SEC23 related diseases

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5 october 2009

Background information

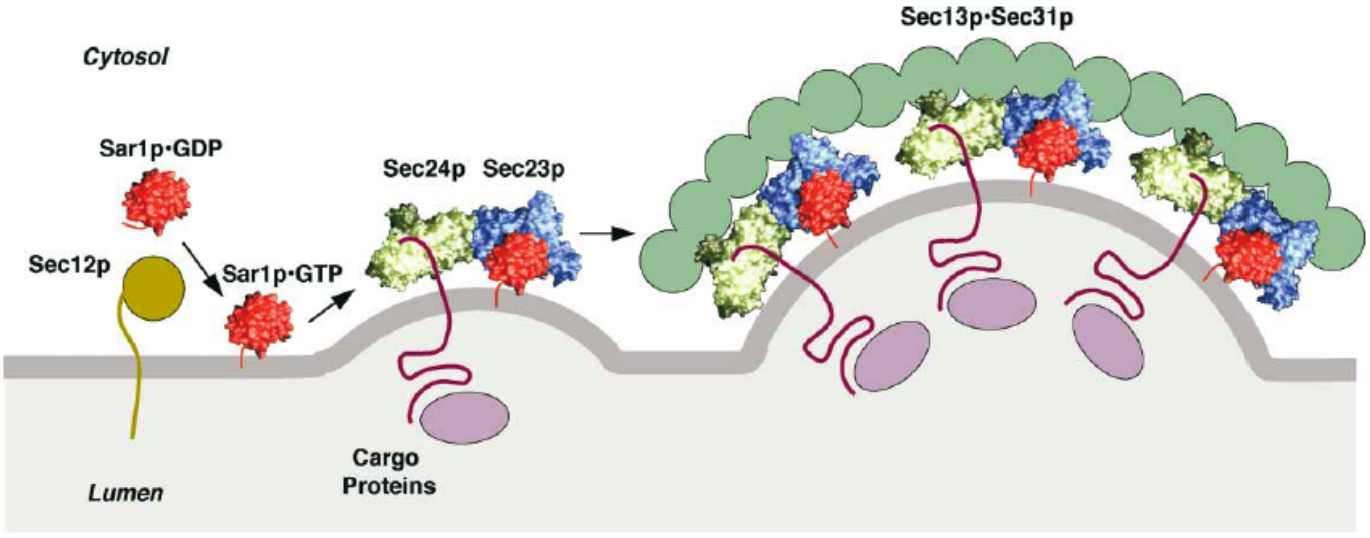


Figure 5. Assembly of COPII

Cytosolic Sar1p•GDP is converted to membrane bound Sar1p•GTP by the transmembrane protein Sec12p. Sar1p•GTP recruits the Sec23p•Sec24p subcomplex by binding to Sec23p, forming the "pre-budding complex". Transmembrane cargo proteins gather at the assembling coat by binding to Sec24p. The Sec13p•Sec31p subcomplex polymerizes onto Sec23p•Sec24p and crosslinks the pre-budding complexes. Cargo proteins are further concentrated. The depictions of Sar1p, Sec23p, and Sec24p are surface representations from the crystal structures of these proteins (Bi et al., 2002). The Sec13p•Sec31p complex is represented as an elongated, five-globular domain structure based on electron microscopy (Lederkremer et al., 2001). Sec16p and Sed4p also participate in the assembly of COPII, but are not represented here because their roles are less well understood. See text for additional details.

Aim of the articles

- Determination of CLSD and CDAII genetic origins.
- Discovery of involved protein (each time an isoform of SEC23).
- Characterization of defects in mutated proteins through *in vitro* and *in vivo studies*.

First paper

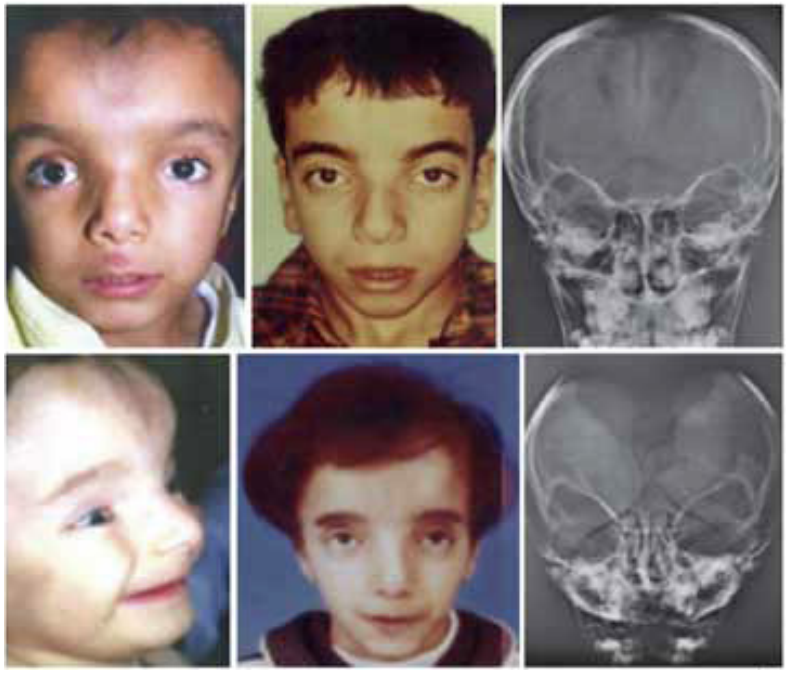
Cranio-lenticulo-sutural dysplasia is caused by a *SEC23A* mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking

Simeon A Boyadjiev[1,2], J Christopher Fromme[3], Jin Ben[4], Samuel S Chong[4], Christopher Nauta[2], David J Hur[1], George Zhang[1], Susan Hamamoto[3], Randy Schekman[3], Mariella Ravazzola[5], Lelio Orci[5] & Wafaa Eyaid[6]

nature genetics

Cranio-lenticulo-sutural dysplasia

Phenotype



- late-closing fontanels
- sutural cataracts
- facial dysmorphisms
- skeletal defects (scoliosis, . . .)

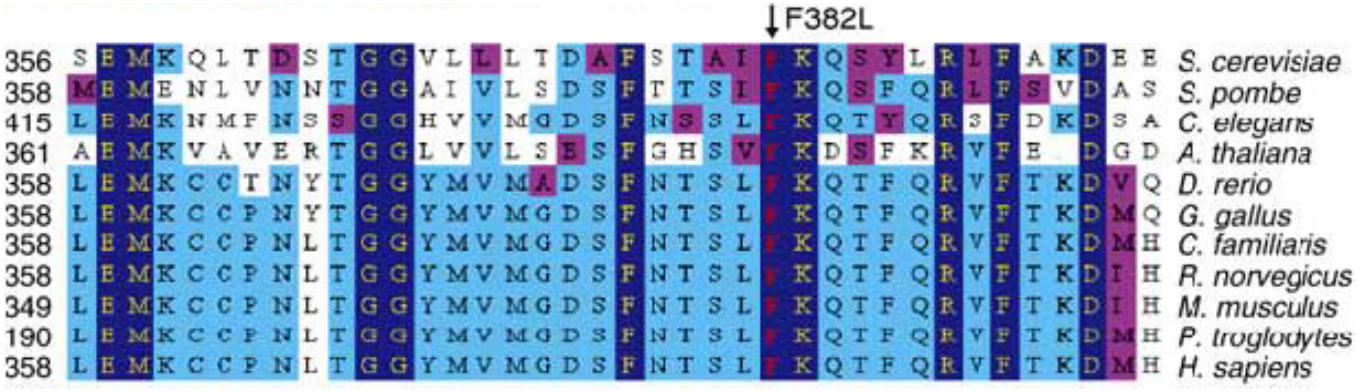
Rare disease characterized first in a consanguineous family of Bedouin descent

Cranio-lenticulo-sutural dysplasia

Genotype

- Genome wide-screen
- Mutation localized in the 14q13–q21 region of the chromosome 14
- Sequencing of genes belonging to this region
- 1144T→C transition in SEC23A only found in affected people

Consequences: shift from F to L at position 382, a highly conserved residue between species



Hypothesis

Because of SEC23A role in the secretion pathway, accumulation of proteins in the RE will be seen.

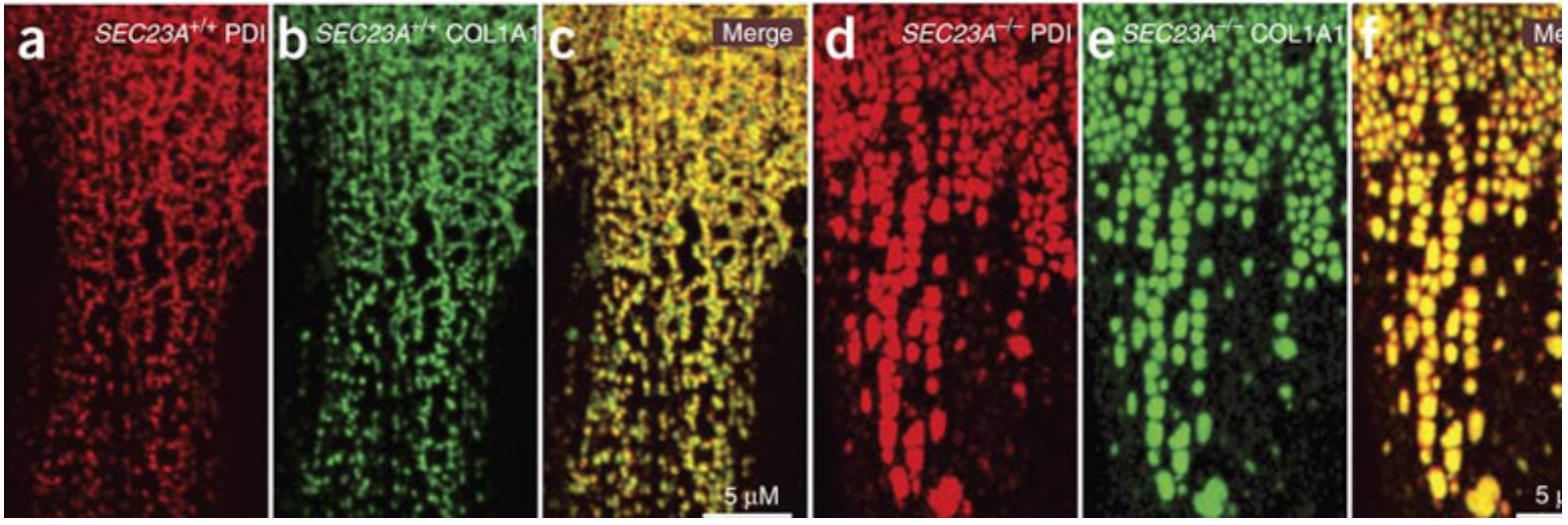
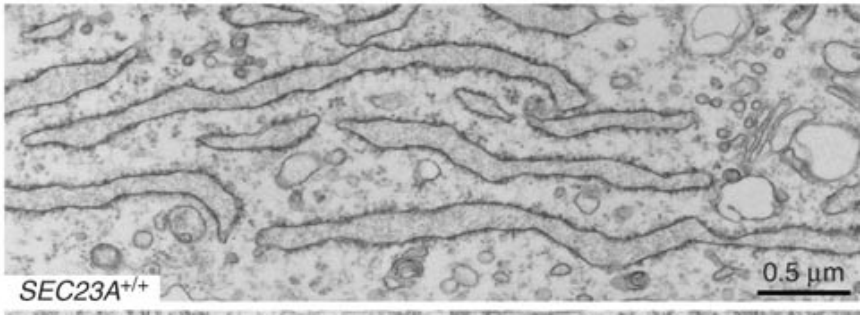
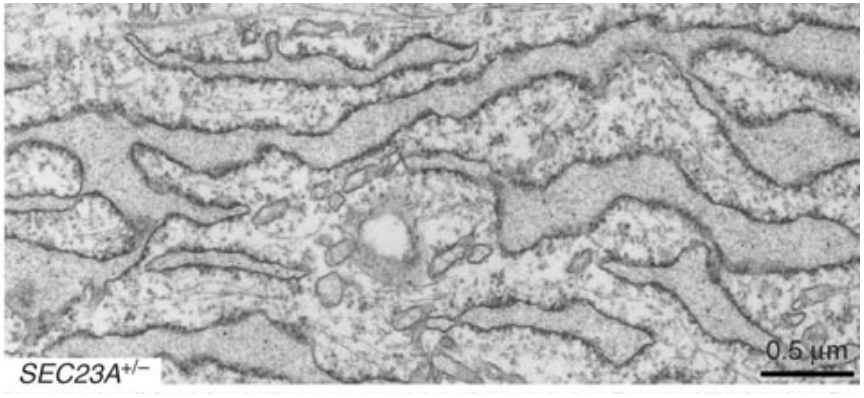
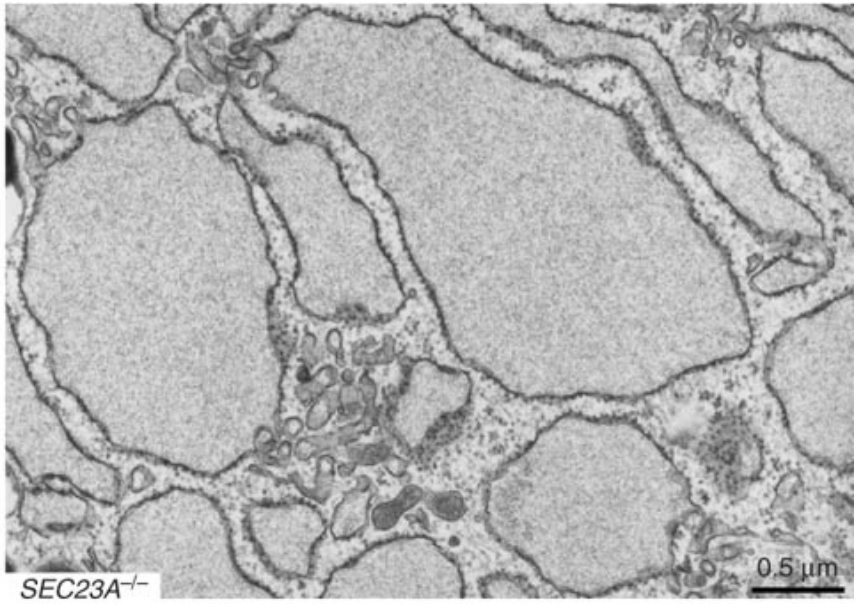


Figure: Immunofluorescence of collagen (secreted protein) and PDI (ER chaperonne) in fibroblasts

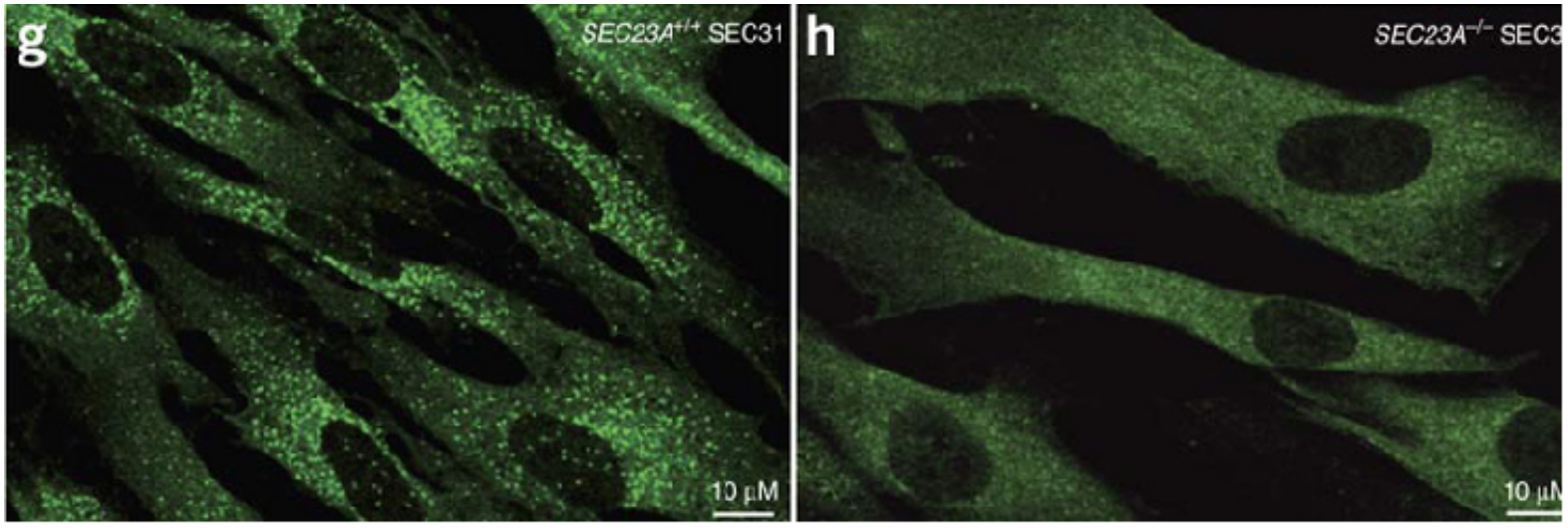
Electronic microscopy of ER fibroblasts







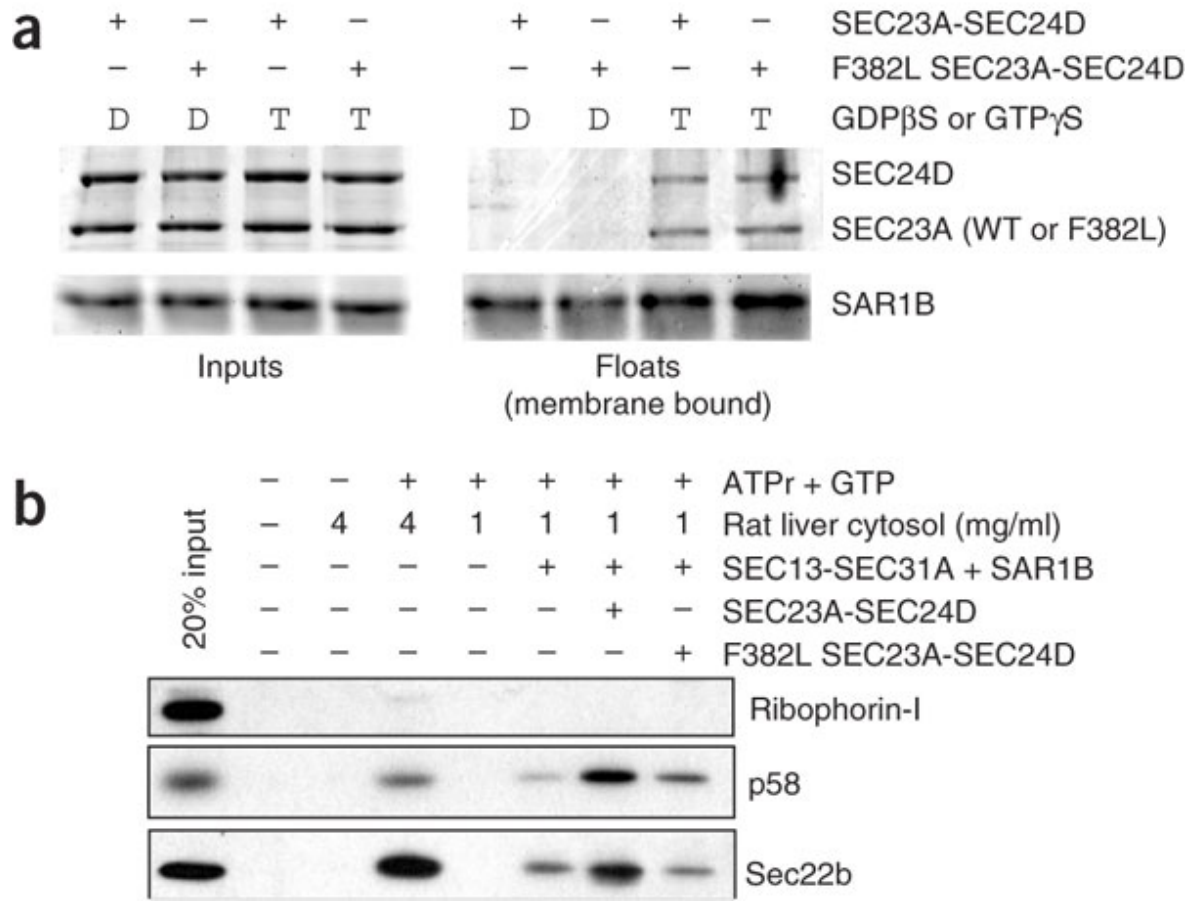
SEC31 is anormaly located in mutant cells



SEC31 (light green) is present all over the cytoplasm in mutant fibroblasts.

In vitro studies of mutant SEC23A

Liposome-binding assay (a) and vesicle formation assay (b)



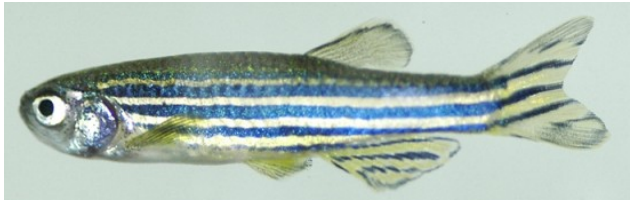
Mutant SEC23A binds Sar1 and SEC24 like WT

Less COPII vesicles bud with mutant SEC23A

In vivo studies

Choice of the model and technique

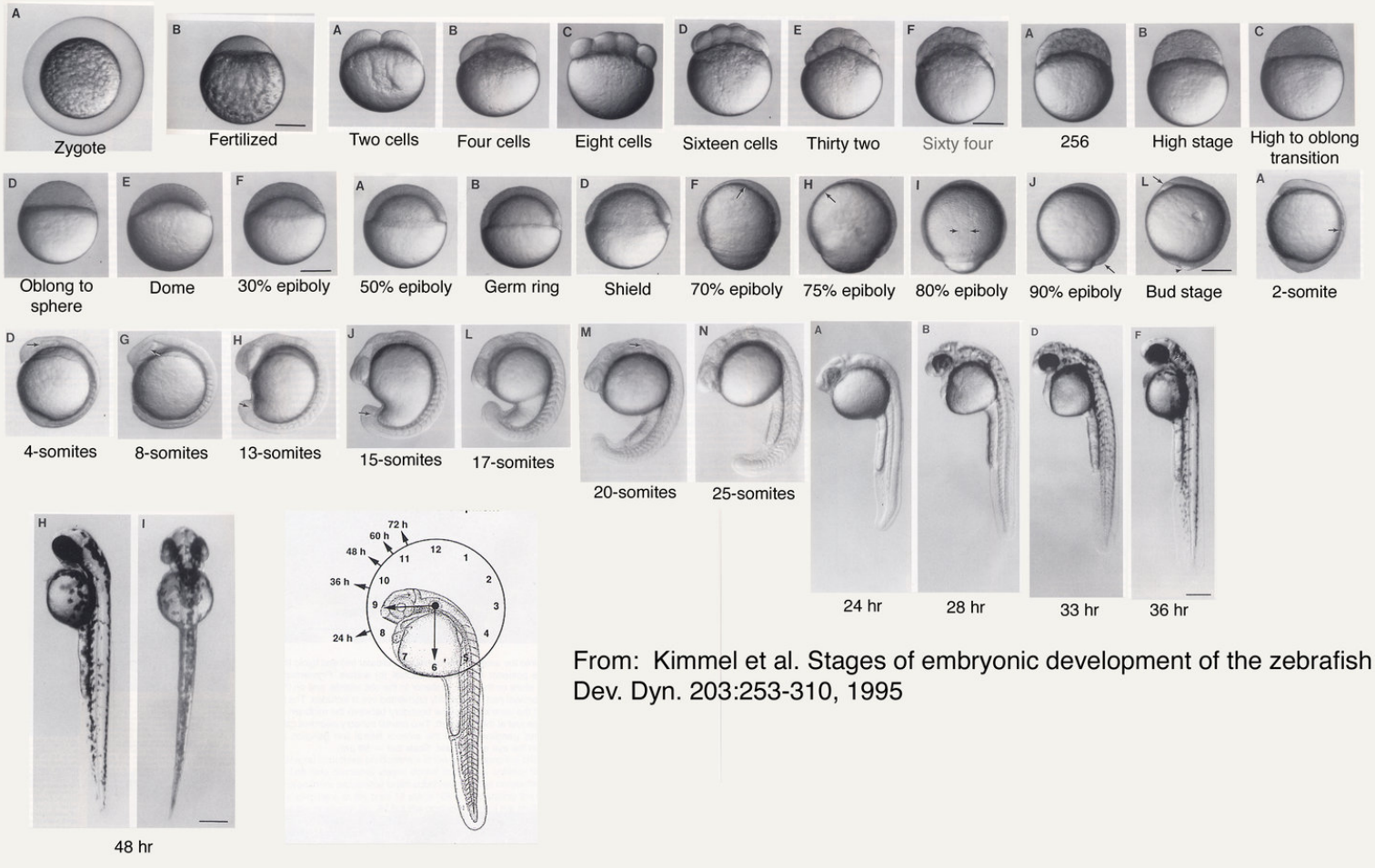
Zebrafish (*Danio Rerio*)



- Vertebrated
- Transparent embryo
- Not too expensive

Technique: 2 different morpholinos injected in embryos targeting only SEC23A

Zebrafish developmental stages

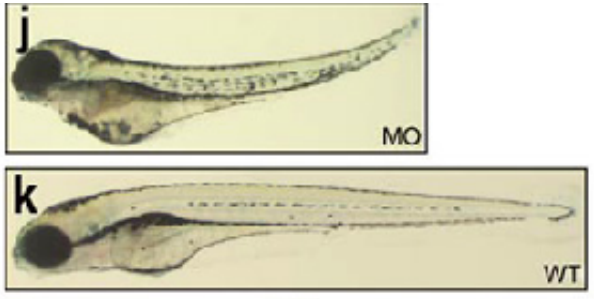


From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

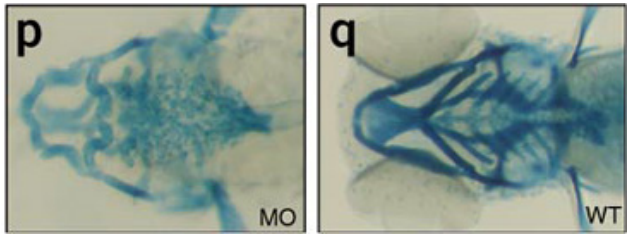
SEC23A inhibition consequences

in situ hybridation (e) and Alcian blue staining (p,q)

SEC23A expression in the notochord is the strongest at 1 dpf (e). Expression of SEC23A is limited to developing head cartilages after 2 dpf



Reduce body length and dorsal curvature without SEC23A at 5 dpf



Malformations off all principal neurocranial cartilage structures at 5 dpf

Death before formation of an ossified skeleton

Conclusion

- Mutation of SEC23A causes CLSD
- Mutated SEC23A leads to abnormal accumulation of proteins in the ER
- Mutated SEC23A retains some functional activity
- There is a decrease of SEC23A –SEC13/31 binding.
- Inhibition of SEC23A in zebrafish causes similar phenotype as affected humans.
- Expression of SEC23A is tissue specific

Discussion

- Clinical symptoms come from accumulation of proteins in the ER (collagen, . . .)
- Tissue specific expression of SEC23A leads to localized defects

Outlook

- Characterization of cargo proteins trapped in the ER because of the mutation
- Origin of cataract formation
- How the mutation is involved in the diminution of proteins interaction

Opinion

- *In vitro*, *in vivo* and *in silico* experiences were performed
- Good characterization of the mutation and good overview of its implications
- No molecular description of SEC23A
- Chosen animal model didn't finish development ; mice may be a better model

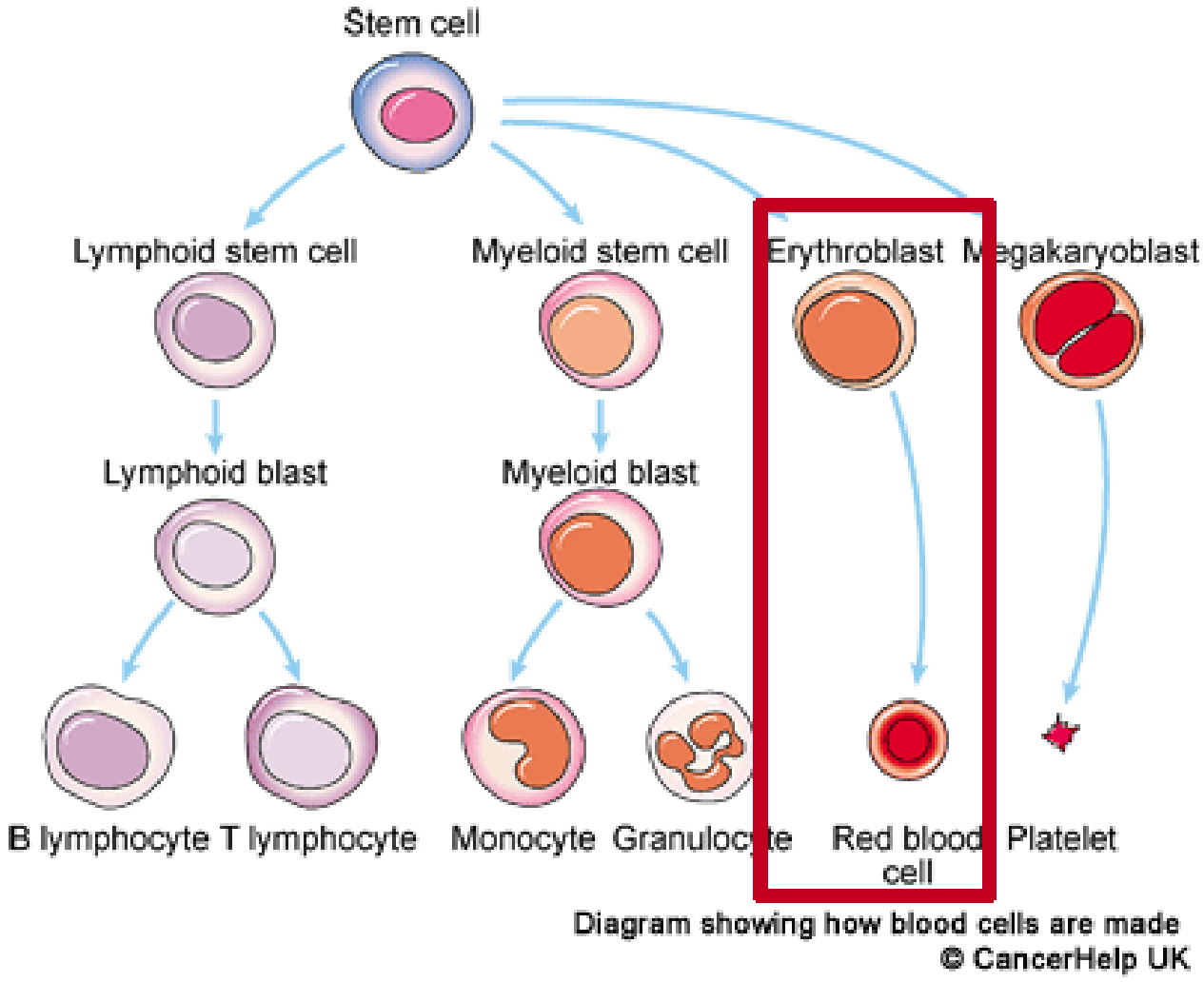
Second paper

Mutations affecting the secretory COPII coat component SEC23B cause congenital dyserythropoietic anemia type II

Klaus Schwarz[1,2,14], Achille Iolascon[3,14], Fatima Verissimo[4], Nikolaus S Trede[5,6], Wyatt Horsley[5,6], Wen Chen[7], Barry H Paw[7], Karl-Peter Hopfner[8], Karlheinz Holzmann[9], Roberta Russo[3], Maria Rosaria Esposito[3], Daniela Spano[3], Luigia De Falco[3], Katja Heinrich[1], Brigitte Joggerst[4], Markus T Rojewski[1,2], Silverio Perrotta[10], Jonas Denecke[11], Ulrich Pannicke[1], Jean Delaunay[12], Rainer Pepperkok[4] & Hermann Heimpel[13]

nature genetics

Erythropoiesis



Erythropoiesis

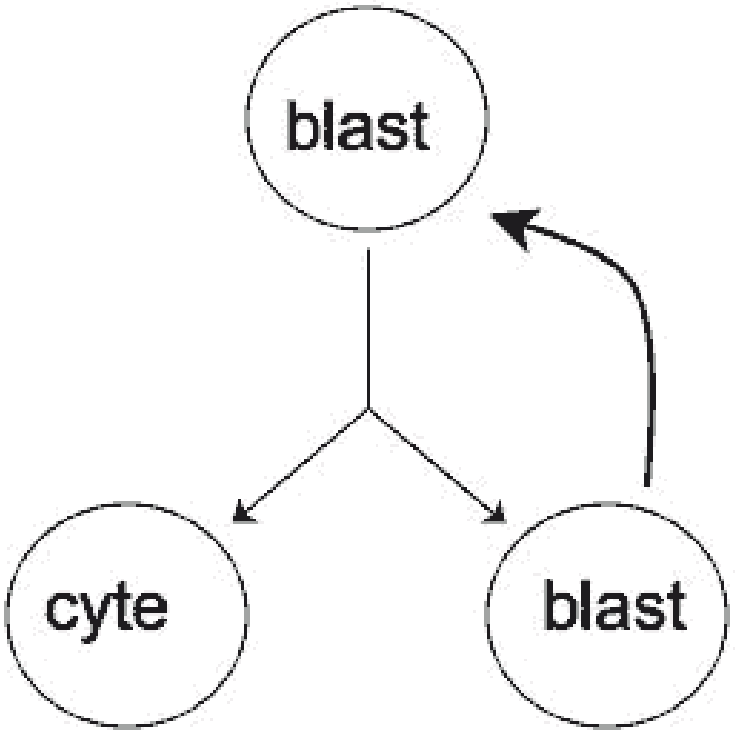


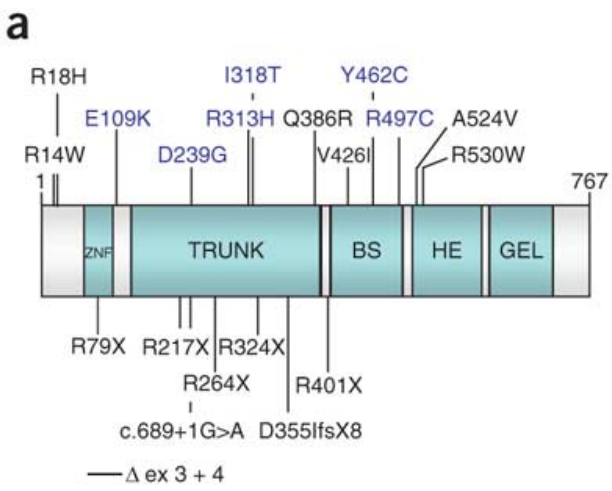
Figure: Erythroblast cell division in bone marrow

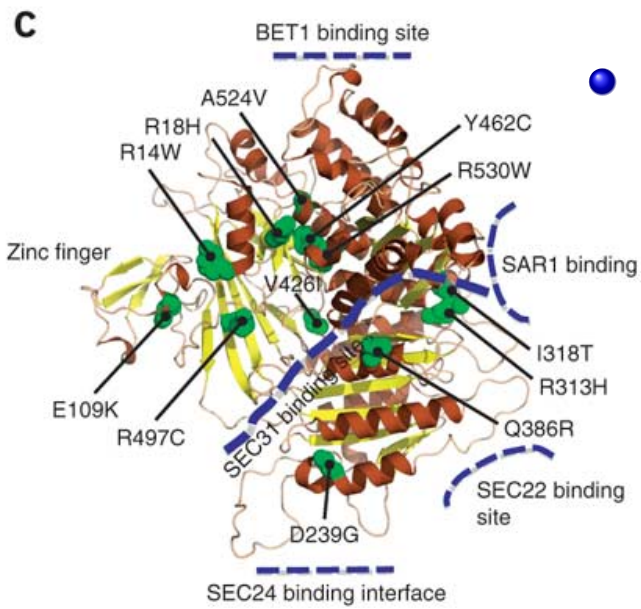
Congenital dyserythropoietic anemia type II (CDAII)

- Hereditary disease
- Incidence : 1/100000 birth
- Most frequent CDA
- Lack of erythrocytes in blood
- Problems with erythropoiesis (multinucleated red cells, anomalous membrane proteins)
- Treatable with sanguine transfusions

CDAII - Genotype

- Genome wide-screen
- Mutation localized in the 20p11.23-20p12.1 region of the chromosome 20
- Sequencing of SEC23B
- 12 different missense mutations, five nonsense mutations, one single-nucleotide, one large deletion and one splice-site mutation
- At least one missense mutation in each affected individual





- Mutations affecting highly conserved residues on all the protein

Quantification of mutated SEC23B in affected individuals

- Measurements by western blots
- Important decrease of SEC23B (-62%) and total SEC23 (-33%)

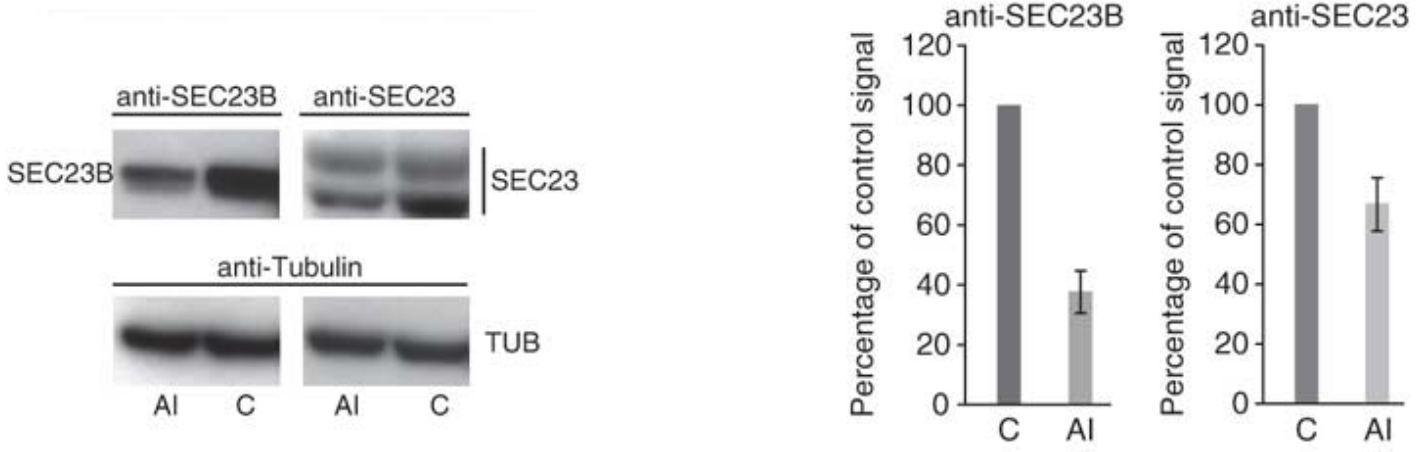


Figure: Affected individual cell line F4P1 (fibroblast)

Quantification of mutated SEC23B in affected individuals

- 50% of the mutations are situated near (R14W) or on (E109K) the zinc finger domain
- As a result, -95% of stable SEC23B

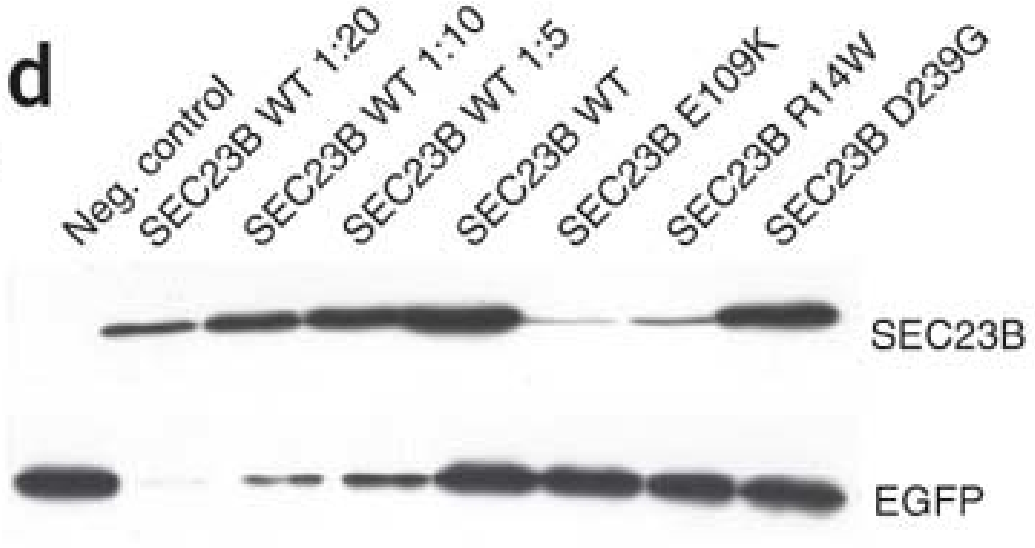


Figure: Western blot of tagged SEC23B in HEK293T cells

Interactions with SEC24

- Coimmunoprecipitation of SEC23B and SEC24D
- Both proteins are seen on the western blot

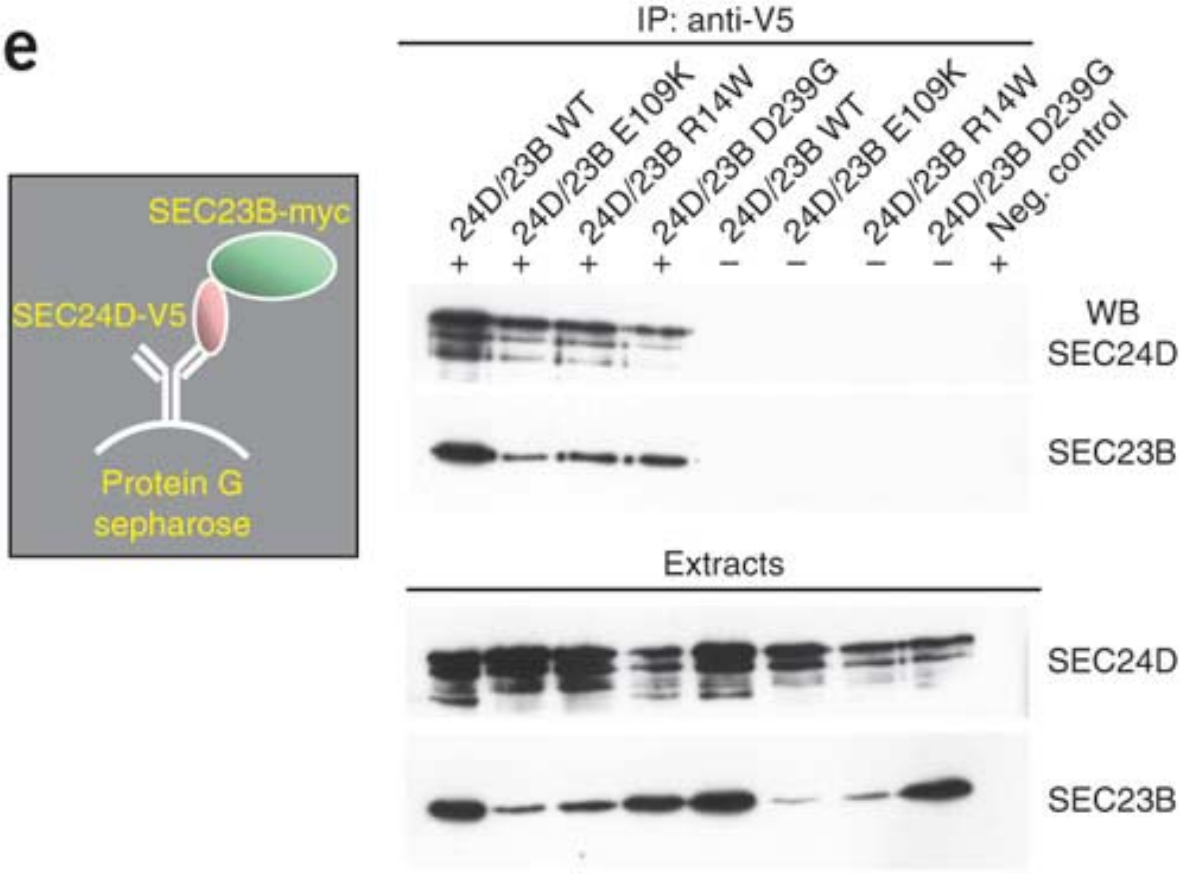
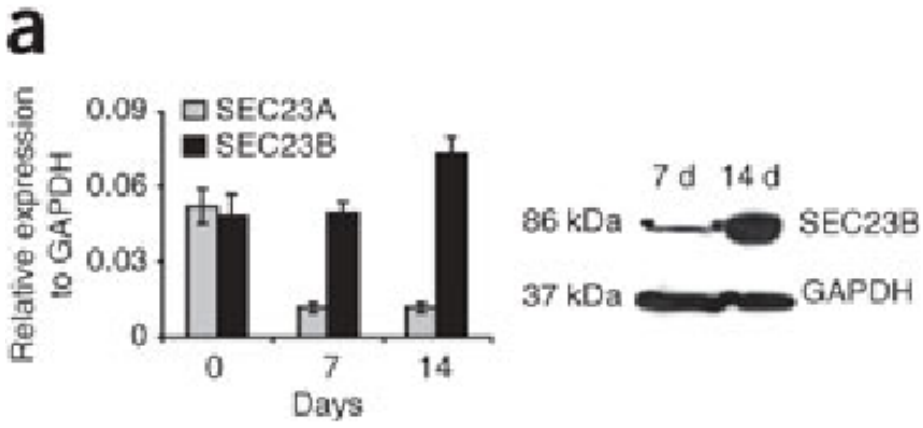


Figure: Presence (+) or absence (-) of the antibody to V5

SEC23 expression is tissue dependant

- In vitro quantification of SEC23 isoforms in erythroid differentiation of $CD34^{+}$ blood cells



- SEC23B is almost the only isoform expressed

SEC23B and cellular cycle

- SEC23B partial inhibition (55.2%) with shRNA leads to an increase of phase M cells (FACS analysis)

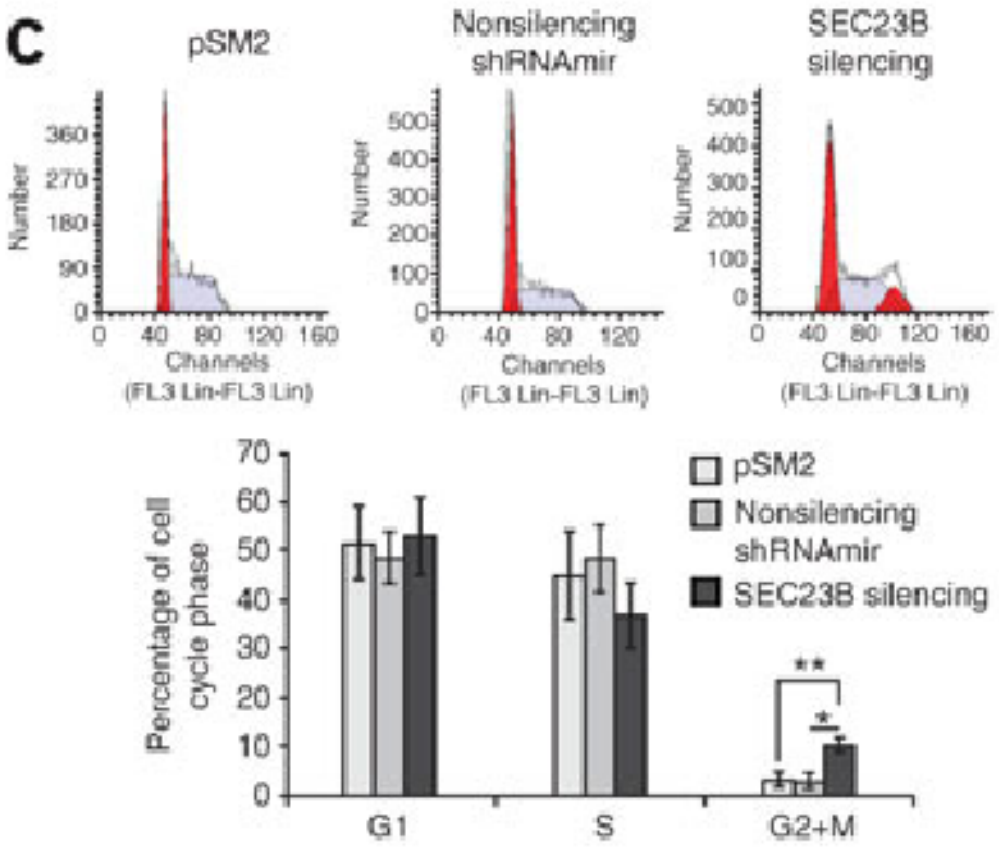


Figure: Erythroleukemic cell line K562

Mutated cells can't do cytokinesis

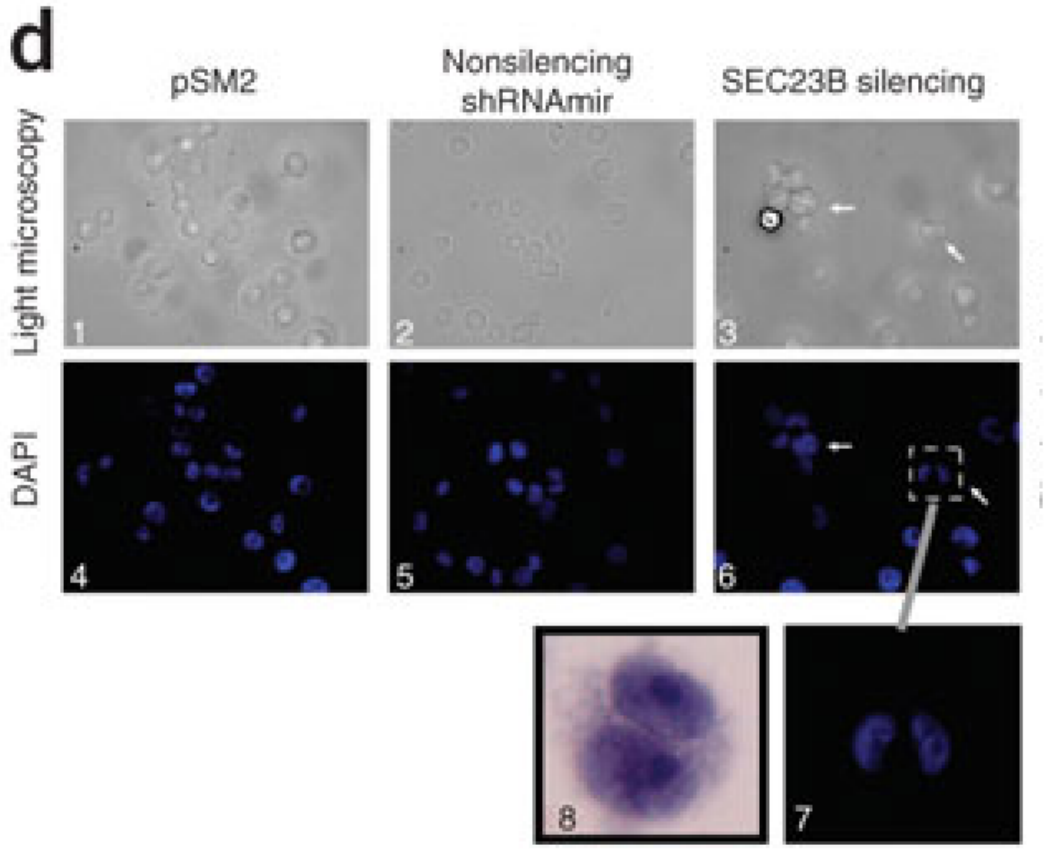
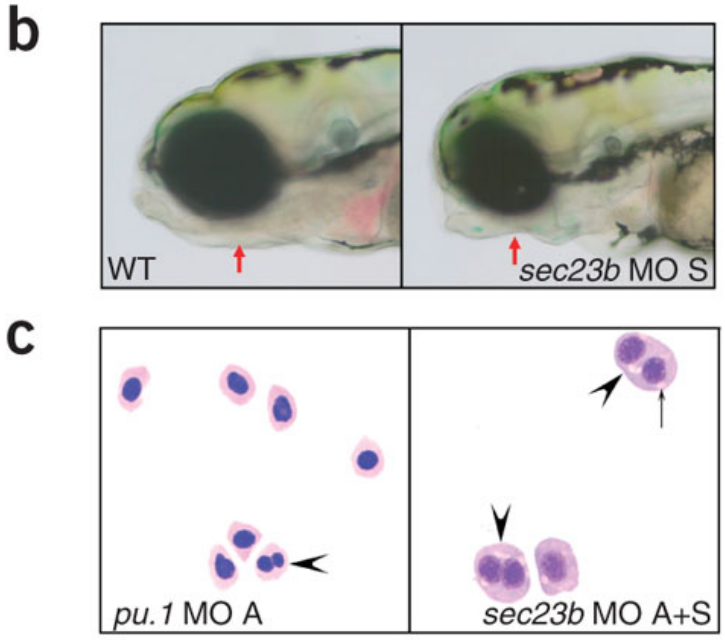


Figure: Erythroleukemic cell line K562

Morpholino zebrafish experiments

- Jaw malformation : redundant function of SEC23A



- Same cytokinesis phenotype observed as in human and *in vitro* experiment

Phenotype differences

- Hypoglycosylation of band3 and duplication of rough ER are two characteristics markers in affected humans but they are not observed in zebrafish

Conclusion

- CDAII can be caused by different SEC23B mutations
- Protein stability is generally decreased
- Binding with SEC24 isn't affected
- SEC23B expression is tissue specific
- Mutations in SEC23B causes cytokinesis failure
- These characteristics are seen in the zebrafish but some other are missing

Discussion

- Zinc finger domain integrity seems essential for SEC23B stability
- SEC23B is active in cytokinesis process
- Tissue specific expression of SEC23B leads to localized defects (erythropoiesis)

Outlook

- Do all mutations of SEC23B leads to the same phenotype ?
- Importance of zinc finger domain in stability ?
- What is the problem in COPII vesicle formation ?
- Are the difference with animal model caused by other involved proteins or an inadequate chosen model ?

Opinion

- Principal cause of the CDAII found (SEC23B)
- No clear explanation of the mutations effects at a molecularly scale
- Analysis of other mutations involvement are lacking

Final words

- Secretion pathway proteins may be involved in diseases
- Isoforms of the same proteins expression can be tissue specific
- This may lead to very different phenotypes and pathologies