SICOM & AOCO 2024
SOMS International Conference on Obesity & Metabolism
in conjunction with Asia-Oceania Conference on Obesity

TOHOKU UNIVERSITY

MYPT1-PP1β phosphatase negatively regulates both chromatin landscape and co-activator recruitment for beige adipogenesis

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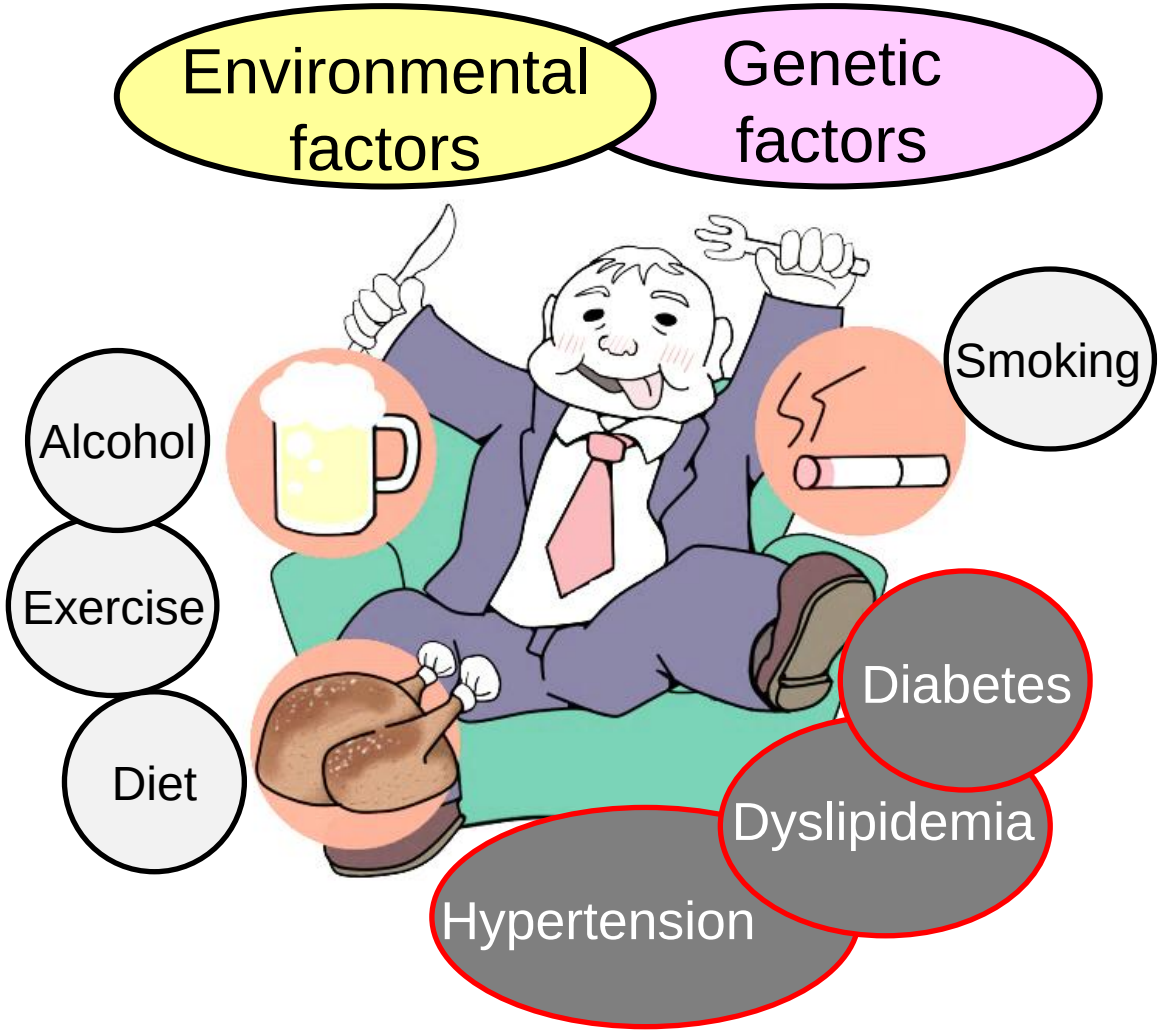
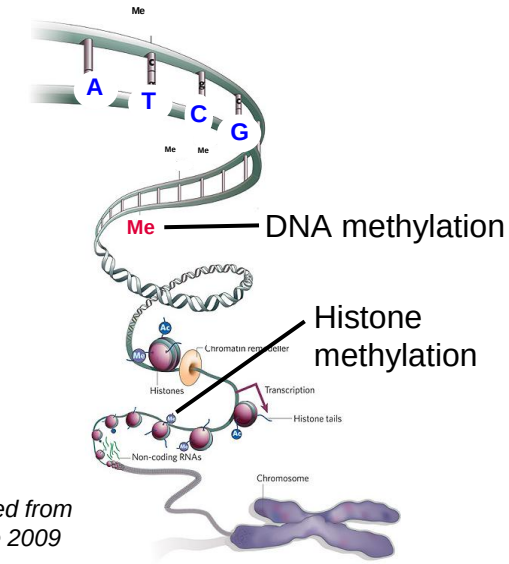
Hosted by SOMS Society for Korean Obesity and Metabolism Studies
Co-Hosted by aoaso Asia Oceania Association for the Study of Obesity

Lifestyle diseases involve both genetic factors and environmental factors



Environmental stimuli

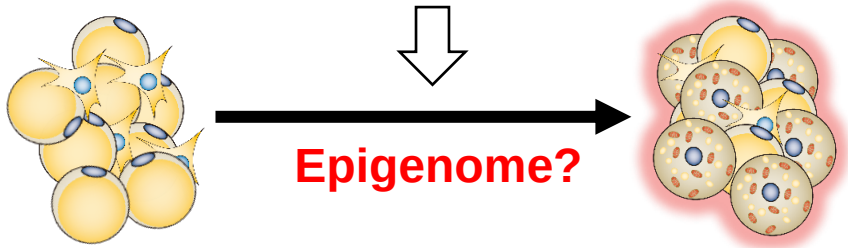


Adapted from Nature 2009

Epigenomic changes

Changes in cellular functions

Chronic cold stress



Epigenome?

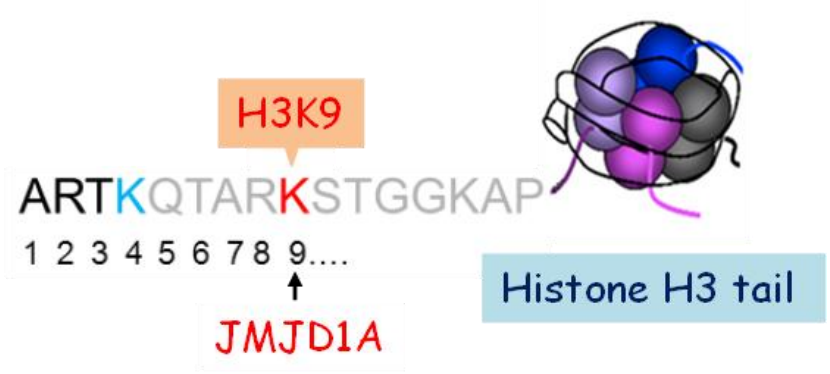
White fat

Beige fat

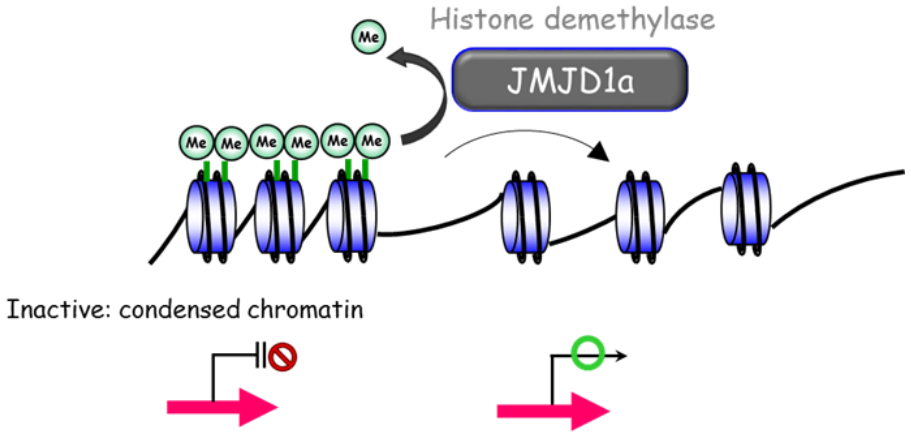
Changes in cellular function

JMJD1A (Jumonji domain-containing 1a)

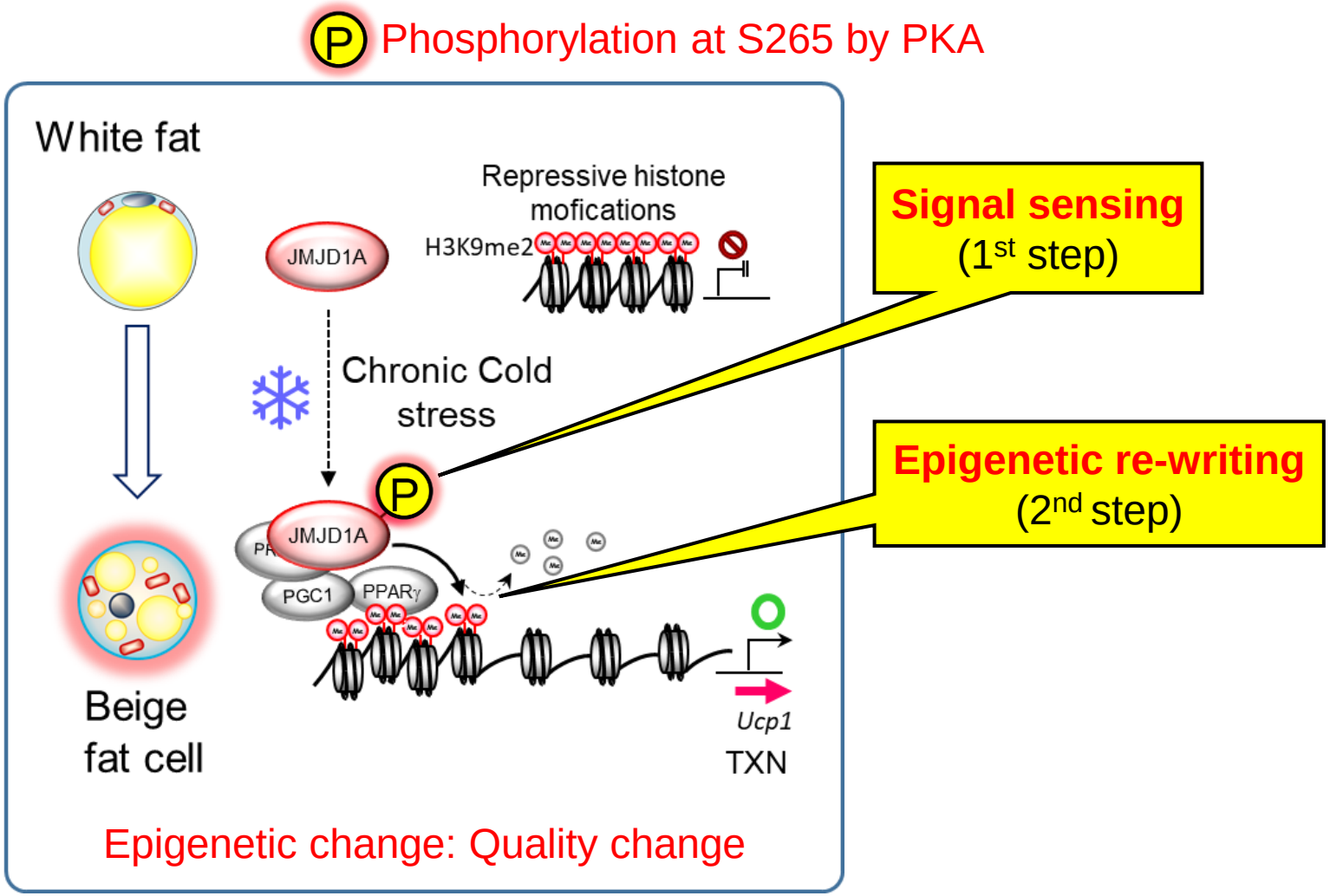
- Histone H3 lysine 9 di-methylation (H3K9me2, repressive histone mark) specific demethylase



- Turn ON gene transcription by removing repressive H3K9me2 mark



JMJD1A promotes beiging through signal sensing (1st step) and epigenetic re-writing (2nd step)

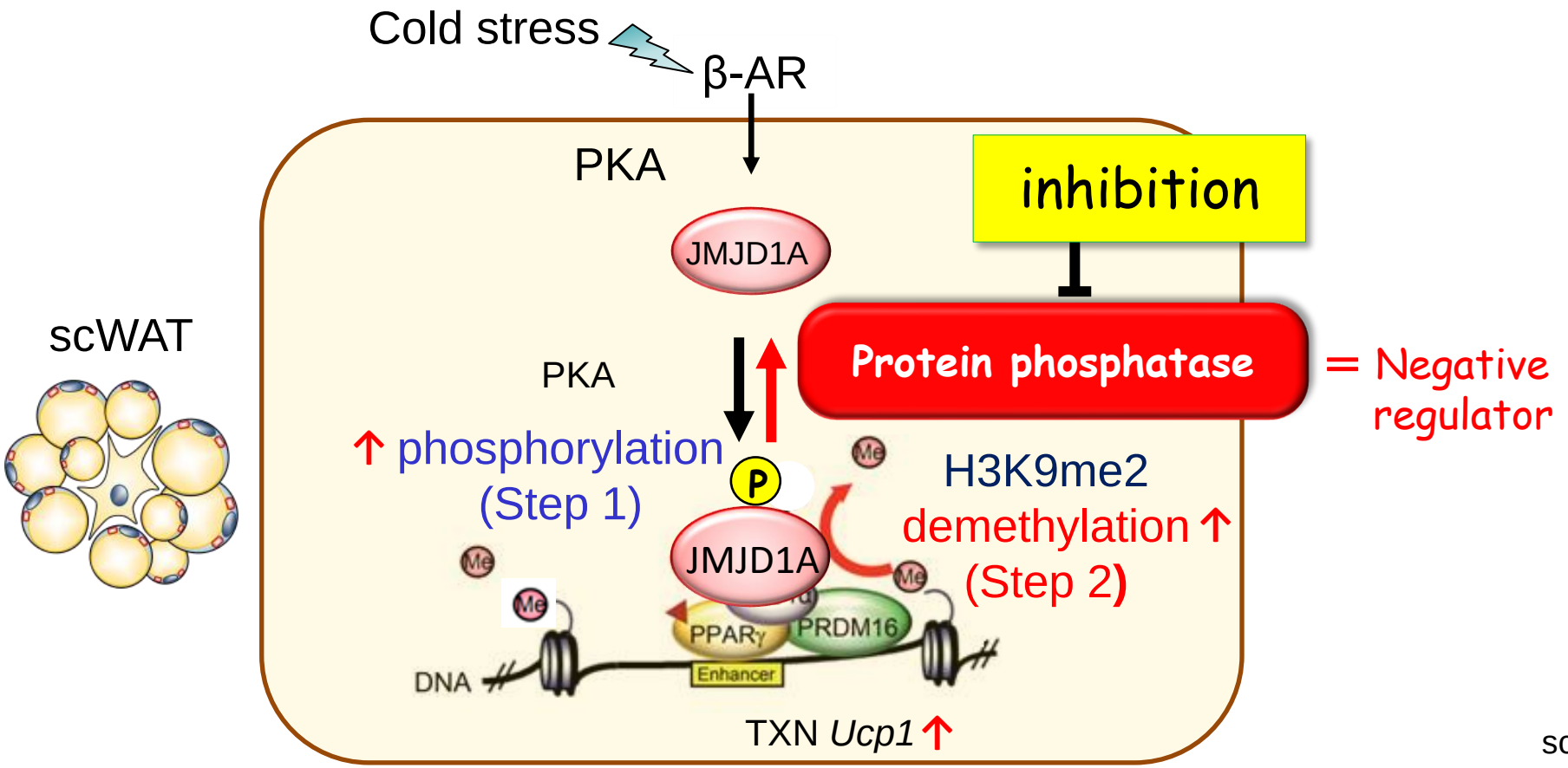


Abe Y, *et al*,. *Nature Commun* (2018)

It has been challenging to artificially activate Step 1 JMJD1A phosphorylation and induce Step 2 epigenomic reprogramming to promote beiging

Hypothesis:

Is it possible to enhance demethylation efficiency (2nd step)
by inhibiting the phosphatase activity toward phospho-JMJD1A (1st step)?

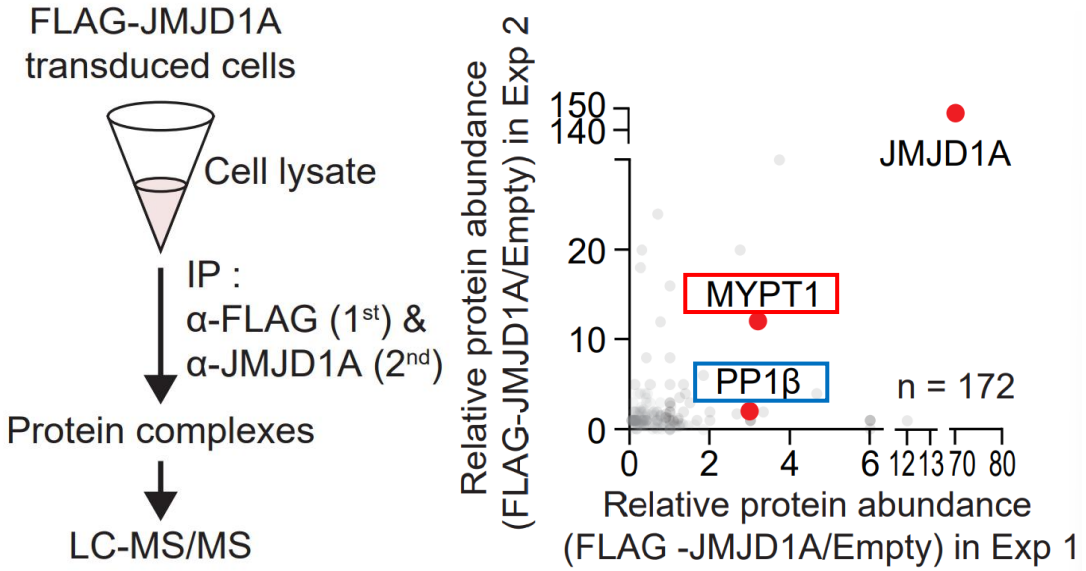


scWAT: subcutaneous white adipose tissue
TXN: transcription
PKA protein kinase A

MYPT1-PP1β : JMJD1A phosphatase

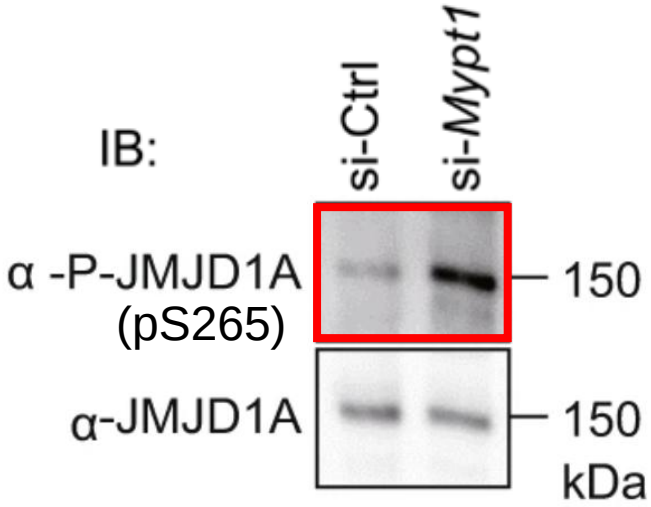
LC-MS/MS

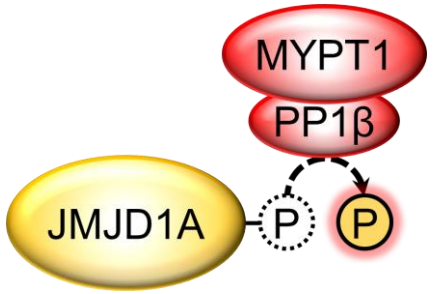
MYPT1-PP1β is identified as an interacting protein of JMJD1A



Phospho-immunoblot

JMJD1A phosphorylation level is increased

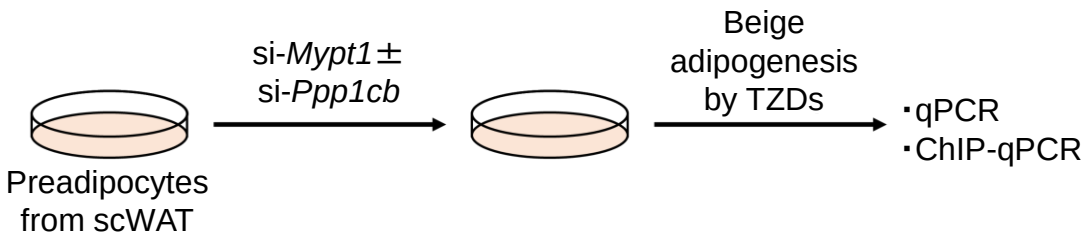




MYPT1: Regulatory subunit

PP1β: Catalytic subunit

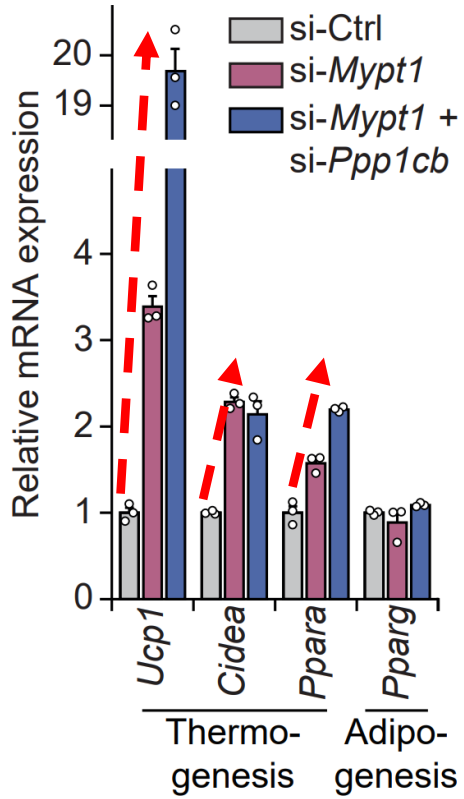
Inhibition of MYPT1-PP1β promotes epigenome rewriting and induces beige adipogenesis



scWAT : subcutaneous white adipose tissue
TZDs : thiazolidinediones
H3K9me2 : repressive histone mark

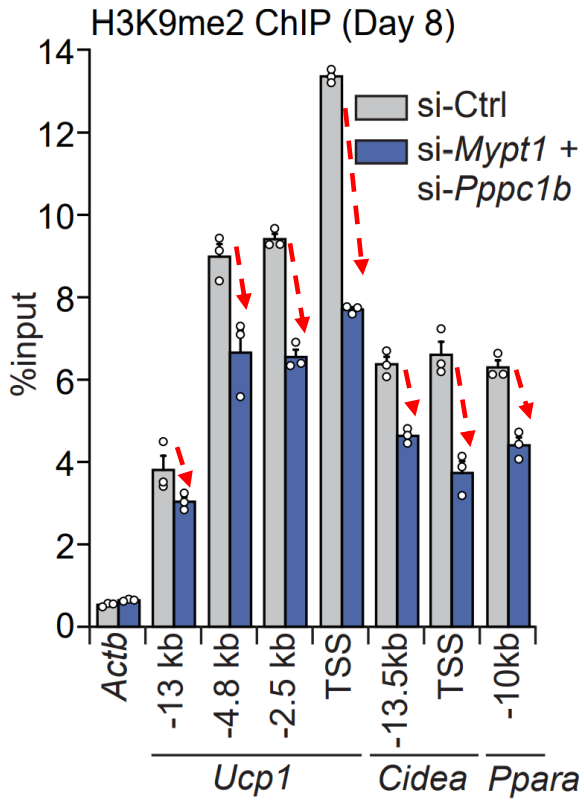
qPCR

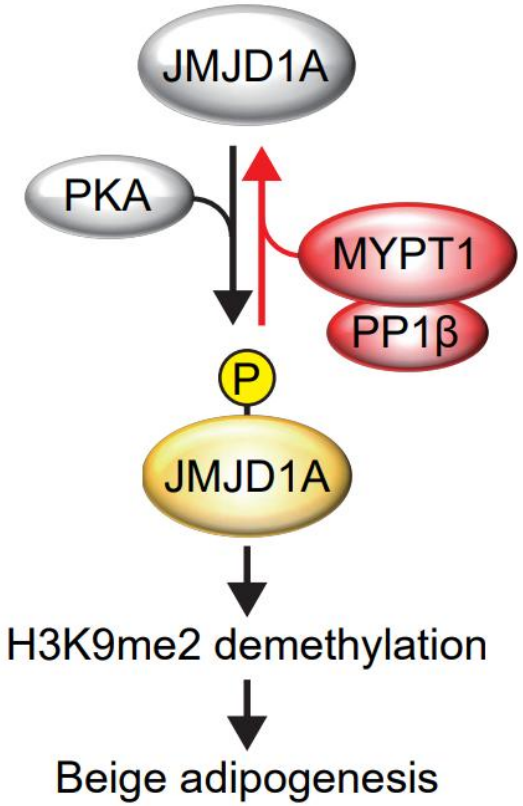
Enhanced thermogenic gene expressions



ChIP-qPCR

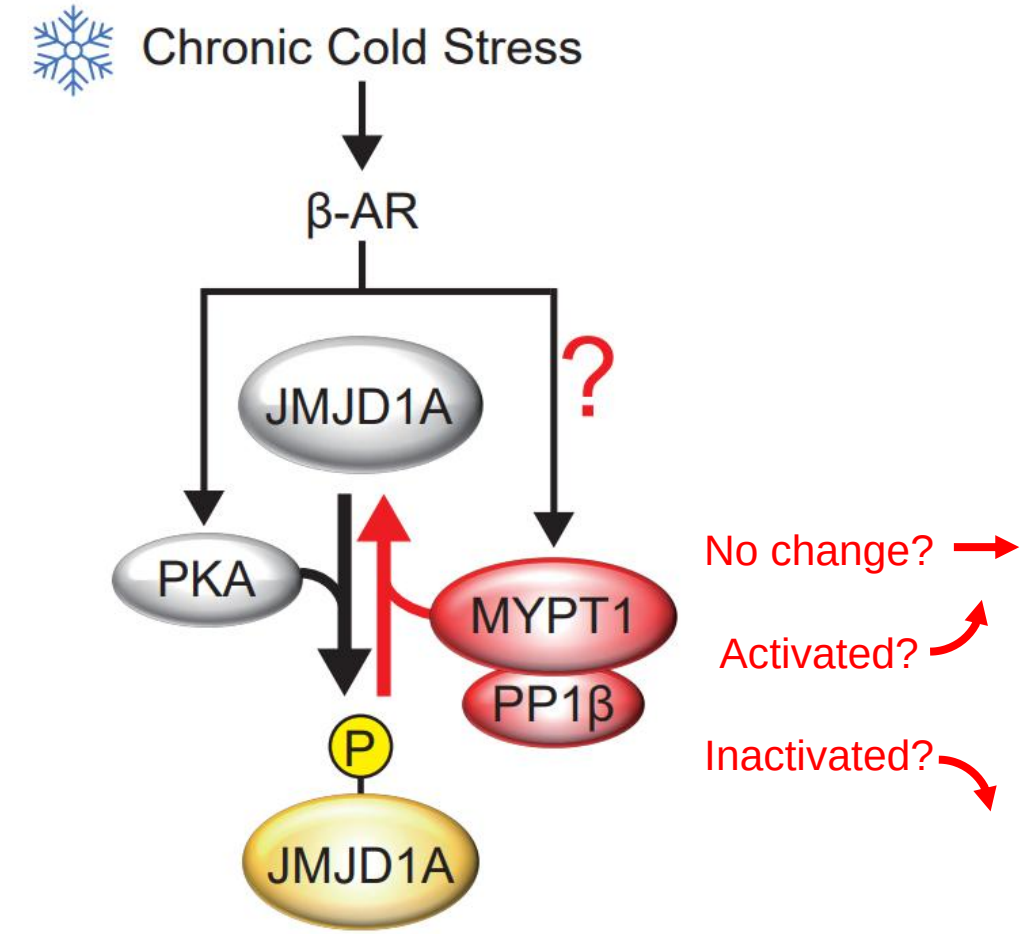
Decreased H3K9me2 levels



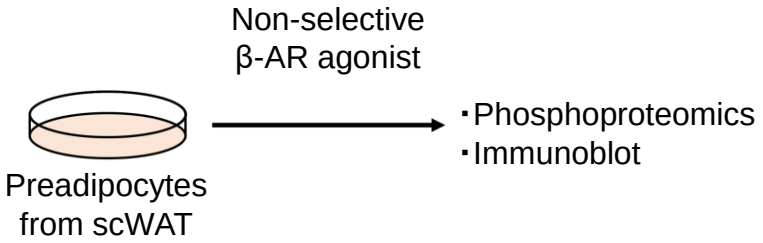


Question:

Is MYPT1-PP1β activity inhibited or upregulated under cold stress?

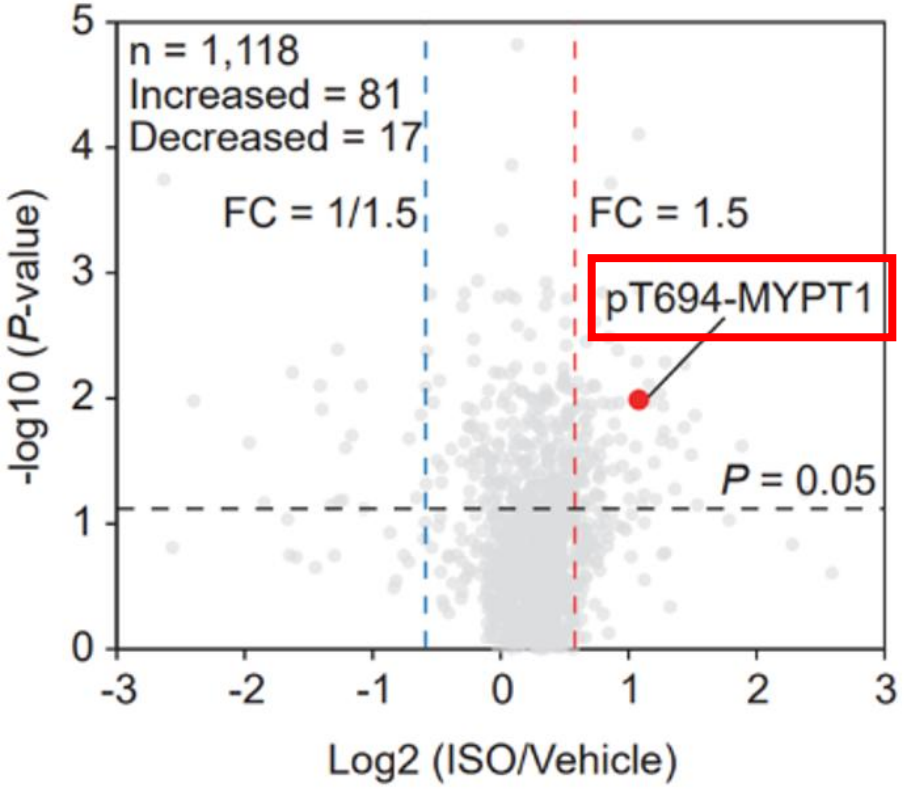


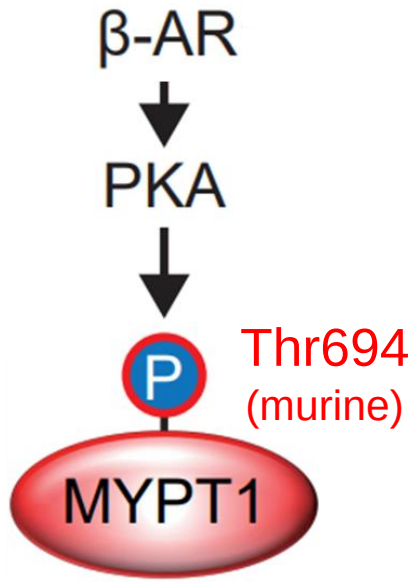
MYPT1 is phosphorylated at Thr694 in response to β-AR activation



Phospho-proteomics

MYPT1 is phosphorylated by β-AR stimulation





ISO : a non-selective β-AR agonist
β-AR: β-adrenergic receptor

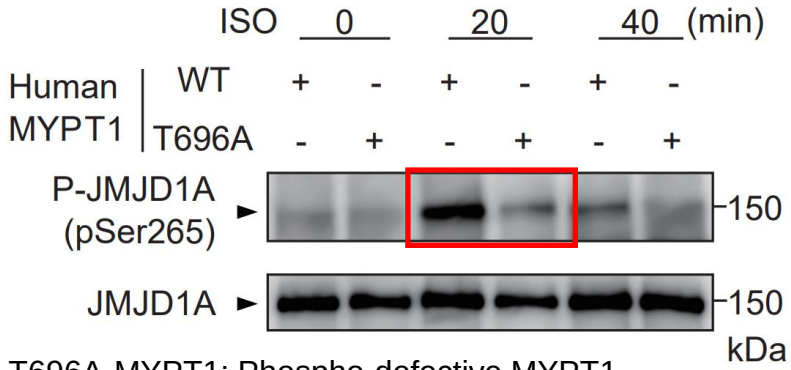
Upon β-AR activation, MYPT1-PP1β is inactivated, leading to the induction of beiging

β-AR → PKA → P → MYPT1

Inactive?

Phospho-immublot

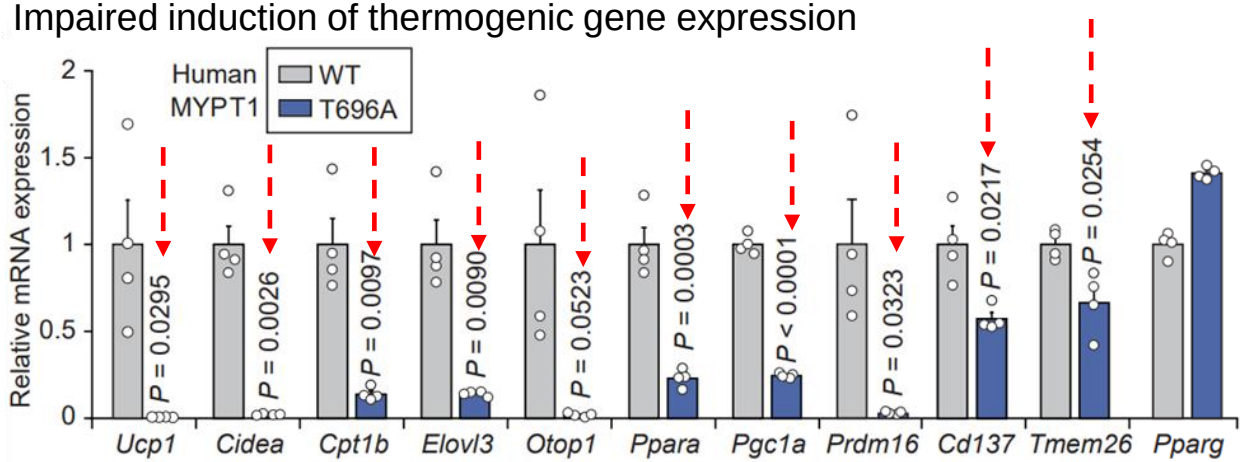
Impaired induction of JMJD1A phosphorylation by β-AR stimulation

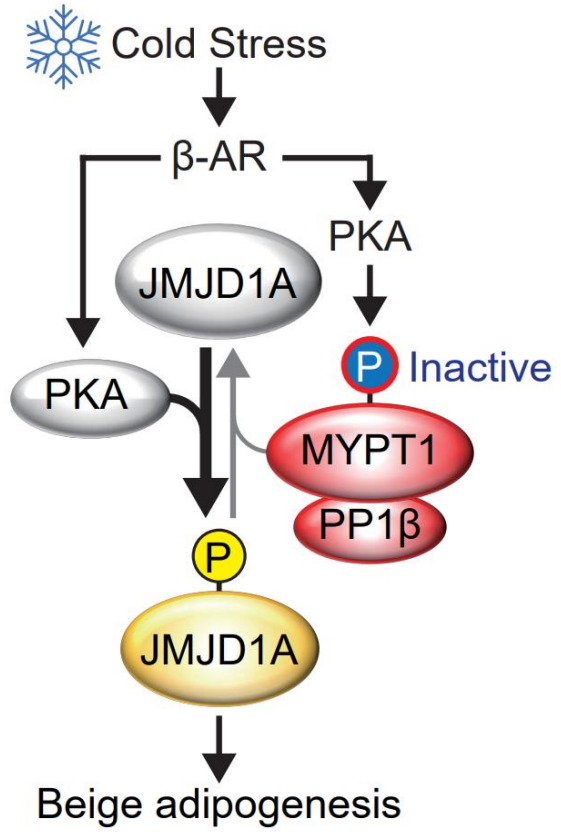


T696A-MYPT1: Phospho-defective MYPT1

qPCR

Impaired induction of thermogenic gene expression



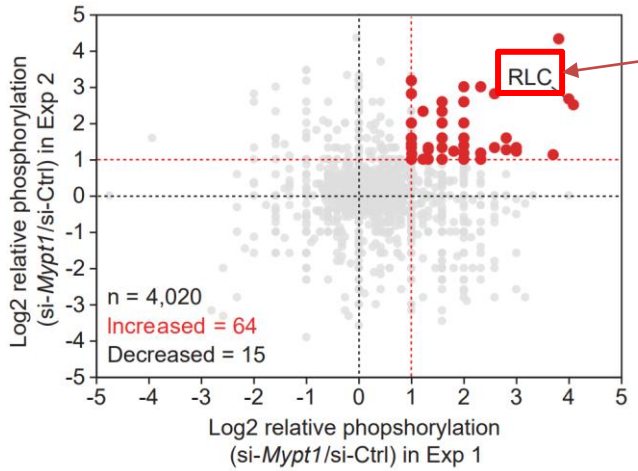


The phospho-defective MYPT1 (T696A) is active

→dephosphorylates JMJD1A, resulting in impaired beige adipogenesis

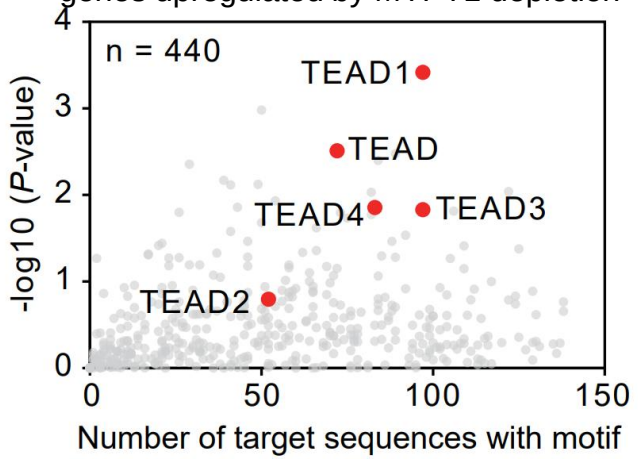
Exploration of transcriptional pathways regulated by MYPT1

Phospho-proteomic anlysis



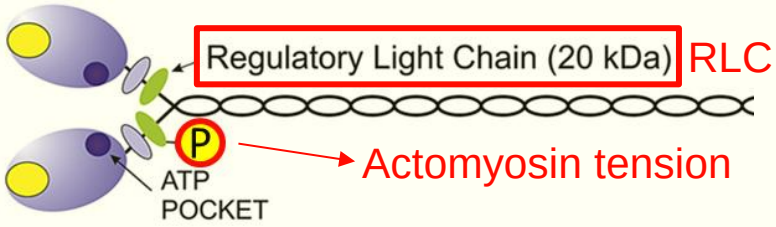
RNA-seq

TEAD motifs are enriched in the upstream of genes upregulated by MYPT1 depletion

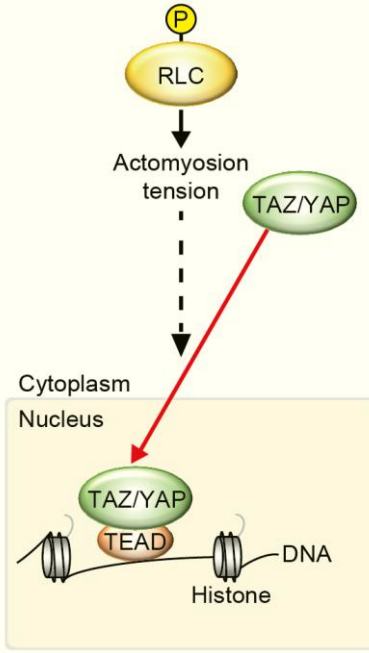


Phospho-RLC is a target of MYPT1

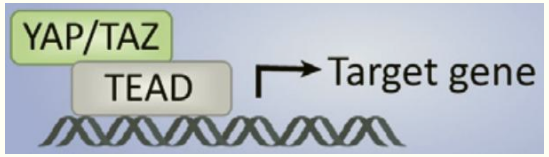
MYOSIN



Core WC, et al,. *Arch Biochem Biophys*. 2011



Lin KC,*et al*,. *Trends Biochem Sci*, 2017

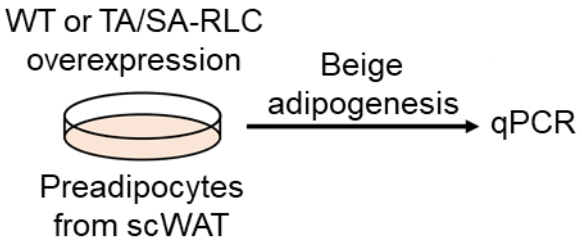


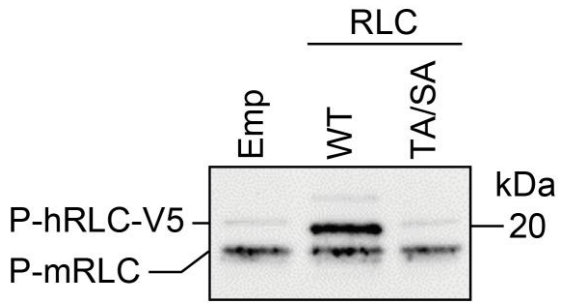
YAP/TAZ:

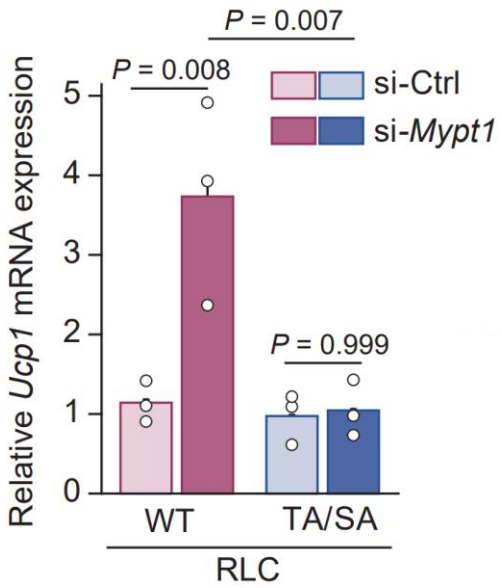
- Mechanosensitive transcriptional co-activator
- Regulating actomyosin tension-mediated gene transcription

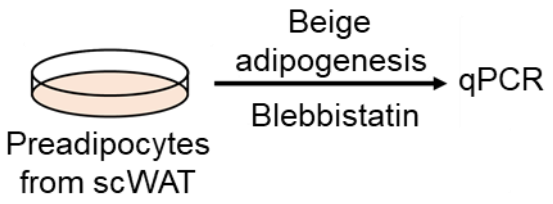
Question:
Is the pRLC-actomyosin-YAP/TAZ transcriptional pathway involved in the regulation of thermogenic gene expression by MYPT1?

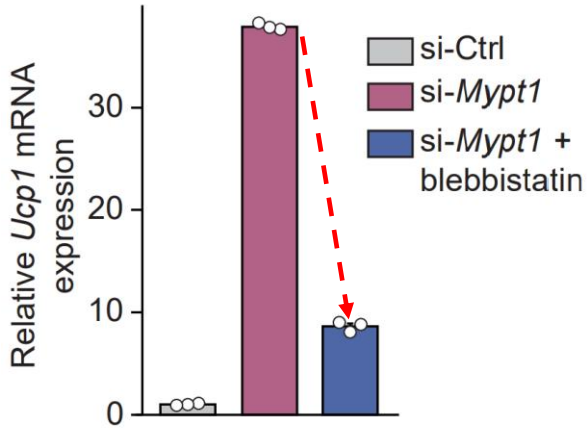
pRLC-actomyosin-YAP/TAZ transcriptional pathway is crucial for the thermogenic gene regulation by MYPT1

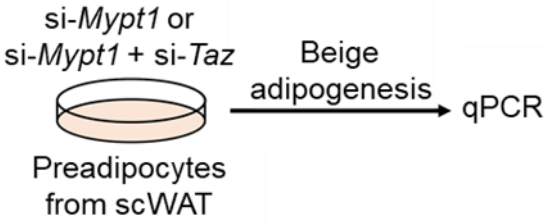


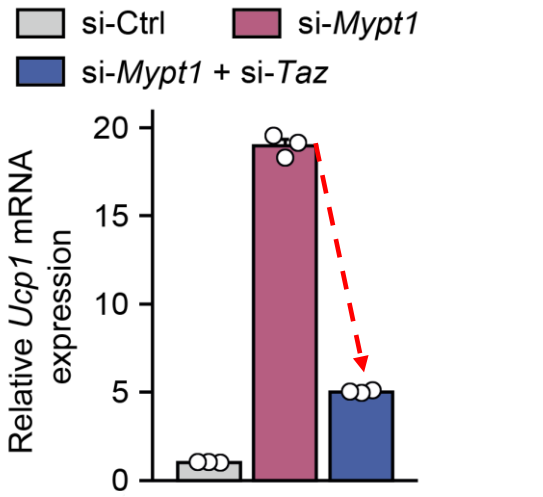








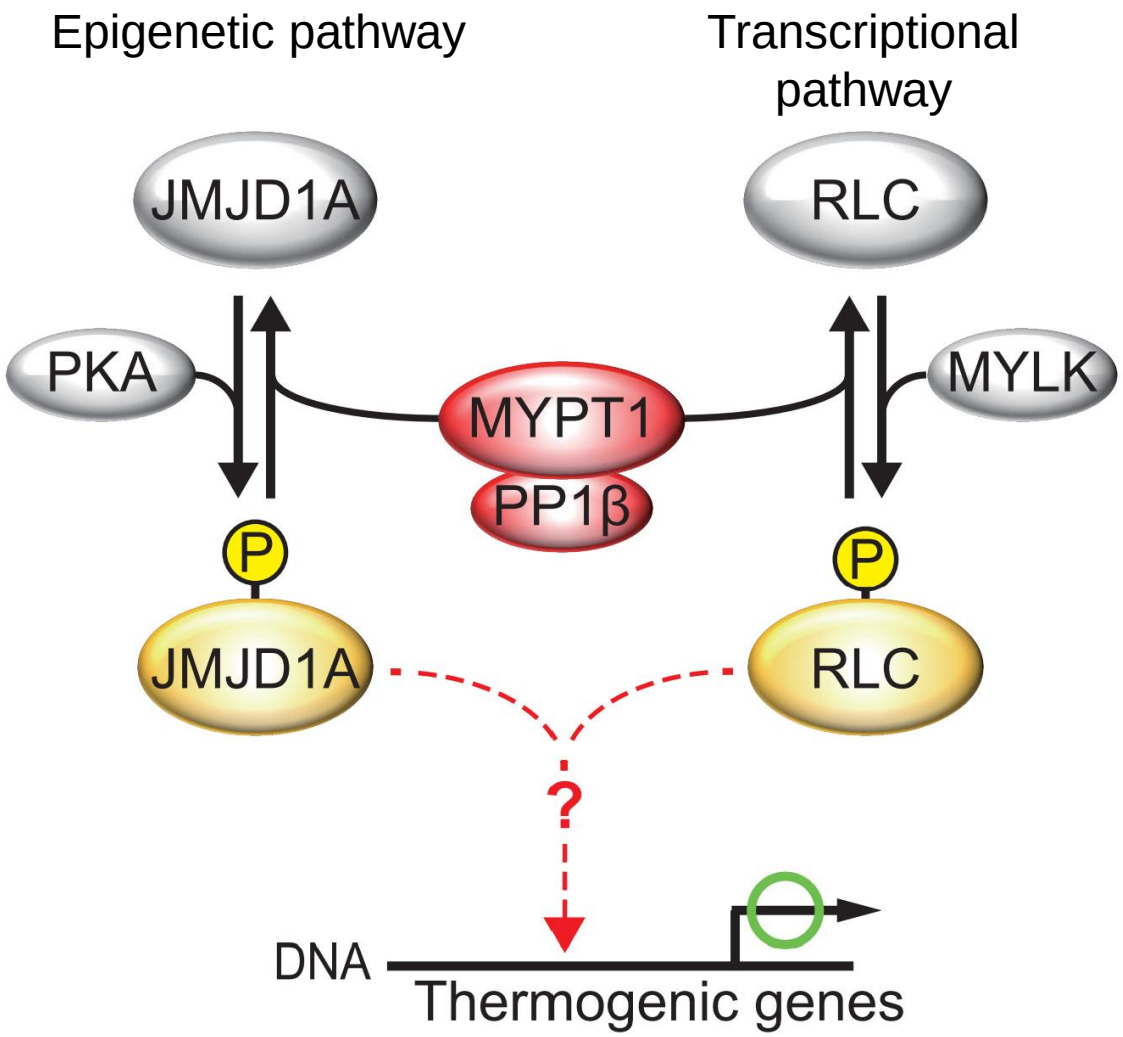




TA/SA : non-phosphorylatable RLC
Blebbistatin: an inhibitor of actomyosin tension

Question:

Do the epigenomic pathway and transcriptional pathway crosstalk?



RLC: regulatory myosin light chain
MYLK: myosin light chain kinase
MYPT1/PP1β: phosphatase for P-JMJD1A

Induction of thermogenic gene expression by MYPT1 inhibition requires prior removal of repressive histone mark by JMJD1A

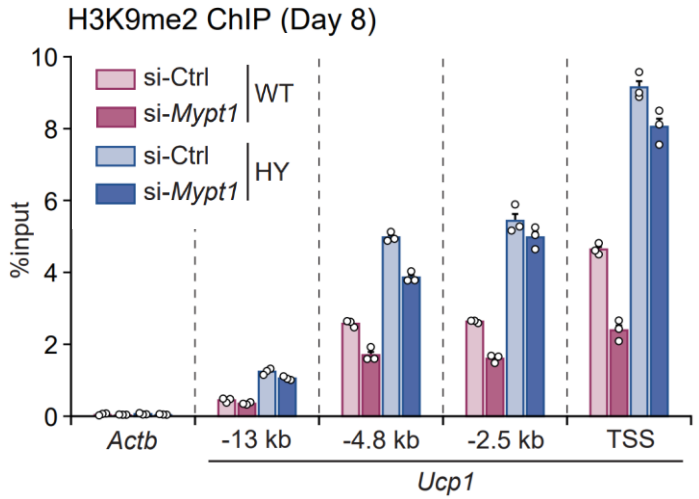
RNA-seq of *Mypt1*-KD & JMJD1A ChIP-seq

JMJD1A is enriched in TEAD motifs on genes upregulated upon MYPT1 depletion

Transcription factor	Motif	*P*-value
TEAD4		1 x 10^{-10}
NF1		1 x 10^{-9}
Fosl2		1 x 10^{-7}
TEAD2		1 x 10^{-6}
Fos		1 x 10^{-5}

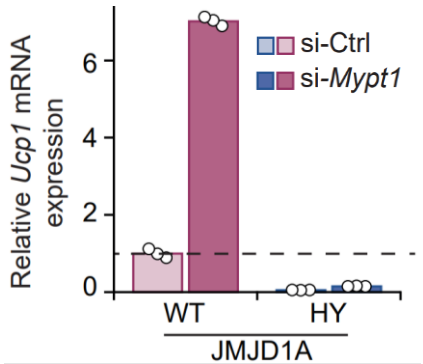
ChIP-qPCR

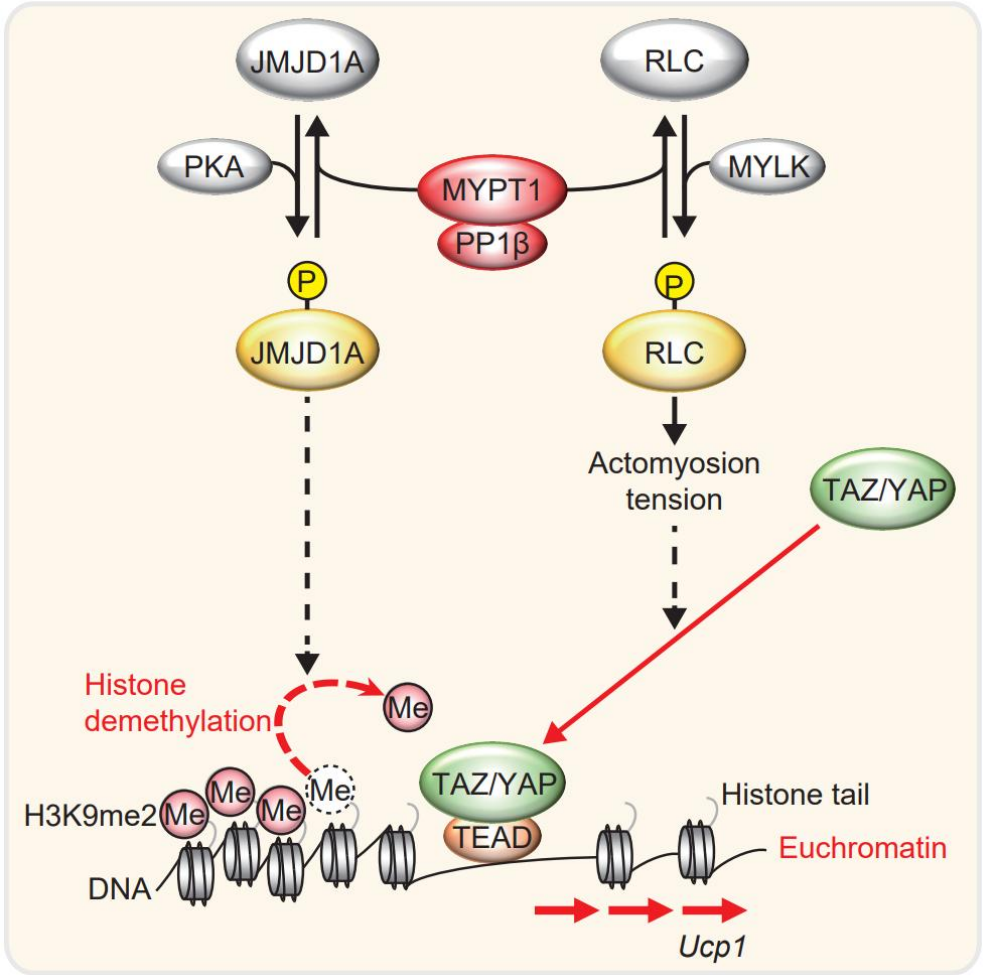
H3K9me2 retains high in HY fat cells



qPCR

Ucp1 reduction in HY fat cells

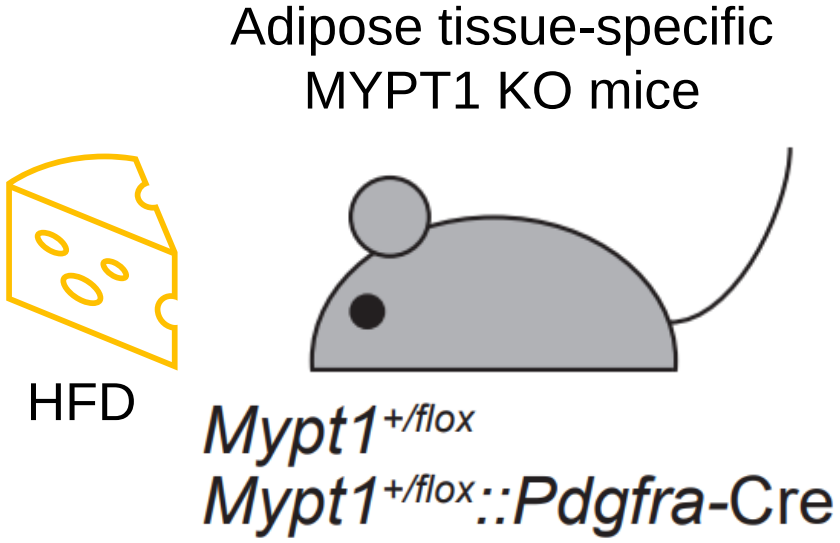




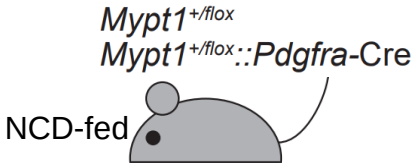
HY-JMJD1A :
H1120Y-JMJD1A、catalytically dead JMJD1A

Question:

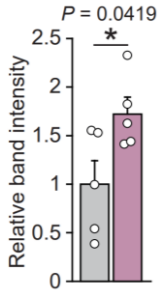
Ameliorating effects on die-induced obesity and glucose intolerance?



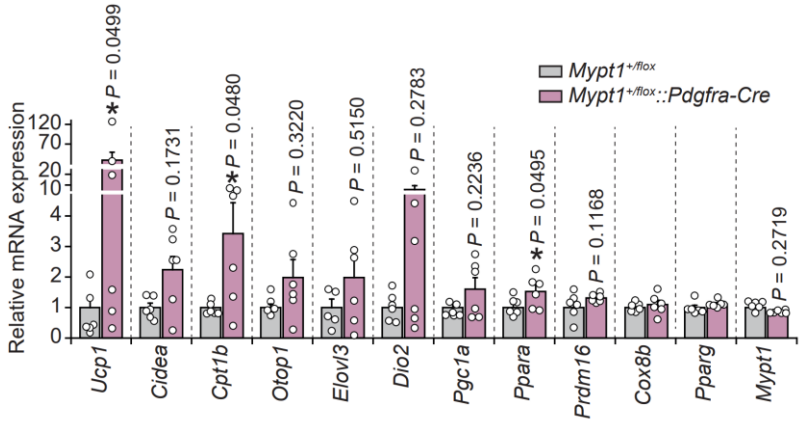
Adipose tissue-specific MYPT1 KO enhances beiging, leading to improved obesity and glucose tolerance



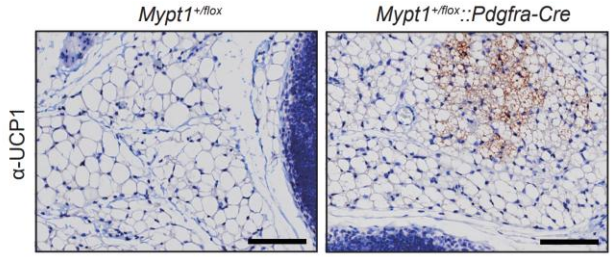
Increased UCP1protein levels
(IB、12℃ for 1 wk, scWAT)

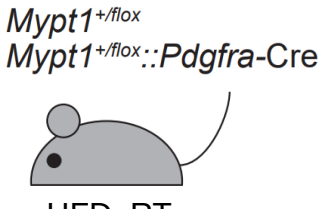


Increased thermogenic gene expression
(qPCR, scWAT, RT)

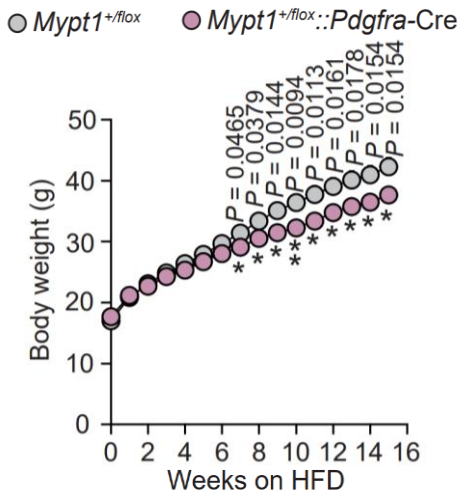


Increased UCP1-positive beige adipocytes
(UCP1 staining, scWAT, RT)

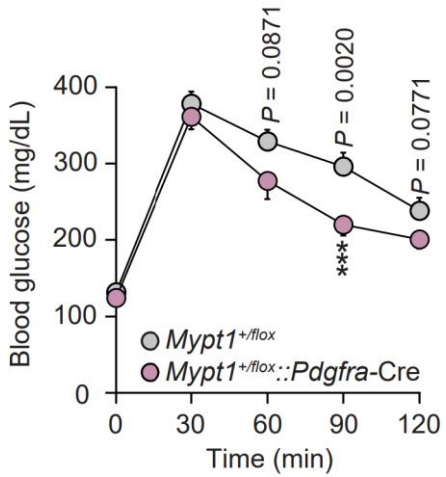




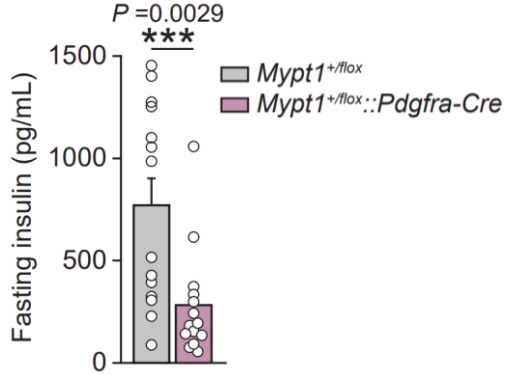
Improved die-induced obesity



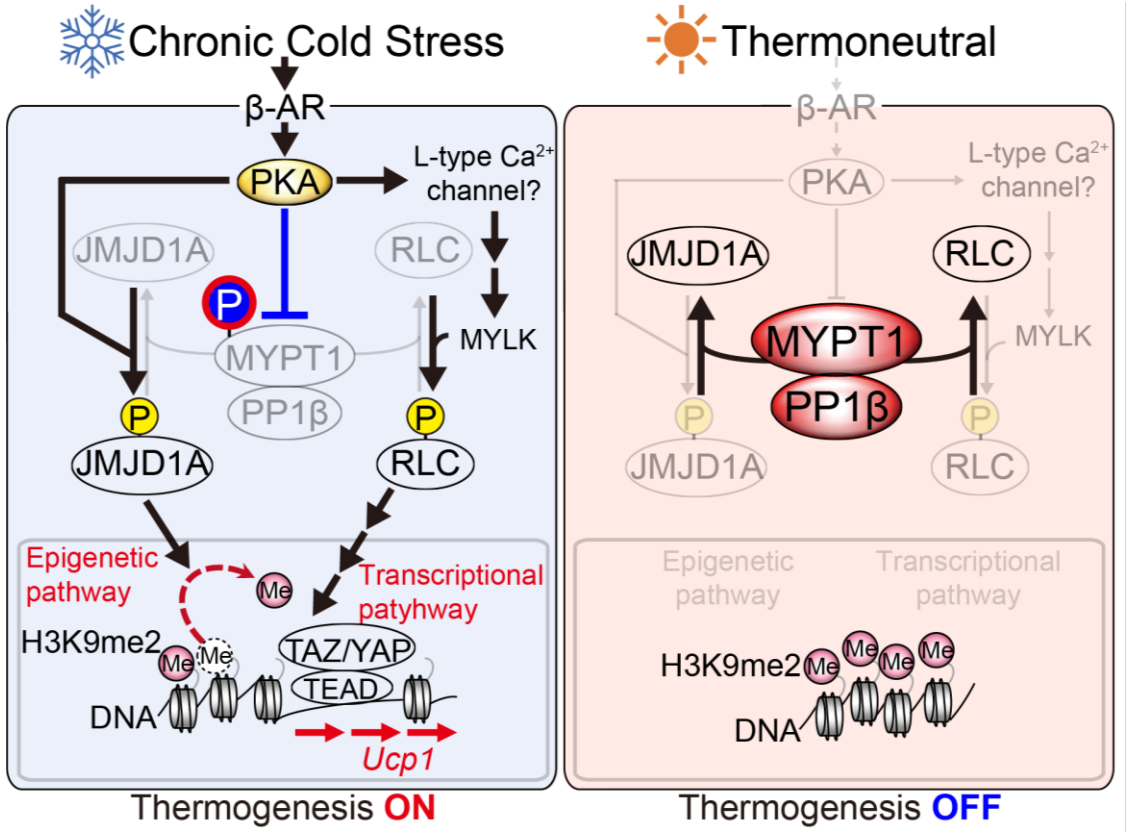
Improved glucose tolerance
(GTT)



Low fasting insulin levels



Summary



Takahashi H, et al,. *Nat Commun*. 2022

- Stabilizing JMJD1A phosphorylation by inhibiting MYPT1-PP1β induces beiging by promoting epigenetic rewriting, ultimately improving obesity and energy metabolism

- We elucidated a concerted mechanism of thermogenic gene activation mediated by epigenomic changes and transcriptional coactivators in response to cold

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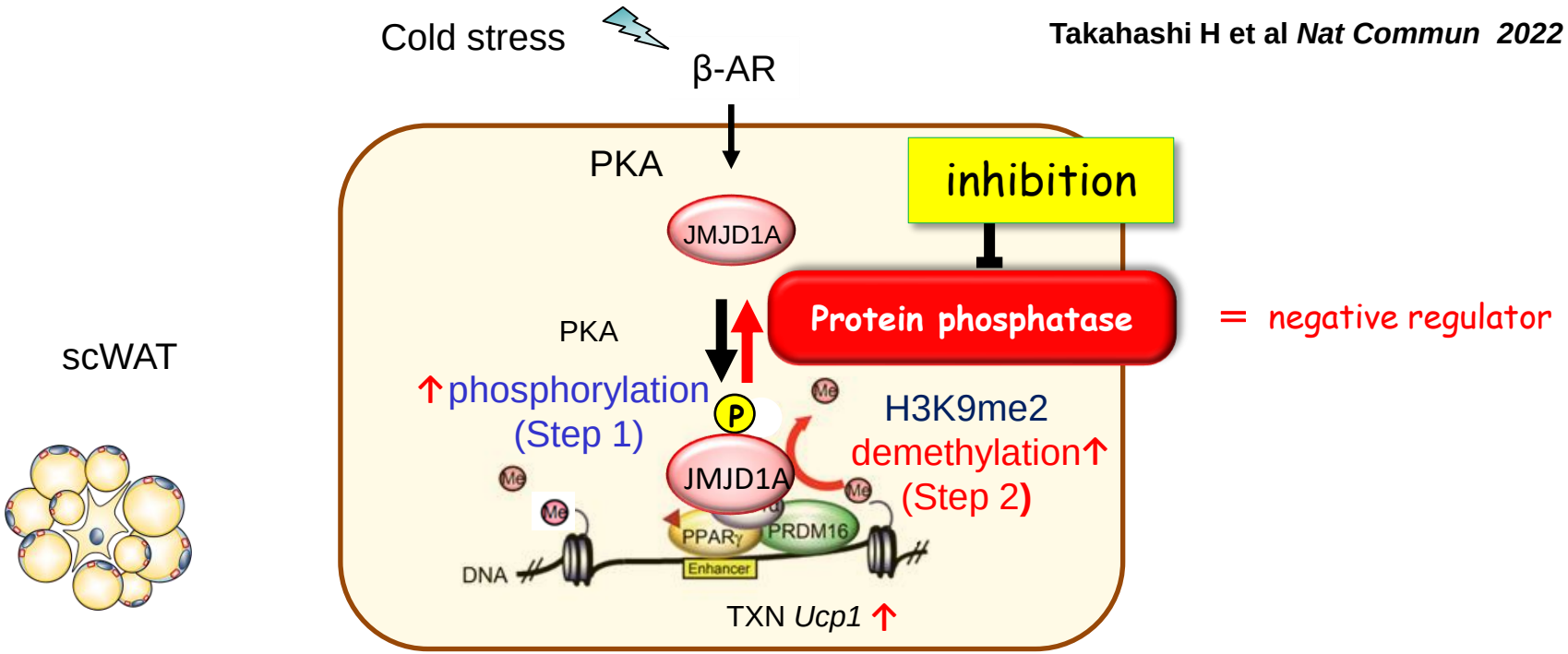
Hiroshi Kimura

(phosphorylation;1st step)

(demethylation; 2nd step)

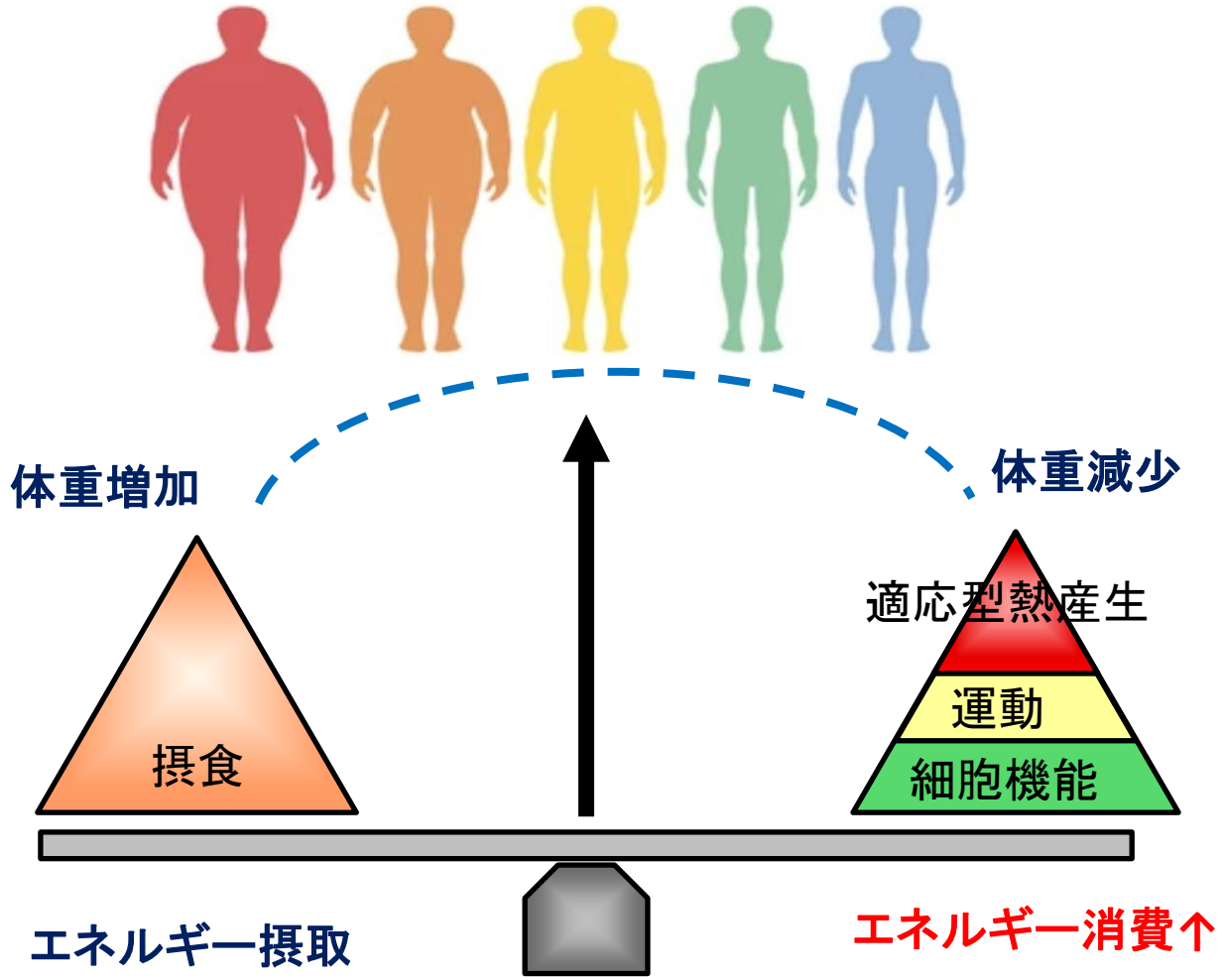
We explored the way to enhance demethylation efficiency (2nd step)
by **inhibiting the phosphatase** of phospho-JMJD1A (1st step)?



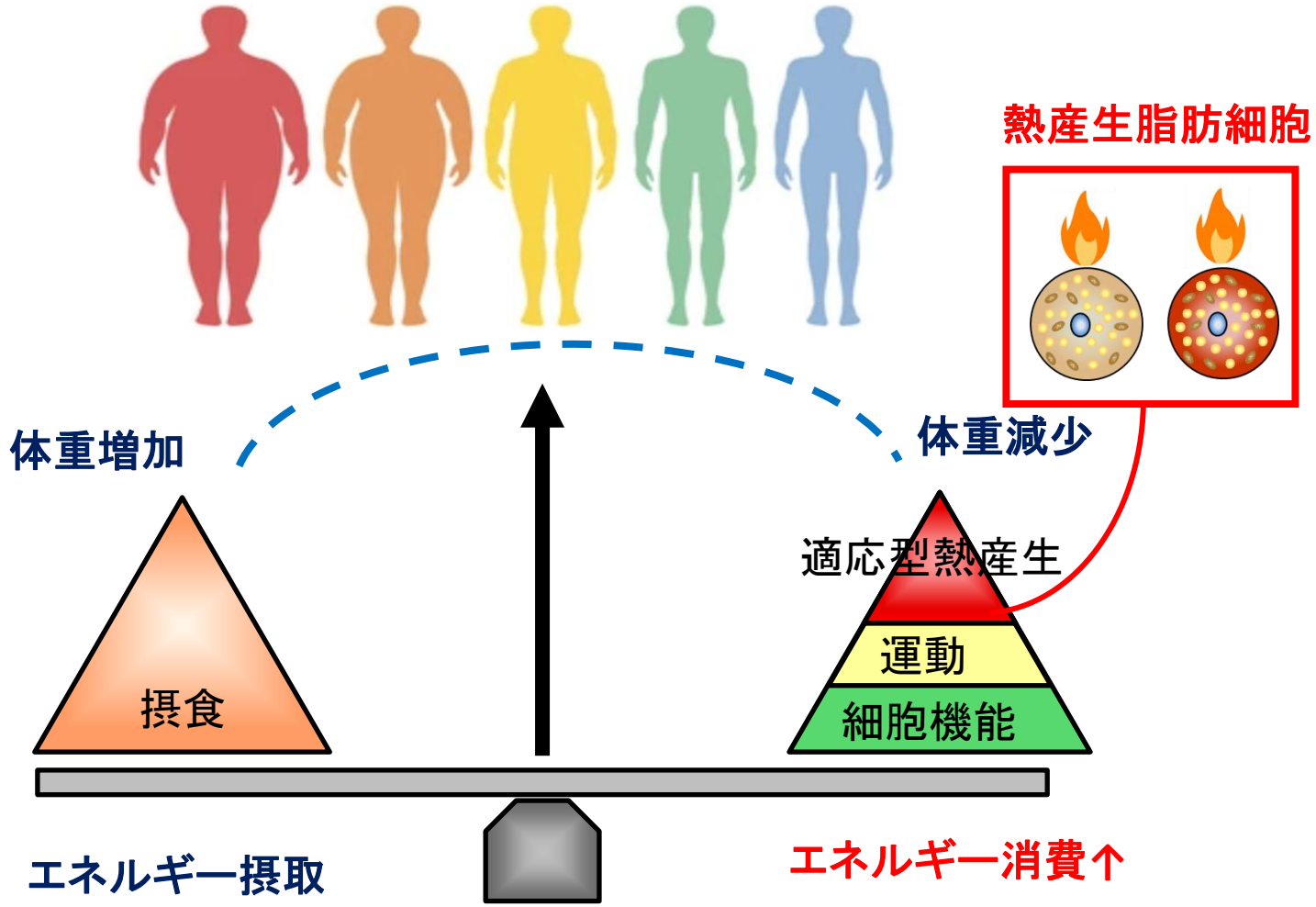
Takahashi H et al *Nat Commun* 2022

TXN: transcription
PKA protein kinase A

肥満はエネルギーバランスの不均衡から生まれる

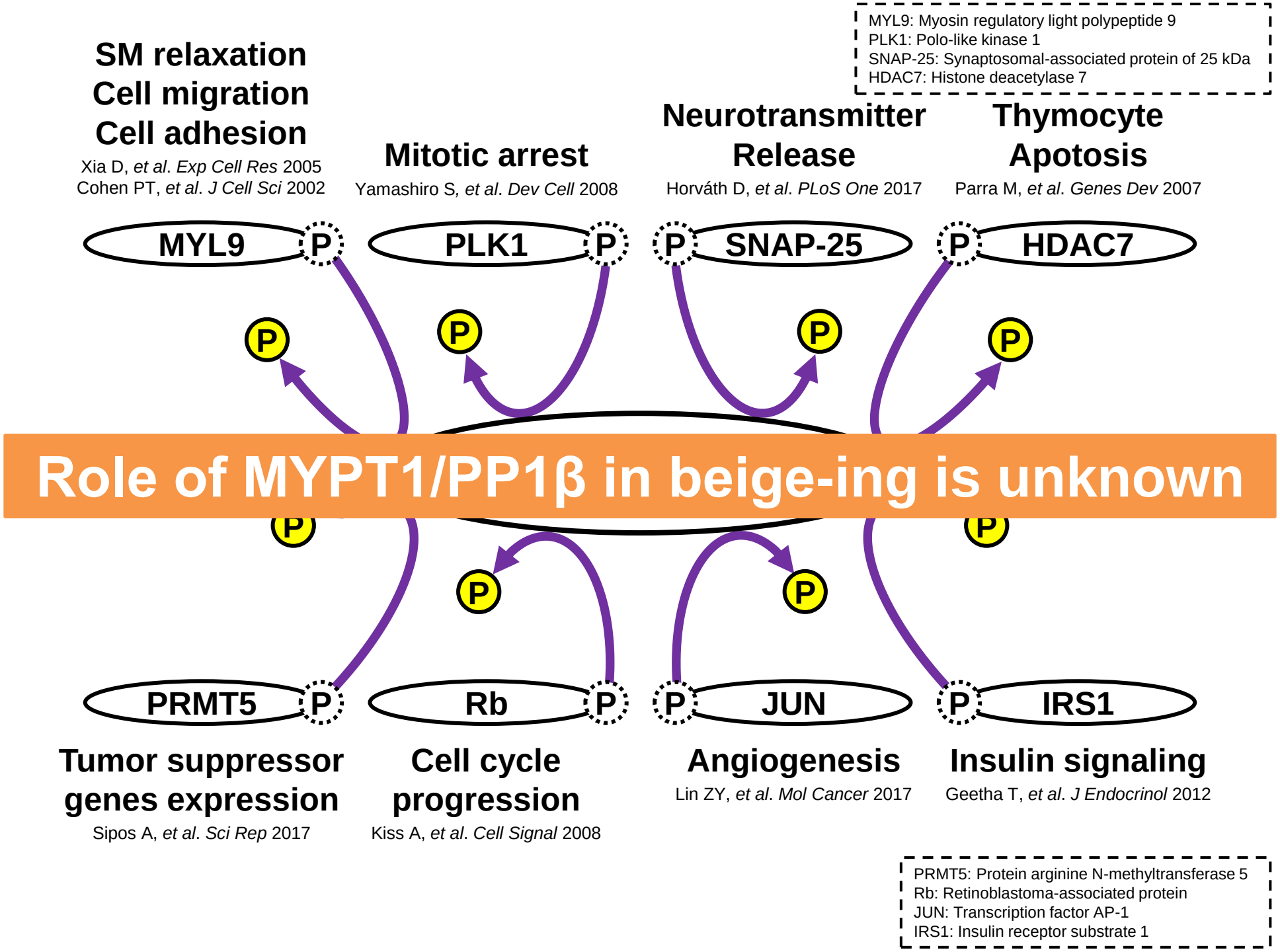


肥満はエネルギーバランスの不均衡から生まれる



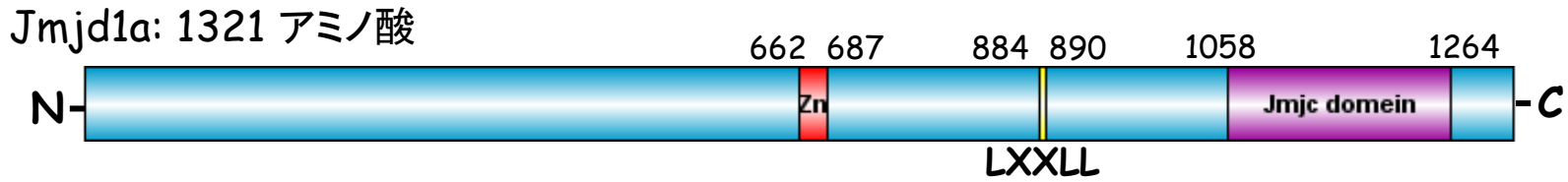
熱産生脂肪細胞は、活発にエネルギーを消費し、負のエネルギーバランスを誘導するから、肥満や生活習慣病の予防治療標的として注目されている

he Nterminal
that P-MLC20
nkyrin repeat
sociates with

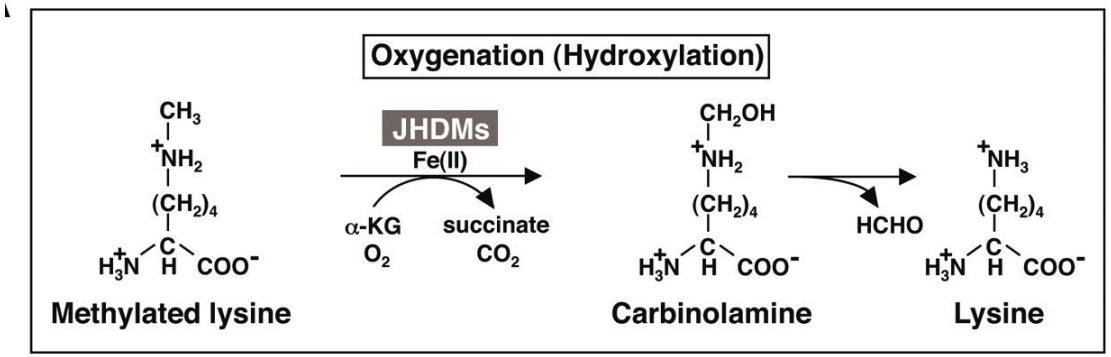


Background 1：ヒストン脱メチル化酵素 Jmjd1a

- 酵素活性に必須な Jumonji C (JmjC) ドメイン，Zn finger モチーフを含有する.
- 核内受容体結合タンパク質に特徴的なアミノ酸配列 LXXLL を持つ.

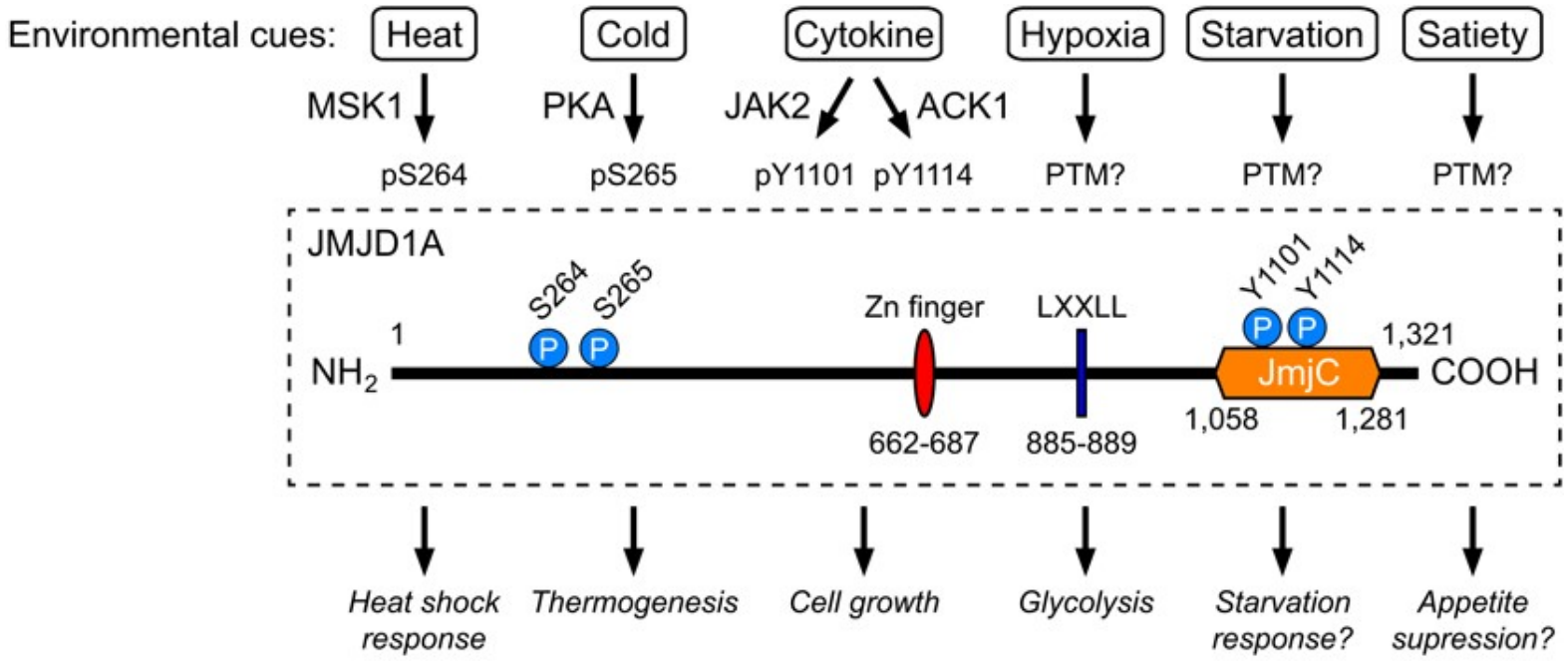


- H3K9 のモノ・ジメチル化を触媒する酵素
- 酵素活性に Fe(II), α-ketoglutarate および O_2 を必要とする.



- Hypoxia によって誘導される (HIF-1α との相互作用).

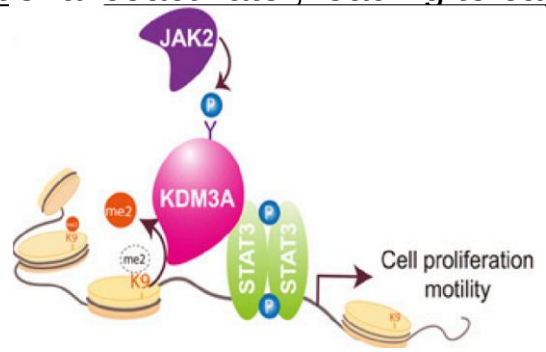
JMJD1A is a signal-sensing epigenetic factor phosphorylated by kinases activated downstream of environmental cues



Matsumura Y, et al,. J Biochem. 2022

013123 021023 Done by Takahas

JMJD1A is phosphorylated at Y1101 by JAK2 upon cytokine stimulation and functions as a STAT3-dependent transcriptional coactivator, leading to cancer cell proliferation

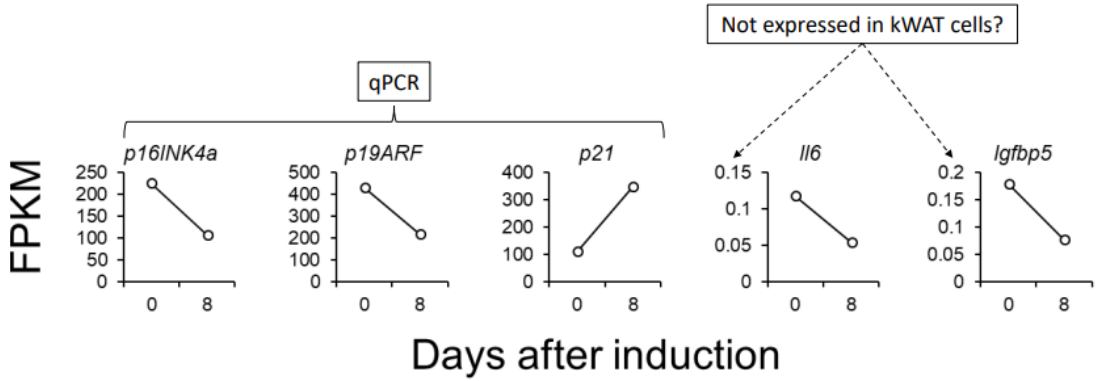


Kim H, et al,. *Proc Natl Acad Sci U S A*. 2018

ENS_ID	Gene_nan	D0_Rosi+_RPK	D4_Rosi+_RPK	D8_Rosi+_RPK	D0_Rosi-_RPK	D4_Rosi-_RPK	D8_Rosi-_RPK	D8_WT_a_RPKM	D8_SA_RPK	D8_WT_b_RPKI	D8_SD_RPK	D8_HYSD_RPK
ENSMUSG00000019907.8	**Ppp1r12a**	17.3077	6.56639	3.50683	12.9623	9.31109	5.91135	9.29345	10.6967	11.5268	11.5667	12.4179

			D8-Empty			D8-shJmjd1a		
Gene De	Probes	Gene ID	0h	1h	2h	0h	1h	2h
Protein phc	1429487_at	Ppp1r12a	244.8	269.7	240.3	299	284.6	266.9
protein phc	1437734_at	Ppp1r12a	161	170	152.1	180.9	168.8	181.1
protein phc	1437735_at	Ppp1r12a	395.8	420.6	346	452.5	418.9	430.8
Protein phc	1444762_at	Ppp1r12a	26.2	26.5	19.5	26.1	20.9	29.9
protein phc	1453163_at	Ppp1r12a	34.7	30.2	34.7	39.9	38.6	36.2

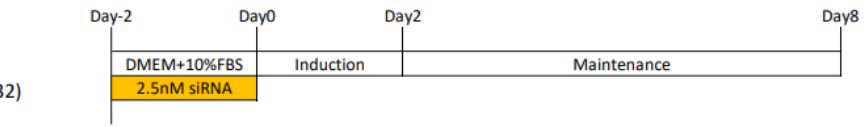
FPKM of cell senescence markers in immortalized scWAT cells

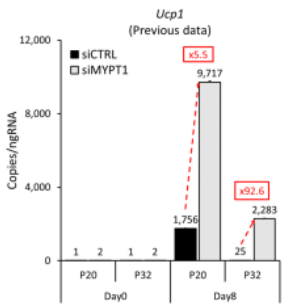


FT059

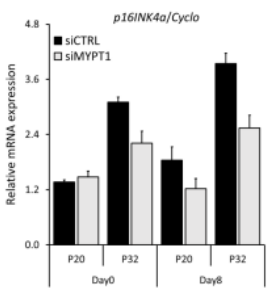
Cells : Immortalized scWAT cells
P20 (⑫Abe⑭HT⑯HT⑱HT20)
P32 (⑫Abe⑭AU⑲AU㉑AU25HT32)

siRNA: NTC Med GC Duplex #2
siMYPT1 #3 (Invitrogen)

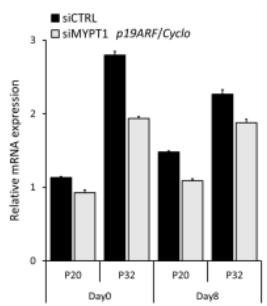


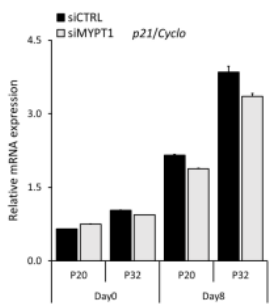


Previous data
021819 Uchida & Takahashi



Slope: -2.73
Ct value: 30.2-31.4
Large standard error

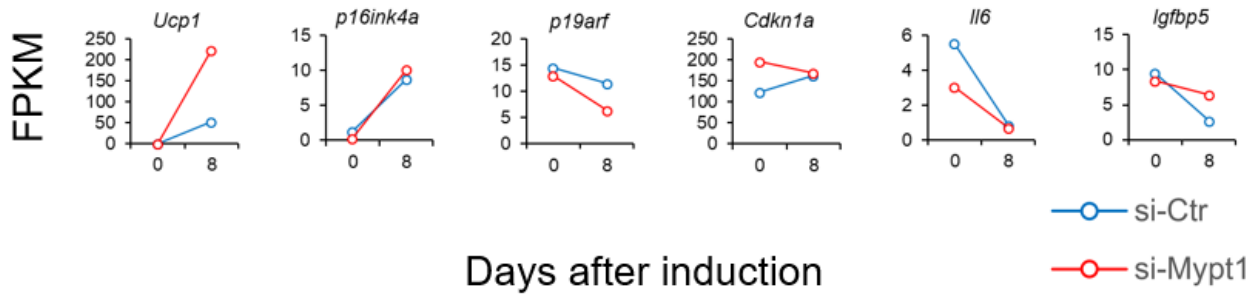




071519 Takahashi & Uchida

- Tendency for P32 cells to express higher levels of markers
- Tendency for siMYPT1 transfected cells to express lower levels of markers (though *Cyclo* is higher in siMYPT1 transfected cells)

Primary scWAT cell



Fwd: Pdgfra 外部 受信トレイ ×

Juro Sakai 酒井寿郎 <jmsakai@med.tohoku.ac.jp> 2022/10/18 15:25

To 高橋宙大, 寒河江, 伊藤, 松村, Makoto

高橋先生
今回使っているPdgfra Creはどこゆらいでしょう？
https://onlinelibrary.wiley.com/doi/10.1002/cne.21730
でしょうか？これですとグリア細胞に今日発現しているようにも見えます。一方、

Nature Communications 返答書に書いた下記は読み間違えており、グリアには発言していないと言ってました。
寒河江さんの結果では、熱産生遺伝子はＷＴと同様にPdgfra Creでも上昇するようでいったいどこに肥満の原因があるのかよくわからないです。

Regarding *Pdgfra*-Cre, recent studies have shown that *Pdgfra* is expressed in progenitor adipocytes, but not in skeletal muscle or liver (Saavedra-Peña, RDM. et al. *J Mol Endocrinol* 68, 179-194, 2022); however, it is expressed in glial cells and gonads (*Journal of Molecular Endocrinology* 68, 179-194, 2022, DOI: https://doi.org/10.1530/JME-21-0260).

Pdgfra 外部 受信トレイ ×

Juro Sakai 酒井寿郎 jmsakai-tky@umin.ac.jp lsbm.org 経由 2022年5月20日(金) 18:56

To Yoshihiro, 米代武司, Tumenjargal, 自分, 伊藤, 酒井寿郎

松村先生、Tumeeさん、米代先生、高橋先生、
先日来、気になっているPdgfra-Creですがこの論文でしらべています。
骨格筋、肝臓にでていない
グリア細胞には発現
Gonads（性腺）に発現（male and female)

ようです。

高橋先生のリバイスでPdgfraはどこにでているかの返答に使えます。
トメさん、筋肉にはでていないよう、一方Gonads and Glia cells in the brain, Pdgfra is expressed.
Interestingly, Adipo-Cre::ERa does not develop obesity in male, however,
Pdgfra-Cre:: ERa mice develop obesity

荒井先生、

Single cell のデータ解析をありがとうございます。
Olig1と共発現していることから、分化途中のオリゴデンドロサイトだと思います（成熟オリゴデンドロサイトはMBP陽性）。
Pdgfraは元々は網膜のグリア細胞で見つかった分子ですが、なぜか前駆脂肪細胞のマーカーとしての知名度が高くなってしまいました。
Jmjd1aflox/flox::Pdgfra-Creマウスの表現型の解釈にあたって、注意しておきたいことですね。

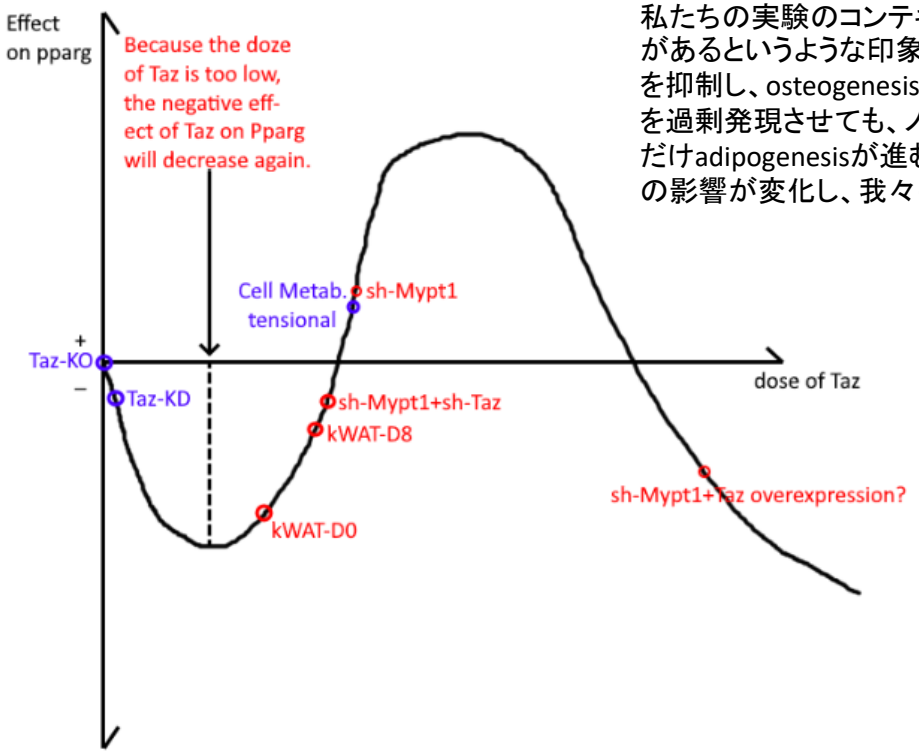
松村

Dear Takahashi-sensei:

I hope this e-mail finds you well. This is Chaoran Yang.

Recently, I read some more papers and found that it is a a long-stand idea that both Yap1/Taz (Wwtr1) are inhibitors of Pparg (and activator of Runx2) and can inhibit adipogenesis by inhibiting Pparg while stimulating osteogenesis by activating Runx2. And a paper published on Cell Reports in 2013 found that **both** Yap1 knockdown and Yap1 overexpression can downregulate Pparg and inhibit the adipogenesis of MSCs.

Therefore, I think maybe the effect of Taz (Wwtr1) on Pparg and thermogenesis genes like Ucp1 is also doze-dependent, as shown in the picture followed. This hypothesis may help to explain why there are both reports that Yap1 can upregulate Ucp1 (that Cell Metabolism story) and Yap1/Taz can downregulate Pparg and inhibit adipogenesis. And, personally, I think maybe we can do a sh-Mypt1+Taz overexpression experiment to test if this hypothesis is right or not.



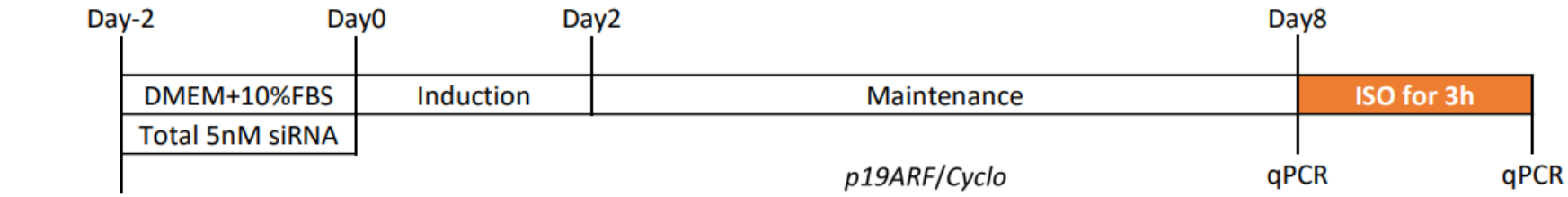
私たちの実験のコンテキストでは、余り、脂肪細胞の分化への影響でベージュ化に変化があるというような印象は受けませんでした。確かに2005のscienceにTAZはadipogenesisを抑制し、osteogenesisを促進すると報告されておりますが、2013年にはcell reportでYAPを過剰発現させても、ノックダウンしてもadipogenesisは抑制され、Mildに発現している時だけadipogenesisが進むといった報告もあり、もしかしますと用量依存性にadipogenesisへの影響が変化し、我々のコンテキストでは影響が見えにくかったのかもしれません。

Thank you for your attention. I have also attached the three articles mentioned for your reference.

Kind regards,
Chaoran Yang

FT068

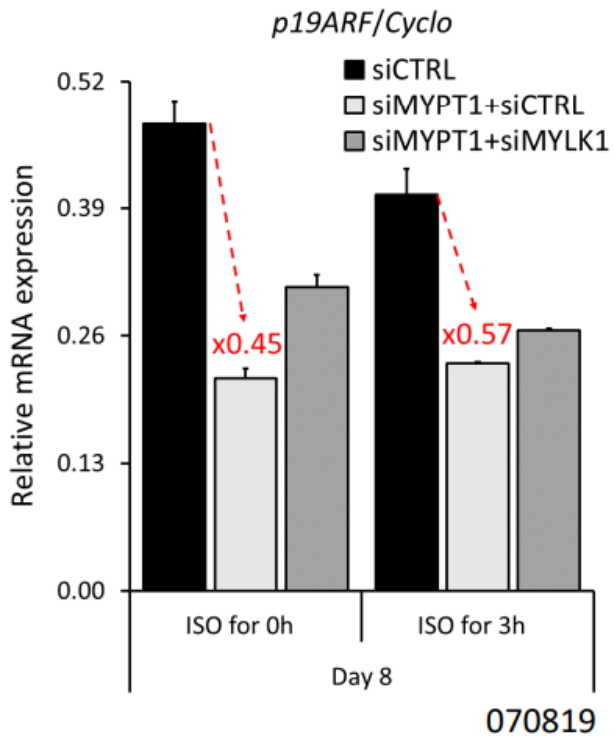
Tendency for siMYPT1 transfected cells to express lower levels of p19ARF



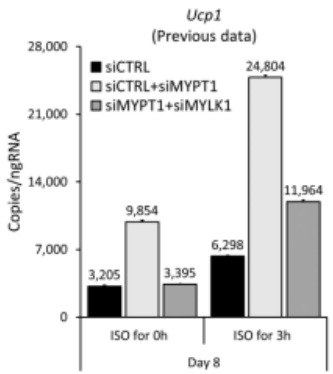
Cells: Immortalized scWAT cells
(P24 (⑫Abe⑭HT⑯HT⑱HT⑳HT24)

Stimulation: 10uM Isoproterenol for 0h, 3h

siRNA: Negative Control Med GC Duplex #2
siMYPT1 #3 (Invitrogen)
siMYLK1 #1,2 (Invitrogen)



070819



Previous data
050919

Posttranslational modifications of proteins such as phosphorylation and dephosphorylation are known mechanisms that operate as a 'molecular switch' to either promote or diminish insulin signaling. Once the IR is activated and appropriate tyrosine residues of IRS1 are phosphorylated (Venable *et al*. 2000), IRS1 interacts with p85 and Grb2, which are affiliated with the PI3K and MAPK pathways, respectively, leading to the activation of a number of downstream kinases, such as Akt/PKB, mTOR, S6K1, GSK3, and MAPK. These downstream kinases can phosphorylate site-specific serine/threonine residues of IRS1, in which most of these phosphorylation events result in reduced insulin action (Gual *et al*. 2005, Sun & Liu 2009, Siddle 2011). Surprisingly, little is known about the role of serine/ threonine phosphatases in IRS1 phosphorylation and insulin-signaling transduction.

Yes-associated protein (YAP) and TAZ (WW domain containing transcription regulator 1, or WWTR1) are paralog transcriptional regulators, able to integrate mechanical, metabolic, and signaling inputs to regulate cell growth and differentiation during development and neoplastic progression. YAP and TAZ hold common and distinctive structural features, reflecting only partially overlapping regulatory mechanisms. The two paralogs interact with both shared and specific transcriptional partners and control nonidentical transcriptional programs. Although most of the available literature considers YAP and TAZ as functionally redundant, they play distinctive or even contrasting roles in different contexts. The issue of their divergent roles is currently underexplored but holds fundamental implications for mechanistic and translational studies. Here, we aim to review the available literature on the biological functions of YAP and TAZ, highlighting differential roles that distinguish these two paralogues.

The osteoblastic and adipocytic lineages arise from mesenchymal stem cells (MSCs), but few regulators of self-renewal and early cell-fate decisions are known. Here, we show that the Hippo pathway effector YAP1 is a direct target of SOX2 and can compensate for the self-renewal defect caused by SOX2 inactivation in osteoprogenitors and MSCs. Osteogenesis is blocked by high SOX2 or YAP1, accelerated by depletion of either one, and the inhibition of osteogenesis by SOX2 requires YAP1. SOX2 favors adipogenesis and induces PPARg, but adipogenesis can only occur with moderate levels of YAP1. YAP1 induction by SOX2 is restrained in adipogenesis, and both YAP1 overexpression and depletion inhibit the process. YAP1 binds b-catenin and directly induces the Wnt antagonist Dkk1 to dampen pro-osteogenic Wnt signals. We demonstrate a Hippo-independent regulation of YAP1 by SOX2 that cooperatively antagonizes Wnt/b-catenin signals and regulates PPARg to determine osteogenic or adipocytic fates.

Major comment 6: *The authors suggested that MYPT1 would play suppressive roles in preadipocytes, not in mature beige adipocytes, which was supported by Adipoq-Cre Mypt1 KO mice model (Supplementary Figs. 5f-i). However, given that PKA signaling, JMJD1A, and MYPT1/PPT1beta are also present in mature adipocytes, appropriate explanations of the different roles of MYPT1/PPT1beta in preadipocytes and adipocytes should be provided.*

Reply to major comment 6: We thank the reviewer for their valuable comments. As per your comments, MYPT1 exerts its main action on JMJD1A during beige adipogenesis. During this period, JMJD1A erases the repressive H3K9me2 from thermogenic genes, converting silenced chromatin to open chromatin, and thus activating transcription. Phosphorylation of JMJD1A forms a -specific complex with transcription factors and nuclear proteins, such as PPAR, PGC1α, and PRDM16, to determine the specificity of the target genes (Abe Y et al, *Nat Commun* 2018). Thus, phosphatase MYPT1/PP1β, which removes this phosphorylation, acts as a repressor during the demethylation period. However, once H3K9me2 is eliminated and beige adipogenesis is completed, JMJD1A is no longer needed and MYPT1/PP1β no longer exhibits a repressive function.

With regards to *Adipoq-Cre::Mypt1*$^{flox/flox}$ mice, they do not exhibit thermogenic phenotype, suggesting that phospho-JMD1A does not promote trans-differentiation from white mature adipocytes to beige adipocytes.

In addition, as a preliminary experiment, primary cultured SVFs were prepared from scWATs of *Mypt1*$^{flox/flox}$ mice and infected with adenovirus carrying Cre recombinase (Adeno-Cre) on the day before (Day 1; pre-adipocyte stage) or seven days after (Day 7; mature adipocyte stage) differentiation to eliminate MYPT1. After differentiation, adipocytes were harvested on day 8 and thermogenic gene expression was examined. The results (**Fig. 3 only for reviewers**) showed that the expression of thermogenic genes was higher in cells infected on day 1 than in those infected on day 7, suggesting that *Mypt1* depletion does not affect the transcription of thermogenic genes in mature beige adipocytes, but only early in differentiation. We acknowledge that this is not a perfect experiment, and there are limitations in the interpretation; however, the results are consistent with the model and it will require significant future studies to clarify these points.

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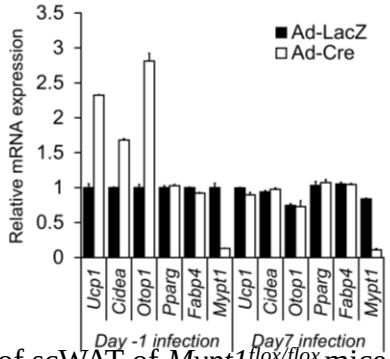


Fig. 3 only for reviewers

Primary preadipocytes isolated from SVF of scWAT of *Mypt1*$^{flox/flox}$ mice were infected with either AxCAN-LacZ (adeno-LacZ) or AxCAN-Cre (Adeno-Cre) that expresses the LacZ and Cre recombinase gene, respectively, under the control of the CAG promoter for 24 h on one day before differentiation (Day -1 infection) or on day 7 (Day 7 infection) during beige adipogenesis. mRNA levels of thermogenic genes, general adipogenic genes, and *Mypt1* on day 8 of differentiation were quantified by RT-qPCR. Data are mean ± SEM of three technical replicates.

Mypt1 deficiency did not influence Ucp1 levels by chronic cold stress in BAT, indicating that MYPT1 may not be essential for BAT thermogenic function at least during the chronic phase of cold adaptation. However, it is also possible that the 50% reduction in MYPT1 was sufficient to reveal a phenotype due to reduction in scWAT but not in BAT where basal levels of Ucp1 are already very high. 脂肪組織特異的MYPT1KOマウスのBATおよび培養細胞でMYPT1ノックダウンの上βARアゴニスト刺激性の熱産生遺伝子発現誘導に差は確認されませんでした。しかし、いづれの実験に於いてもMYPT1の減少は50%程度であったためよりdrasticにMYPT1を減少させてあげることができれば差が見えてくるかもしれません。2015年にもリン酸化JMJD1Aはスキャホルディングタンパクとして機能することでβARアゴニスト刺激性の熱産生遺伝子発現誘導に関与することを報告しておりますので、またアクトミオシンの張力がYAP/TAZ転写経路を介してBAT熱産生に関与することも報告されているため、JMJD1Aリン酸化とRLCリン酸化を制御するMYPT1がBATにおいて機能している可能性は十分ありうると思います。(tharpのことも)
scWATではクロマチンが閉じているためMYPT1 KDによるJMJD1Aの機能活性化はdrasticにフェノタイプに影響する一方r、BATでは元々熱産生遺伝子領域はオープンで、元々UCP1発現が高いBATではJMJD1A axisが活性化されても、他の転写経路の寄与に埋もれてtaUCP1の増加は見にくいのかもしれません。
Question 1: In other tissues where PKA signaling is activated, do you think MYPT1 and JMJD1A is phosphorylated and similar downstream signaling is activated?
Answer: 脂肪細胞でしか確認していませんが他の交感神経支配されている器官においてもβアドレナリン受容体シグナル下流でJMJD1Aがリン酸化され、これにMYPT1が拮抗する機構が存在しているかもしれません。ベージュ化のコンテキストにおいては、リン酸化されたJMJD1AはPRDM16,PGC1A,PPARGといった転写因子、転写共役因子と相互作用することで、熱産生遺伝子の発現を制御しますが、他の組織、器官においてはそれぞれの組織特異的な転写因子、転写共役因子と相互作用することで、特異的な遺伝子の発現制御に関与しているのかもしれません。コンテキストdependentに細胞毎にコファクターが変わって特異性が変わる

Question 2: What is the biological significance of inducing actomyosin tension in beige adipogenesis?
Answer:
Thermogenic adipocytes maintain an extensive cytoskeletal network that supports and organizes multilocular lipid droplets and numerous mitochondria, whereas white adipocytes are
comprised of a unilocular lipid droplet. we hypothesized that brown/beige adipose generates cytoskeletal stiffness requisite to maintain or promote differentiation status.
ベージュ脂肪細胞は、多房性の脂肪滴やミトコンドリアリッチな構造であり、アクトミオシン骨格がミトコンドリアのサイズや数、融合等に影響を及ぼすという報告もありますようで、この構造を機械的に維持し、ベージュ化状態を維持するために、アクトミオシンのテンションが必要なのかもしれません。

Adrenergic stimulation has also been identified to promote mitochondrial fragmentation in brown adipocytes (Wikstrom et al., 2014), which leads us to postulate that mitochondrial dynamics may require mechanical support from actomyosin. Actin dynamics have only recently been identified to modulate mitochondrial function in mammalian cells (Beck et al., 2012), and ER-associated mitochondrial divisions appear to require force generated by the actin cytoskeleton (Hatch et al., 2014; Korobova et al., 2013). While our data suggest a significant role for mechanoregulation in mitochondrial function during thermoregulation, further study is necessary to establish a mechanistic explanation of how respiration is regulated by cellular elasticity

Here, we analyzed the impact of actin on neuronal mitochondrial size and localization。F-actin enhanced mitochondrial size and mitochondrial number in neurites and growth cones. In contrast, raising G-actin resulted in mitochondrial fragmentation and decreased mitochondrial abundance. In this study, we demonstrate that SRF-cofilin-actin signaling affects mitochondrial dynamics (i.e., size, subcellular distribution to neurites and growth cones, mitochondrial energy metabolism).
. In this Commentary, we present a mechanistic model for mitochondrial fission in which actin and myosin contribute in two ways; firstly, by supplying the force for preconstriction and secondly, by serving as a coincidence detector for Drp1 binding
MYPT1遺伝子の変異が人における肥満等の形質と相関しているかどうかというのは大変興味深いところですが、まだ調べていないので是非今後の課題とさせて頂きたいです　（MYPT1はアクトミオシン張力を介した細胞機能（平滑筋の収縮）に関与するため、変異がホモだとリーサルになりかねないが」）

適応熱産生は、寒冷誘発性熱産生と食事誘発性熱産生から成り、食事誘発性熱産生は主に褐色脂肪組織（brown adipose tissue; BAT ）が担っている。これまで一般的に、食事摂取により交感神経系が活性化されることでBAT熱産生が亢進すると考えられてきたが、例えば、栄養素のうち交感神経系を活性化するのは炭水化物のみであること、交感神経系をβ遮断薬でブロックしても食後早期の熱産生は抑制できないことなどから、その分子機構には不明な点が多かった。筆者らは、カテコラミンだけではなく、摂食によって分泌される消化管ホルモンが直接BATを活性化するのではないかと考え、検証を試みた。

はじめに筆者らは、BATにおける消化管ホルモン受容体の発現を検討し、セクレチン受容体の発現が突出して高いことを突き止めた。次に初代培養褐色脂肪細胞を用いて、セクレチンがUCP1依存性の熱産生を強力に誘導すること、その活性はセクレチン受容体に依存するが、アドレナリン受容体には依存しないことを明らかにした。またセクレチンによる熱産生亢進がcAMP-PKA経路を介する脂肪分解に依存すること、さらにセクレチンの腹腔内投与によって野生型マウスでは熱産生が亢進するが、UCP1欠損マウスではその効果は認められないことを示した。

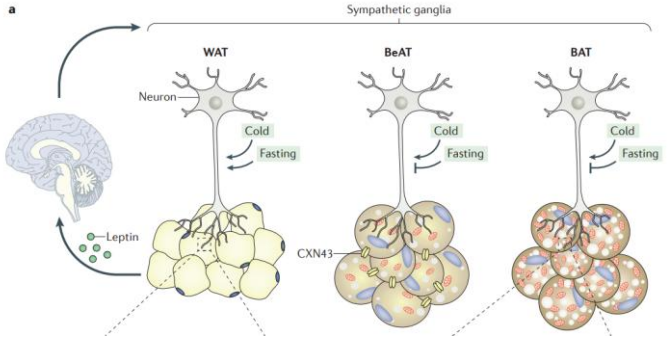
血漿セクレチン濃度は絶食で低下したが再摂食で顕著に上昇し、BAT温と相関した。以前からセクレチン投与が食欲減退につながることが知られていたが、筆者らはこの効果がUCP1欠損マウスでは消失することを示し、セクレチンがBAT熱産生を介して食欲減退効果を発現することを明らかにした。セクレチン投与は、視床下部において食欲抑制に働くPOMC発現を増加させるとともに、食欲増進に働くAgRP発現を減少させたが、UCP1欠損マウスではこれらの変化は認められなかった。セクレチンは弓状核のPOMCニューロンに発現するTRPV1の発現も上昇させたことから、BAT熱産生による体温上昇をTRPV1が感知し、POMCニューロンが活性化されることで食欲抑制につながる経路が示唆された。

筆者らはさらに、セクレチンに対する中和抗体の投与が食後早期のBAT温上昇を抑制し、食事摂取量を増加させること、逆にセクレチンアナログを肥満マウスに投与することで、一過性にエネルギー消費が亢進することも示した。またヒトにおいても、食前食後の血清セクレチン上昇レベルが、食後エネルギー消費量およびBATへの脂肪酸取り込み量の増加と正に相関することを示し、セクレチンからBAT熱産生を介して飽食感につながる経路がマウス、ヒトで保存されていることを示した。

脱リン酸化酵素阻害剤は脱リン酸化酵素基質特異性が広く中々開発が難しいと思いますがMYPT1-JMJD1A複合体の三次元構造を解析しJMJD-AtoMYPT1の相互作用を特異的に阻害する小分子を同定することができればJMJD1Aのリン酸化を特異的に増加させることができれば、JMJD1A-S265リン酸化は寒冷センサーなので、寒冷刺激によるシグナルを増幅し、熱産生遺伝子の特異的な活性化を増強できるかもと思います。アクトミオシン骨格を介した細胞機能に影響してしまうので副作用が強くなってしまうかもしれません。
ただ単にMYPT1を阻害しても(pan-MYPT1阻害剤)アクトミオシン骨格を介した細胞機能が阻害され副作用。g9a阻害剤も全てのg9aターゲット遺伝子で影響が出るが、JMJD1A-s265リン酸化を増強できれば、熱産生遺伝子のみを活性化できる。

adrenergic stimulation leads to both increased lipolysis and thermogenesis.ベージュや褐色脂肪へのSNSのトーンは、食事により増加し、bARが活性化され、食事誘導熱産生が起きると思われるが、食事誘導熱産生はbARを介するので、cold induced thermogenesisと同様に、食事誘導熱産生においても同様の機構でMYPT1-JMJD1Aが機能していると考えられる。Coldだろうが、dietだろうがNAの分泌によるbARの活性化なので、基本的に同じような下流が動くのか？カテコラミンだけでなく、食後に十二指腸のS細胞から分泌される消化管ホルモンセクレチンも食事誘導性熱産生に関与することが報告されている。セクレチンによる熱産生亢進がcAMP-PKA経路を介する脂肪分解に依存するということで、セクレチン受容体の下流で活性化されたPKAにより、cold-bAR-cAMP-PKAの場合と同様な機構を介して、食事誘導熱産生にJMJD1A-MYPT1が関与する可能性も考えられる。もしくは、HSLのリン酸化をMYPT1が制御している（既報であるかもしれないが知らない）か, またはJMJD1AやYAP/TAZによりlipolysis関連遺伝子が制御される場合には、セクレチン受容体の下流で活性化されたPKAによる脂肪分解を介した熱産生にも関与するかもしれない。しかしMYPT1KOマウスで食事誘導性熱産生が亢進しているかどうかは確認していない。

LipolysisはbARの活性化で、誘導されるが、PKA→pHSL axisで制御されるため、JMD1A-MYPT1の上流若しくは並列レベルでの関係であり、例えば、MYPT1 KDでlipolysisに影響するとすれば、JMJD1AやYAP/TAZによりlipolysis関連遺伝子が制御されるか（詳しく確認していない）、もしくはHSLのリン酸化をMYPT1が制御している（既報であるかもしれないが知らない）かということになるかと思う



さらに、本研究から JMJD1A を介した細胞非自律的制御やエピゲノム-RNA 修飾軸の分子基盤が解明され ることで、抗肥満薬の創薬標的として JMJD1A を活性化する薬剤の可能性が考えられる。申請者は、これ までに JMJD1A の抑制因子として脱リン酸化酵素の調節サブユニットである MYPT1 を特定しており、こ れの阻害剤が JMJD1A を活性化する薬剤になり得ると考えられる。またH3K9 メチル化酵素である G9A の 阻害剤は、JMJD1A の作用を増大させる薬剤になると考えられ、肥満や 2 型糖尿病をはじめとした生活習 慣病の治療薬として発展し得ると考えられる

インスリン感受性がMYPT1KOで改善するのは、ベージュ化促進により、肥大化脂肪細胞が少なくなり、遊離脂肪酸、TNFa, レジ巣チン、IL6といったインスリン抵抗性惹起因子（IRチロシンキナーゼ活性やPI3Kの活性を抑制）やm1マクロファージを郵送するケモカインMCP1の肥大化脂肪細胞からの分泌が減少し、炎症が減り、インスリンシグナルが阻害されないから？

Thermoneutralではどのように考えているか
このように転写経路とエピゲノム経路が協調的に遺伝子制御することは知られているのか？
寒冷刺激でMYPTY1活性化面白く、それであれば、細胞骨格状態は実際に寒冷刺激で変化しているのでしょうか。
JMJD1AとMYPT1の発現制御
YAPTAZ脂肪細胞分化
YAP/TAZ ぱらろぐ　メバロンさん

Adipo-Creで差が見えなかった為、少なくとも、transdifferentiationやmature beigeの熱産生行進よりもdenovo beige adipogenesis変化による寄与が大きいのではという風に考えております。

Our analysis revealed negatively correlation between *JMJD1A* expression and BMI, waist circumference, hip circumference, serum triglycerides, and serum cholesterol (Figures 7A–7F). This implies that JMJD1A may play a role in enhancing energy metabolism in adipose tissue and potentially preventing obesity in humans.

Cold-induced Yes-associated-protein expression through miR-429 mediates the browning of white adipose tissue

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Targeting the white-to-brown fat conversion is important for developing potential strategies to counteract metabolic diseases; yet the mechanisms are not fully understood. Yes-associated-protein (YAP), a transcription co-activator, was demonstrated to regulate adipose tissue functions; however, its effects on browning of subcutaneous white adipose tissue (sWAT) are unclear. We demonstrated that YAP was highly expressed in cold-induced beige fat. Mechanistically, YAP was found as a target gene of miR-429, which downregulated YAP expression *in vivo* and *in vitro*. In addition, miR-429 level was decreased in cold-induced beige fat. Additionally, pharmacological inhibition of the interaction between YAP and transcriptional enhanced associate domains by verteporfin dampened the browning of sWAT. Although adipose tissue-specific YAP overexpression increased energy expenditure with increased basal uncoupling protein 1 expression, it had no additional effects on the browning of sWAT in young mice. However, we found age-related impairment of sWAT browning along with decreased YAP expression. Under these circumstances, YAP overexpression significantly improved the impaired WAT browning in middle-aged mice. In conclusion, YAP as a regulator of sWAT browning, was upregulated by lowering miR-429 level in cold-induced beige fat. Targeting the miR-429-YAP pathway could be exploited for therapeutic strategies for age-related impairment of sWAT browning.

aging, browning, miR-429, UCP1, YAP

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