Diamond-Blackfan anemia pathophysiology in 2024

Lydie Da Costa
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Paris-Saclay University
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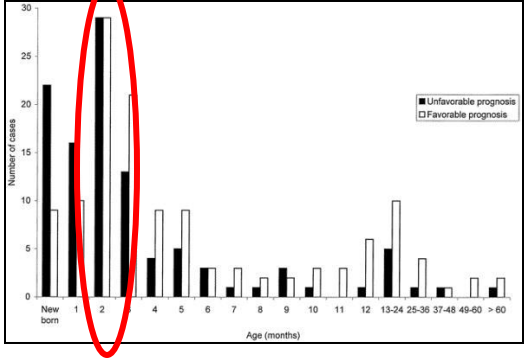
France

Reconnue par le Ministère de la Santé



Diamond-Blackfan anemia (DBA) :

Rare disease – 7 to 12 cases/million life birth

One of the inherited bone marrow failure

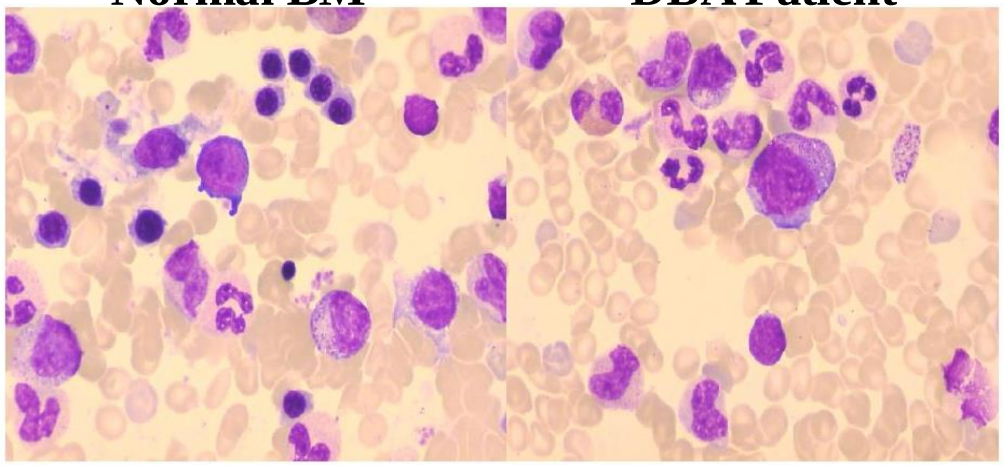
Intrinsic defect in erythropoiesis

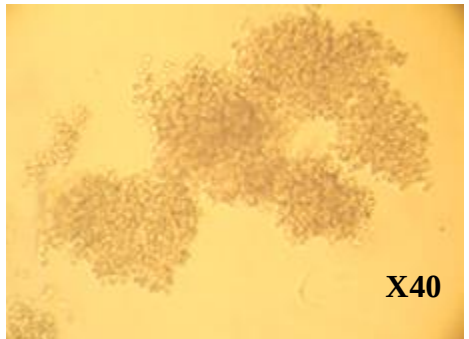
First ribosomopathy described – 24 genes

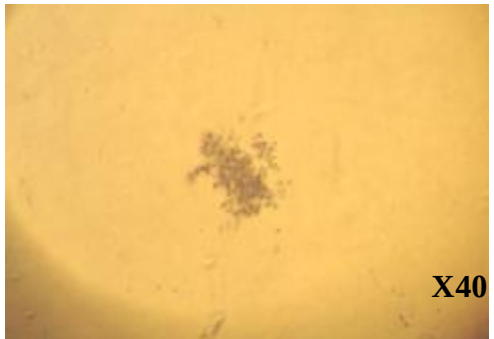
Various malformations (50% of cases)

Erythroblastopenia/pure hypoplastic anemia

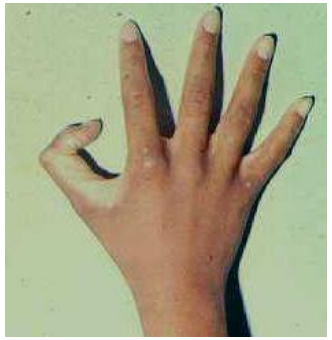
Normal BM **DBA Patient**



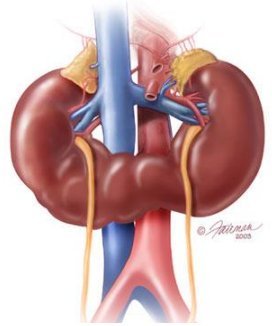




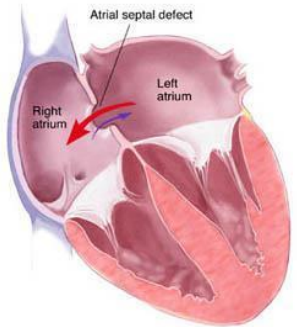
Extremities (thumbs+++)



Kidney & tractus



Heart & vessels



Cephalic area

The DBA guidelines, just been published

THE LANCET
Haematology

REVIEW | VOLUME 11, ISSUE 5, E368-E382, MAY 2024 Download Full Issue

Diagnosis, treatment, and surveillance of Diamond-Blackfan anaemia syndrome: international consensus statement

Marcin W Wlodarski, MD PhD * • Prof Adrianna Vlachos, MD * • Jason E Farrar, MD * •
Prof Lydie M Da Costa, MD PhD • Prof Antonis Kattamis, MD • Irma Dianzani, MD PhD • et al. Show all authors •
Show footnotes

Published: May, 2024 • DOI: https://doi.org/10.1016/S2352-3026(24)00063-2 • Check for updates

Diagnostic criteria

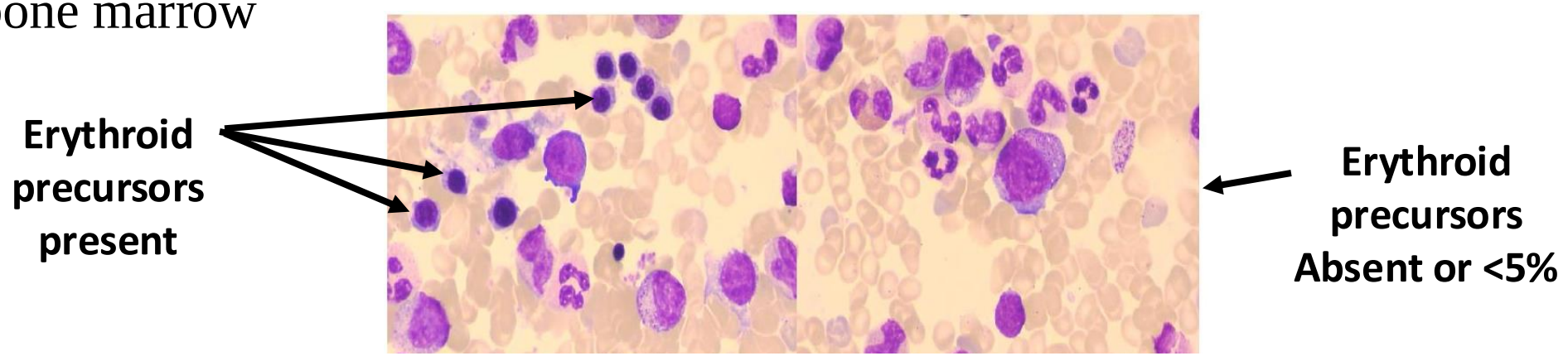
- Pathogenic or likely pathogenic mutation in a Diamond-Blackfan anaemia (DBA) syndrome gene (appendix p 4); or
- Haematological features consistent with DBA syndrome: macrocytic anaemia* with reticulocytopenia and bone marrow erythroblastopenia; absence of dysplasia, dyserythropoiesis†, and sideroblasts; and exclusion of known differential diagnoses (see below)

Typical findings (not mandatory for diagnosis)‡

- Patients are younger than 1 year at onset of disease
- Elevated eADA activity (before first transfusion, in patients who have not received a transfusion, or in parents of patients)
- Elevated HbF (reliably assessed in patients older than 6 months)
- Positive family history or unexplained history of anaemia during infancy or childhood
- Congenital abnormalities (appendix p 5)
- Abnormal rRNA processing in patient cells§

“Classical” DBA diagnosis

- Moderate to severe, often macrocytic aregenerative anemia
- The other cell lineages are usually normal
- Bone Marrow smear = Erythroblastopenia with <5% erythroid precursors in an otherwise normocellular bone marrow

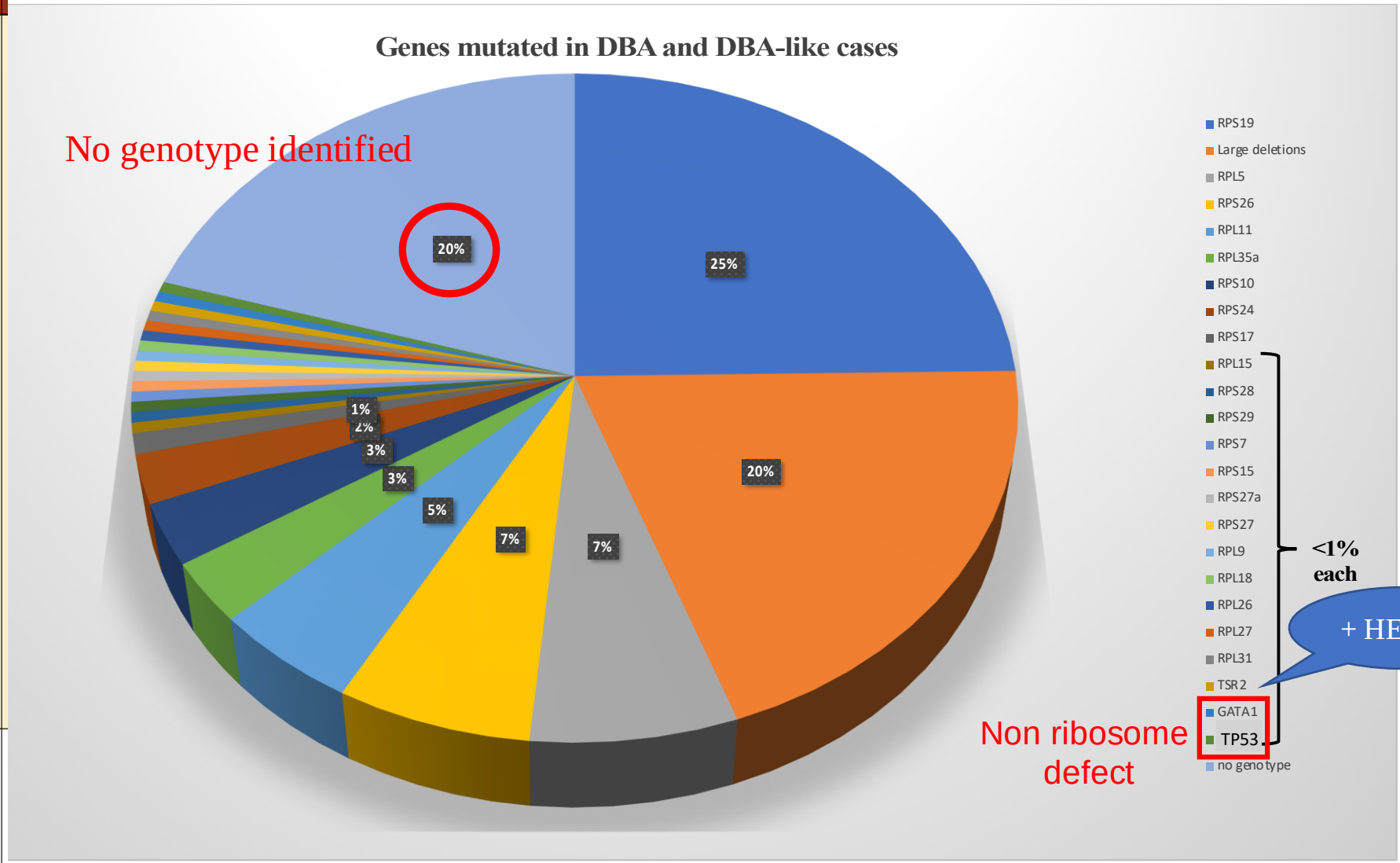


Bone marrow smear control Erythroblastopenia

- Elevated erythrocyte adenosine deaminase activity
- Fetal erythropoiesis features (elevated HbF)
- Very High EPO level
- Identification of one mutation in a gene involved in DBA
- No evidence for another cause of bone marrow failure

DBAS genotype

Mutated gene	RP	Incidence in DBA population
Genes involved in DBA*		
RPS19	eS19	25%-30%
Large deletions		10%-20%
RPL5	uL18	7%-12%
RPS26	eS26	6.6%-9%
RPL11	uL5	5%-7%
RPL35a	eL33	2%-3%
RPS10	eS10	1%-3%
RPS24	eS24	2.4%-3%
RPS17	eS17	1%-3%
RPL15	eL15	1 case
		6 cases
RPS28	eS28	2 families
RPS29	uS14	2 families
RPS7	eS7	1 case
RPS15	uS19	1 case
RPS27a	eS31	1 case
RPS27	eS27	1 case
RPL9	uL6	1 case
RPL18	eL18	1 family
RPL26	uL24	1 case
RPL27	eL27	1 case
RPL31	eL31	1 case
TSR2 (X linked)†		1 family
Genes involved in DBA-like diseases		
GATA1 (X linked)‡		5 families
EPO		1 case
ADA2§		9 individuals



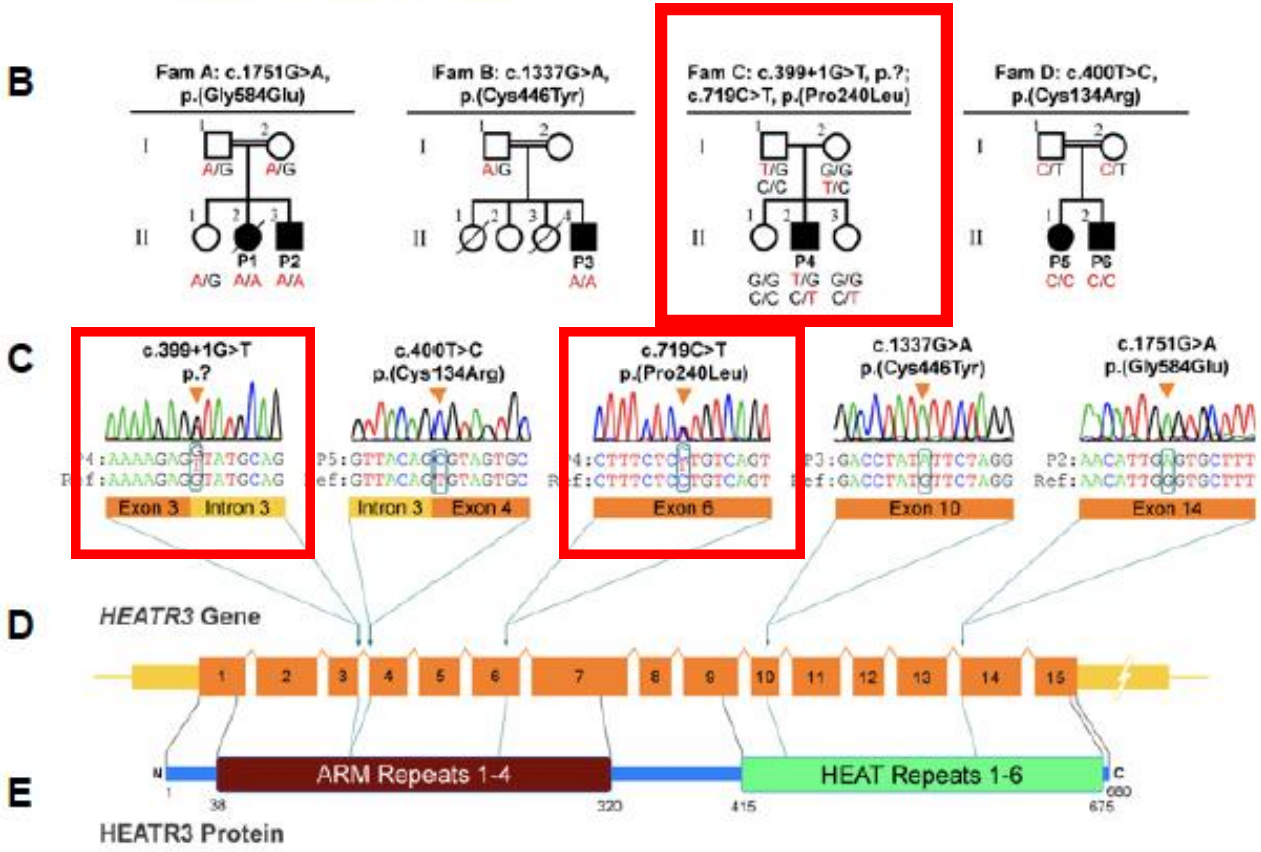
Da Costa L, Leblanc T, Mohandas N, Blood 2020

Identification of a new candidate gene, which is a chaperone of RPL5: *HEATR3*

A

homozygous	homozygous	compound heterozygous	homozygous
anemia brachydactyly short stature mild intellectual disability P1 died of osteosarcoma	anemia brachydactyly short stature intellectual disability	Anemia short stature no intellectual disability	anemia, transient thrombocytopenia (P6) preaxial polydactyly, brachydactyly, mild intellectual disability (P5)

With parents' authorization



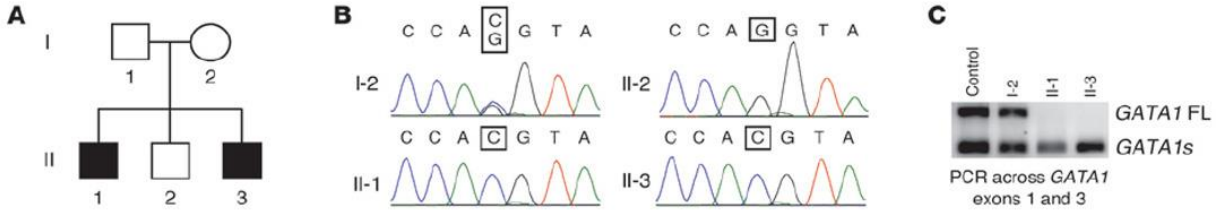
MF O'Donohue, L Da Costa*, Marco Lezzerini*, Sule Unal*, … PE Gleizes, DLJ Lafontaine, AW MacInnes, Blood, 2022*

DBA – like and borderline DBA cases

➢ *GATA-1 gene mutation:* X-linked

Sankaran et al., J Clin Invest, 2012
Weiss et al., J Clin Invest, 2012
Parella et al., Pediatr Blood cancer, 2014
Klar et al., Br J Hematol, 2014

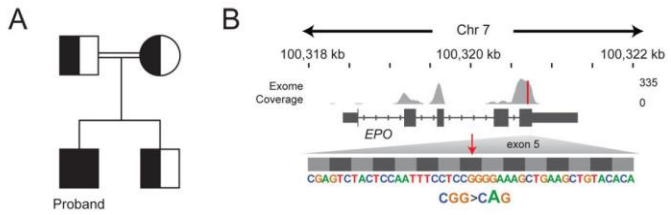
Mutation in exon 2:
c.220G>C ; p.(Leu74Val)



Loss of the 83 first aa
Loss of the long form of GATA1 (GATA1 FL)

➢ *EPO gene mutation*

KIM et al., Cell 2017



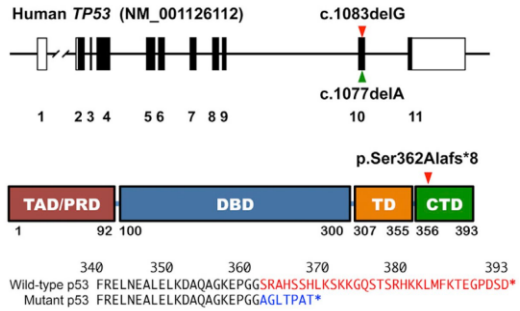
Mutation - exon 5 :

g.100,320,704G>A
p.(Arg150Gln)

➢ *TP53 gene mutation:*

Toki et al., Am J Hum Genet, 2018

Borderline DBA/DKC?

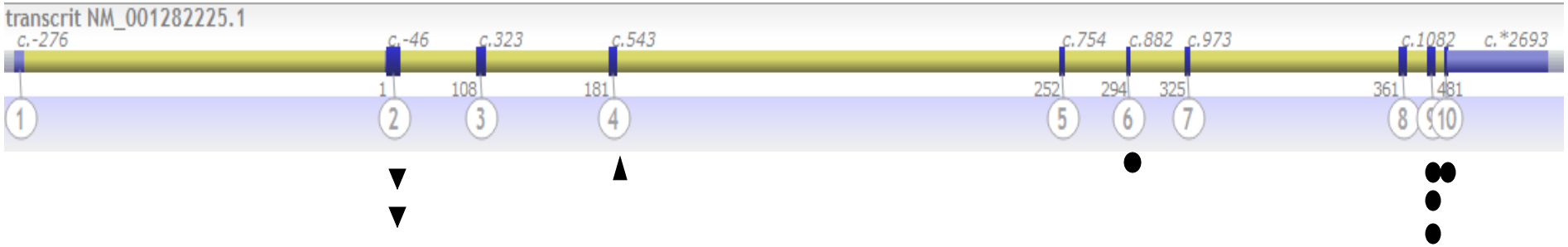


Mutation in exon 10:
c.1083delG or c.1077delA
p.(Ser362Alafs*8)

! Gain of function

Apart DBA: Mutation in *CECR1 (ADA2)* gene (ADA2 deficiency)

- 7 patients mutated in *CECR1* in the French registry



- **Features of the ADA2 deficiency French registry DBA patients :**
  - Middle east and north Africa Origins
  - Autosomal recessive inheritance
  - Severe Aregenerative Anemia from birth, normal MCV
  - Isolated erythroblastopenia
  - No IUGR, no growth retardation, no malformations
  - Normal eADA
  - **Hypogammaglobulinemia (IgA)**
  - Response to Steroid ; need to → HSCT

New DBA genes identified in DBAS :

Recurring mutations in *RPL15* are linked to hydrops fetalis and treatment independence in Diamond-Blackfan anemia

Marcin W. Wlodarski,[1,2] Lydie Da Costa,[3,4,5,6] Marie-Françoise O'Donohue,[7] Marc Gastou,[3,4,8] Narjesse Karboul,[3,5] Nathalie Montel-Lehry,[7] Ina Hainmann,[1] Dominika Danda,[1,9] Amina Szvetnik,[1] Victor Pastor,[1,10] Nahuel Paolini,[11] Franca M. di Summa,[11] Hannah Tamary,[12,13] Abed Abu Quider,[14] Anna Aspesi,[15] Riekelt H. Houtkooper,[16] Thierry Leblanc,[17] Charlotte Niemeyer,[1,2] Pierre-Emmanuel Gleizes[7] and Alyson W. MacInnes[16]

770–787 Nucleic Acids Research, 2020, Vol. 48, No. 2 *Published online 4 December 2019*
doi: 10.1093/nar/gkz1042

Ribosomal protein gene *RPL9* variants can differentially impair ribosome function and cellular metabolism

Marco Lezzerini[1,†], Marianna Penzo[2,†], Marie-Françoise O'Donohue[3,†], Carolina Marques dos Santos Vieira[4,†], Manon Saby[5,†], Hyung L. Elfrink[1,6], Illja J. Diets[7], Anne-Marie Hesse[8], Yohann Couté[8], Marc Gastou[9,10,11], Alexandra Nin-Velez[12], Peter G.J. Nikkels[13], Alexandra N. Olson[4], Evelien Zonneveld-Huijssoon[14,15], Marjolijn C.J. Jongmans[14,16], GuangJun Zhang[12], Michel van Weeghel[6], Riekelt H. Houtkooper[1], Marcin W. Wlodarski[17,18], Roland P. Kuiper[14], Marc B. Bierings[16], Jutte van der Werff ten Bosch[19], Thierry Leblanc[20], Lorenzo Montanaro[2], Jonathan D. Dinman[4], Lydie Da Costa[5,9,10,21], Pierre-Emmanuel Gleizes[3] and Alyson W. MacInnes[1,*]

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ARTICLE | VOLUME 103, ISSUE 6, P930-947, DECEMBER 06, 2018

The Genetic Landscape of Diamond-Blackfan Anemia

Jacob C. Ulirsch • Jeffrey M. Verboon • Shideh Kazerounian • ... Ron Do • Vijay G. Sankaran [18] • Hanna T. Gazda [18] • Show all authors • Show less • Show footnotes

Open Archive • Published: November 29, 2018 • DOI: https://doi.org/10.1016/j.ajhg.2018.10.027 •

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REPORT | VOLUME 105, ISSUE 5, P1040-1047, NOVEMBER 07, 2019

RPL13 Variants Cause Spondyloepimetaphyseal Dysplasia with Severe Short Stature

Cedric Le Caignec [21] • Benjamin Ory [21] • François Lamoureux [21] • ... Pierre-Emmanuel Gleizes [22] • Marc Baud'huin [22] • Bertrand Isidor [22] • Show all authors • Show less • Show footnotes

genes MDPI

Article

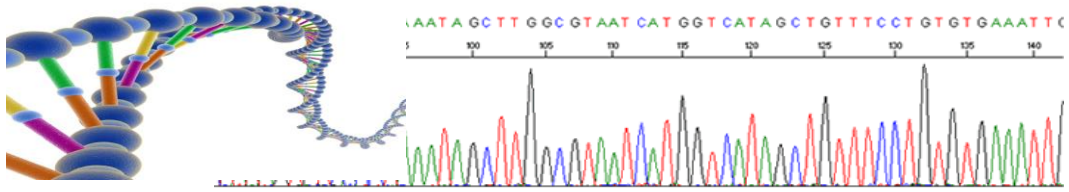
GATA-1 Defects in Diamond–Blackfan Anemia: Phenotypic Characterization Points to a Specific Subset of Disease

Birgit van Dooijeweert [1,2], Sima Kheradmand Kia [3,4], Niklas Dahl [5], Odile Fenneteau [6], Roos Leguit [7], Edward Nieuwenhuis [8], Wouter van Solinge [1], Richard van Wijk [1], Lydie Da Costa [6] and Marije Bartels [2,8,*]

JCI insight 2024

An atypical form of 60S ribosomal subunit in Diamond-Blackfan anemia linked to *RPL17* variants

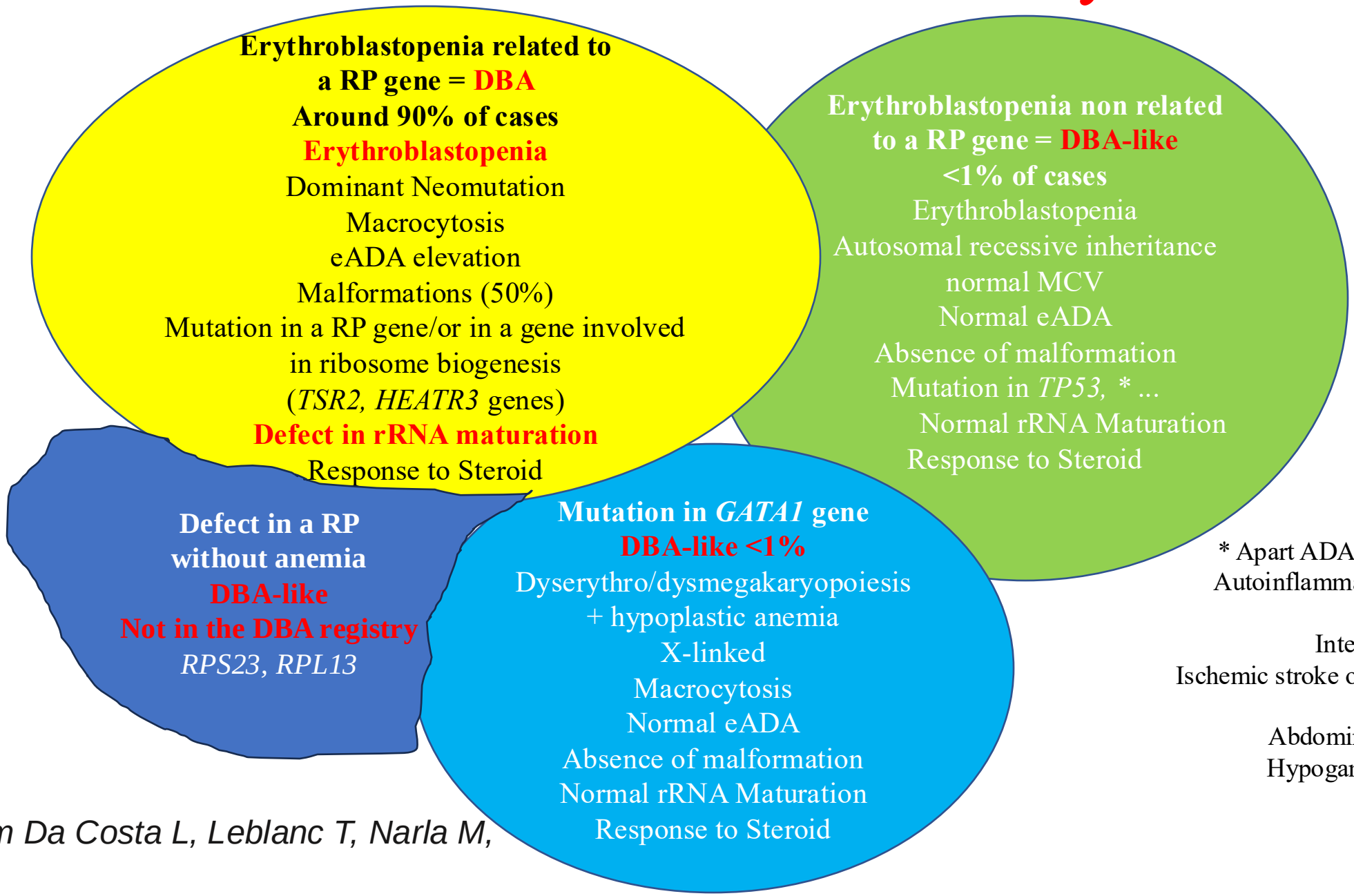
Florence Fellmann, … , Erica E. Davis, Pierre-Emmanuel Gleizes



Vanlerberghe C, …Da Costa L, Petit F. 2024
RPL26 variants: a rare cause of Diamond-Blackfan Anemia Syndrome with multiple congenital anomalies at the forefront. Genet Med. 2024

New definition

DBA+DBA-like = DBA syndrome



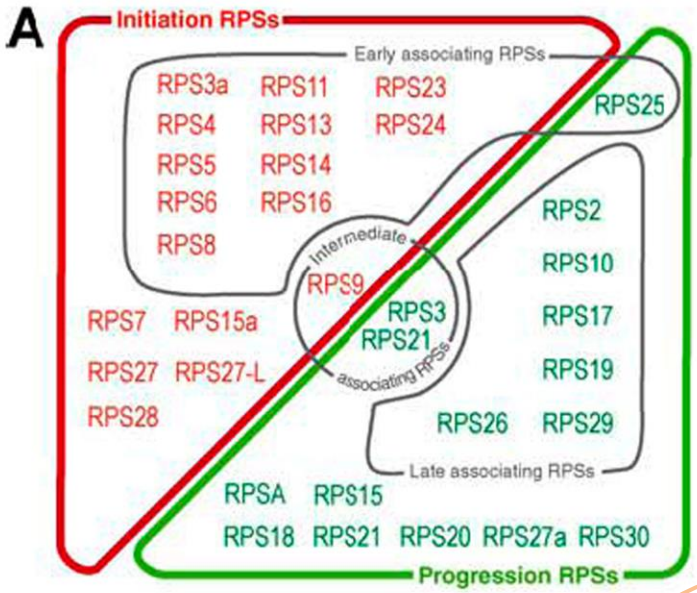
* Apart ADA2 deficiency :
Autoinflammatory disorder
Vasculitis
Intermittent fever
Ischemic stroke or hemorrhage
Arthralgia
Abdominal pain crisis
Hypogammaglobulins

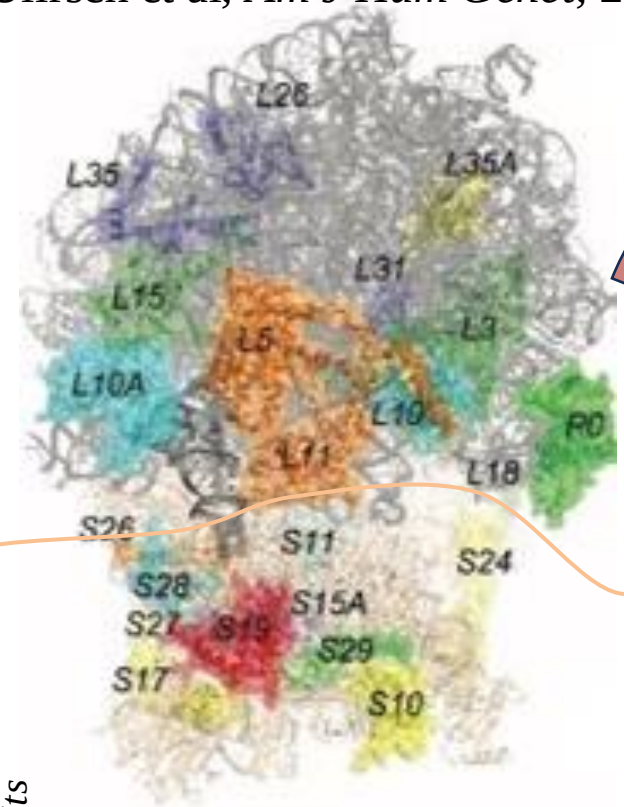
Modified from Da Costa L, Leblanc T, Narla M, Blood, 2020

DBA, the first ribosomopathy described

Ribosome assembly

Ulirsch et al, *Am J Hum Genet*, 2018

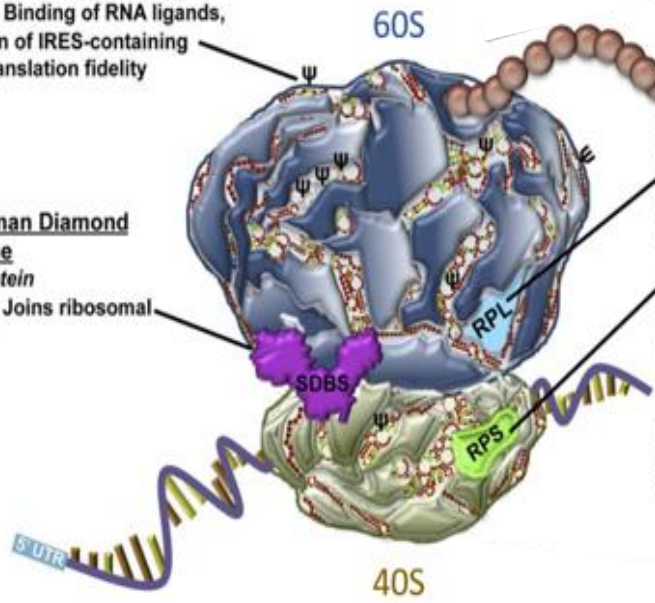




Mutations

Dyskeratosis Congenita
Dyskerin protein: Converts Uridine ➡ Pseudouridine (ψ)
Function: Binding of RNA ligands, translation of IRES-containing mRNA, translation fidelity

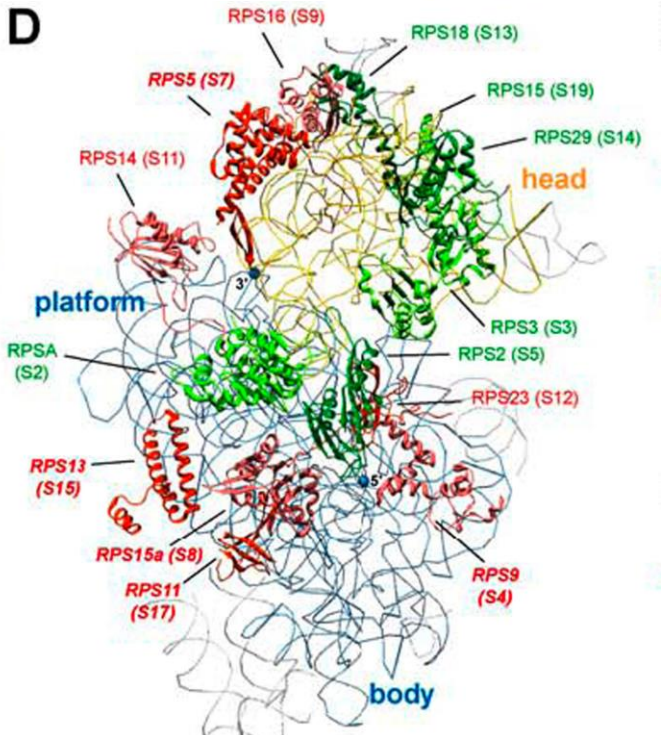
Shwachman Diamond Syndrome
SDBS protein
Function: Joins ribosomal subunits



Diamond Blackfan Anemia
Ribosomal proteins
RPs mutated in 60S: RPL5, RPL11, RPL15, RPL26, RPL35A

RPs mutated in 40S: RPS7, RPS10, RPS17, RPS19, RPS24, RPS26

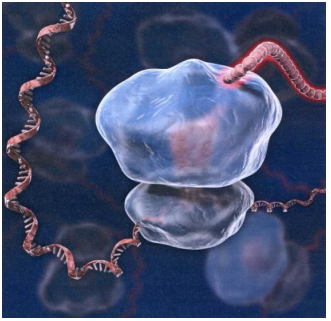
Function: Involved in ribosome biogenesis, protein synthesis, and translation of specific mRNAs



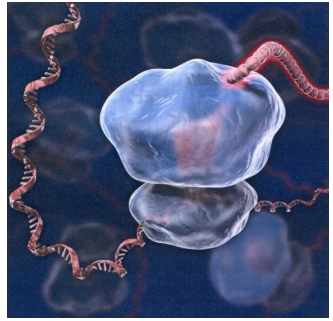
O'Donohue, JCB, vol 190, 2010

Composition of both ribosome subunits

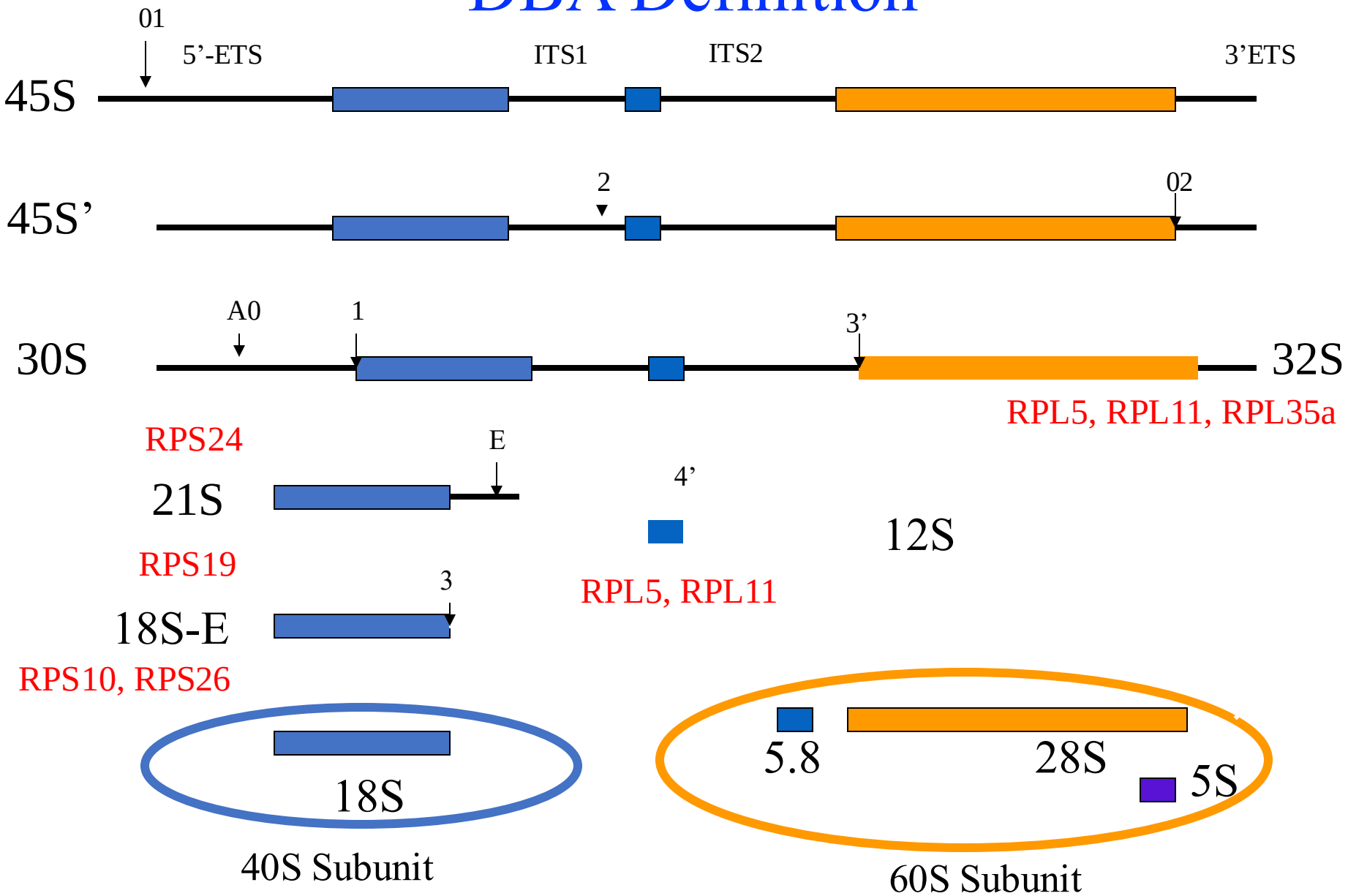
	Eukaryotes
Large subunit	
Sedimentation	60S
Number of proteins	42
rRNA	5S 5.8S 28S
Small subunit	
Sedimentation	40S
Number of proteins	32
rRNA	18S



DBA, a defect in rRNA maturation = DBA Definition



McCann and al., Science, 2013



Ribosome Dysfonction

Adapted from Fumagalli et al., Nature cell biology, 2009

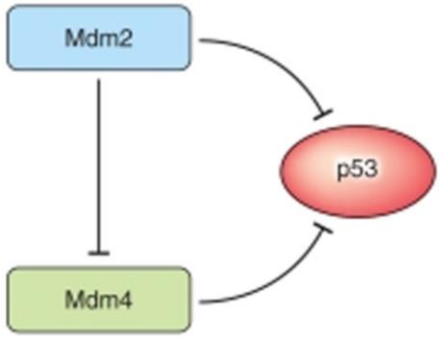
Oncogenic Activation

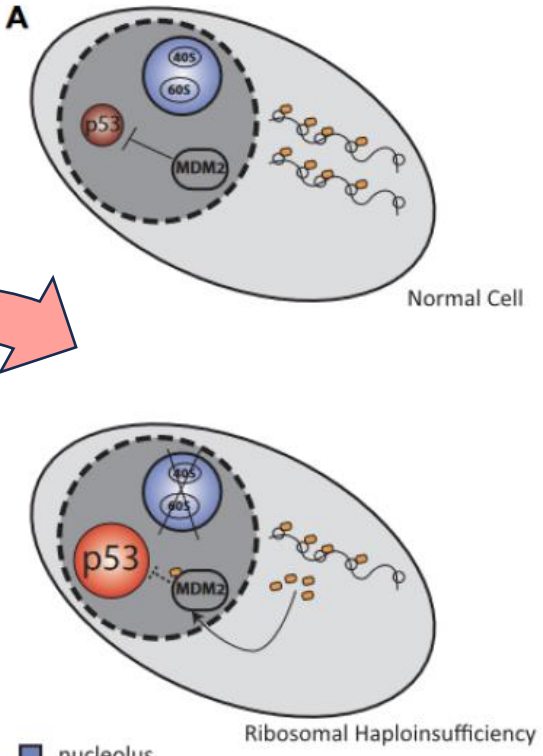
Radiation

p53

Apoptosis
Cell cycle arrest
Senescence

➢ Homeostasis





➢ Ribosomal or nucleolar Stress

Ribosomal Stress

S27a **S27** **S3** **S7** **L11** **L5** **L23**

MDM2

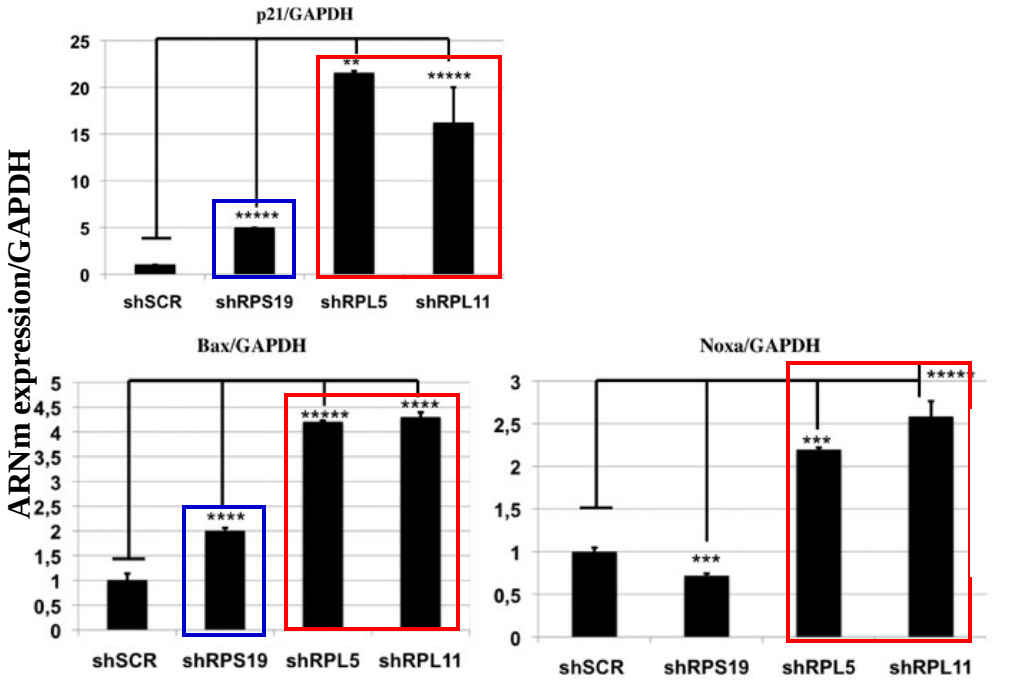
Stabilization or p53 activation

p53 p53 p53 p53 p53 p53 p53

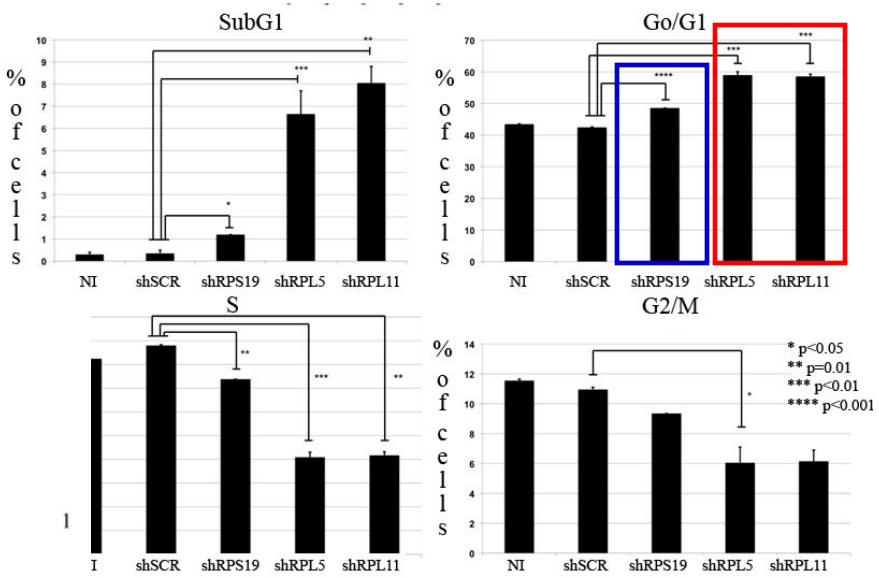
Cell cycle arrest

Apoptosis

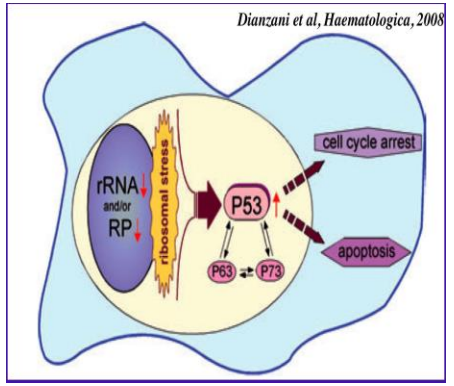
Increased p53 target mRNA



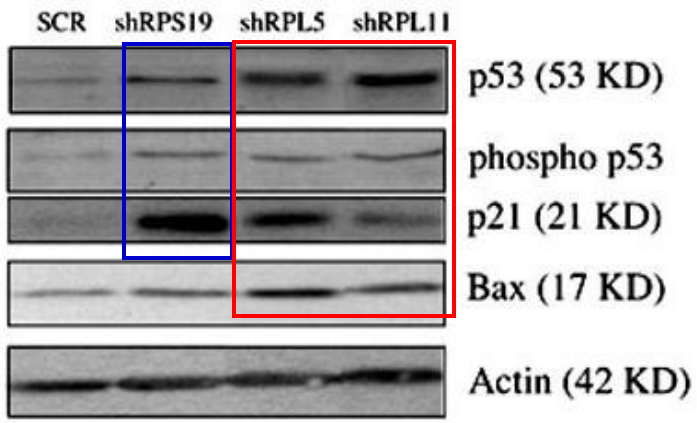
Cell cycle arrest in G_0/G1



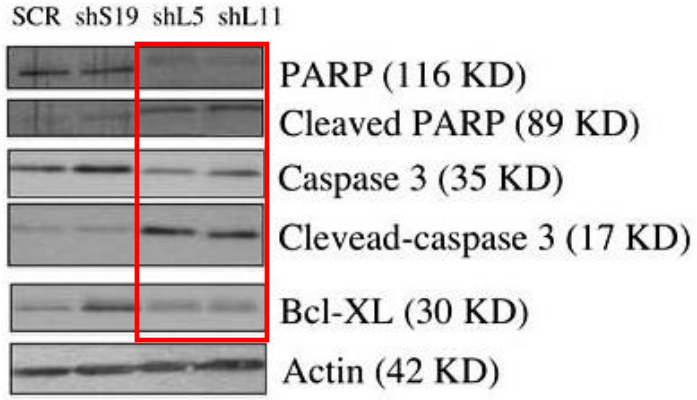
p53 is activated in DBA



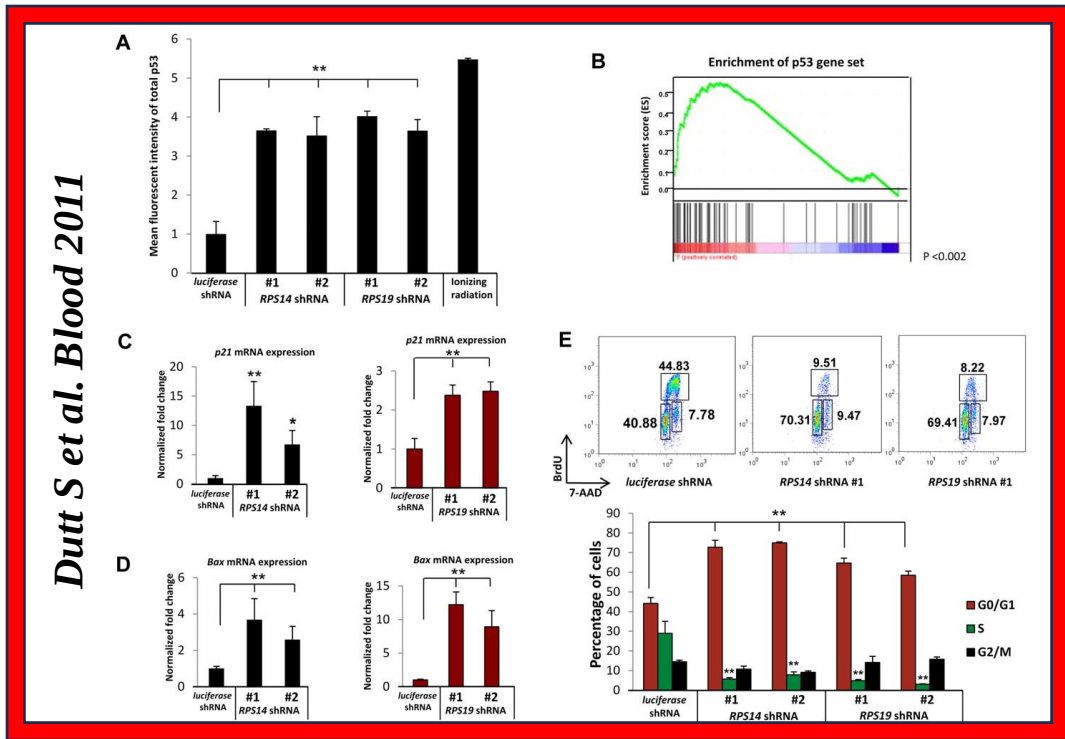
Increased p53/P-p53 and targets



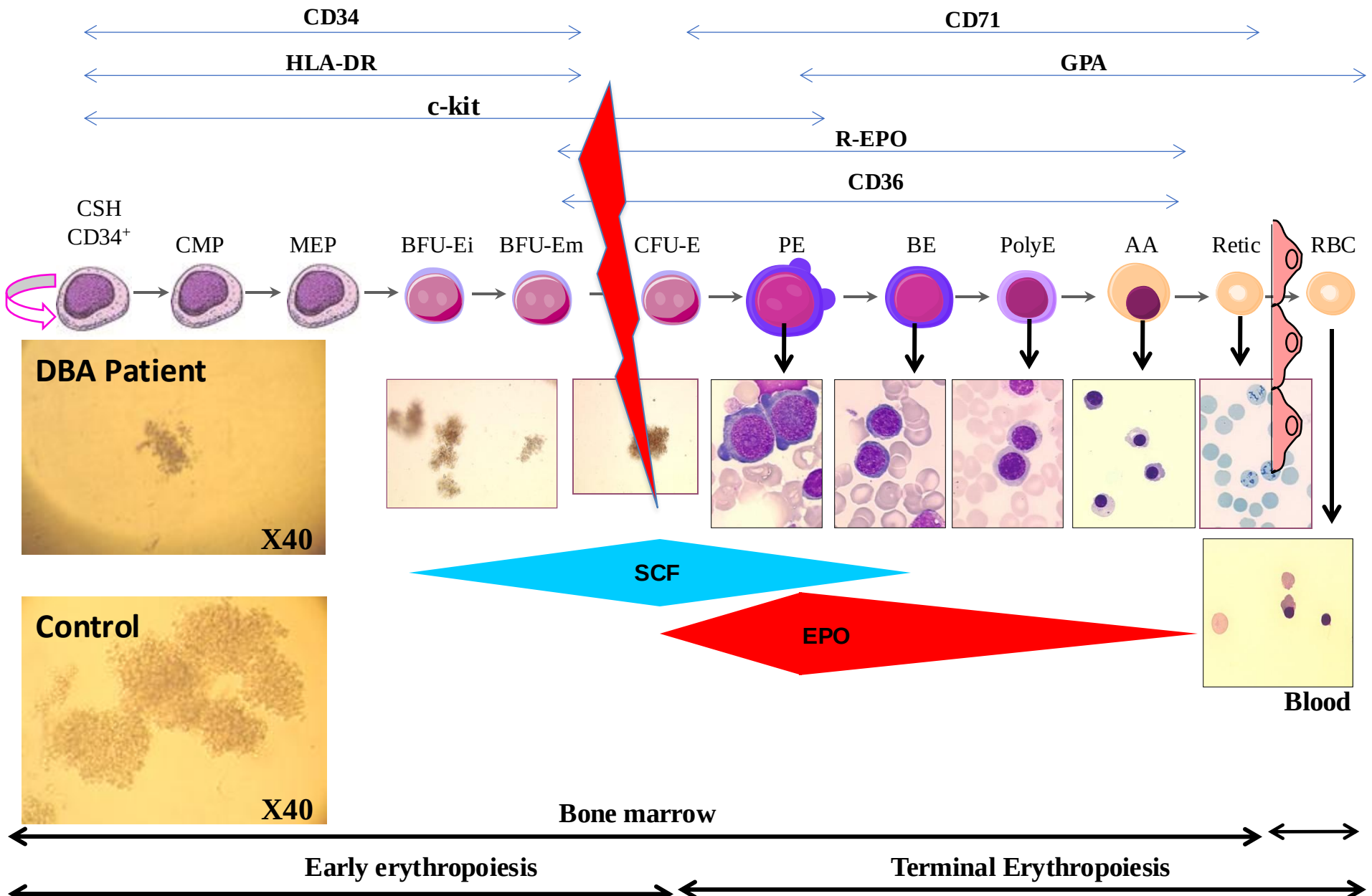
Increased apoptosis



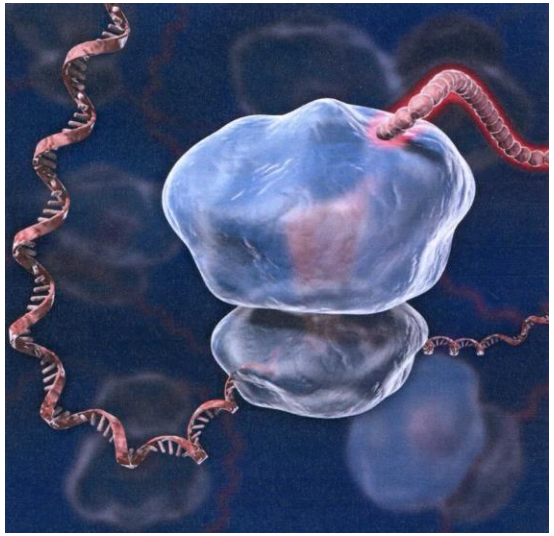
H. Moniz et al., Cell Death and disease, 2012

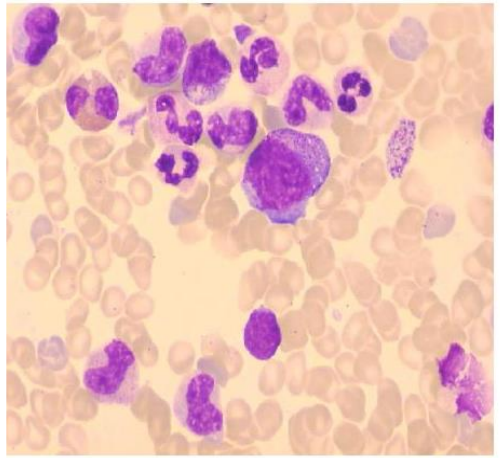


DBA, an intrinsic defect of erythropoiesis



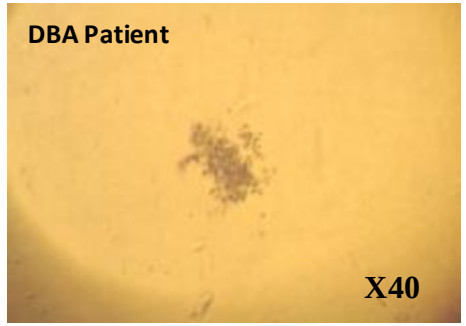
Diamond-Blackfan Anemia :
Why an erythroid tropism in a ribosomopathy?

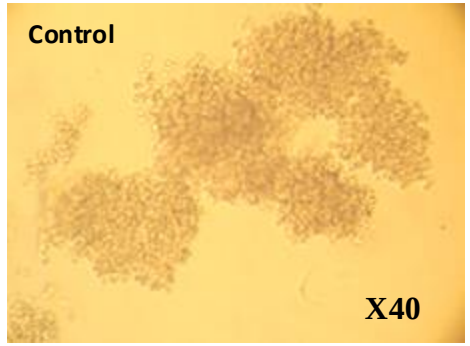




DBA - Erythroblastopenia

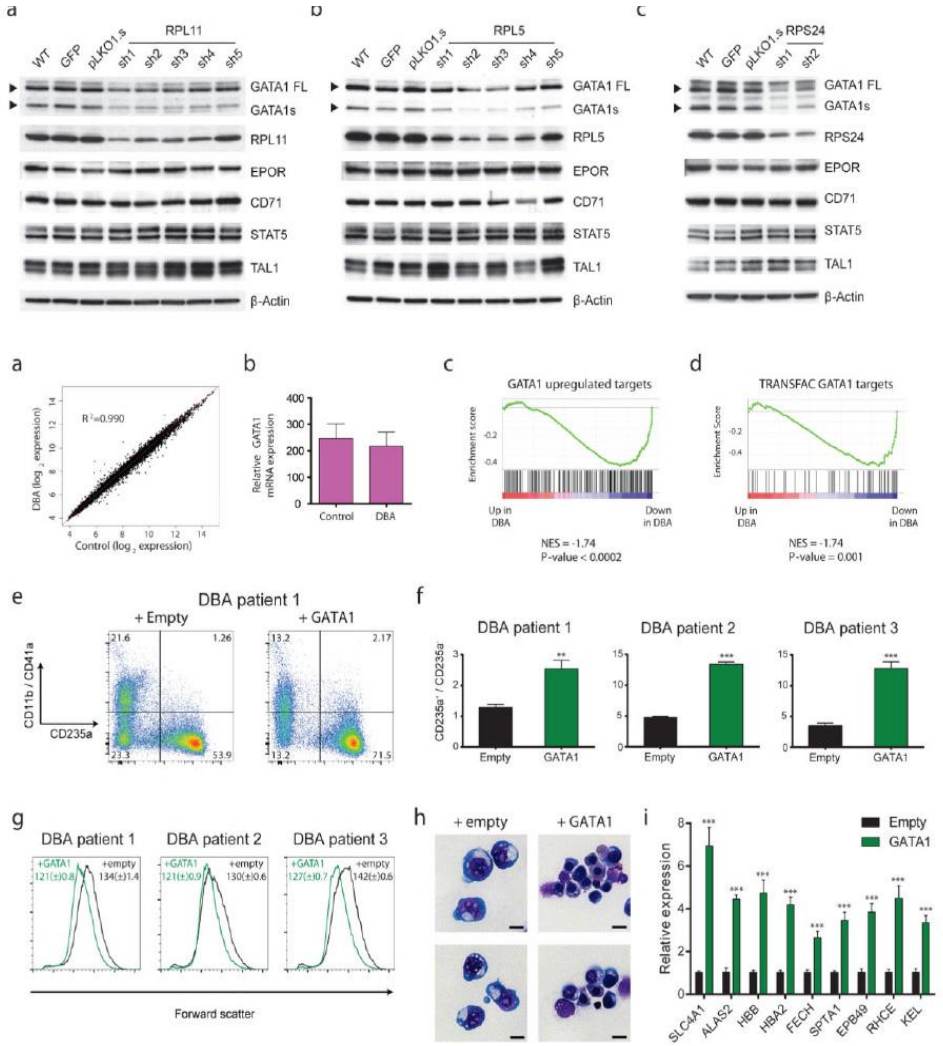
D7 erythroid culture





And GATA1 in DBA pathophysiology ?

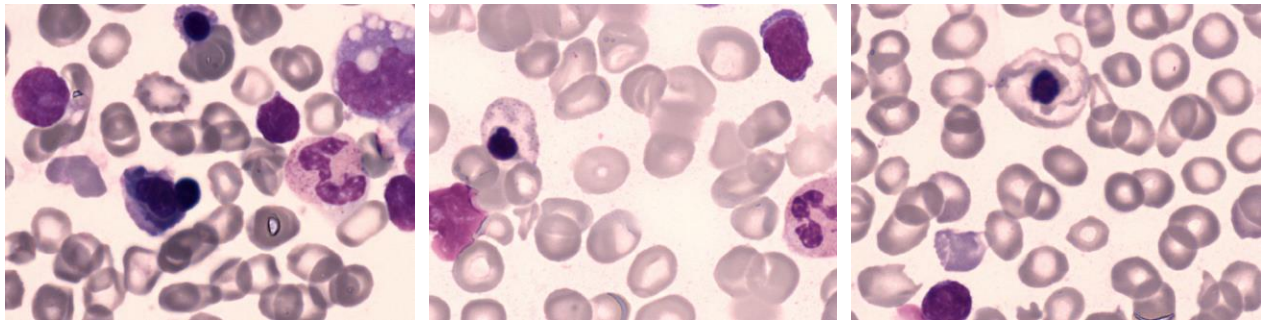
Alteration of GATA1 translation in cases of RP gene mutation



Lutwig et al., Nature Med, 2014

GATA1 gene mutation

However, …

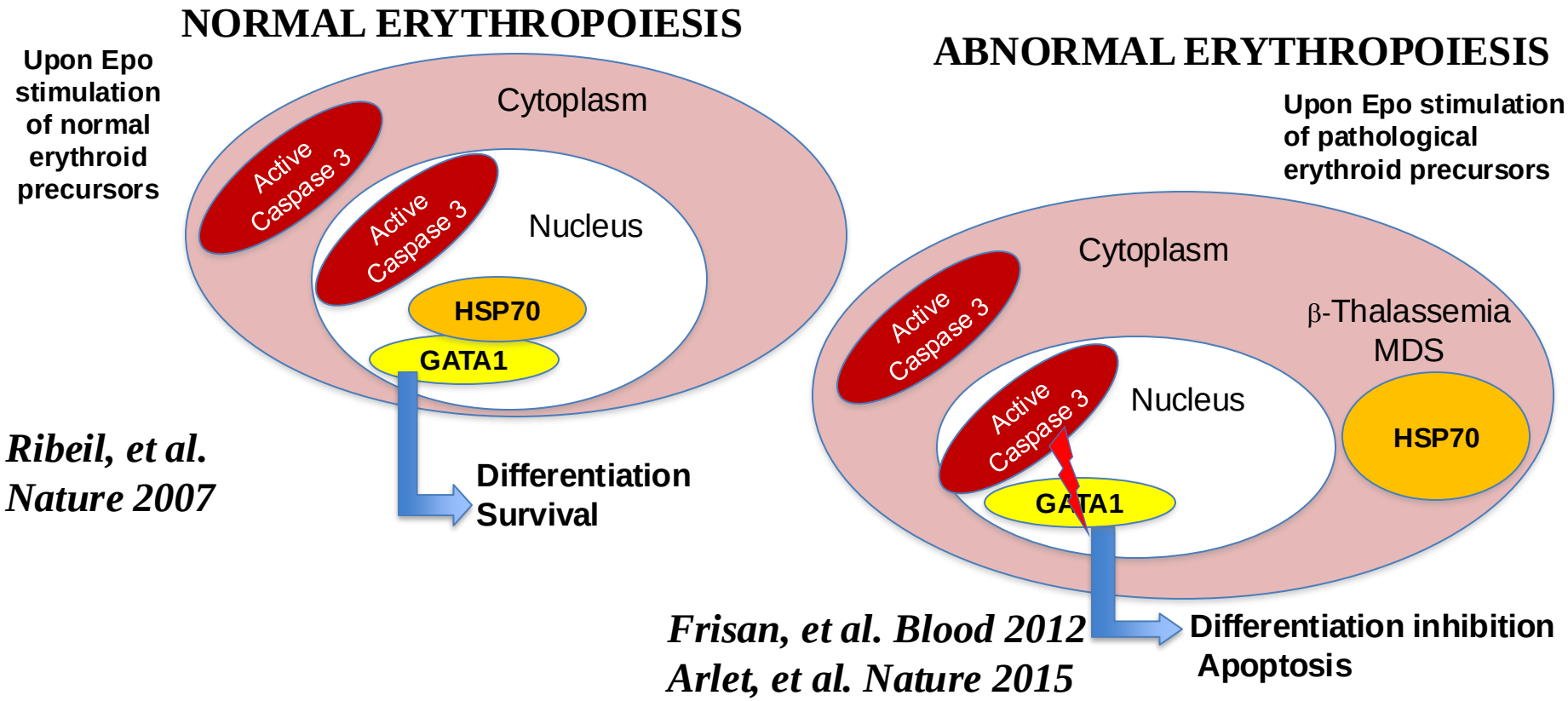


c.220 +2T>C
p.?
Class 4

Birgit van Dooijeweert et al., Genes, 2022

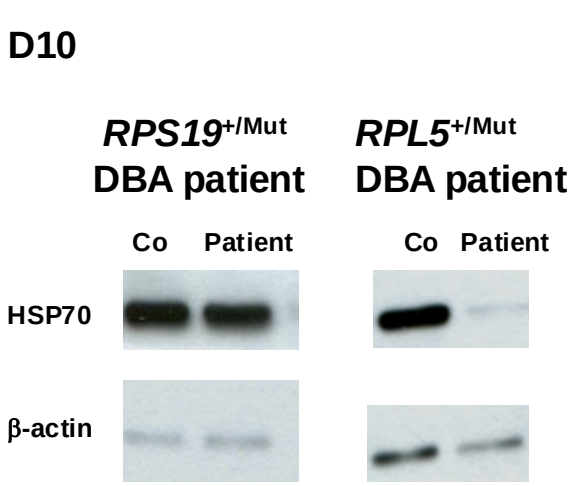
HSP70 ?

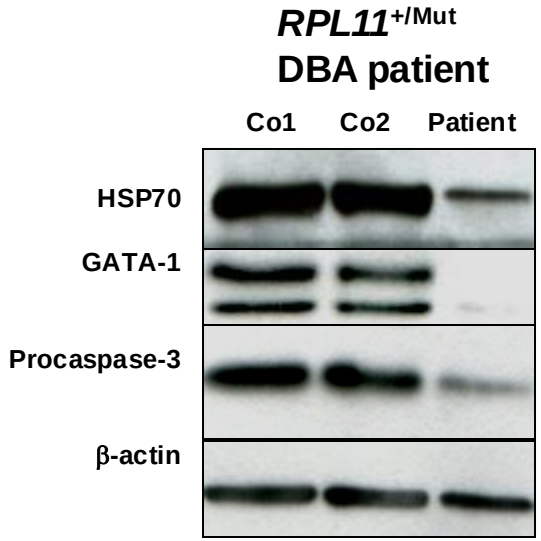
Hypothesis

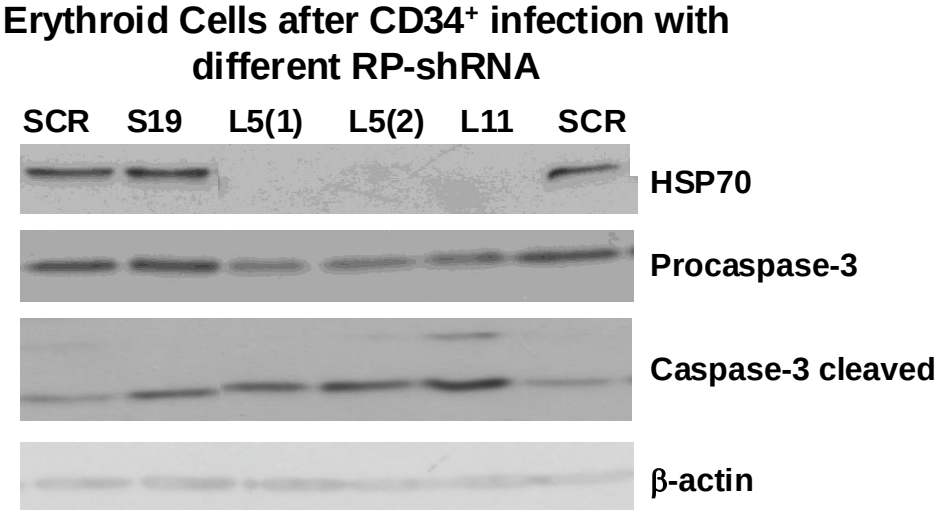


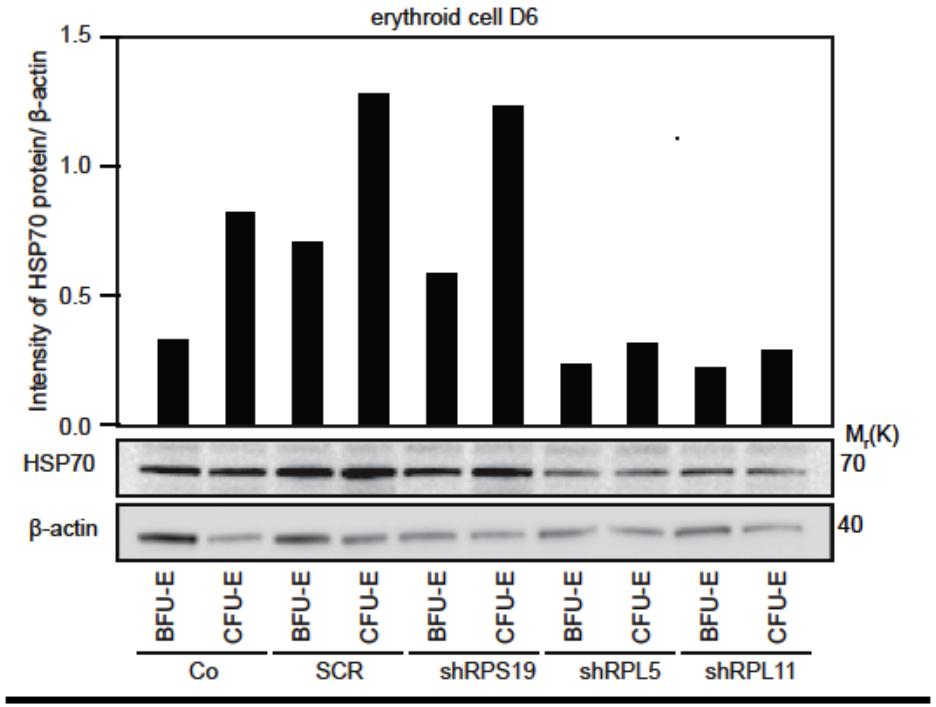
Does a defective protection of GATA1 by HSP70
also account for DBA-associated erythroblastopenia ?

HSP70 protein expression level is decreased in *RPL5* and *RPL11* haploinsufficient primary erythroid cells as soon as BFU-e stage



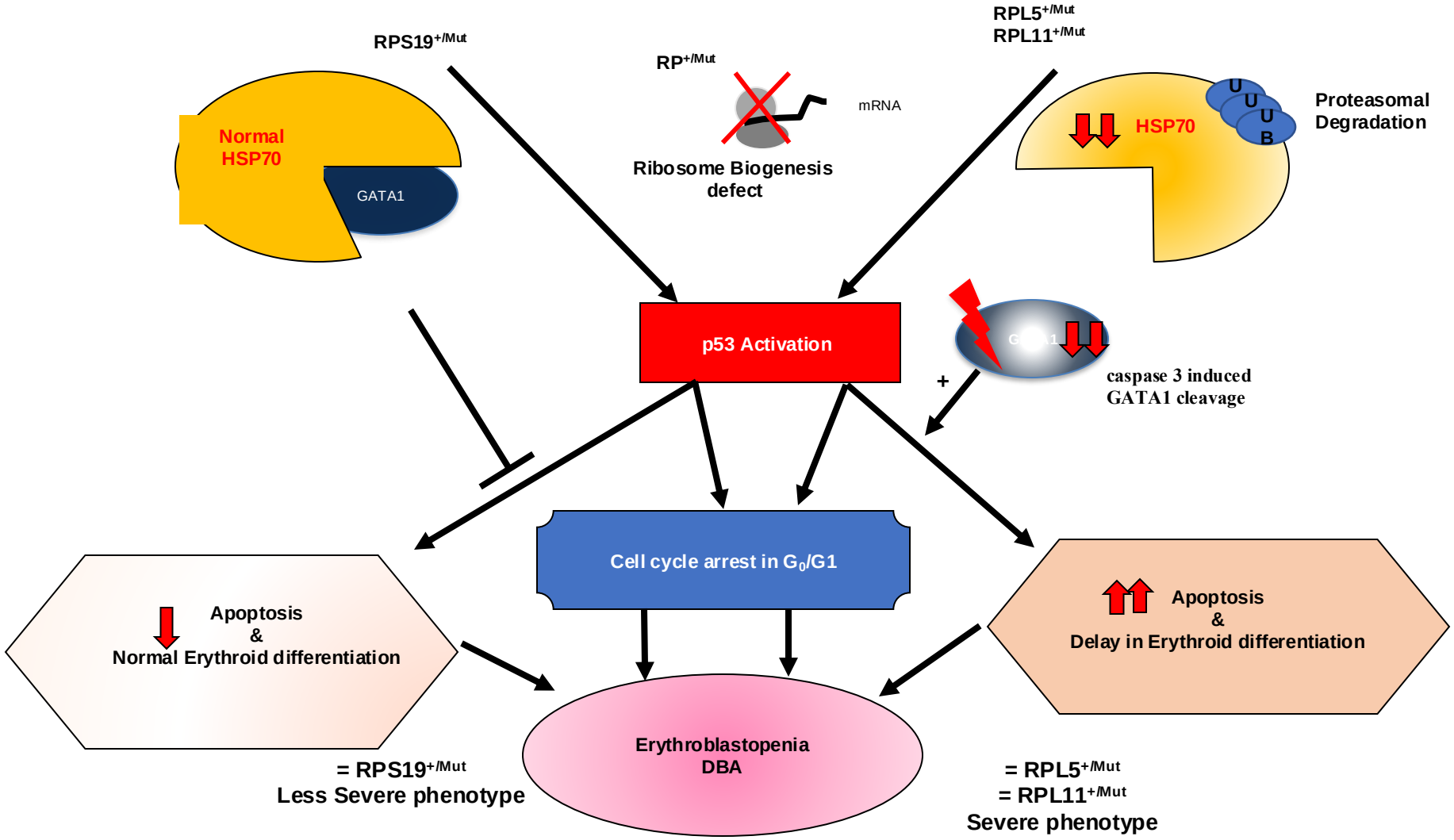






Marc Gastou et al., Blood Advances 2017

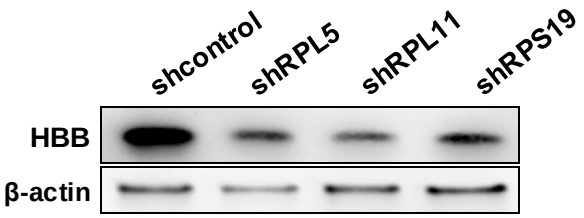
HSP70 is involved in DBA erythroid defect

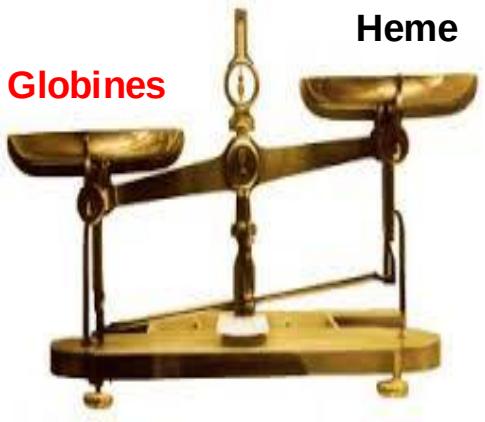


Gastou M. et al., Blood Advances 2017
Moniz H et al., Cell Death and Dis, 2012

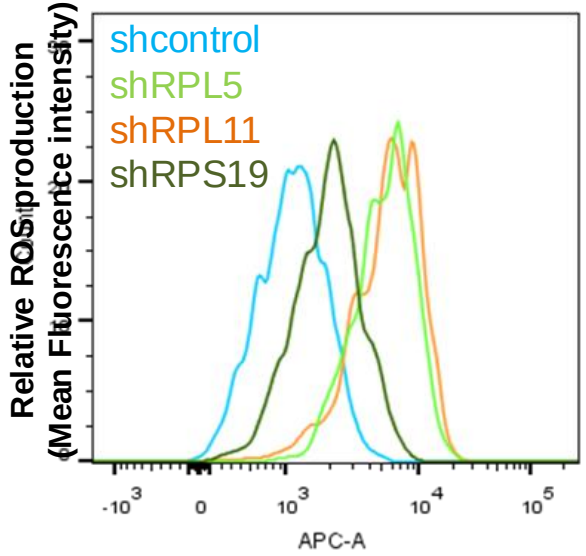
GATA-1/HSP70 and free heme overload in DBA

D9 erythroid differentiation

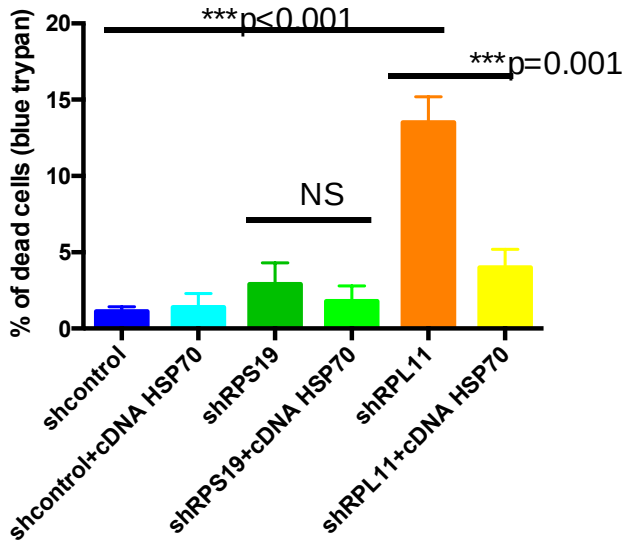


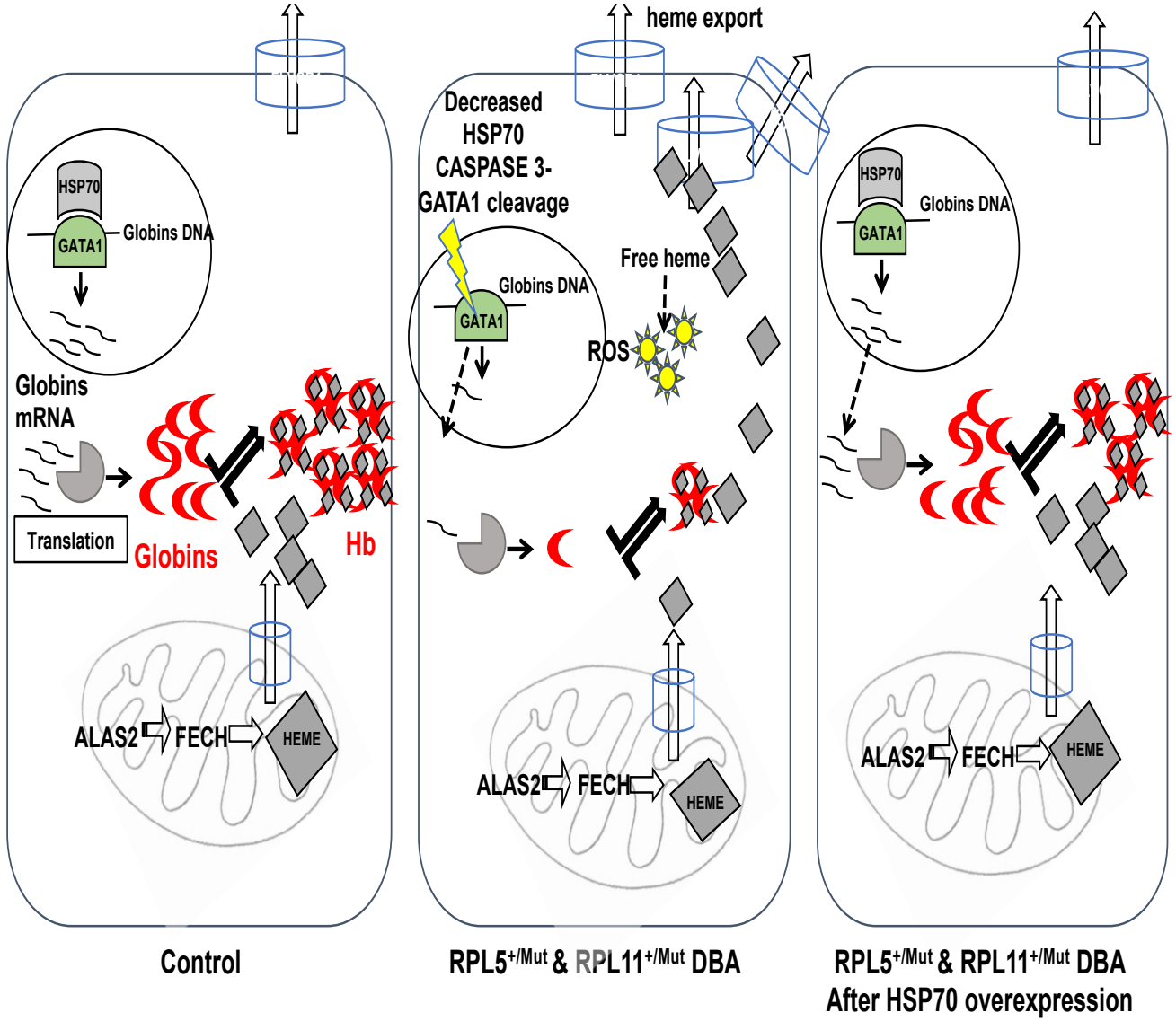


ROS production at D9



Cell death





Control

Normal erythroid proliferation and differentiation

RPL5$^{+/Mut}$ & RPL11$^{+/Mut}$ DBA

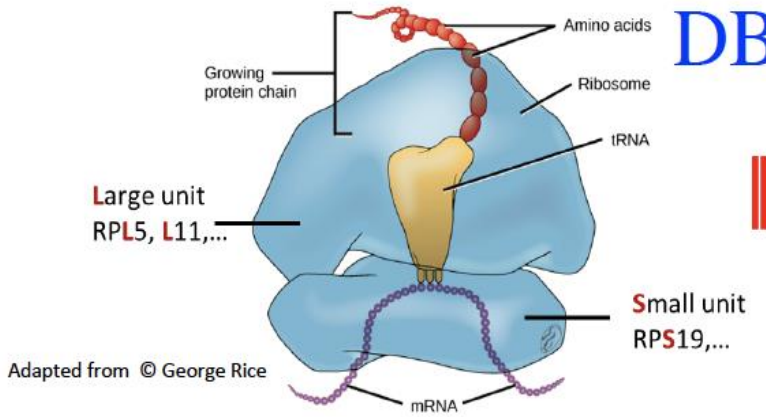
Apoptosis and delay in erythroid differentiation

RPL5$^{+/Mut}$ & RPL11$^{+/Mut}$ DBA After HSP70 overexpression

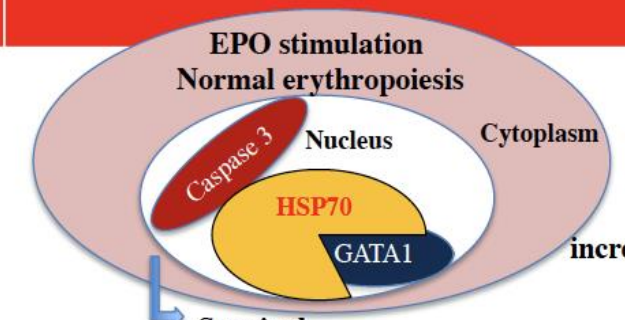
Rescued erythroid proliferation and differentiation

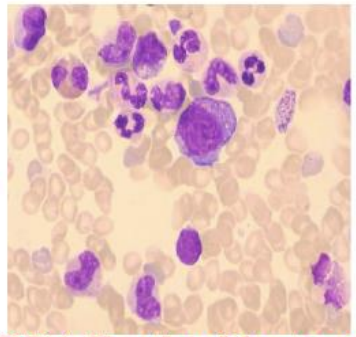
S. Rio, M. Gastou* et al., Blood 2019*

DBA pathophysiology



?





DBA Erythroblastopenia

(decreased proliferation, delayed differentiation, increased apoptosis, ROS production, cell cycle arrest)

RP haploinsufficiency

1 **p53 activation**

Decreased ribosome amount

2 **Decreased specific mRNA translation**

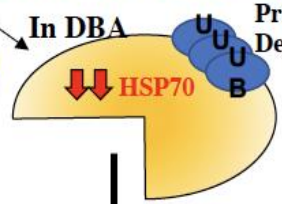
other gene translation, (may affect GATA-1 translation as consequence)

GATA-1 translation

?

Decreased HSP70 protein

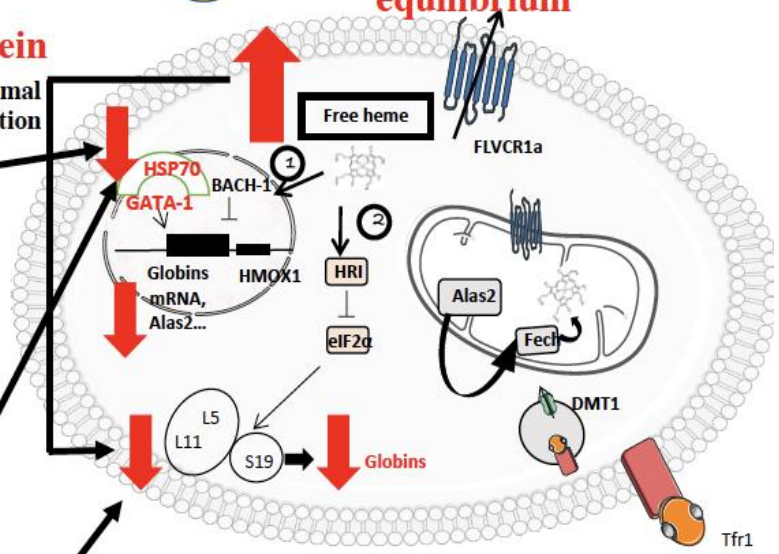
3 In DBA



Proteasomal Degradation

GATA-1 protein

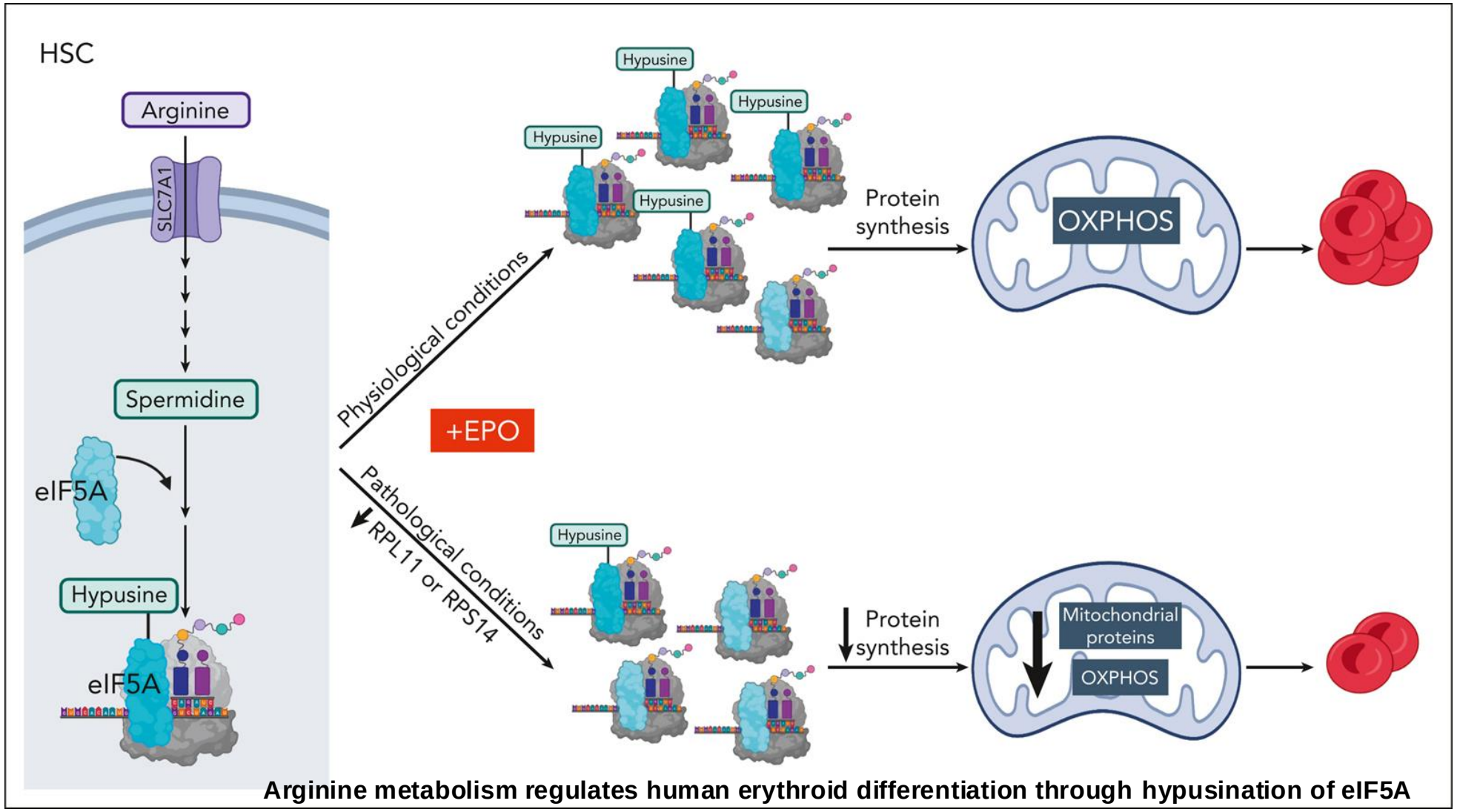
4 **Globin/Heme unbalanced equilibrium**



Da Costa L, Leblanc T, Mohandas N, Blood 2020

Lutwig et al., Nature Med, 2014
Boussaid, I et al. Haematologica, 2021
Gastou M. et al, Blood Advances 2017
S. Rio, M. Gastou* et al., Blood 2019*
Doty et al., Blood 2019

New Mechanism of erythroid defect in DBA ?
Default in hypusination and mitochondrial RP



Arginine metabolism regulates human erythroid differentiation through hypusination of eIF5A

ANR ErythroHyp –
Collaboration Sandrina Kinet, Naomi Taylor

Pedro Gonzalez-Menendez et al. Blood, 2023

Take home message

- DBA a rare constitutional erythroblastopenia
- First described ribosomopathy
- Not easy to diagnose
- New definition = DBA syndrome (DBAS) including
  - The “classical” DBA (90% of the cases) but also
  - The DBA-other (DBA-like)(related to *GATA1*, *TP53* genes)
  - The RP mutated gene related to DBA phenotype without anemia
- New guidelines published !

Wlodarski MW, et al., Lancet Haematol. 2024 May;11(5):e368-e382. PMID: 38697731

- Large progress in the mechanistic understandings of the DBA pathophysiology: p53, GATA1, HSP70, free heme excess, hypusination, and…..

Thank you for your attention

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Michael Dussiot
Olivier Hermine
Exome sequencing platform (C. Bole, C. Masson, P. Nitschke)

MaRIH
Filière de santé Maladies Rares Immuno-Hématologiques

Regis Peffault de la Tour
Flore Sicre de Fontbrune
And all the MDs involved in DBA cares

Saint Louis iuh
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