LOSS OF SPECIFIC CHAPERONES INVOLVED IN MEMBRANE GLYCOPROTEIN BIOSYNTHESIS DURING THE MATURATION OF HUMAN ERYTHROID PROGENITOR CELLS

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SUPPLEMENTAL DATA

EXPERIMENTAL PROCEDURES

In vitro co-immunoprecipitation of AE1 - To confirm that AE1 is able to interact with various molecular chaperones, *in vitro* co-immunoprecipitation of AE1 was performed. Wild type and SAO AE1 were synthesized using the Promega TNT® Quick Coupled Transcription Translation System (L1170) supplemented with Canine Pancreatic Microsomal Membranes (Y4041) in the presence of [^{35}S]Methionine. Microsomes were pelleted and solubilized in 1% digitonin in PBS and co-immunoprecipitation was performed using antibodies for various chaperones as describe in '*antibodies*'. 7.5% SDS-PAGE and autoradiography was then used to identify co-immunoprecipitated AE1.

In vivo co-immunoprecipitation of AE1 - Human Embryonic Kidney cells (HEK-293) were transfected by calcium phosphate method as previously described (1). 48 hours post-transfection, cells were lysed in 1% digitonin supplemented with proteasome inhibitors for 30 min on ice. Cell lysates were centrifuged (16,000g, 10min) and the supernatant was collected. To confirm glycosylation of AE1, cell extracts were treated with 1,000 units of endoglycosidase H (endo H; New England Biolabs) to remove high mannose oligosaccharides. Co-immunoprecipitation was then performed and immunoblotting identified co-immunoprecipitated AE1. Pulse chase experiments were performed as described previously (2).

REFERENCES

1. Popov, M., Li, J., and Reithmeier, R.A. (1999) *Biochem J* **399** (Pt 2), 269-79
2. Quilty, J.A., Li, J., and Reithmeier, R.A. (2002) *Am J Physiol Renal Physiol* **282**(5), F810-20

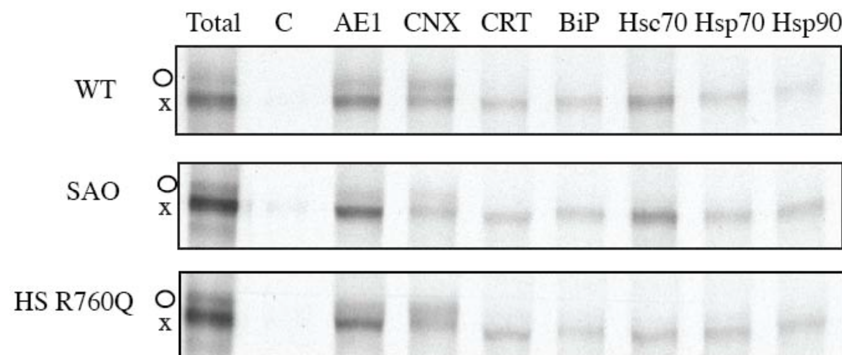

Fig. S1. AE1 interacts with various chaperones *in vitro*. Cell-free translation of AE1 was carried out in the presence of [^{35}S]Methionine and microsomal membranes. Microsomes were pelleted, solubilized in 1% digitonin and subjected to immunoprecipitation. Samples were run on 7.5% SDS-polyacrylamide gels containing 1 mg/mL concanavalin A and exposed to autoradiography. Close circles indicate the position of glycosylated AE1, and the open circles indicate the position of the non-glycosylated AE1. Lanes 4-9 show the co-immunoprecipitation of AE1 with various chaperones.

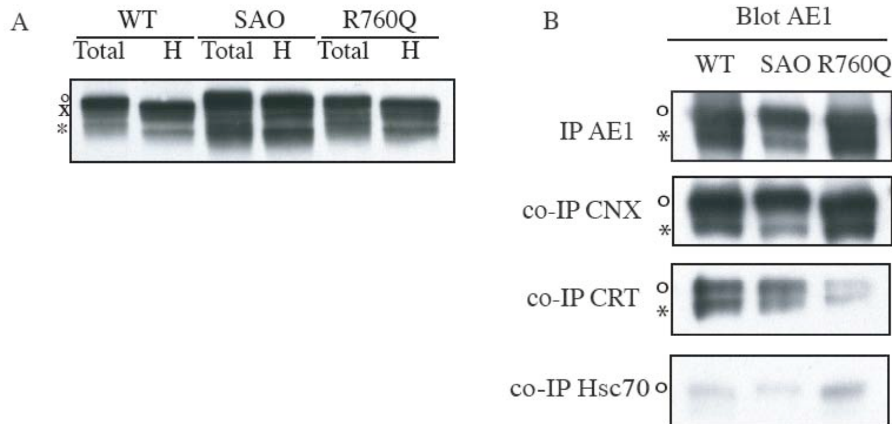

Fig. S2. AE1 interacts with various molecular chaperones *in vivo*. (A) HEK-293 cells were transiently transfected with AE1 constructs containing an HA tag in the 3rd extracellular loop. Endoglycosidase H (H) digestion reveals that AE1, SAO and R760Q contains a high mannose oligosaccharide(o). A truncated isoform can also be seen at a low molecular weight(*). (B) HEK-293 cells, 48 hours post-transfection, were lysed in 1% digitonin and subjected to immunoprecipitation for AE1 or co-immunoprecipitation for molecular chaperones, calnexin (CNX), calreticulin (CRT), or Hsc70. Immunoprecipitated proteins were run on 8% SDS-polyacrylamide gels and immunoblotted using an anti-HA antibody to detect AE1.

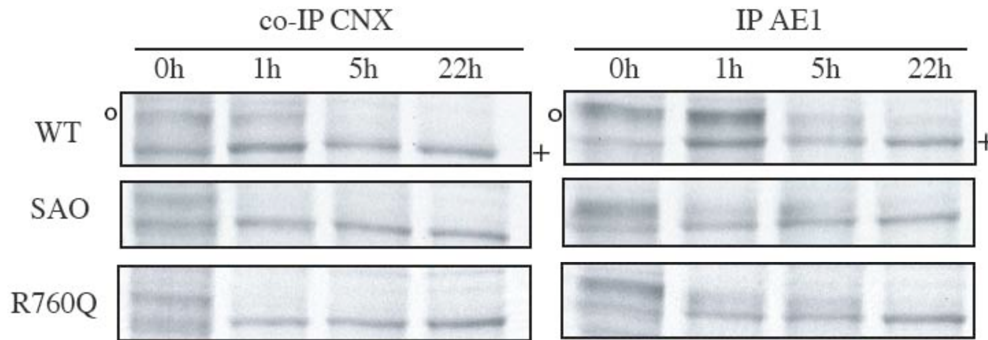

Fig. S3. Calnexin interacts transiently with wild type and mutant AE1. Transfected HEK-293 cells were pulsed with 0.2mCi/mL of [^{35}S]Methionine for 20 min. Cells were collected at 0, 1, 5, 22 h of the chase and AE1 was immunoprecipitated (right) or co-immunoprecipitated with calnexin (left). Radiolabelled proteins (oAE1, +CNX) were visualized by SDS-PAGE and autoradiography.