Age- and diet-dependent requirement of DJ-1 for glucose homeostasis in mice with implications for human type 2 diabetes

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Elderly patients often suffer from multiple age-related diseases. Here we show that the expression of DJ-1, an antioxidant protein with reduced expression in the central nervous system of patients with Parkinson’s disease, is reduced in pancreatic islets of patients with type 2 diabetes mellitus (T2DM). In contrast, under non-diabetic conditions, DJ-1 expression increases in mouse and human islets during aging. In mouse islets, we show that DJ-1 prevents an increase in reactive oxygen species levels as the mice age. This antioxidant function preserves mitochondrial integrity and physiology, prerequisites for glucose-stimulated insulin secretion. Accordingly, DJ-1-deficient mice develop glucose intolerance and reduced β cell area as they age or gain weight. Our data suggest that DJ-1 is more generally involved in age- and lifestyle-related human diseases and show for the first time that DJ-1 plays a key role in glucose homeostasis and might serve as a novel drug target for T2DM.

Keywords: diabetes, pancreatic islets, DJ-1, mitochondria

Introduction

DJ-1 is a multifunctional, highly conserved antioxidant protein encoded by Parkinson’s disease (PD) gene park7 (Bonifati et al., 2003; Dawson and Dawson, 2003; Guzman et al., 2010; Hong et al., 2010). In neurons, DJ-1 is upregulated upon oxidative stress and protects neurons from mitochondrial dysfunction (Hao et al., 2010; McCoy and Cookson, 2012). Interestingly, in mouse pancreatic islets and mouse insulinoma cells (MIN6), which have a low expression of antioxidant proteins (Tiedge et al., 1997; Robertson and Harmon, 2006; Wiederkehr and Wollheim, 2006; Lenzen, 2008), DJ-1 was recently shown to be specifically upregulated under hyperglycemic conditions in vitro and in vivo (Waanders et al., 2009; Inberg and Linial, 2010).

Pancreatic islets are aggregates of endocrine cells of which β cells form the majority (Maedler, 2008; Collombat et al., 2010). An increase in blood glucose concentration stimulates the β cells to secrete insulin, which lowers blood glucose to normal levels. Pancreatic islets and neurons have many features in common (van Arensbergen et al., 2010; Soltani et al., 2011), and recently it was shown that diabetics have a 40% increased risk of developing PD (Xu et al., 2011), suggesting a possible link between type 2 diabetes mellitus (T2DM) and PD. Here, we studied the role of DJ-1 in pancreatic islet function, glucose homeostasis, and T2DM.

Results

Age-dependent upregulation of DJ-1 in human pancreatic islets and reduced DJ-1 expression in islets from T2DM patients

We first showed that, in the human pancreas, DJ-1 is strongly expressed in islets compared with the surrounding exocrine tissue (Figure 1A–D). In addition, DJ-1 protein expression almost doubled in human pancreatic islets under hyperglycemic conditions (Figure 1E). When comparing islets from 38 to 56 years old and 68 to 81 years old, we also discovered that the mRNA expression of DJ-1 significantly increased in the islets of non-diabetic humans during aging (Figure 1F and Supplementary Table S1). In contrast, DJ-1 expression was significantly reduced on both the mRNA and protein levels in the islets from T2DM patients compared with islets from non-diabetic controls (Figure 1G and H, and Supplementary Tables S1 and S2). The decline in DJ-1 protein expression might have been even larger when age-matched T2DM patients and non-diabetic controls

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observed in islets isolated from DJ-1-deficient mice at the age of 12–13 weeks of age (Figure 2E and F). Although DJ-1 mRNA expression remained unchanged up to the age of 8 weeks, the expression of DJ-1 increased from 8 to 20 weeks of age (Supplementary Figure S1). In contrast, there was no age-dependent change in the mRNA level for any other antioxidant protein we studied, i.e., superoxide dismutase 1 and 2 (sod1, sod2), glutathione peroxidase 1 (gpx1), and uncoupling protein 2 (ucp2) (Supplementary Figure S2).

Since the loss of DJ-1 was previously shown to increase mitochondrial reactive oxygen species (ROS) production in neurons (Andres-Mateos et al., 2007; Guzman et al., 2010), we next studied whether DJ-1 deficiency increased ROS in mouse pancreatic islets. Surprisingly, no significant increase in ROS levels was observed in islets isolated from DJ-1-deficient mice at the age of 8 weeks (Figure 2G), showing that DJ-1 was not constitutively required for lowering ROS levels in islets. In contrast, DJ-1 was explicitly required for lowering ROS levels in mouse islets when the mice were 12–13 weeks of age (Figure 2G, I, and J), suggesting that DJ-1 keeps ROS at constant levels as islets age. Moreover, the levels of ROS could be normalized by culturing DJ-1-deficient islets in the presence of the redox modulator N-acetyl-L-cysteine (NAC) (Figure 2H and K).

Reduced expression of DJ-1 increases mitochondrial ROS production in mouse insulinoma cells

Next, to determine the cellular compartment where ROS accumulated in the absence of DJ-1, we transfected glucose-responsive mouse insulinoma cells (MIN6), a model cell line for β cells, with a redox-sensitive variant of green fluorescent protein (roGFP2) containing a mitochondrial matrix targeting sequence, or with a cytoplasmic roGFP2 (Hanson et al., 2004). Comparison of untreated MIN6 cells with fully reduced [dithiothreitol (DTT)-treated] and fully oxidized (Aldrithiol-treated) cells suggested that MIN6 cells normally contain low levels of ROS in both mitochondria and cytosol (Figure 3A and B). We then lowered DJ-1 protein expression in these cells using two different small interfering RNAs (siRNA) (Supplementary Figure S3). Importantly, a reduced expression of DJ-1 protein in MIN6 cells significantly increased ROS levels in mitochondria as determined in living cells using Laser Scanning Microscopy (LSM) (Figure 3C, and Figure 3E–G vs H–J and K–M). We confirmed the specificity of the knockdown by showing that normal ROS levels could be restored in DJ-1-silenced cells by transfection with a DJ-1 cDNA

had been available for the analysis of human islet lysates. In summary, our data show that DJ-1 expression normally increases in pancreatic islets during aging, but that, in the islets of patients with T2DM, its expression decreases.

Age-dependent requirement of DJ-1 for reducing reactive oxygen species in pancreatic islets

To investigate the role of DJ-1 in vivo, we turned to the mouse and showed that in the mouse pancreas as in the human pancreas, DJ-1 is more strongly expressed in islets compared with the surrounding exocrine tissue (Figure 2A–D). As in humans, mouse DJ-1 expression was significantly higher in islets at both the mRNA and protein levels during aging, as shown by comparing islets taken from mice at 8 and 12–13 weeks of age (Figure 2E and F). Although DJ-1 mRNA expression remained unchanged up to the age of 8 weeks, the expression of DJ-1 increased from 8 to 20 weeks of age (Supplementary Figure S1). In contrast, there was no age-dependent change in the mRNA level for any other antioxidant protein we studied, i.e., superoxide dismutase 1 and 2 (sod1, sod2), glutathione peroxidase 1 (gpx1), and uncoupling protein 2 (ucp2) (Supplementary Figure S2).

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In contrast, cytoplasmic ROS were not significantly increased (Figure 3D). The data suggest that DJ-1 is specifically required for reducing ROS in the mitochondria of insulin-secreting β cells.

Age-dependent requirement of DJ-1 for mitochondrial integrity, function, and glucose-stimulated insulin secretion from pancreatic islets

Since elevated ROS levels can result in mitochondrial fragmentation (Mai et al., 2010; Nikic et al., 2011), we studied whether DJ-1 was required for normal mitochondrial morphology in pancreatic β cells. To this end, we compared the mitochondria of DJ-1-deficient β cells with those of controls in situ (Figure 4A–H). A marked fragmentation was found to accompany the increased ROS production in pancreatic β cells (Figure 4B vs F). While the majority of wild-type mitochondria formed networks in the primary β cells, the mitochondria in DJ-1-deficient β cells were often fragmented (Figure 4A–D vs E–H). Moreover, significantly more MIN6 cells displayed fragmented mitochondria when DJ-1 expression was reduced using siRNA (Supplementary Figure S4A–D and I). However, in the presence of the redox modulator NAC, mitochondrial morphology was rescued (Supplementary Figure S4E–H and J), suggesting that mitochondrial fragmentation arose from defects induced by ROS accumulation in β cells silenced for DJ-1.

As a measure of mitochondrial function, we then determined adenosine triphosphate (ATP) levels in DJ-1-deficient islets as well as in MIN6 cells silenced for DJ-1. In β cells, ATP was shown to be a key metabolic signal from mitochondria that translates elevated nutrient levels into increased insulin secretion (Maechler and Wollheim, 2001; Ashcroft, 2005). Interestingly, ATP levels were not altered in DJ-1-deficient islets under low-glucose conditions (Figure 4I, white columns). In contrast, ATP levels were significantly reduced in DJ-1-deficient MIN6 cells (Figure 4I, black columns).
levels in response to stimulatory high-glucose concentrations were significantly lower in DJ-1-deficient islets compared with controls (Figure 4I, black columns), and addition of NAC partially rescued the ATP production under high-glucose conditions (Figure 4J). Similar results were obtained using MIN6 cells silenced for DJ-1 (Supplementary Figure S4K and L).

Since ATP is an important signal for glucose-stimulated insulin secretion (GSIS; Lowell and Shulman, 2005; Leibiger and Berggren, 2006; Muoio and Newgard, 2008), we studied whether the reduction of ATP production led to reduced GSIS from DJ-1-deficient islets. As shown in Figure 4K, GSIS was not significantly changed in islets isolated from DJ-1-deficient mice at the age of 8 weeks, showing that DJ-1 was not constitutively required for GSIS. In contrast, DJ-1-deficient islets from 12 to 13 weeks old mice showed a significant reduction in GSIS compared with controls (Figure 4K), which was not due to lower insulin content (Figure 4L).

To demonstrate that DJ-1 is required for GSIS at this age because of its effect on mitochondria and the glucose-stimulated increase in ATP levels, we tested glibenclamide-induced insulin secretion from DJ-1-deficient islets taken from 12 to 13 weeks old mice (Figure 4M). Glibenclamide, a sulphonylurea, is one of the two oral anti-diabetic drugs in the WHO list of essential medicines for treatment of T2DM. Like other sulphonylureas, it closes the KATP channels of b cells independent of an increase in ATP levels (Ashcroft, 2005). Importantly, glibenclamide was found to induce insulin secretion from pancreatic islets regardless of the presence of DJ-1 (Figure 4M, grey columns), suggesting that DJ-1 acts upstream of KATP channels in pancreatic b cells. Since islets harbor endocrine cells other than b cells (Thorel et al., 2010), we also used MIN6 cells to corroborate our results obtained with pancreatic islets (Supplementary Figure S5). In these cells, DJ-1 was similarly required for glucose-stimulated rather than glibenclamide-induced insulin secretion (Supplementary Figure S5A and B).

Age- and diet-dependent glucose intolerance and reduced b cell area in the absence of DJ-1

Glucose intolerance can be a consequence of b cell dysfunction and/or reduced b cell area (Ueki et al., 2006; Poy et al., 2009; Sachdeva et al., 2009; Liew et al., 2010; Esterhazy et al., 2011). Since we observed reduced b cell function in DJ-1-deficient animals, we evaluated glucose tolerance and b cell area in
these animals (Figure 5). Consistent with the age-dependent changes in islet physiology, DJ-1-deficient mice did not show any defect in glucose-stimulated plasma insulin (Figure 5A), glucose tolerance (Figure 5B), and β cell area (Figure 5C) at the age of 8 weeks. At 12–13 weeks of age, however, DJ-1-deficient mice had significantly lower levels of plasma insulin after glucose challenge (Figure 5D), displayed glucose intolerance (Figure 5E), and had a small but significant reduction in β cell area (Figure 5F).

To investigate whether glucose intolerance could also be induced in younger animals, we placed 6 weeks old mice on a high-fat diet for 2 weeks. DJ-1 mRNA and protein levels were elevated in the islets from these mice compared with islets from mice kept on a chow diet (Supplementary Figure S6). In contrast, there was no diet-dependent change in mRNA level for any other antioxidant protein we studied (Supplementary Figure S6A). Importantly, plasma insulin was significantly reduced upon glucose challenge in the 8 weeks old mice that had been kept

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**Figure 4** Age-dependent requirement of DJ-1 for mitochondrial integrity, function and GSIS from mouse pancreatic islets. (A–H) LSM images of islets in the pancreas of control (A–D) and DJ-1-deficient mice (E–H) stained with DAPI (A and E), anti-VDAC1 antibodies (B and F), and anti-insulin antibodies (C, G). Merged images are shown in D and H. Scale bars, 10 μm. (I and J) ATP production in islets from control and DJ-1-deficient mice. Data are presented as percentage of basal control in the absence (I) or presence of NAC (J). n = 3. (K) Insulin secretion (normalized to total insulin content) from mouse islets at a low (2 mM) and a high (25 mM) glucose concentration. Islets were isolated from DJ-1-deficient mice and controls at 8 and 12–13 weeks of age. n = 3. (L) Total insulin content (normalized to total protein content) of islets isolated from DJ-1-deficient mice and controls at 8 and 12–13 weeks of age. n = 3. (M) Insulin secretion (normalized to total insulin content) from mouse islets at a low-glucose concentration (2 mM), a high-glucose concentration (25 mM), and at a low-glucose concentration (2 mM) in the presence of 1 μM glibenclamide. n = 3. *P < 0.05 (two-tailed Student’s t-test). All values are means ± SD.
onset of PD (Bonifati et al. 2003). In neurons, DJ-1 deficiency was shown to increase ROS as well as mitochondrial fragmentation and dysfunction (Surmeier et al. 2011). DJ-1 is a highly conserved protein that is expressed in bacteria, plants, and animals (Lin et al. 2011). A few different scenarios have been proposed for how DJ-1 reduces high ROS levels or oxidative stress in cells. For example, DJ-1 has a conserved cysteine residue (Cys106), which allows DJ-1 to act both as an ROS scavenger and sensor of cellular redox homeostasis (Canet-Aviles et al. 2004). It has also been suggested that DJ-1 acts as a transcriptional co-activator (Inberg and Linial 2010), stabilizes the NF-E2-related factor (Nrf2) (Clements et al. 2006), and is an integral mitochondrial protein that maintains the activity of mitochondrial complex I in mammalian cells (Hayashi et al. 2009). In plant cells, it was recently shown that DJ-1 interacts with glutathione peroxidase (gpx) and superoxide dismutase 1 (SOD1), and activates the latter (Xu et al. 2010).

We have shown previously that the DJ-1 protein is significantly upregulated in the islets of mice when they are fed with a high-fat diet for 2 weeks (Figure 5A vs G). Additionally, glucose intolerance was detected (Figure 5B vs H), and the β cell area of the fat mice was reduced to a small but significant extent compared with that of the age-matched controls that had been kept on a chow diet (Figure 5C vs I). This metabolic phenotype was observed in male mice (Figure 5) and, to a lesser but significant extent, in female mice (Supplementary Figure S7). The data show that DJ-1 is specifically required for GSIS, normal β cell area and glucose tolerance during aging and on a high-fat diet.

**Discussion**

DJ-1 is an antioxidant protein, and its loss leads to an early onset of PD (Bonifati et al. 2003). In neurons, DJ-1 deficiency was shown to increase ROS as well as mitochondrial fragmentation and dysfunction (Surmeier et al. 2011). DJ-1 is a highly conserved protein that is expressed in bacteria, plants, and animals (Lin et al. 2011). A few different scenarios have been proposed for how DJ-1 reduces high ROS levels or oxidative stress in cells. For example, DJ-1 has a conserved cysteine residue (Cys106), which allows DJ-1 to act both as an ROS scavenger and sensor of cellular redox homeostasis (Canet-Aviles et al. 2004). It has also been suggested that DJ-1 acts as a transcriptional co-activator (Inberg and Linial 2010), stabilizes the NF-E2-related factor (Nrf2) (Clements et al. 2006), and is an integral mitochondrial protein that maintains the activity of mitochondrial complex I in mammalian cells (Hayashi et al. 2009). In plant cells, it was recently shown that DJ-1 interacts with glutathione peroxidase (gpx) and superoxide dismutase 1 (SOD1), and activates the latter (Xu et al. 2010).

We have shown previously that the DJ-1 protein is significantly upregulated in the islets of mice when they are fed with a high-fat diet.
diet and become hyperglycemic (Waanders et al., 2009). In addition, DJ-1 was found to be upregulated in mouse islets cultured in vitro under hyperglycemic conditions (Waanders et al., 2009; Inberg and Linial, 2010). However, the role of DJ-1 in maintaining pancreatic islet function and glucose homeostasis in vivo remained unknown. Furthermore, no information was available on the role of DJ-1 in islets during aging and on a high-fat diet. The data of this study now suggest that DJ-1 is required to maintain physiological ROS levels in an age- and diet-dependent manner (Figure 5).

Physiological levels of ROS are important to maintain glucose homeostasis, whereas prolonged exposure to high ROS levels results in chronic oxidative stress and impaired glucose homeostasis (Robertson, 2004; Wang and Hai, 2011). Importantly, pancreatic islets are particularly vulnerable to oxidative stress as they express low levels of antioxidant proteins (Lenzen et al., 1996; Tiedge et al., 1997). In addition, increased ROS levels are observed in animal models of T2DM (i.e. db/db mice, GK rats, and ZDF rats) and human T2DM patients (Ihara et al., 1999; Kaneto et al., 1999; Tanaka et al., 1999).

Mitochondria are a major source of ROS, and mutations in mitochondrial DNA in humans and β-cell-specific deletion of mitochondrial genes in animal models cause T2DM in humans and mice, respectively (Silva et al., 2000; Maassen et al., 2004). Our data point to mitochondria as a main site of elevated ROS production in pancreatic β cells during aging, and we show that DJ-1 is required to reduce mitochondrial fragmentation in pancreatic β cells during aging and on a high-fat diet. This fragmentation might contribute to the reduced ATP levels and impaired GSIS observed in DJ-1-deficient islets, as mitochondrial fission proteins have been shown to influence ATP levels and GSIS in pancreatic islets and β cell lines (Park et al., 2008; Twig et al., 2008).

Oxidative stress and mitochondrial dysfunction are also the hallmarks of PD (Surmeier et al., 2011), and a recent report indicates an ~40% increase in prevalence of PD in T2DM patients (Xu et al., 2011). In turn, several studies reveal glucose intolerance in patients with PD (Aviles-Olmos et al., 2012). The link between PD and T2DM might lie in the disruption of common molecular mechanisms. Alternatively, PD may simply result from hyperglycemia, hyperinsulinemia, or both. In support of the first scenario, we showed that DJ-1 is not only expressed in the substantia nigra, which gets functionally impaired during PD (Surmeier et al., 2011), but is also expressed in human pancreatic islets, which become dysfunctional during T2DM. Similar to the situation in PD where the concentration of DJ-1 decreases in the cerebrospinal fluid (Hong et al., 2010), we found that in the islets of elderly T2DM patients, DJ-1 expression is decreased compared with age-matched controls. A possible reason for a lower expression of DJ-1 during T2DM might be an epigenetic silencing, since several genes have been shown to be epigenetically downregulated in the islets from T2DM patients (Ling et al., 2008; Yang et al., 2011).

Since DJ-1 regulates the androgen receptor (Niki et al., 2003) that binds to the tyrosine hydroxylase (TH) promoter, active in both the neurons of the substantia nigra and the beta cells of pancreatic islets, we also studied whether gender-specific differences in DJ-1 gene expression could be detected. Interestingly, 74 ± 7 years old females expressed significantly more DJ-1 in their pancreatic islets than 79 ± 3 years old males (Supplementary Table S3), a finding that might help us to explain gender-specific differences in the incidence and phenotype of PD and T2DM, respectively (Ding et al., 2006; Wirdefeldt et al., 2011).

Based on our findings, we now propose that DJ-1 expression increases in pancreatic β cells upon aging and a high-fat diet to prevent a pathological increase in mitochondrial ROS levels (Figure 5), right panel). This function helps in preserving mitochondrial morphology and physiology during aging and weight gain, thus maintaining normal ATP-dependent GSIS and glucose tolerance (Figure 5), right panel). In contrast, when DJ-1 expression fails to increase, ROS levels begin to increase beyond a normal level, thereby altering mitochondrial morphology and function in pancreatic islets. This results in glucose intolerance, a first step in the development of T2DM (Figure 5), left panel). Since we identified T2DM as a human disease where DJ-1 expression is significantly reduced in elderly humans, similar to the situation observed in PD, our data point to a more general role of the oxidative stress modulator DJ-1 in age- and lifestyle-related human diseases.

Finally, our findings suggest that DJ-1 expression could be targeted for the treatment of T2DM. Along this line, it has recently been shown that the histone deacetylase inhibitor, phenylbutyrate increases the expression of DJ-1 by 300% in the N27 dopamine cell line and that it is useful for the treatment of both PD and glucose intolerance (Ozcan et al., 2006; Xiao et al., 2011; Zhou et al., 2011). Besides phenylbutyrate, substances have been developed to specifically increase the activity of DJ-1 by preventing its hyperoxidation and inactivation (Inden et al., 2011; Kitamura et al., 2011). Since we showed that upregulation of DJ-1 in pancreatic islets is key to maintain GSIS and blood glucose levels during aging and weight gain, our study now warrants investigations on compounds that increase DJ-1 expression or activity for the treatment of T2DM, the most common metabolic disease worldwide.

Materials and methods
Human islet isolation and analysis of DJ-1 expression
Human pancreatic islets were prepared with the approval of the local ethics committees from the pancreases of multi-organ donors by collagenase digestion followed by density gradient purification. Then, the islets were maintained in M199 culture medium, containing 5.5 mM glucose, supplemented with 10% (v/v) serum and 100 U/ml penicillin, 100 mg/ml streptomycin, 50 mg/ml gentamicin, and 750 ng/ml amphotericin B (Sigma-Aldrich). Total RNA was extracted, quantified, and its integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies). For semiquantitative RT–PCR experiments, 1 µg total RNA was reverse transcribed with iScript cDNA Synthesis Kit (Bio-Rad Laboratories), and the following primers were used for PCR: DJ-qPCR_huRP1 (5′-GACCACATACGCTACT-3′) and DJ-1-qPCR_huFP1 (5′-GAGCTGAGATTAAGGCAC-3′). The level of mRNA for DJ-1 was quantified using an Applied Biosystems 7700 Bioanalyzer (Applied Biosystems) and normalized to the housekeeping gene hypoxanthine-guanine phosphoribosyltransferase. DJ-1 and β-actin protein levels were measured in human islet lysates using specific enzyme-linked immunosorbent assay
Cell culture and RNA interference

MIN6 cells (Miyazaki et al., 1990) were used from passage 29–35 and were electroporated via nucleofection (Lonza) according to the manufacturer’s instructions with either pEGFP-DJ-1 or pc-DNA3-FLAG-DJ-1 plasmid (kindly provided by Dr Hiroyoshi Ariga at the University of Hokkaido, Japan) and/or siRNA. siRNA against DJ-1 was prepared as previously described (Nikolova et al., 2006; Konstantinova et al., 2007), and knockdown efficiencies were tested using western blots.

Insulin secretion and content

Islets and MIN6 cells were maintained at 37°C for 1 h in 2 mM glucose, 25 mM glucose, or 2 mM glucose with 1 μM glibenclamide in Krebs-Ringer HEPES buffer. The amount of insulin secreted into the buffer was measured using an ultrasensitive rat insulin ELISA (Crystal Chem.). Total insulin content was determined in lysates of islets or MIN6 cells using the same ELISA kit.

Measurement of ROS levels

ROS levels were measured in islets using the ROS-sensitive fluorescent dye CM-H2DCFDA (Invitrogen). After starvation in 2 mM glucose, islets were stimulated with 25 mM glucose containing 10 μM CM-H2DCFDA. Islets were homogenized and after a centrifugation step, the fluorescence was measured in the supernatant using a microplate reader (Tecan, infinite M1000).

The fluorescence intensity was also detected in intact pancreatic islets using an LSM 710 confocal microscope with an excitation wavelength of 488 nm and emission wavelength at 520 nm. In addition, ROS levels were measured in MIN6 cells co-transfected with roGFP2 plasmid (kindly provided by the University of Oregon, Eugene, OR, USA) and siRNA against DJ-1 or control siRNA. Cells were starved for 1 h in KRH buffer containing 2 mM glucose and then treated with 25 mM glucose, 25 mM glucose containing 10 mM DTT to reduce or 500 μM Alidri-thiol-2 (Ald) to oxidize the samples. The fluorescence intensities (I) of cells were measured at excitation wavelengths of 405 and 488 nm and emission wavelengths of 530 nm using an LSM 710 confocal microscope. The fluorescence intensities were determined using ImageJ, and the degree of oxidation of roGFP2 was determined using fluorescence measurements after fully reducing the samples with DTT and then fully oxidizing with Alidri-thiol-2 using

\[ \text{OxD}_{\text{roGFP2}} = R - R_{\text{red}} / [I_{488,\text{ox}} / I_{488,\text{red}} (R_{\text{ox}} - R) + (R - R_{\text{red}})] \]

(Meyer and Dick, 2010), where R is the ratio of the fluorescence intensities of the sample at the excitation wavelengths of 405 and 488 nm, R_{red} is the ratio of the intensities of the fully reduced sample at the excitation wavelengths of 405 and 488 nm, and R_{ox} is the ratio of the intensities of the fully oxidized sample at the excitation wavelengths of 405 and 488 nm. I_{488,\text{ox}} and I_{488,\text{red}} are the fluorescence intensities at the excitation wavelength of 488 nm of the fully oxidized and fully reduced sample, respectively.

Measurement of cellular ATP levels

Cellular ATP levels were measured using the ApoSENSOR ATP assay kit (Biovision) according to the manufacturer’s instructions. Luminescence was determined using an LB9507 luminometer (Berthold Technologies) and normalized to total protein levels.

Supplementary material

Supplementary material is available at Journal of Molecular Cell Biology online.

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Conflict of interest: none declared.

References


Role of DJ−1 in type 2 diabetes


