Title

Evidence for a role for the putative *Drosophila* hGRX1 orthologue in copper homeostasis

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Abstract

Glutaredoxins are a family of small molecular weight proteins that have a central role in cellular redox regulation. Human GRX1 (hGRX1) has also been shown to play an integral role in copper homeostasis by regulating the redox activity of the metalated sites of copper chaperones such as ATOX1 and SOD1, and the copper efflux proteins ATP7A and ATP7B. To further elucidate the role of hGRX1 in copper homeostasis, we examined the impact of RNA interference-mediated knockdown of CG6852, a putative *Drosophila* orthologue of hGRX1. CG6852 shares ~41% amino acid identity with hGRX1 and key functional domains including the metal-binding CXXC motif are conserved between the two proteins. Knockdown of *CG6852* in the adult midline caused a thoracic cleft and reduced scutellum, phenotypes that were exacerbated by additional knockdown of copper uptake transporters *Ctr1A* and *Ctr1B*. Knockdown of *CG6852* in the adult eye enhanced a copper-deficiency phenotype caused by *Ctr1A* knockdown while ubiquitous knockdown of *CG6852* resulted a mild systemic copper deficiency. Therefore we conclude that CG6852 is a putative orthologue of hGRX1 and may play an important role in *Drosophila* copper homeostasis.

Keywords

Drosophila, hGRX1, Copper, CTR1, ATP7

Abbreviations

SOD1 - superoxide dismutase 1; Cu - copper, BCS - bathocuproine disulfonate, PBS – phosphate buffered saline; COX – cytochrome c oxidase; *Pannier* (*PNR*); GSH – Glutathione.

Introduction

Copper is an essential trace element which functions as a cofactor for many enzymes, such as cytochrome c oxidase (COX), Cu, Zn-superoxide dismutase (SOD1), lysyl oxidase and dopamine β -monooxygenase (Linder 2002). The role of copper as an enzymatic cofactor stems from its ability to easily switch between cuprous (Cu^+) and cupric (Cu^{2+}) forms, making copper an efficient mediator of electron transfer (Kaim and Rall 1996). Despite being critical for the activity of a number of enzymes involved in a broad range of cellular processes, uncontrolled accumulation of free copper ions within cells is known to be toxic, due to the propensity of unbound copper to generate damaging free radicals. Therefore sophisticated homeostatic mechanisms have evolved to allow the required amount of copper to be delivered to cuproproteins while excess copper is either safely bound or removed from cells in order to protect against copper-induced oxidative stress (Valko, Morris et al. 2005).

Proteins involved in maintaining copper homeostasis have been well conserved throughout evolution, as seen by the similarity of copper transport proteins in yeast, insect and mammalian systems (Camakaris, Voskoboinik et al. 1999). Copper uptake into cells is mediated primarily by the Ctr family of proteins. Once copper enters the cytosol it is bound by copper chaperones which deliver copper to specific intracellular organelles or proteins. For instance in human cells, CCS delivers copper to SOD1 in the cytosol. ATOX1, in contrast, is required to deliver copper to the P-type ATPase proteins ATP7A and ATP7B which are localized to the *trans* Golgi network under basal copper conditions where they supply copper to the cuproenzymes of the secretory pathway. Copper transport to the mitochondria, required for the metalation of cytochrome c oxidase, is facilitated by chaperones such as COX17 and SCOI (Huffman and O'Halloran 2001, Walker 2002, Luk, Jensen et al. 2003, Cobine, Pierrel et al. 2006).

To date, three Ctr proteins have been identified in *Drosophila melanogaster* (Ctr1A, Ctr1B and Ctr1C), with Ctr1A and Ctr1B both having critical roles in copper uptake (Zhou, Cadigan et al. 2003, Southon, Burke et al. 2004, Turski and Thiele 2007). Most mammalian copper chaperones also have a direct *Drosophila* orthologue (Southon, Burke et al. 2004). *Drosophila* has only one ATP7A / ATP7B homologue, ATP7, which has been shown to have a copper efflux function analogous to that of its mammalian counterparts and is critical for normal *Drosophila* development (Norgate 2005, Southon, Palstra et al. 2010).

hGRX1 is a small (12kDa) protein that belongs to the thioredoxin family of cellular redox regulators. One of the main roles of GRX proteins is to reactivate proteins by de-glutathionylation of key cysteine thiols, allowing these proteins to once more bind their targets. This process of addition and removal of glutathione (GSH) from target proteins acts as a molecular switch and is essential in preventing the irreversible oxidation of these cysteine thiols (Dalle-Donne, Rossi et al. 2007). Glutathionylation is therefore a post-translational modification of proteins, similar to phosphorylation.

hGRX1 is referred to as a dithiol oxidoreductase and like other classical glutaredoxins, catalyses GSH-disulphide oxidoreductions via two redox active cysteines that are always separated by two other amino acids, giving the CXXC motif (Fernandes and Holmgren 2004, Lillig and Berndt 2013). More than 200 mammalian proteins have been found to undergo post-translational modification via thiol/disulphide exchange with GSH (Fratelli, Gianazza et al. 2004, Mieyal and Chock 2012) and changes in glutathionylation have been linked with a growing list of diseases. For instance, increased glutathionylation has been demonstrated in Alzheimer's disease (Dinoto, Deture et al. 2005, Newman, Sultana et al. 2007, Domenico, Cenini et al. 2009), Parkinson's disease (Bharath, Hsu et al. 2002) and a number of inflammatory diseases (Shelton, Chock et al. 2005, Janssen-Heininger, Aesif et al. 2010, Coppo and Ghezzi 2011).

hGRX1 is required to de-glutathionylate ATP7A and ATP7B by removing GSH from the cysteine residues of the metal-binding sites of these proteins. Interaction of hGRX1 with the N-termini of ATP7A and ATP7B was found to be copper dependent (Lim, Cater et al. 2006). Targeted knockdown of *hGRX1* in HEK293T cells inhibited copper-stimulated translocation of ATP7A and ATP7B from the Golgi apparatus to the plasma membrane, resulting in increased accumulation of copper within the cytosol (Singleton, McInnes et al. 2010) and indicating that glutathionylation is critical in regulating the copper efflux activity of these proteins. Furthermore, overexpression of *hGRX1* has been found to provide cells with an increased tolerance to high copper levels, indicating that hGRX1 may be involved in binding intracellular copper (Cater, Materia et al. 2014, De Benedetto, Capo et al. 2014), for which it has a very high affinity (Hatori, Clasen et al. 2012, Brose, La Fontaine et al. 2014).

As the role of hGRX1 in copper regulation is yet to be examined *in vivo*, we sought to characterise the function of one of the two possible *Drosophila hGRX1* homologues, the as-yet-unnamed gene *CG6852*. Targeted RNAi-mediated knockdown of *CG6852* resulted in

phenotypes previously associated with copper deficiency. When combined with genetically induced copper deficiency, these phenotypes were further exacerbated. We therefore propose that the *Drosophila* gene *CG6852* encodes the orthologue of hGRX1. Our results support the notion that hGRX1 may be an important mediator of intracellular copper metabolism.

Materials and Methods

Drosophila stocks

The following fly stocks were used: *w*¹¹¹⁸ (BL (Bloomington *Drosophila* Stock Centre, Bloomington, IN, USA, 3605); *GMR-GAL4* (BL9146); *Pannier (PNR)-GAL4* (BL3039); *TUB-GAL4*, *ACTIN-GAL4* and *DA-GAL4*; overexpression lines used included *UAS-ATP7^{FLAG}*, *UAS-Ctr1A^{FLAG}* and *UAS-Ctr1B^{FLAG}* which have been described previously (Norgate 2005, Binks, Lye et al. 2010) and RNAi lines obtained from the Vienna *Drosophila* RNAi Center included V26001 (*CG6852*), V8315 (*ATP7*), V46757 (*Ctr1A* – strong (s)), V46758 (*Ctr1A* – weak (w)) and V5805 (*Ctr1B*).

Drosophila maintenance

All *Drosophila* stocks and crosses were maintained on standard medium at 25°C unless stated otherwise. Standard medium was supplemented with either 500 µM bathocuproine disulfonate (BCS; Sigma-Aldrich) to make copper-deficient food medium or copper sulphate (CuSO₄·5H₂O; Sigma-Aldrich) to make copper-supplemented medium.

Drosophila survival experiments

Crosses were set up in cages with 50 virgin females and 20-25 males. For adult survival experiments, replicates of 50 first instar larvae were placed onto basal media or media supplemented with copper or BCS. Successful adult survival was classed as the ability of the adults to eclose from their pupal cases and if not otherwise mentioned, the experimental genotypes were normalised to the *w*¹¹¹⁸ (WT) mean survival on each food type. For pupal and adult survival assays, n≥3 for each food type.

Microscopy

For phenotypic analysis, adult flies were partially dissected, then mounted directly onto plasticine and monitored with a Leica MZ6 stereomicroscope. All images were recorded with Leica DC300 digital camera using Leica Application Suite software.

Quantitation of RNAi knockdown efficacy

mRNA was extracted using TRIsure Reagent (Bioline) from 10 whole adult flies of the following genotypes: (1) *DA>w*¹¹¹⁸ and (2) *DA>CG6852^{RNAi}*. cDNA was generated using the Tetro cDNA synthesis kit (Bioline). *CG6852* transcript levels were determined by semi

quantitative RT-PCR using equal concentrations of cDNA from *DA>w¹¹¹⁸* and *DA>CG6852^{RNAi}* cDNA. PCR reactions were assembled using GoTaq® Green Master Mix (Promega). Housekeeping gene *Rp49* was used as an endogenous control in both experiments. Band intensity quantitation was completed using Image J Fiji gels utility, which allows one to plot and measure the peak area of each respective band on a gel. Initially the peak area of *DA>CG6852^{RNAi}* and *DA>w¹¹¹⁸* were normalised against the peak area of the *Rp49* house keeper gene for each respective genotype. The peak area values of each genotype were then normalised against the average peak area of *DA>w¹¹¹⁸*. Oligonucleotide sequences available upon request.

Statistical analysis

Statistical analyses were conducted with Prism 6 (GraphPad Software). Quantitative data is presented as the mean with S.E.M and $p < 0.05$ was considered to be statistically significant.

Results

The key domains for hGRX1 function are conserved between hGRX1 and its putative *Drosophila* orthologue, CG6852

The *Drosophila* genome encodes two proteins homologous to hGRX1, the previously named Grx-1 (CG7975) and the unnamed CG6852. Protein sequence alignments revealed CG6852 to be marginally more conserved with hGRX1 than CG7975; while both fly proteins were equally similar to hGrx1, CG6852 shares 41.3% identity compared to CG7975's 39.1%. The CPYC motif containing the redox-active cysteines is conserved in both CG6852 and CG7975, whereas the thioredoxin domain is completely conserved in CG6852 but slightly divergent in CG7975 (Fig. 1). Importantly, high-throughput gene expression analysis shows CG6852 to have a broad expression pattern, in contrast to CG7975 whose expression is limited to the testes (dos Santos, Schroeder et al. 2014). Therefore CG6852 is more likely to be the true *Drosophila* orthologue of hGRX1 which is also expressed extensively in mice and humans and we believe the previous naming of CG7975 as Grx-1 is misleading.

Knockdown of CG6852 in both the adult thoracic midline and the adult eye results in copper deficiency-associated phenotypes

To investigate any *in vivo* functions of CG6852, a UAS-RNA interference (RNAi) line targeting this gene was used in conjunction with a number of ubiquitous and tissue-specific GAL4 lines. Semi-quantitative RT-PCR analysis showed that CG6852 knockdown reduced CG6852 mRNA levels to ~20% of control levels in whole adults (Supp. Fig. 1). Strong ubiquitous knockdown of CG6852 with both *TUB-GAL4* and *ACTIN-GAL4* caused a fully penetrant lethality, primarily at the pupal stage of development.

Targeted suppression of CG6852 in the adult thoracic midline using *PNR-GAL4* caused a prominent thoracic cleft and reduced scutellum (Fig. 2B) in contrast to *PNR-GAL4*-only control flies (Fig. 2A). To investigate whether the *PNR*>*CG6852*^{RNAi} phenotype was a result of copper dyshomeostasis, genetic interaction crosses were set up with knockdown and overexpression lines for known copper transporters. Co-expression of *CG6852*^{RNAi} with *ATP7*^{RNAi} (Fig. 2C) resulted in an additive phenotype, showing both the thoracic cleft caused by *CG6852*^{RNAi} and the hypo-pigmentation phenotype previously demonstrated for *PNR*>*ATP7*^{RNAi} flies (Fig. 2D) (Binks, Lye et al. 2010). A combination of *CG6852*^{RNAi} and *ATP7*^{FLAG} resulted in a worsened thoracic cleft than either *CG6852*^{RNAi} or *ATP7*^{FLAG} alone

with complete loss of the scutellum (Fig. 2E) in comparison to *PNR>ATP7^{FLAG}* flies which exhibited hypopigmentation and a mild cleft (Fig. 2F) as previously published (Norgate 2005, Binks, Lye et al. 2010). When *CG6852^{RNAi}* was combined with knockdown of either *Ctr1A* (*Ctr1A^{wRNAi}* (Binks, Lye et al. 2010)) or *Ctr1B^{RNAi}*, in both cases this resulted in an exacerbation of the thoracic cleft and a smaller scutellum (Fig. 2G, Fig. 2I). As previously reported (Binks, Lye et al. 2010), moderate RNAi knockdown of *Ctr1A* resulted in hypopigmentation down the thorax and abdomen, whereas knockdown of *Ctr1B* caused no observable defects (Fig. 2H and J). Overexpression of *Ctr1B* caused no phenotype and did not alter the phenotype of *CG6852^{RNAi}* flies (Fig. 2L, K).

Knockdown of *CG6852* in the adult eye, using *GMR-GAL4* (Fig. 3B), caused a very mild disruption to the normal ommatidial arrays observed in the wild type adult eye (Fig. 3A). Copper deficiency in the adult eye, induced by knockdown of *Ctr1A* using a strong (s) RNAi line, has been previously shown to result in a smaller, flattened eye with necrotic patches (Binks, Lye et al. 2010), which was replicated in this study (Fig. 3C). A combination of *CG6852* and *Ctr1A* knockdown resulted in a dramatically smaller eye, with patches of necrosis, a glazed appearance and some loss of pigmentation (Fig. 3D). This result indicated a possible genetic interaction between *CG6852* and *Ctr1A*.

Weak ubiquitous suppression of *CG6852* induces a susceptibility to copper deficiency

As described above, strong ubiquitous suppression with both *TUB-GAL4* and *ACTIN-GAL4* of *CG6852* causes pupal lethality. In order to further evaluate the role of *CG6852* in *Drosophila* systemic copper homeostasis, the weak ubiquitous GAL4 driver *DA-GAL4* was used. *DA>CG6852^{RNAi}* flies had a mean survival rate to adulthood on basal media (NF) of $75 \pm 8\%$ when normalised to the survival of control *DA>w¹¹¹⁸* animals (Fig. 4). The survival rate of *DA>CG6852^{RNAi}* flies was reduced on BCS-supplemented media and elevated by copper supplementation but only the comparison between low- and high-copper survival rates showed a significant difference. These results indicate that knockdown of *CG6852* may result in a decrease in copper accumulation, leading to increased sensitivity to copper deficiency.

Discussion

The major factors involved in the control of intracellular copper levels are well established, particularly in relation to the trade-off between the requirement of copper as an enzymatic co-factor and the prevention of toxic accumulation of unbound intracellular copper (Maehara, Ogasawara et al. 1983, Klinman 2005). There is, however, considerable uncertainty surrounding the question of how copper is delivered to intracellular copper chaperones.

Copper transfer from the Ctr proteins to chaperones could be direct, or mediated via an additional factor. Glutathione (GSH) has been shown to facilitate copper uptake (Maryon, Molloy et al. 2013); therefore it is plausible that the glutathionylation machinery, which includes hGRX1, may have a role as a key mediator of intracellular copper levels. In this study we show that knockdown of the putative *Drosophila* orthologue of *hGRX1*, *CG6852*, results in the exacerbation of copper deficiency phenotypes, providing evidence that loss of *CG6852* activity *in vivo* may result in intracellular copper dyshomeostasis.

There are two putative *Drosophila* orthologues of hGRX1 protein, *CG6852* and *CG7975* (dos Santos, Schroeder et al. 2014); we contend that *CG6852* is the more plausible candidate due to its broader expression pattern and slightly higher conservation with hGRX1. Both fly hGRX1 homologues could be classified as classical glutaredoxins as they both possess a CXXC motif, which is predicted to catalyse GSH-disulphide oxidoreductions via the two redox-active cysteines (Fernandes and Holmgren 2004, Lillig and Berndt 2013). The close homology (68.1% identity) and distinct expression patterns of *CG6852* and *CG7975* suggest they may be playing similar cellular roles in specific tissues.

The thoracic cleft and reduced scutellum phenotypes resulting from *CG6852* knockdown with *PNR-GAL4* are similar to the copper deficiency phenotypes caused by *ATP7* overexpression (Norgate 2005, Binks, Lye et al. 2010), albeit without the typical loss of pigmentation seen in *PNR>ATP7^{FLAG}* flies (Binks, Lye et al. 2010). Genetic interaction experiments combining *CG6852* knockdown with *ATP7* over-expression or knockdown both produced hybrid phenotypes that could be considered additive, providing no clear evidence of direct interactions between these two genes. However the considerable exacerbation of the thoracic cleft observed when *Ctr1A* or *Ctr1B* knockdown was combined by *CG6852* knockdown is indicative of a genetic interaction; alone, mild *Ctr1A* knockdown only confers loss of pigmentation whereas *Ctr1B* knockdown has no phenotypic effect whatsoever. Further

evidence is seen in the strong exacerbation of the *Ctr1A* knockdown phenotype in the adult eye upon *CG6852* knockdown.

Two interpretations of the interactions between *CG6852* and *Ctr1A/B* are possible: 1) *CG6852* knockdown is causing a mild / moderate reduction in cell viability which is aggravated by the copper deficiency resulting from reduced copper uptake; or 2) *CG6852* is contributing to cellular copper uptake or distribution. Our observation that ubiquitous *CG6852* knockdown sensitizes larvae to systemic copper deficiency supports the second notion, that this *Grx1* orthologue plays a role in copper accumulation or distribution.

CG6852 may be involved in the copper uptake process via *Ctr1A* and *Ctr1B*. GSH has been shown to be required for facilitating copper uptake into mammalian cells via *Ctr1* (Maryon, Molloy et al. 2013). Furthermore, overexpression of hGRX1 *in vitro* can cause an increase in *Ctr1* protein levels, possibly due to increased copper sequestration by hGRX1 (De Benedetto, Capo et al. 2014). Therefore it is plausible that in *Drosophila*, the glutathionylation machinery, *CG6852* included, could be controlling the flow of copper into cells based on their redox environment. The exact mechanism by which GSH facilitates copper uptake has not been elucidated, as there is currently no evidence that *Ctr* proteins undergo glutathionylation.

Alternatively, *CG6852* could be acting as a mediator of intracellular copper distribution. It has been previously proposed that GSH balance within human cells, which requires hGRX1 function, is a key factor in the regulation of the copper transport machinery (Hatori, Clasen et al. 2012). hGRX1 regulates the copper binding activity of the chaperone Atox1 by catalysing the oxidation of the redox active cysteines in Atox1's CXXC motif (Brose, La Fontaine et al. 2014). Grx1 has also been shown to be involved in reducing the disulphide bond of human SOD-1 in a CCS independent manner (Carroll, Girouard et al. 2004, Bouldin, Darch et al. 2012). Therefore it is possible that *Drosophila CG6852* may be functioning in a similar fashion, regulating intracellular copper distribution by modifying the activity of central copper chaperones.

The absence of pigmentation defects in the *CG6852* knockdown thorax and abdomen implies that only a subset of cellular copper is affected by loss of *CG6852* and that sufficient Golgi copper is retained in affected cells to maintain normal pigment-forming activity of the cuproenzyme laccase 2. We have previously shown that reducing copper transport to the mitochondria via knockdown of the chaperone *Sco1* results in a thin-bristle phenotype typical

of dominant loss of function mutations in ribosomal proteins (Binks, Lye et al. 2010). Bristle morphology also appears normal under *CG6852* knockdown conditions, implying that mitochondrial copper is also unaffected.

The thoracic cleft / reduced scutellum phenotypes are indicative of loss of underlying epithelial cells due to apoptosis, suggesting that *CG6852* knockdown may be resulting in insufficient copper levels in a particular cellular compartment or pathway that is critical for cell survival. While caution must be taken in comparing between cell types and / or organisms, cultured MEFs from *Grx1* knockout mice were found, in contrast, to have increased intracellular copper levels and a consequent sensitivity to copper supplementation (Cater, Materia et al. 2014). One way to reconcile these apparently conflicting data would be to envisage a situation where reduction in *Grx1* causes a redistribution of cellular copper; a localized deficiency would then trigger an increased uptake response in murine MEFs leading to higher copper levels and increased copper sensitivity. In the developing fly thorax and eye, such compensatory uptake mechanisms may not exist or there may be insufficient levels of copper in the circulation to redress the localized deficiency. Weak ubiquitous knockdown of *CG6852* resulted in a sensitivity to copper depletion, a result consistent with genetic interactions seen between *Ctr1A/B* and *CG6852* and indicating that loss of *CG6852* may be causing a mild systemic copper deficiency.

The results presented in this study provide evidence that the *Drosophila* Grx protein, *CG6852* is the true orthologue of hGRX1 and shed light on the potentially important role of hGRX1 as a mediator of copper distribution within cells.

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Figure Legends

Figure 1. Key functional domains of hGRX1 are conserved in *Drosophila* Grx protein CG6852 and *Drosophila* Grx-1 protein

Polypeptide sequence alignment between hGRX1, the putative *Drosophila* Grx1 orthologue CG6852 and the *Drosophila* Grx-1 protein revealed conservation of the CXXC motif (red) and Thioredoxin (blue) domain.

Figure 2. Knockdown of CG6852 results in copper deficiency-associated phenotypes in the adult thorax.

Adult male *Drosophila*, anterior to top containing *PNR-GAL4* alone (A) and in combination with: (B) *CG6852^{RNAi}*, causing a cleft thorax and shrunken scutellum; (C) *CG6852^{RNAi}* and *ATP7^{RNAi}*, causing an additive phenotype of (B) and (D); (D) *ATP7^{RNAi}*, causing hypopigmentation on the midline of the thorax and abdomen; (E) *CG6852^{RNAi}* and *ATP7^{FLAG}*, showing an additive phenotype of both (B) and (F); (F) *ATP7^{FLAG}*, causing a mild cleft thorax and hypopigmentation on the midline of the thorax and abdomen; (G) *CG6852^{RNAi}* and *CtrlA^{wRNAi}*, causing an exacerbation of cleft thorax and a decrease in size of the scutellum; (H) *CtrlA^{wRNAi}*, causing a mild hypopigmentation on the midline of the thorax and abdomen; (I) *CG6852^{RNAi}* and *CtrlB^{RNAi}*, causing an exacerbation of the thoracic cleft; (J) *CtrlB^{RNAi}*, generating a wildtype appearance, (K) *CG6852^{RNAi}* and *CtrlB^{FLAG}*, causing a cleft thorax and shrunken scutellum seen with *CG6852^{RNAi}* alone; and (L) *CtrlB^{FLAG}*, generating a wildtype appearance.

Figure 3. Knockdown of CG6852 in the adult eye exacerbates a copper-deficiency phenotype caused by *CtrlA* knockdown

Adult male *Drosophila* heads, anterior to left containing *GMR-GAL4* alone (A) and in combination with: (B) *CG6852^{RNAi}*, causing a very mild rough eye; (C) *CtrlA^{sRNAi}*, causing a smaller eye with a flattened appearance and patches of necrosis; and (D) *CG6852^{RNAi}* and *CtrlA^{sRNAi}* together, causing a shrunken eye, with a glazed appearance, some loss of pigmentation and patches of necrosis.

Figure 4. Ubiquitous knockdown of *CG6852* causes an increased tolerance to elevated copper levels

Survival to adulthood of *DA>CG6852^{RNAi}* flies raised from early first instar under density-controlled conditions on various media types: NF = normal food; BCS = 500 μ M BCS; Cu = 1mM CuSO₄. Survival is expressed relative to survival of *DA>w¹¹¹⁸* control flies on the same food type. There was a significantly higher relative survival on copper-supplemented food compared with BCS-supplemented media (n $\geq$ 5, ** p-value <0.01).

Supplementary Material

Figure S1. *CG6852* RNAi line effectively decreases *CG6852* transcript levels

Weak ubiquitous RNAi-mediated suppression of *CG6852* using *DA-GAL4* (*DA>CG6852^{RNAi}*) significantly decreased *CG6852* expression levels compared with wild type (*DA>w¹¹¹⁸*). $n \geq 3$, *** p-value < 0.001 . 1-3: *DA>CG6852^{RNAi}*, *CG6852* primer pair. 4-6: *DA>w¹¹¹⁸*, *CG6852* primer pair. 7: *DA>CG6852^{RNAi}*, RP49 primer pair. 8: *DA>w¹¹¹⁸*, RP49 primer pair.

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GLRX1_HUMAN -----MAQEFVNCKIQPGKVVVFIKPT**CPYC**RRAQEILSQLPIKQG
DmCG6852 MGAVGSALRSPI--VDMSTKQAKFVENTIASNKVVIFSKTY**CPYC**TMAKEPFKKLNVDAT
DmGrx-1 MGTVVSTLQRPTLYVSMDSHAQFVRDTISGNKVVIFSKSY**CPYC**SMAKEQFRKINVKAT
Consensus MG V S L P V M A F V T I N K V V I F S K Y **CPYC** MAKE F K L N V K A T

GLRX1_HUMAN LLEFVDITATNHTNEIQDYLLQL**TGARTVPRVFI**GKDCIGGCSDLVSLQQSGELLTRLKQ
DmCG6852 IIE---LDGNPDGNEIQAVLGEI**TGARTVPRVFI**DGKFIGGGTDIKRMFETGALQKYFQ-
DmGrx-1 VIE---LDQRDDGNEIQAVLGEM**TGSRTVPRCFI**DGKFVGGGTDVKRLYEQGILQKYFQ-
Consensus IE LD DGNEIQAVLGE **TGARTVPRVFI**DGKFIGGGTD KRL E G LQKYFQ

GLRX1_HUMAN IGALQ
DmCG6852 -----
DmGrx-1 -----

GMR>W1118

GMR>CG6852^{RNAi}

GMR>Ctr1A^{RNAi}

*GMR>CG6852^{RNAi} +
Ctr1A^{RNAi}*

A

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