Presentation

Delete Nuclear Export Signal (NES) of human Activation-induced cytidine deaminase (hAID)

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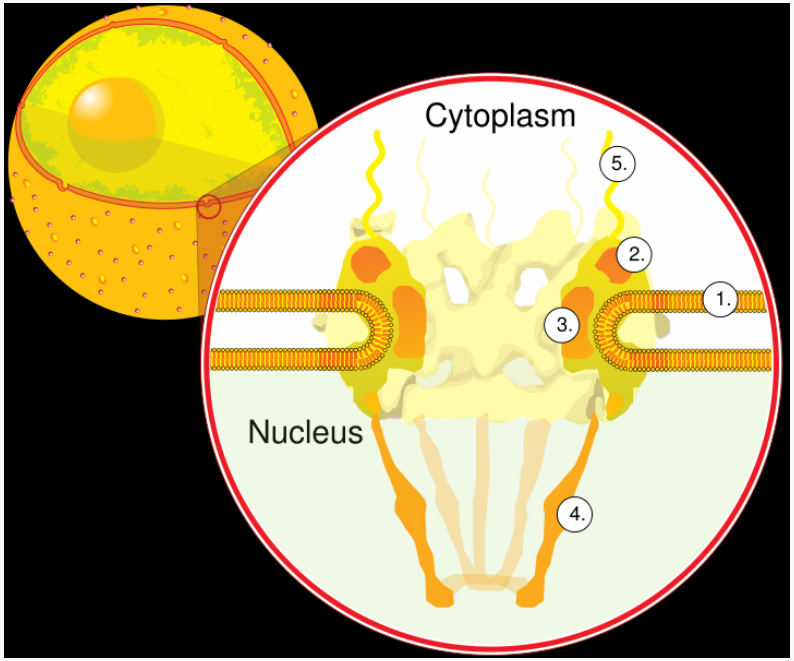
NES (Nuclear Export Signal)

- ashort amino acid sequence of 5-6 hydrophobic residues in a protein that targets it for export from the cell nucleus to the cytoplasm through the nuclear pore complex

- be recognized and bound by exportins

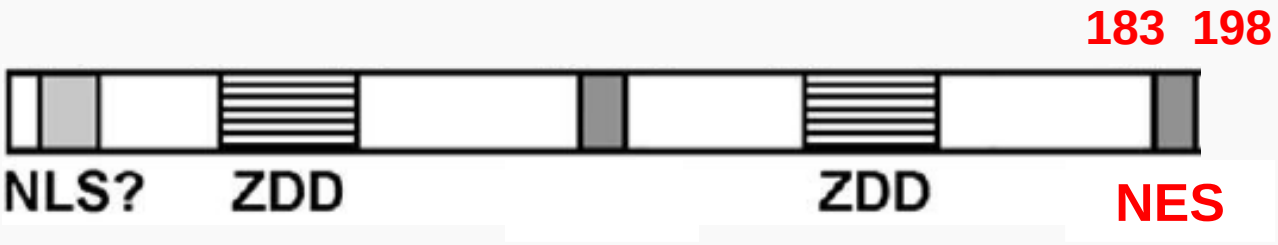


AID: Nucleo-cytoplasmic Trafficking Protein

- AID has C-terminal leucine-rich NES domain

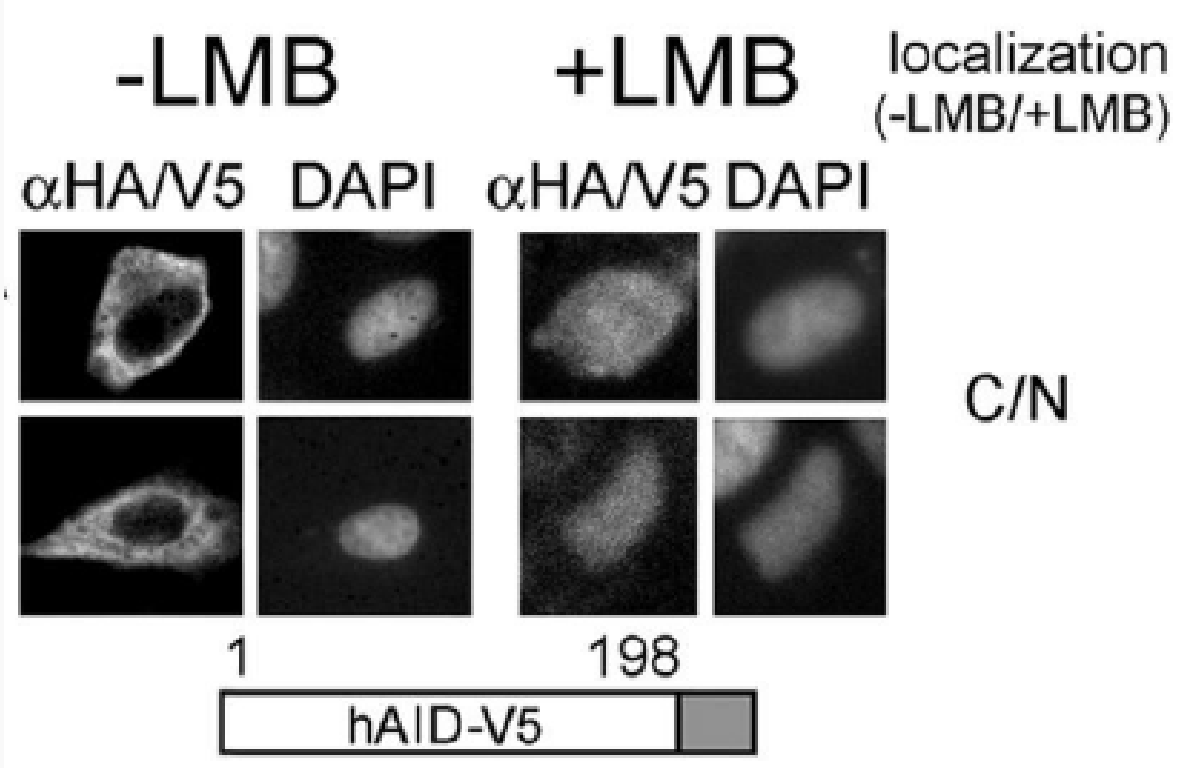
NES homologies

hAPOBEC-1	173-	LWMMLYA-LEL-182
hAID	189-	LRDAFRT-LGL-198
hA3G-NT	185-	LHIMLGEILRH-195
hA3G-CT	371-	LSGRLRAILQN-380
consensus		Lx(2,3)[LIVFM]x(2,3)Lx[LI]



R.P.Bennett *et al* (2006)

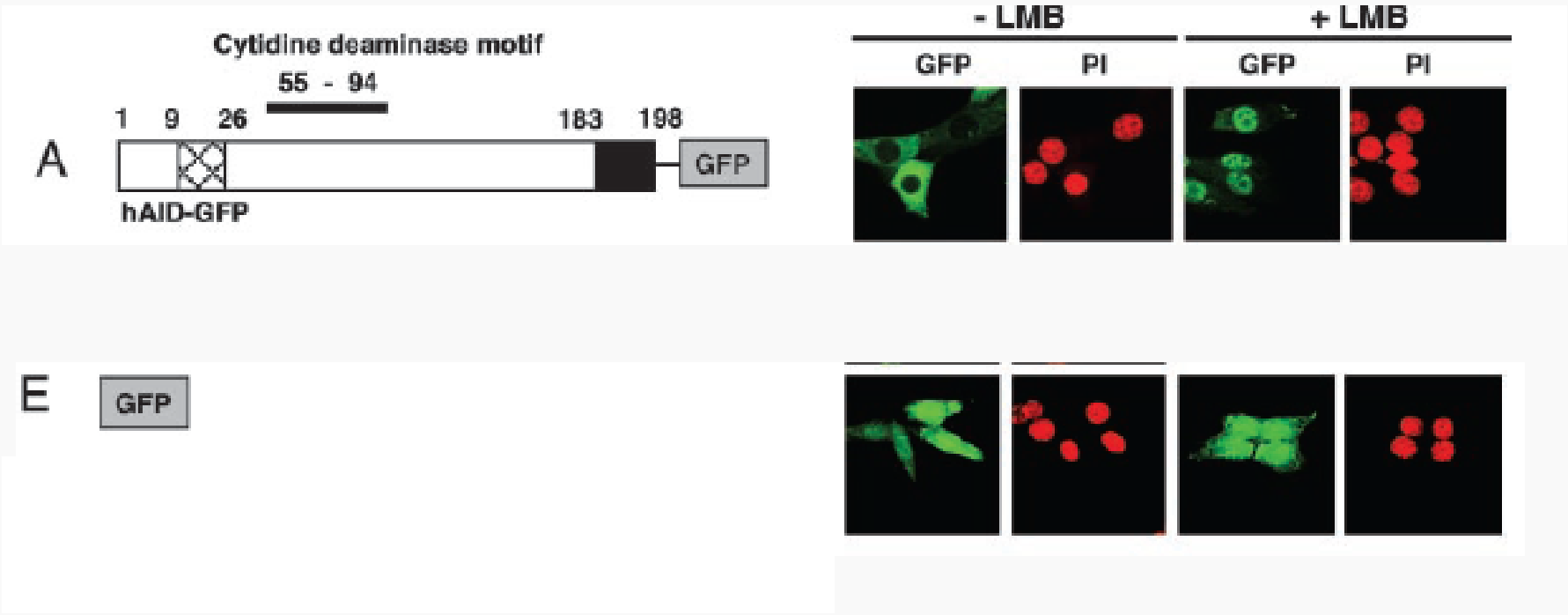
Cellular Distribution of AID



Leptomycin B (LMB, inhibit exportin1-dependent nuclear export) treatment resulted in nuclear accumulation of AID

R.P.Bennett *et al* (2006)

AID is a nucleocytoplasmic shuttling protein



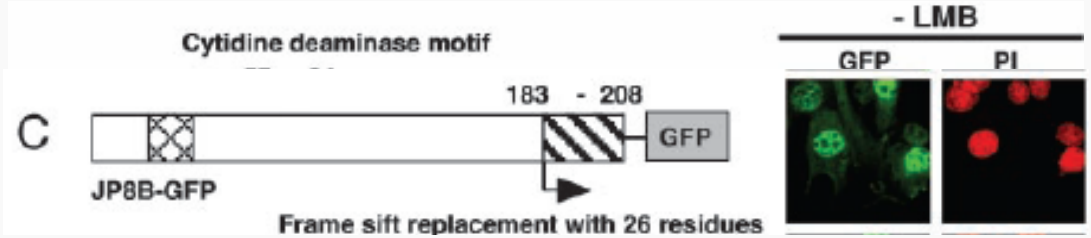
Exportin1 recognizes a leucine-rich NES on a target protein and exports it from the nucleus

S. Ito *et al* (2003)

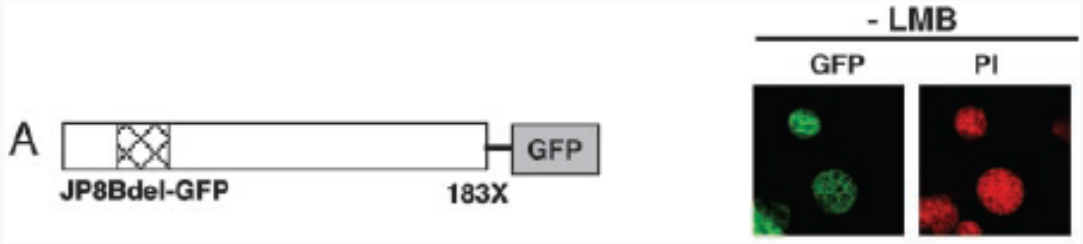
Mutations cause AID accumulate in Nucleus

P20: a 34-aa insertion at residue 182

JP8B: a frame-shift mutation at residue 183



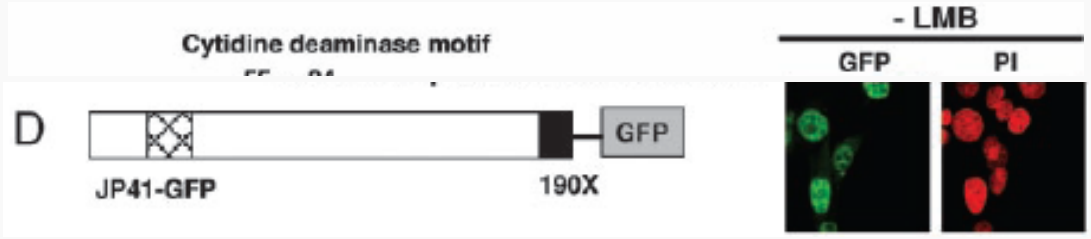
183X: artificial truncation at resident 183



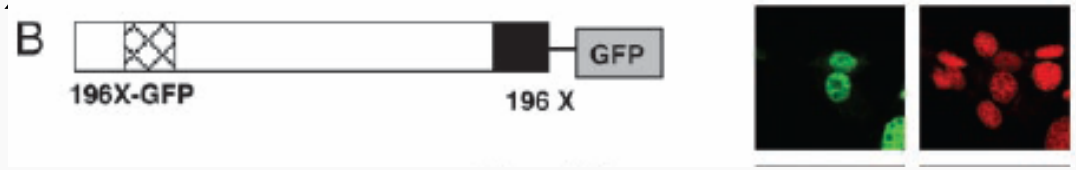
S. Ito *et al* (2003)

Mutations cause AID accumulate in Nucleus

JP41(190X): C-terminal deletion mutant

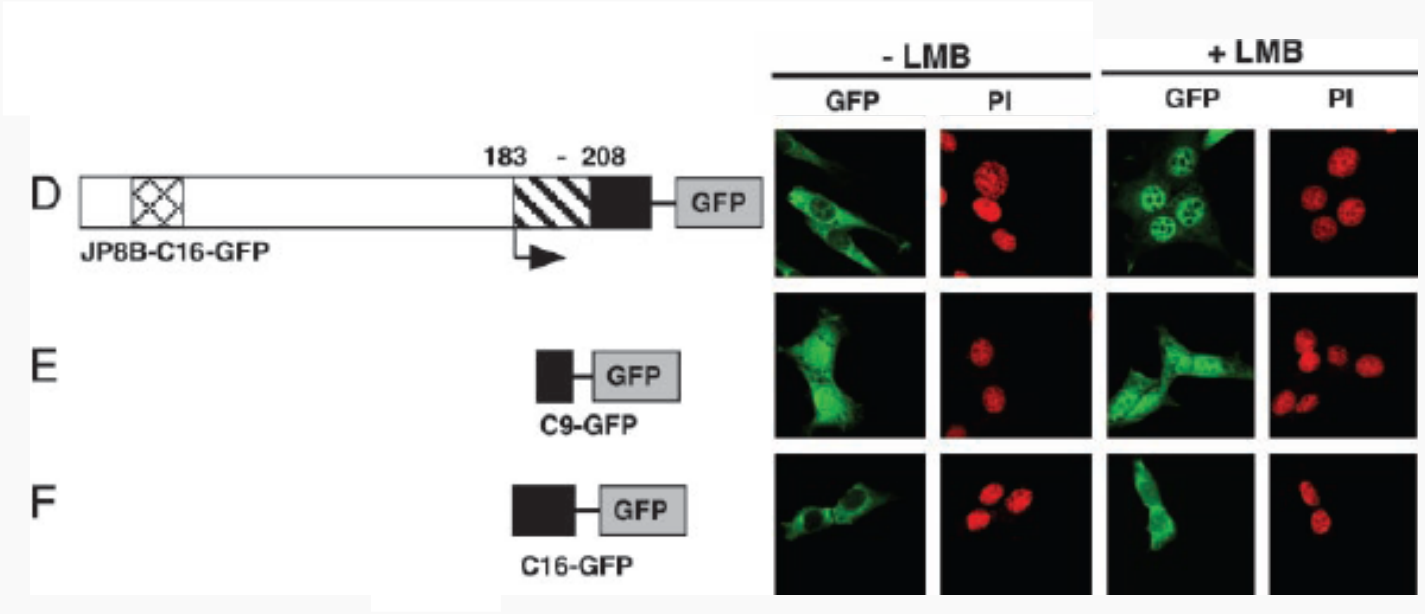


196X(&193X)



S. Ito *et al* (2003)

GOF: functional NES at C183-198 for AID



Another leucine-rich NES candidate 172-183 shows no indications of NES activity

S. Ito *et al* (2003)

Influence to mutation

C-terminal 17 aa of AID: critical for CSR; not for SHM and GC

Table 1. Mutation frequency induced by mutant AID-GFP fusion protein

Sequence	Clone mutated/total	Mutation (del. or ins.)	Total bases	Frequency per 10^4	*P* values versus* None	*P* values versus* hAID
196X	7/10	16 (1)	4,760	33.6	<0.001	<0.001
193X	5/10	12 (0)	4,760	25.2	<0.001	0.006
JP8Bdel	8/10	20 (3)	4,730	42.2	<0.001	<0.001
ΔN5JP8Bdel	7/10	20 (1)	4,714	42.4	<0.001	<0.001
hAID	7/33	12 (2)	15,679	7.7	0.005	
None	1/27	1 (0)	12,851	0.8		

*Fisher's exact test for mutation/bases. del., deletion; ins., insertion.

Efficient export from nucleus is not critical for induction of SHM

S. Ito *et al* (2003)

Summary

Proved AID-nucleus-accumulation causing mutants:

Natural: JP8B
Artificial: 183X, JP41(190X), 196X, 193X

Effect
not critical for induction of SHM; even higher mutation frequency

Question
Is it actually suitable for our system?

Thank you for attention☺