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Protein-Protein Interaction Between the Short and Long Form of Grg Family of Co-Repressors

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Abstract

The Grg family of corepressor proteins lacks DNA-binding domain and these are recruited to the promoter region by interacting with DNA-binding transcription factors. Recruitment to the promoter by DNA-binding transcriptional factors results in transcriptional repression. There are five member of Grg proteins namely Grg1-5. Grg1-4 are the long forms and Grg5 is the short form of Grg family of corepressor. It has been reported that Grg proteins make tetramers to mediate the repression. In a collocatiozation assay, the full length myc-mGrg3 was transfected in COS7 cells along with mGrg5 and mGrg1Δ280 fused with GFP. The colocalization assay indicates that full length myc-mGrg3 interacts with short form mGrg5-GFP by changing the localization pattern of mGrg5-GFP from cytoplasm to nucleus. In addition, we have also shown that Grg proteins interact with each other via N-terminal end, since myc-mGrg3 could not alter the translocation of non nuclear truncated form mGrg1Δ280-GFP from cytoplasm into the nucleus. The inability of myc-mGrg3 to translocate mGrg1Δ280-GFP indicates that Grg protein interact with each other only via N-terminal end. In conclusion, results we show here suggest that Grg protein interact with each other via N-terminal end and that this interaction alters the localization pattern of interacting Grg proteins.

Keywords: *COS7 cells, Grg proteins, Colocalization assay.*

Introduction

The groucho family of repressors is named after the groucho gene first identified in Drosophila melanogaster (Paroush, Finley et al. 1994; Payankaulam and Arnosti 2009). In mammals, they were independently named 'Transducin-like Enhancer of split, abbreviated to TLEs, or Groucho-related-genes (Grgs) (Pinto and Lobe 1996; Imai, Kurokawa et al. 1998). Five members of the Grg family exist, four full-length versions, Grg1-4, and one short form Grg5, which is a c-terminally truncated form (Sekiya and Zaret 2007; Santisteban, Recacha et al. 2010). Grg1-4 contain five domains, namely, in order from the N-terminus, Q, GP, CcN, SP and WD domains (Li 2000). The Q domain is rich in glutamine amino acids, and this domain is highly conserved across the Grg family of corepressors (Li

2000). The Q domain also has two putative leucine zipper sequences, which are believed to mediate tetramerization (Chen, Nguyen et al. 1998; Song, Hasson et al. 2004). The GP domain, which is rich in glycine and proline amino acids has been implicated in the recruitment of HDACs, which is why the GP domains of Grg proteins are required for its repressor functions (Pickles, Roe et al. 2002). CcN domain contains a nuclear localization sequence (NLS), as well as putative Cdc2 and casein kinase 11 (protein kinase CK2) phosphorylation sites (Nuthall, Joachim et al. 2004). The SP domain is rich in serine and proline amino acids(Li 2000). WD domain also known as WD-40, because it contains a 40 amino acid tandem repeat including conserved tryptophan (W) and aspartate (D) residues(Li 2000).

Grg proteins mediate repression by silencing the chromatin structure (Sekiya and Zaret 2007; Patel, Bhumbra et al. 2012). Grg proteins are involved in development and diseases (Chen and Courey 2000). Grg proteins mediate embryonic segmentation, neurogenesis, dorsal-ventral patterning, and Notch and Wnt signaling. Member of Grg family have been shown to mediate their role in cancer progression (Chen and Courey 2000). Recently it has been shown that Aes the small variant of Grg family can function as metastasis suppresser, Aes prevents local tumor invasion through inhibition of notch signaling (Allen, van Tuyl et al. 2006; Sarma and Yaseen 2011; Xia, Li et al. 2013). Grg proteins mediate these functions by recruiting the transcription factors which have binding sites for Grg proteins. These binding sites are known as WRPW motif (Seo, Tanaka et al. 2012) and engrailed like motif (Copley 2005).

It has been shown that Grg protein form tetramers by interacting with each and these oligomer structures are required for the repressor function of Grg proteins(Chen, Nguyen et al. 1998; Song, Hasson et al. 2004). Quite recently it has also been shown that both the short form and long form colocalize in nucleus(Formaz-Preston, Ryu et al. 2012). However, the abilities of Grg protein to interact with each other and change the localization pattern have not been extensively studied.

We therefore set out to determine whether full length form of Grg3 has the ability to interact with the short form Grg5. We found that Grg3 can indeed interact with Grg5. Consistent with this we found that localizatiuon patterns of Grg3 and Grg5 were similar when coexpressed in COS7 cells.

Material and Methods

Cell Culture

Cell lines (COS-7) were cultured in Dulbecco's modified Eagle's medium (D-MEM GIBCO Invitrogen) containing 10% Fetal Bovine Serum (GIBCO Invitrogen), 0.5% pencilinestreptomycine (GIBCO Invitrogen), 1% L-Glutamate 200mM (GIBCO Invitrogen) and 0.5% non-essential amino acids 100X (GIBCO Invitrogen), in humidified 5% CO^2 incubator at 37C.

Cell Transfection by Electroporation

One to six million cells were transfected using a 4mm cuvette (Cell Projects) at 210 voltage for 50 milli seconds (ECM 830 electroporater (BTX)). After electroporation the cells were seeded onto cover slips (VWR International) that had been coated with 50µg of Poly-D-lysin hydro-bromide (sigma) in a 6-well culture plates. Cells were then incubated for 20 hours at 37^0C and then processed for immunostaining.

Immunostaining of cells

Cells were washed twice with PBS for 10 minutes each. Cells were fixed in 4% PFA/PBS for 10 minutes, permeabilized with 0.2% Triton (X-100 Sigma) for 20 minutes, incubated in a blocking solution (10% BSA (Sigma), 0.1% Triton X-100 in PBS) for 30 minutes, and then incubated with

primary antibodies. Anti-MYC 9E10 (hybridoma bank) 1/20 for 1 hour at room temperature. Cells were then washed twice with 0.1% Triton X-100/PBS and incubated with fluorescently tagged secondary antibodies for 1 hour at room temperature. After three washes with 0.1% triton X-100 in PBS the cells were mounted in Vecta-shield with DAPI (Vector). Fluorescence signals were observed under epifluorescence and recorded using a digital camera (Nikon).

Results

Figure 1 shows the list of the constructs used for this study. mGrg3 was fused with myc-tag peptide at the N-terminal end, myc-mGrg3 (Figure 1.A) and mGrg5, which is the natural short variant of Grg protein was fused with GFP at the C-terminal end, mGrg5-GFP (Figure 1 B), the same way mGrg1Δ280, which is the truncated form of full length mGrg1 was also fused with GFP at the C-terminal end mGrg1Δ280-GFP (Figure 1 C). In truncated form, the first 280 amino acids were removed from C-terminal end that's why the construct is named mGrg1Δ280 (Figure 1 C).

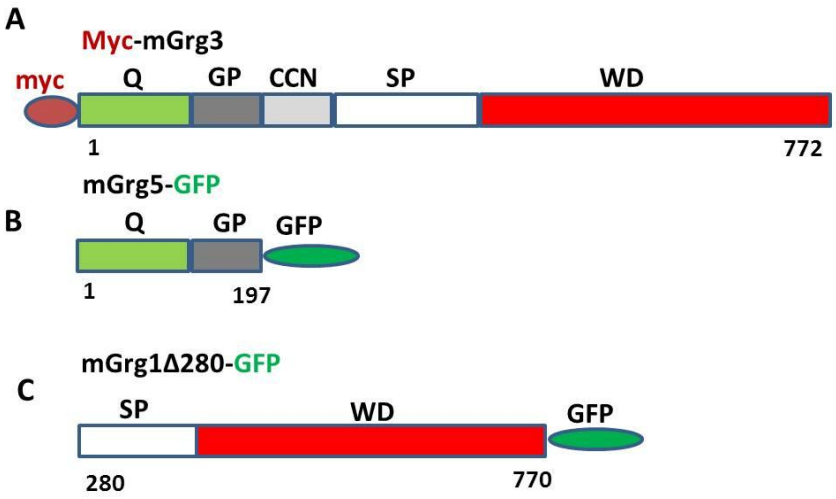
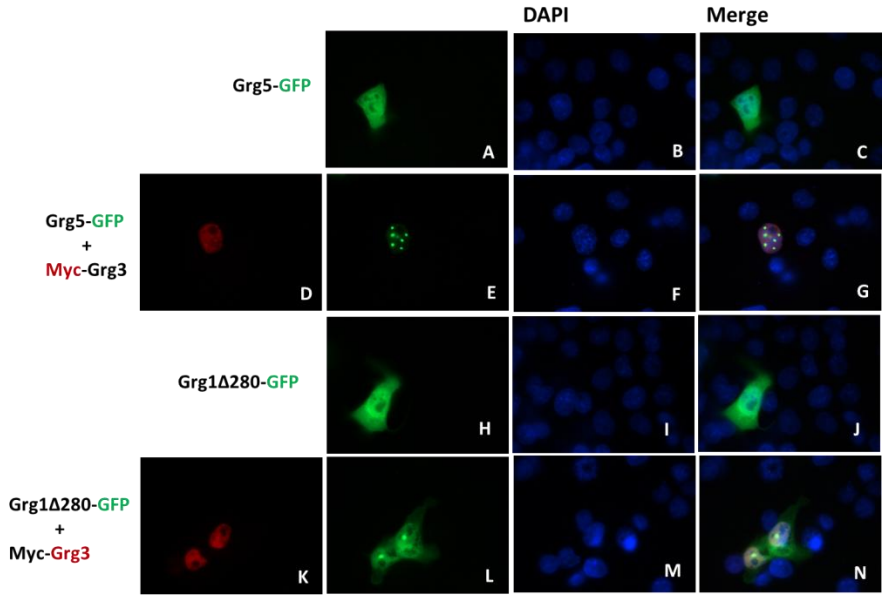


Figure 1. Diagrammatic representation of various Grg constructs used in the study

The transient expression of mGrg5-GFP in COS-7 cells showed the localization of Grg5-GFP was in both the nucleus and cytoplasm (Figure 2 A). Expression of myc-Grg3 in COS7 cells and staining it with anti-myc antibody reveals those myc-mGrg3 forms dotted structures in the nucleus. Co-expression of myc-mGrg3with mGrg5-GFP altered the localization of mGrg5-GFP into the nucleus, as seen in the merged image; both myc-Grg3 and Grg5-GFP are localized inside the nucleus in the dotted structures (Figure 2 G). The ability of myc-mGrg3 to translocate mGrg5-GFP was observed in 100% of transfected cells, further, anti-myc antibody revealed the nuclear distribution of mGrg5-GFP was dotted (Figure 2 G). This suggests that the full length form of Grg protein Grg3 has the ability to change the localization of non-nuclear, short variant of Grg protein, Grg5 into the nucleus in the form of dotted structures.



Fi**gure 2.** Co-localization assay demonstrates the interaction of myc-Grg3 with Grg5-GFP.

COS7 cells were transiently transfected with mGrg5-GFP, myc-mGrg3 and mGrg1Δ280-GFP. Transfected cells were grown on cover slips for 18 hours. Myc-mGrg3 was stained with anti-myc followed by texas red-conjugated secondary antibody. Nuclei were stained with DAPI (blue). Images were merged with Photoshop element. A-C) Over expression of mGrg5-GFP alone shows the distribution of mGrg5-GFP into the nucleus and cytoplasm D-G) Coexpression of myc-mGrg3 with caused the translocation of mGrg5-GFP into the nucleus. H--J) Overexpression of mGrg1Δ280-GFP alone shows the distribution of mGrg1Δ280-GFP in the cytoplasm and in the nucleus. K-N) Coexpression with myc-mGrg3 did not alter the subcellular translocation of mGrg1Δ280-GFP in the nucleus

We next tested whether full length myc-mGrg3 could also translocate the C-terminal form of mGrg1, mGrg1Δ280-GFP. M Grg1Δ280 represents the deletion of 280 amino acids at N-terminal part of the Grg1 protein (Figure 1 C). Since myc-mGrg1Δ280-GFP lacks an NLS, when over expressed in COS7 cells it was detected mainly in the cytoplasm, however, some mGrg1Δ280-GFP was also detected in the nucleus (Figure 2H-J). To find out if myc-mGrg3could also interact with the C-terminal end of Grg family of corepressor, myc-mGrg3 was co expressed in COS7 cells in combination with mGrg1Δ280-GFP, which has the WD and the SP domain and like mGrg5 lacks an NLS. Co-staining of transfected COS7 cells showed that myc-mGrg3 failed to tranlocatemGrg1Δ280-GFPinto the nucleus (Figure 2 K-N). However, the nuclear distribution of mGrg1Δ280-GFP was changed into the dotted structures. This result provides evidence that Grgprotein interacts with each only via N-terminal end and that might be possible through dimerizing domain present at the N-terminal end of Grg proteins.

Discussion

Nuclear translocation assay is widely used for observing the protein-protein interaction. The data we have collected suggest that the full length myc-mGrg3 interact with the short form mGrg5, this

interaction causes the translocation of mGrg5 into the nucleus. This data is consistent with the study, where it has been reported that full length TLE1, which is human homolog of Grg1 colocalizw with AES form of Grg family, TLE1 colocalize with and Grg5 was observed in the nucleus (Formaz-Preston, Ryu et al. 2012). However, we have found that translocation does not always bring these granules and speckled appearance. This speckled might be due to oligomerization, which resulted from tetramers formation of Grg proteins by interacting with each via LZL motif (Chen, Nguyen et al. 1998). Number of studies suggests that Grg proteins mediate the repression by spreading along the chromatin, forming tetramers, this suggests that more Grg proteins required at the site of repression (Winkler, Ponce et al. 2010; Patel, Bhumbra et al. 2012). mGrg5-GFP does not form these dotted appearances in the nucleus when expressed alone, mGrg5-GFP only localizes in these dotted structures when coexpressed with Grg3. These findings are not conclusive, however these still put an insight into understanding the mechanism of repression, altered localization of mGrg5-GFP into the nucleus by the long form Grg3 might bring change in the action of Grg5 protein, which needs to be confirmed in the future.

Conclusion

We conclude that the member of Grg family interact with each other via their N-terminal domain. We further conclude that mGrg3 and mGrg5 colocalize in the granular structures. However, we have been unable to give a conclusive model whether this colocalization brings repression or depression of target genes.

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