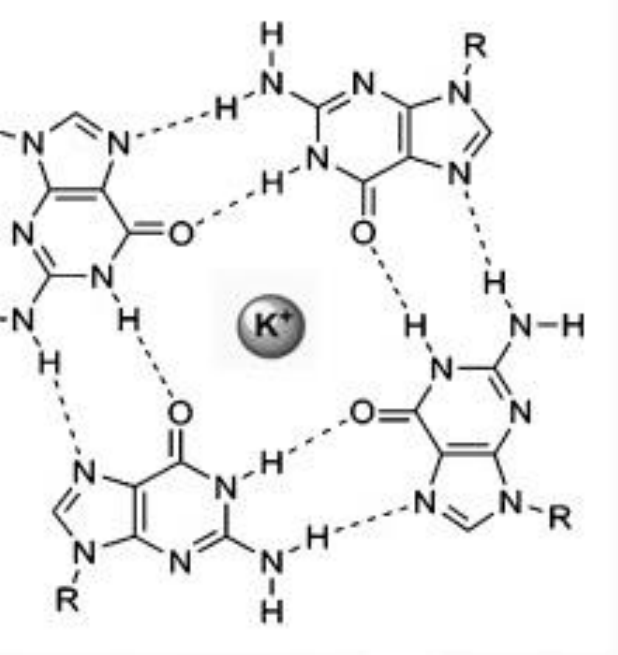
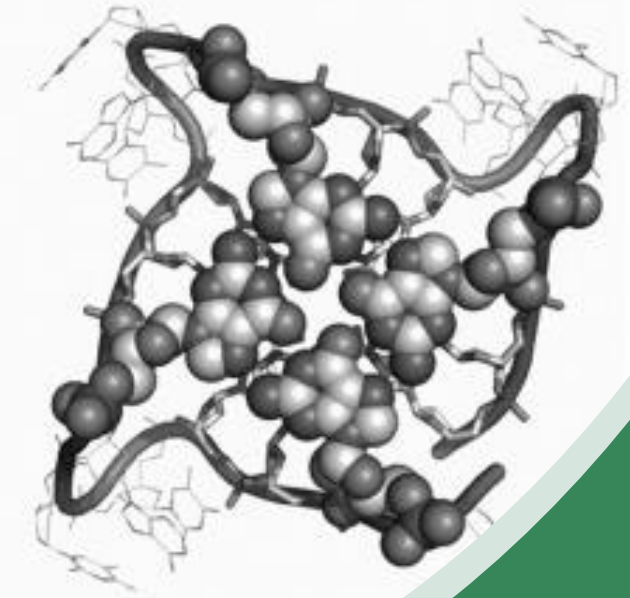




G-quadruplexes in RNAs and DHX36

2019/11/21

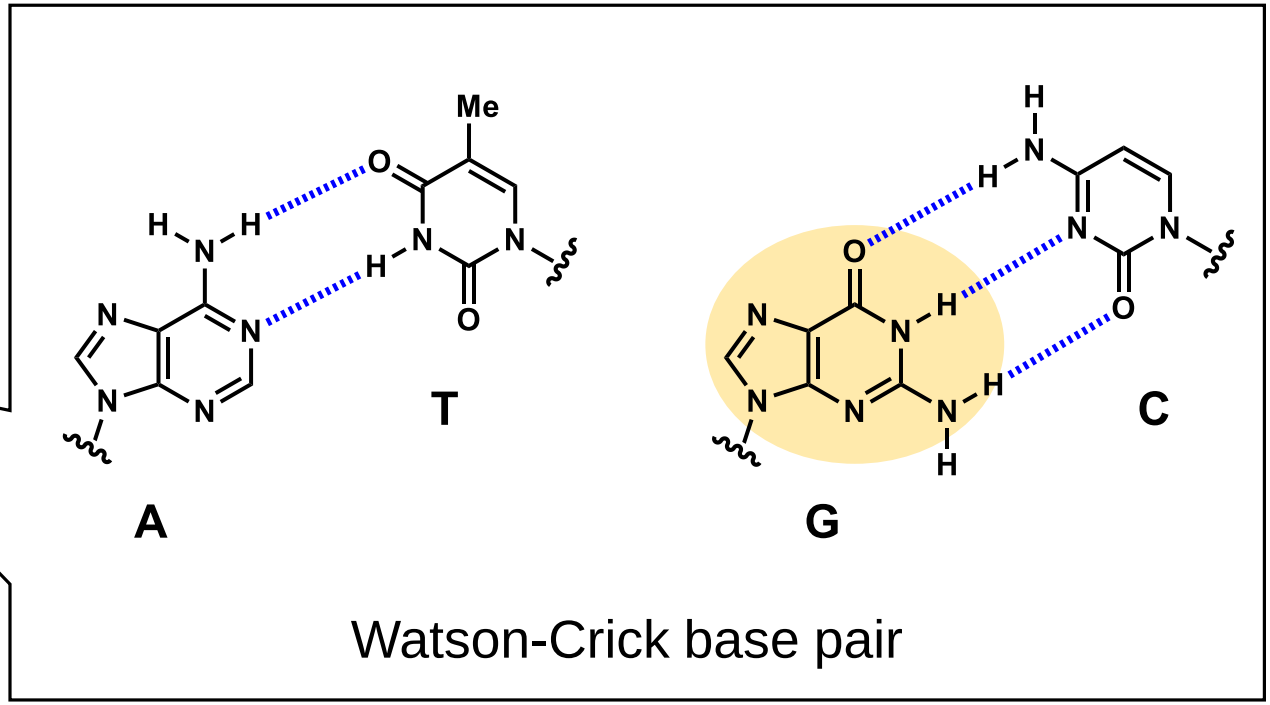
M2 Furuta

Contents

Contents

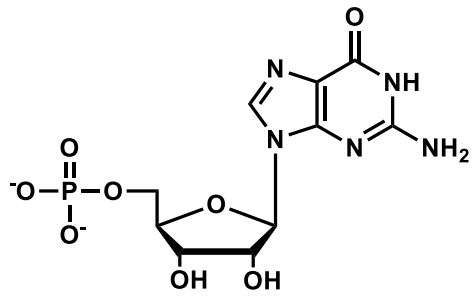
Guanine in DNA

DNA double helix



Watson, J. D. & Crick, F. H. *Nature.* ***1953***, *171,* 737.

G-quartet



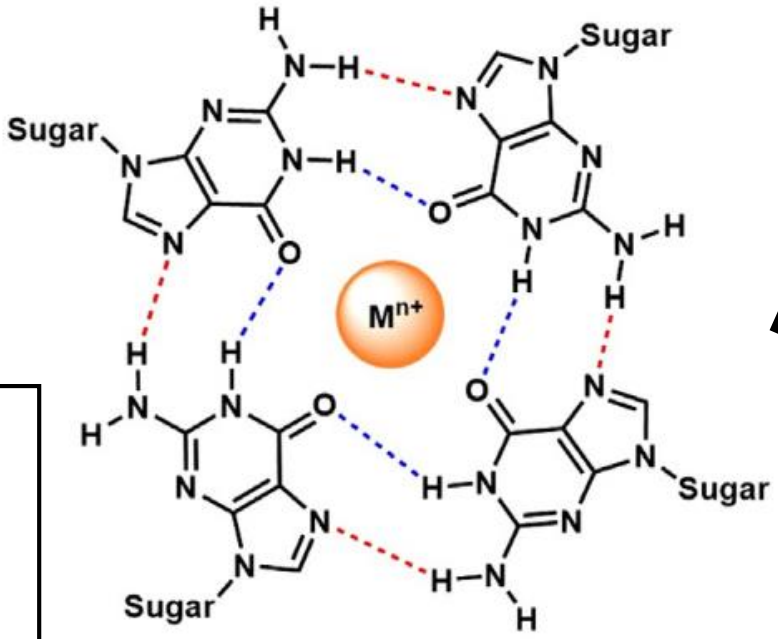
guanylic acid

(solution)

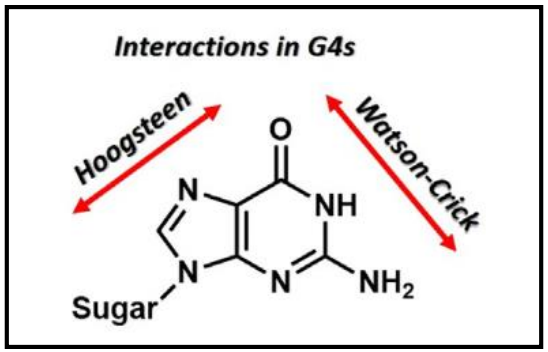
conc.

gelatinize

X-ray crystal structure analysis



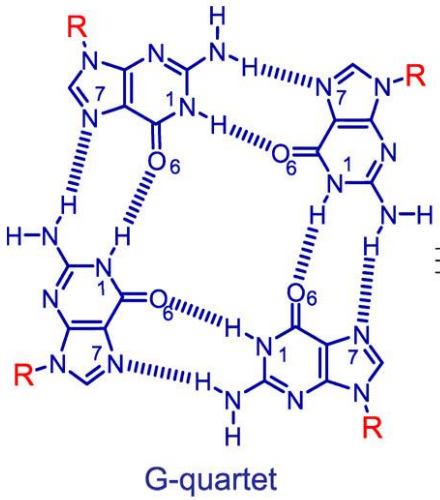
G-quartet

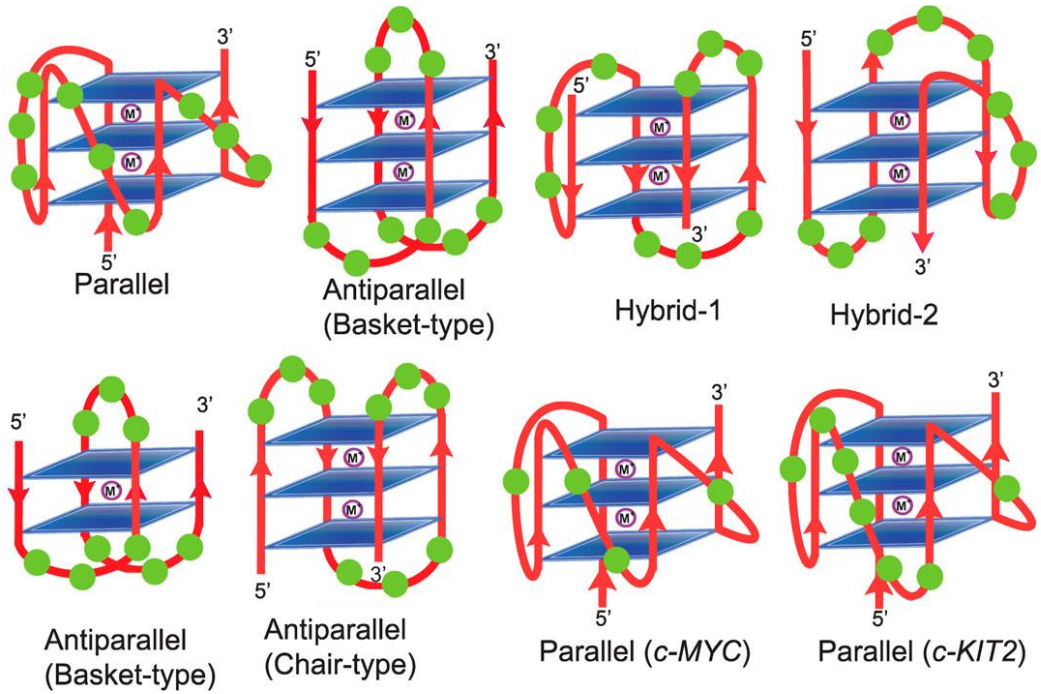


✓ Self-association in water

Davies, R. D. *et. al., Proc. Natl. Acad. Sci. USA* **1962**, *48*, 2013.

G-quadruplex (oligonucleotide)

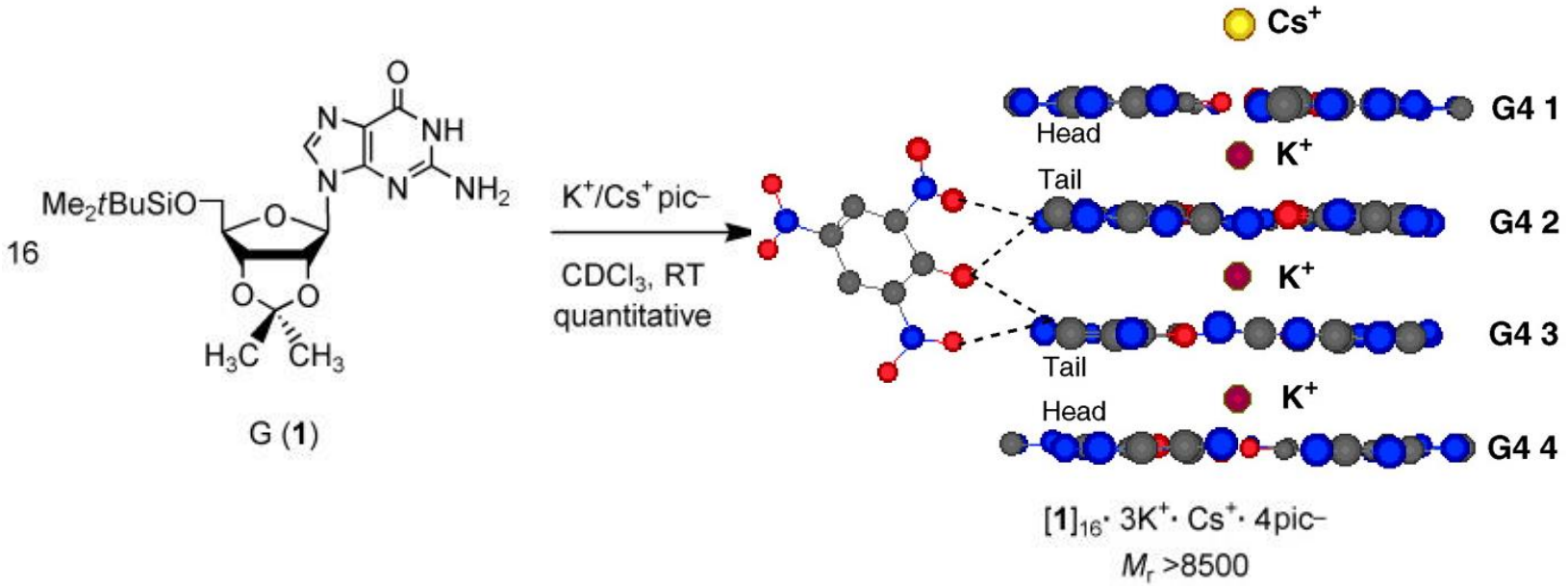




- ✓ Stacking of two or more quartets
- ✓ Stabilized by monovalent metal ions

Pradeepkumar, P. I., *et. al., ACS Chem. Biol.* **2019**, *14*, 2114.

Synthetic G-quadruplex



- ✓ Functional… ionophore
- ✓ Chiral… diastereotopic faces (Tail & Head)

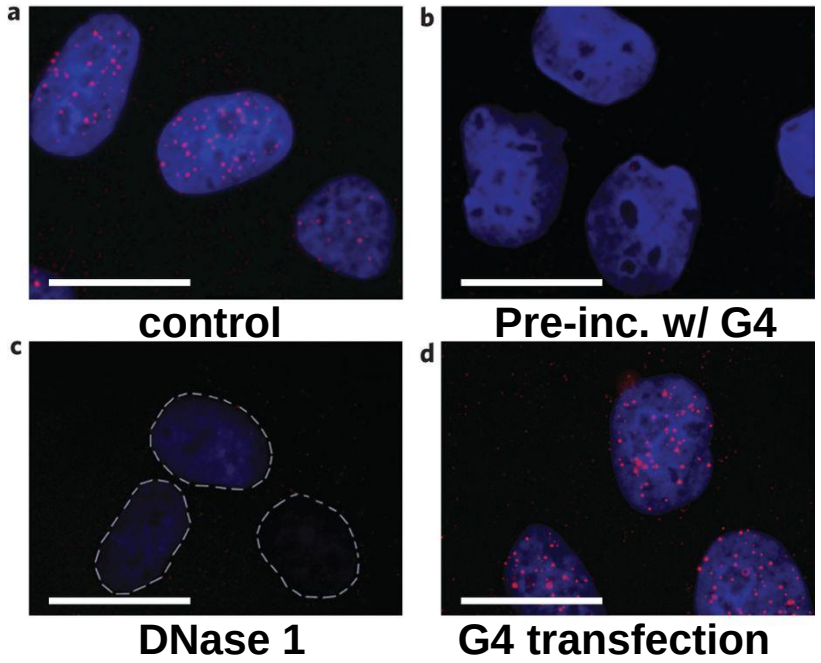
Hierarchical self-assembly process

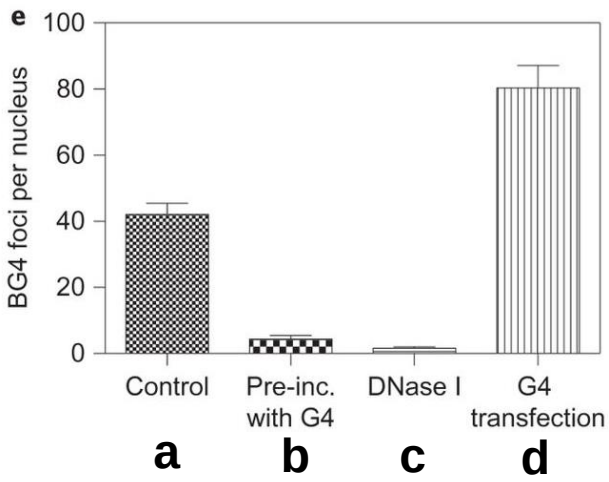
1. Hydrogen-bonded G-quartet
2. G_8-M^+ octamer formed by cation-dipole interactions and π stacking
3. Hexadecameric G-quadruplex with anion-nucleobase H-bonds

Davis, J. T., et. al., *J. Am. Chem. Soc.* **2000**, *122*, 4060.
Davis, J. T. *Angew. Chem. Int. Ed.* **2004**, *43*, 668.

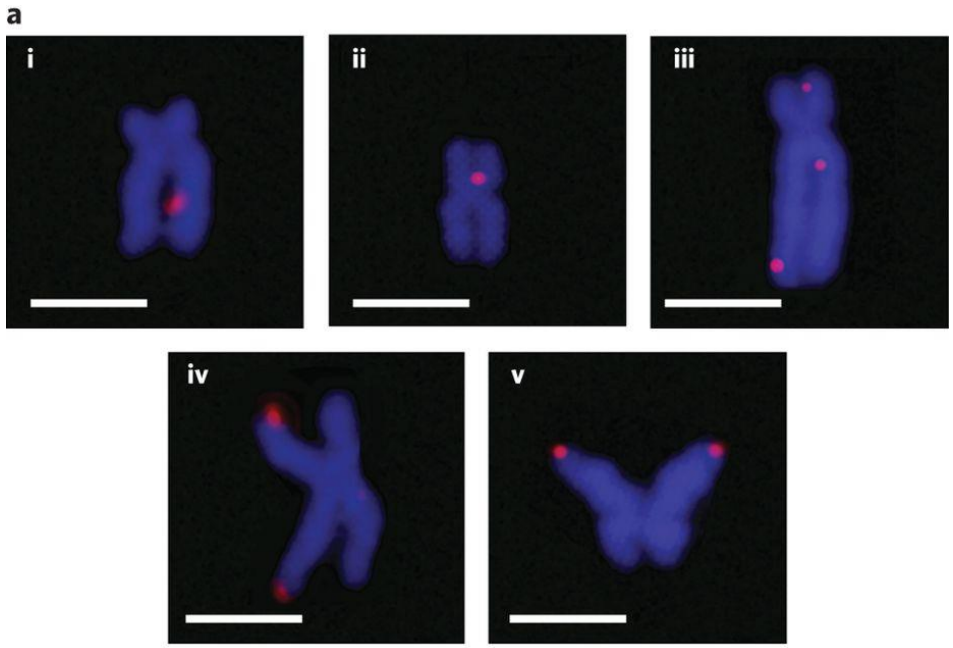
DNA G-quadruplex in cell (1)

In nuclei of human cancer cells





In chromosomes

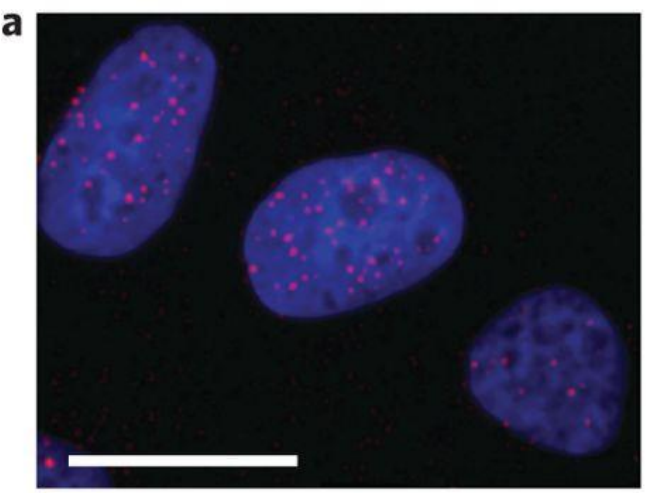


i - iii non-telomeric regions
iv - v telomeres (G-rich regions)

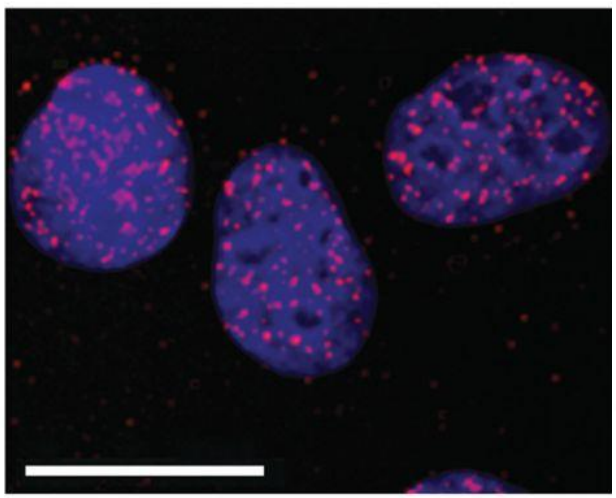
BD4… antibody for G-quadruplex structures
(generated by *in vitro* selection with phage display)

Balasubramanian, S., *et. al., Nat. Chem*. **2013**, *5*, 182.

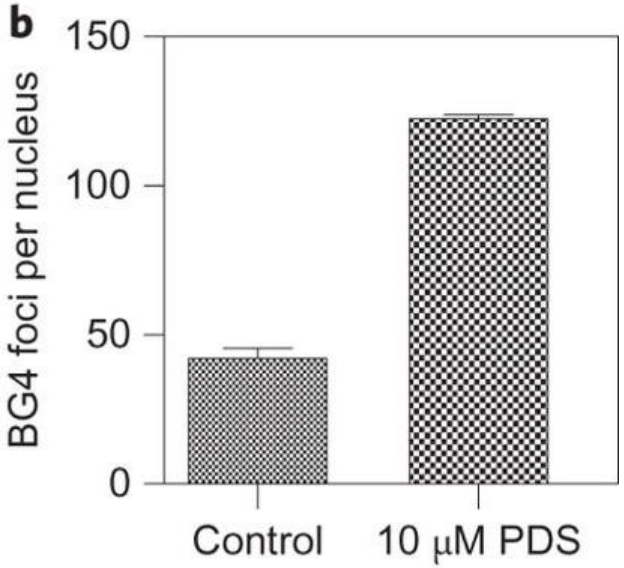
DNA G-quadruplex in cell (2)

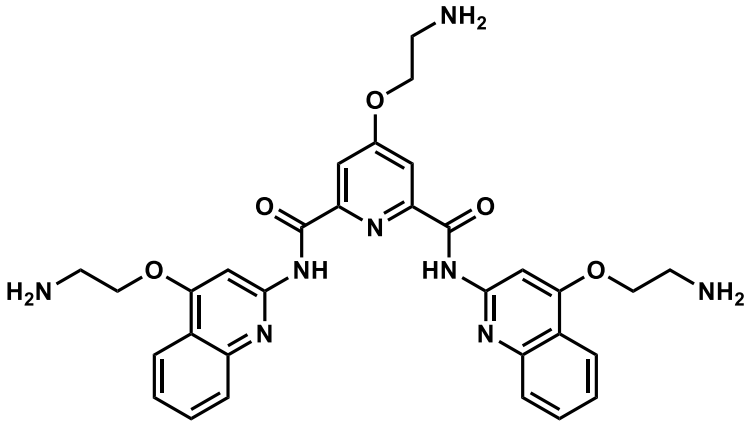


Control



10 uM PDS



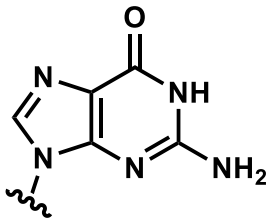


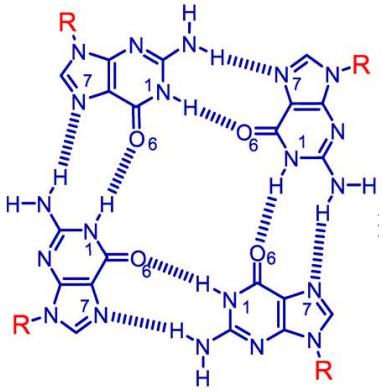
Pyridostatin (PDS)

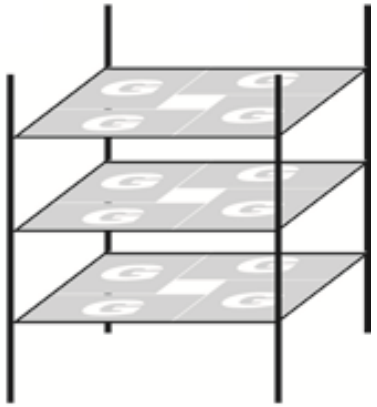
- ✓ Small molecule ligand can stabilize G-quadruplex structure even in cells.

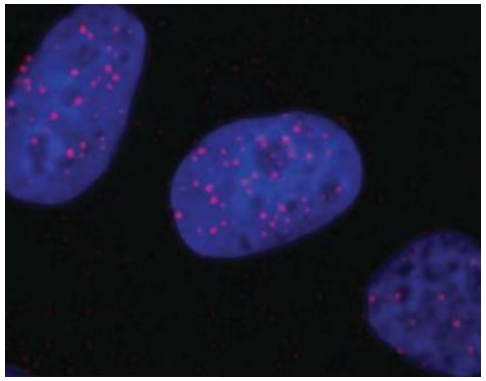
Balasubramanian, S., *et. al., Nat. Chem*. **2013**, *5*, 182.

G-quadruplex in many fields









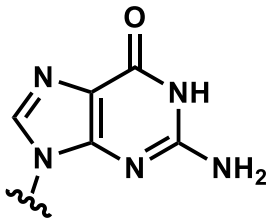
Nano-technology

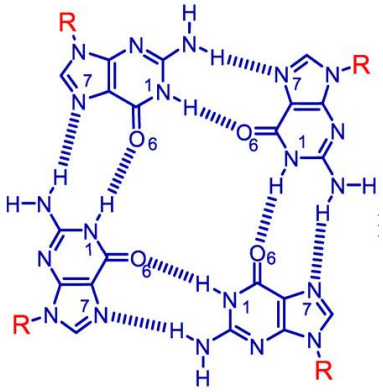
Supramolecular chemistry

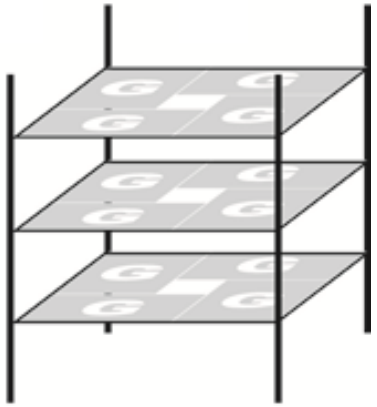
Molecular biology

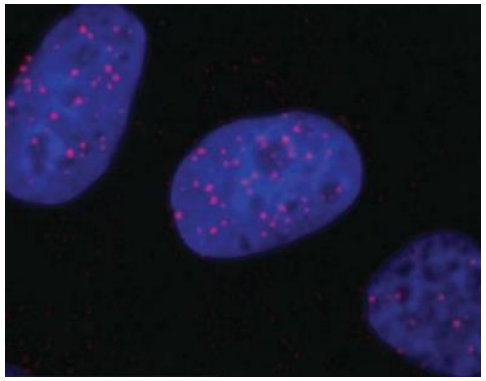
Medicinal chemistry

G-quadruplex in many fields









Nano-technology

Supramolecular chemistry

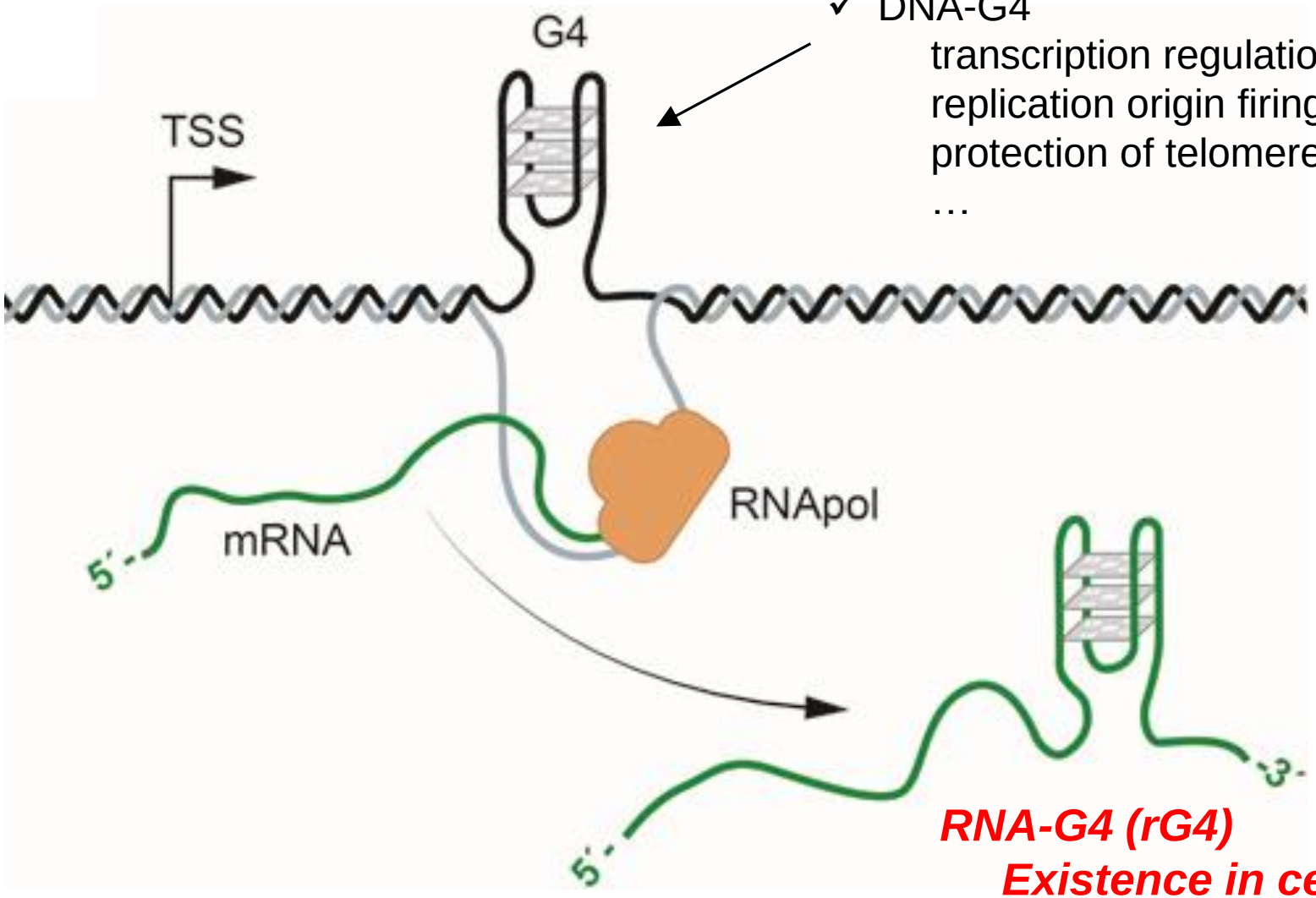
Today's topics

Molecular biology

Medicinal chemistry

Contents

DNA-G4 and RNA-G4

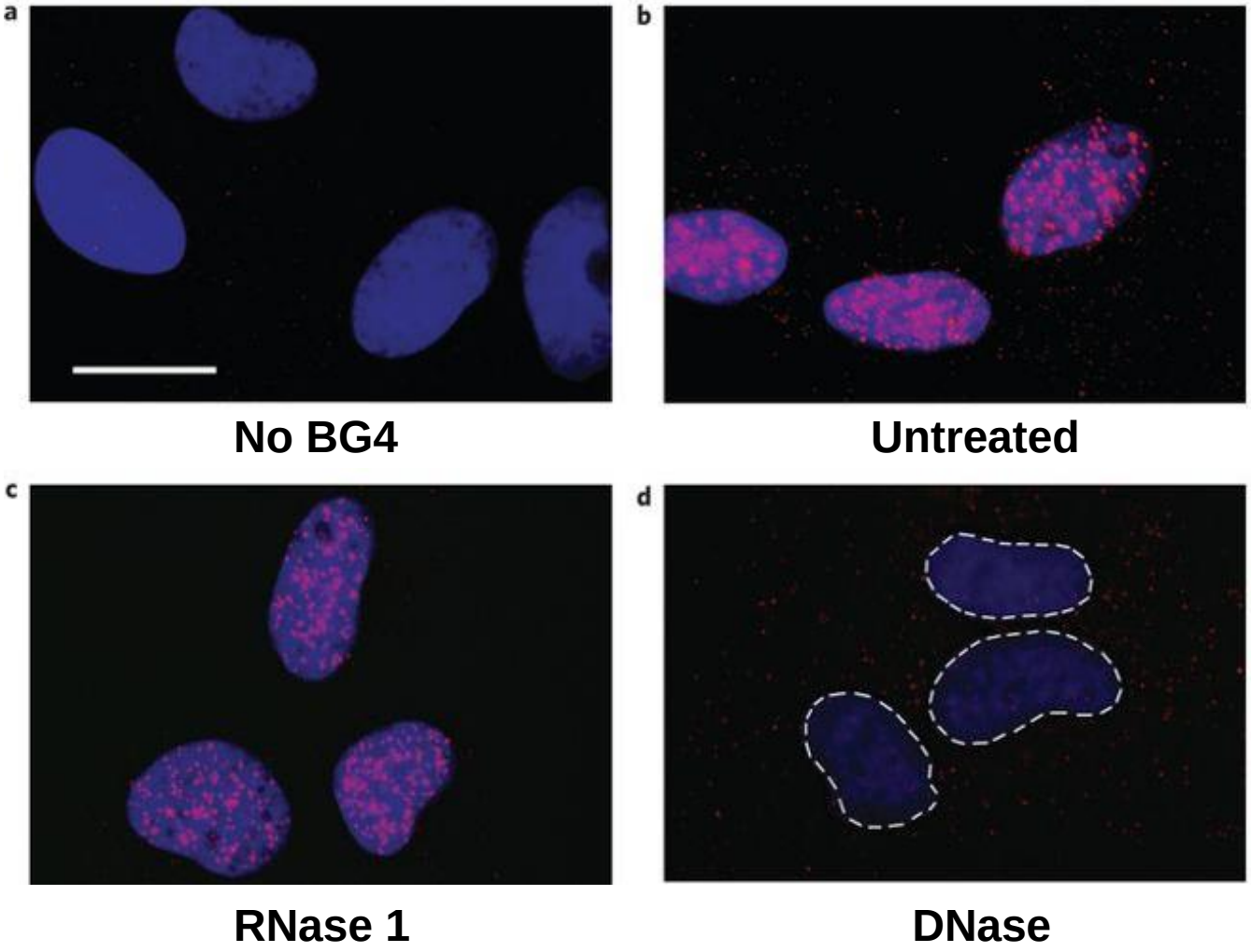


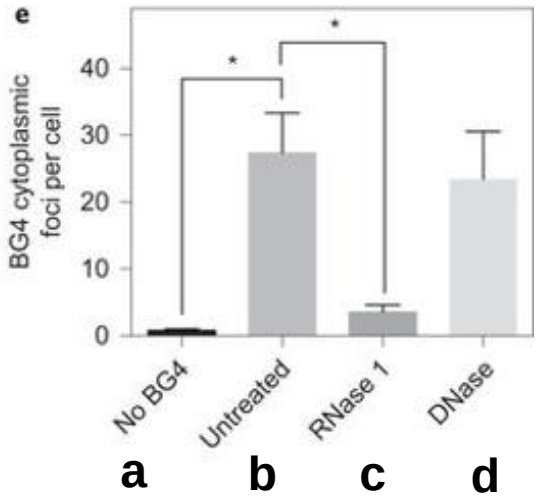
✓ DNA-G4
transcription regulation
replication origin firing
protection of telomeres
…

RNA-G4 (rG4)
Existence in cells?
Function?

Lipps, H. J., *et. al., Nucleic Acids Res.* **2015**, *43*, 8627.

Visualization of rG4s in cells (1)

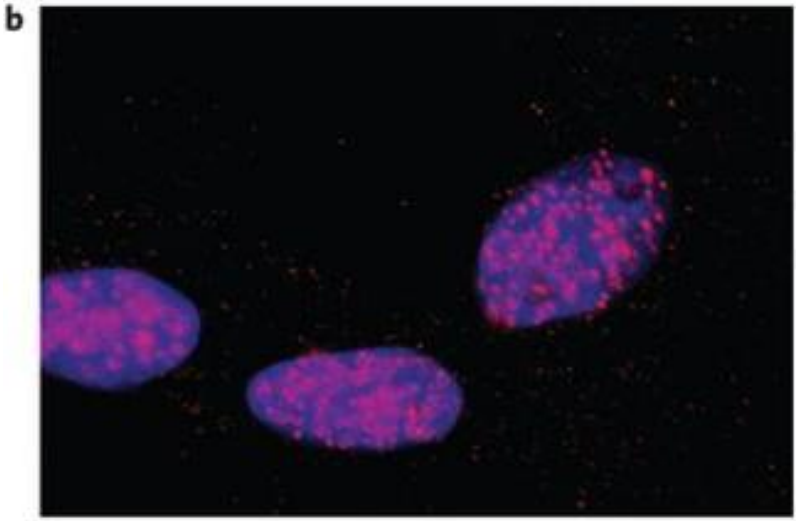




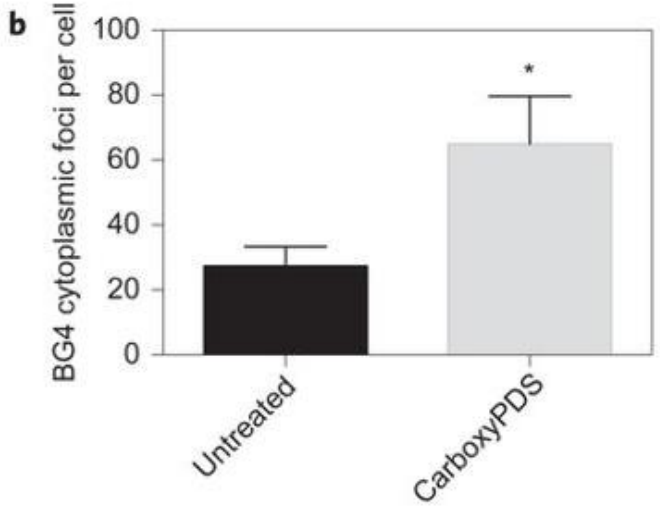
✓ On longer exposure, staining was also distributed throughout the cytoplasm.

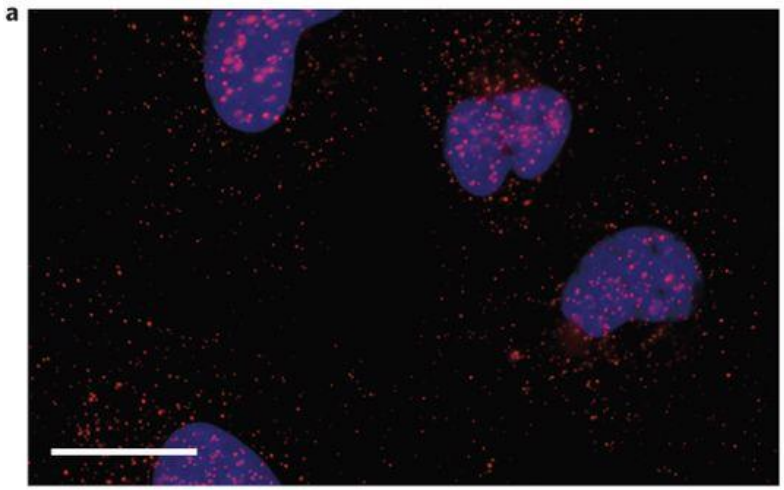
Balasubramanian, S., *et. al., Nat. Chem*. **2014**, *6*, 75.

Visualization of rG4s in cells (2)

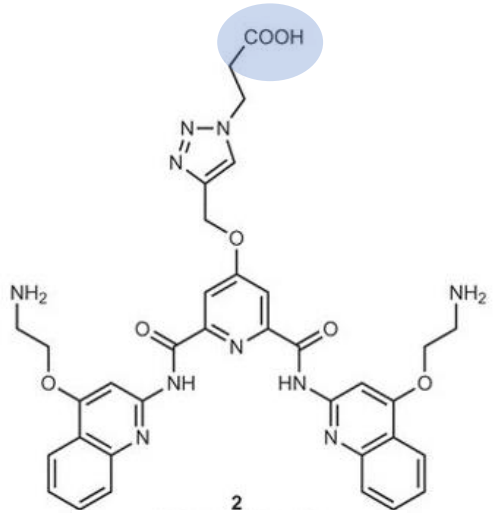


Untreated





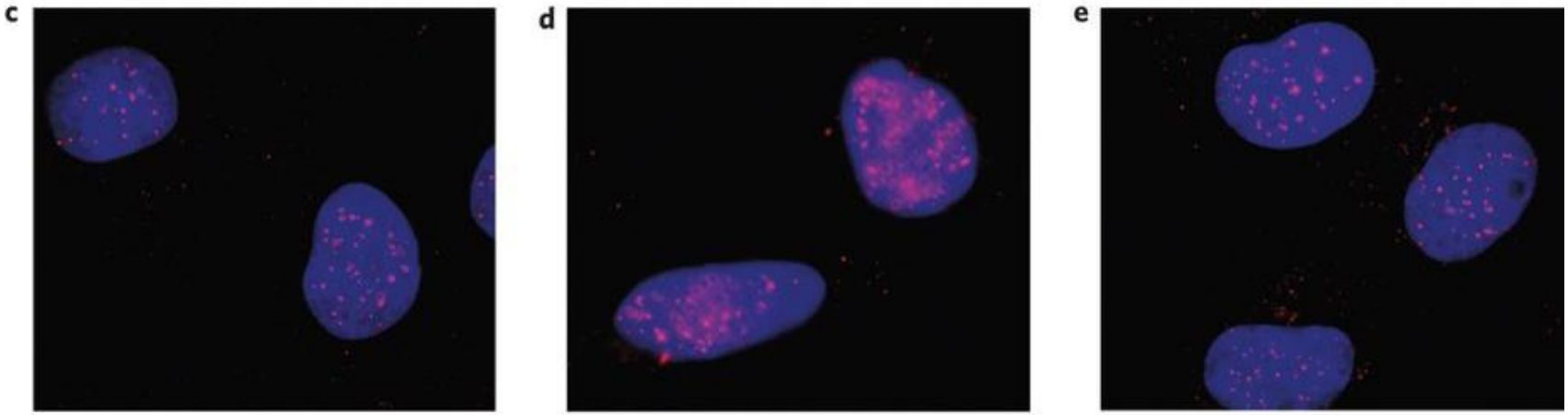
CarboxyPDS



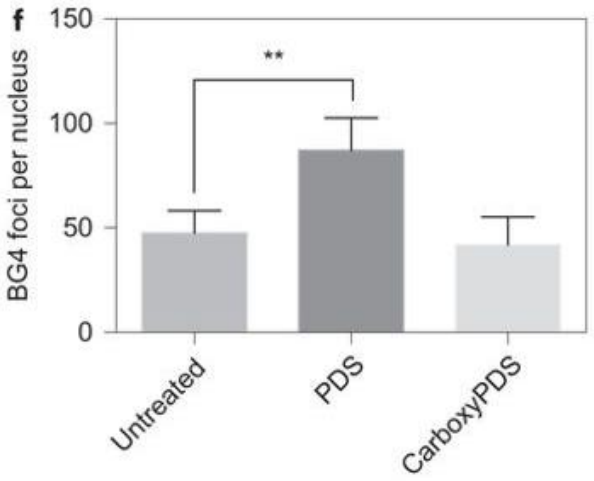
CarboxyPDS

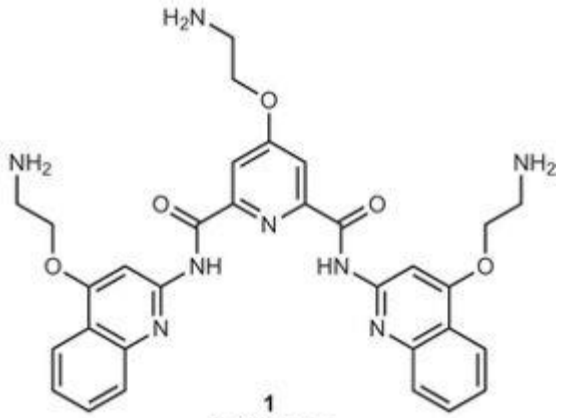
Balasubramanian, S., *et. al., Nat. Chem*. **2014**, *6*, 75.

Visualization of rG4s in cells (3)

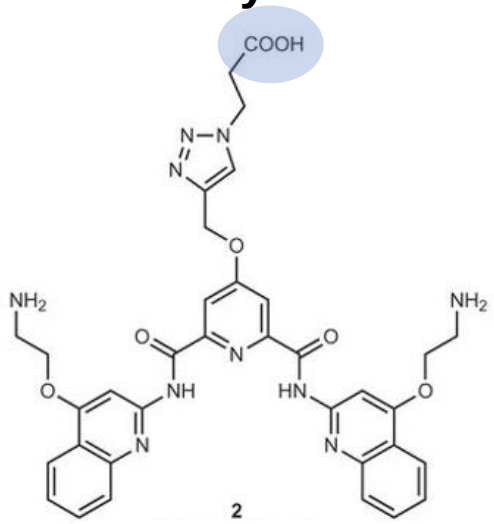


Untreated **PDS** **CarboxyPDS**





PDS

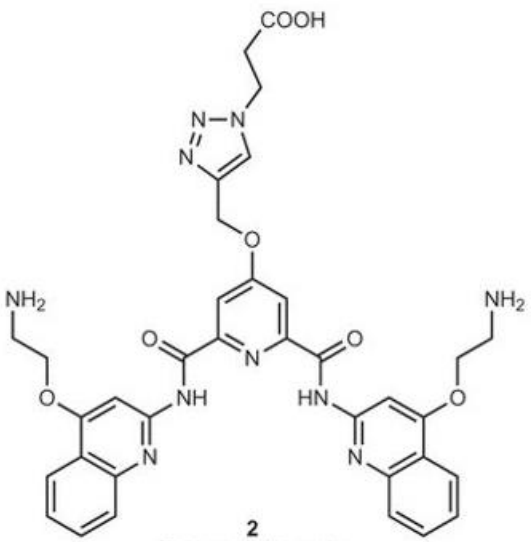


CarboxyPDS

✓ Selective stabilization of endogenous RNA G4s with a RNA G4-specific ligand

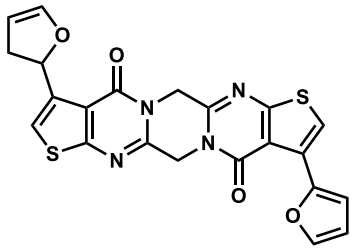
Balasubramanian, S., *et. al., Nat. Chem*. **2014**, *6*, 75.

RNA G4 selectivity (v.s. DNA G4)

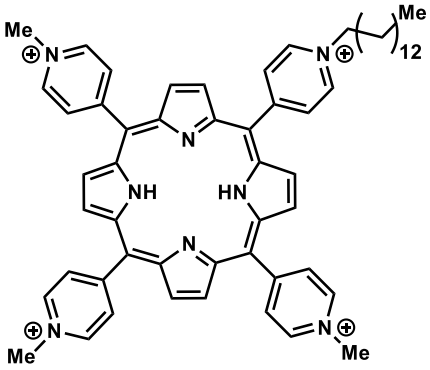


CarboxyPDS

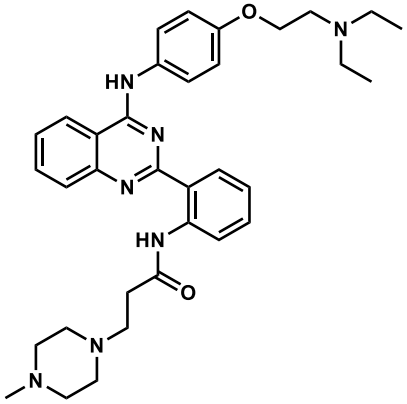
Other reported RNA-G4-selective ligands



RGB-1



TMPPyP4-C14

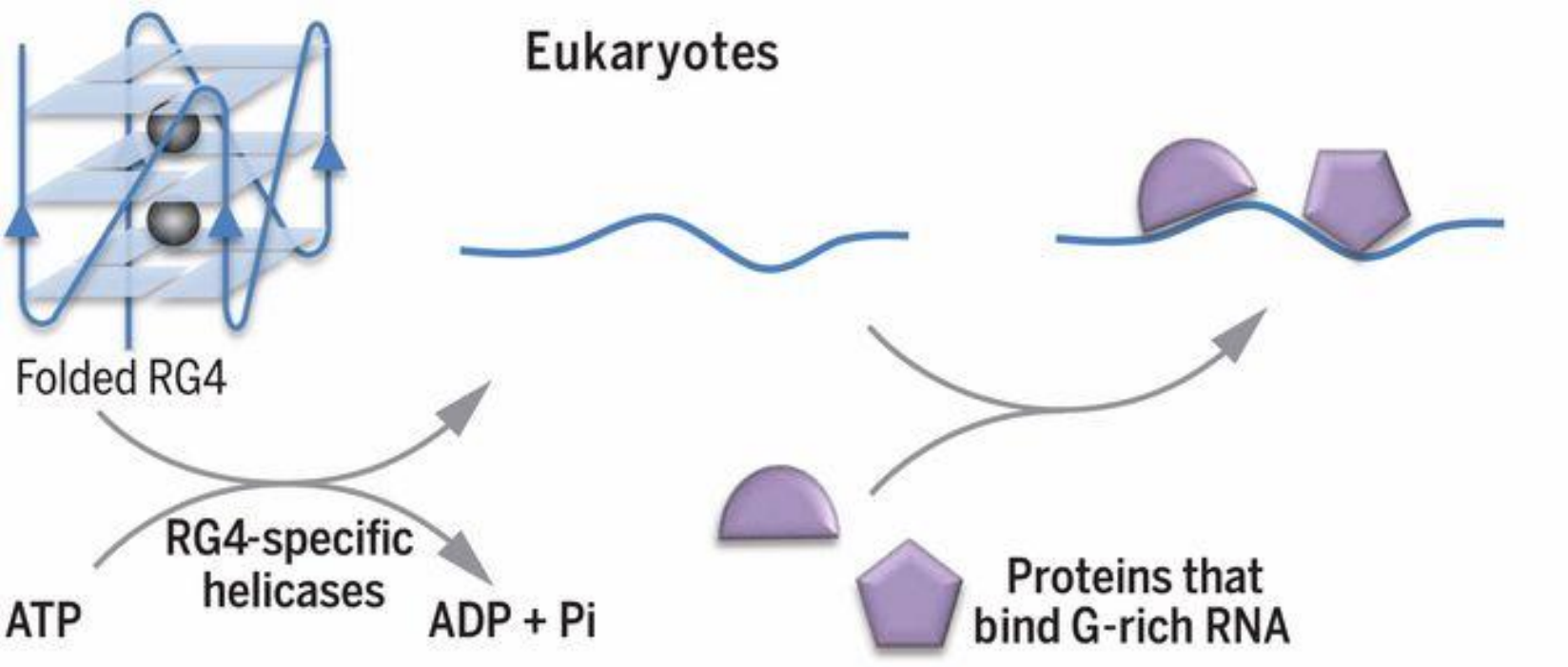


quinazoline derivative

- ✓ Hydrogen bond with 2'-OH group on the ribose
- ✓ Multiple hydrogen-bond receptor sites
- ✓ Accumulation in the cytoplasm of cells

Balasubramanian, S., *et. al., Angew. Chem. Int. Ed.* **2012**, *51*, 11073.
Zhou, X., *et. al., Chem* **2018**, *4*, 1314.

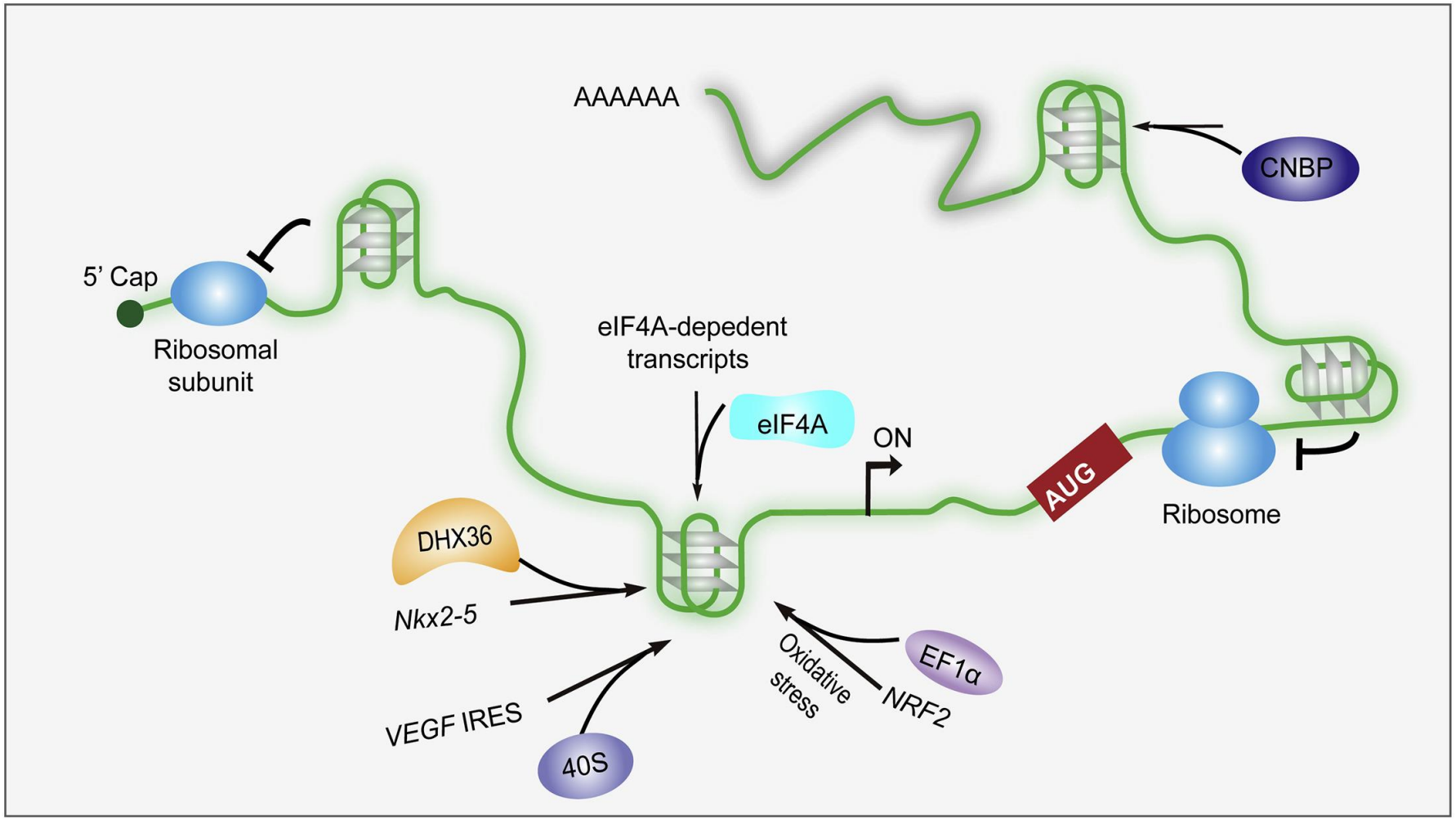
rG4 formation in eukaryotic cells



- ✓ In eukaryotic cells, rG4 are globally unfolded though thousands of sites in the transcriptome form stable rG4s in vitro.

A specialized machinery regulating rG4s formation in cells?

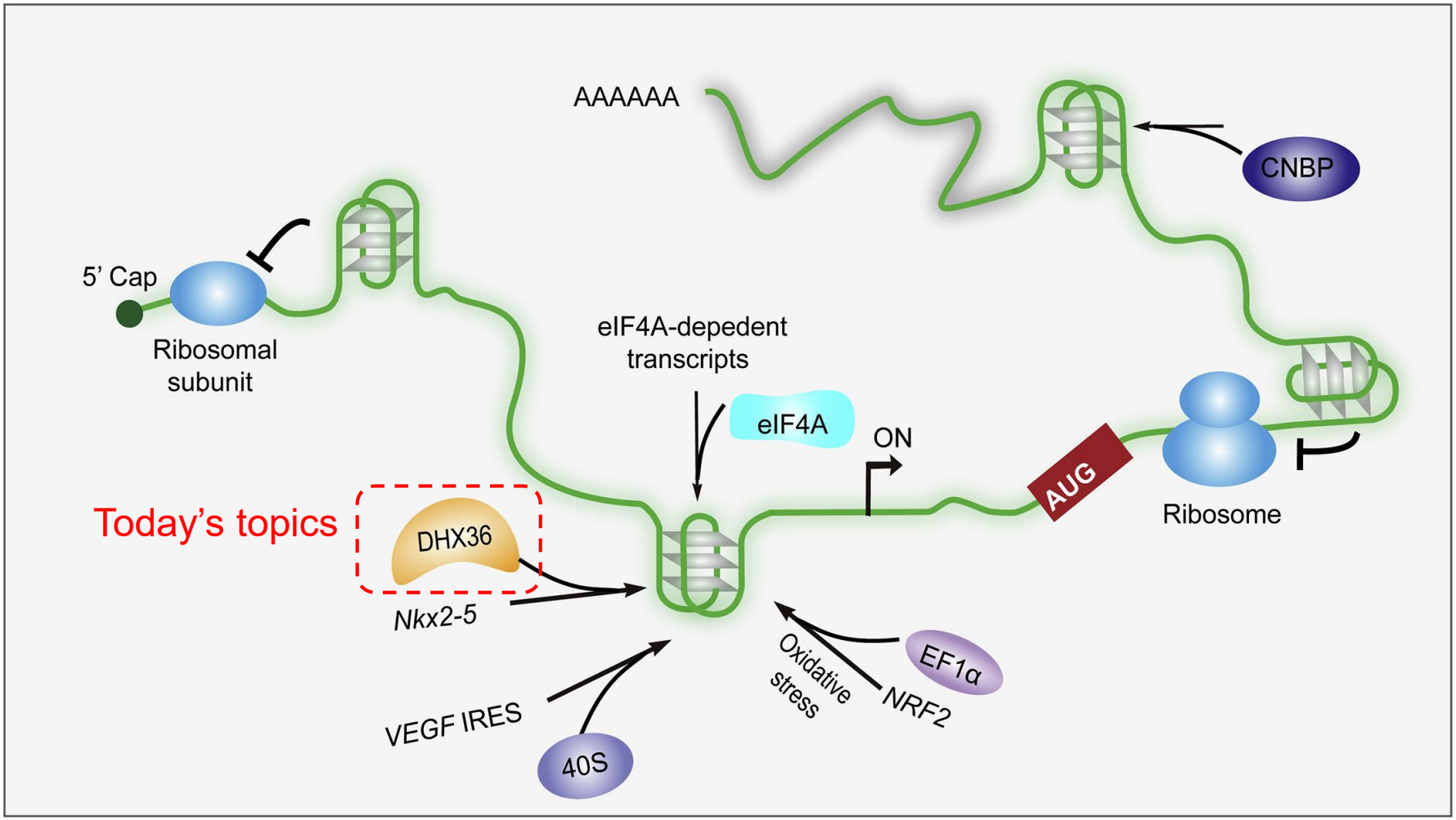
Bartel, D. P., *et. al., Science* **2016**, *353*, aaf5371.



✓ Complex factors are involved in mRNA-translation regulation.

Zhou, X., *et. al., Chem* **2018**, *4*, 1314.

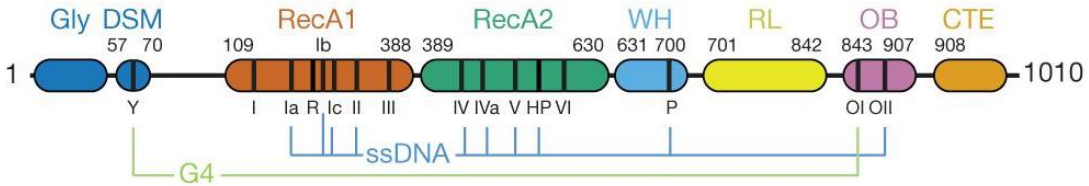
RNA G4s in mRNA

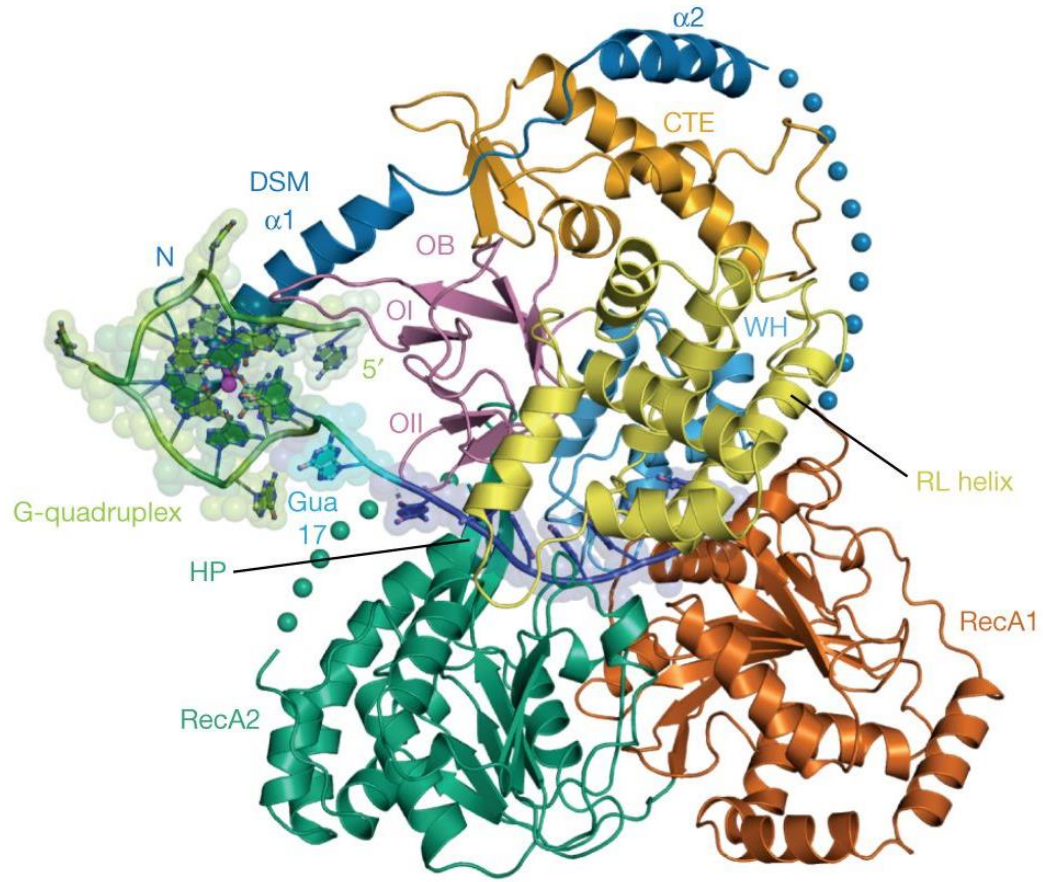


✓ Complex factors are involved in mRNA-translation regulation.

Zhou, X., *et. al., Chem* **2018**, *4*, 1314.

DHX36 (1)





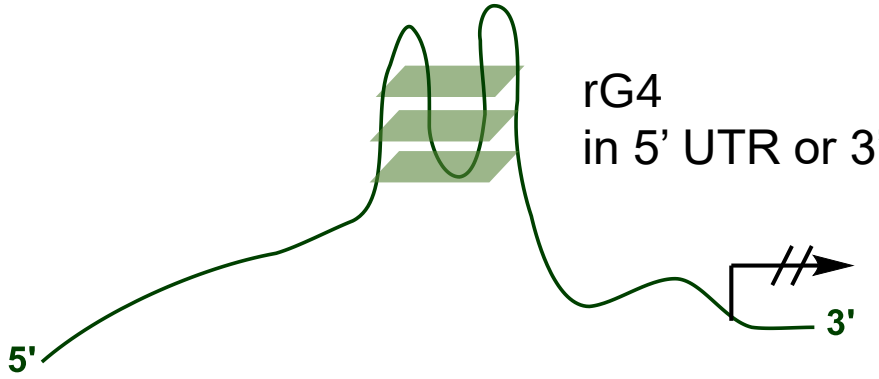
Structural charasteristics

- ✓ DSM(DHX36-specific motif) and OB subdomain specifically recognizes G4s.
- ✓ DSM forms a hydrophobic core, which surface stacks on the top of the bound G4.

Ferré-D'Amaré, A. R., *et. al., Nature* **2018**, *558*, 465.

DHX36 (2)

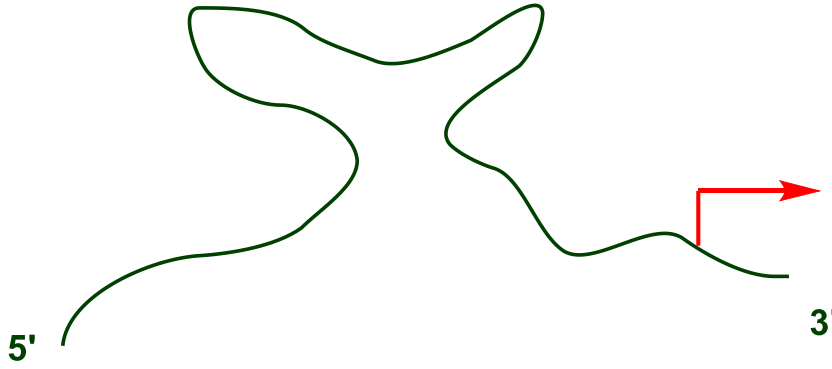
Functional characteristics… RNA helicase which binds and unwinds rG4 (ATP-dependently)



rG4
in 5' UTR or 3' UTR (untranslated region)

Translational regulation

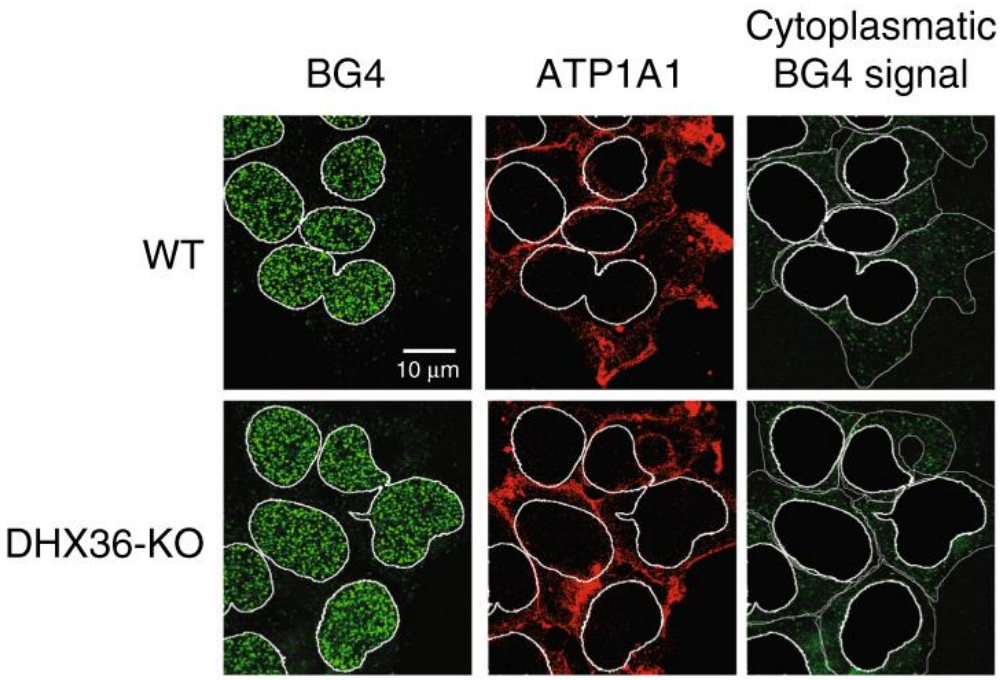
Examples of mRNAs
Nkx2-5 (TF related to heart development)
YY1 (regulating gene expression)
PITX1 (tumor suppressor gene)
…

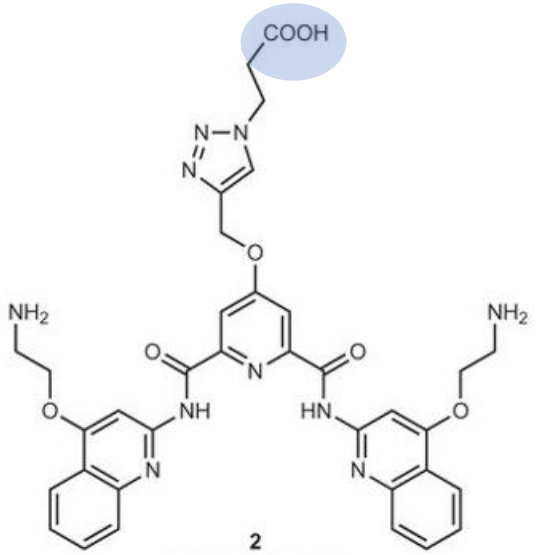


Translationally active

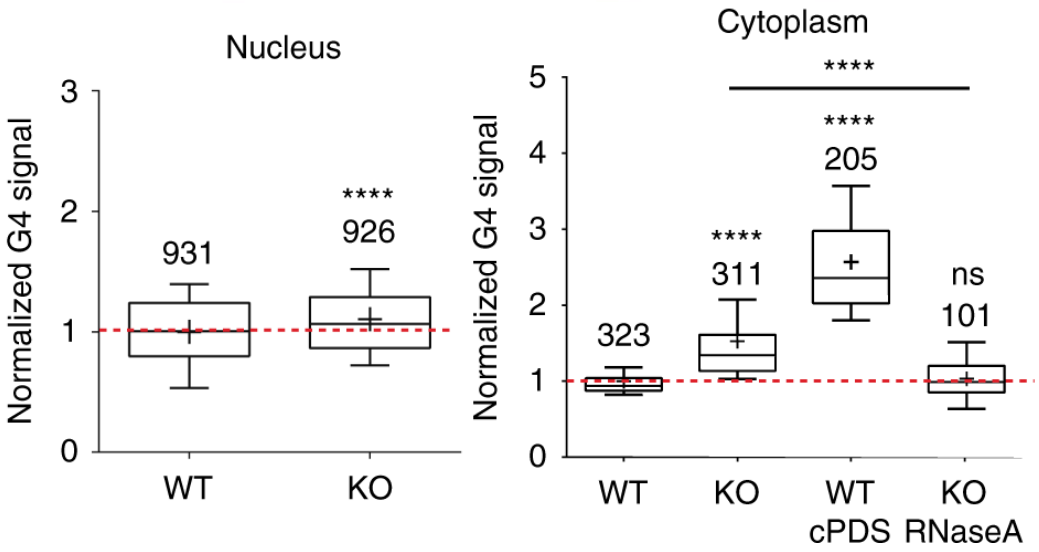
Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

DHX36 (3)





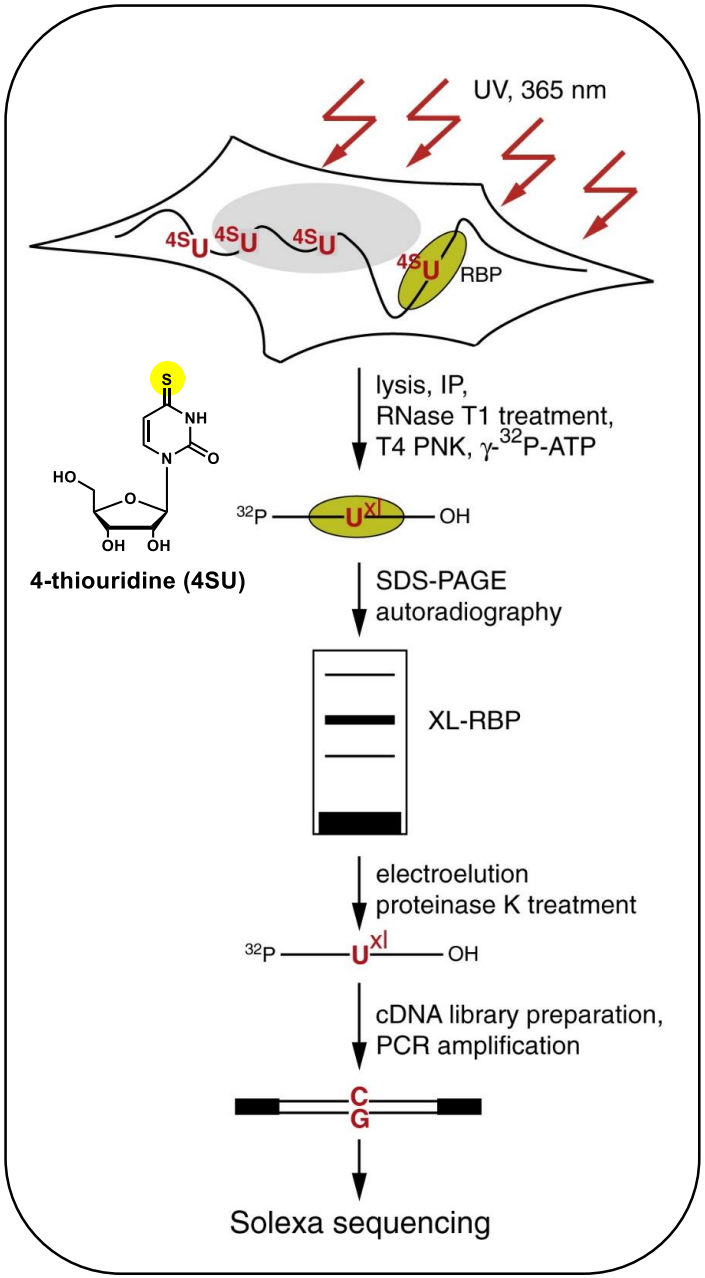
CarboxyPDS (cPDS)
rG4-selective ligand



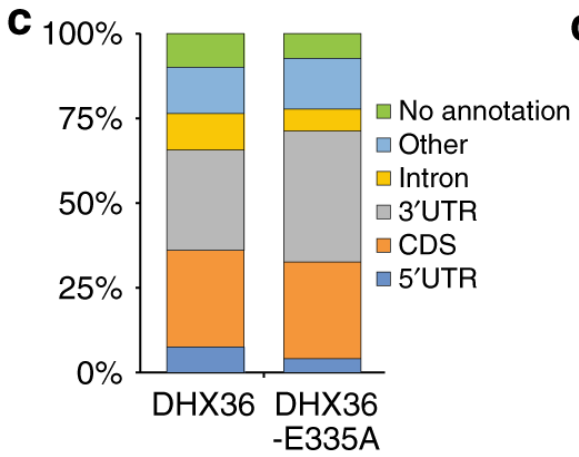
✓ DHX36-KO resulted in an increase in rG4 formation in living cells.

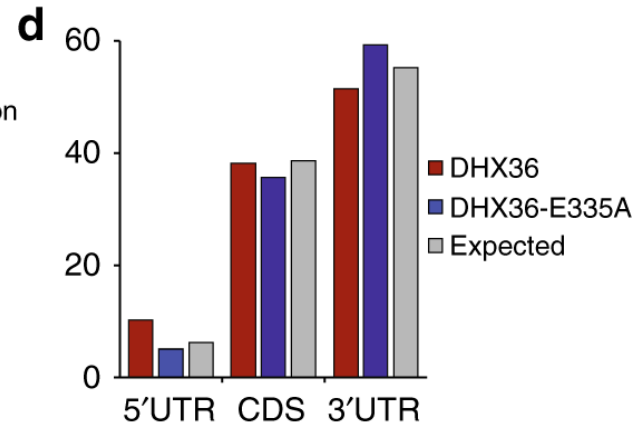
Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

Systems-wide analysis of DHX36 (1)



PAR-CLIP … mapping RNA-interactome of a protein
(Photoactivatable-ribonucleoside-enhanced crosslinking and immuno-precipitation)





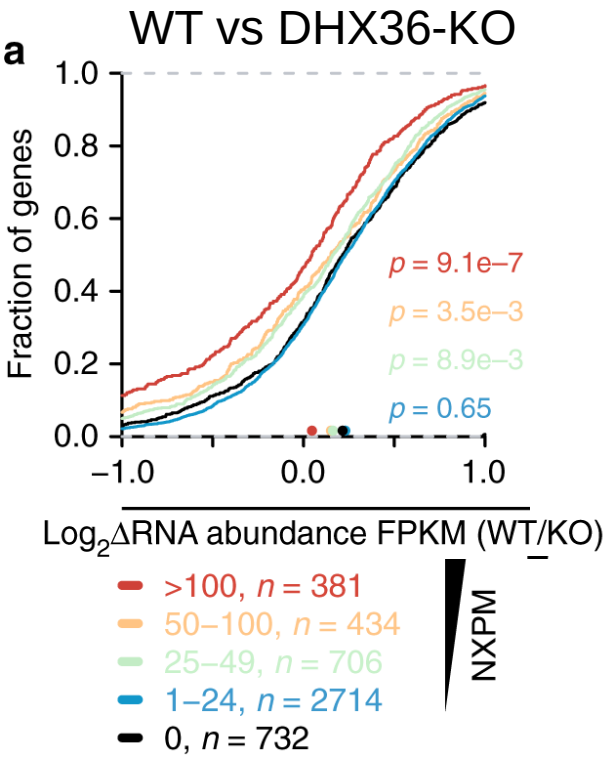
(CDS: coding sequence)
(E335A… catalytic dead)

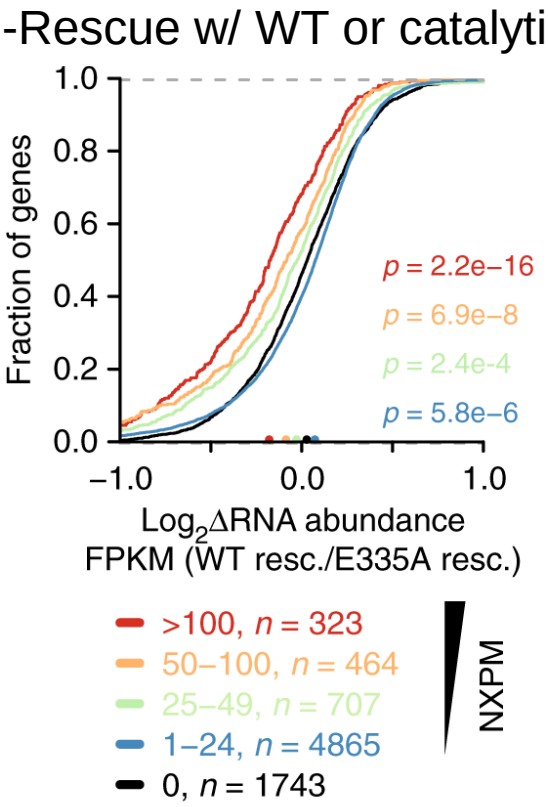
✓ Any preference for binding sites in exons were observed compared to chance.

Tuschl, T., *et. al., Cell* **2010**, *141*, 129.
Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

Systems-wide analysis of DHX36 (2)

RNA-seq





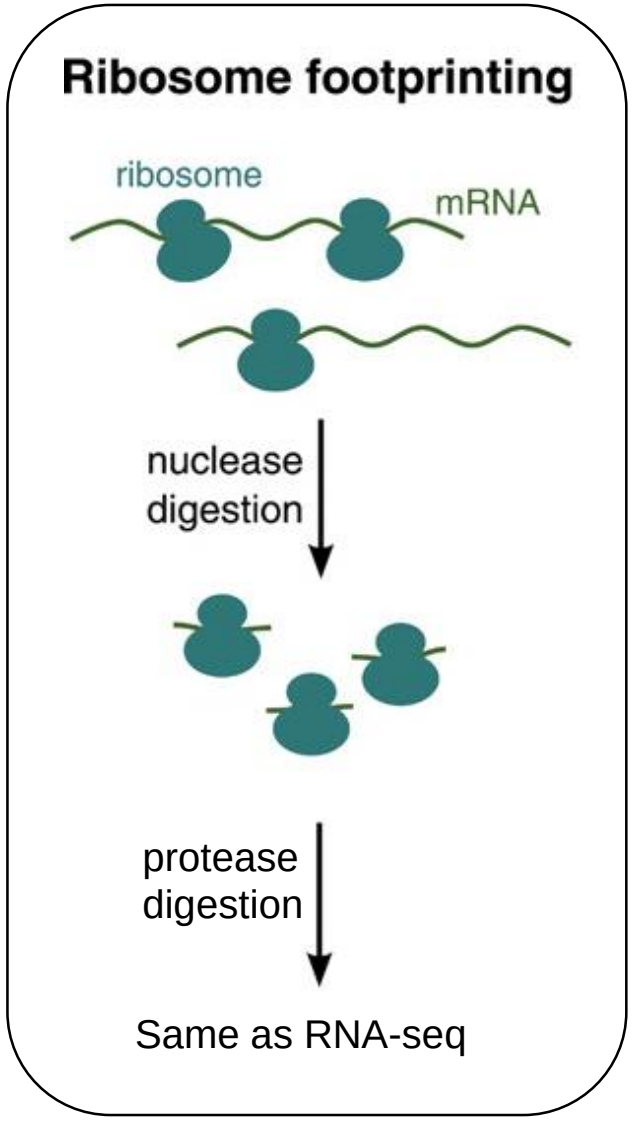
(NXPM : normalized crosslinked reads per million in PAR-CLIP)

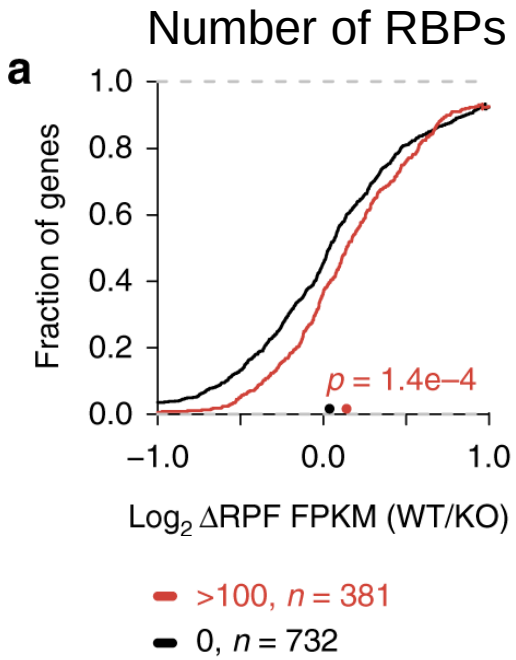
- ✓ DHX36 regulates gene expression in a post-transcriptional manner.
- ✓ DHX36's loss results in the stabilization of target mRNAs in a helicase-dependent manner.

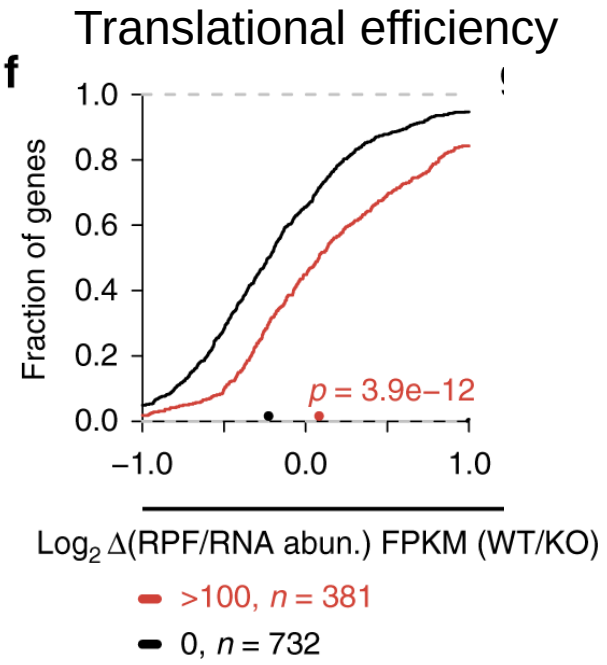
Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

Systems-wide analysis of DHX36 (3)

Ribo-seq (ribosome-footprinting) : measures a change in translational activity







(RPF : ribosome-protected fragments)

✓ DHX36 increases translational efficiency of its targets.

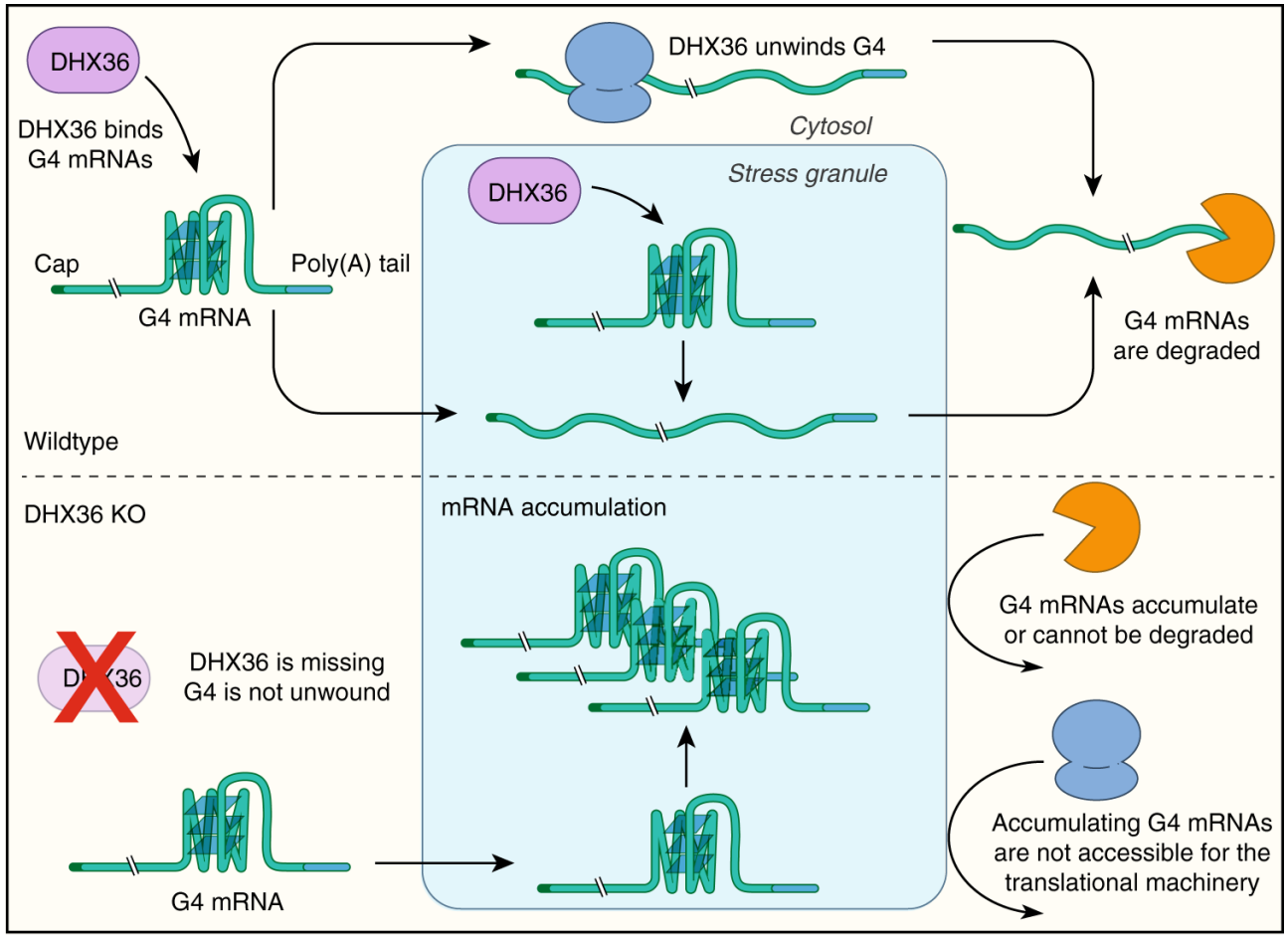
Weissman, J. S., *et. al., Science.* **2009**, *324*, 218.
Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

rG4-dependent SGs formation (1)

✓ DHX36 binding in 3' UTR was as efficient in promoting translation as binding in 5' UTR.

→ rG4 resulted in their sequestration into translationally inactive subcellular compartments? (such as stress granules (SG) or P-bodies)

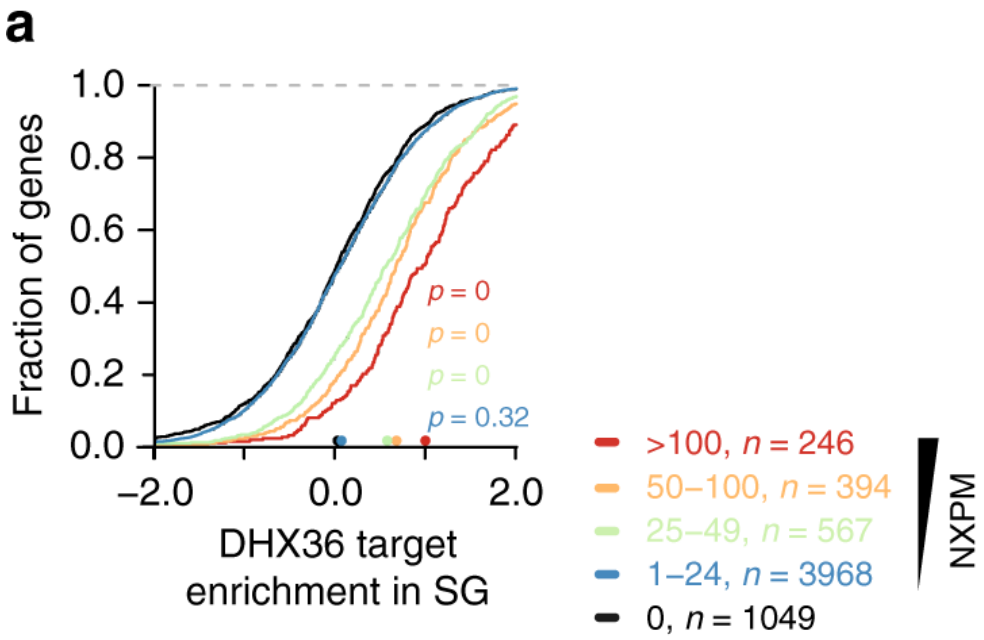
Hypothesis



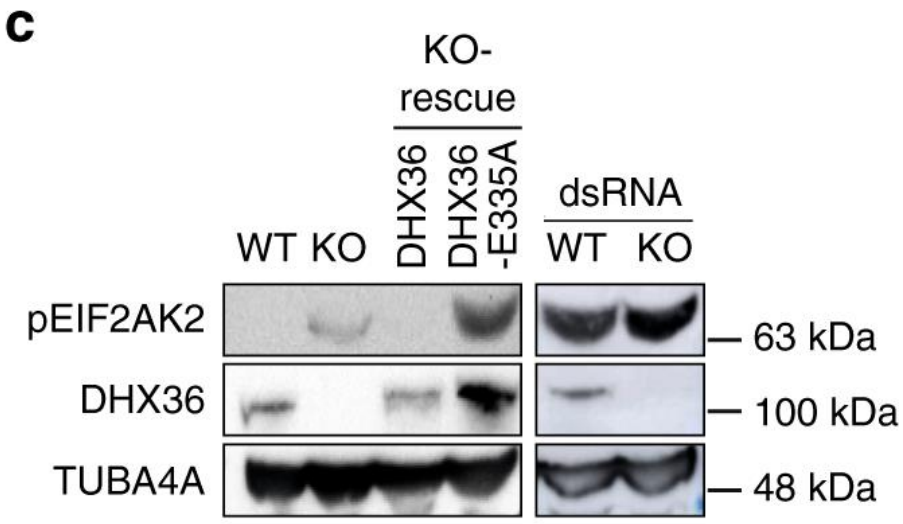
Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

rG4-dependent SGs formation (2)

Cross-reference in dataset of PAR-CLIP & transcripts enriched in SGs



Western-blotting analysis

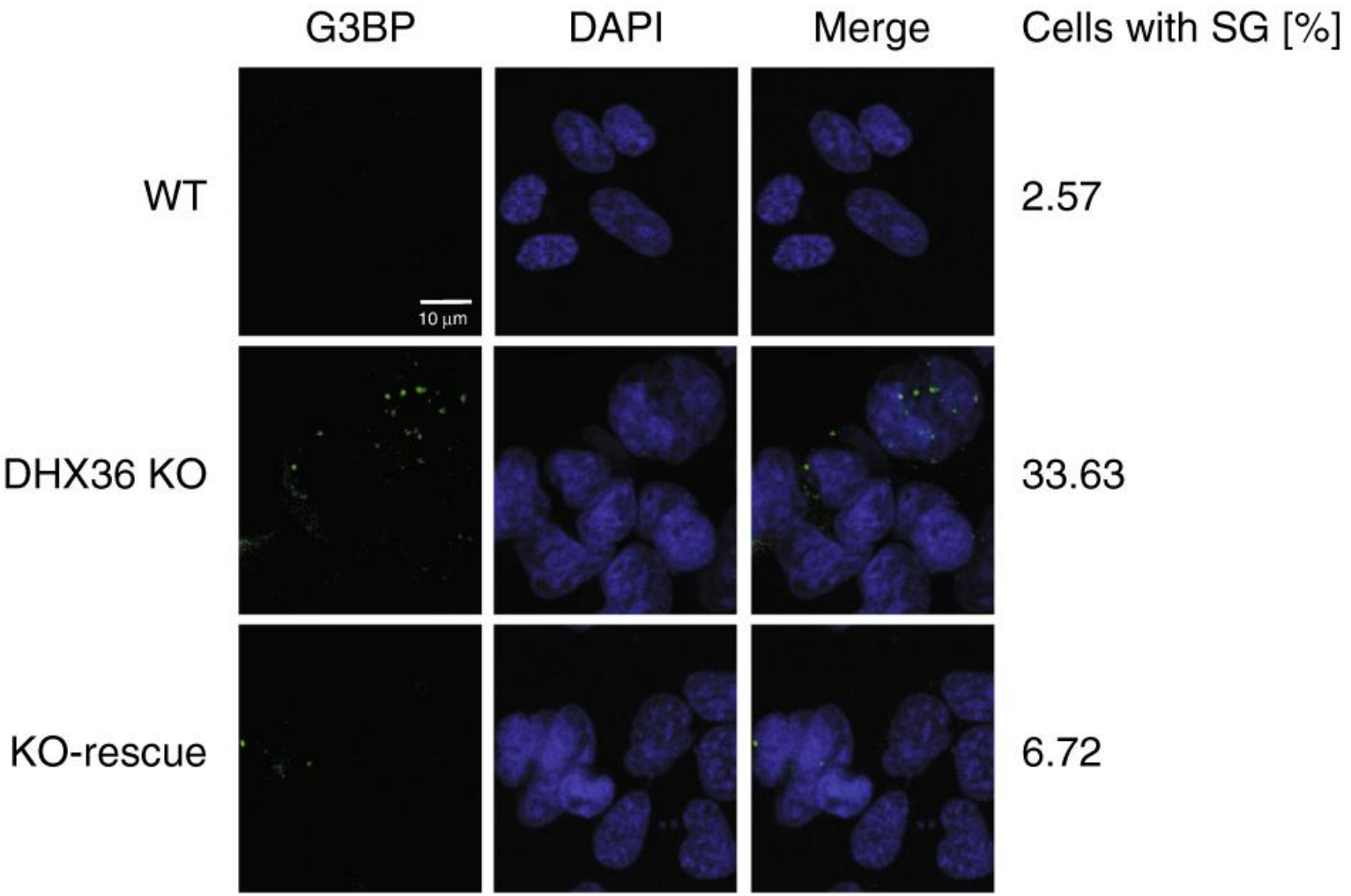


(pEIF2AK2… marker of cellular stress response)

- ✓ DHX36 mRNA targets are enriched in SGs.
- ✓ DHX36-KO induced stress response.

Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

rG4-dependent SGs formation (3)



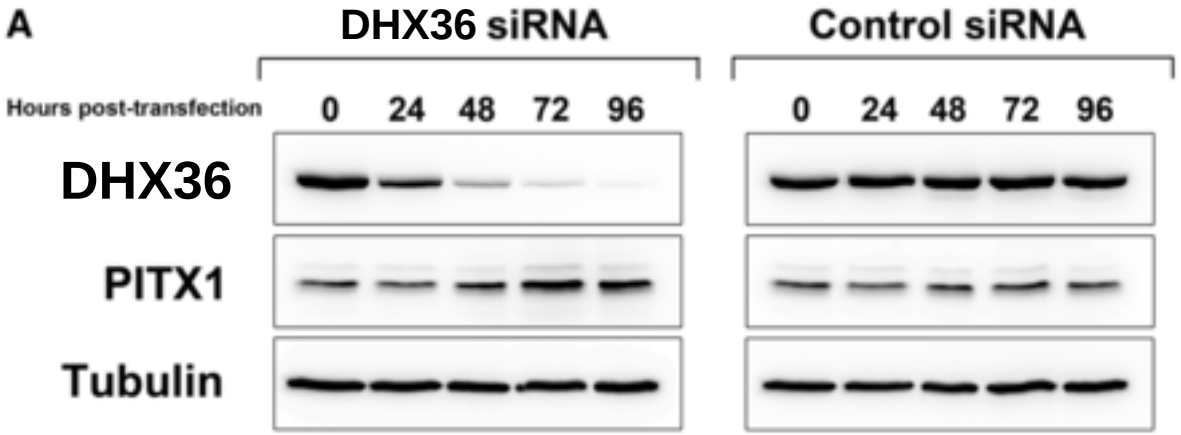
(G3BP… stress granules)

✓ Formation of SGs dependent on rG4 was suggested.

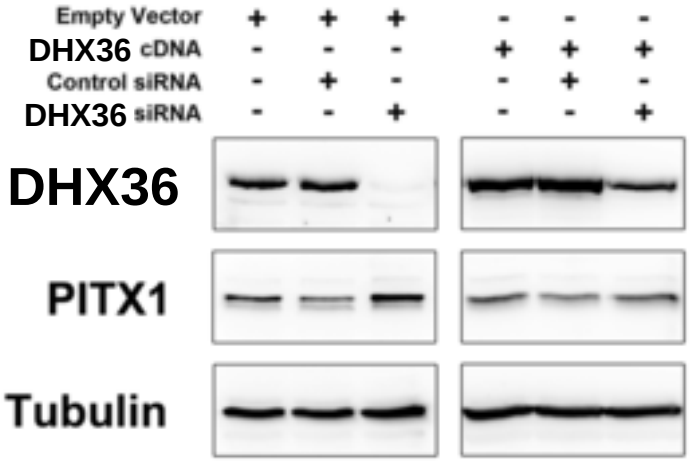
Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

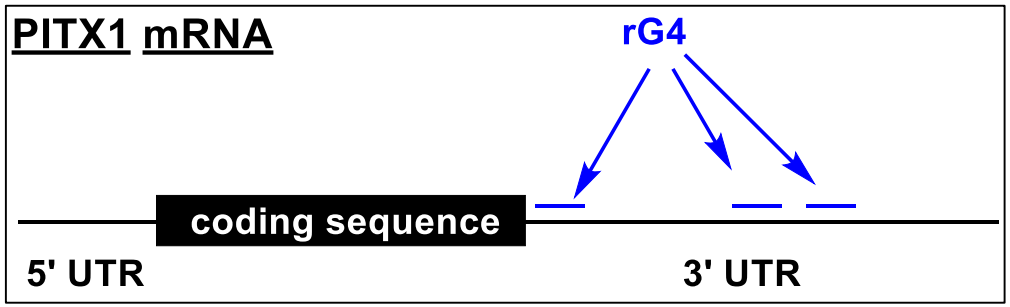
Suppression of PITX1 by DHX36 (1)

DHX36 knockdown



Rescue experiment



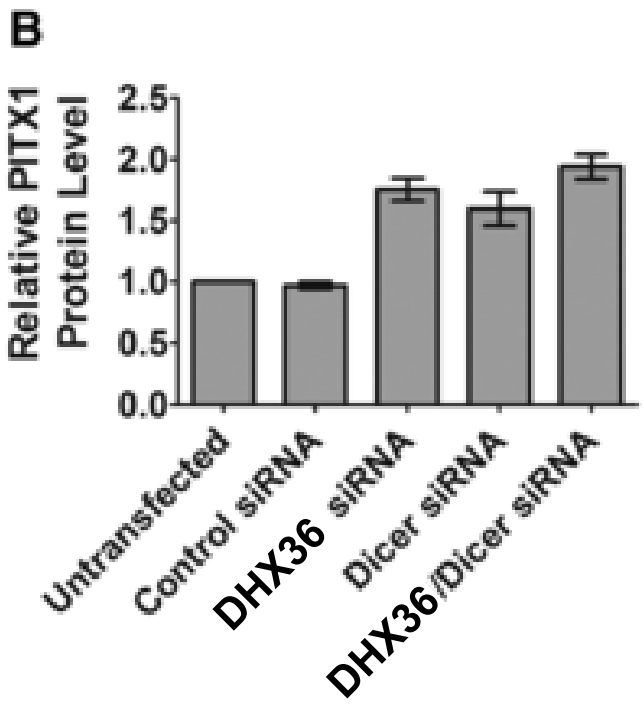


✓ DHX36 is a negative regulator of PITX1 protein expression.

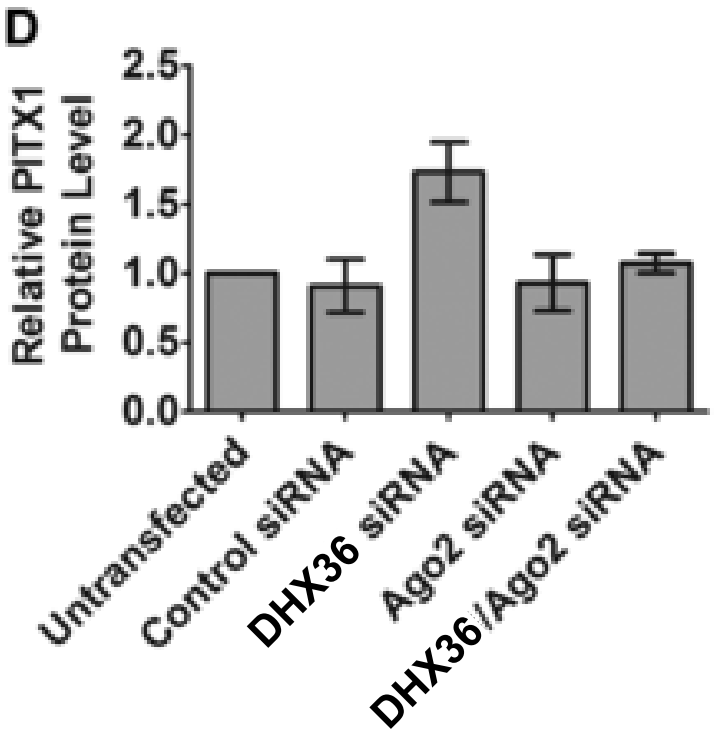
McKenna, S. A., *et. al., Nucleic Acids Res.* **2014**, *42*, 3346.

Suppression of PITX1 by DHX36 (2)

Dicer knockdown



Ago2 knockdown



Dicer and Ago2... proteins related to miRNA-mediated RNA silencing
(see appendix for details)

- ✓ There is a link between DHX36 and miRNA-mediated regulation of PITX1.
- ✓ However, further study is necessary to determine the precise interplay.

McKenna, S. A., *et. al., Nucleic Acids Res.* **2014**, *42*, 3346.

Contents

Long non-coding RNA (lncRNA)

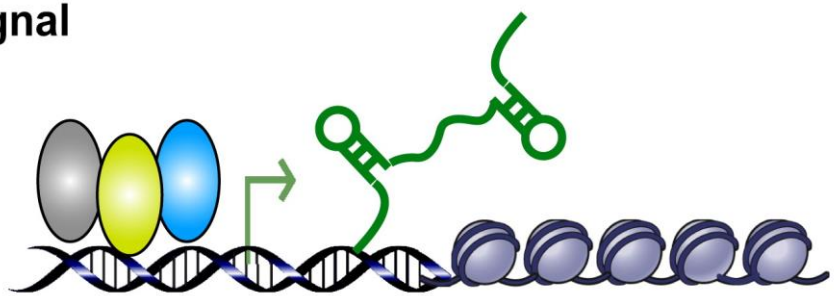
✓ Non-coding RNA(ncRNA)… RNAs which is not translated into proteins

Long non-coding RNA(lncRNA)…ncRNA with lengths exceeding 200 nucleotides

Mechanisms of functions

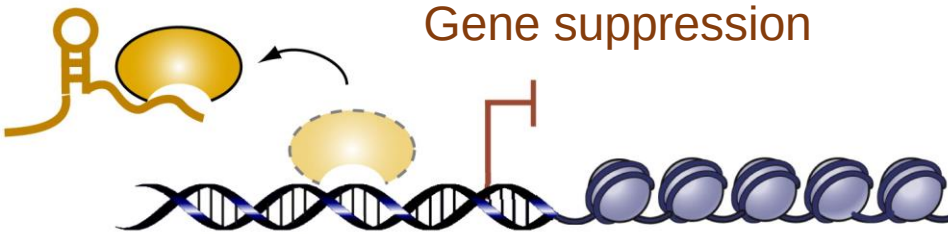
I. **Signal**

Gene activation



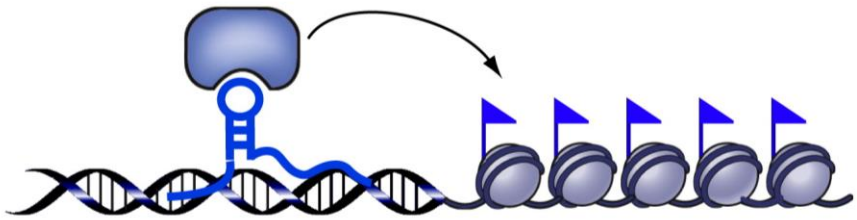
II. **Decoy**

Gene suppression



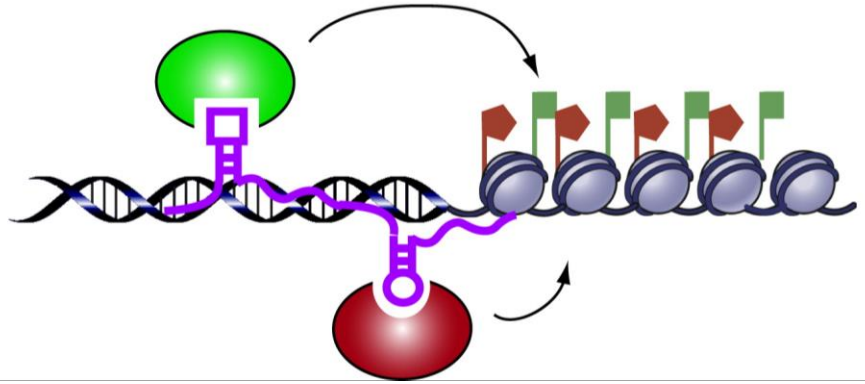
III. **Guide**

Chromatin modification



IV. **Scaffold**

Act on chromatin structure



Chang, H. Y., *et. al., Mol. Cell* **2011**, *43*, 904.

Long non-coding RNA (lncRNA)

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Mechanisms of functions

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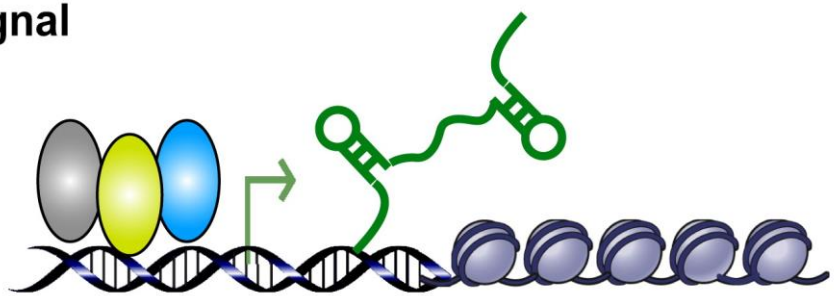

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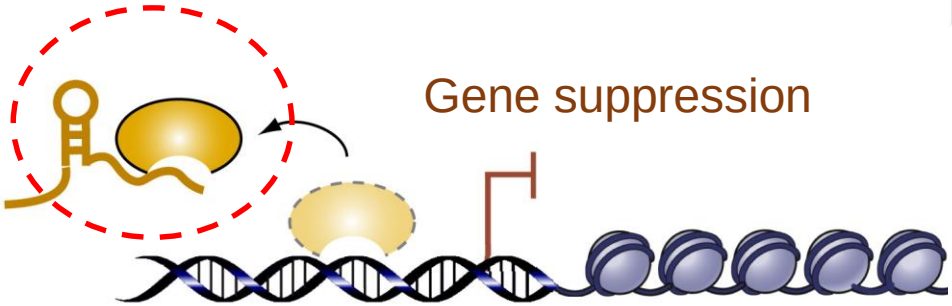
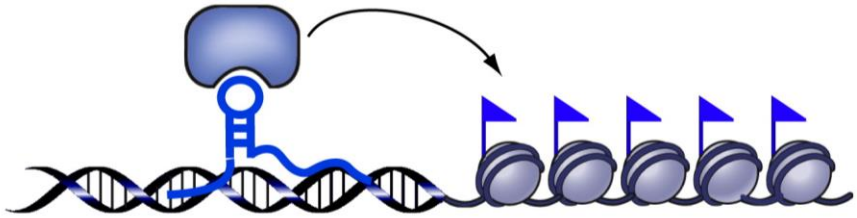


Interaction of RNA and protein
… rG4 & DHX36

III. **Guide**

Chromatin modification



IV. **Scaffold**

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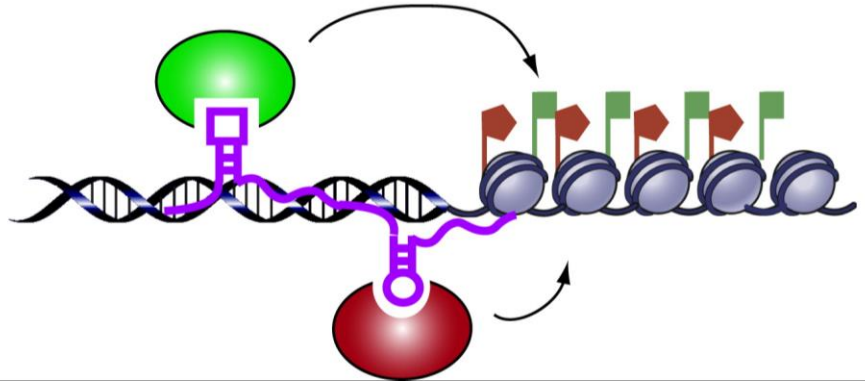
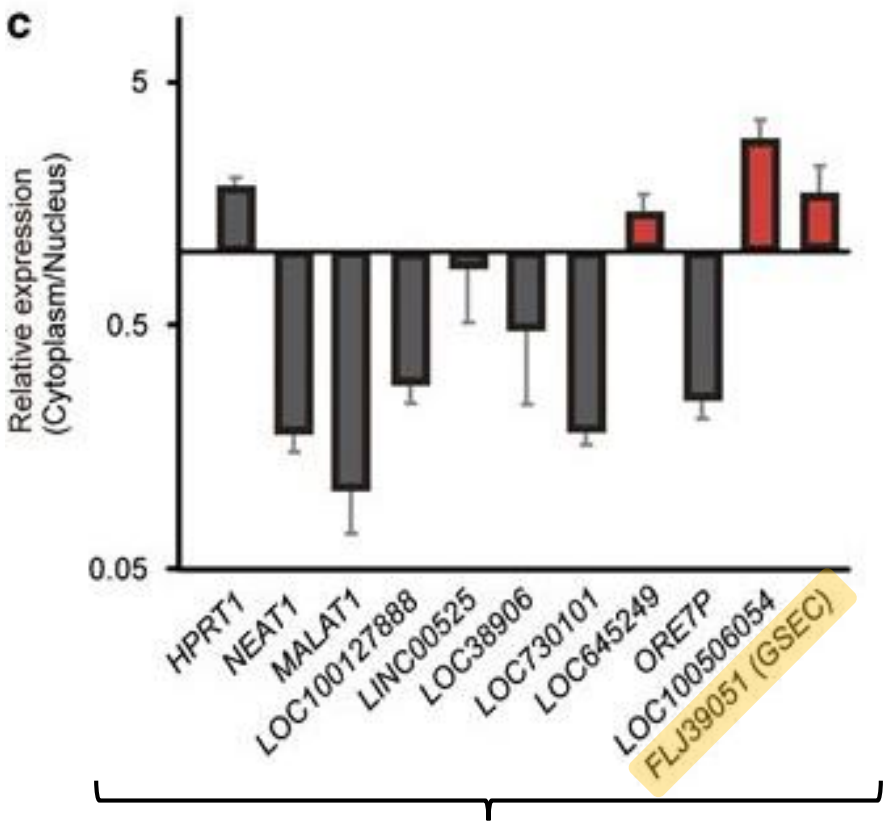
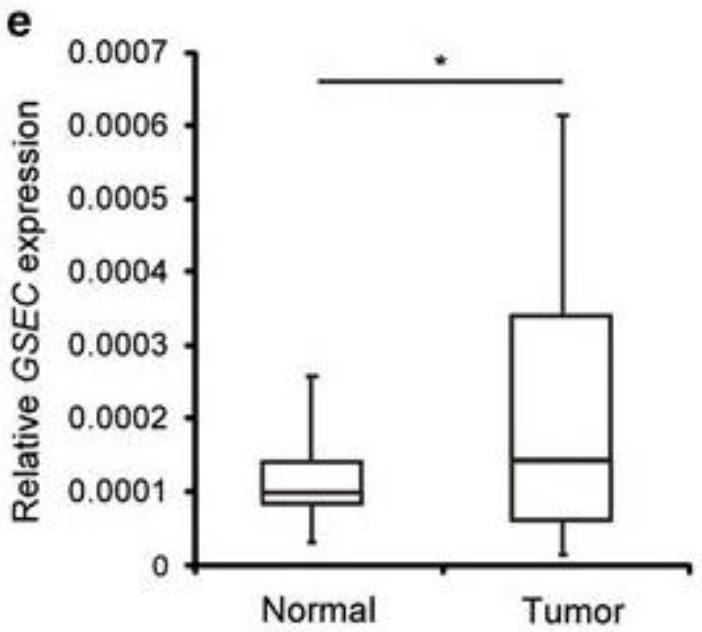


Chang, H. Y., *et. al., Mol. Cell* **2011**, *43*, 904.

lncRNA *GSEC* (1)

GSEC (FLJ39051)… G-quadruplex-forming sequence containing lncRNA





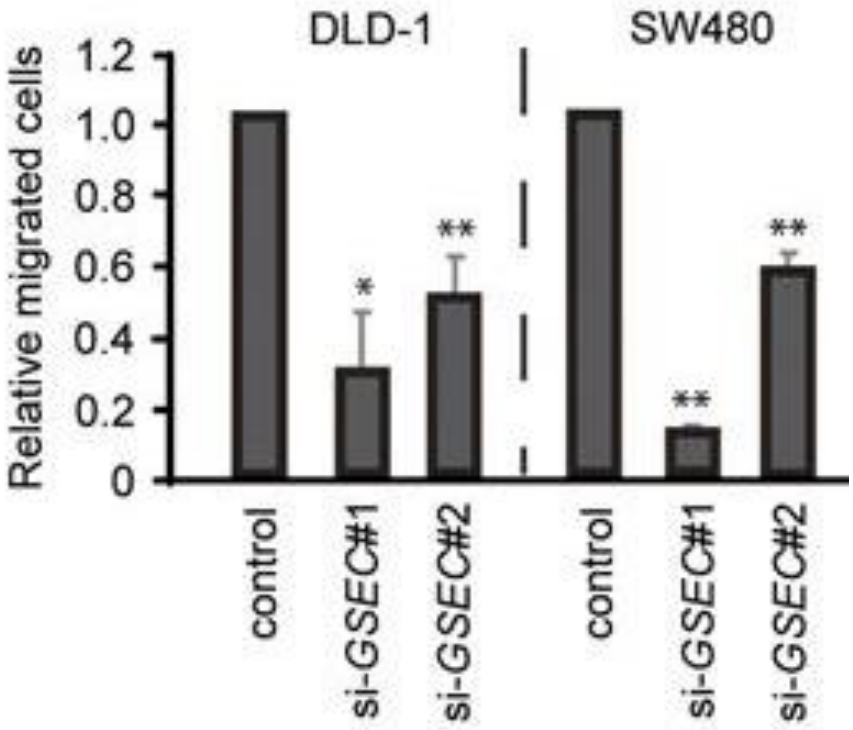
lncRNA upregulated in colon tumor tissues

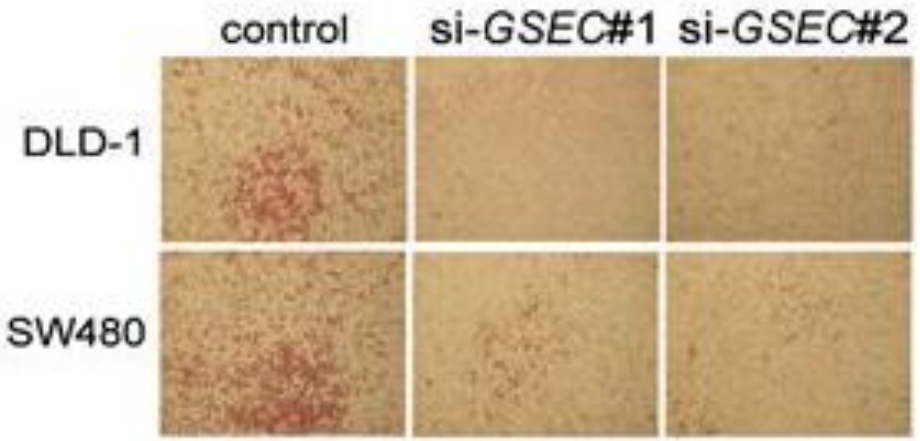
- ✓ *GSEC* was identified as a lncRNA upregulated in colorectal cancer.
- ✓ lncRNA *GSEC* showed enrichment in the cytoplasmic fraction.

Akiyama, T., *et. al., Oncogene* **2017**, *36*, 1191.

lncRNA *GSEC* (2)

Transwell migration chamber assay … examine cell motility (細胞遊走性)



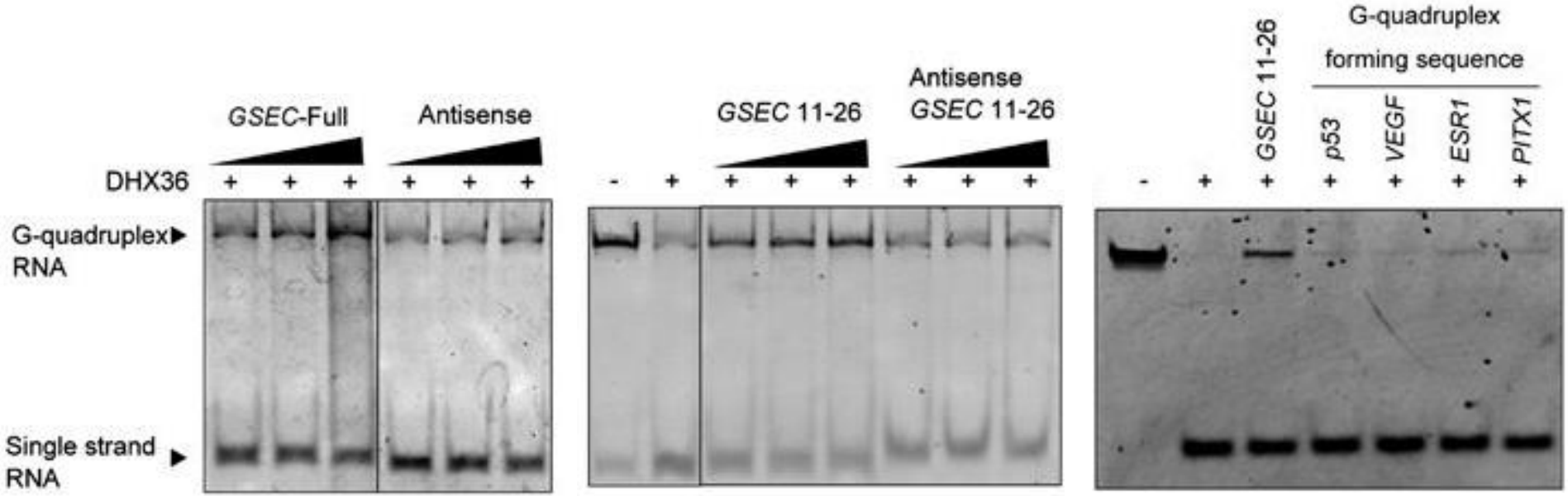


✓ *GSEC* is required for the motility of colon cancer cells.

Akiyama, T., *et. al., Oncogene* **2017**, *36*, 1191.

Antagonistic effect of *GSEC* (1)

rG4 resolution assay



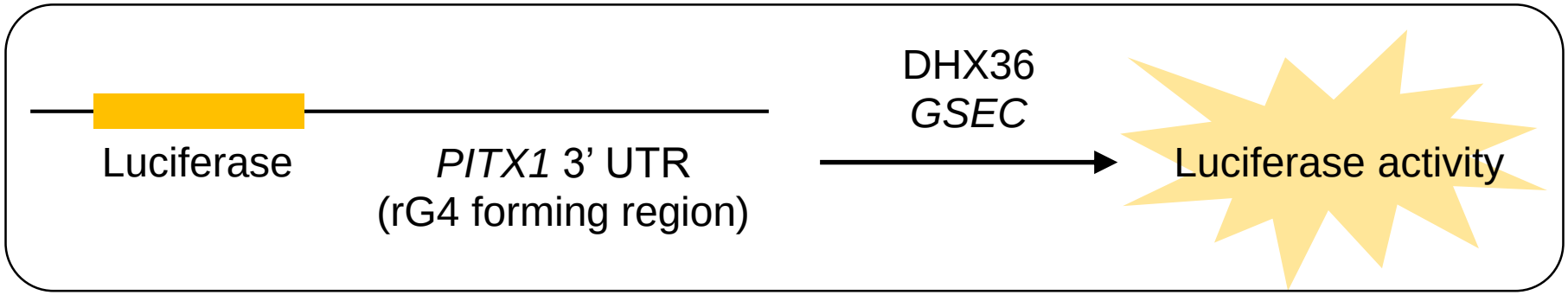
(*GSEC* 11-26… rG4 forming region)

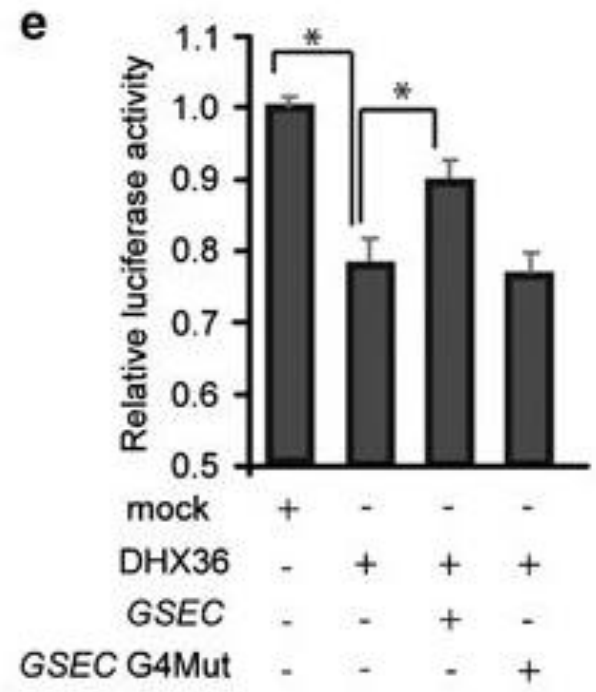
✓ *GSEC* inhibits the function of DHX36.

Akiyama T., *et. al., Oncogene* **2017**, *36*, 1191.

Antagonistic effect of *GSEC* (2)

Luciferase reporter assay



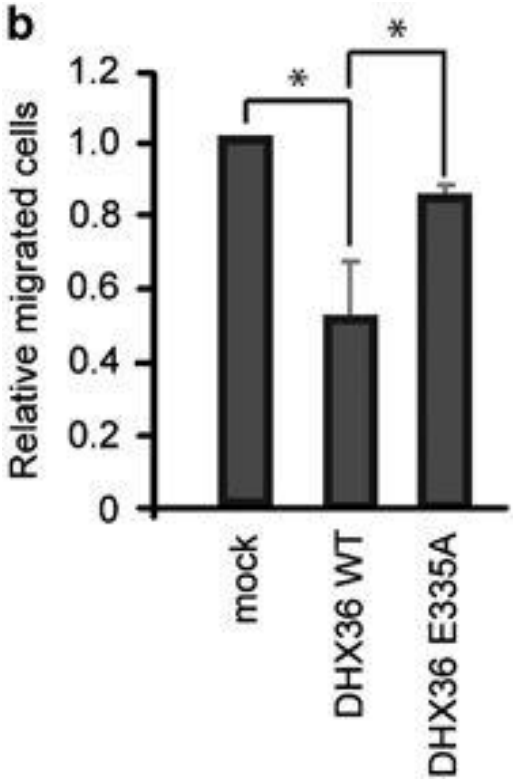


- ✓ It is possible that *GSEC* is also involved in miRNA-mediated regulation of gene expression.

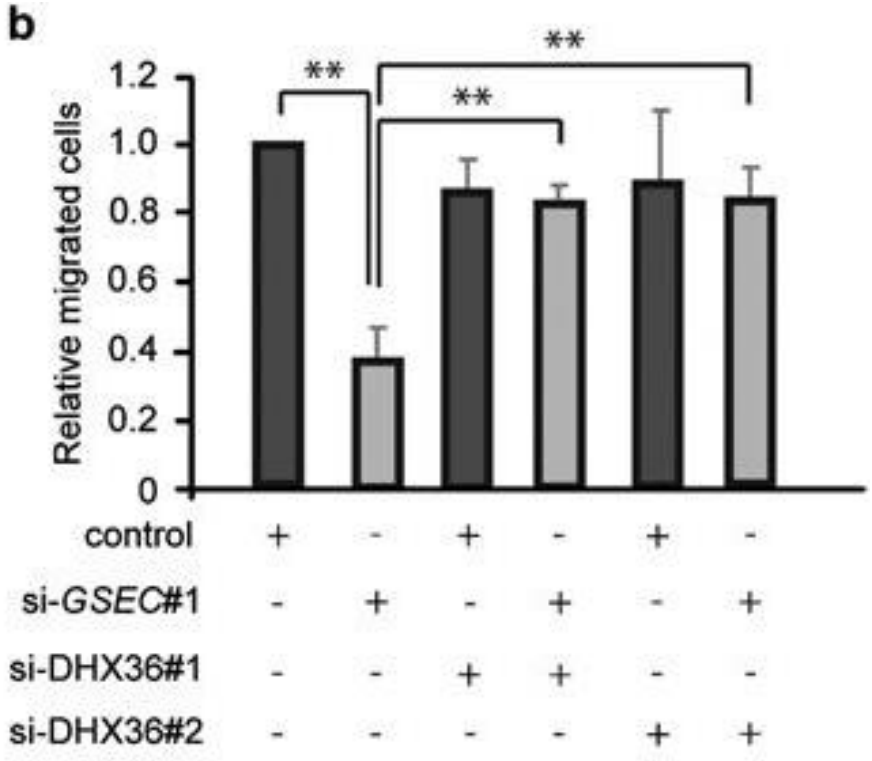
Akiyama, T., *et. al., Oncogene* **2017**, *36*, 1191.

Antagonistic effect of *GSEC* (3)

Overexpression of DHX36



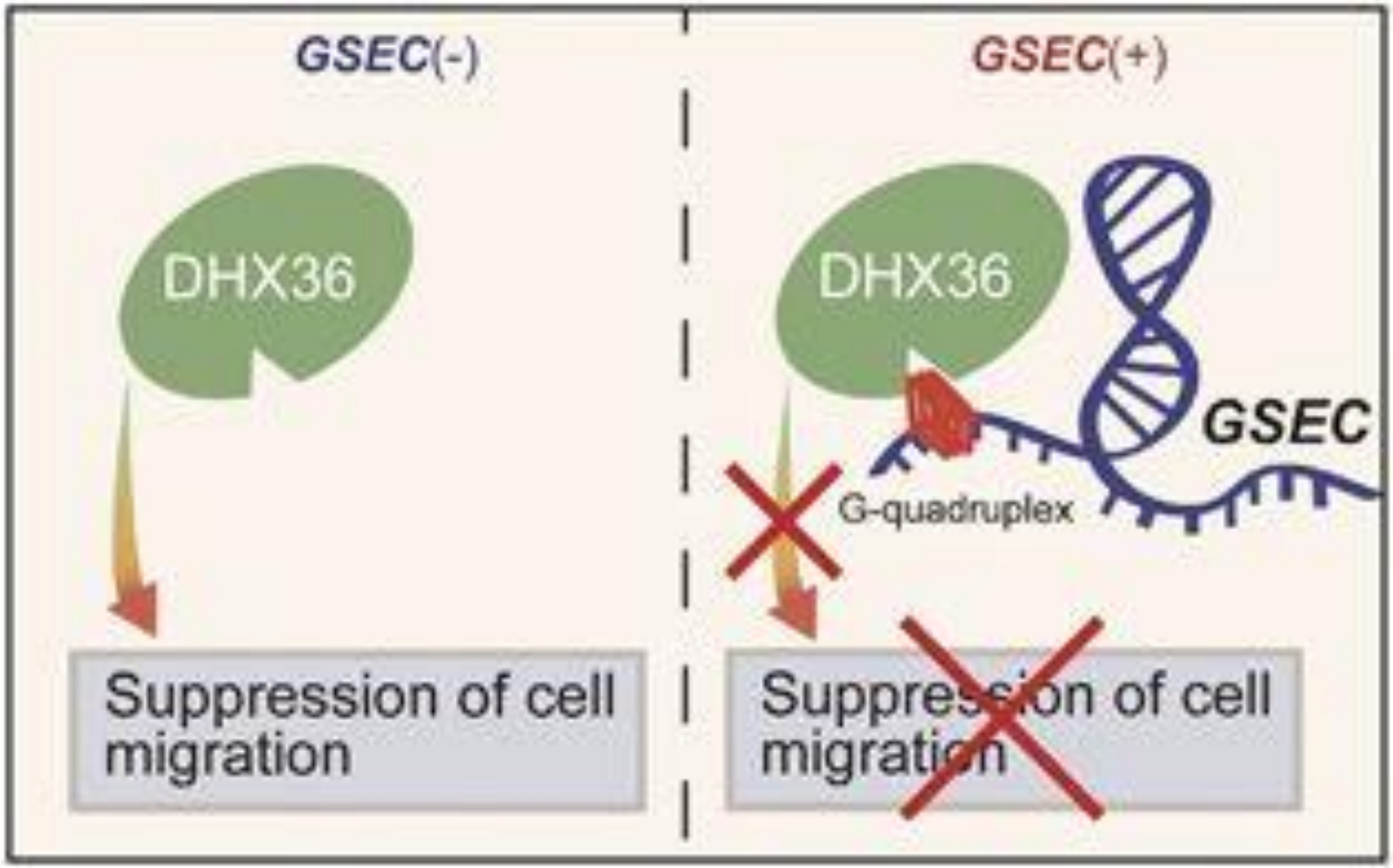
Knockdown of DHX36 and *GSEC*



- ✓ DHX36 decreases cell motility in a helicase activity-dependent manner.
- ✓ *GSEC* enhances cell motility by inhibiting the function of DHX36.

Akiyama, T., *et. al., Oncogene* **2017**, *36*, 1191.

Schematic model of *GSEC* functions



✓ GSEC binds to DHX36 via rG4 and inhibits rG4-resolution activity.

Akiyama, T., *et. al., Oncogene* **2017**, *36*, 1191.

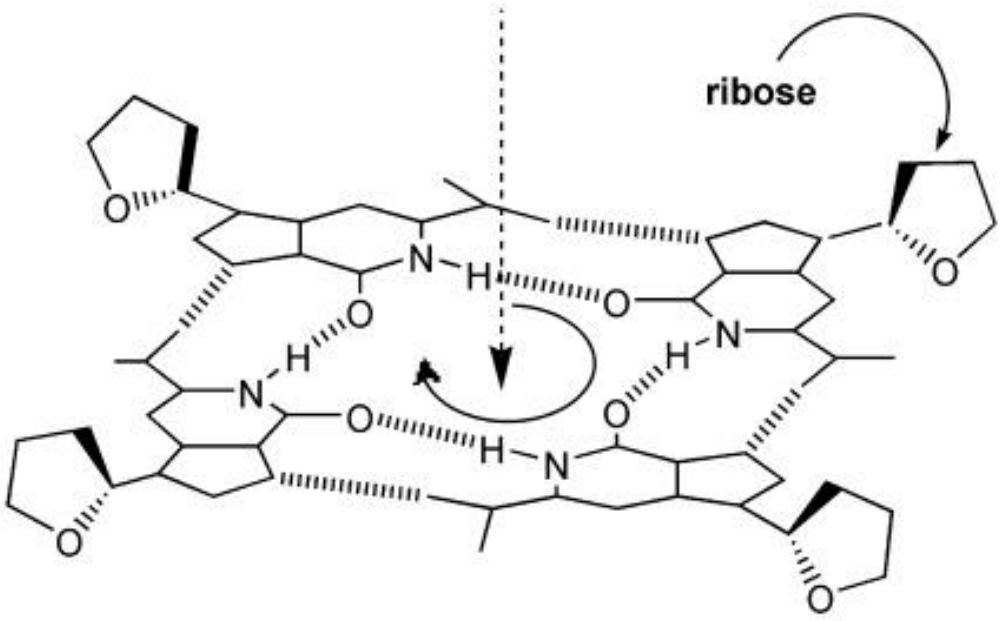
Contents

Summary

- ✓ RNA G-quadruplexes (rG4s) are thought to be regulated by complex factors, which includes DHX36, RNA helicase.
- ✓ DHX36 mainly gives a positive regulation to the translation of mRNAs via its helicase activity.
- ✓ Long non-coding RNA (lncRNA) *GSEC* acts as a decoy for DHX36 binding to prevent the action of DHX36 on other targets.

Appendix

Diastereotopic faces of G-quartet



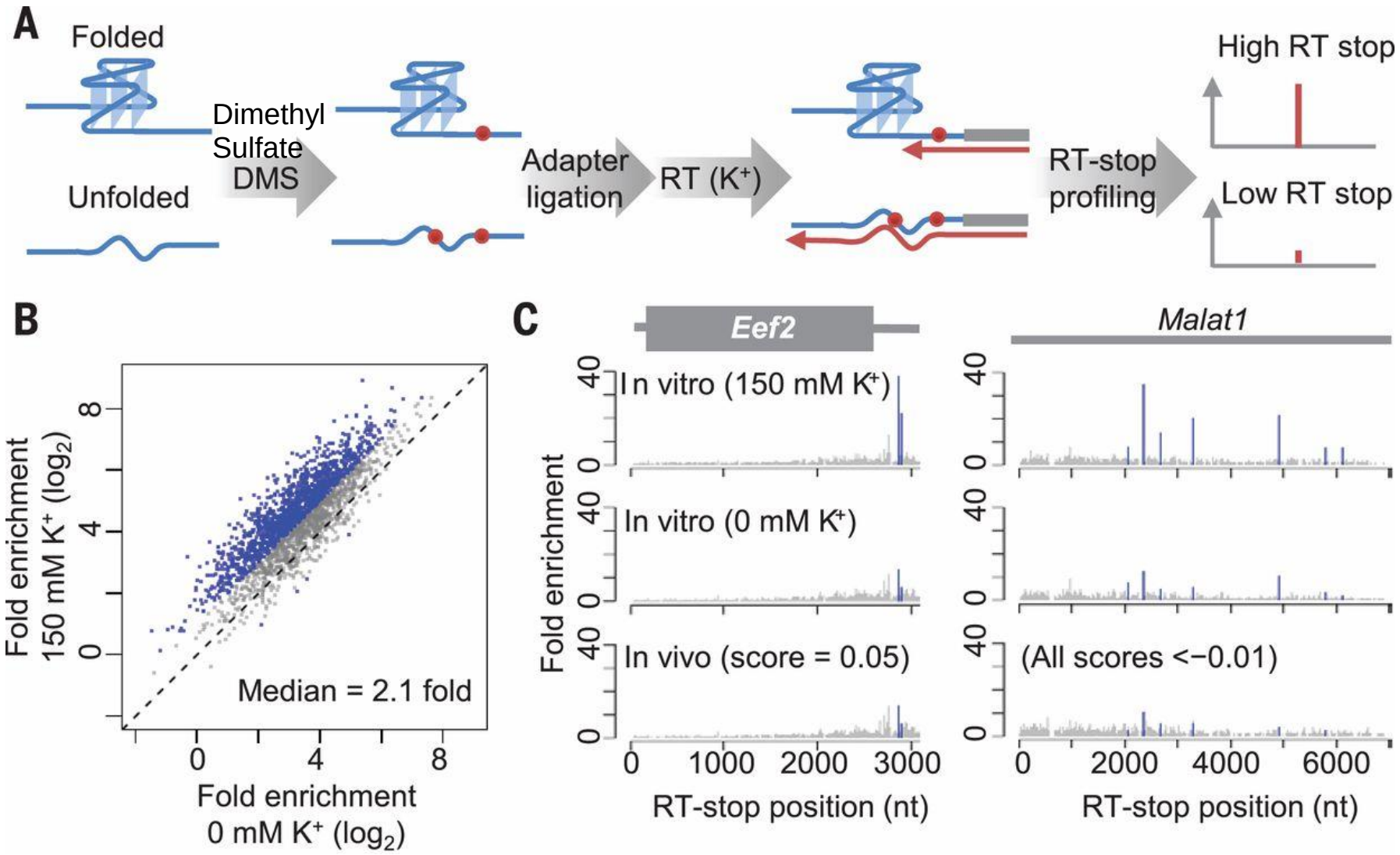
NH''''C=O H-Bond Direction is Clockwise

||

"Head" face

- ✓ The attached sugars result in the chiral G-quartet having diastereotopic faces, a "head" and a "tail".

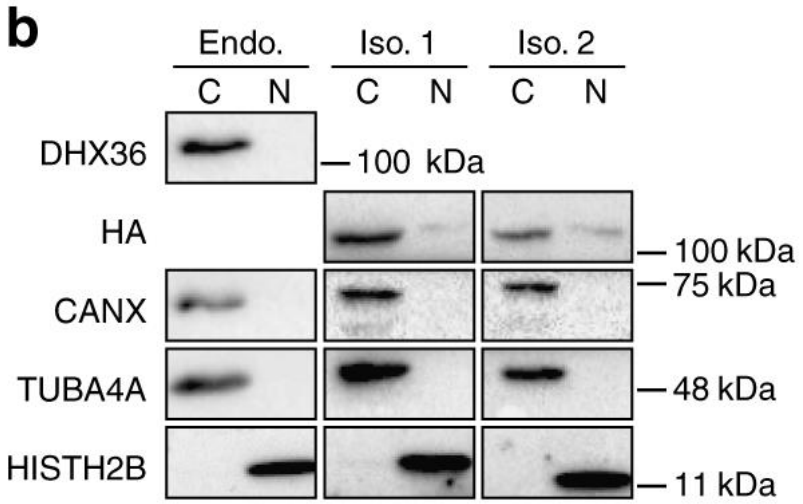
Global unfolding of rG4s



Bartel, D. P., *et. al., Science* **2016**, *353*, aaf5371.

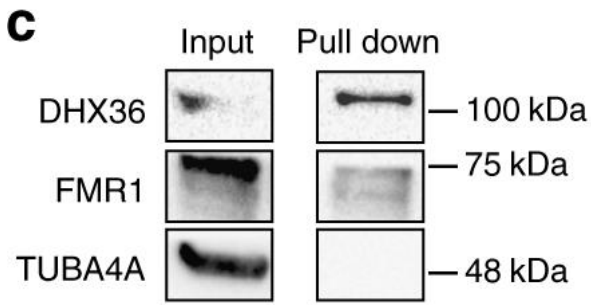
Localization of DHX36

FlagHA-DHX36(iso1 or iso2) expressed HEK293 cells



- ✓ DHX36 is a cytoplasmic helicase.
- ✓ Isoforms 1 and 2 shows no differences in localization.

Purification of polyadenylated RNA after crosslinking

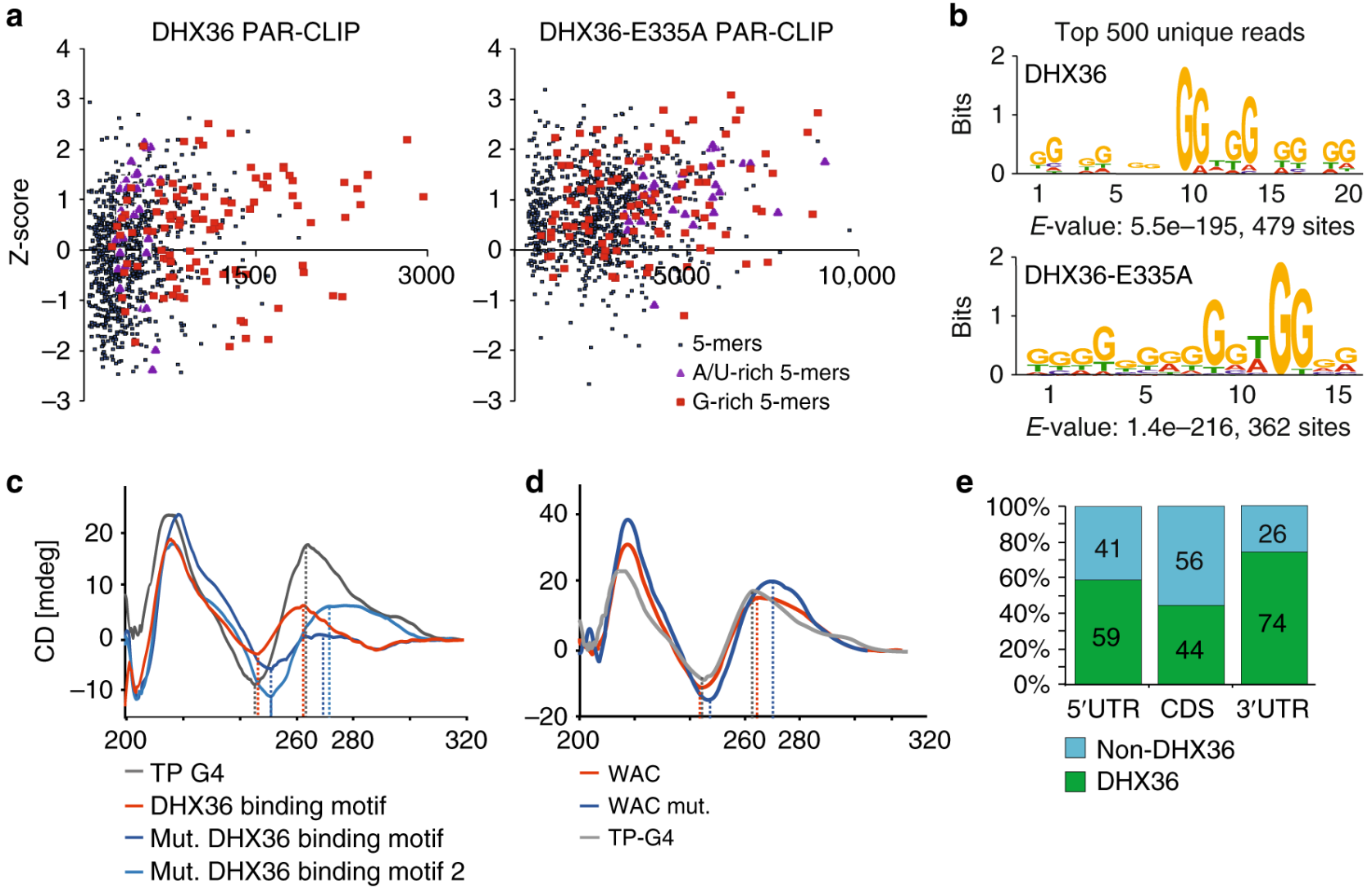


- ✓ DHX36 interacts with mRNA.

✓ DHX36's main targets are cytoplasmic mRNAs.

Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

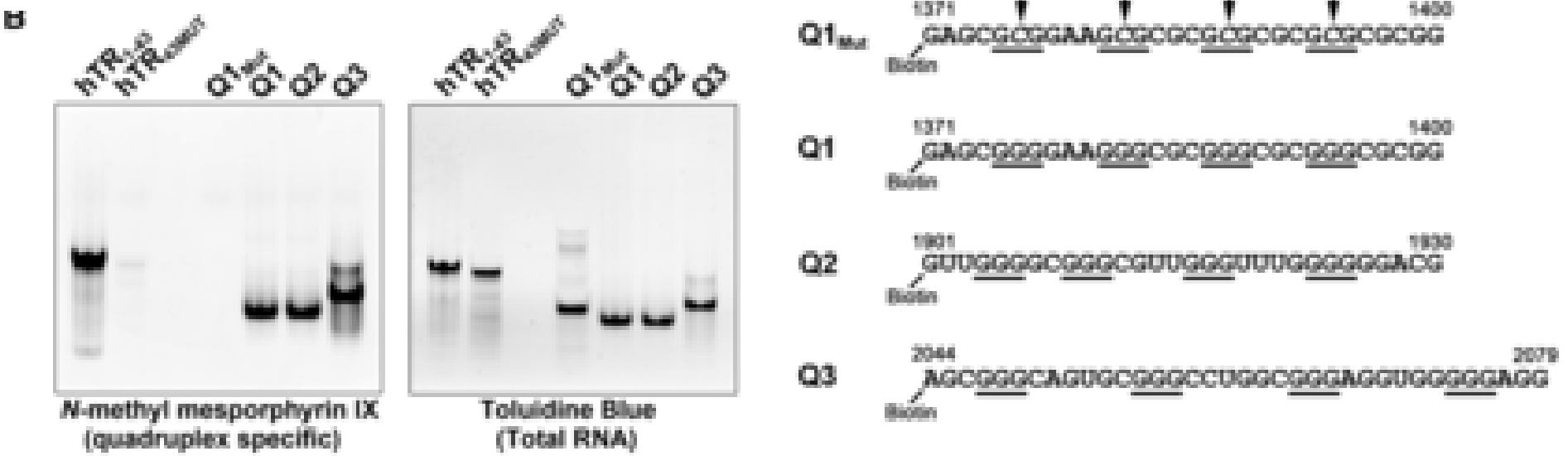
RNA-seq analysis of DHX36 binding

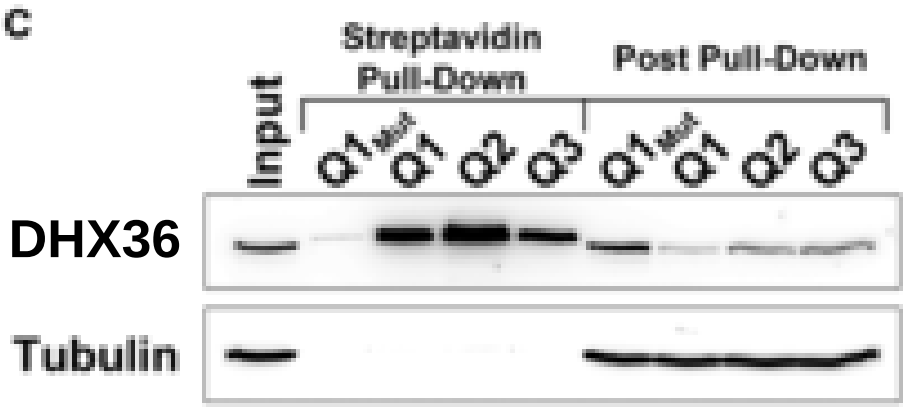


✓ DHX36 binds G-rich target mRNAs in cells that form rG4s in vitro.

Paeschke, K., *et. al., Nat. Commun.* **2019**, *10*, 2421.

rG4s in the PITX1 mRNA 3'-UTR

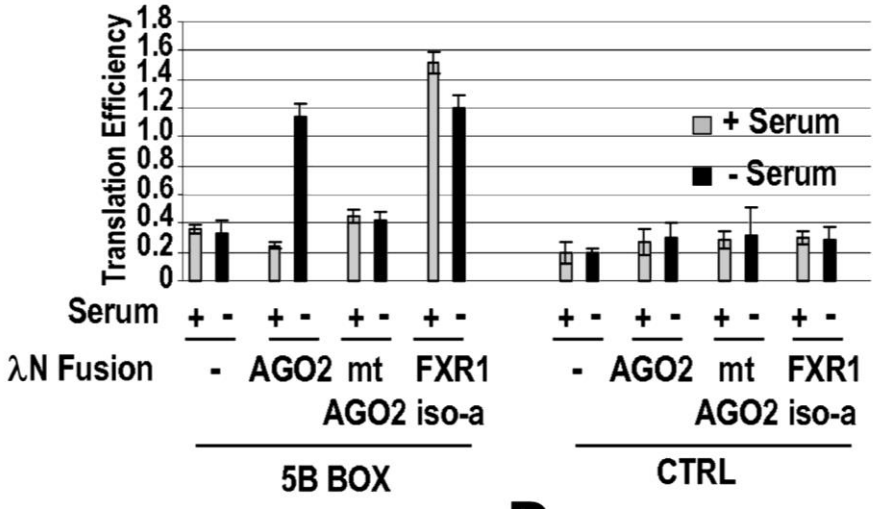


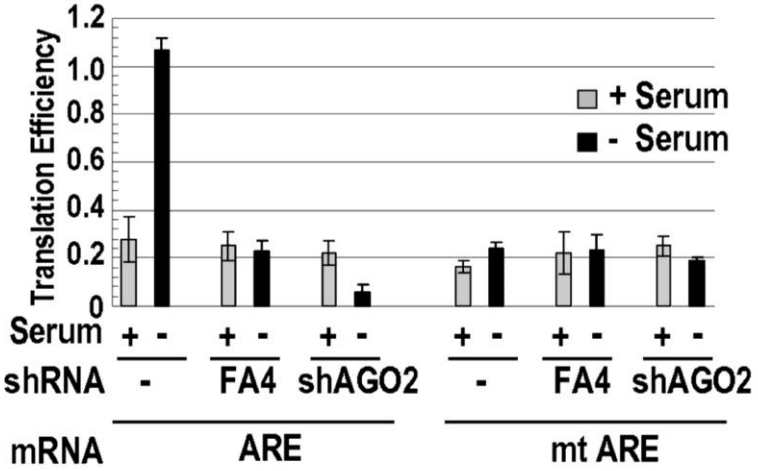


- ✓ PITX1 3'-UTR contains three rG4s capable of interacting with DHX36.

McKenna, S. A., *et. al., Nucleic Acids Res.* **2014**, *42*, 3346.

DHX36 and miRNA machinery (1)



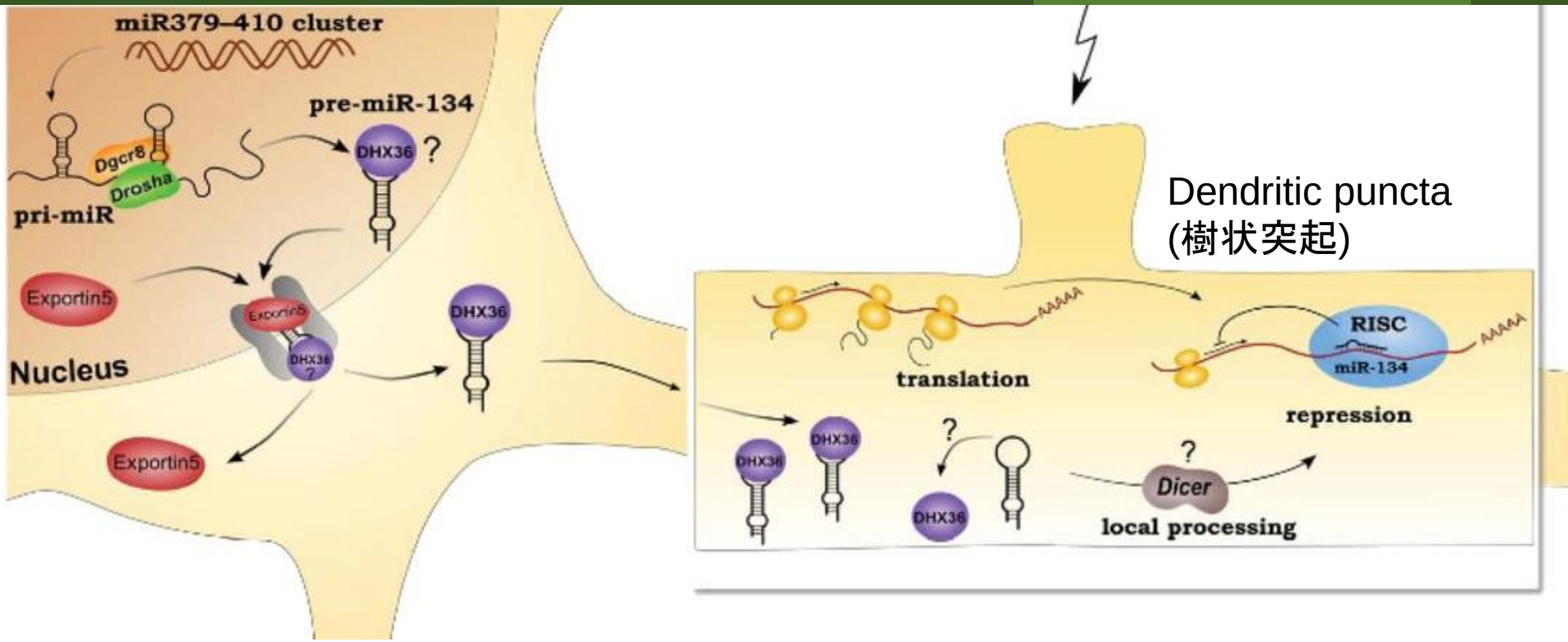


- ✓ Ago2 acts also as a translational upregulator dependent on the context of 3'UTR and bound cofactors.

Steiz, J. A., *et. al., Cell.* **2007**, *128*, 1105.

- ✓ This literature could explain in part the result that Ago2 knockdown abrogated DHX36 siRNA-mediated PITX1 upregulation.

DHX36 and miRNA machinery (2)



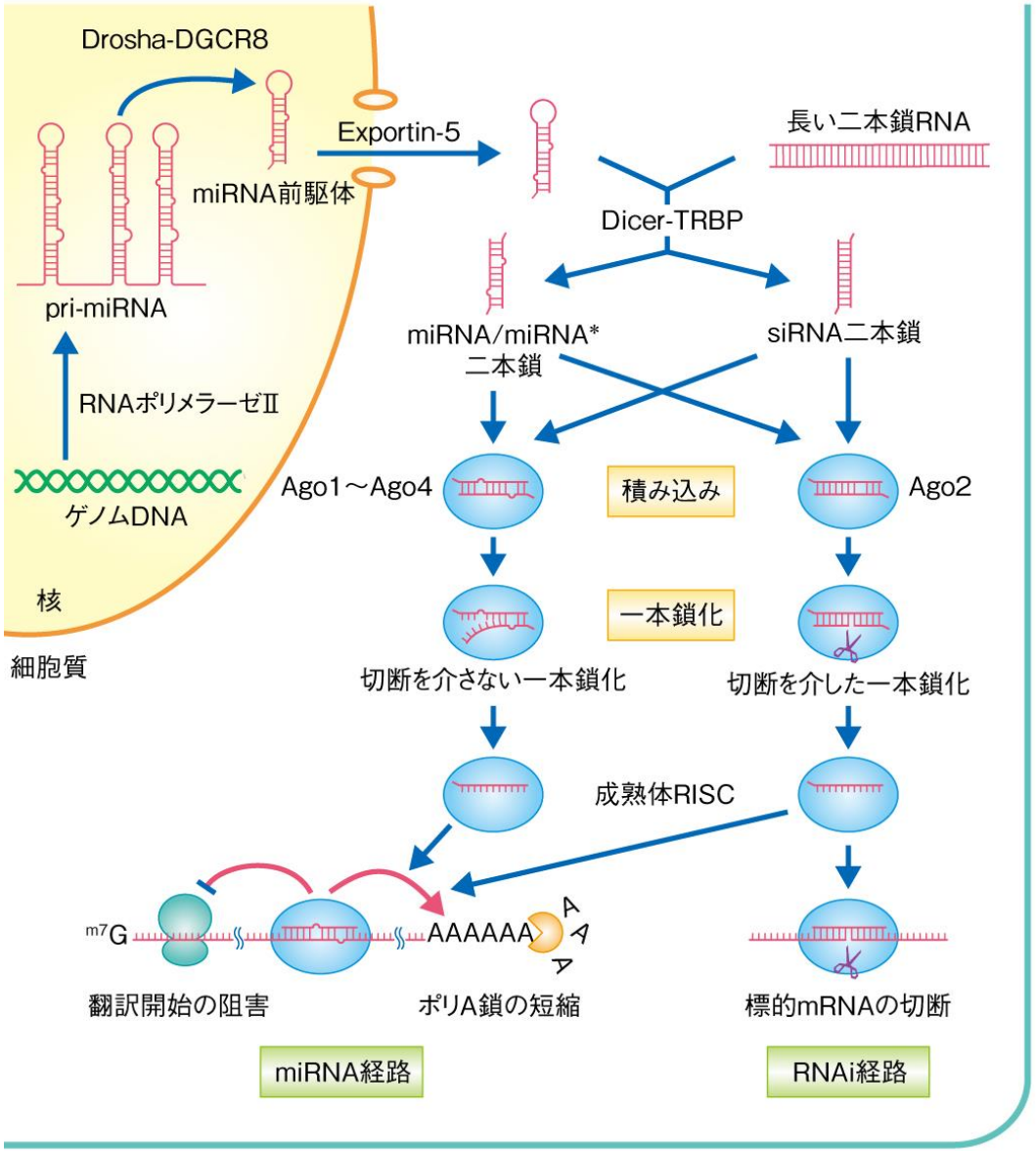
- ✓ DHX36 acts as a specific binding partner of the miR-134 microRNA in neuronal cells.
- ✓ It demonstrates a role in microRNA trafficking.

Schratt, G., *et. al., Genes Dev.* **2013**, *27*, 991.

<u>Hypothesis of miRNA-mediated translational regulation mechanism</u>

1. The interaction between DHX36 and PITX1 mRNA is mediate by a microRNA.
2. In certain context, DHX36 may be involved in the sub-cellular localization of PITX1 mRNA.

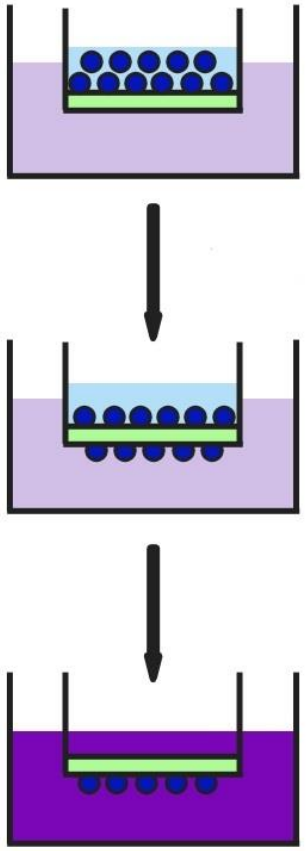
Regulation mechanism by miRNA



- ✓ Dicer… Rnase which produces RNA duplex (21-24 bp)
- ✓ Ago protein… cleave RNA duplex into single strand RNA
- ✓ Ago + ssRNA → RISC (RNA-induced silencing complex)

http://leading.lifesciencedb.jp/1-e001

Transwell migration chamber assay



細胞懸濁液を上側チャンバーに添加する。

インキュベーション

遊走細胞のみがポリカーボネート膜を通過し，底面に接着する。非遊走細胞は上側チャンバーに残る。

非遊走細胞を除去した後，遊走細胞を染色し定量する。

細胞

培地／化学誘引物質

血清フリー培地

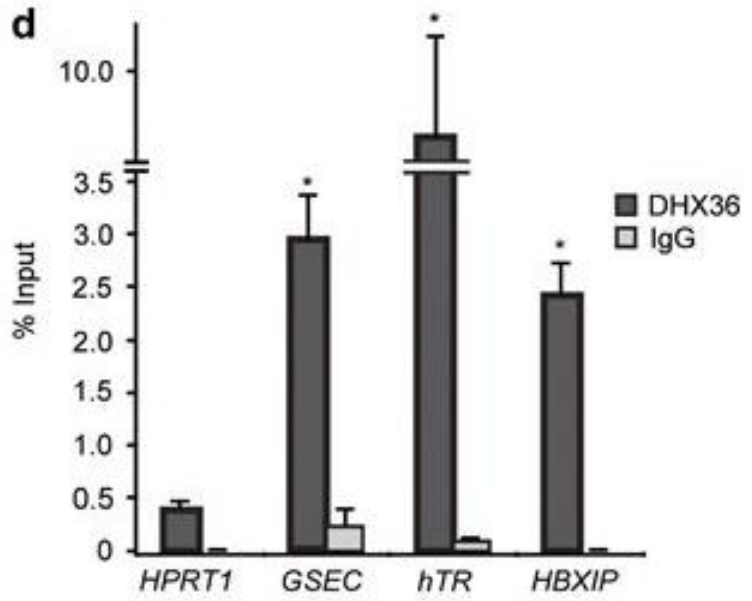
染色試薬

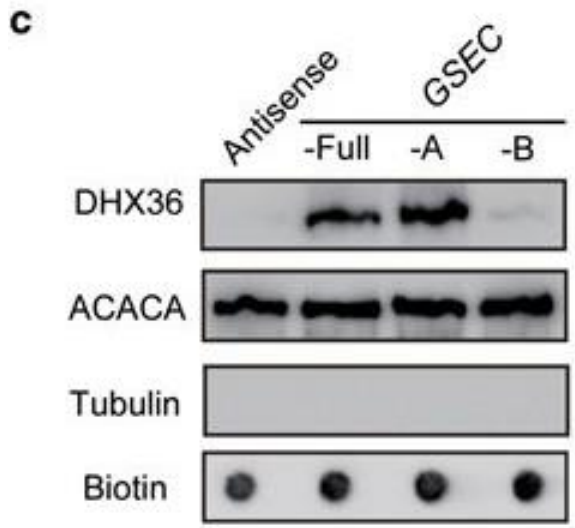
ポリカーボネート膜

https://www.funakoshi.co.jp/contents/64388

GSEC binding to DHX36 via rG4 (1)

RIP(RNA immunoprecipitation) & qRT-PCR

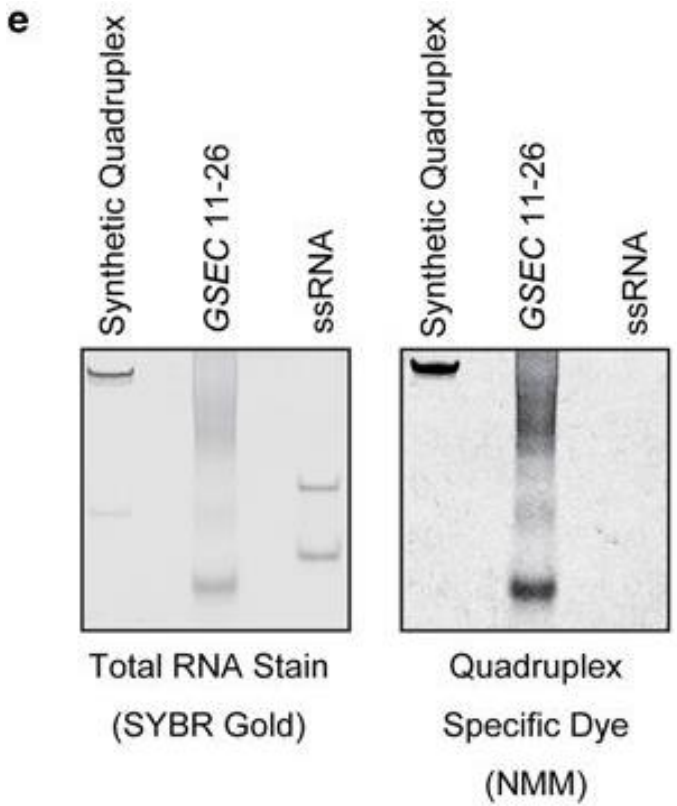


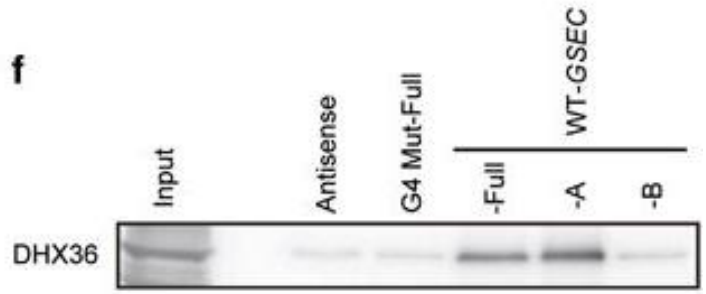


✓ *GSEC* binds to DHX36 even in living cells.

Akiyama, T., *et. al., Oncogene* **2017**, *36*, 1191.

GSEC binding to DHX36 via rG4 (2)





✓ *GSEC* directly interacts with DHX36 through a G-quadruplex structure.

Akiyama, T., *et. al., Oncogene* **2017**, *36*, 1191.