in inhibition of ATP hydrolysis in this site. As a consequence the physiological activity of Drg1, i.e. the release of Rlp24 from pre-60S particles, is blocked, and further progression of cytoplasmic preribosome maturation is prevented. Our results identify the first target of an inhibitor of ribosome biogenesis and provide the mechanism of inhibition of a key step in large ribosomal subunit formation.

Ribosomes translate the genetic information into the amino acid sequence of proteins and are composed of one large and one small subunit containing ribosomal RNAs (rRNA) and ribosomal proteins. In eukaryotic cells, the formation of ribosomes involves >200 trans-acting factors (nonribosomal proteins), most of which are essential (for a recent introduction to ribosome biogenesis see Ref. 1). Ribosome biogenesis starts with the transcription of precursors of ribosomal RNA (pre-rRNA) in the nucleolus. Joining of ribosomal and nonribosomal proteins with pre-rRNA results in formation of precursor particles for the large and small subunits. A complex maturation pathway driven by transiently joining nonribosomal proteins leads to export-competent particles that are transported through the nuclear pore complex into the cytoplasm where final maturation steps take place. These include release and recycling of shuttling maturation and export factors and incorporation of late joining ribosomal proteins (for review, see Ref. 2). We and others demonstrated recently that the AAA2 protein Drg1 is a key factor in cytoplasmic pre-60S maturation in yeast (3–6). Drg1 is composed of an N domain and the two ATPase domains D1 and D2 and forms hexamers in the presence of ATP (7–9). The protein is recruited to cytoplasmic pre-60S particles by direct interaction with the shuttling protein Rlp24 (3). The C-terminal domain of Rlp24 then stimulates ATP hydrolysis in both AAA domains of Drg1. ATP hydrolysis in D2 is essential and triggers the release of Rlp24 from pre-60S particles whereas ATP hydrolysis in D1 is required for the subsequent dissociation from Rlp24. The release of Rlp24 by Drg1 represents a critical event in pre-60S maturation and is a prerequisite for downstream maturation steps like the release of other shuttling factors and association of late joining cytoplasmic proteins (3).

We showed previously that the drug diazaborine blocks ribosome biogenesis in yeast (10). Diazaborine is a heterocyclic boron-containing compound with strong antimicrobial activity (11). In Gram-negative bacteria the drug blocks fatty acid biosynthesis by inhibiting the enoyl-ACP reductase FabI (12, 13). In yeast, fatty acid biosynthesis is not affected by diazaborine (9). Instead, the drug specifically interferes with large ribosomal subunit formation (10). Resistance to diazaborine in yeast is mediated by certain allelic forms of DRG1 (8). However, the exact relationship between Drg1 and the drug remained elusive, because the basal ATPase activity of Drg1 was not inhibited by diazaborine in vitro, and direct binding of radiolabeled inhibitor could not be demonstrated using equilibrium dialysis (9).

We show here that diazaborine binds to Drg1 and specifically blocks ATP hydrolysis in the D2 domain, thereby preventing Rlp24 release from pre-60S particles. Thus, our results identify the target and describe the mechanism of action of the first specific inhibitor of the ribosome biogenesis pathway.

*This work was supported by grants from the European Union Seventh Framework Programme (FP7/2007–2013) under Grant 289330 (to E. K.) and Austrian Science Fund (FWF) Grants P21991 and P26136 (to H. B.).

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**The abbreviations used are: AAA, ATPases associated with diverse cellular activities; AMP-PNP, adenosine 5′-(β,γ-imino)triphosphate; DSF, differential scanning fluorometry; res, diazaborine-resistant; TAP, tandem affinity purification; YPD, yeast extract peptone dextrose.
Diazaborine Inhibits Drg1

**TABLE 1**

<table>
<thead>
<tr>
<th>Yeast strains</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azr1-TAP</td>
<td>Ma tua u3 his3 trp1</td>
<td>(5)</td>
</tr>
<tr>
<td>Azr1-TAP</td>
<td>ARX1-TAP:TRIPIMX6</td>
<td>(5)</td>
</tr>
<tr>
<td>M2</td>
<td>MATa tua u3 his3 leu2 trp1</td>
<td>(9)</td>
</tr>
<tr>
<td>FWY111</td>
<td>MATa tua u3 leu2 his3 ade2 trp1</td>
<td>(9)</td>
</tr>
</tbody>
</table>

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Growth Conditions**—The yeast and bacterial strains used in the present study are listed in Table 1. All plasmids used are listed in Table 2. Chromosomal deletions or gene fusions were generated by homologous recombination using PCR products to transform the respective yeast strain as described (14). Strains were grown either in yeast extract peptone dextrose (YPD) complex medium or for plasmid maintenance, in synthetic dextrose complete medium supplemented with the appropriate amino acids.

**Tandem Affinity Purification (TAP)**—Preribosomal particles were purified according to the standard TAP protocol (15, 16) from 4 liters of yeast culture. Strains were treated for 1 h with 370 μM diazaborine were carried out in a bead mill (Merck & Co.) in the presence of 0.6-mm glass beads for 4 min with CO2 cooling. Fusion proteins were expressed in *Escherichia coli* BL21 codon plus (Table 1). GST–Rlp24C was purified via the GST tag using GSH-agarose beads (Sigma) as described previously (3). All expression plasmids are listed in Table 2.

**Protein-Protein Interaction**—Interaction assays of Drg1 and Rlp24C in the absence or presence of 370 μM diazaborine were carried out as described in Ref. 3. The eluates were then analyzed by SDS-PAGE and Coomassie staining.

**ATPase Activity Assay**—ATPase activity was determined using the Malachite Green phosphate assay from BioAssaySystems through measurement of the formation of free inorganic phosphate (P_i) as described previously (3). If not otherwise stated, a concentration of 1 mM ATP was used. All samples

**Subsequent purification on calmodulin beads was performed as described (5).**

**Fluorescence Microscopy**—Strains were grown in YPD to early log phase and treated with 370 μM diazaborine for 1 h. Cells were then inspected for GFP/YFP fluorescence with a Zeiss Axioskop fluorescence microscope using a suitable band pass filter for GFP or YFP, respectively. For immune fluorescence microscopy, an Rlp24-TAP-tagged strain was used. After diazaborine treatment, cells were fixed using buffered formaldehyde. After washing, cells were spheroplasted with zymolyase and as secondary antibody a goat anti-rabbit-AB conjugated toethanol. Spheroplasts were spotted on polylysine-treated slides, washed with PBS containing 0.1% Triton X-100, and incubated with BSA to block nonspecific adsorption. As primary antibody a polyclonal anti-protein A antibody (Sigma) and as secondary antibody a goat anti-rabbit-AB conjugated with rhodamine (Amersham Biosciences) were used. Chromatin was stained with DAPI (Invitrogen).
were measured in triplicate of at least two independent experiments. The enzymatic parameters were calculated with GraphPad Prism.

In Vitro Release Assay—Pre-60S particles, stalled at an early cytoplasmic maturation step by incubation of the Arx1-TAP drg1-ts strain at 37 °C for 1 h, were purified from 12 liters of late log phase cultures using the TAP protocol described above. Subsequent release reactions were performed as described previously (3) in the presence of Drg1 variants, His₆Nup116 fragment, and ATP. When indicated, 370 μM diazaborine was added, or ATP was replaced by AMP-PNP. After incubation for 45 min at room temperature, the supernatants containing released proteins were collected by centrifugation and analyzed by SDS-PAGE and Western blotting using polyclonal antibodies.

Differential Scanning Fluorometry (DSF)—Differential scanning fluorometry was performed in a Corbett Rotor-Gene 6000 device with a constant fluorescence measurement at each stage (excitation 470 nm/emission 555 nm) based on the method described in Ref. 18. Samples contained 1.8 μM Drg1 and different concentrations of ATP and/or diazaborine in DSF buffer (20 mM HEPES-KOH, pH 7.0, 150 KΟAc, 5 mM Mg(OAc)₂, 1 mM DTT). SYPRO® Orange (Sigma) was added to a final dilution of 1:1000, and the reaction mixtures were incubated on ice for 30 min. 40-μl samples were subjected in duplicate to thermal unfolding using a gradient from 25 to 99 °C. The temperature was raised 1 °C per stage with a delay of 5 s after each step. The results were analyzed using the Rotor-Gene 6000 Series software 1.7.

Generation of a Homology Model of Drg1—The model of Drg1 was built with the automatic homology modeling module (19) of the YASARA program (20). Initially, 10 models were built using the two closest templates in the Protein Data Bank (1R7R and 3CF3, which are both crystal structures of murine p97 protein) and five slightly different alignments per template (21). The modeling procedure involved the SCWRL algorithm (22) for side chain rotamer prediction and hydrogen bonding network optimization (23), as well as an energy minimization with explicit solvent shell (19) to generate the final models. Surprisingly, the models based on template 3CF3 (24) scored consistently better, even though 1R7R was solved at higher resolution (3.6 versus 4.25 Å). The best model obtained a structure validation Z-score (24) of −2.2 (average of dihedral and packing Z-scores), which is good considering the low resolution template. A Protein Data Bank file of the model is available from the authors upon request.

RESULTS

Diazaborine Blocks Cytoplasmic Pre-60S Maturation—We reported previously that certain drg1 mutants are defective in the release of shuttling proteins from cytoplasmic pre-60S particles (3, 5, 6). Most shuttling proteins are released and recycled into the nucleus soon after nuclear export of pre-60S particles into the cytoplasm. Therefore, these proteins show low abundance in purifications of late preribosomal particles and a mainly nuclear steady-state localization. In contrast, inhibition of shuttling factor release by Drg1 inactivation results in their increased occurrence in preparations of late pre-60S particles and a mislocalization into the cytoplasm (3, 5, 6) (Fig. 1B). Furthermore, the failure to release these proteins prevents the binding of later joining factors to these pre-60S particles (3, 5).

The resistant phenotype of certain drg1 alleles prompted us to investigate whether diazaborine triggers phenotypes similar to those caused by functional inactivation of Drg1. To investigate whether diazaborine also affects shuttling factor release, we purified late pre-60S particles by TAP using Arx1 as bait protein. As shown in Fig. 1A, drug treatment of the wild-type strain resulted in increased levels of the shuttling proteins Rlp24 and Nog1 and of the export factors Mex67 and Mtr2 in these pre-60S purifications. As expected, due to its near stoichiometric presence on Arx1 particles, the levels of Nmd3 showed only a minor increase relative to the bait protein. Concomitantly, decreased levels of the late joining cytoplasmic proteins Sqt1 and Rpl10, and to a lesser extent Rei1, were detected in pre-60S preparations upon diazaborine treatment. In contrast, no alterations in the composition of late pre-60S particles were observed upon diazaborine treatment of the resistant drg1-1 mutant, which carries a V725E exchange in the polypeptide chain. These results suggest a block of shuttling protein release from the pre-60S particles in the presence of diazaborine.

We next asked whether this blockage takes place in the cytoplasm as is the case in drg1-ts mutants (5, 6). For this purpose GFP or YFP fusions of representative shuttling factors were inspected by fluorescence microscopy after diazaborine treatment. The shuttling proteins Arx1, Nog1, Bud20, and Rlp24, which showed a predominantly nuclear localization in untreated cells, accumulated in the cytoplasm after treatment with diazaborine (Fig. 1, B and C). In contrast, no cytoplasmic mislocalization was observed in the resistant drg1-1 strain or after diazaborine treatment of strains expressing a YFP fusion of the strictly nuclear pre-60S maturation factor Nop7. The phenotype of diazaborine-treated cells strongly resembled that observed in a drg1-ts mutant shifted to the restrictive temperature (Fig. 1B and Ref. 6).

Together, these results demonstrate that the drug causes a failure to release shuttling factors from pre-60S particles, which leads to their accumulation in the cytoplasm in pre-60S bound form. Notably, as Drg1 was still associated with pre-60S particles after treatment with diazaborine, the drug does not prevent association of the AAA-ATPase with pre-60S particles in vivo.

Diazaborine Inhibits the Release of Rlp24 from Pre-60S Particles—Targeting of Drg1 to preribosomal particles as well as activation of ATP hydrolysis are mediated by the C-terminal domain of the shuttling factor Rlp24 (3). To investigate whether diazaborine affects binding of Drg1 to Rlp24C, we analyzed their interaction using GST pulldown assays. As shown in Fig. 2A, diazaborine had no effect on the in vitro interaction between Drg1 and Rlp24. This is consistent with the presence of Drg1 on pre-60S particles from the diazaborine-treated strain (Fig. 1A).

Because the physiological function of Drg1 is to release Rlp24 from pre-60S particles, we investigated whether diazaborine interferes with this activity using our recently developed in vitro release assay (3) (Fig. 2B). Briefly, pre-60S particles purified from a drg1-ts mutant were bound to calmodulin beads and incubated with ATP and Drg1 in the absence or presence of
Diazaborine Inhibits Drg1

diazaborine. As our previous work showed that the nucleoporin Nup116 is required for the *in vitro* release of Rlp24 from pre-60S particles by Drg1 (3) it was also added to the reaction mixture. The amounts of released Rlp24 in the supernatants were then analyzed by Western blotting. As shown in Fig. 2C, addition of diazaborine prevented the release of Rlp24 almost completely (95.0 ± 3.0% inhibition compared with the untreated control). A similar result was obtained with the nonhydrolyzable nucleotide analog AMP-PNP (96.7 ± 1.9% inhibition), which could be an indication that diazaborine interferes with ATP hydrolysis of Drg1. To compare the effects of diazaborine treatment with those caused by inactivation of ATP hydrolysis
in the two individual AAA domains, we investigated mutant variants defective for ATP hydrolysis in the first (DRG1 EQ1) or the second (DRG1 EQ2) AAA domain. As shown in Fig. 2D, DRG1 EQ1 was still able to release Rp24 from purified pre-60S particles, although to a lesser extent than the wild-type protein (inhibition of 57.2 ± 10.8%). In contrast, a much stronger inhibition of release was observed when DRG1 EQ2 was used (89.0 ± 4.5%). Thus, diazaborine blocks Rp24 release to a similar extent as inactivation of ATP hydrolysis in the D2 domain of DRG1. These results are consistent with in vivo data showing that only ATP hydrolysis in D2 is essential for growth and for the release of Rp24 (3).

Binding of Diazaborine to DRG1 Requires Loading of ATP in D2—The inhibition of the physiological activity of DRG1 by diazaborine suggests direct interaction of the drug with DRG1. We therefore measured binding of diazaborine to DRG1 by DSF (18). This method makes use of the stain SYPRO® Orange, which emits fluorescence only when bound to hydrophobic regions of proteins that become exposed upon denaturation. This property enables the recording of melting curves of proteins. The melting temperature can then be deduced from the first derivative of this curve. Binding of low molecular weight compounds to proteins frequently results in an increase of their melting temperature (25). Therefore, plotting the increase in melting temperature against the compound concentration allows estimation of quantitative binding parameters.

When diazaborine was added in the absence of ATP, no alteration of the melting curve was detected (Fig. 3A). However, when 5 mM ATP and 740 μM diazaborine were simultaneously present in the sample, a shift of the melting curve to higher temperatures was observed compared with the sample with ATP alone (Fig. 3B). We conclude that diazaborine binds directly to DRG1 and that this interaction requires ATP. The order of drug and nucleotide addition did not influence the melting temperature. In addition, increasing ATP concentrations did not inhibit binding of the drug to DRG1, suggesting that diazaborine does not compete with the nucleotide for binding to DRG1. We next asked whether ATP binding to the D1 or D2 domain is required to allow interaction with the inhibitor. For this purpose, we made use of the DRG1 KA1 and DRG1 KA2 variants, which are unable to bind ATP in the first and second AAA domain, respectively. In the presence of ATP, the DRG1 KA1 variant showed an additional peak in the melting curve when diazaborine was present in the sample (Fig. 3C). Thus, nucleotide binding to the D1 domain is not required for diazaborine binding. In contrast, the melting curve of the DRG1 KA2 variant was not influenced by diazaborine, demonstrating that ATP binding into the D2 domain of DRG1 is a prerequisite for interaction with the inhibitor (Fig. 3D).

To obtain quantitative binding parameters, we recorded melting curves of DRG1 in the presence of 5 mM ATP and...
increasing diazaborine concentrations. As shown in Fig. 3E (left panel), a concentration-dependent shift of the melting temperature was observed. Plotting the increase in melting temperature (relative to the sample containing ATP alone) against the drug concentration resulted in a hyperbolic drug binding curve with a $K_D$ of $122.3 \pm 4.1 \, \mu M$ diazaborine (mean of three biological replicates, right panel). In contrast to the wild-type, the protein from the diazaborine-resistant Drg1-1 variant did not show a change in melting temperature even at high drug concentrations (Fig. 3F). We conclude that the resistance of the Drg1-1 protein to diazaborine arises from the failure of the inhibitor to bind to this variant.

**Resistance Is Caused by Amino Acid Exchanges in the Nucleotide Binding Pocket of D2**—The $drg1^{-1}$ allele was originally identified together with several other alleles in a random mutagenesis screen for diazaborine-resistant mutants (8) (Fig. 4A). However, the mechanism of how these alleles mediate resistance was unknown. As indicated in Fig. 4B, all exchanges identified in the mutants are located in the second AAA domain of Drg1. To better understand the mechanism of resistance, we...
calculated a model of Drg1 based on the crystal structure of the closely related AAA-ATPase p97. p97 shows 45% sequence identity with Drg1 and is the closest relative for which a crystal structure is available. As shown in Fig. 4C, all amino acid exchanges conferring resistance to diazaborine cluster around the nucleotide binding pocket of the D2 domain, suggesting that the inhibitor binds in close proximity to the nucleotide.

**Diazaborine Specifically Inhibits ATP Hydrolysis in the D2 Domain of Drg1**—The data shown above demonstrate that diazaborine binding to Drg1 requires the presence of nucleotide. Next we tested whether the drug blocks ATP hydrolysis by Drg1. However, consistent with previous data, the basal ATPase activity of Drg1 was largely unaffected by the inhibitor (Ref. 9 and Fig. 5A). Because the ATPase activity of Drg1 is stimulated by the C-terminal domain of Rlp24 (3) (Rlp24C), we also tested the effect of diazaborine on the stimulated ATPase activity. Indeed, in the presence of saturating Rlp24C concentrations, the wild-type protein was inhibited by 370 μM diazaborine by ~25%. Higher drug concentrations (i.e. 740 μM) did not further decrease the measured activity. No inhibition of the resistant Drg1-1 protein was found even with the highest tested drug concentration (Fig. 5A). Notably, when the wild-type Drg1 protein was stimulated with lower concentrations of Rlp24C in the range of the \( K_D \) (28 nM (3)), we observed a more pronounced inhibition (~60%) in the presence of diazaborine (Fig. 5B). In contrast, no inhibition of the resistant Drg1-1 variant was observed throughout all tested Rlp24 concentrations (Fig. 5C).

The fact that ATP hydrolysis was not completely abolished by the inhibitor suggests that diazaborine only targets one of the two AAA domains of Drg1 and that in the presence of saturating Rlp24 concentrations the activity of the insensitive domain is prevailing. Because we have previously shown that Rlp24 stimulates ATP hydrolysis in both AAA domains of Drg1 (3), we aimed to identify which of the two domains is targeted by the drug. For this purpose we purified the Drg1EQ1 and Drg1EQ2 variants. The fact that these proteins can hydrolyze ATP only in either the first (EQ2) or the second (EQ1) ATP binding site allowed us to dissect the influence of the drug on the individual AAA domains. In the presence of saturating Rlp24 concentrations, diazaborine inhibited the ATPase activity of Drg1EQ1 by 80%. Additionally, the basal activity of Drg1EQ1 was also reduced by the drug by 60 ± 11%, showing that diazaborine specifically blocks ATP hydrolysis in the second AAA domain (Fig. 5D, inset). In contrast, the ATPase activity of the first AAA domain was not inhibited in the presence of Rlp24, as the Drg1EQ2 variant was insensitive to the inhibitor. Notably, the basal ATPase activity observed with this variant, which is 4-fold higher than that of the wild-type (Fig. 5D and Ref. 3), was reduced by diazaborine. However, our data clearly demonstrate that the inhibitor specifically targets the second AAA domain of Drg1 (Figs. 3 and 4). We therefore suggest that in Drg1EQ2, the observed inhibition of ATP hydrolysis in D1 in the absence of Rlp24 is likely an indirect consequence of diazaborine binding into D2. Together with the elevated basal activity of Drg1EQ2 compared with the wild-type protein, this result indicates a pronounced communication from the D2 to the D1 domain.

We have demonstrated previously that the two AAA domains of Drg1 exhibit different affinities for the nucleotide (9). To test the influence of ATP loading into D2 on the inhibition of Drg1, we measured the enzymatic activity of wild-type Drg1 and the Drg1EQ1 variant in the presence of Rlp24 and different ATP concentrations (Fig. 5, E and F). At the lowest tested nucleotide concentration of 0.2 mM ATP, similar ATP hydrolysis was measured with the wild-type protein in the presence and in the absence of the drug, suggesting that under these conditions only the diazaborine-insensitive D1 domain is active (Fig. 5E). In the presence of higher nucleotide concentrations, the inhibitor caused a pronounced reduction of ATP hydrolysis. In contrast to the wild-type protein, the Drg1EQ1 variant displayed reduced ATP hydrolysis rates in the presence of...
Diazaborine Inhibits Drg1

Diazaborine at all tested nucleotide concentrations, even at 0.2 mM (Fig. 5F; compare first data points in Fig. 5, E and F). Taken together, these results suggest that the D2 domain represents the low affinity ATP binding site which is specifically targeted by diazaborine.

**DISCUSSION**

Ribosome biogenesis in eukaryotic cells involves the coordinated activity of >200 trans-acting factors. Because most of
these factors are essential, ribosome biogenesis provides a promising target for antimicrobial and anti-tumor chemotherapy. In this study, we investigated the mechanism of action of the first specific inhibitor of this pathway, the heterocyclic boron-containing compound diazaborine. Our data demonstrate that the AAA-ATPase Drg1 is the direct target of diazaborine. Drg1 is only found in eukaryotic cells and was previously shown to be a key factor of pre-60S ribosome maturation in yeast. Accordingly, treatment with the drug leads to the same phenotypic consequences as those caused by the functional inactivation of Drg1, i.e. a failure to release Rlp24 from pre-60S particles and a block in downstream maturation (Fig. 1, compare with Ref. 3).

What is the mechanism of inhibition of the drug? Our work shows that diazaborine specifically binds into the second AAA domain of Drg1 and thereby inhibits ATP hydrolysis in this site. However, the drug does not act as a competitive inhibitor for ATP. Instead, ATP binding to D2 is a prerequisite for diazaborine. Diazaborine shows that diazaborine specifically binds into the second AAA domain of Drg1 and thereby inhibits ATP hydrolysis in this site. In this study, we investigated the mechanism of action of diazaborine Inhibits Drg1.

Structural studies showed that in the active site of FabI, the boron atom of diazaborine forms a covalent bond with the 2’-OH of the nicotinamide ribose moiety of the NAD+ cofactor (26, 27). It is however currently unknown whether or not binding of diazaborine to Drg1 also involves the formation of a covalent bond between ATP and the inhibitor. Our homology model of Drg1 shows that all up to now identified amino acid exchanges causing resistance to diazaborine are located in close proximity to the nucleotide in the D2 domain of the protein (Fig. 4C). This observation supports the possibility of a close contact between ATP and diazaborine within the D2 domain of Drg1. Therefore, formation of covalent intermediates or at least tight binding between a nucleotide cofactor and the inhibitor could be a general principle for the mode of action of diazaborine.

Our structural model also offers an explanation for the resistance mechanism: The mutations resulting in the resistant phenotype in the 

\[\text{drg1-1, drg1-3, and drg1-4} \]

alleles introduce amino acid residues with bulkier side chains than those present in the wild-type and therefore may reduce the accessibility of the binding pocket for the drug. As diazaborine is unable to bind to the Drg1-1 protein, the resistance mechanism may involve the occlusion of diazaborine from its binding site near the D2 nucleotide binding pocket of Drg1.

Taken together, our work shows that ribosome biogenesis in general and particularly energy-consuming enzymes within this pathway may represent promising targets for the development of novel inhibitors. These enzymes include additional AAA-ATPases, like Rix7 and Rea1, but also a number of RNA helicases and GTPases (1). Furthermore, AAA-ATPases have emerged as interesting new targets for low molecular weight inhibitors in recent years. For example, significant efforts were undertaken to identify inhibitors for p97/VCP (28-31), which is closely related to Drg1 but involved in different cellular pathways. The mechanistic insights gained from our study will therefore be helpful for the rational design of novel inhibitors of other AAA-ATPases.

Acknowledgments—We thank Kerstin Klein and Anke Loregger for expert help during early stages of this work; Micheline Fromont-Racine, Bernard L. Trumpower, Juan P. Ballesta, Arlen W. Johnson, Ed Hurt, and Sabine Rospert for generously supplying reagents; and Tamsyn Stanborough for critically reading the manuscript.

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Diazaborine Inhibits Drg1

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doi: 10.1074/jbc.M113.536110 originally published online December 26, 2013

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