Increased expression of α-synuclein is a primary cause of PD

A limited increase in WT α-synuclein expression can cause both familial and sporadic forms of PD:

- Duplications and triplications of the WT *SNCA* gene lead to autosomal dominant PD.
- Polymorphisms in the *SNCA* locus increasing *SNCA* transcription confer high risk for sporadic PD.
- α-synuclein mRNA levels are increased in dopaminergic neurons of sporadic PD patients.
- Overexpression of WT α-synuclein leads to neurodegenerative syndromes in animal models.

However, the transcriptional regulation of α-synuclein is poorly understood.

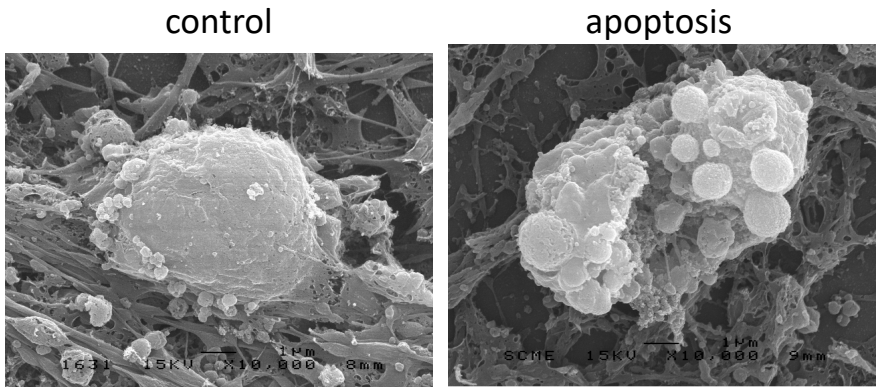
TRIM17, a crucial E3 ubiquitin-ligase for neuronal apoptosis

★ *TRIM17* is highly induced in early apoptotic neurons

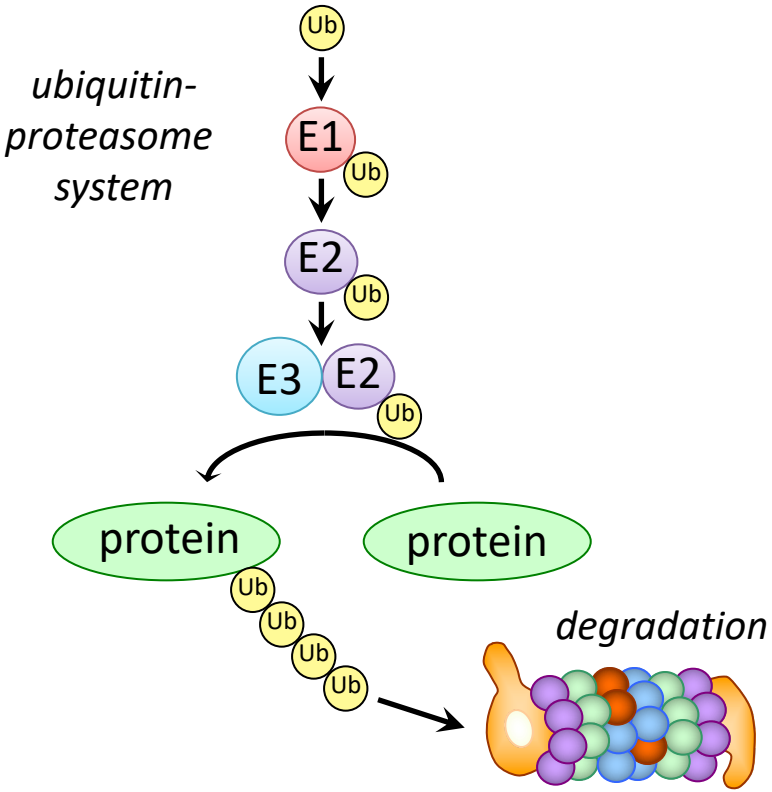
Desagher et al. (2005) *J. Biol. Chem.* 280: 5693-5702.

★ TRIM17 is both sufficient and necessary for neuronal apoptosis

Lassot et al. (2010) *Cell Death Differ.* 17: 1928-1941



primary cultures of cerebellar granule neurons

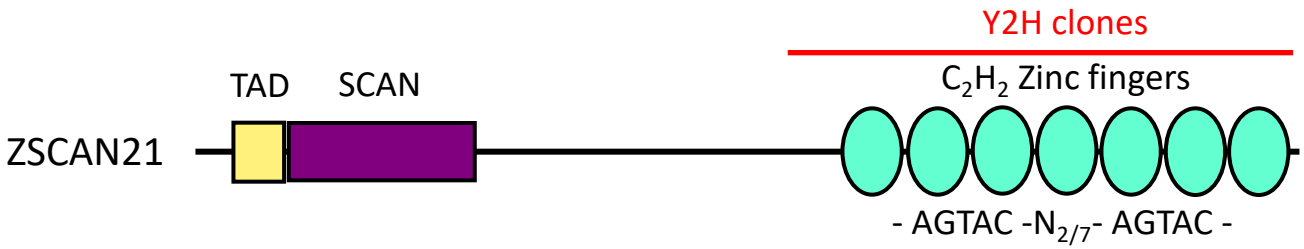


★ TRIM17 belongs to the TRIM family of E3 ubiquitin-ligases

ZSCAN21, a transcription factor that regulates α-synuclein expression

Yeast two hybrid screen (Hybrigenics) for proteins binding to TRIM17:

➡ 19 independent partner candidates, including ZSCAN21 and TRIM41

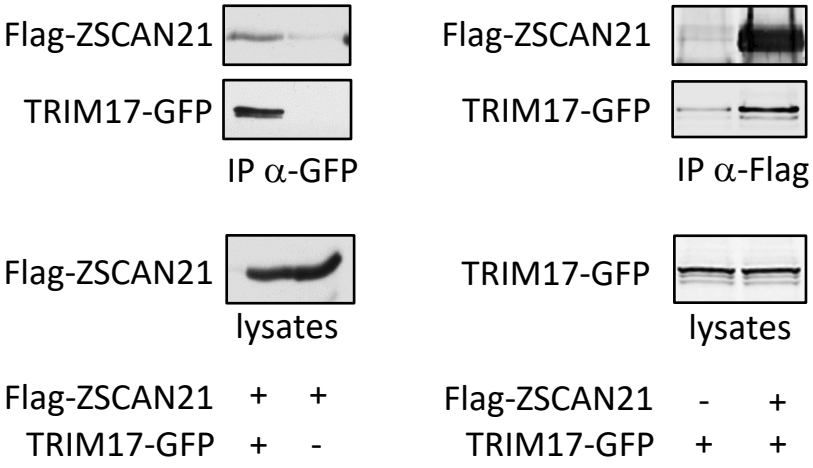


★ ZSCAN21 is involved in the transcriptional regulation of α-synuclein

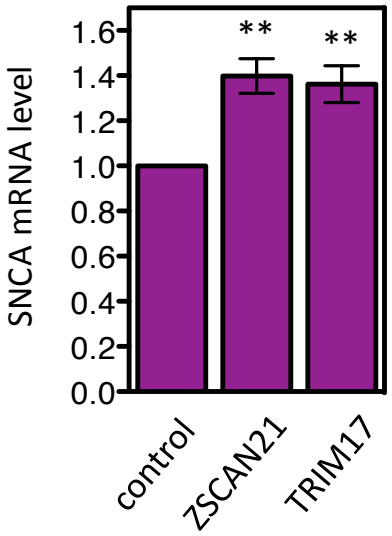
(Clough et al. 2009 *J. Neurochem* 110: 1479-149; Wright et al. 2013 *Mol Cell Neurosci* 57: 33-41; Dermentzaki et al. 2016 *J Biol Chem* 291: 8756-8772)

TRIM17 and ZSCAN21 interact with each other and increase α-synuclein expression

co-immunoprecipitation

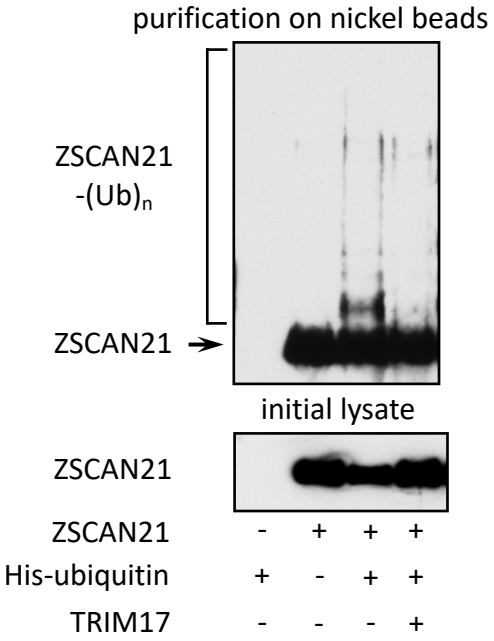


quantitative PCR using SH-SY5Y cells after co-transfection with GFP and FACS sorting

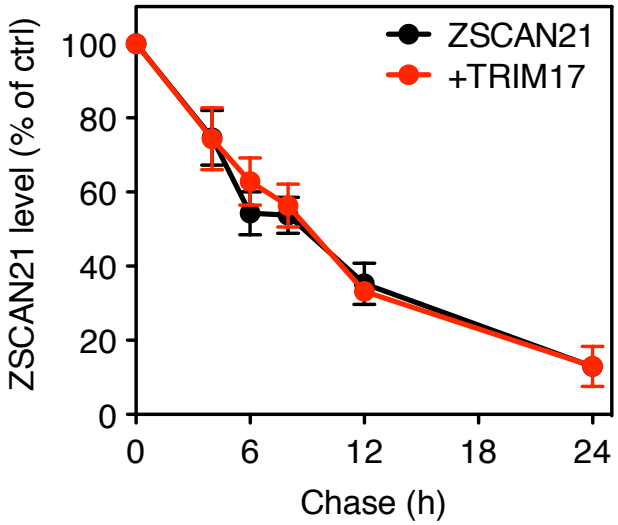


Regulation of ubiquitination/degradation of ZSCAN21 by TRIM17

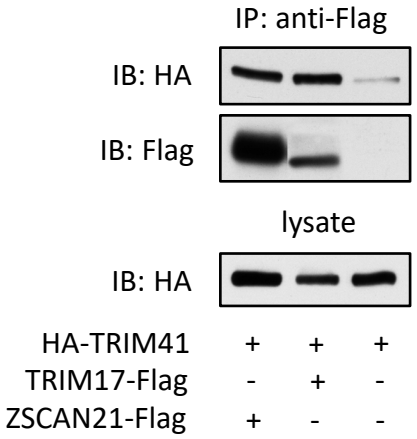
ubiquitination levels in cells



pulse-chase



co-immunoprecipitation

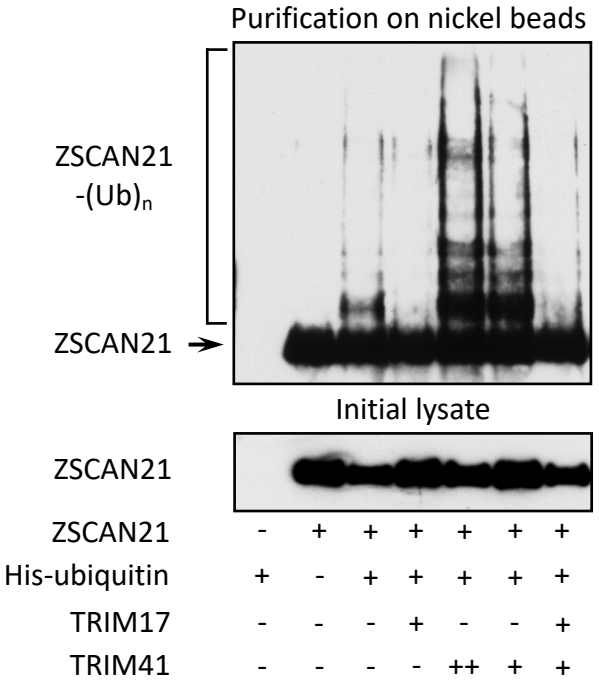


- Overexpression of TRIM17 does not significantly modify ZSCAN21 ubiquitination/degradation.

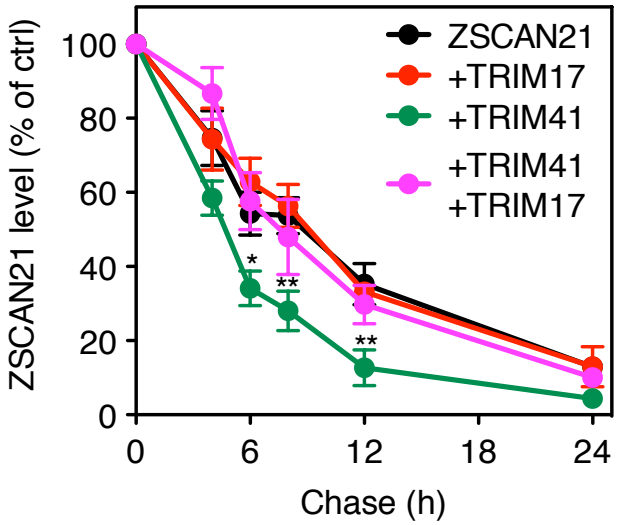
➡ TRIM17 is not an E3 ubiquitin-ligase for ZSCAN21.

Regulation of ubiquitination/degradation of ZSCAN21 by TRIM41 and TRIM17

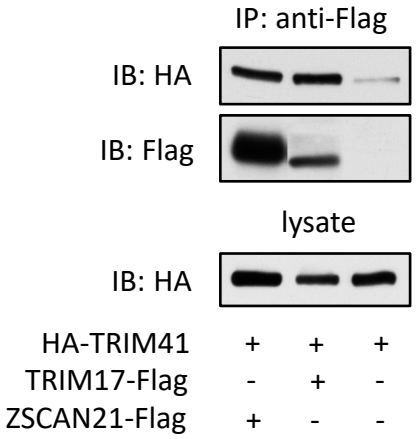
ubiquitination levels in cells



pulse-chase



co-immunoprecipitation



- Overexpression of TRIM17 does not significantly modify ZSCAN21 ubiquitination/degradation.

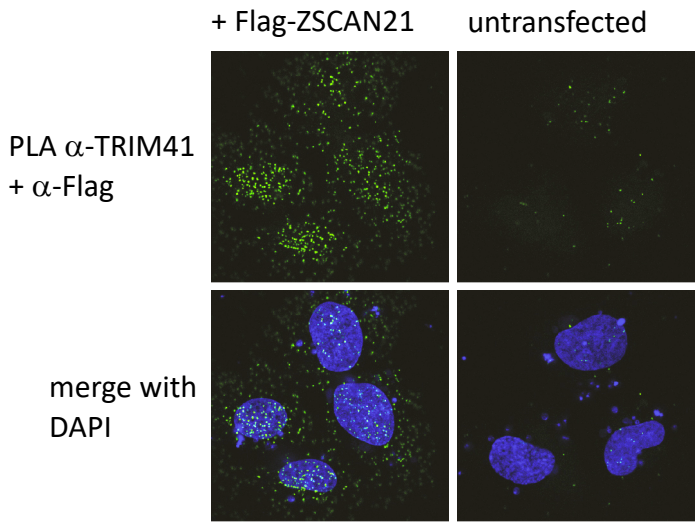
➔ TRIM17 is not an E3 ubiquitin-ligase for ZSCAN21.

- TRIM41 strongly increases ZSCAN21 ubiquitination/degradation in cells.

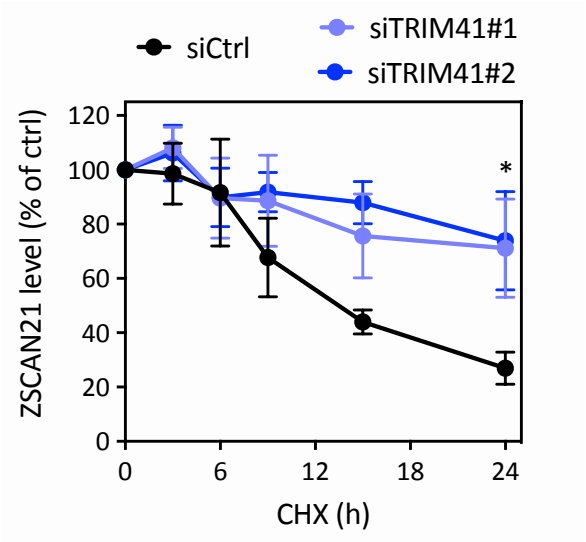
- The effects of TRIM41 are abolished by TRIM17.

TRIM41 is an E3 ubiquitin-ligase for ZSCAN21 that targets it for degradation

Proximity Ligation Assay (Duolink)

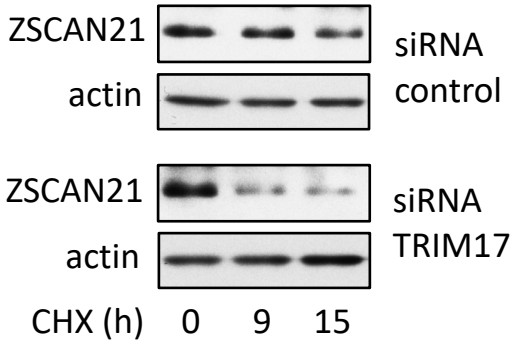


Silencing of TRIM41 stabilizes ZSCAN21

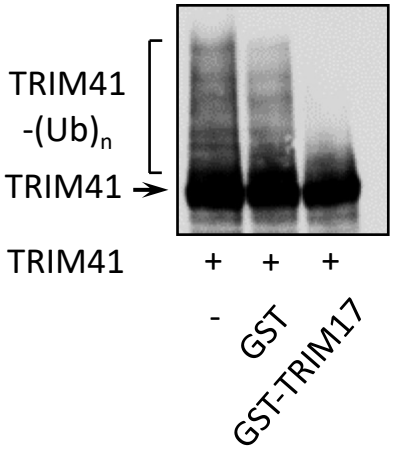


TRIM17 inhibits TRIM41-mediated ubiquitination/degradation of ZSCAN21

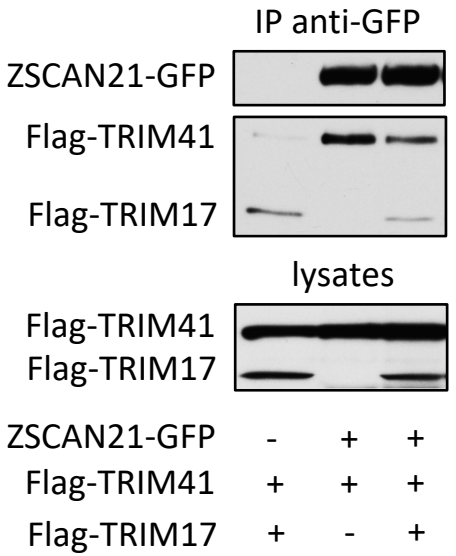
silencing of TRIM17 decreases ZSCAN21 levels



in vitro auto-ubiquitination

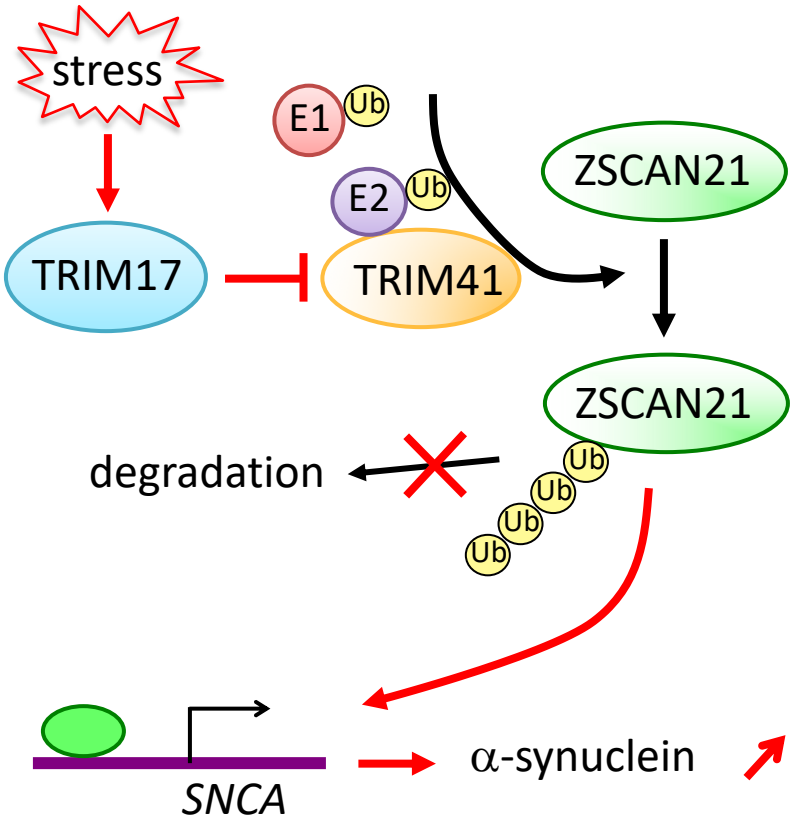


co-immunoprecipitation



➡ by reducing both the E3 ubiquitin-ligase activity of TRIM41 and the interaction between ZSCAN21 and TRIM41

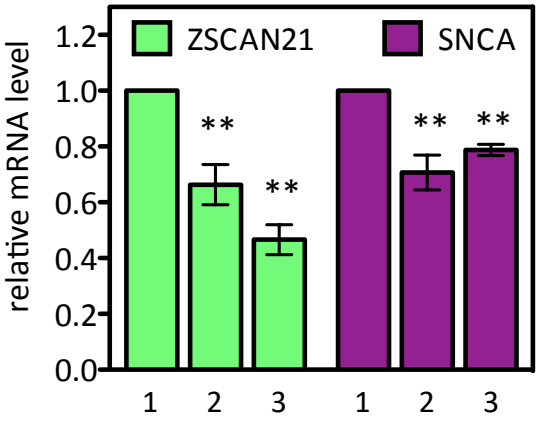
Working hypothesis: TRIM17 stabilizes ZSCAN21 by inhibiting TRIM41

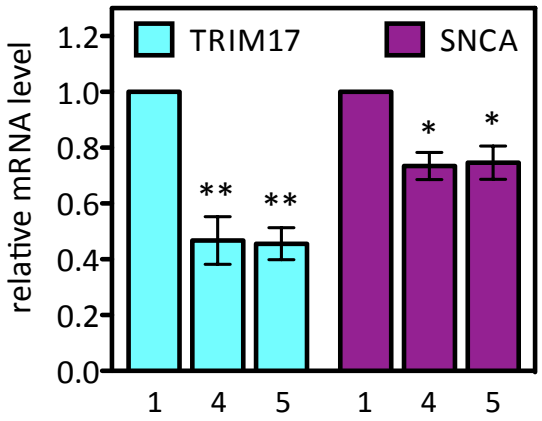


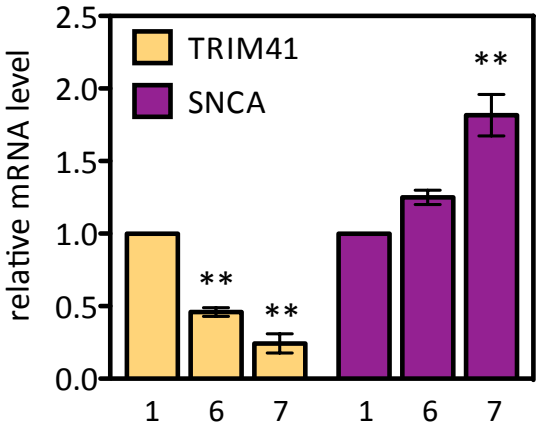
Lassot et al. (2018) *Cell Reports* 25: 2484-2496.

Regulation of α-synuclein expression by TRIM17, TRIM41 and ZSCAN21

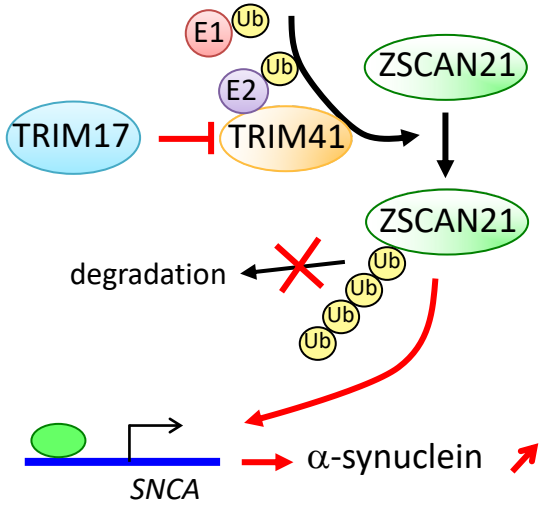
quantitative PCR , SH-SY5Y cells after transduction with specific shRNAs







1: shRNA ctrl
2: shRNA ZSCAN21#1
3: shRNA ZSCAN21#2
4: shRNA TRIM17#1
5: shRNA TRIM17#2
6: shRNA TRIM41#1
7: shRNA TRIM41#2

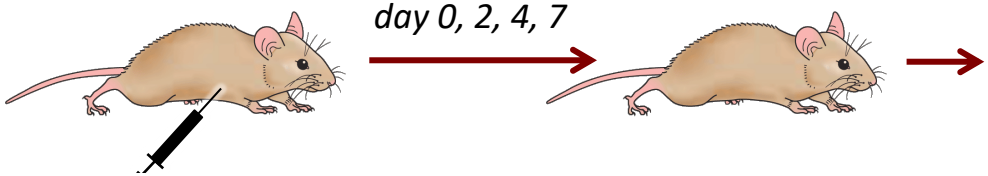


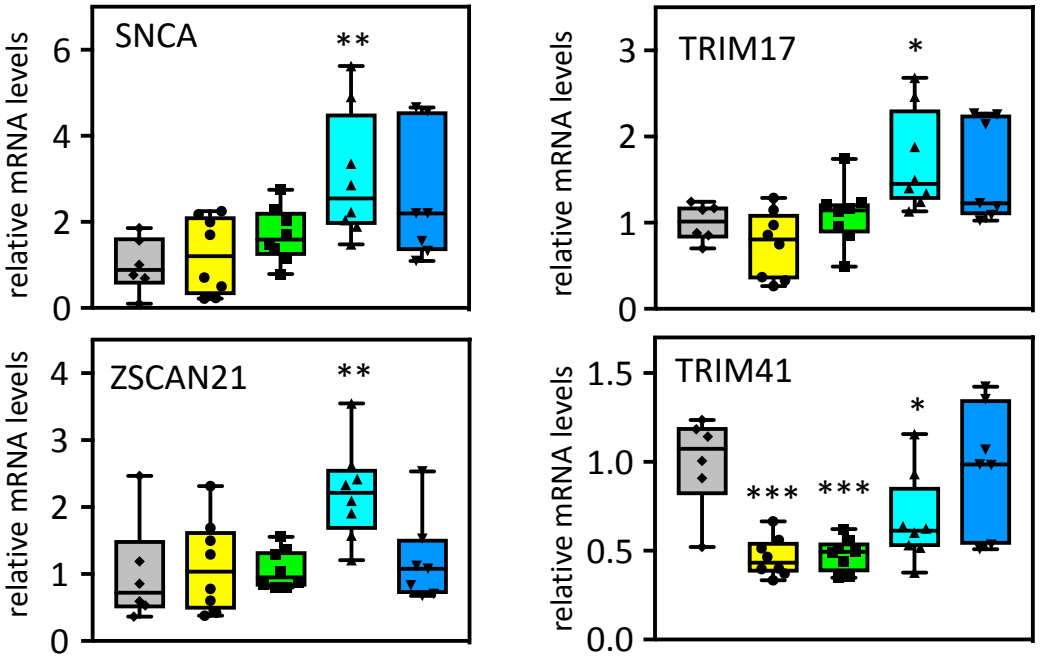
Lassot et al. (2018) *Cell Reports* 25: 2484-2496.

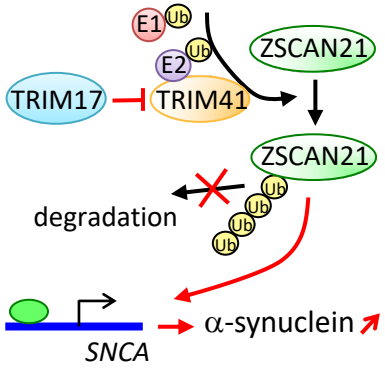
SNCA, TRIM17, ZSCAN21 and TRIM41 expression in midbrains of MPTP-treated mice

systemic injection of saline
or MPTP (subacute protocol)

collaboration with the lab of
Miquel Vila (Barcelona)





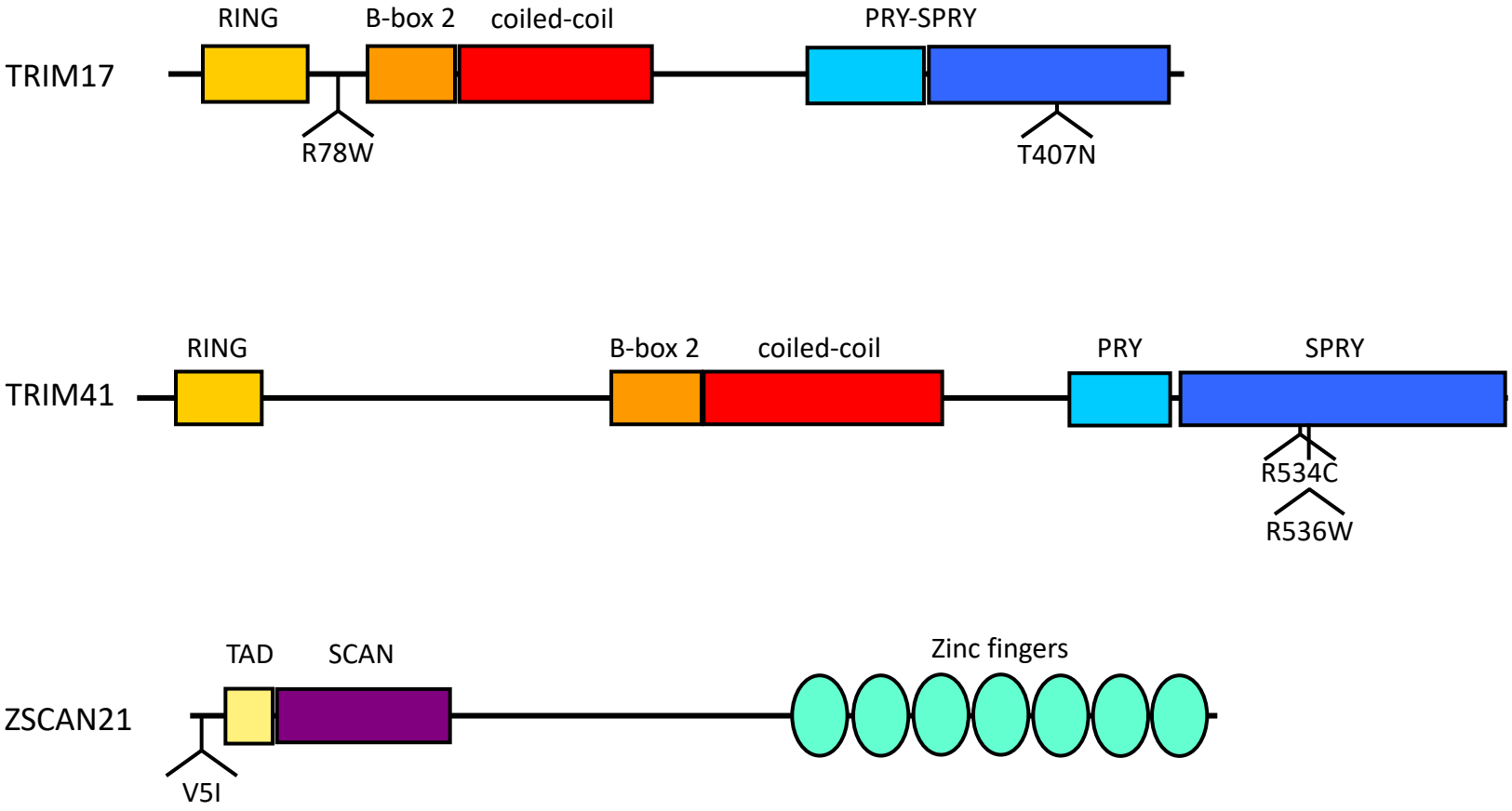


Support the possible implication of the TRIM17/TRIM41/ZSCAN21 pathway in the increased α-synuclein expression in Parkinson's disease.

Lassot et al. (2018) *Cell Reports* 25: 2484-2496.

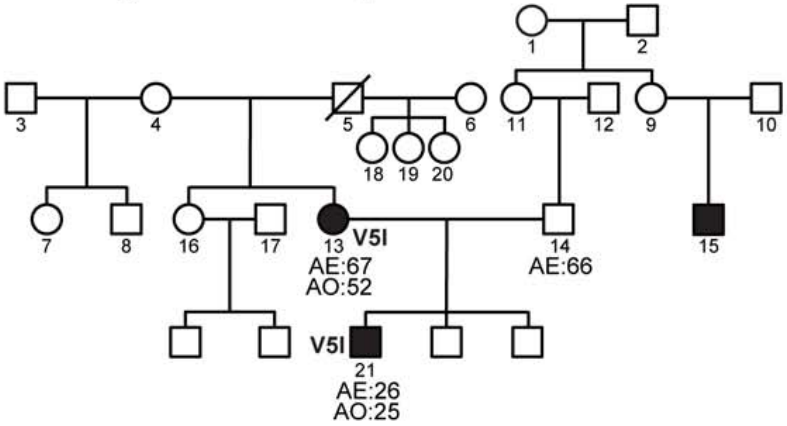
Genetic variations in *TRIM17*, *TRIM41* and *ZSCAN21* in PD patients

Sequencing of *TRIM17, TRIM41* and *ZSCAN21* in 200 index cases from families with autosomal dominant Parkinson's disease and 200 healthy controls (collaboration with Suzanne Lesage, ICM).

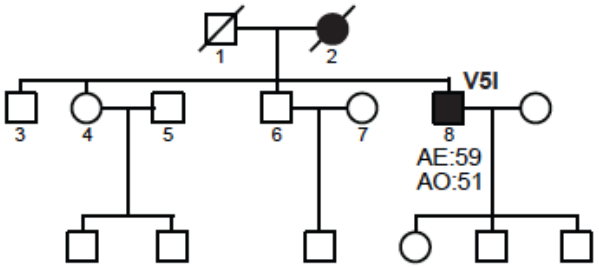


ZSCAN21 p.V5I and TRIM41 p.534C variants co-segregate with Parkinson's disease

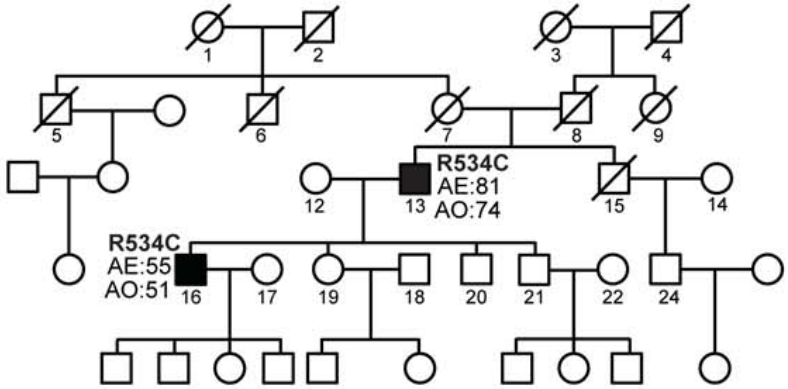
Family A: ZSCAN21 p.V5I



Family B: ZSCAN21 p.V5I



Family C: TRIM41 p.R534C

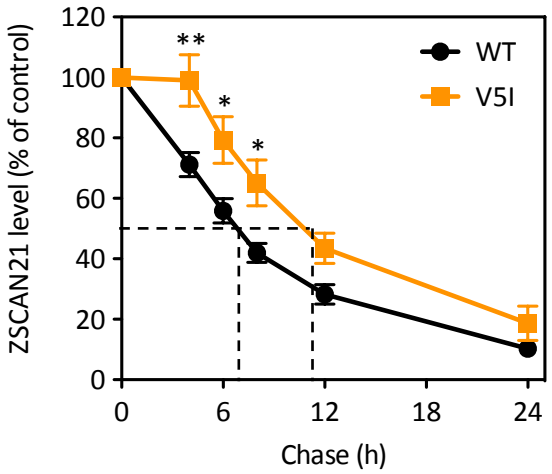


AE: age at examination
AO: age at onset

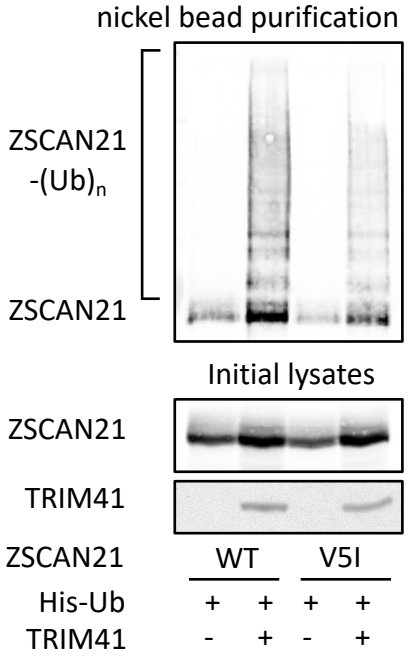
A dysregulation of the TRIM17/TRIM41/ZSCAN21 pathway may be involved in PD.

The variant ZSCAN21 p.V5I is more stable

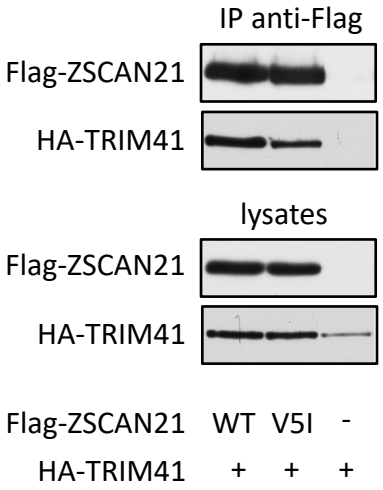
pulse chase



ubiquitination levels in cells



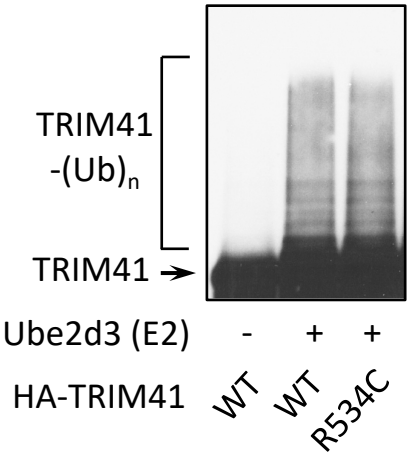
co-immunoprecipitation



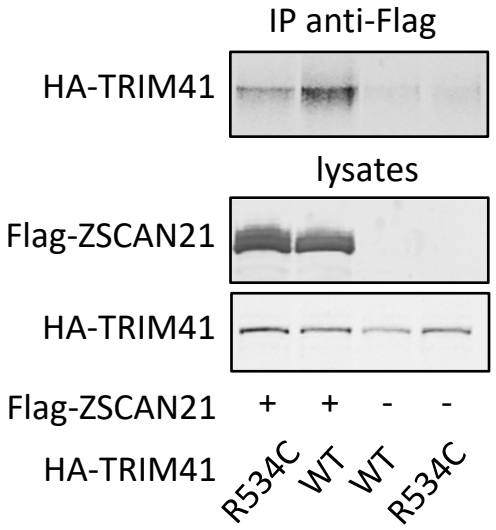
➡ ZSCAN21 p.V5I may increase the expression of α-synuclein in patients by being more stable.

The variant TRIM41 p.R534C has a lower affinity for ZSCAN21

in vitro auto-ubiquitination

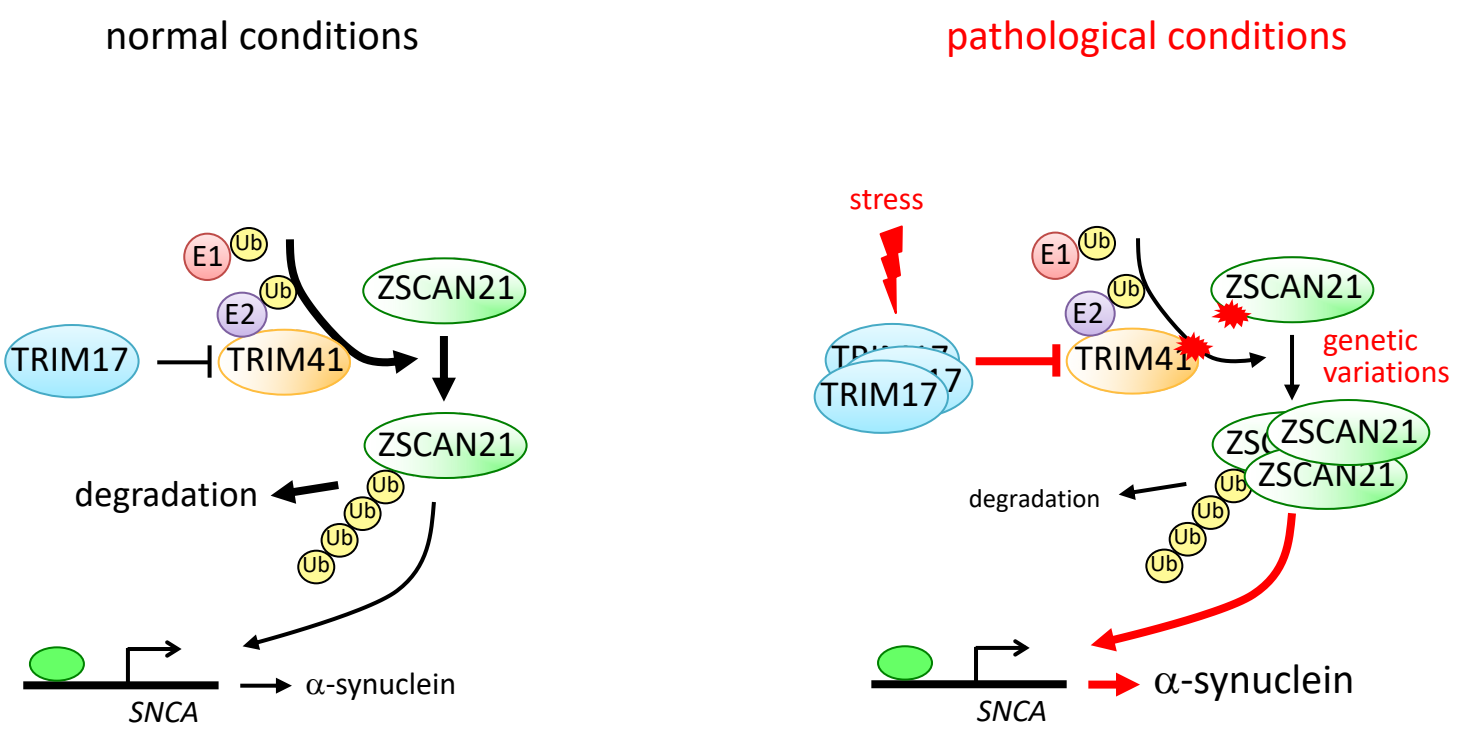


co-immunoprecipitation



TRIM41 p.R534C may increase the expression of α-synuclein in patients by targeting ZSCAN21 for degradation with a lower efficiency.

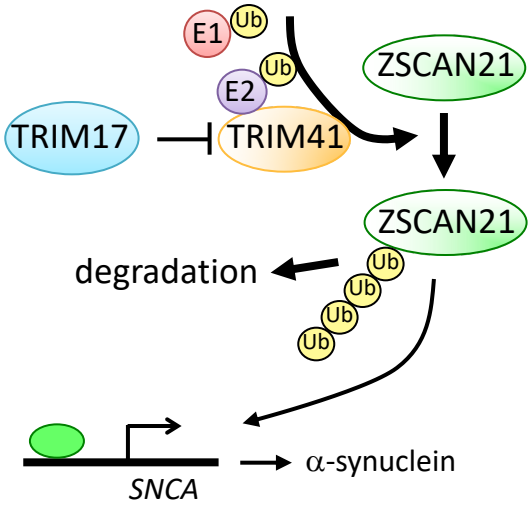
A dysregulation of the TRIM41/TRIM17/ZSCAN21 pathway may be involved in PD



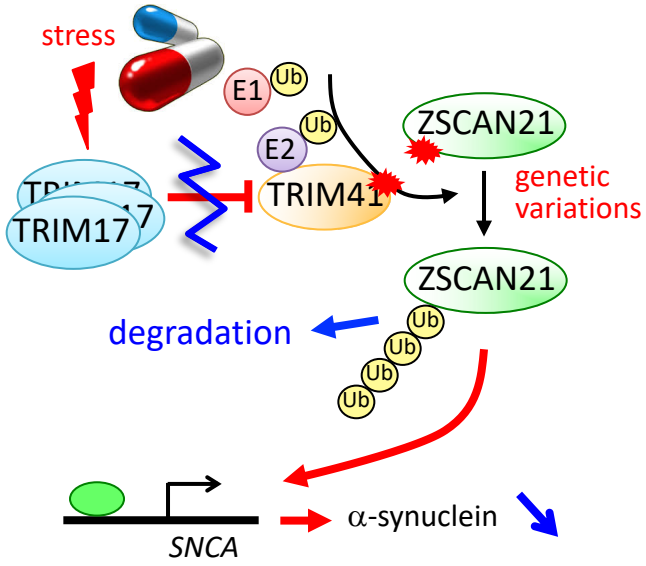
Lassot et al. (2018) *Cell Reports* 25: 2484-2496.

Therapeutic perspectives

normal conditions



pathological conditions



Acknowledgements

The team “Molecular Mechanisms of Apoptosis Regulation”

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- **Iréna Lassot**
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- Meenakshi Basu
- Alina Kozoriz
- Sahra Tasdelen

former members:
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- Barbara Zieba
- Emmanuelle Coque
- Piotr Bossowski
- Barbara Mojsa
- Anne-Sophie Dumé
- Alessandra Damiano
- Caroline Soulet

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(Olga Corti’s laboratory)

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PROJET SOUTENU PAR FONDATION DE FRANCE