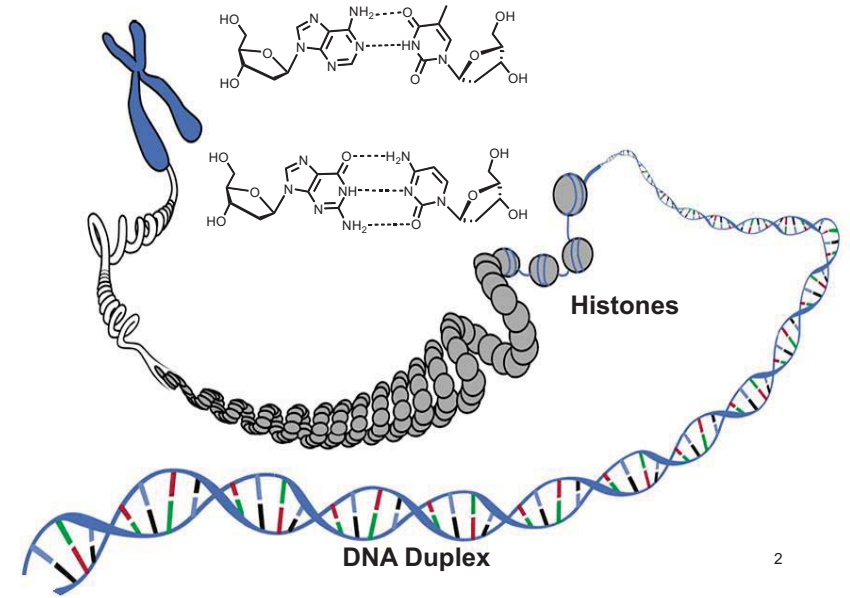
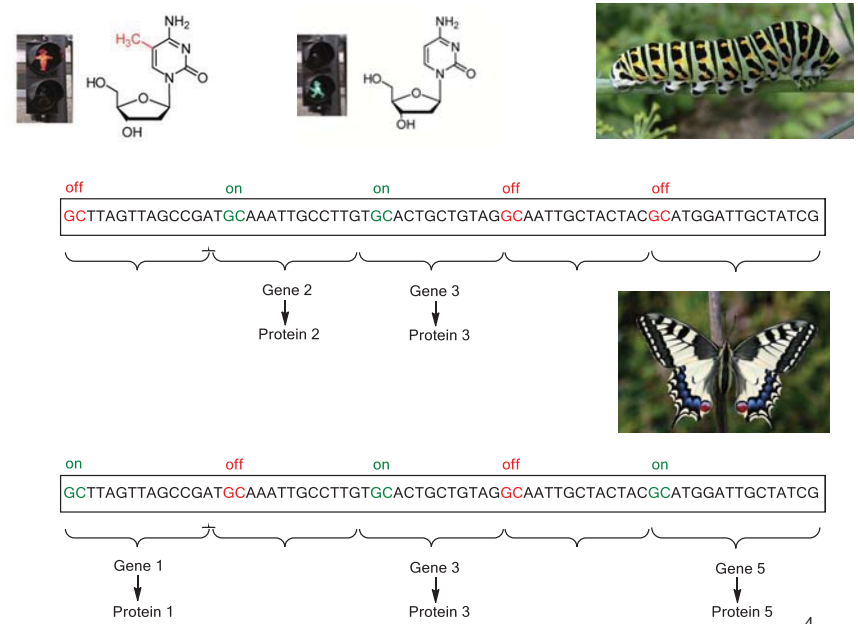
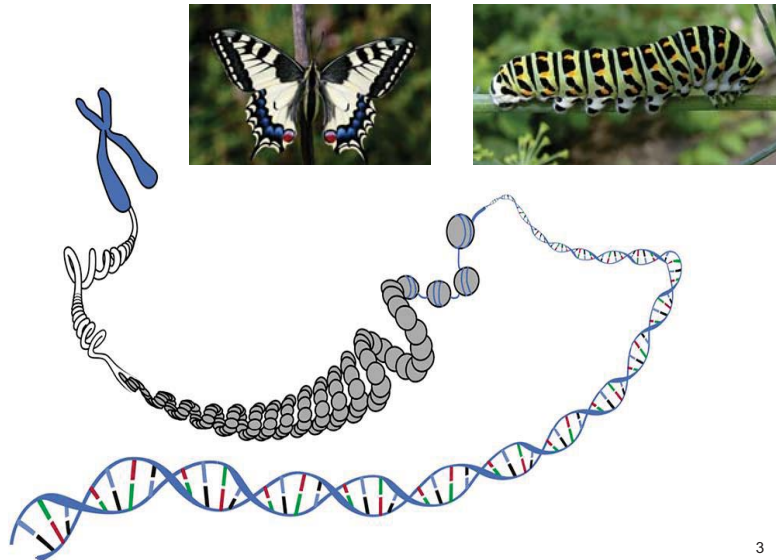


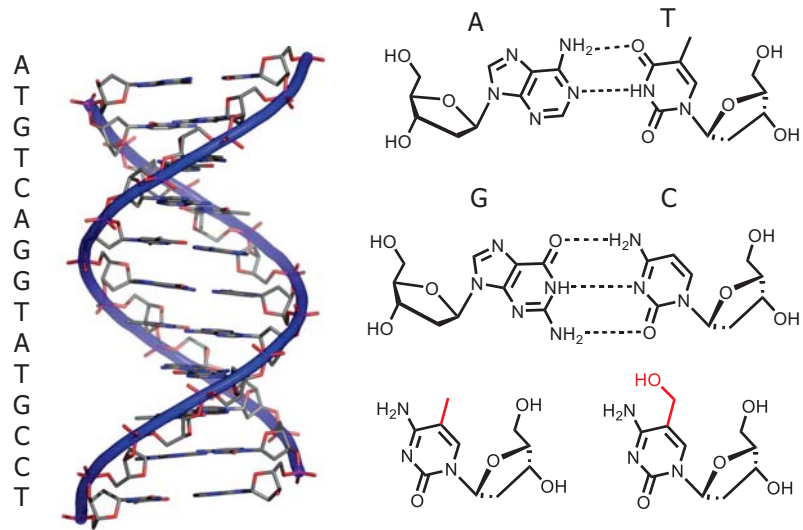
The complexity of DNA



Epigenetic control of transcription



The molecules of the genetic code



S. Kriaucionis, et al. *Science*, **2009**, 324, 929. M. Tahiliani et al. *Science*, **2009**, 324, 930

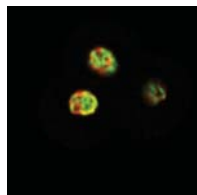
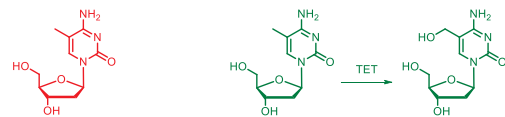
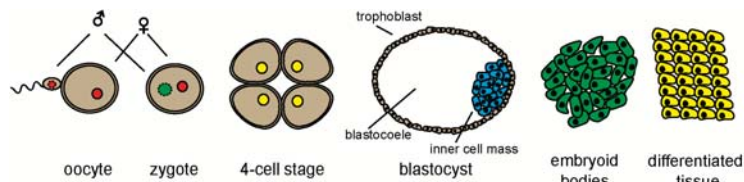
5

Reprogramming the epigenome



Exhibition
„Körperwelten“

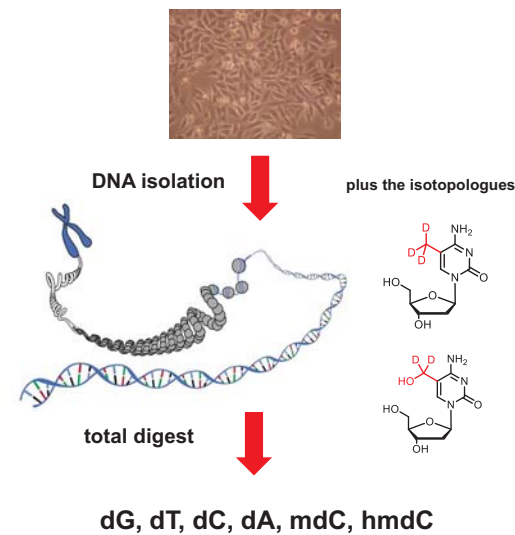
Chemistry in zygotes



Green: hmC Red: mC

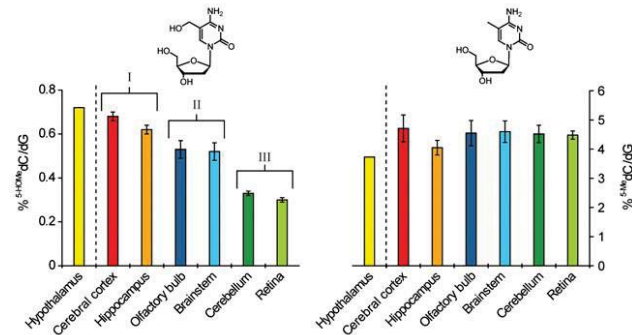
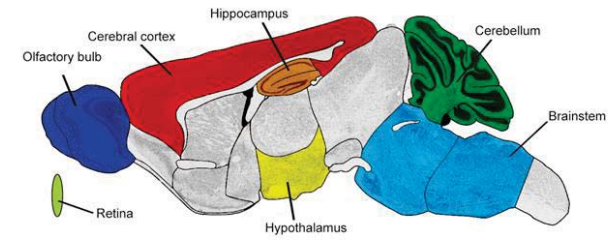
Images: *PNAS* **2011**, 3642 – 3647.

The isotope standardized LC-MS quantification method (*is*-LC-MS)



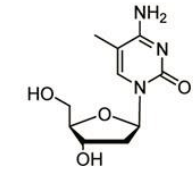
8

Quantification in brain tissues

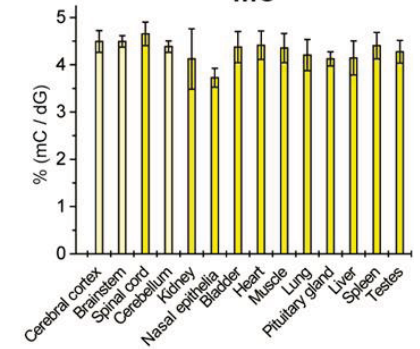


9
M. Münzel & T. C. ACIE 2010, 49, 5375.

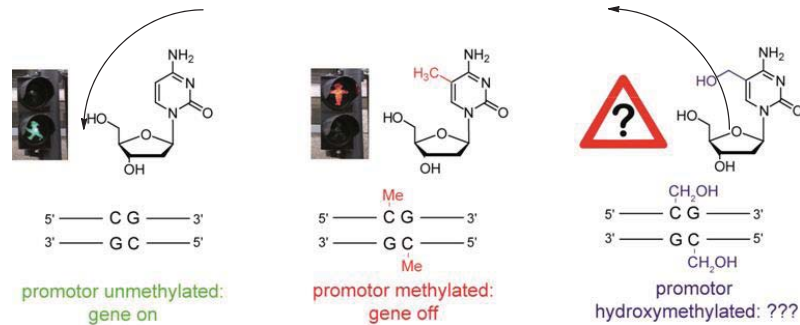
Distribution of mC and hmC in various tissues



mC

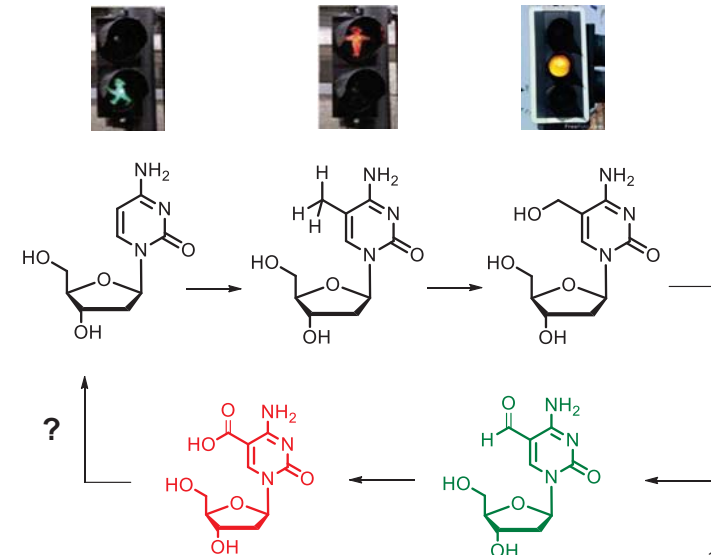


10
D. Globisch & T.C. PLoS One, 2010, 5, e15367



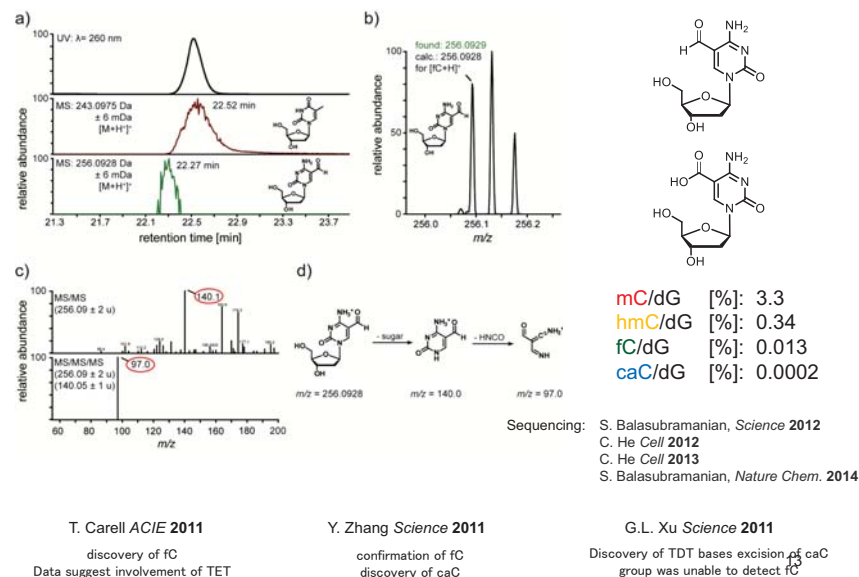
11

The function of hmC, fC and caC

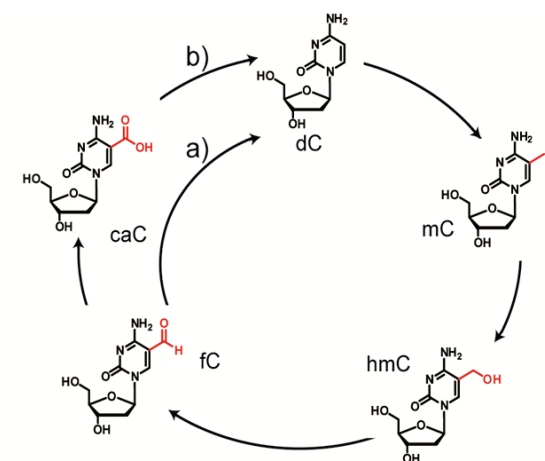


12

Discovery of fC and caC in ESC



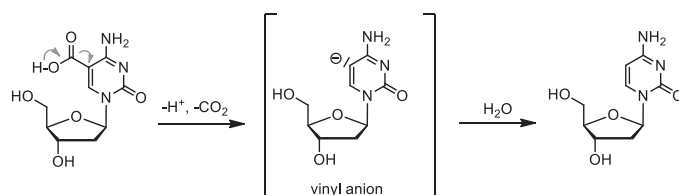
Potential chemistry of active demethylation



T. Carell *ACIE* 2011, Y. Zhang *Science* 2011 and G.L. Xu *Science* 2011

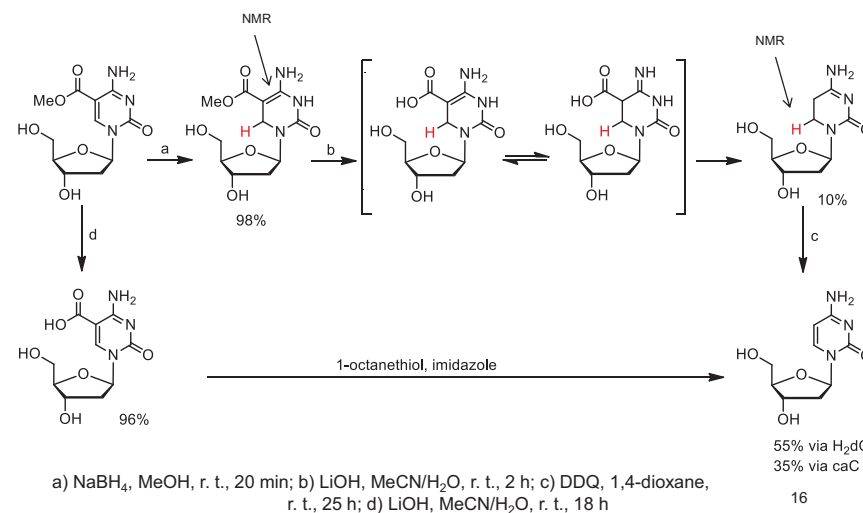
14

Chemical mechanism of caC decarboxylation



15
S. Schiesser & T.C. *JACS* 2013, 135, 6516-6520

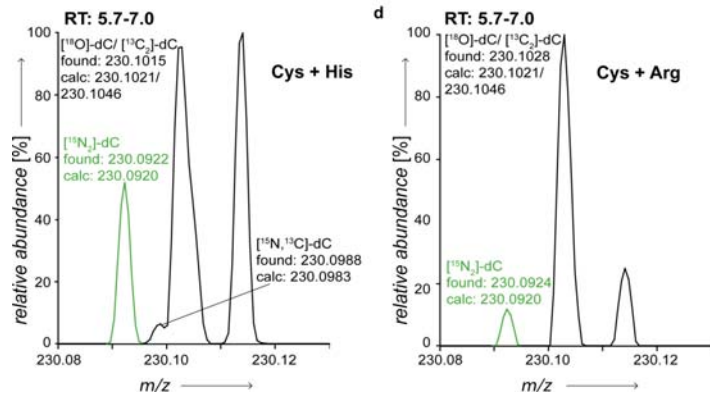
Chemical mechanism of caC decarboxylation



5'-GAGCATCCGGTCACCGTCCCTTcaCGGAAAGG-3' ODN1

addition of amino acids

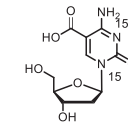
5'-GAGCATCCGGTCACCGTCCCTTGGAAAGG-3' digest
 $[^{15}\text{N}_2]\text{-dC}$
 $[^{15}\text{N}_2]\text{-caC}$



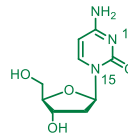
DNA plus amino acids (half saturated solution), water, 50°C, 12 h

17
 S.Schiesser & T.C.ACIE 2012, 51, 6516-6520

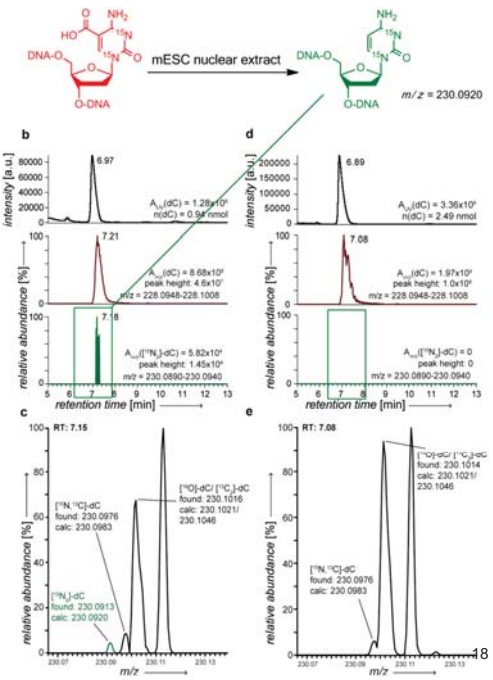
Experiments with stem cell extracts



?



S.Schiesser & T.C.ACIE 2012, 51, 6516-6520



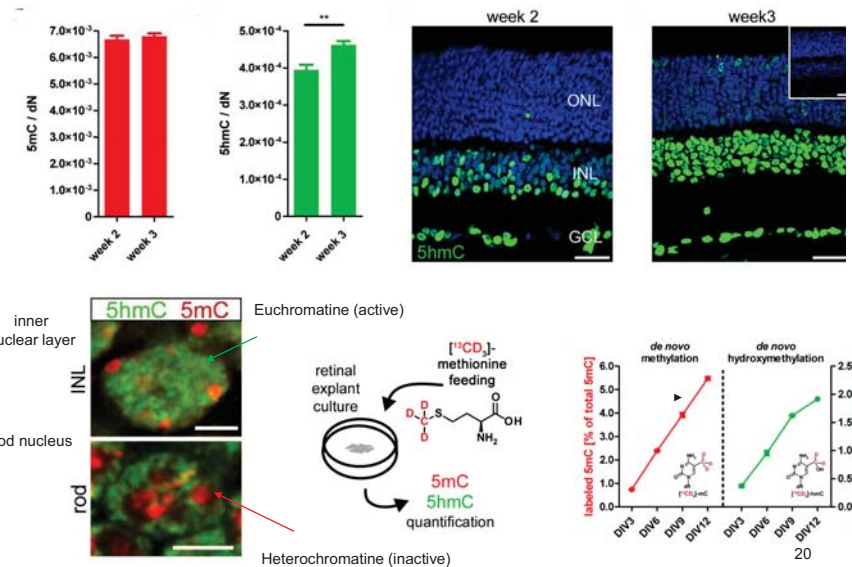
Analysis of the function of hmC

eye opening in mice occurs between week 2 and 3
 and goes in hand with large genomic changes in the
 neuroreceptor cells of the retina



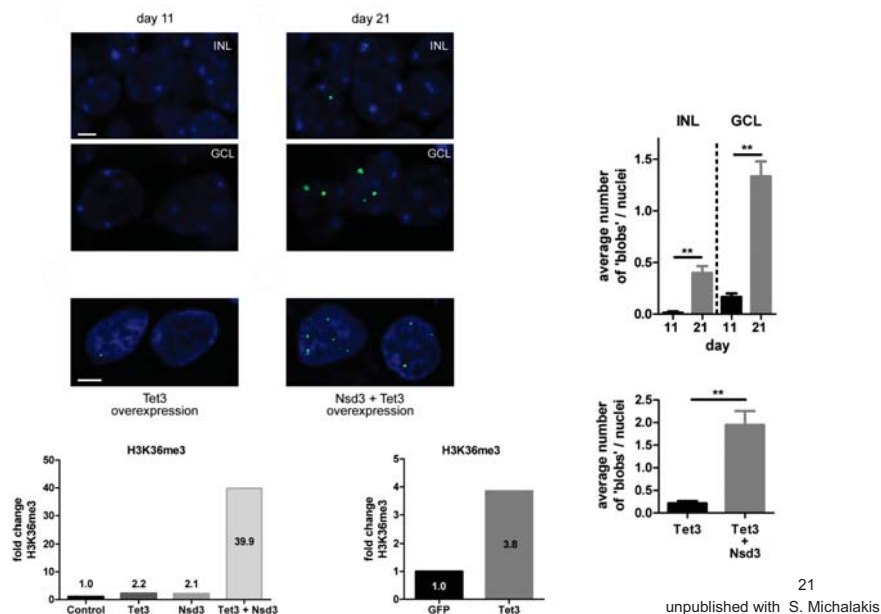
19

Studies of brain development shows that hmC is associated with active transcription

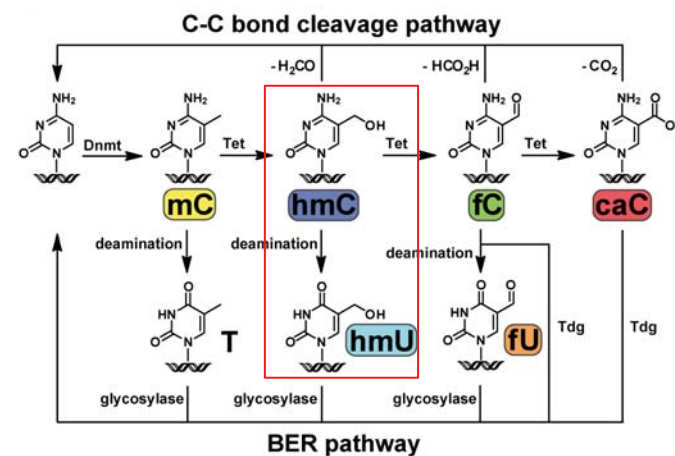


unpublished with S. Michalakakis

Upregulation of expression leads to increased H3K36me³



Discussed mechanisms of active demethylation



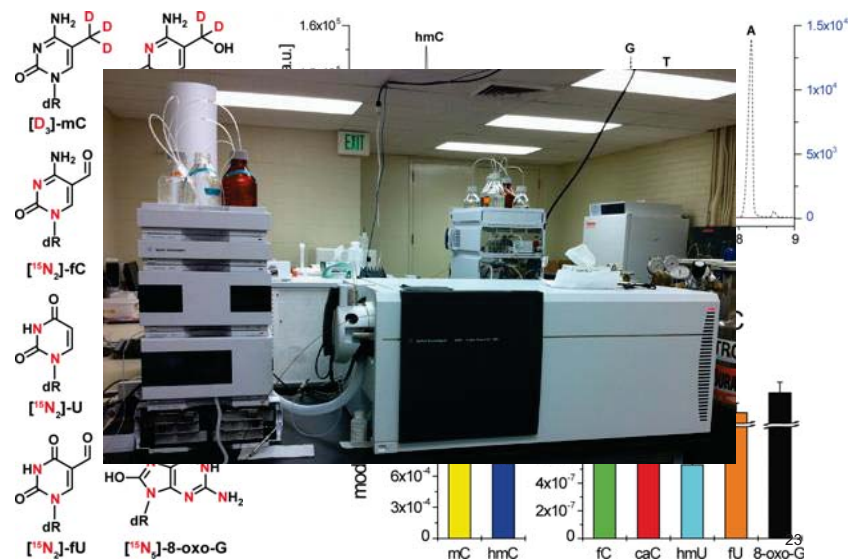
D. Globisch & T. C. *PLoS One*, 2010, 5, e15367

H. Song, *Cell*, 2011, 145(3), 423-434.

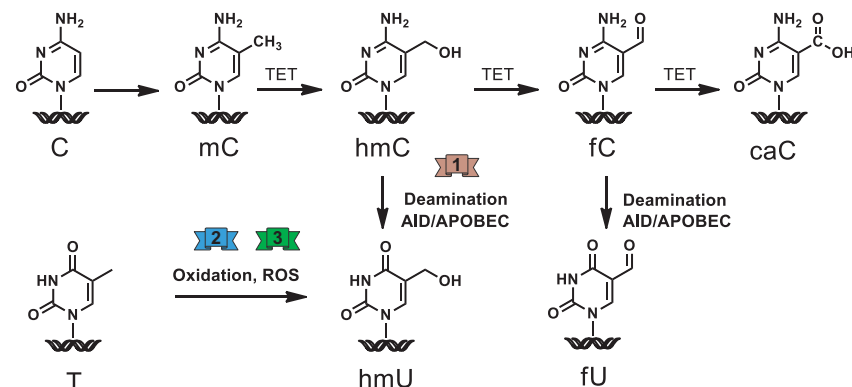
R. M. Kohli, *Nature Chem. Biol.* 2012, DOI: 10.1038/NCHEMBIO.1042

22

Parallel quantification shows presence of hmU, fU and 8-oxo-G



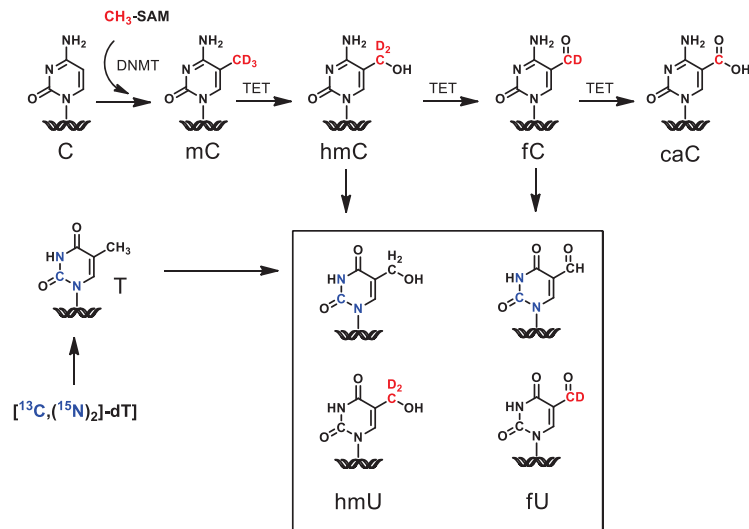
What is the origin of hmU?



T. Pfaffeneder, T. C. *Nature Chem. Biol.* 2014

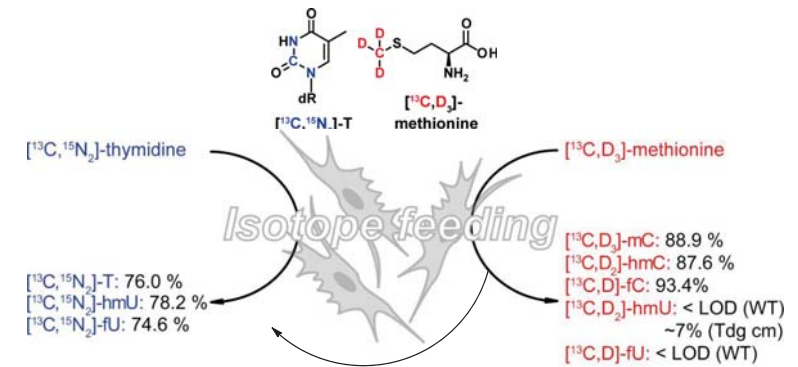
24

Labelling schemes



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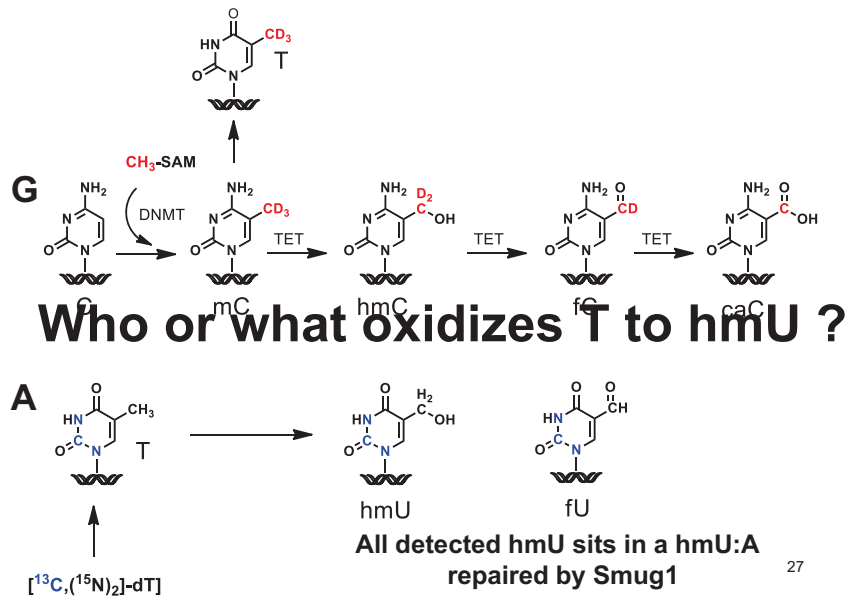
Isotope tracing shows hmU is a dT derivative



All hmU that is detected in wt mESC is generated from T and is situated in an hmU:A base pair

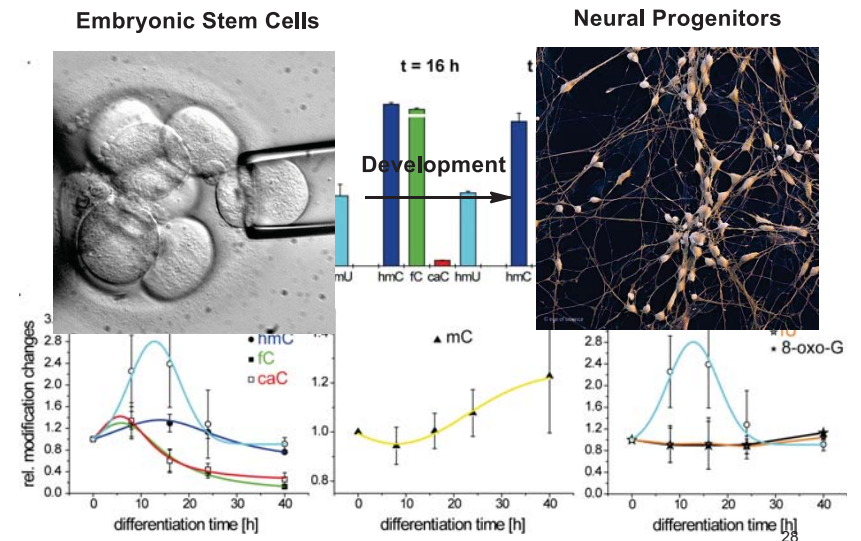
26

Isotope tracing reveals the origin of the new bases

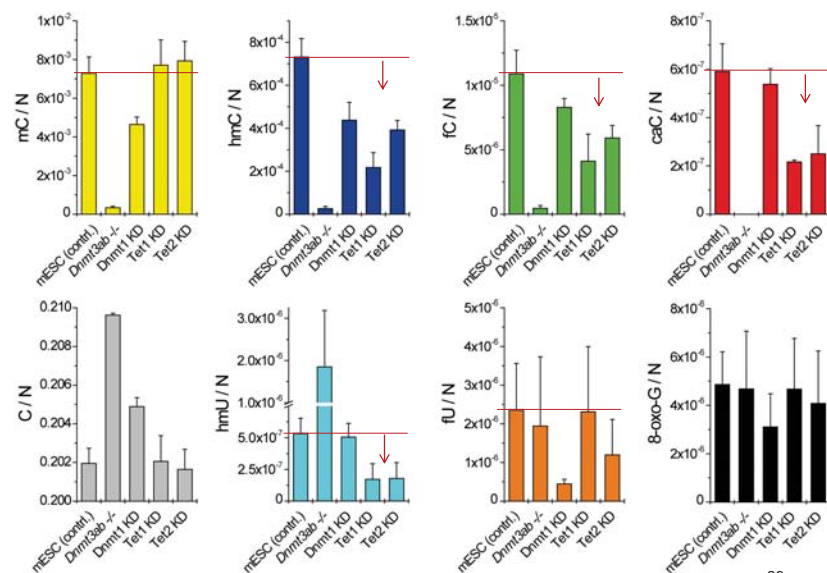


27

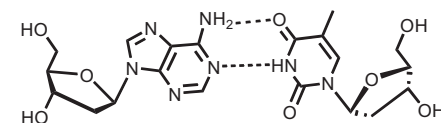
Kinetic analysis shows that hmU is a new base



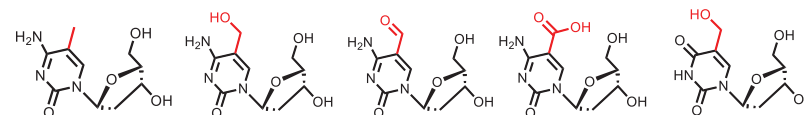
Tet enzymes oxidize T to hmU



29



What are the functions of hmC, fC, caC and hmU?



Sequencing:

S. Balasubramanian, *Science* **2012**

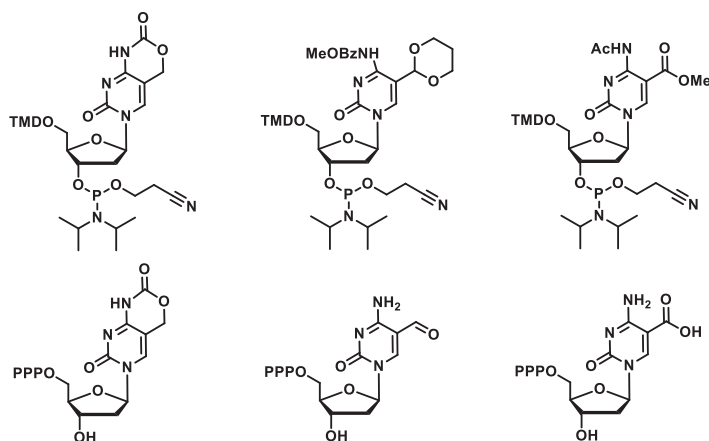
C. He *Cell* **2012**

C. He *Cell* **2013**

S. Balasubramanian, *Nature Chem.* **2014**

30

Building blocks for the synthesis of hmC, fC and caC containing oligonucleotides and PCR products



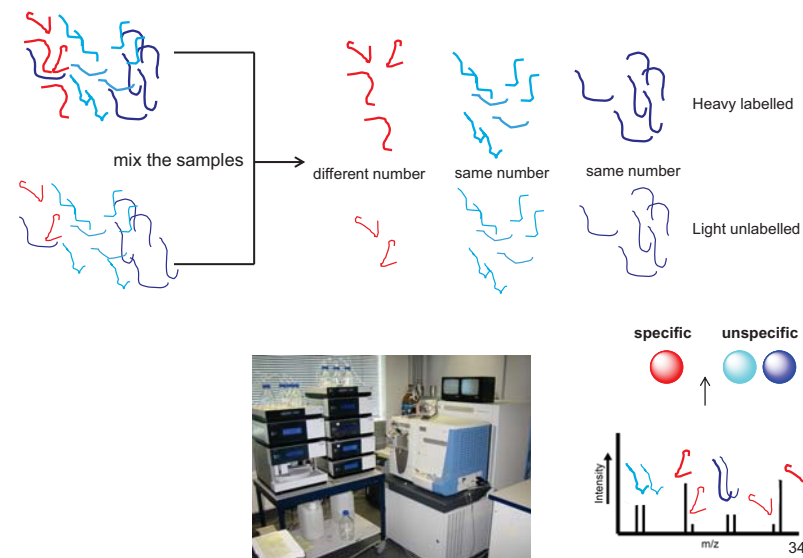
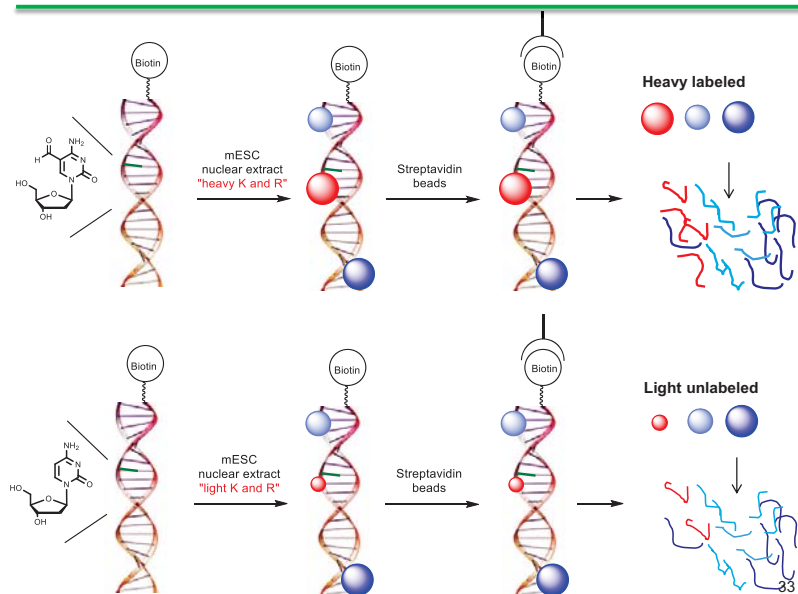
M. Münzel, A. Schröder & T. C.: *Org. Lett* **2010, 2013**, *ACIE* **2014**. Available from Glen Res.

31

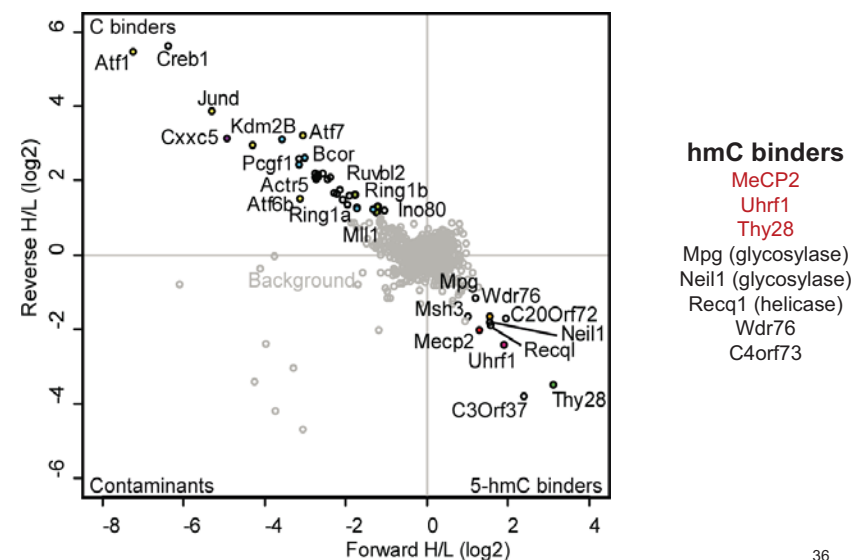
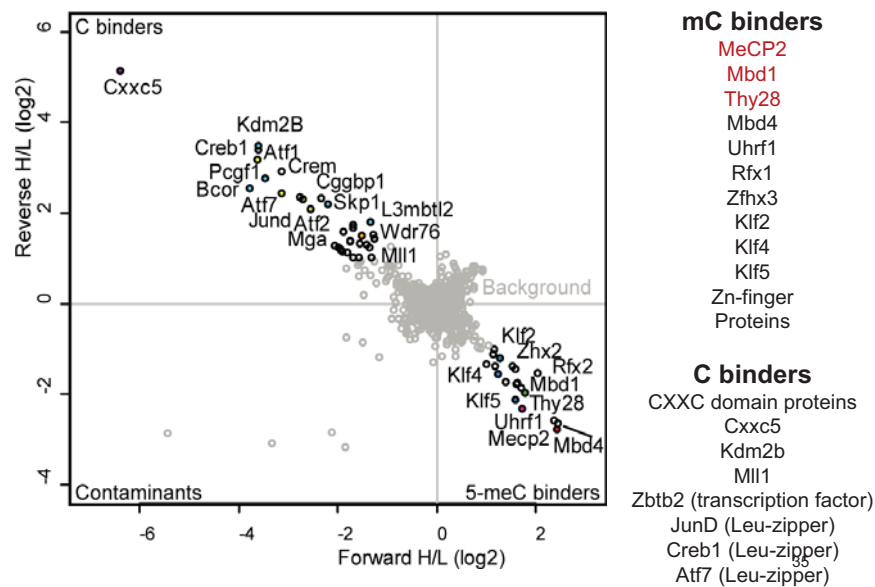


32

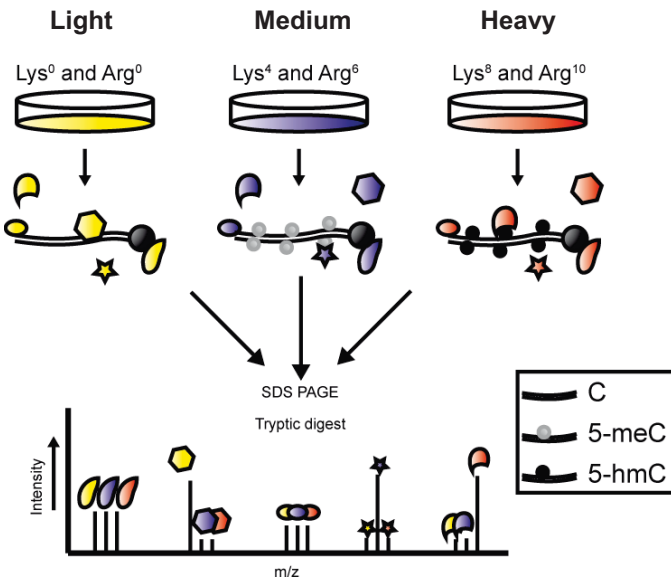
Which proteins bind to hmC, fC and caC *in vivo* ?



SILAC derived 5-hmC binders

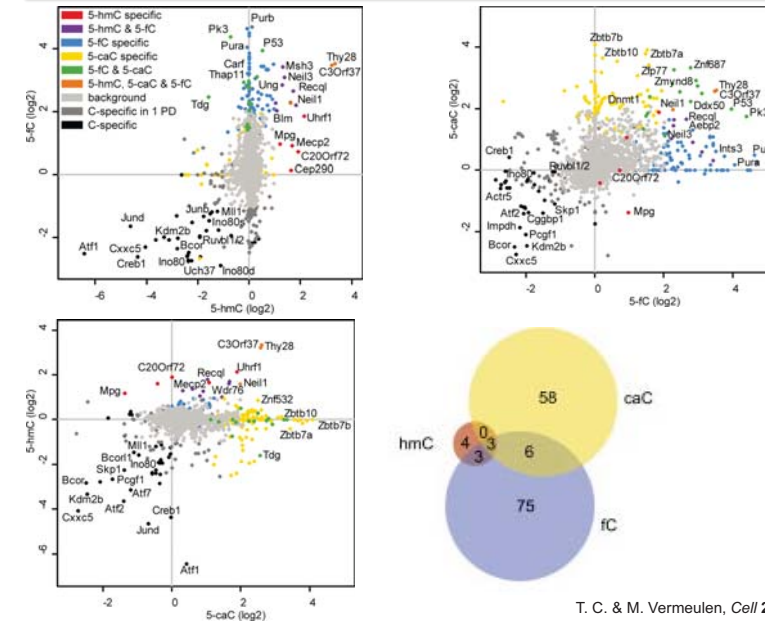


Triple-SILAC (M. Mann, M. Vermeulen)



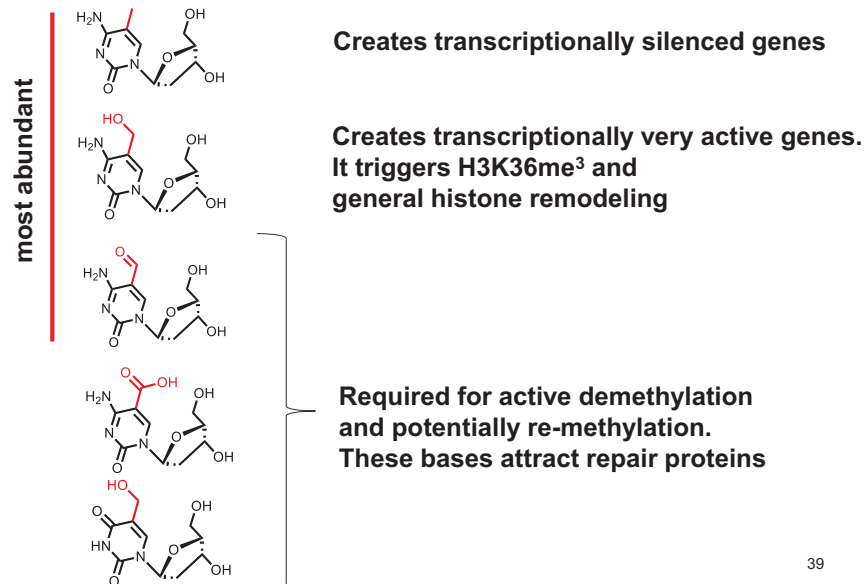
37
T. C. & M. Vermeulen, *Cell* **2013**, 152, 1-14.

Triple SILAC data

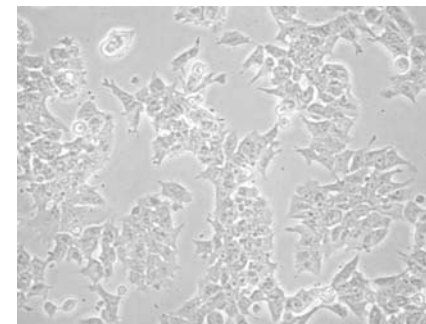


38
T. C. & M. Vermeulen, *Cell* **2013**, 152, 1-14.

Proposed functions of the new bases



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Axolotl

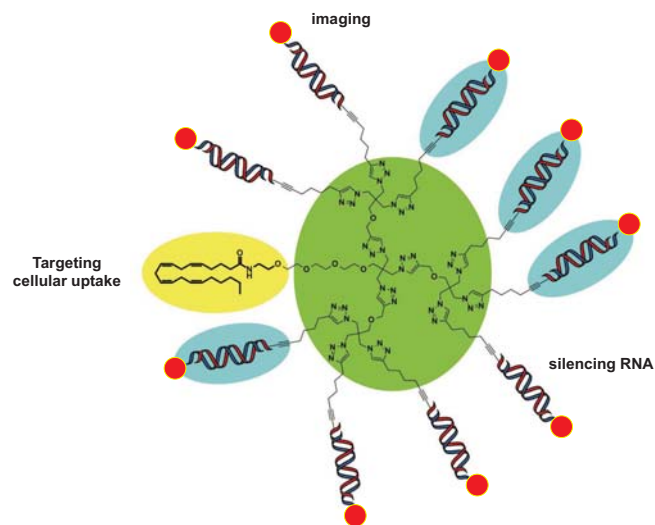


Nanog, Oct4, Sox2, Ssea1 (cMyc, KLF4)

mmu-miRNA-302a,b,c,d
mmu-mirRNA-367

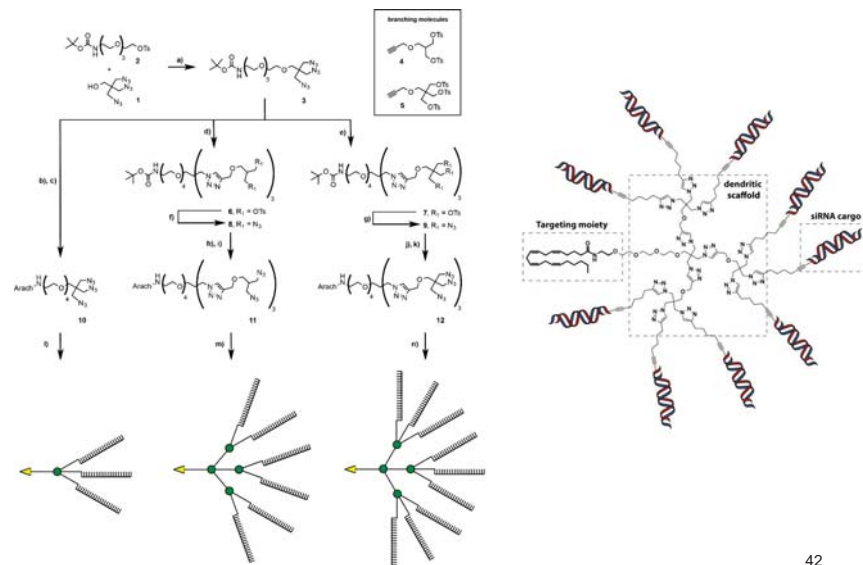
40
E. E. Morrissey *Cell Stem Cell*, **2011**, 8, 376.

siRNA dendrimers to control stem cell faith assembled by „Click-Chemistry“



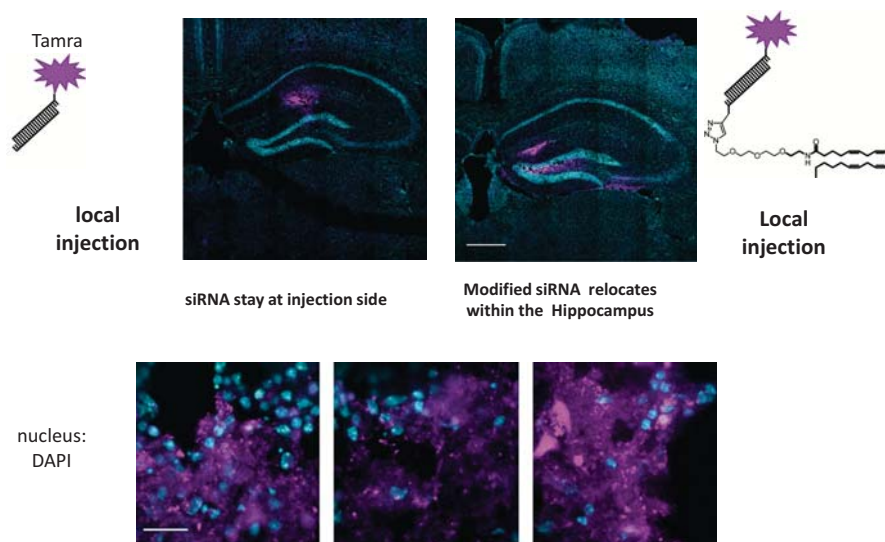
41

siRNA dendrimers created by „Click Chemistry“



42

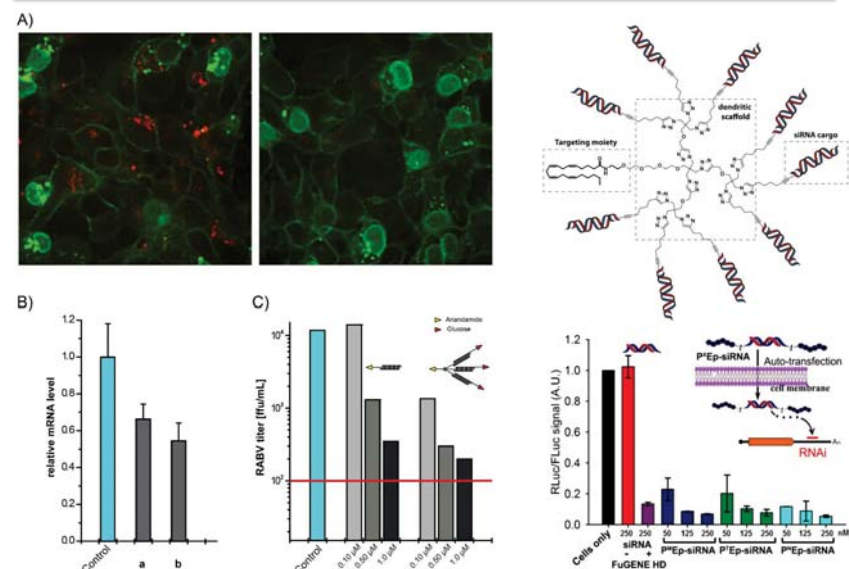
Arachidonic acid modified siRNA



60-time enlargement: Z-scan demonstrates cellular uptake of (AEA)-siRNA into brain cells

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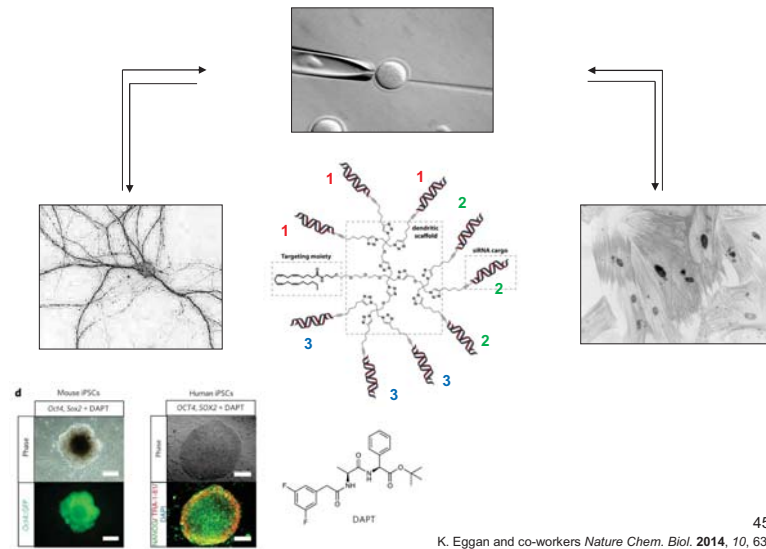
The molecules of the genetic code



J. Willibald, T. C. JACS 2012, 134, 12330-3.

K. Matyjaszewski & S. Das, JACS 2014, 135, 12508
K. Matyjaszewski & S. Das, ACIE 2014, in press

The molecules of the genetic code



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