FKBP36 has been previously shown to be a crucial factor in spermatogenesis because of its interplay with the synaptonemal complex protein SCPI. Here we show that beyond this function, FKBP36 forms complexes with glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12) and Hsp90. Both proteins bind independently to different sites of the FKBP36 tetratricopeptide repeat domain. The interaction between FKBP36 and GAPDH directly inhibits the catalytic activity of GAPDH. In addition, FKBP36 expression causes a significant reduction of the GAPDH level and activity in COS-7 cells. Particularly in the cytosolic fraction, GAPDH was depleted by FKBP36 expression. Thus, FKBP36 diminishes GAPDH activity by direct interaction and down-regulation of GAPDH, which represents a previously unknown mechanism of GAPDH regulation and a novel function of FKBP36 in testis-specific signaling.

FKBP36 (FK506-binding protein 36; FKBP6) belongs to the human immunophilins and is predominantly found in testes. FKBP6 was initially identified as one of the genes on chromosome 7 whose deletion is linked to Williams-Beuren syndrome (1). Williams-Beuren syndrome is a dominant autosomal disorder, which may include supravalvular aortic stenosis, multiple peripheral pulmonary arterial stenoses, elfin face, mental and statural deficiency, characteristic dental malformation, and infantile hypercalcemia (2).

FKBP6 is predominantly expressed in testes and plays an important role in spermatogenesis. The loss of FKBP36 causes a significant reduction of the size of testes and the infertility of male mice. The testis-specific FKBP was identified as a component of the synaptonemal complex, which forms between two homologous chromosomes during meiosis (3). The synaptonemal complex participates in chromosome pairing, synapsis, and recombination (4).

FKBP36 contains an N-terminal peptidyl-prolyl cis/trans isomerase domain and three tetratricopeptide repeat (TPR)3 motifs in its C-terminal segment. Furthermore, FKBP36 displays a high sequence homology to the regulated FKBP38, and the absence of FKBP36 activity might suggest a similar activation scenario (5). The TPR domains of FKBP38, FKBP51, and FKBP52 have been shown to be involved in the heat shock protein Hsp90 (5, 6). The interactions between the different FKBP and Hsp90 are mediated by the Hsp90 C90 domain. Complexes of FKBP51 and FKBP52 with Hsp90 have been reported to interact with client molecules, e.g. steroid hormone receptors, to modify the activity, stability, or subcellular localization of the client proteins (7). Within these complexes, the FKBP51 interacts with both the receptor and Hsp90 (8).

In the present study, we show that FKBP36 interacts with Hsp90 via the TPR domain. A second binding site in the TPR domain of FKBP36 mediates the interaction with GAPDH, leading to GAPDH-FKBP36-Hsp90 complexes, which are comparable to complexes formed by other TPR-containing immunophilins. Furthermore, our experiments showed that FKBP36 controls GAPDH activity either by direct interference with binding of the enzyme NAD+ or down-regulation of GAPDH levels. Hence, FKBP36 represents a novel non-metabolic and multifunctional inhibitor of GAPDH.

**EXPERIMENTAL PROCEDURES**

Sources of enzymes used in the experiments, recombinant human GAPDH and recombinant human Hsp90 C90 (Hsp90627–731), were recombinantly expressed by using a pET28a vector in *Escherichia coli* Rosetta™ cells. Glutathione S-transferase (GST) fusion FKBP36 variants were expressed by using a pGEX4T1 vector in *E. coli* Rosetta™ cells. The C-terminal Hsp90 peptides Hsp90C20 (Hsp90727–731, PDEIPPLEG-DEDASRMEEVD), Hsp90C7 (Hsp90725–731, SRMEEVD), and Hsp90C5 (Hsp90727–731, MEEVD) were produced by solid-phase synthesis using the Fmoc (N-(9-fluorenylmethoxycarbonyl) strategy.

**Rabbit muscle GAPDH** was purchased from Sigma. The FKBP36 antibody was a section 4 polyclonal antibody from rabbit against purified FKBP36. Additional antibodies were used were polyclonal rabbit anti-actin (Sigma), monoclonal mouse anti-His6 (Qiagen), monoclonal mouse anti-GST (Sigma), monoclonal rat anti-Hsp90 (Stressgen), polyclonal rabbit anti-Hsp90 (Stressgen), polyclonal rabbit anti-Hsp 90, polyclonal rabbit anti-GAPDH (Sigma), and polyclonal rabbit anti-H3 (Cell Signaling Technology, Danvers, MA). MG115, NAD+, and glyceraldehyde 3-phosphate (GA-3-P) were obtained from Sigma.

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2 The abbreviations used are: TPR, tetratricopeptide repeat; GA-3-P, glyceraldehyde 3-phosphate; GST, glutathione S-transferase; FKBP, FK506-binding protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight.
GAPDH Activity Assay—GAPDH activity was measured with an HP 8452A spectrophotometer by following the reduction of NAD⁺ at 340 nm. The reaction mixture in a volume of 1.5 ml contained 10 mM sodium arsenate, 2.5 mM EDTA, 2.5 mM dithiothreitol, 150 mM NaCl (pH 8.5).

Unless mentioned otherwise, 25 µl of COS-7 crude cell extract, 1 mM NAD⁺, and 1 mM GA-3-P were used in all assays. One unit of enzyme activity is expressed as the amount of enzyme that forms 1 µmol of NADH/min at 25 °C.

Protein Interaction Assays—For GSH-Sepharose-binding assay, 30-µl volume of GSH-Sepharose was pre-equilibrated in 25 mM Tris-HCl buffer (pH 7.5) 150 mM NaCl, 1 mM dithiothreitol and saturated with GST or GST fusion proteins. The beads were then washed twice with buffer B (25 mM Tris-HCl, (pH 7.5), 150 mM NaCl, 1 mM dithiothreitol) and subsequently incubated for 2 h with 100 µl of rat testis cytoplasm. In some experiments, 50 µM Hsp90 C90 or 1 mM NAD⁺, NADH + H⁺, GA-3-P, or arsenate was added. Beads were centrifuged, washed three times, and mixed with an equal volume of Lammli sample buffer. Samples were then subjected to 12.5% SDS-PAGE and analyzed by Western blotting using monoclonal mouse anti-His5 (Pharmingen), monoclonal mouse anti-Hsp90, monoclonal rat anti-Hsp90 (Stresgen), and polyclonal rabbit anti-GAPDH antibody.

Co-immunoprecipitation was performed with 5 ml of rat testis cytosol (pre-cleaned with 200 µl of Protein G-Sepharose™ 4 Fast Flow for 1 h at 4 °C). The samples (3 x 1.5 ml) were subjected to 5 µg of polyclonal goat anti-Hsp90 (Santa Cruz Biotechnology) and 5 µg of polyclonal goat anti-GAPDH antibody (Santa Cruz Biotechnology) or buffer, respectively. After a 3-h pre-incubation at 4 °C, Protein G Sepharose™ 4 Fast Flow (50 µl) (GE Healthcare) was added and incubated for 2 h at 4 °C. Subsequently, samples were centrifuged at 12,000 x g for 1 min, washed five times with 200 µl of buffer B, boiled with 50 µl of sample buffer, subjected to SDS-PAGE, and analyzed by Western blotting.

Cell Culture—For FKBP36 expression, transformed African green monkey kidney fibroblast cells (COS-7 line) (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) were used. The cells were cultured in Dulbecco’s modified Eagle’s medium (Biochrom, Berlin, Germany) supplemented with 2 mM l-glutamine and 10% (v/v) heat-inactivated fetal calf serum in a humidified incubator at 37 °C in 10% CO₂. COS-7 cells were transfected with pcDNA3.1 or pcDNA-FKBP36 and seeded at 5 x 10⁶ cells/ml in 6-well plates.

To analyze GAPDH proteasomal degradation, COS-7 cells were transfected with either pcDNA3.1 or pcDNA-FKBP36 constructs. After 48 h, cells were treated with 0, 25, and 50 µM MG115 for 5 h. Subsequently, cells were harvested and analyzed by Western blot.

To prepare COS-7 crude cell extract, cells were harvested and centrifuged for 10 min at 2,000 x g, and cell pellets were resuspended in the same volume of hypotonic lysis buffer (50 mM Tris-HCl, (pH 7.4), 20 mM EDTA, 1 mM dithiothreitol, and 1% Triton X-100), followed by sonication. The crude extract was centrifuged for 10 min at 800 x g. The pellet was used for the preparation of nuclei (9). The supernatant was centrifuged again for 45 min at 100,000 x g to prepare cytosol and membrane fractions. The preparation of rat testis cytoplasm was described previously (10).

RESULTS

FKBP36 Interacts with GAPDH and Hsp90—The TPR domains of several immunophilins have been shown to mediate interactions with Hsp90 (7). Hence, we tested whether FKBP36 is able to interact with Hsp90 as well.

We performed a GST pulldown experiment loading GST-FKBP36 on GSH-Sepharose and applying rat testis lysate to the matrix. Several endogenous proteins from rat testis cells bound to the FKBP36 affinity matrix, Hsp90, Hsp70, tubulin, actin, GAPDH, and GST (Fig. 1A), as identified by MALDI-TOF mass spectrometry (data not shown). However, Hsp70, tubulin, and actin were also found in the control experiment using only a GST matrix. Thus, only Hsp90 and GAPDH can be considered as specific binders of FKBP36. To confirm these results, a second interaction assay was performed using FKBP36 with Strep-Tag II fusion immobilized on a StrepTactin matrix. The FKBP36-affinity matrix was incubated with rat-testis lysate in parallel to the previous experiment, and bound proteins were analyzed by Western blotting with anti-GAPDH and anti-Hsp90 antibodies (Fig. 1B). GAPDH and Hsp90 only interacted with the FKBP36 affinity matrix, whereas no binding was observed to the matrix alone.
The FKBP36 TPR Domain Binds GAPDH and Hsp90—To test whether FKBP36 binds directly to GAPDH, we made use of purified GAPDH from rabbit muscle, which was previously utilized in several similar studies (11–13). Isolated GAPDH was incubated with the GST-FKBP36 affinity matrix. Fig. 2A shows that GAPDH bound specifically to FKBP36, demonstrating direct interaction between both proteins.

Next, several GST-FKBP36 variants were applied to GSH-Sepharose and incubated with rat testis lysate to investigate which segment of FKBP36 mediates the interaction with GAPDH. Western blot analysis using anti-GAPDH antibody showed that GAPDH bound only to the full-length FKBP36 and to the FKBP36\textsuperscript{145–327} variant containing the three TPR motifs (Fig. 2B) and a C-terminal segment.

A similar interaction pattern was observed for Hsp90 using the different FKBP36 variant affinity matrices. Thus, the C-terminal FKBP36 segment, which contains the three TPR motifs, interacts with both Hsp90 and GAPDH. Further experiments using a FKBP36 variant lacking the C-terminal extension were carried out to investigate the contribution of the FKBP36 C terminus to the interaction with either GAPDH or Hsp90. Fig. 2C shows that both GAPDH and Hsp90 bound similarly to the FKBP36 variants independent of the presence of the C-terminal FKBP36 extension. Hence, only the FKBP36 segment that contains the TPR motifs mediates the interactions with GAPDH and Hsp90.

The Hsp90 MEEVD Segment and GAPDH Interact with Different Binding Sites in the FKBP36 TPR Domain—Given the interaction between the FKBP36 TPR domain and Hsp90, we tested if this interaction is mediated by the C90 domain of Hsp90 in parallel to other immunophilin-Hsp90 complexes. To this end, we incubated FKBP36 with either a C90-GST affinity matrix or a GST matrix (Fig. 3A). Indeed, the C90 domain sufficed to interact with FKBP36. Hence, the interaction between FKBP36 and Hsp90 is mediated by the TPR domain and the C90 domain similar to other FKBP-Hsp90 complexes.

Furthermore, we investigated using a FKBP36 affinity matrix which C-terminal segments of Hsp90 can compete with endogenous rat Hsp90 and thus mediate the interaction with FKBP36. The results in Fig. 3B show that the different segments of the C90 domain including the very C-terminal MEEVD motif competed with endogenous Hsp90 from testis lysate for FKBP36 binding. Thus, the interaction between Hsp90 and FKBP36 is mediated by the C-terminal MEEVD motif of Hsp90, which is similar to previously described interactions between Hsp90 and human FKBP.

Far-UV CD spectroscopic measurements show changes in the secondary structure elements of either FKBP36 or the C90 domain because of complex formation (Fig. 3C). Interestingly, these changes of the protein ellipticity in the far-UV CD spectra were not observed for interactions between FKBP36 and the MEEVD peptide, suggesting either structural changes in the C90 domain upon FKBP36 binding or additional binding sites between Hsp90 and FKBP36.

Furthermore, we analyzed whether Hsp90 and GAPDH compete for the same interaction site in the FKBP36 TPR domain or perhaps can be found together in a complex with FKBP36. Thereto, we applied purified proteins to a FKBP36

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**FIGURE 2. The FKBP36 TPR domain binds GAPDH and Hsp90.** A, direct binding between FKBP36 and GAPDH was investigated by incubation of purified GAPDH from rabbit muscle with GST-FKBP36- or GST-pre-loaded GSH-Sepharose. The samples were analyzed by Coomassie-stained SDS-PAGE (12.5%). Samples without GAPDH served as a control. B, binding of GAPDH and Hsp90 to different domains of FKBP36 was tested by incubation of rat testis lysate with GSH-Sepharose pre-loaded with different GST-FKBP36 variants or GST alone. Bound proteins were analyzed by SDS-PAGE and Western blotting using anti-GAPDH and anti-Hsp90 antibodies. Eluted GST and GST-FKBP36 variants were analyzed by Western blotting using anti-GST antibodies (lower panel). C, endogenous GAPDH and Hsp90 from rat testis lysate bound to GSH-Sepharose loaded with GST-FKBP36 variants was analyzed by SDS-PAGE and Western blotting using anti-GAPDH antibodies. The Western blot analysis of eluted GST-FKBP36 variants using anti-GST antibodies is displayed in the lower panel.
affinity matrix that was incubated with testis lysate (Fig. 3D).

The presence of additional C90 domain had no effect on the binding of GAPDH to the FKBP36 affinity matrix. Further addition of purified GAPDH (5 μM) did not influence the FKBP36/Hsp90 interaction either. The lack of competition between Hsp90 C90 or purified GAPDH from rabbit muscle cells. Input and eluate were subjected to SDS-PAGE and subsequently analyzed by Western blotting using anti-Hsp90 and anti-GAPDH antibodies. The presence of Hsp90 C90 was analyzed using anti-His antibody. E, the presence of GAPDH-FKBP36-Hsp90 complexes was studied by co-immunoprecipitation of endogenous proteins from rat testis cytosol using goat anti-Hsp90 or goat anti-GAPDH antibodies. Antibody-protein complexes were bound to Protein G Sepharose. After washing precipitates, input and samples were subjected to SDS-PAGE and analyzed by Western blotting using rat anti-Hsp90 antibody, rabbit anti-GAPDH antibody, and rabbit anti-FKBP36 antibody. As a control, cell lysate pre-incubated with Protein G-Sepharose was used.

To analyze the presence of protein complexes containing FKBP36, GAPDH, and Hsp90 in spermatocytes, we performed a co-immunoprecipitation using cytosol from rat testis cells (Fig. 3E). Endogenous FKBP36 bound either Hsp90 or GAPDH, and in addition, Hsp90 was detected in the fraction of GAPDH-interacting proteins and vice versa, suggesting the presence of GAPDH-FKBP36-Hsp90 complexes in testis cells. Further-
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more, we incubated cell lysate of COS-7 cells either expressing FKBP36 or lacking the human FKBP with a GST-Hsp90 C90 affinity matrix (supplemental Fig. 1). GAPDH only interacted with the C90 affinity matrix in the presence of FKBP36, demonstrating the requirement of FKBP36 for the formation of the trimeric complex.

FKBP36 Inhibits GAPDH Activity—Next, GAPDH activity measurements were performed in the presence of FKBP36 to investigate the role of FKBP36 in complexes with GAPDH. FKBP36 had only a slight effect on GAPDH activity at 1 mM NAD⁺, reducing the residual activity to about 90% compared with the absence of FKBP36 (Fig. 4A). FKBP36-mediated GAPDH inhibition was not influenced by GA-3-P concentrations in the range of 0.01–1 mM. However, the inhibitory effect of FKBP36 on GAPDH activity was increased significantly at lower NAD⁺ concentrations of 0.01 mM. Under these conditions, FKBP36 reduced the GAPDH activity in a concentration-dependent manner to less than 40% residual activity at 2.5 μM FKBP36 with a Kᵢ of 1.5 ± 0.45 μM. Thus, we analyzed whether FKBP36 competes with NAD⁺ for GAPDH binding. The Eadie-Hofstee plot (Fig. 4B) shows deviations from the linear function, which are caused by negatively cooperative NAD⁺ binding at four binding sites in a GAPDH tetramer (14–16). The FKBP36-mediated GAPDH inhibition differs from the pattern of a competitive inhibitor by indicating different Vₘₐₓ and thus indicates a mixed form of inhibition that is not solely caused by competition with NAD⁺. Interactions between FKBP36 and NAD⁺ can be excluded because the fluorescence spectra of both FKBP36 and NAD⁺ remain unaltered in the presence of each other (supplemental Figs. 2 and 3). Furthermore, the interference of GAPDH substrates and effectors with the interaction between GAPDH and FKBP36 was tested using the FKBP36 affinity matrix (Fig. 4C). In the presence of NAD⁺ or NADH + H⁺, the amount of FKBP36-bound GAPDH was significantly reduced, which corresponds to the kinetic measurements. However, neither GA-3-P nor arsenate influenced the FKBP36/GAPDH interaction, demonstrating interference only between FKBP36/GAPDH interactions and NAD⁺ binding.

FKBP36 Reduces GAPDH Levels and Activity in Cells—Next, we studied the influence of FKBP36 on endogenous GAPDH activity to get inside the physiological effect of this interaction. Thus, the testis-derived cell lines NTERA-2, F9, TM3, and TM4 were analyzed for FKBP36 and GAPDH expression levels. However, the FKBP36 levels in the different cell lines were significantly lower than in testis lysate. In the absence of a cell system reflecting the physiological FKBP36 concentration, COS-7 cells were, thus, transfected with a pcDNA-FKBP36 construct, and the GAPDH activity in transfected cells was compared with that in normal cells. Fig. 5A shows that FKBP36 reduced the GAPDH activity by about 70 units/g of total cell protein, which is 32% of the GAPDH activity in these cells. To test whether the reduction of GAPDH activity is caused by an altered GAPDH concentration or by direct inhibition because of FKBP36 binding, the GAPDH protein levels were analyzed. Interestingly, the presence of FKBP36 reduced the GAPDH protein level by about 33% in COS-7 cells based on the densitometric analysis, which is likely to be the major cause of the reduction of GAPDH activity (Fig. 5, B and C). Furthermore, COS-7 cells expressing FKBP36 were fractionized, and the GAPDH localization was compared with the pattern observed in wild-type cells (Fig. 5D). GAPDH was found in the cytosol and the membrane fraction of COS-7 cells both in presence and absence of FKBP36. However, FKBP36 expression significantly reduced the GAPDH levels in both cell fractions. In fact, the
FKBP36 is a GAPDH Inhibitor

FIGURE 5. FKBP36 expression reduces GAPDH protein levels in COS-7 cells. A, GAPDH activity measurements using cell lysate of either untransfected COS-7 cells or COS-7 cells transfected with pcDNA3.1 or pcDNA-FKBP36 constructs, respectively. Data were statistically analyzed by a non-paired Student’s t test and are represented as mean ± S.D. from six replicates. *, p < 0.05. B, Western blot analysis of proteins present in COS-7 cells either not transfected or transfected with pcDNA3.1 or pcDNA-FKBP36 constructs, using anti-GAPDH, anti-actin, and anti-FKBP36 antibodies. C, densitometric analysis of the GAPDH levels of the Western blot analysis depicted in B. GAPDH levels are displayed as percent of endogenous protein found in untransfected COS-7 cells. Data are represented as mean ± S.D. from four replicates. *, p < 0.05. D, Western blot analysis of a cell fractionation of COS-7 cells were either transfected with pcDNA3.1 or pcDNA3.1 FKBP36 construct using anti-FKBP36, anti-GAPDH, and anti-histone antibodies. E, influence of FKBP36 on proteasomal GAPDH degradation analyzed by treatment of pcDNA3.1 or pcDNA-FKBP36-transfected COS-7 cells with 0, 25, or 50 μM MG115 for 5 h and subsequent analysis of crude cell extracts by anti-GAPDH, anti-FKBP36, and anti-β-actin antibodies.
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cytosolic GAPDH concentration seems to be affected in particular by FKBP36 expression.

GAPDH was previously found to be degraded by the proteasome (17). Thus, we analyzed if the presence of FKBP36 increases GAPDH degradation using the proteasome inhibitor MG115. Fig. 5E shows a significant reduction of GAPDH levels in the presence of FKBP36. However, this reduction was not prevented by 50 μM MG115. On the other hand, FKBP36 was found at slightly higher levels in the presence of the proteasome inhibitor. Thus, FKBP36 does not increase the GAPDH degradation rate, but is likely reduces GAPDH expression.

DISCUSSION

FKBP36 is a multifunctional immunophilin, which has been reported to interact with components of the synaptonemal complex. Here, we show interactions of this testis-specific FKBP with Hsp90 and GAPDH, suggesting additional cellular functions. The interaction between FKBP36 and the C-terminal domain of Hsp90 is mediated by its TPR domain (Figs. 2B and 3A) and is thus similar to interactions between Hsp90 and other immunophilins (e.g. FKBP52) (7). The FKBP52 residues Lys2842, Lys354, and Arg358 were reported to assemble a dicarboxylate clamp, which binds the Hsp90 C-terminal MEEVD motif to form the FKBP52-Hsp90 complex (18). FKBP36 features identical residues in the corresponding positions, and the MEEVD pentapeptide was sufficient for the interaction with FKBP36 (Fig. 3C), implying a similar binding mechanism. Changes in the far-UV CD spectrum (Fig. 3C) suggest either additional binding sites between FKBP36 and Hsp90 or structural changes in the C90 domain upon FKBP36 binding. However, the competition between the MEEVD peptide suggests that the latter possibility is more likely.

GAPDH also binds to the TPR domain of FKBP36, but GAPDH and Hsp90 interact with different sites in FKBP36 because both proteins do not compete for FKBP36 binding (Fig. 3D). In fact, the results of binding studies with rat-testis proteins and COS-7 cell lysate containing or lacking FKBP36 suggest the presence of the GAPDH-FKBP36-Hsp90 complexes in the cytosol, where all three proteins are present. These interactions occurred with recombinant human proteins, purified rabbit GAPDH, or endogenous rat proteins, suggesting similar interactions in different mammalian organisms perhaps based on the high similarity of the interaction partners in different mammals. Such GAPDH-FKBP36-Hsp90 complexes might be similar to steroid hormone receptor complexes. Thus, the FKBP36-mediated GAPDH control is perhaps comparable with the reduction of steroid hormone receptor activity induced by high cellular FKBP51 concentrations (19), but presents a unique mechanism, because GAPDH, in contrast to receptor molecules, is not a client protein of Hsp90 and direct interactions between both proteins were not observed. Nevertheless, Hsp90 might interact with GAPDH in complex with FKBP36 or recruit further interaction partners that could participate in the regulation of GAPDH activity. Endothelial nitric-oxide synthase, for instance, is activated by Hsp90 and S-nitrosylation at Cys149 and is known to inhibit GAPDH activity (20).

FKBP36 also participates in the control of GAPDH in an Hsp90-independent manner via direct interaction with the NAD+/binding site of GAPDH. Even though only minor FKBP36-mediated effects on GAPDH activity were observed at NAD+ saturation, significant GAPDH inhibition was found at lower NAD+ concentrations. Similar effects are caused by the interaction between GAPDH and adenine nucleotides or RNA (21). Based on our results, FKBP36-mediated GAPDH inhibition might play an important role in spermatocytes because the NAD+ levels in spermatocytes are significantly lower than in COS-7 cells and comparable with low NAD+ concentrations in the kinetic measurements (Fig. 4) because of lactate-based nutrition (22).

FKBP36 expression furthermore reduced the GAPDH concentration in COS-7 cells (Fig. 5), demonstrating a control of GAPDH protein levels. GAPDH is likely diminished by FKBP36-mediated reduction of GAPDH expression because the use of MG115 showed no involvement of proteasomal degradation in reduced GAPDH levels. Similar down-regulation of GAPDH levels have been reported previously. However, formerly identified regulators of GAPDH expression are nutrients (e.g. amino acids and ethanol) or hormones such as insulin (21, 23, 24). FKBP36, by contrast, is the first described interaction partner that affects the GAPDH expression level. Due to the FKBP36-mediated reduction of GAPDH expression, the GAPDH activity in COS-7 cell lysate was diminished. Moreover, the FKBP36 expression changed the subcellular GAPDH distribution by reducing the cytosolic GAPDH population in particular, which might potentiate the FKBP36-mediated GAPDH inhibition in the cytosol of spermatocytes. Hence, FKBP36 controls GAPDH activity in a combination of direct inhibition and reduction of the GAPDH concentration, particularly in the cytosol. Previous reports also describe a testis-specific GAPDH isoform, which is expressed in late spermatid stages (25, 26). However, glyceraldehyde-3-phosphate dehydrogenase, spermatogenic (GAPDS) isoform is not regulated by FKBP36 because the FKBP is only present in early stages of spermatocytes, not in spermatids (3).

GAPDH participates in glycolysis by catalyzing the oxidative phosphorylation of GA-3-P into 1,3-diphosphoglycerate. GAPDH is expressed ubiquitously, but the cellular concentrations vary significantly from cell type to cell type (27). Interestingly, particularly low GAPDH concentrations were identified in testes (28). Thus, only in spermatogenic cells, GAPDH is limiting for the glycolysis (29). The interaction between GAPDH and the testis-specific FKBP36 may participate in glycolysis and several other processes because GAPDH was found to promote membrane-membrane fusion processes, modulate the cytoskeleton, and be involved in apoptosis control, DNA repair, DNA replication, and tRNA export (30–33). On the basis of the bifunctional reduction of GAPDH activity by FKBP36 and the expression pattern of both proteins, an influence of FKBP36 on GAPDH-dependent processes in testes is likely and might be involved in the regulation of physiological processes that depend on GAPDH activity.

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