Control of mammalian cell differentiation by feedback and noise

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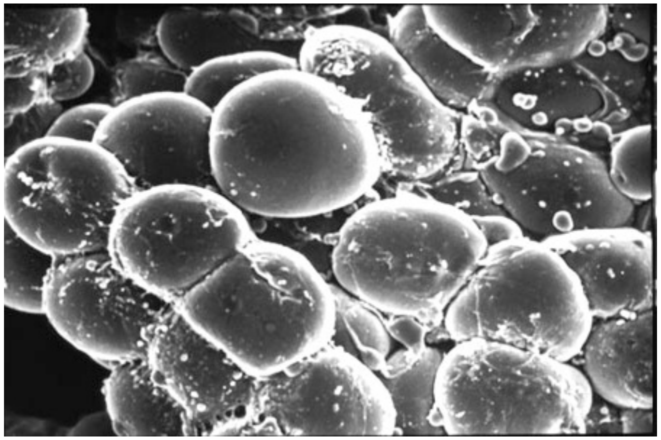
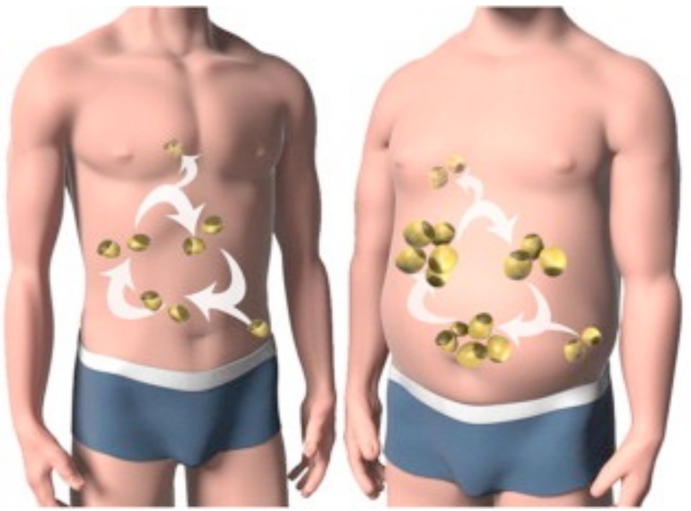
Functioning fat cells are essential



- Regulates glucose levels in the bloodstream
- Stores energy - fat provides as much as 80% to 90% of your body's energy requirements.
- Acts as our body's largest endocrine organ. Produces and secretes key hormones: leptin, adiponectin, IL6, TNF-alpha, angiotensin, resistin
- **Functioning fat cells prevent diabetes, cardiovascular disease and cancer (breast, colon, liver)**

Adipocytes turnover rapidly all throughout adulthood



~ 10% of a person’s fat mass is renewed each year

Adipogenesis occurs continually and controls the number of cells in adipose tissue.

Three parts

1) Fat or not fat: breaking the code of a key cellular decision process

2) Controlling tissue size with feedback and stochastic noise

3) Transcription factor dynamics reveals a circadian code for cell differentiation

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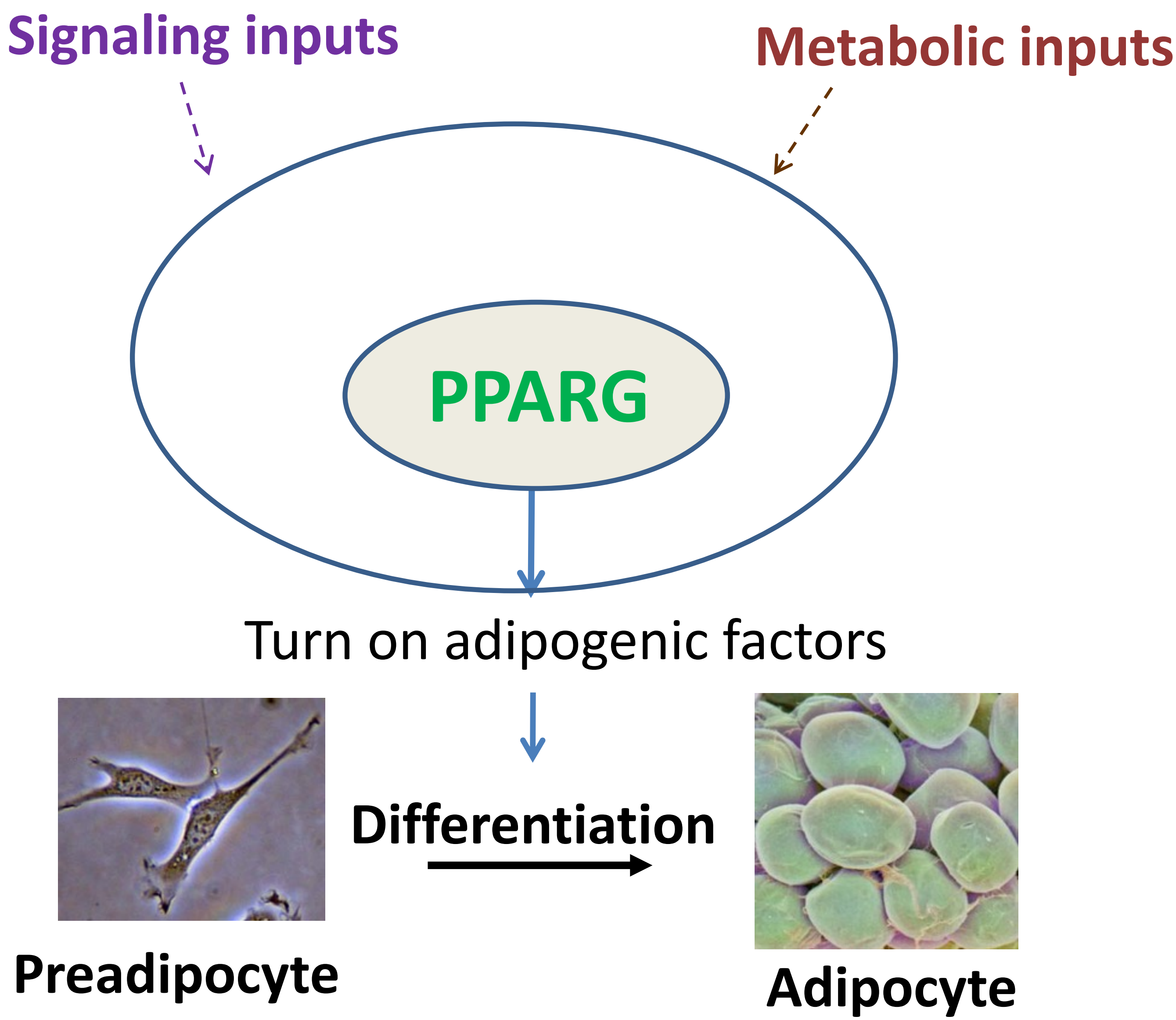
1) Fat or not fat: breaking the code of a key cellular decision process

2) Controlling tissue size with feedback and s noise

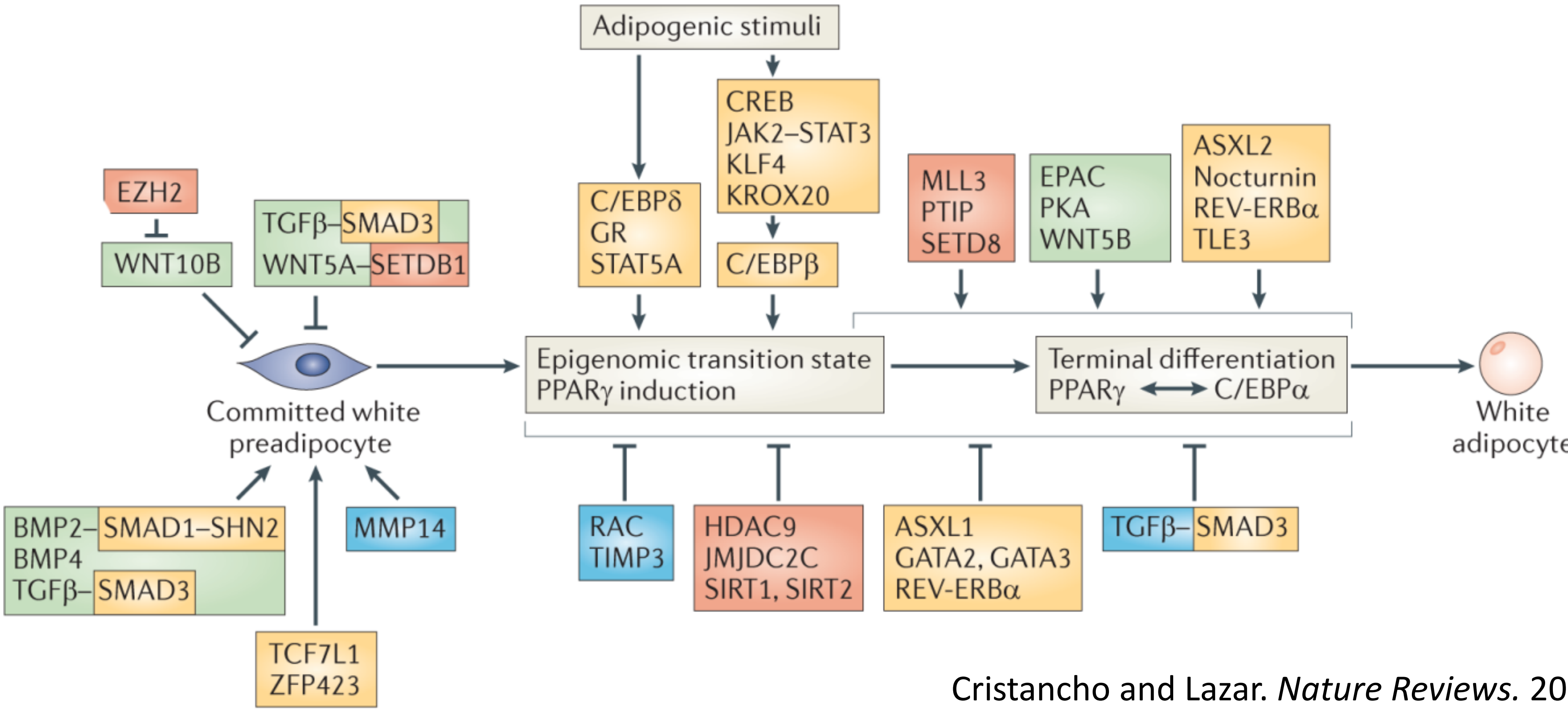
Byung Ouk Park

3) Transcription factor dynamics reveals a circadian code for cell differentiation

Adipocyte (fat cell) differentiation is driven by the expression of PPARG



Many regulatory factors and connections have been implicated in adipogenesis



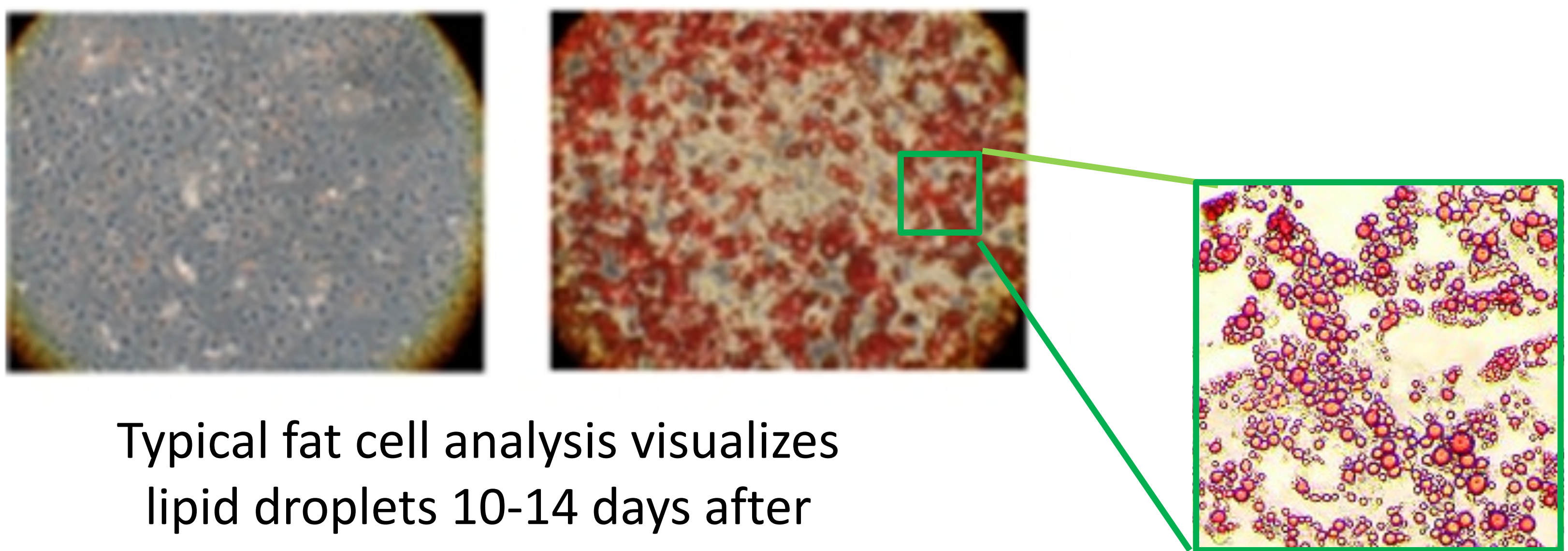
Cristancho and Lazar. *Nature Reviews.* 2011.

Questions:

1) Is there a ***distinct*** terminal differentiation state?

2) If so, what is it and where, when, and how is it created?

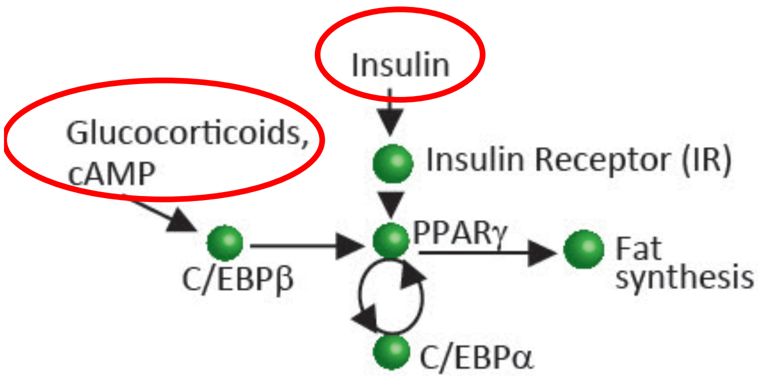
Previous assays for differentiation were often qualitative

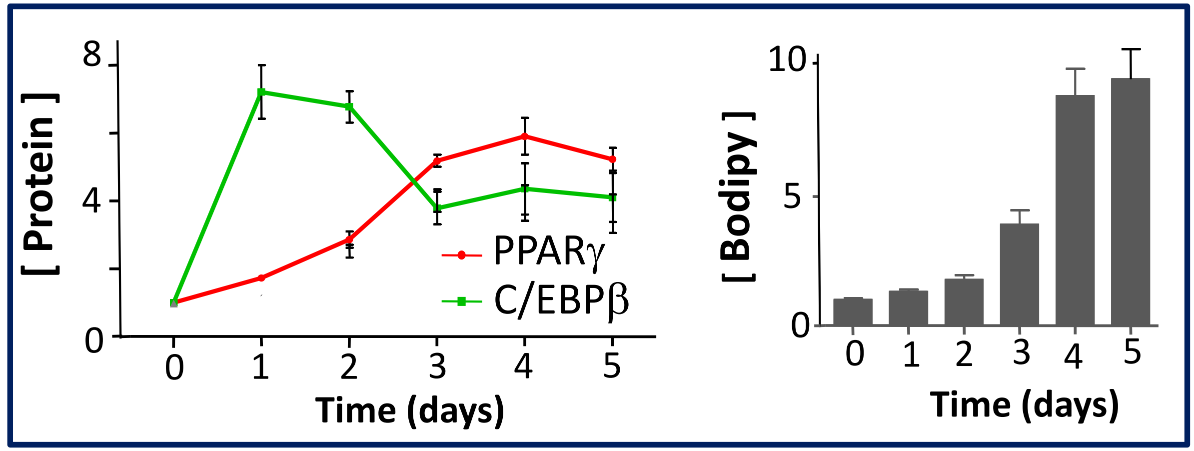


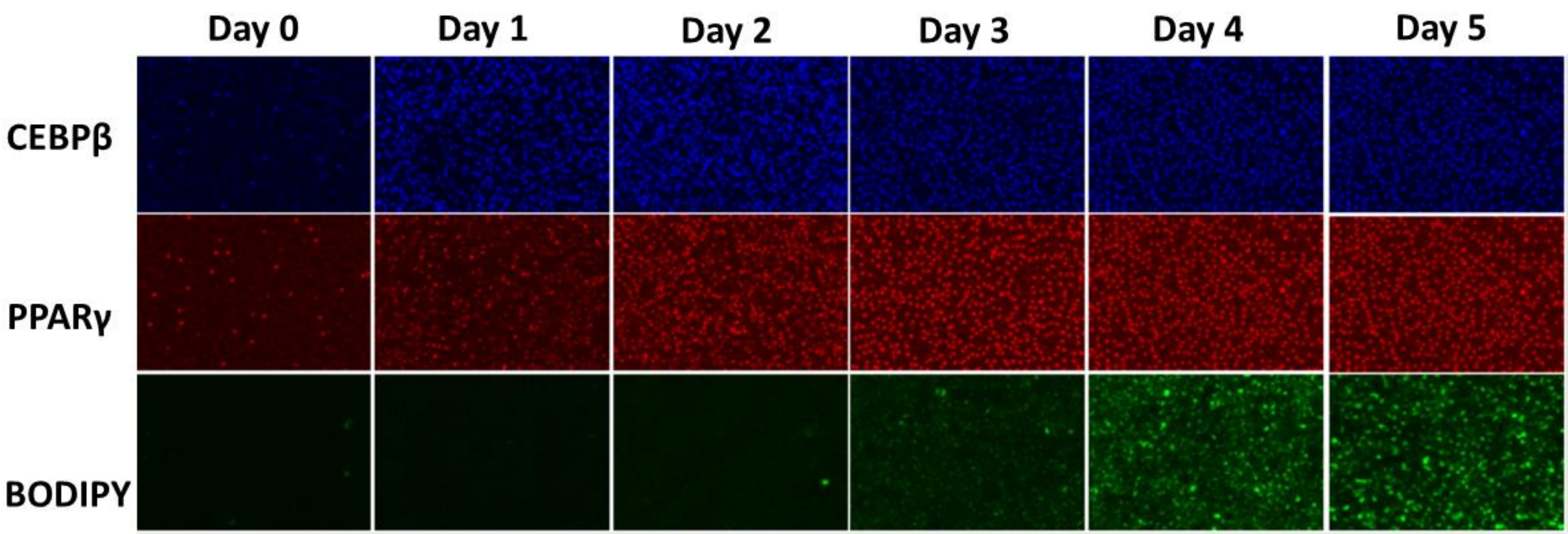
Typical fat cell analysis visualizes lipid droplets 10-14 days after inducing differentiation

A quantitative analysis and model is needed to understand such a complex system.

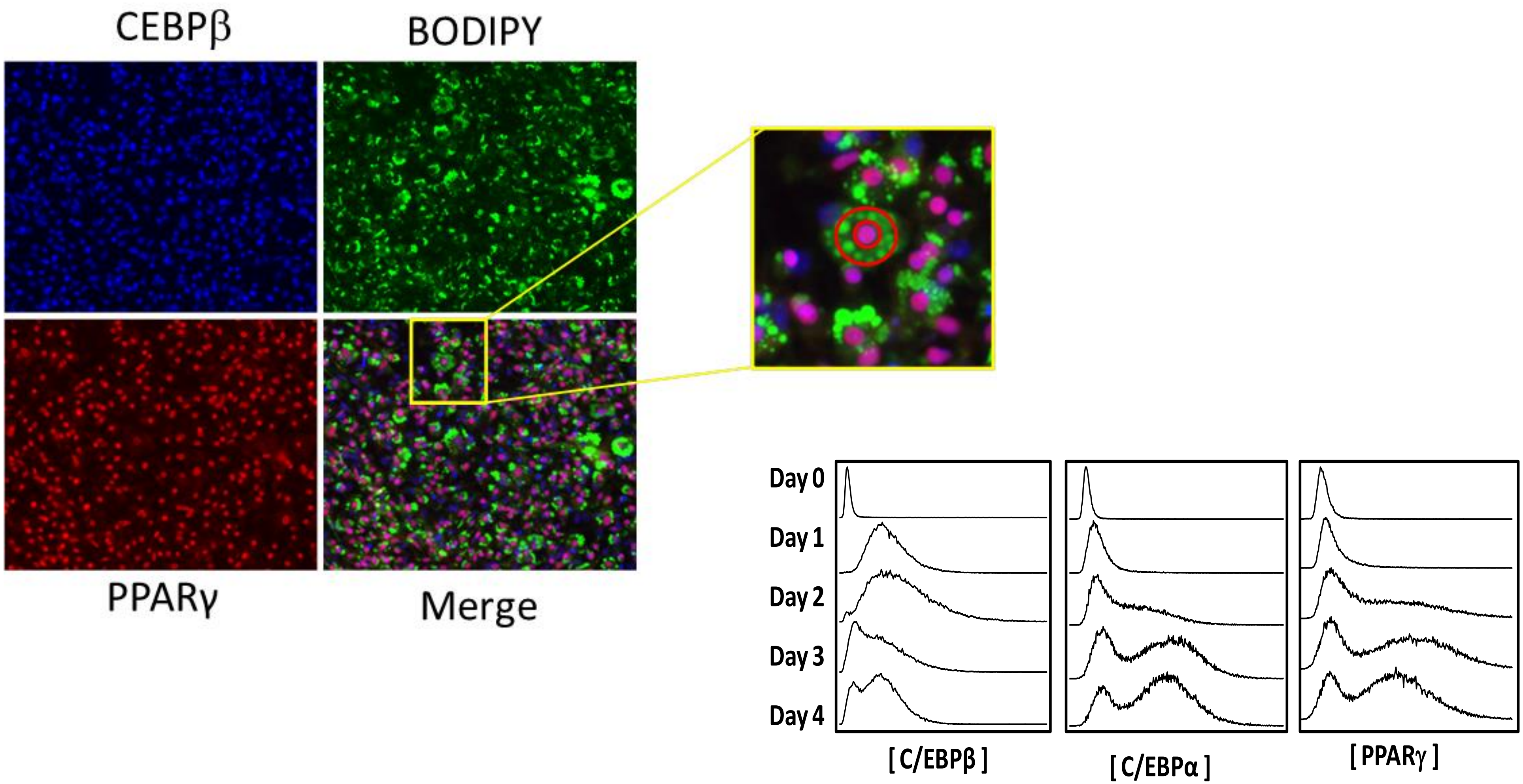
Measuring transcription factor expression and lipogenesis over the timecourse of differentiation



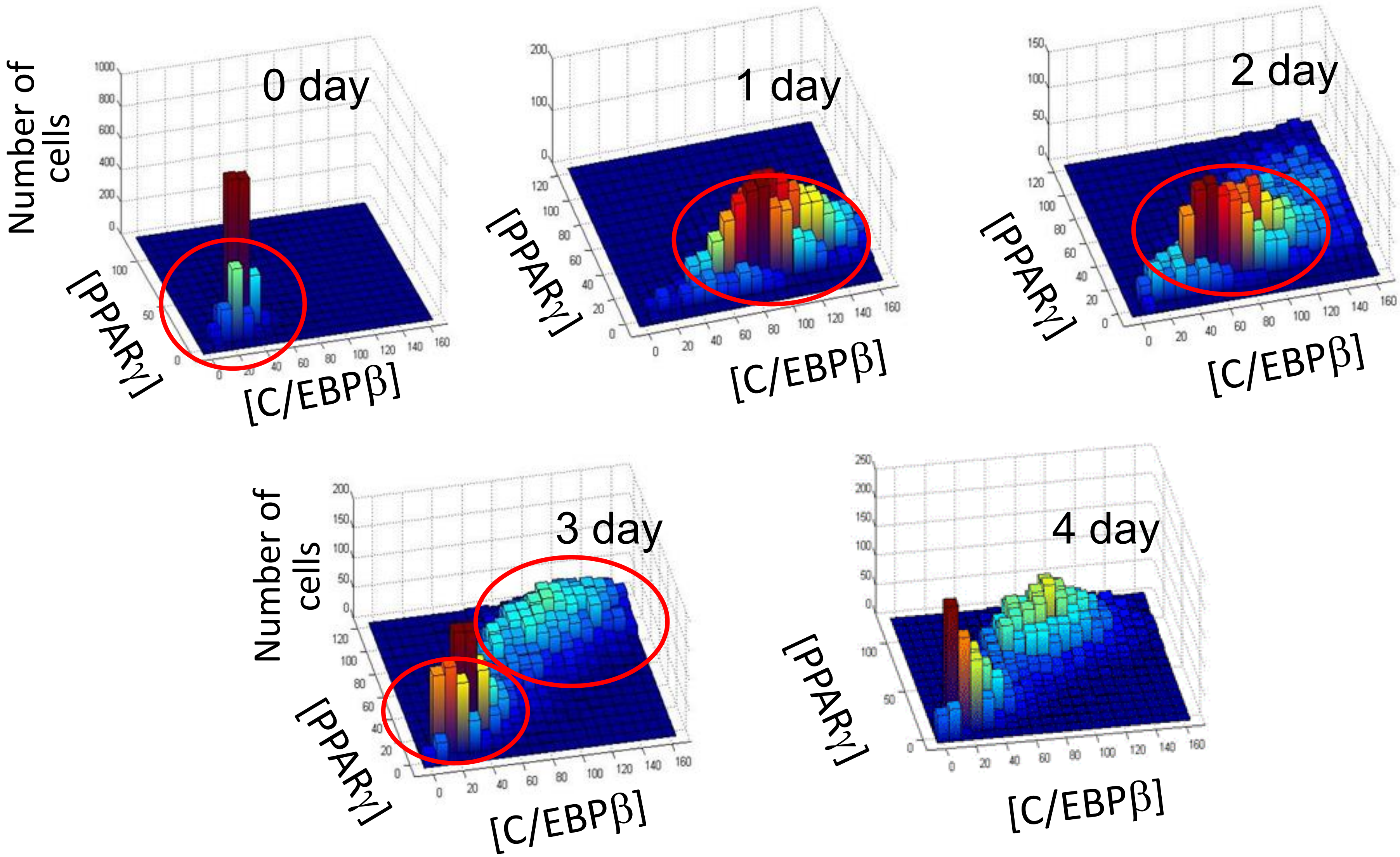




Single-cell, multi-parameter, image-based analysis of the fat cell differentiation process

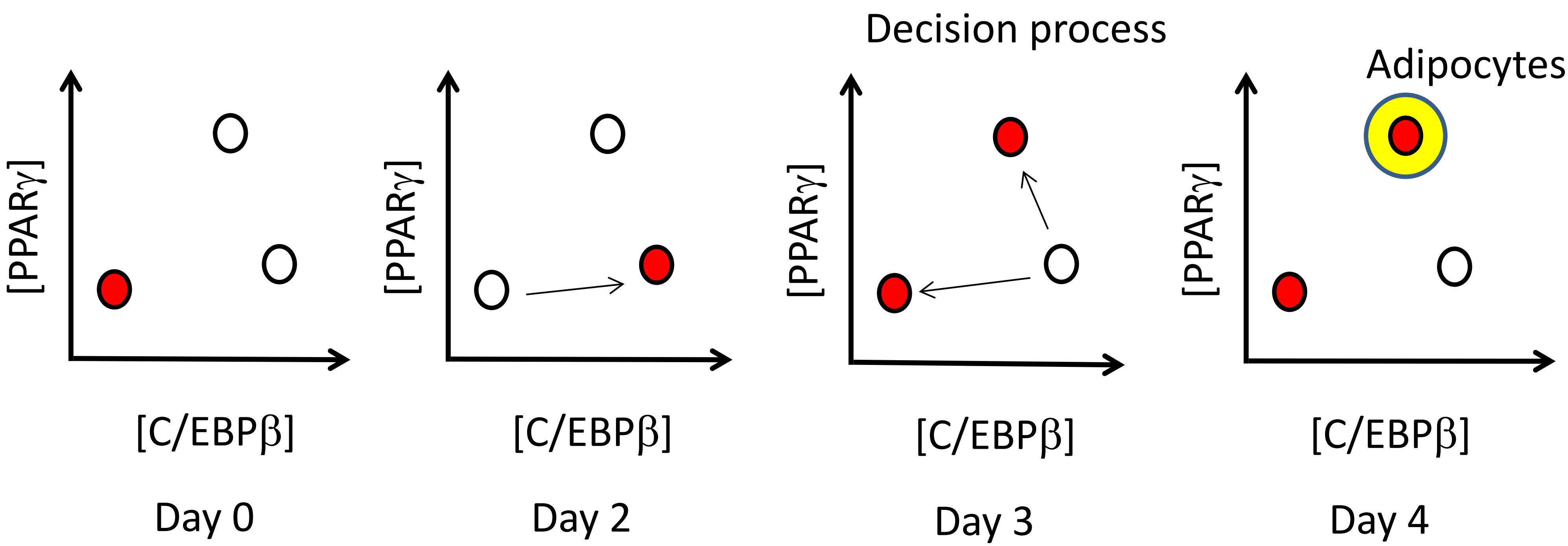


Fat or no fat: breaking the code of a key cellular decision process



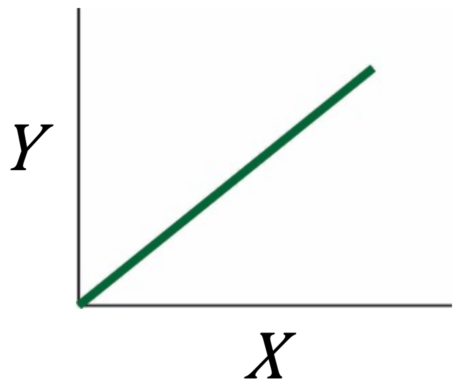
Park,…,Teruel, *Cell Reports* 2012

The cells undergo state transitions early in adipogenesis before lipid accumulation occurs

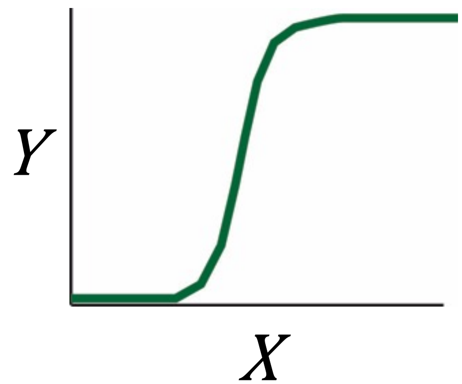


Need to have cooperativity and positive feedback to get two stable states

Cooperativity



$$Y = \beta X$$

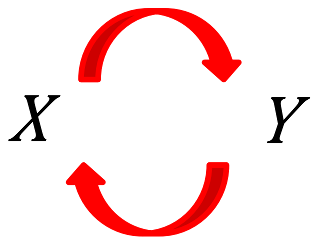


$$Y = \frac{\beta X^n}{EC50^n + X^n}$$

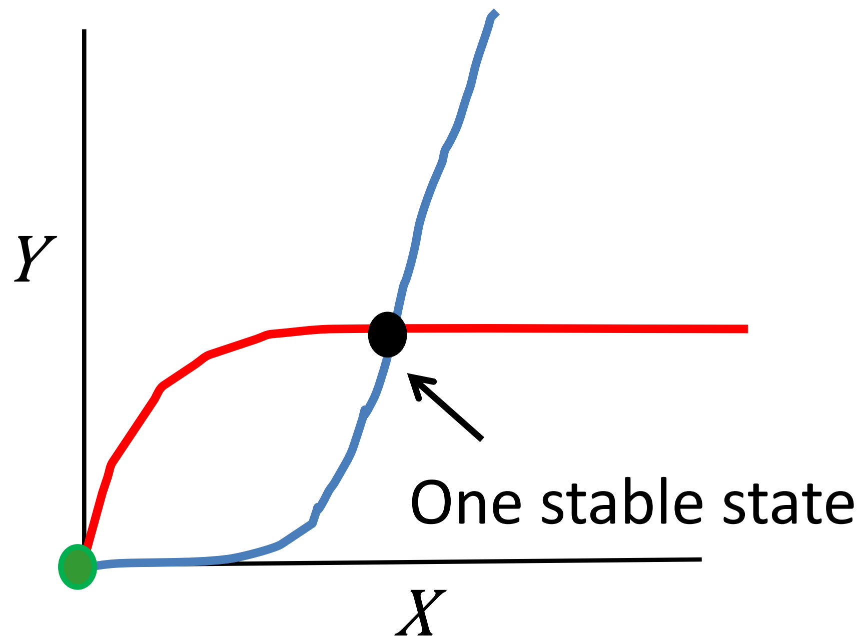
Hill equation

Cooperativity filters small signals out, allowing the system to have a stable off-state

Positive feedback



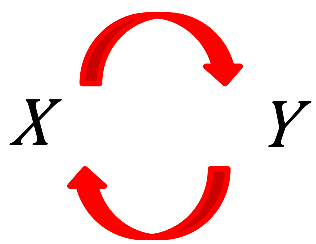
Positive feedback: makes it so the system cannot rest in intermediate states

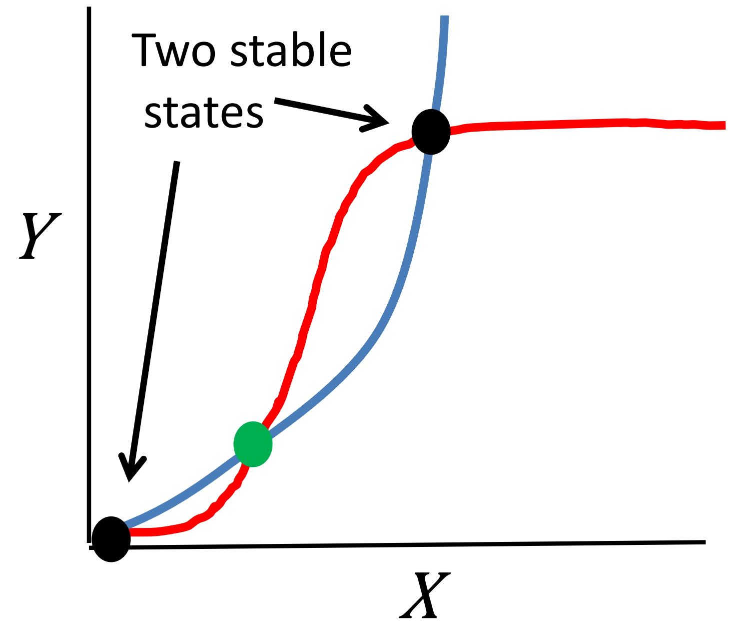


$$\frac{dX}{dt} = \frac{Y}{1+Y} - k_1 X \quad \Longrightarrow \quad X = \frac{1}{k_1} * \left(\frac{Y}{1+Y}\right)$$

$$\frac{dY}{dt} = \frac{X}{1+X} - k_2 Y \quad \Longrightarrow \quad Y = \frac{1}{k_2} * \left(\frac{X}{1+X}\right)$$

Positive feedback + cooperativity





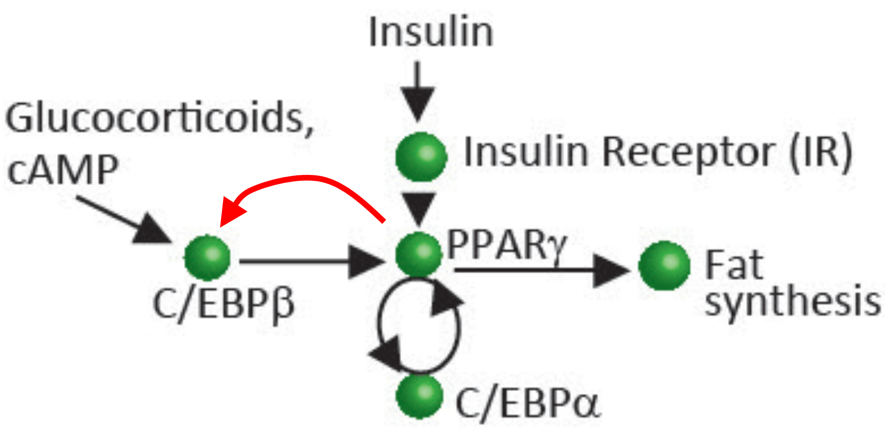
$$\frac{dX}{dt} = \frac{Y}{1+Y} - k_1 X \quad \Rightarrow \quad X = \frac{1}{k_1} * \left(\frac{Y}{1+Y}\right)$$

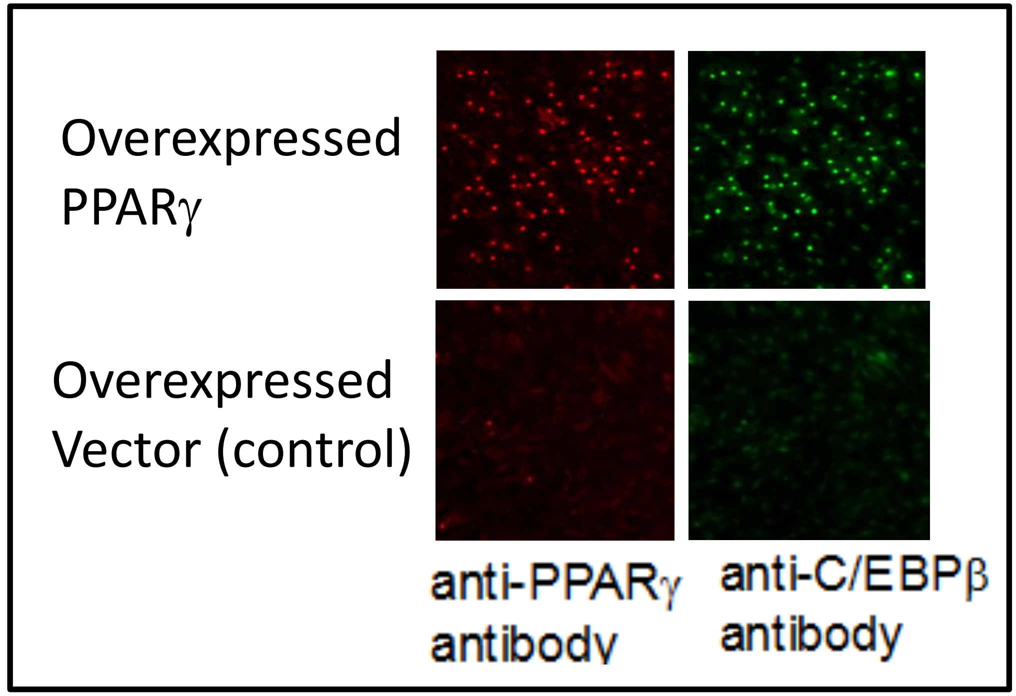
$$\frac{dY}{dt} = \frac{\beta X^n}{EC50^n + X^n} - k_2 Y \quad \Rightarrow \quad Y = \frac{1}{k_2} * \left(\frac{\beta X^n}{EC50^n + X^n}\right)$$

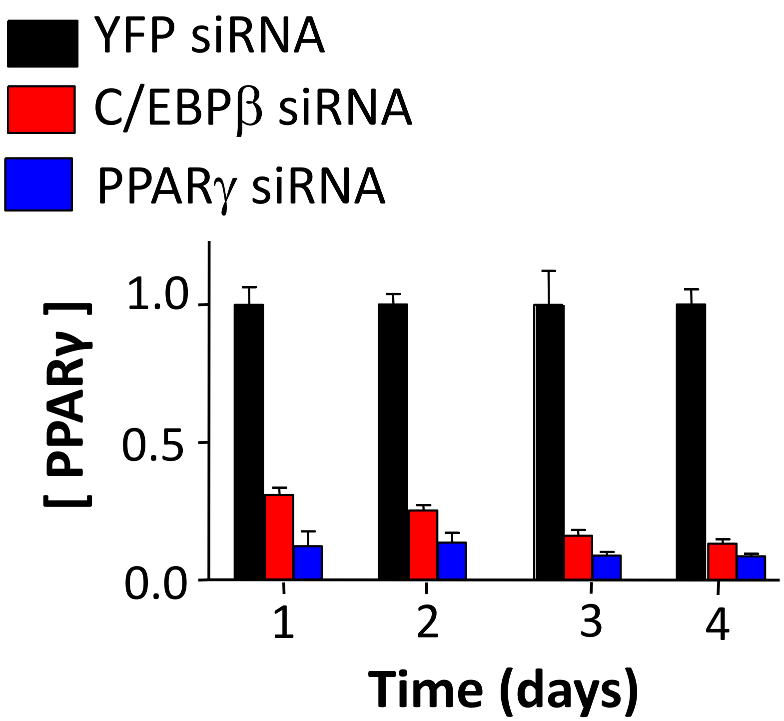
Positive feedback: makes it so the system cannot rest in intermediate states

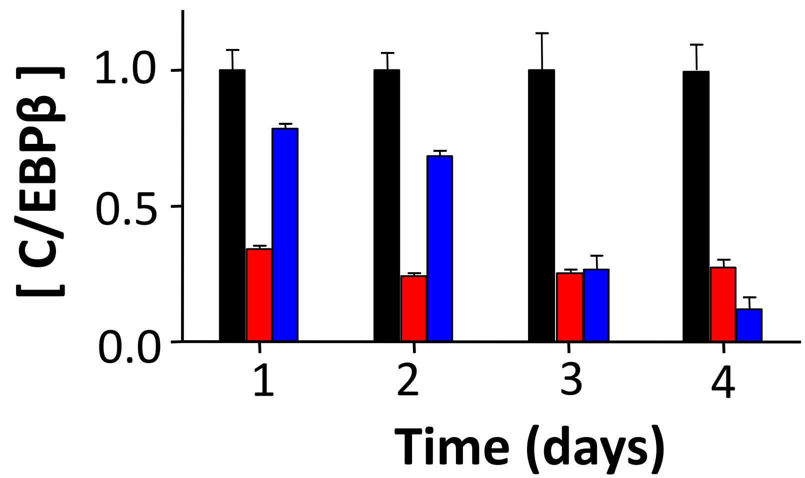
Cooperativity: filters small signals out of the feedback loop, allowing the system to have a stable off-state

Identification of a positive feedback loop from PPARγ to CEBPβ

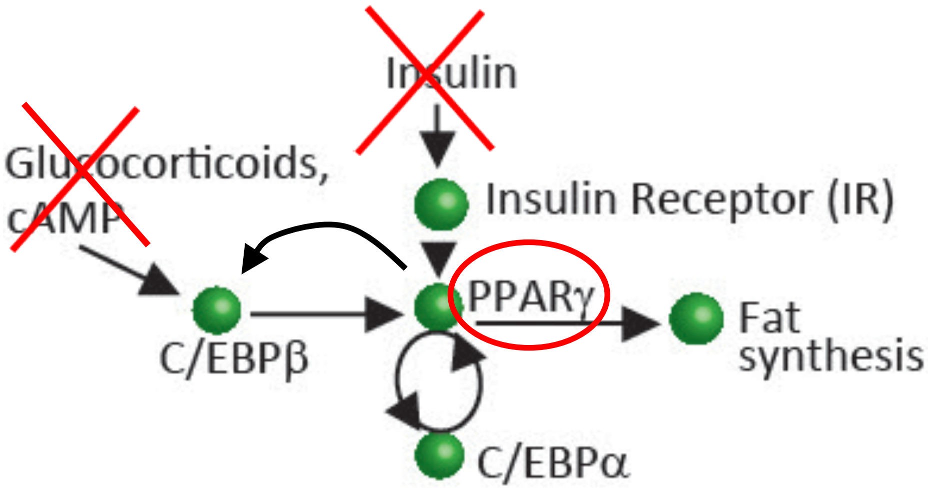


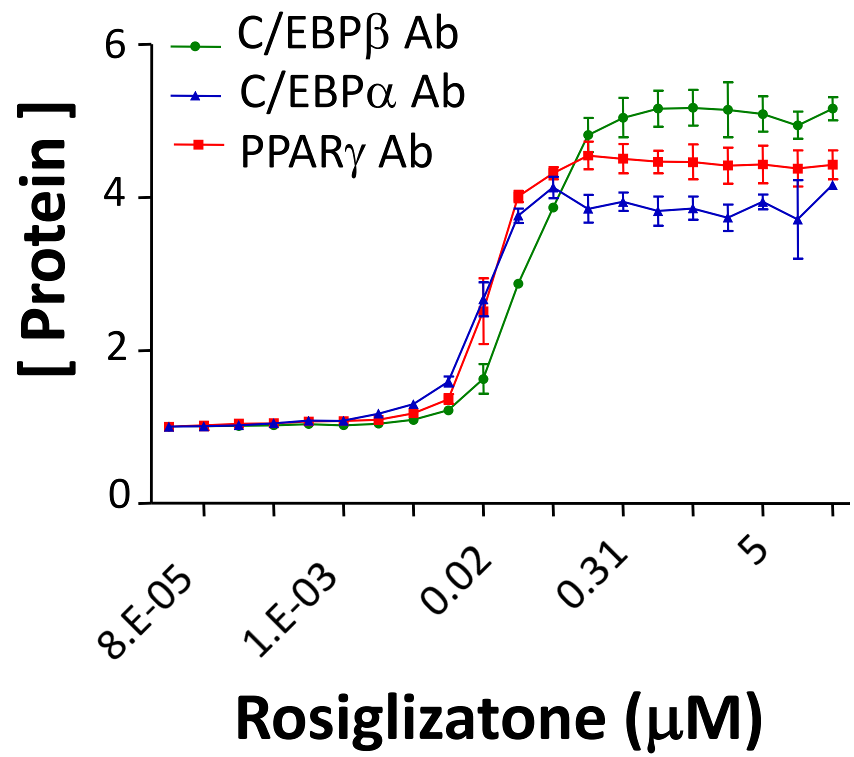


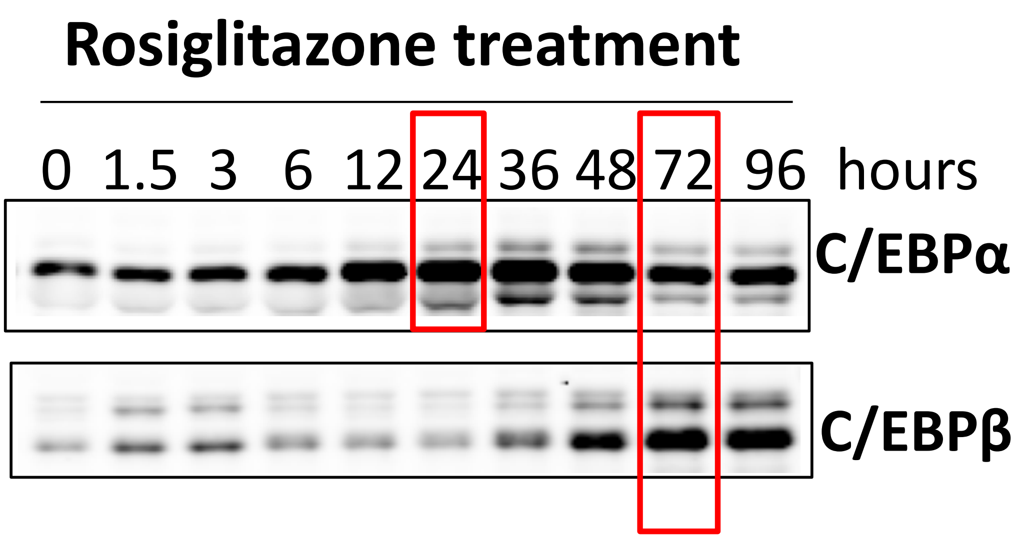




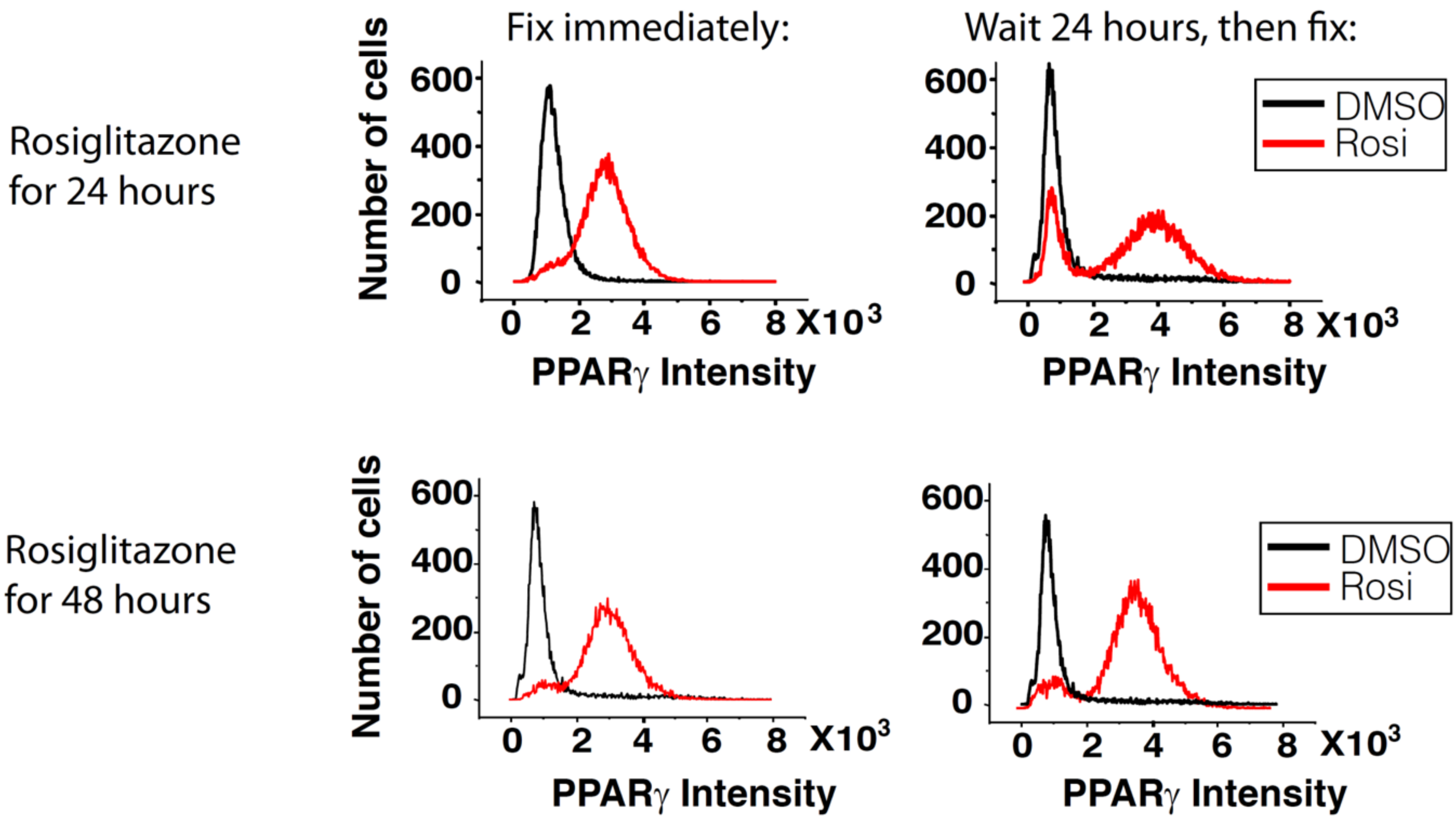
The feedback loops are cooperative and operate at different timescales



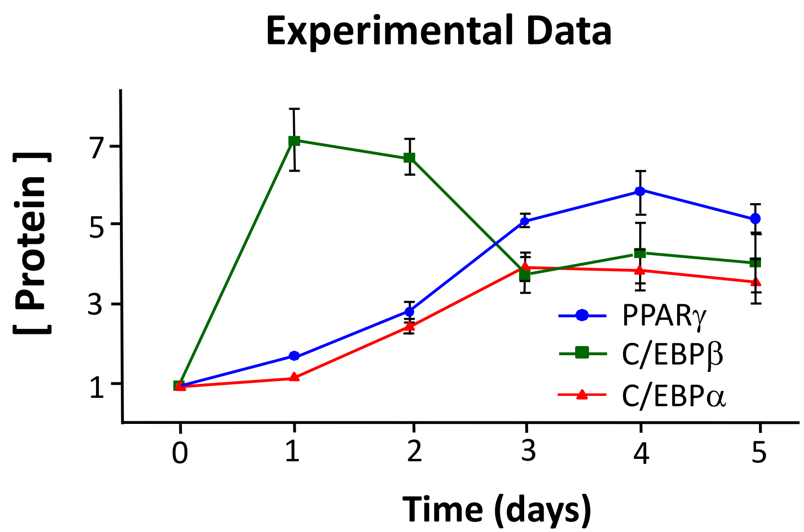


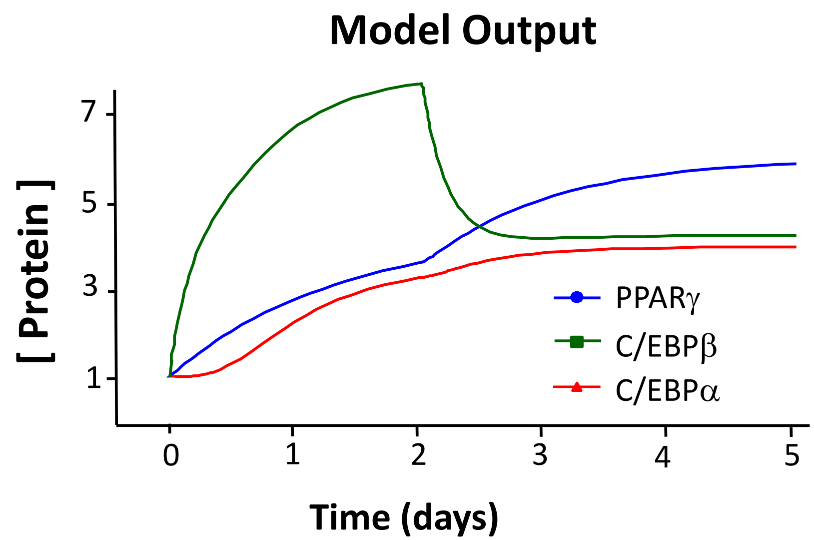


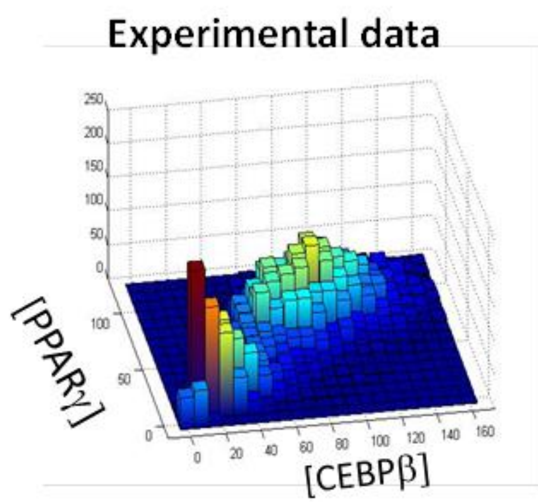
Multiple, consecutive positive feedback loops make the differentiation decision robust and prevent accidental triggering

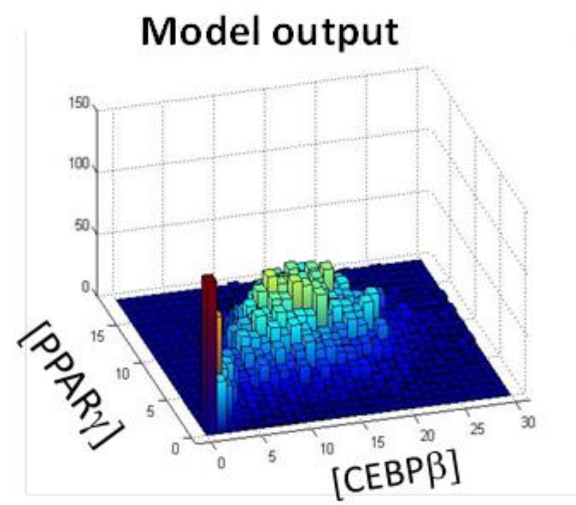


We developed a quantitative molecular model of adipogenesis based on cooperative positive feedback









Summary of Part 1

- We found that fat cell differentiation is an irreversible bistable switch in PPARγ levels triggered early in the differentiation process, well before accumulation of lipid.

- The bistable switch is driven by positive feedback between PPARγ-C/EBPβ and PPARγ-C/EBPα.

- Using our experimental data, we developed the first quantitative molecular model showing how cooperative positive feedback makes adipocyte differentiation robust and irreversible.

Three parts

1) Fat or not fat: breaking the code of a key cellular decision process

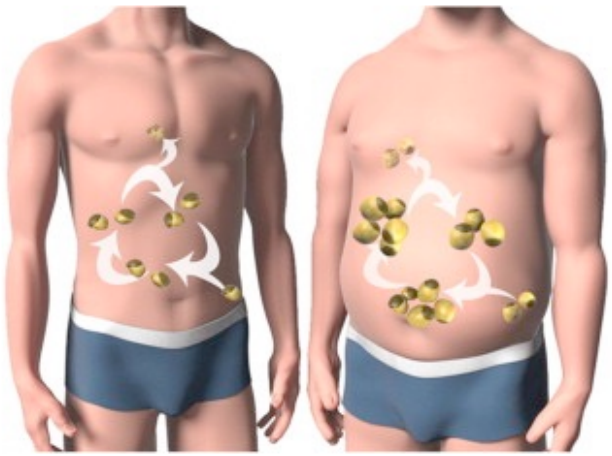
2) Controlling tissue size with feedback and stochastic noise

3) Transcription factor dynamics reveals a code for cell differentiation

Robert Ahrends

Adult mammalian tissues are regenerating themselves at very low rates

Cardiomyocytes renew at a rate of ~1% each year



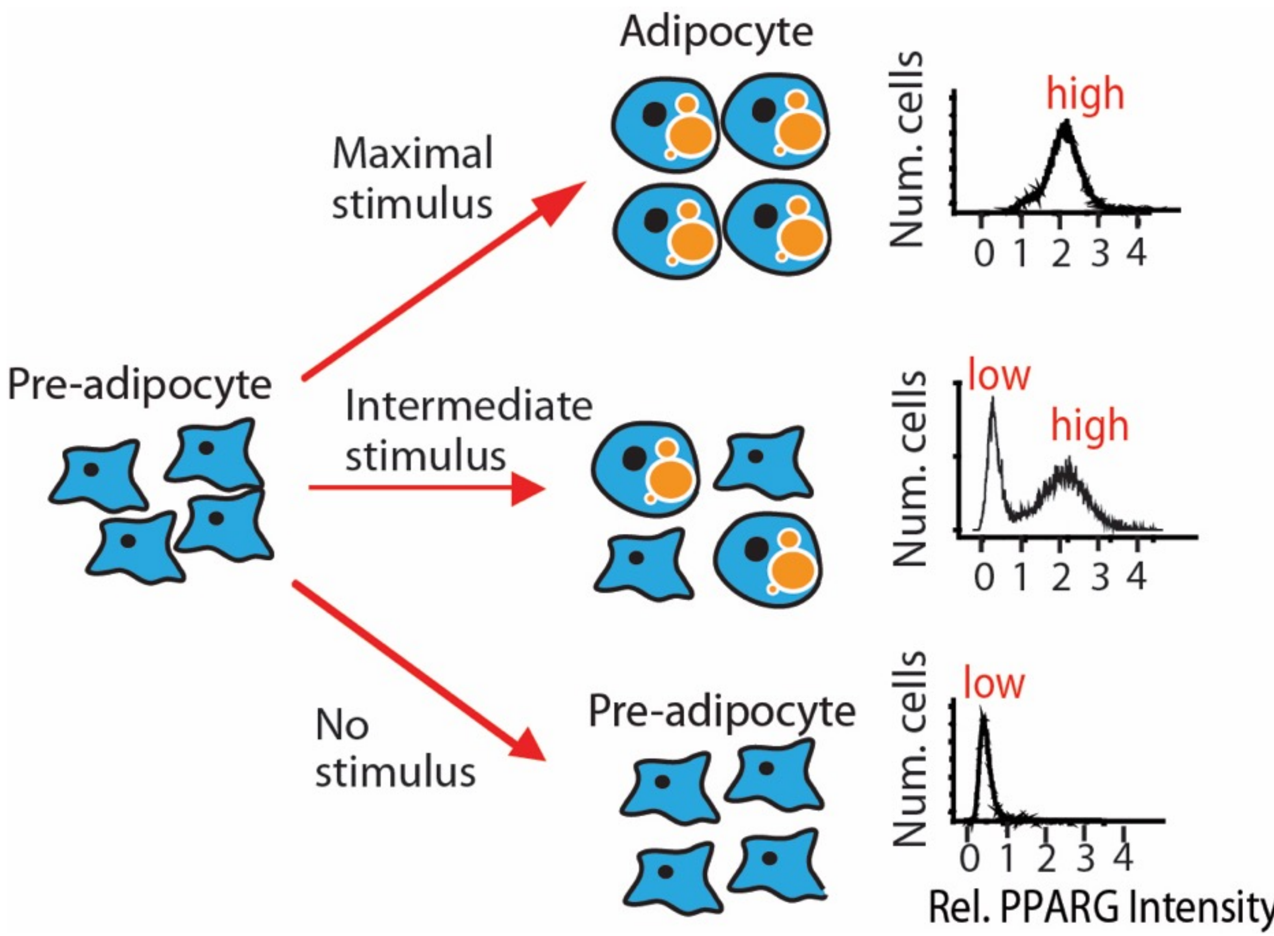
Fat cells (adipocytes) renew at a rate of ~10% each year

(Spalding et al., Nature 2008)

Too low or too high a rate can cause aging and disease

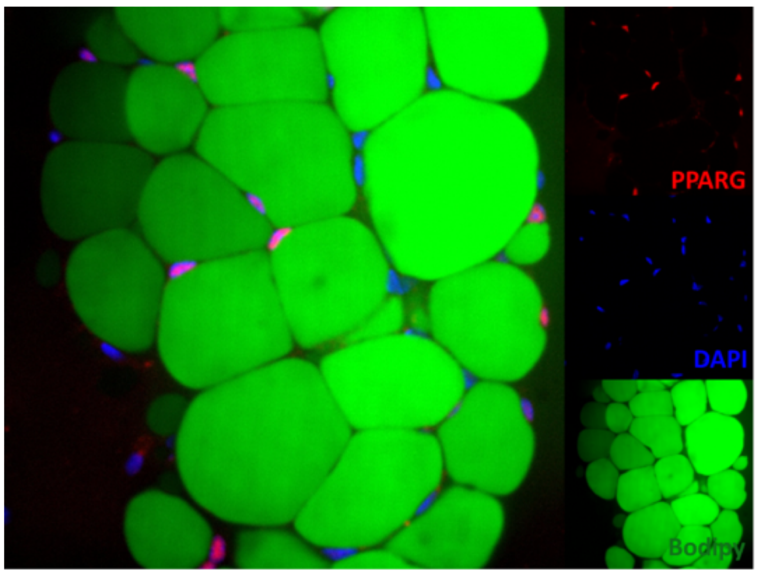
What enables a constant, low-rate of cell differentiation in humans?

The conversion of preadipocyte to adipocyte occurs via a bistable switch

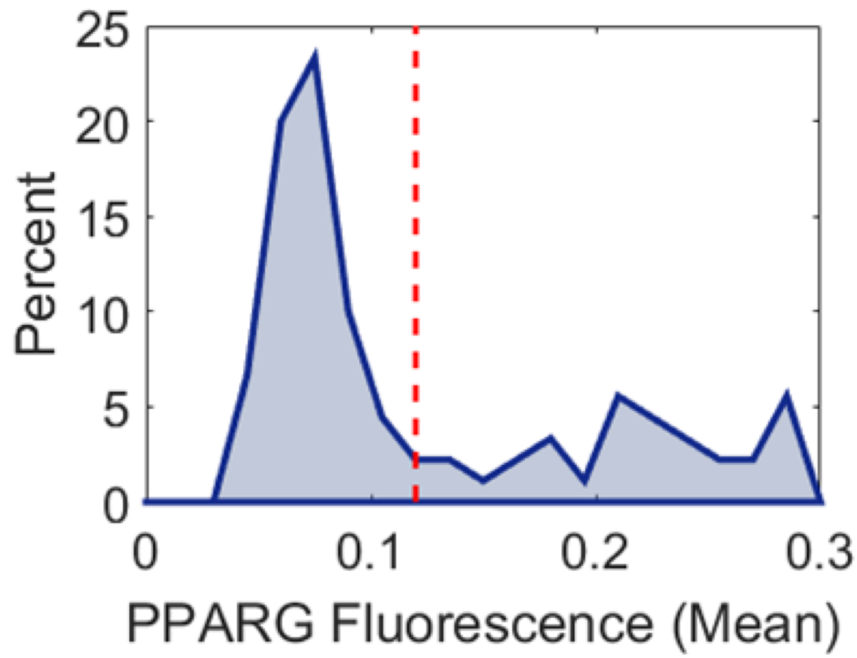


Park,…,Teruel, *Cell Reports* 2012

Preadipocytes differentiate via a bistable switch *in vivo*

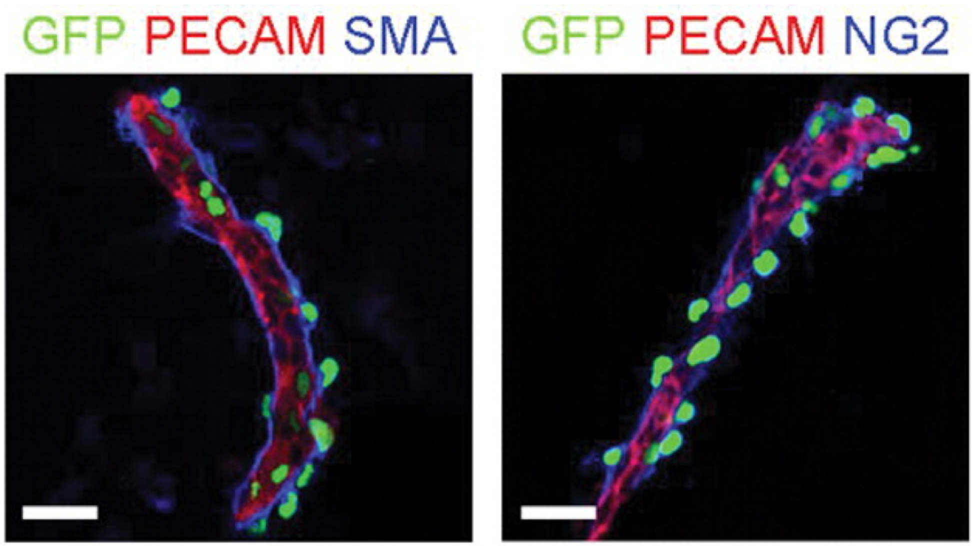


de novo fat pad from a mouse
5 weeks post- injection



Adipocyte precursor cells (preadipocytes) reside in the fat tissue along the vasculature

….and there are a lot of them!



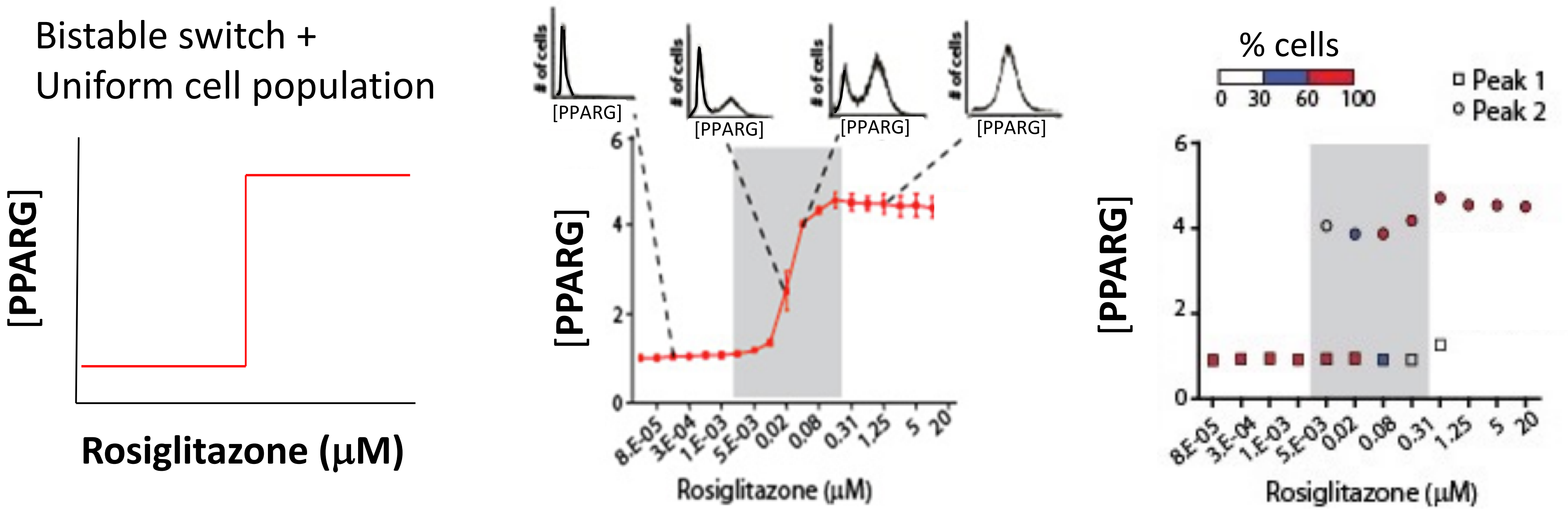
GFP = preadipocytes
PECAM (red) = endothelial cell marker

~1 preadipocyte to 5 adipocytes

Tang,…Graff, *Science* 2008

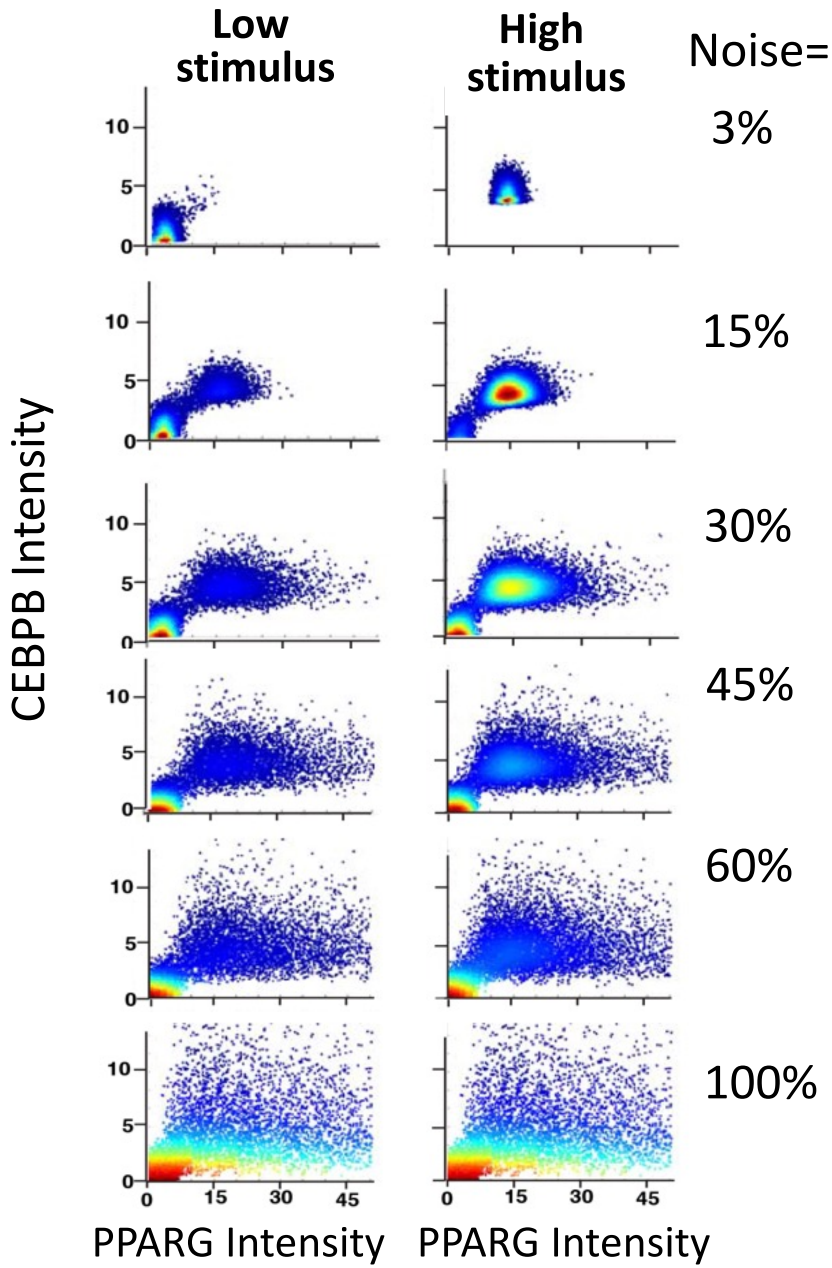
Since fat cell differentiation is a bistable process, why do only 10% of our fat cells turn over each year?

Shouldn't all our cells either differentiate or stay undifferentiated for the same given stimulus?



What controls the fraction of precursor cells that differentiate?

Computer simulations show that cell-to-cell variation (noise) controls the number of cells that differentiate



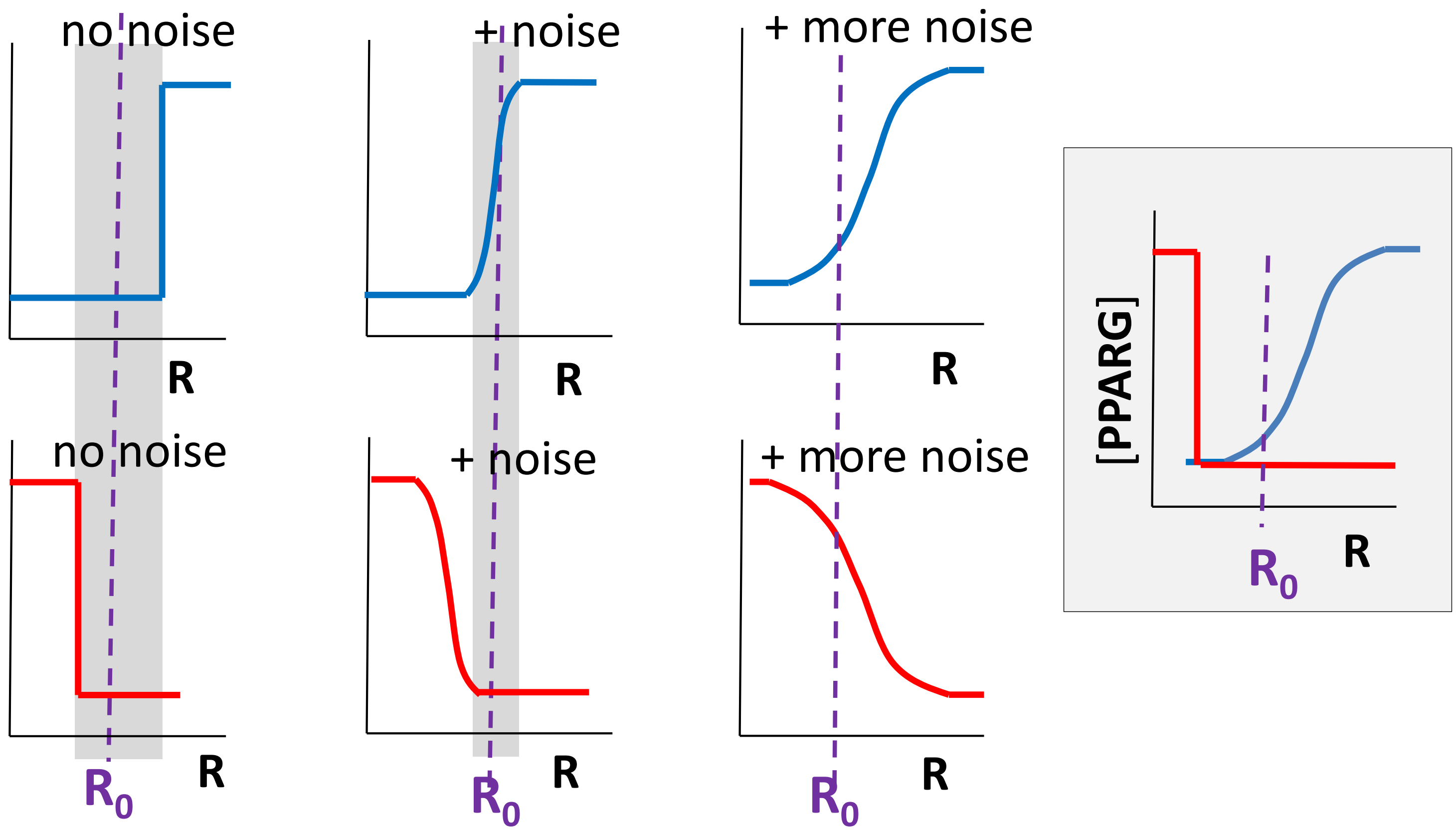
Noise has to be in the right range to enable optimal control of differentiation.

Park,…,Teruel, *Cell Reports* 2012

A fundamental problem in maintaining tissue size: how to obtain the right amount of noise:

Fraction of precursor cells that differentiate

Dedifferentiation: Fraction of adipocyte cells that lose the differentiated state

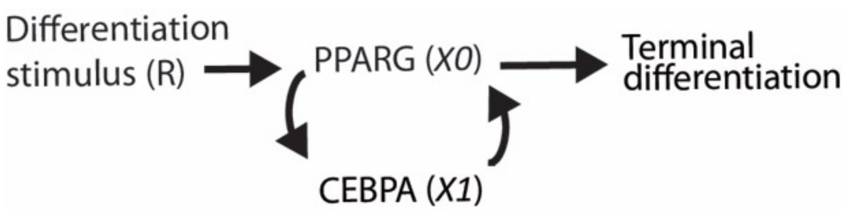


With too little noise, impossible to control by receptor stimulus the fraction of cells that differentiate.

With too much noise, impossible to create a bistable system that is irreversibly locked in a differentiated state.

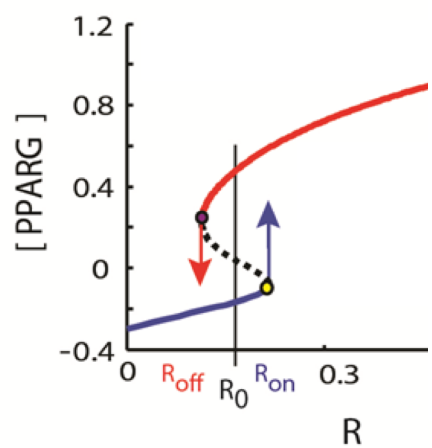
What system architecture are cells using to maintain tissue size?

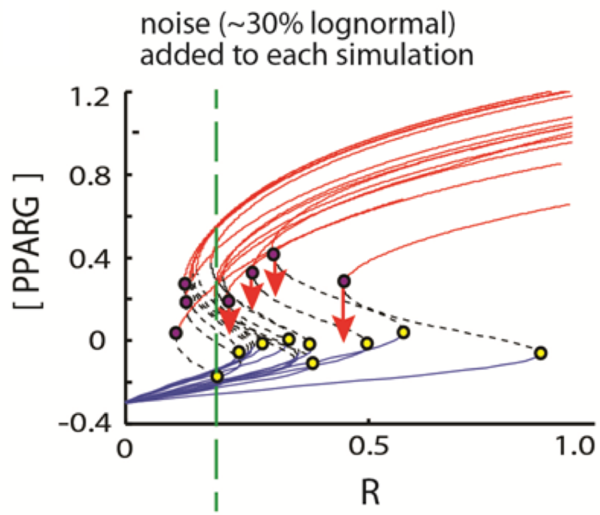
A one-feedback loop model

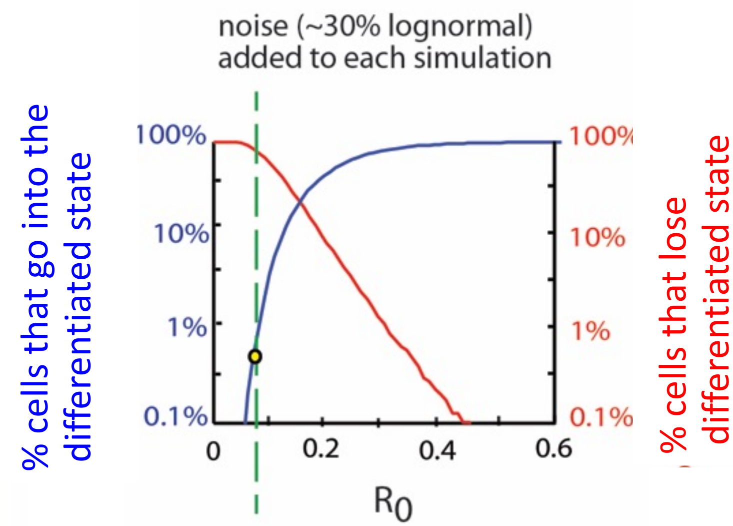


$$\frac{dX_0}{dt} = \varepsilon_0 * R * \left(1 + \alpha * \frac{X_1^3}{1 + X_1^3}\right) - X_0$$

$$\frac{dX_1}{dt} = \varepsilon_1 * X_0 - X_1$$

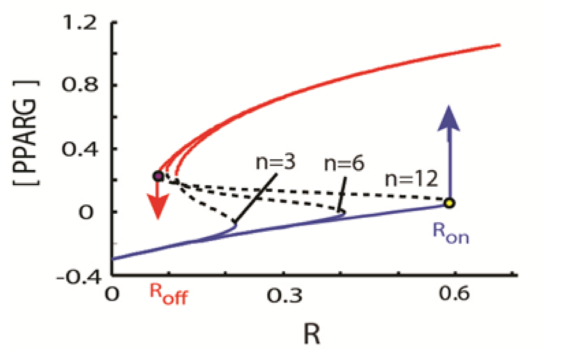


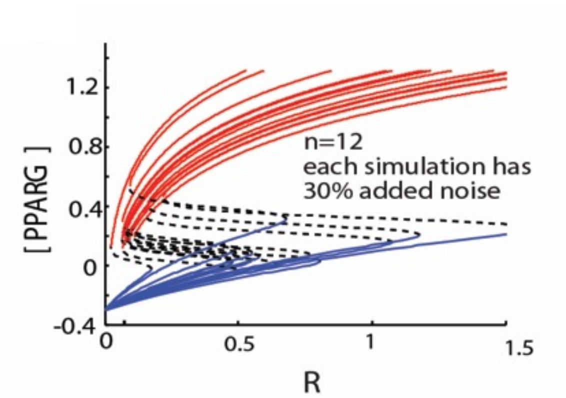




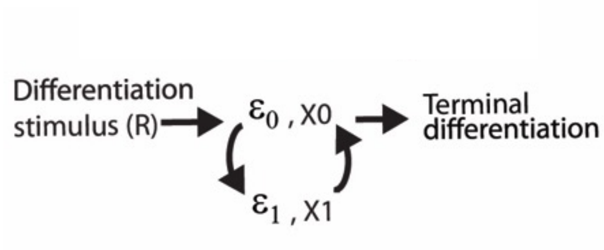
Ahrends,...,Teruel, *Science* 2014.

How are cells solving the optimization problem?



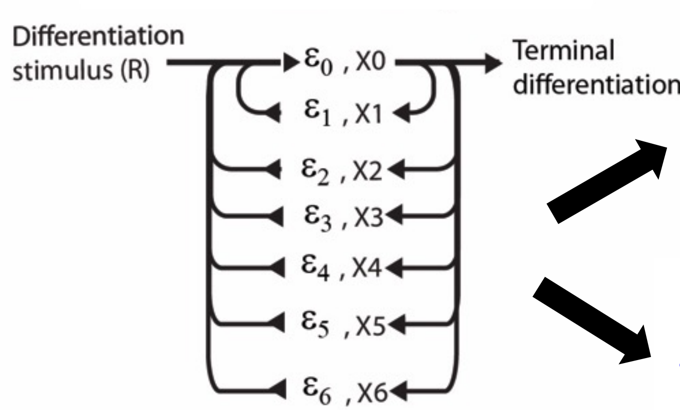


Increasing the cooperativity helps, but still does not solve the optimization problem!



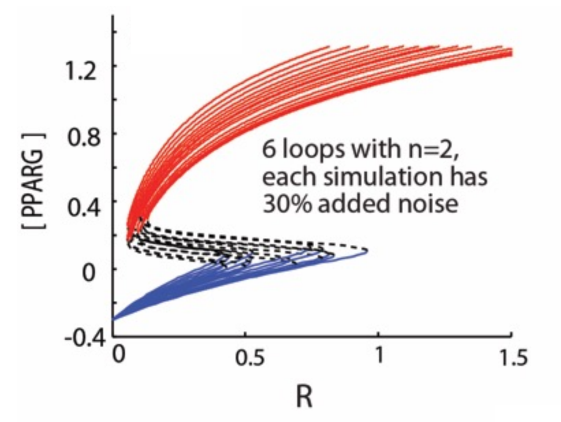
Cooperativity, n=12

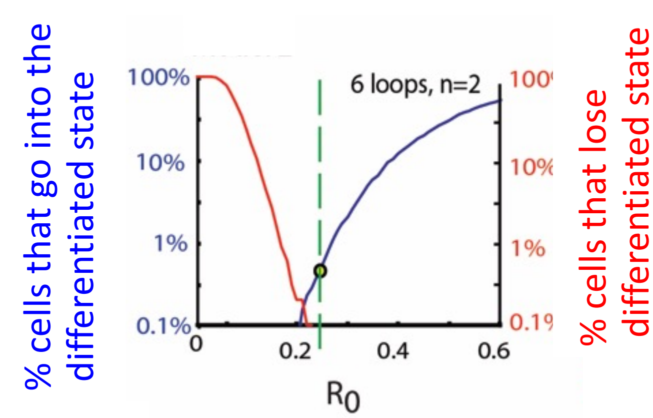
total noise = 12 * ε_0



Cooperativity, n=12

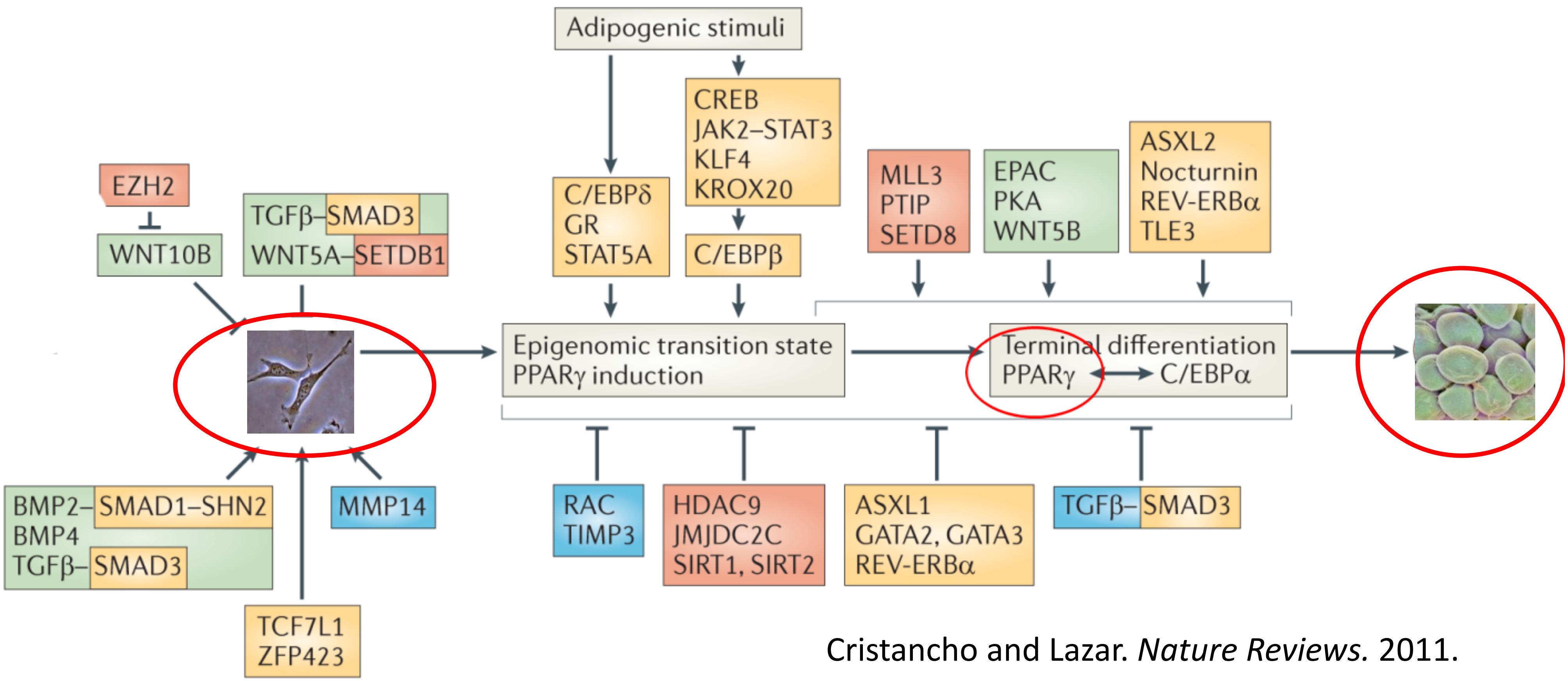
total noise = 4.9 * ε_0





Systems with ultra-high feedback can solve the optimization problem

Over a hundred factors have been implicated in regulating fat cell differentiation

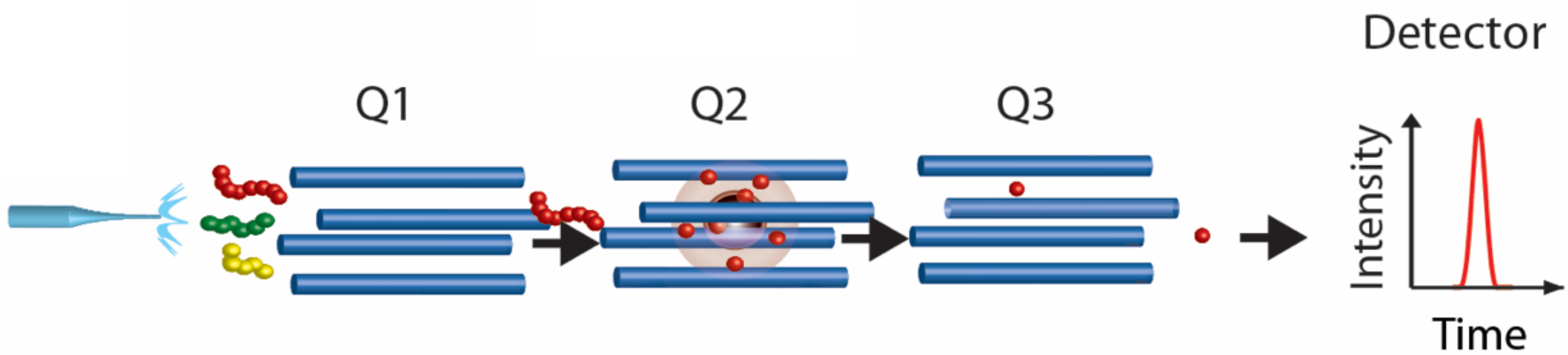


Cristancho and Lazar. *Nature Reviews.* 2011.

How can we systematically identify feedback loops in a protein network??

Expanded on targeted proteomics methods that we developed in Abell,…,Teruel, *PNAS* 2011

Selective reaction monitoring (SRM) using a triple-quadropole mass spectrometer

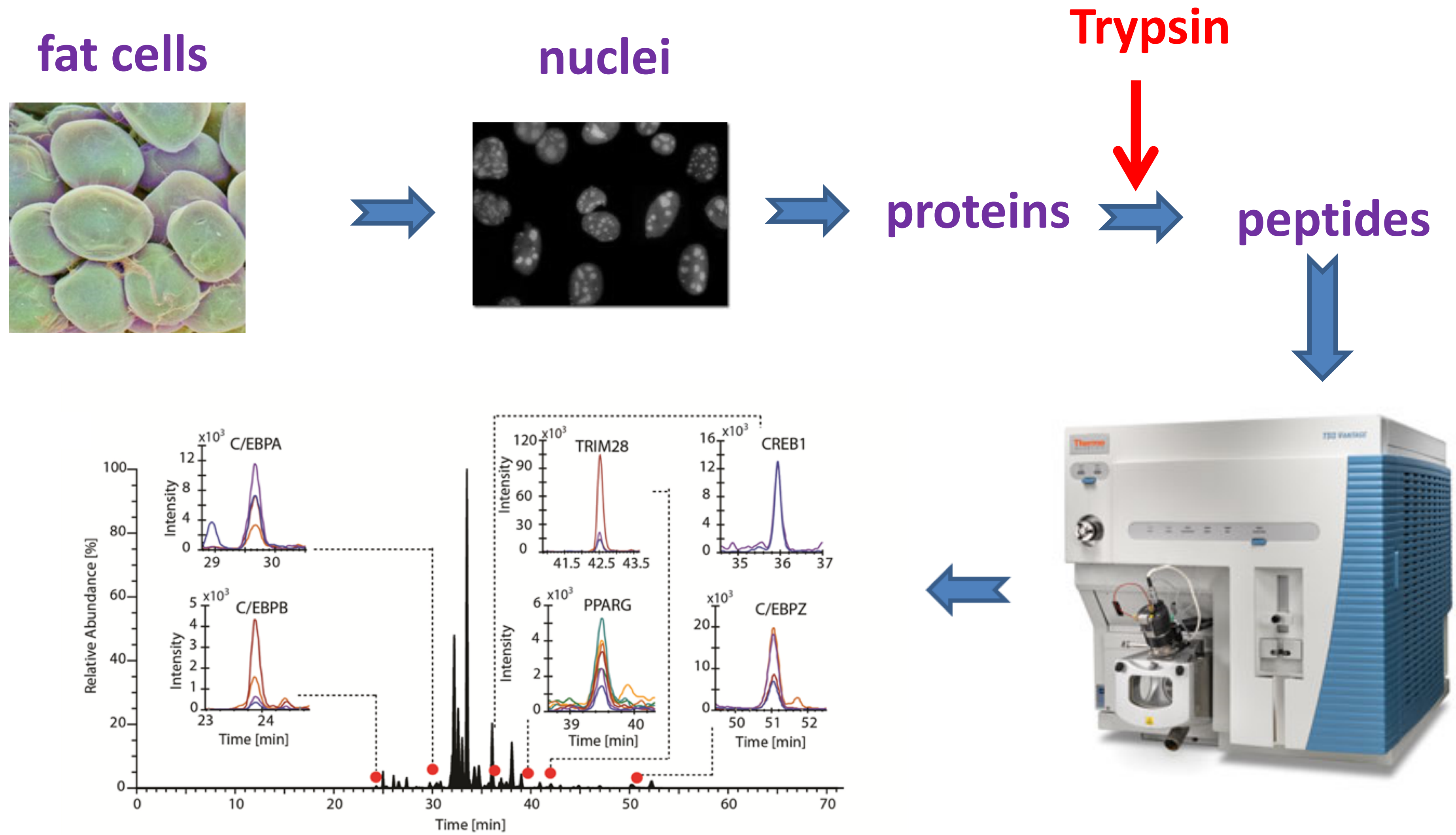


You need to know what peptides to look for !

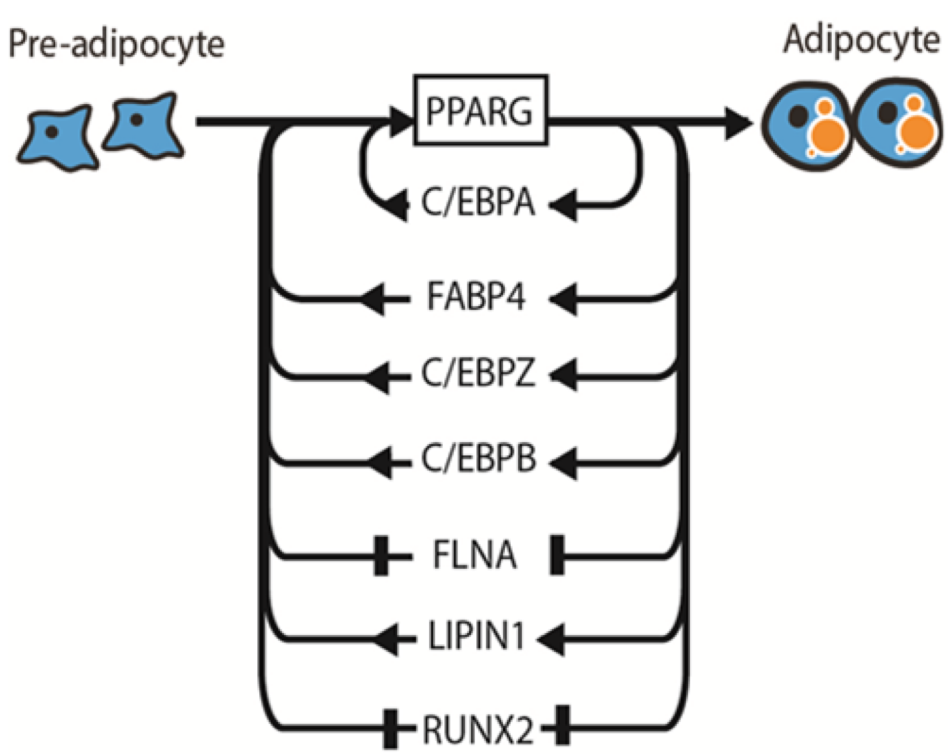
Proteotypic peptide should be:

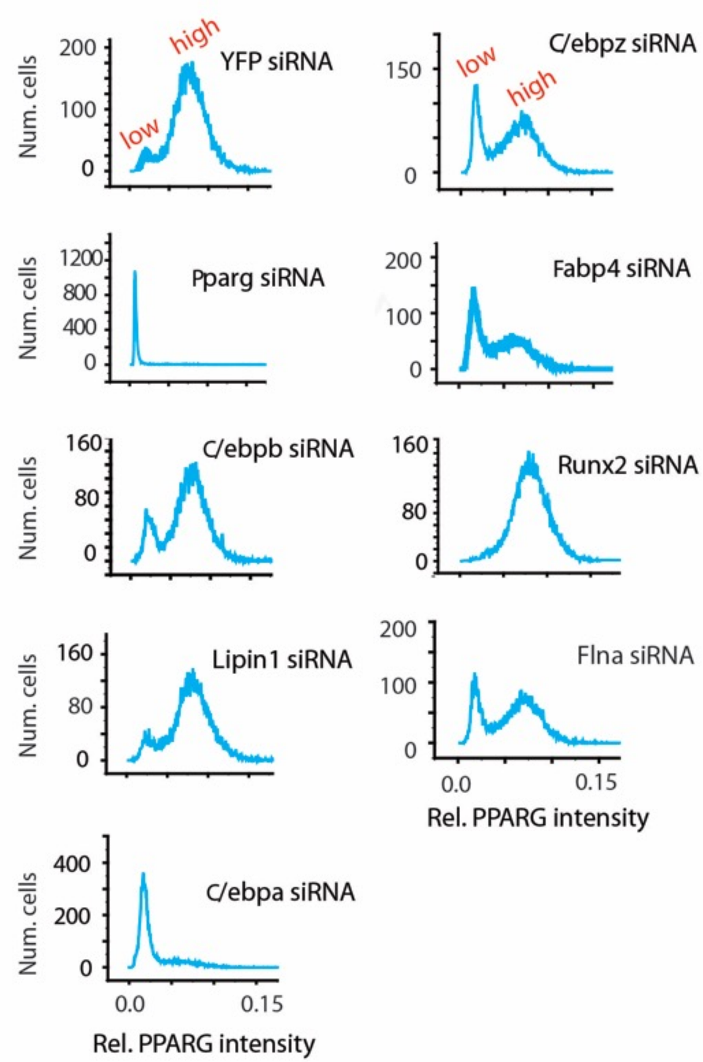
1) Unique to your protein
2) "Flies" well in the mass spectrometer
3) No posttranslational modifications, chemical-induced modifications, missed-cleavage

Using SRM mass spectrometry to simultaneously measure 100 key, but low-abundant, adipogenic regulators in a single sample



Protein abundance noise acts within a network of at least 7 positive feedbacks to permit preadipocytes to differentiate at very low rates





Ahrends,…,Teruel, *Science* 2014.

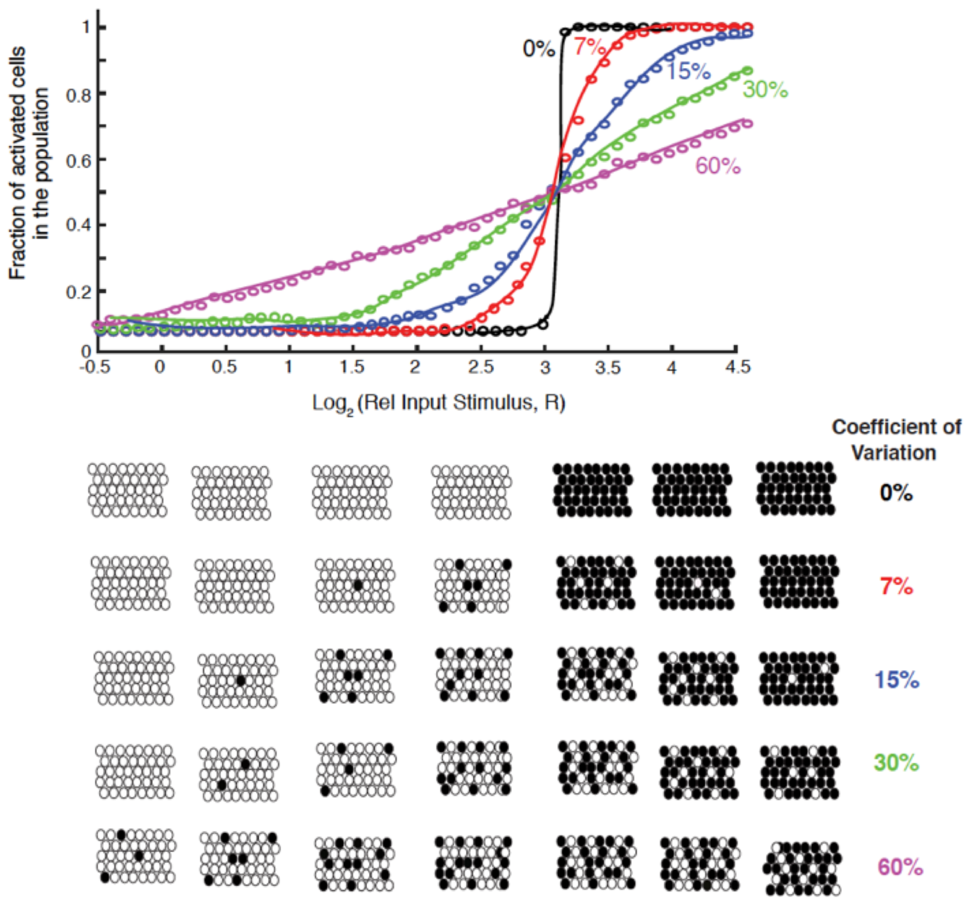
Summary of Part 2

- Theoretical noise analysis argues that highly connected multi-feedback systems can resolve the challenge to control at the same time low rates of differentiation and also lock differentiated cells in the differentiated state.

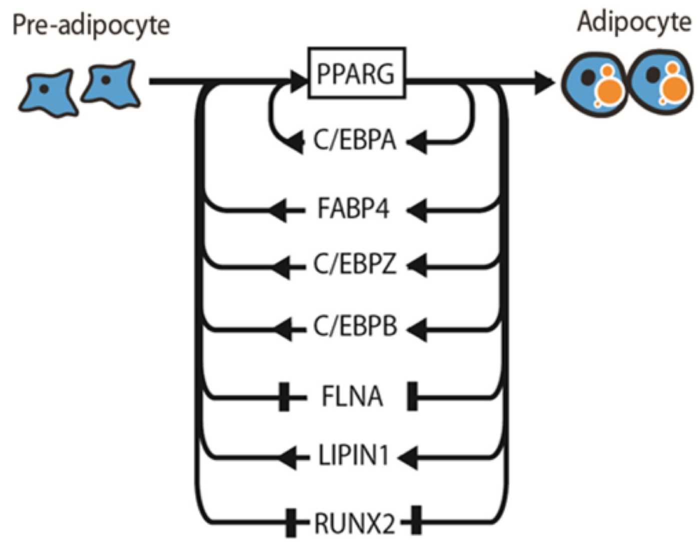
- Using highly-sensitive and quantitative selected reaction monitoring (SRM) mass spectrometry, we showed that adipogenesis is driven by at least 7 interconnected positive feedback loops.

- Together, these results provide a conceptual framework of how organisms use noise to effectively control low rates of differentiation without sacrificing the robustness of the differentiated state.

To control which fraction of a population makes an all-or-none decision, alot of noise is good

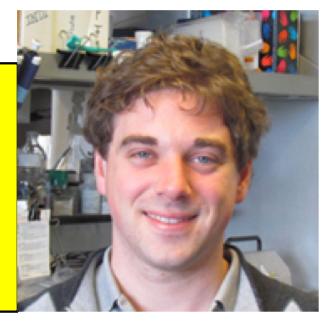


But too much noise can cause cells to drop out of the differentiated state



Ahrends,…,Teruel, *Science* 2014.

Kovary,…,Teruel, “Expression variation and covariation impair analog and enable binary signaling control”, *Molecular Systems Biology,* May 2018.



Three parts

1) Fat or not fat: breaking the code of a key cellular decision process

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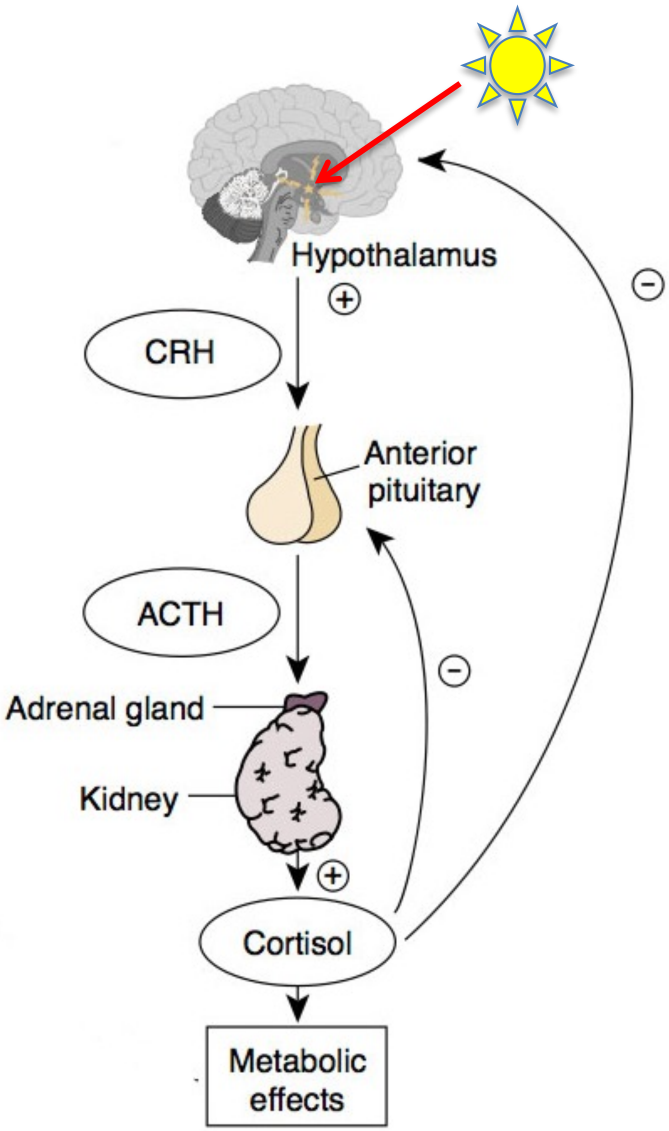
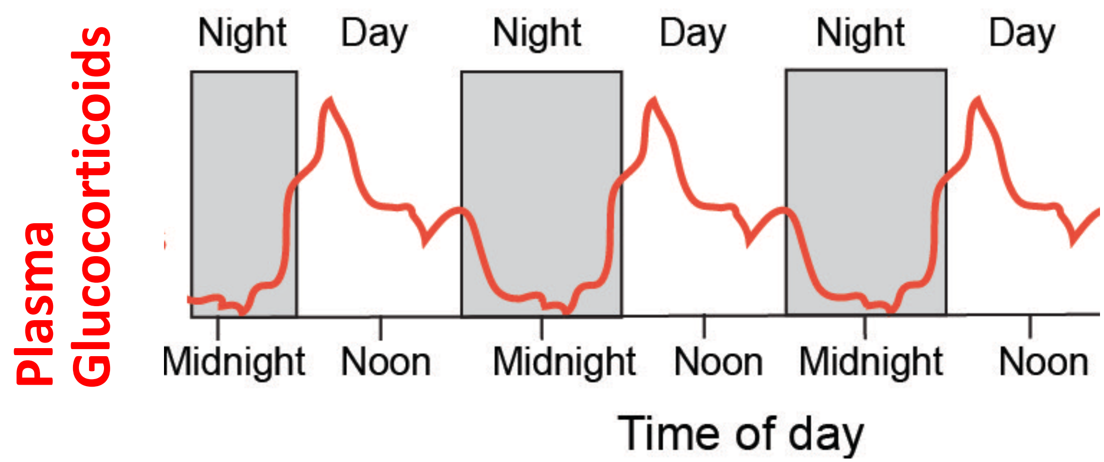
3) Transcription factor dynamics reveals a circadian code for cell differentiation


Zahra Bahrami-Nejad and Michael Zhao

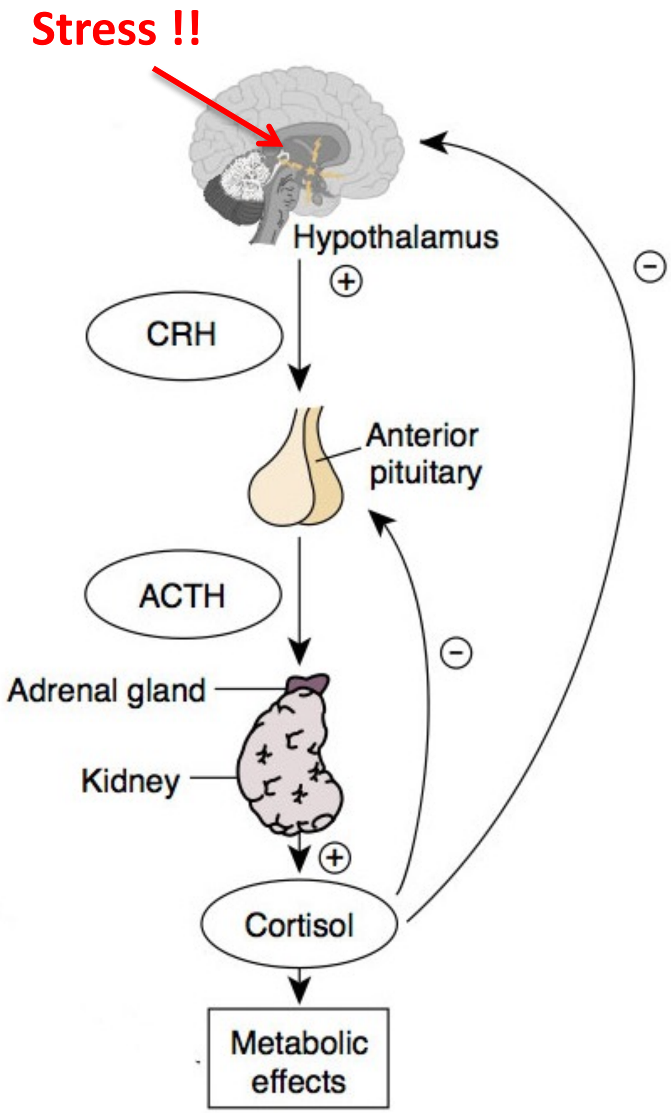
Glucocorticoids are secreted in regular circadian rhythms





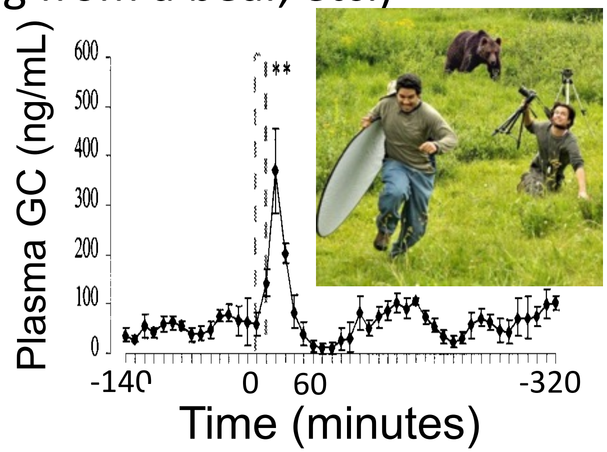
The daily increase in glucocorticoid levels produces a wake-up signal, turning on appetite and physical activity.

Stress also induces glucocorticoid secretion…

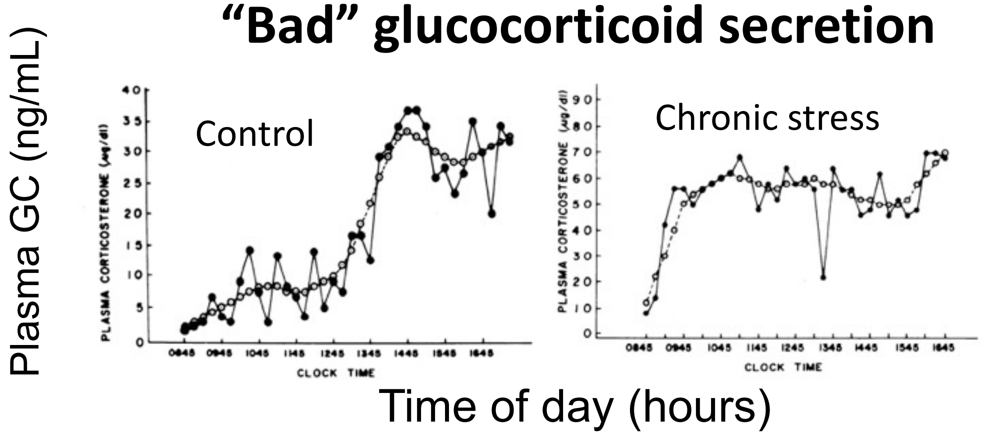


“Good” glucocorticoid secretion

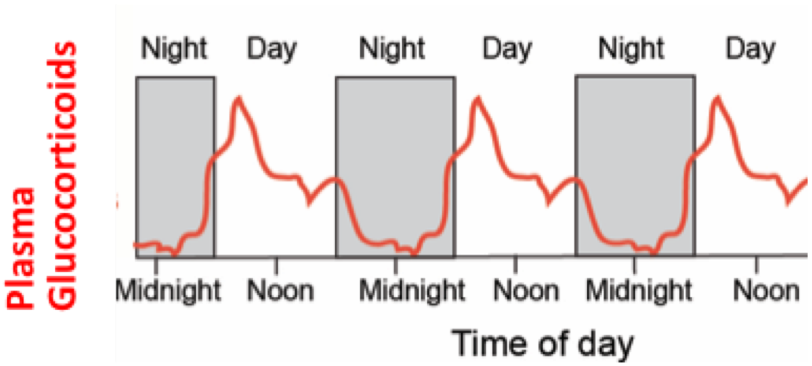
- After exercise
- In response to cold (i.e. going outside in winter)
- When sitting or standing up (upright posture)
- To deal with anxiety (help with focusing for a test, running from a bear, etc.)

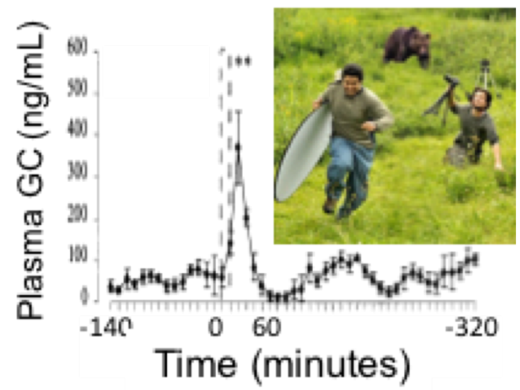


“Bad” glucocorticoid secretion

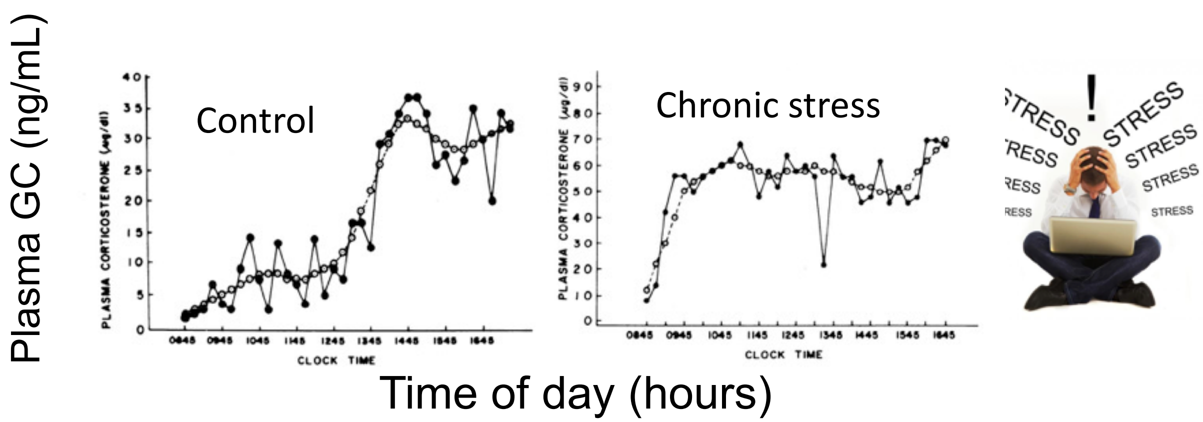


How can low rates of adipogenesis be maintained despite daily oscillations and healthy, but unpredictable, spikes in glucocorticoid levels?

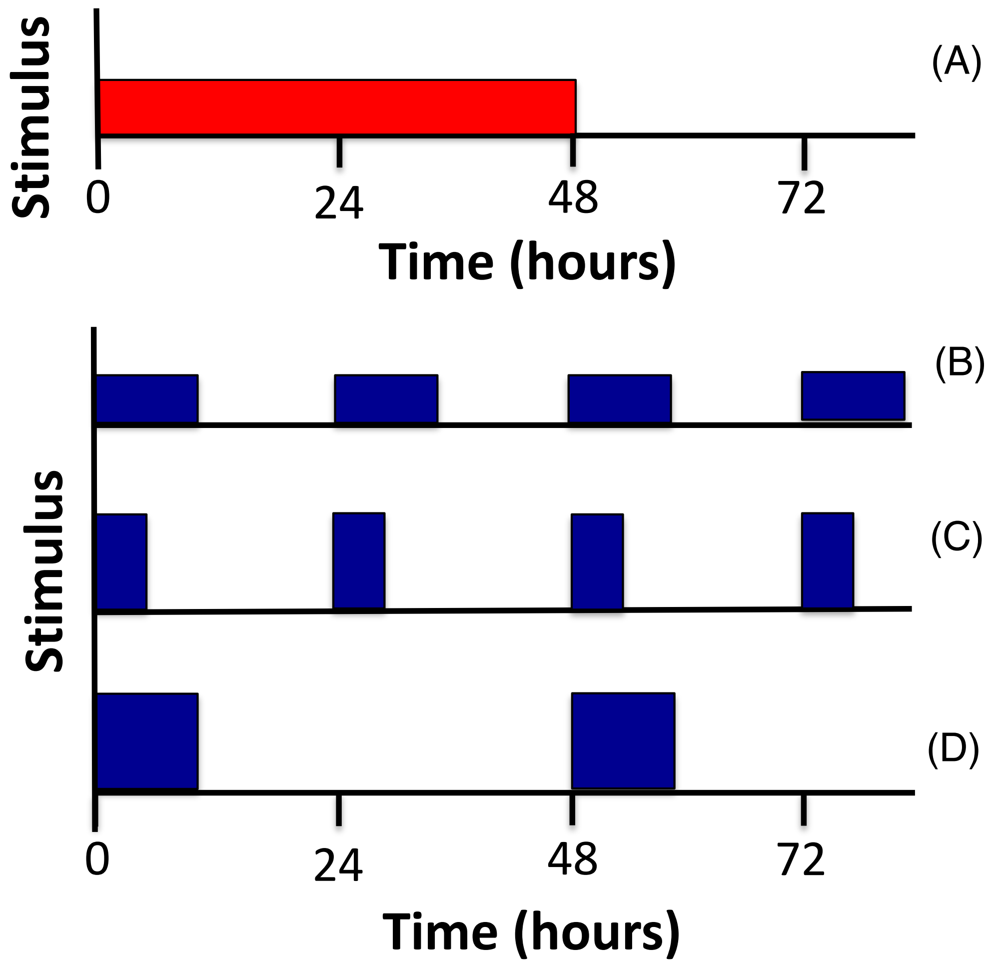


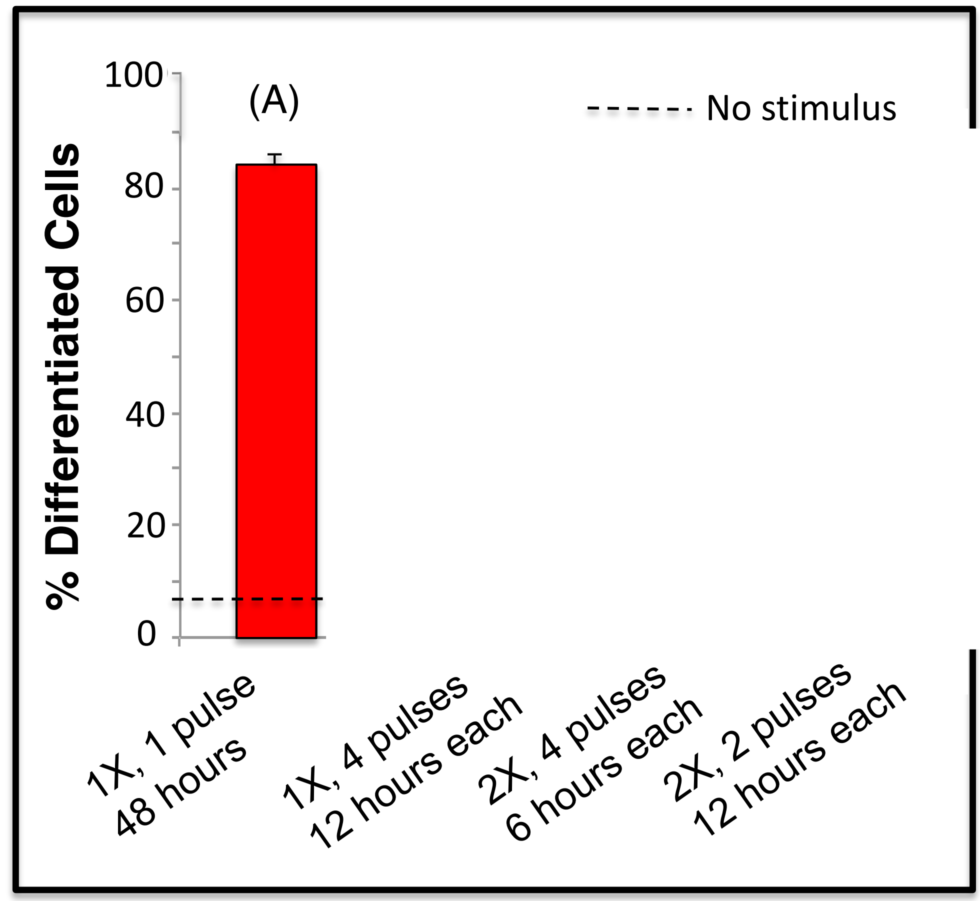


And why is losing pulsatility in glucocorticoid secretion so closely linked with obesity?

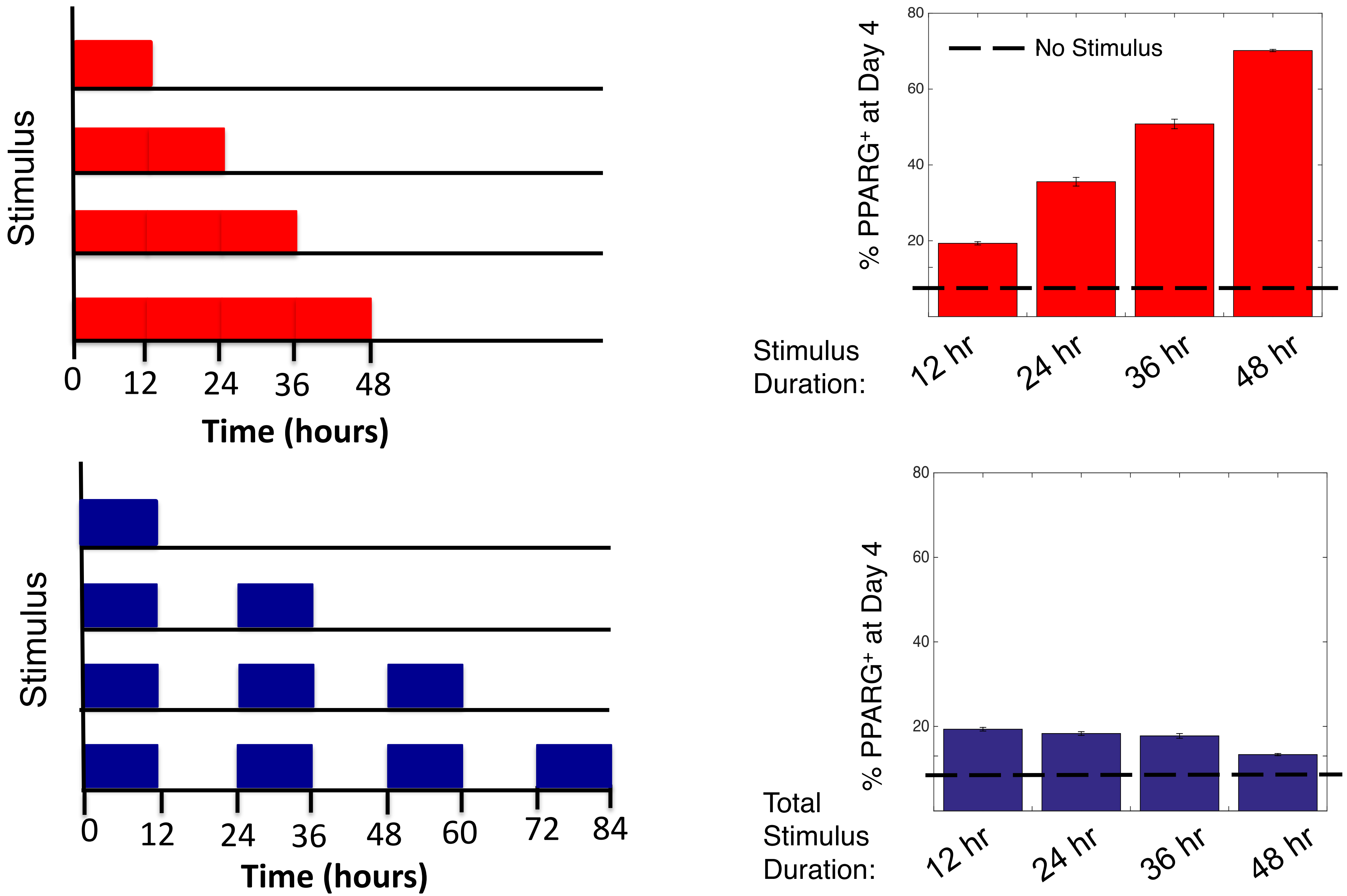


The same TOTAL glucocorticoid stimulus given over 4 days has dramatically different outcomes depending on how it is applied

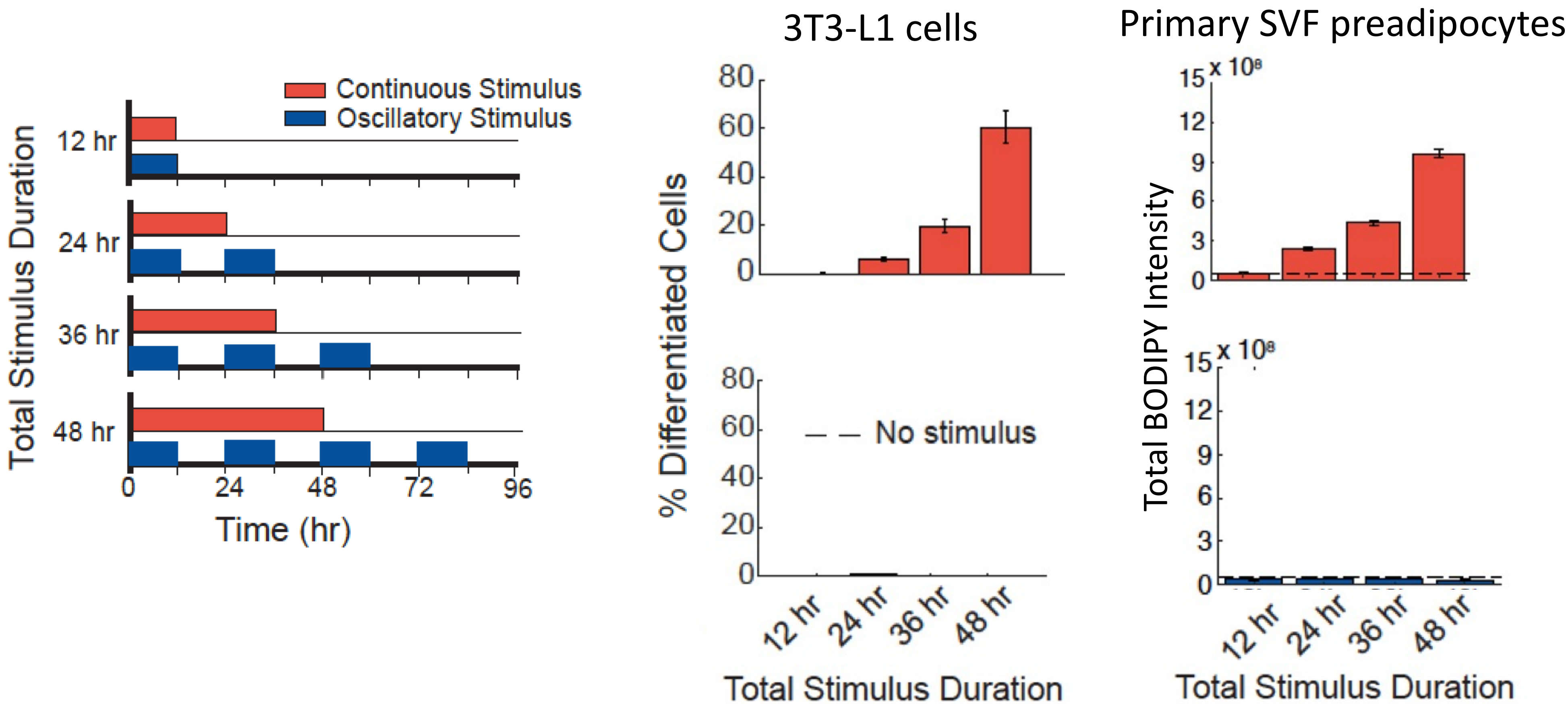




Stimuli longer than 12 hours increase adipogenesis while oscillatory, circadian inputs are rejected

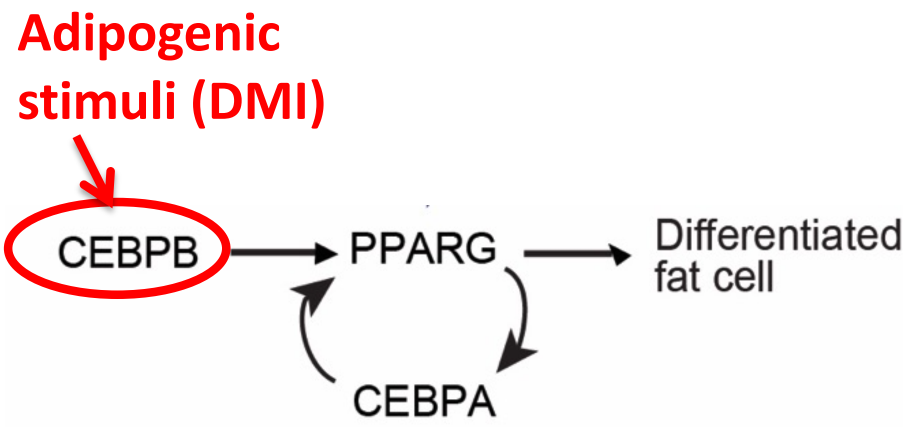


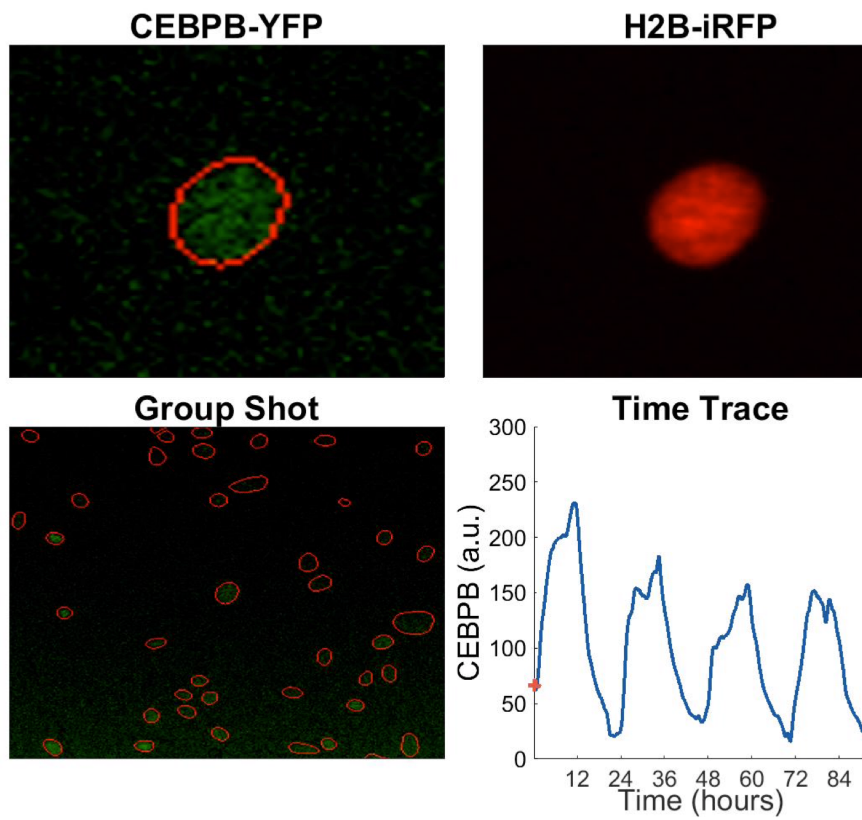
The same rejection of oscillating hormone pulses is observed in 3T3-L1 and primary SVF preadipocytes



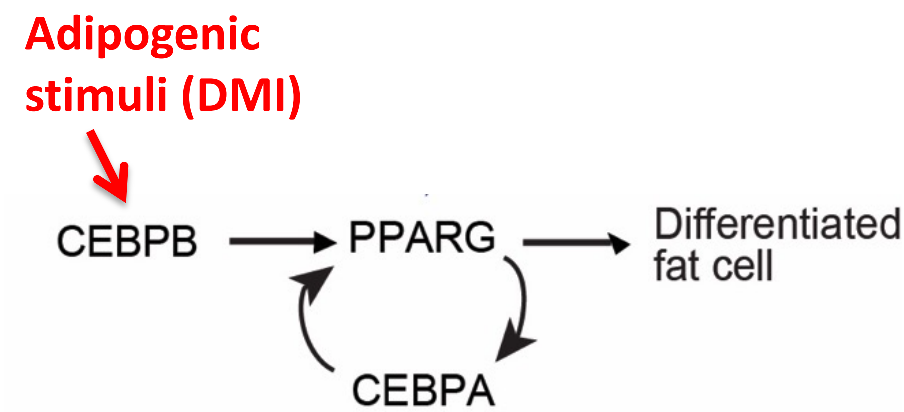
Filtering mechanism occurs irrespective of amplitude:
Even increasing or decreasing pulse amplitudes 10-fold for oscillating conditions does not cause differentiation

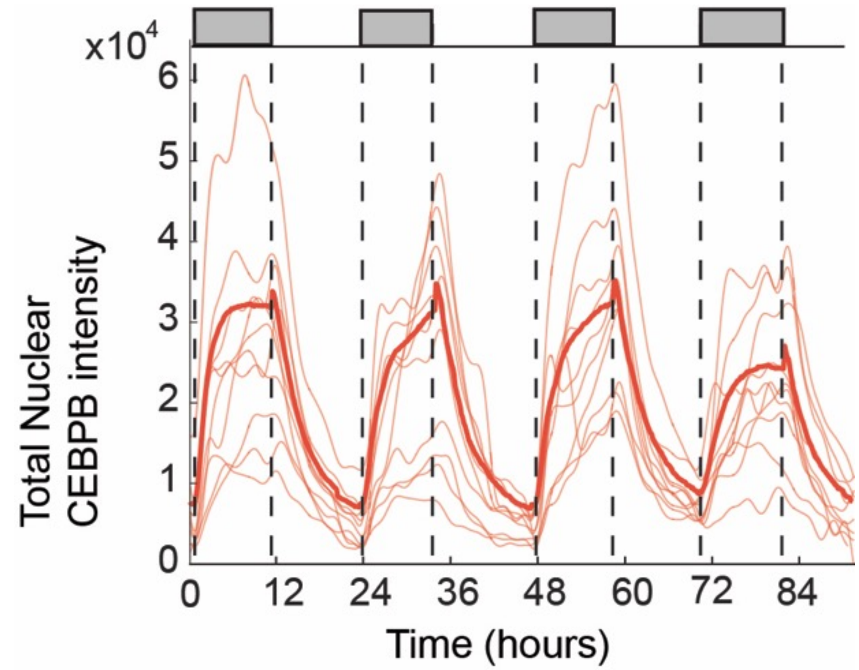
We used CRISPR-mediated genome editing to tag CEBPB with YFP(citrine)





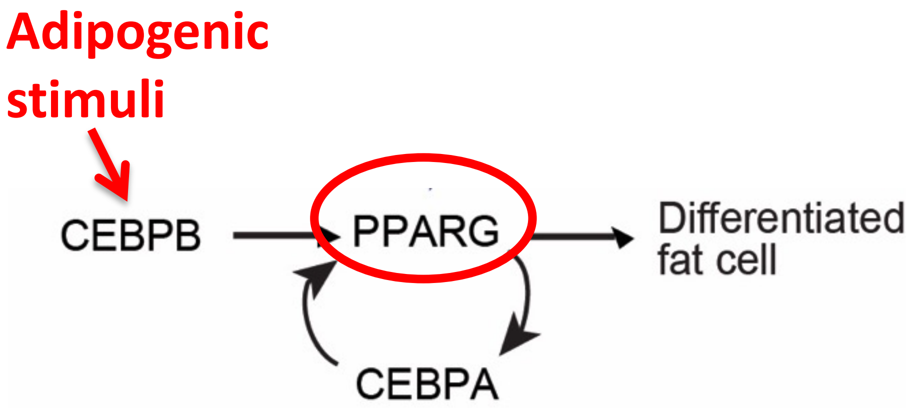
CEBPB nuclear expression closely mirrors the hormonal input stimuli

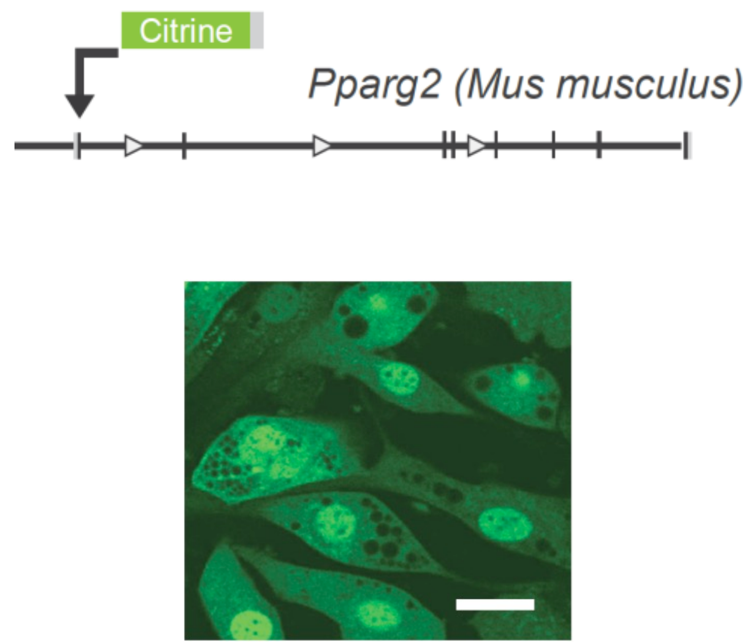




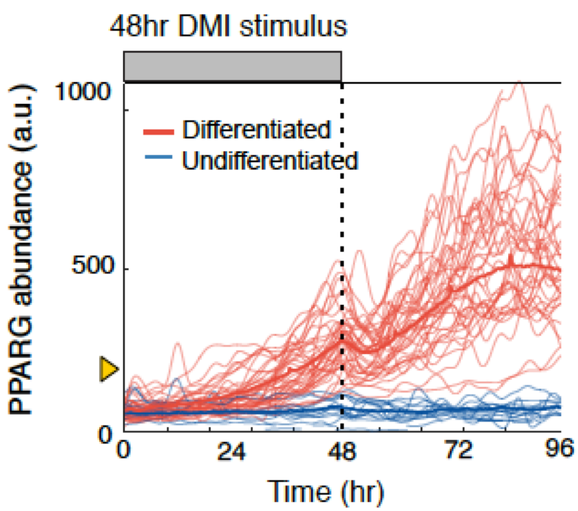
CEBPB mRNA and protein rapidly decay (half-lives ~2 and ~3 hours respectively)

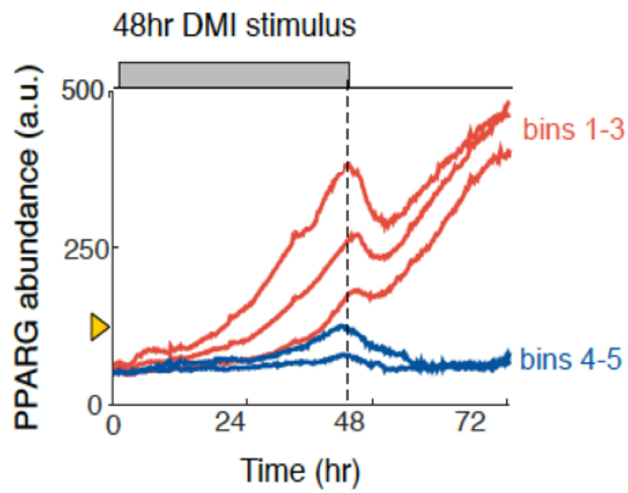
We created a live cell sensor for adipogenesis by tagging endogenous PPARG with Citrine (YFP)

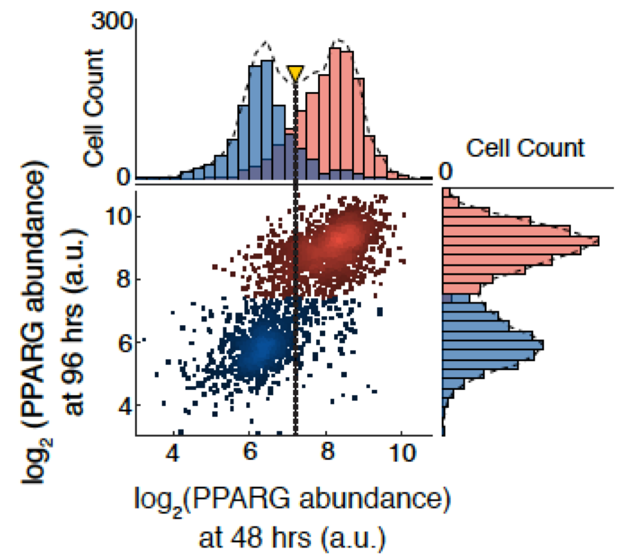


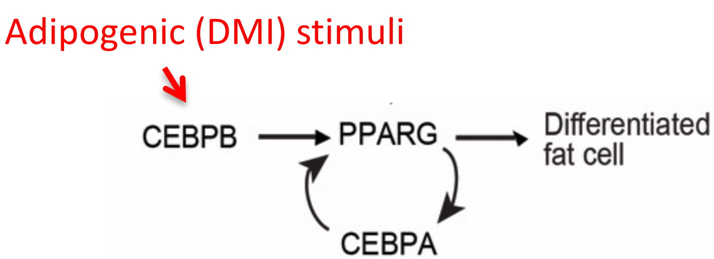


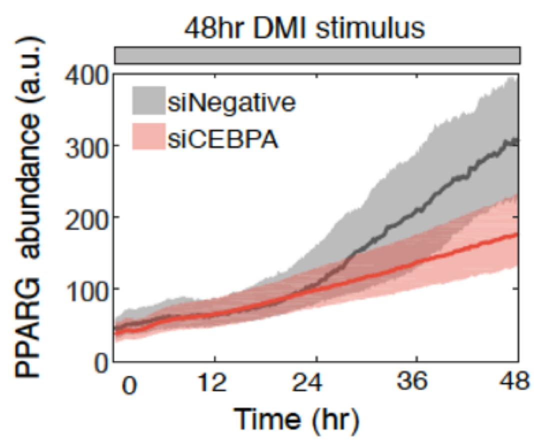
Live-cell imaging directly shows the existence of a bistable switch with a threshold in PPARG that determines whether or not a cell will differentiate



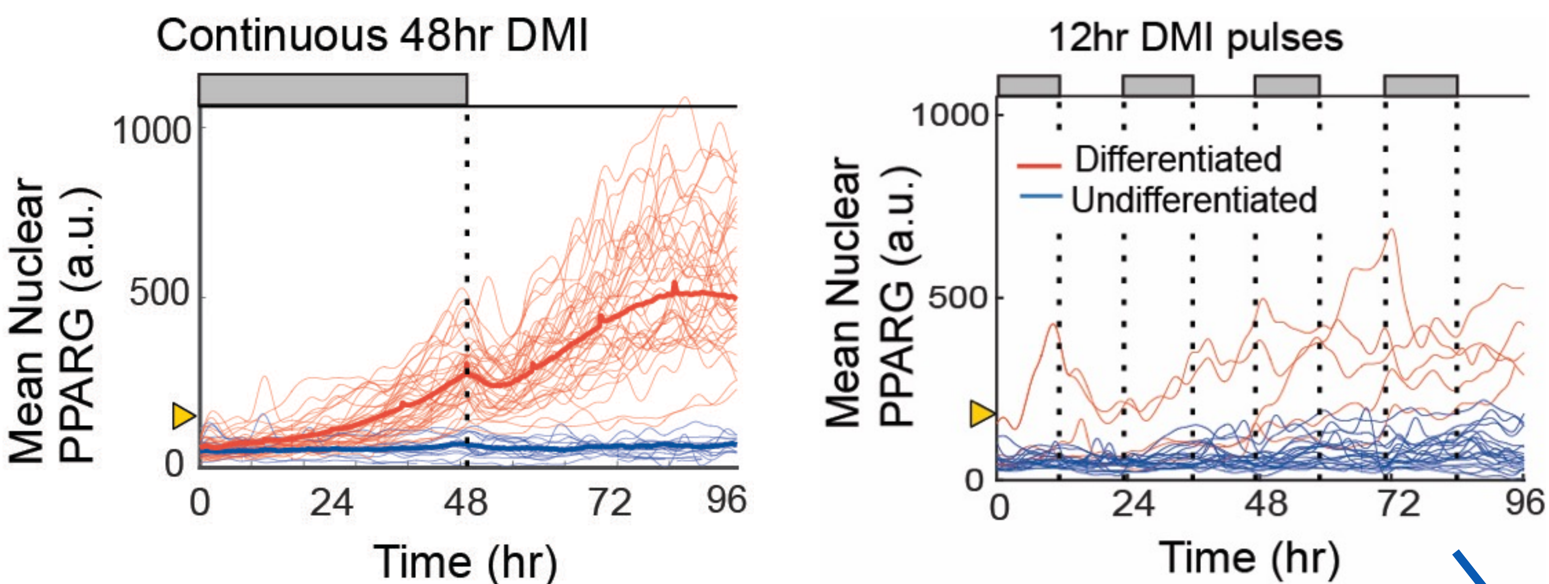


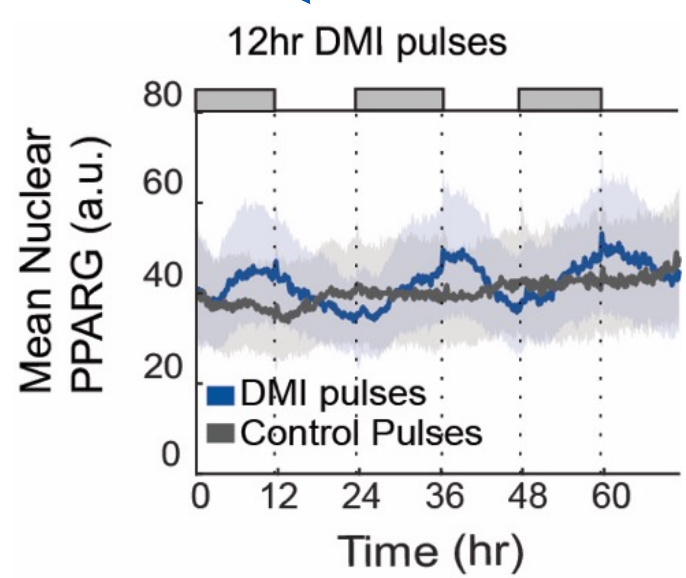






Pulsing the input stimulus prevents PPARG from reaching the threshold in most cells

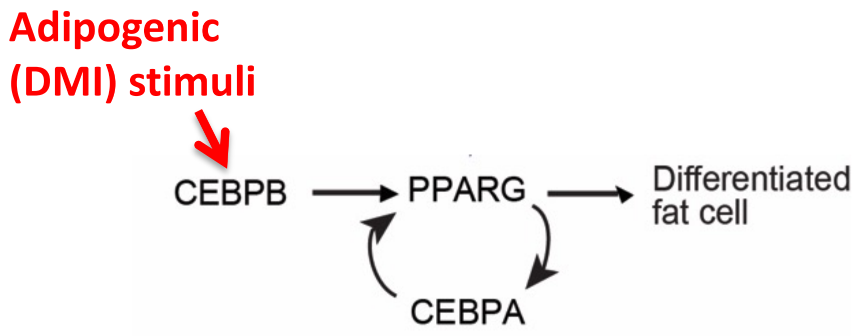




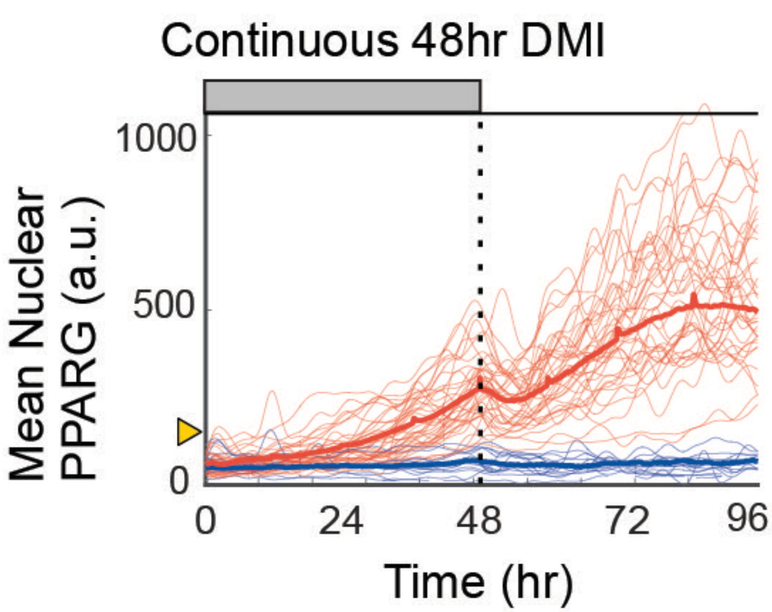
The adipogenic network is built around a highly unstable central protein!

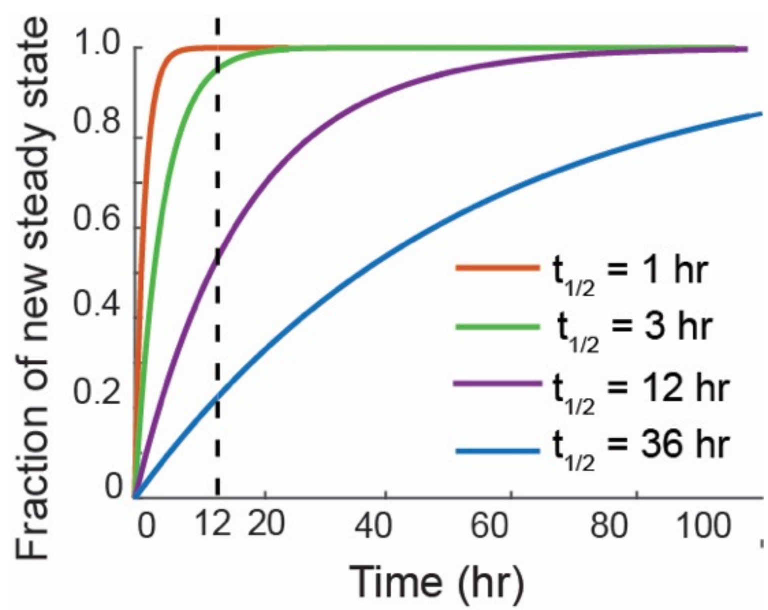
PPARG protein and mRNA decay rapidly (half-lives of ~ 1 hour)

We were faced with a conundrum: fast-degrading proteins cannot increase steady-state levels over days!

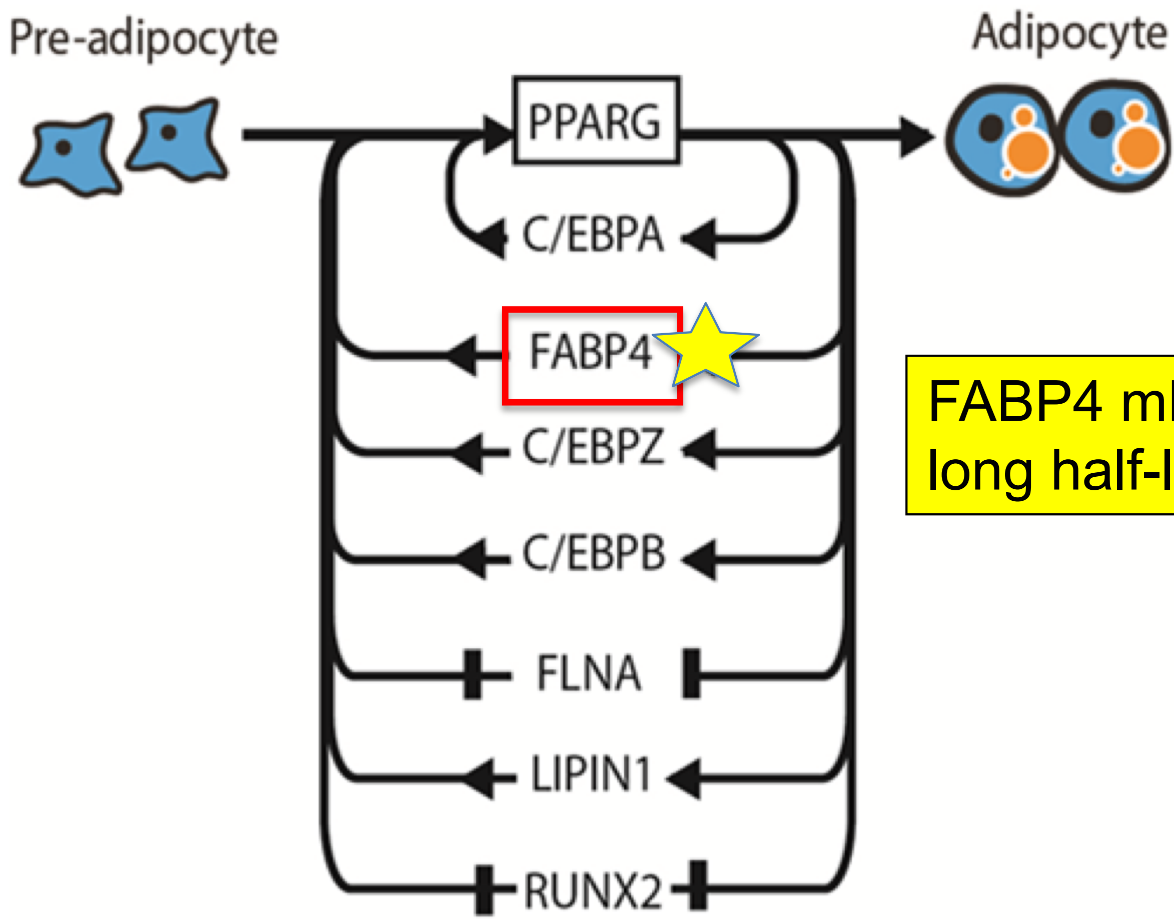


CEBPB, CEBPA, and PPARG protein and mRNA degrade rapidly (in < 3 hours)





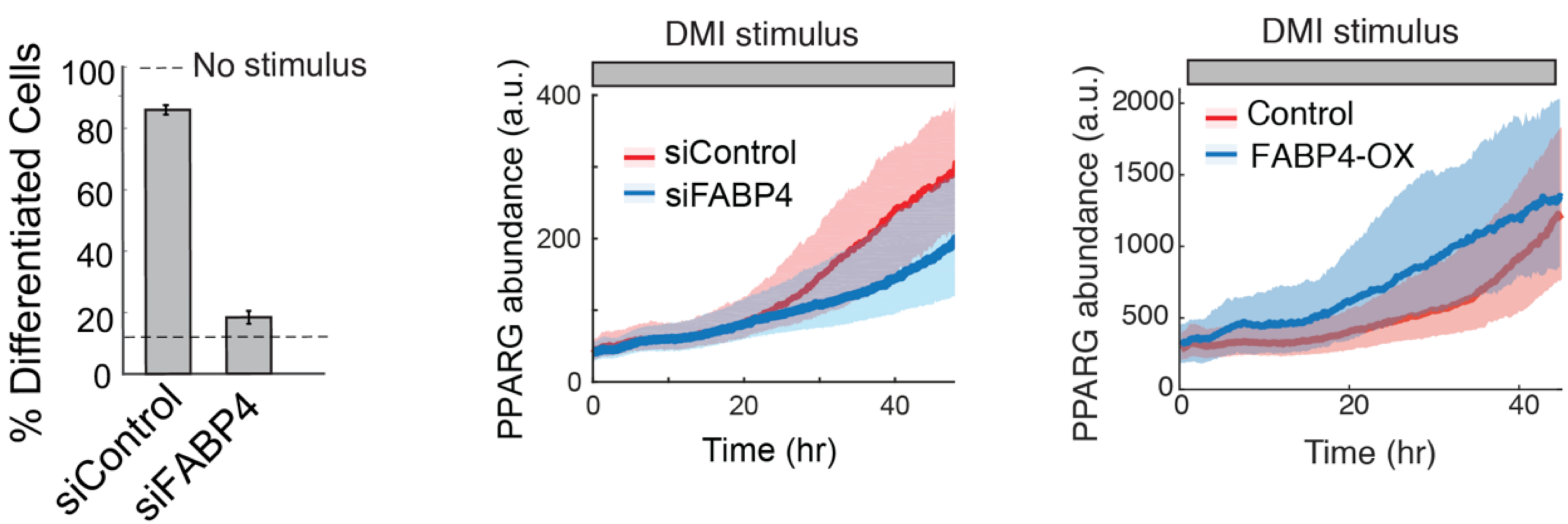
There needs to be a slow regulator of PPARG in the system!



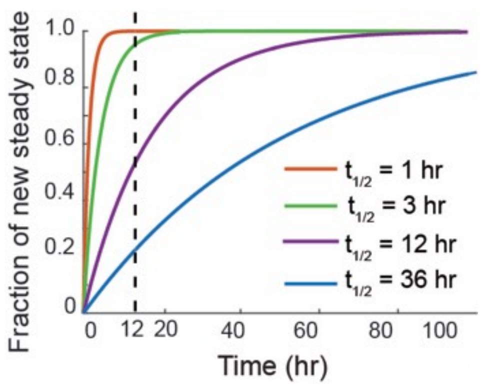
FABP4 mRNA has a long half-life (> 30 hours)

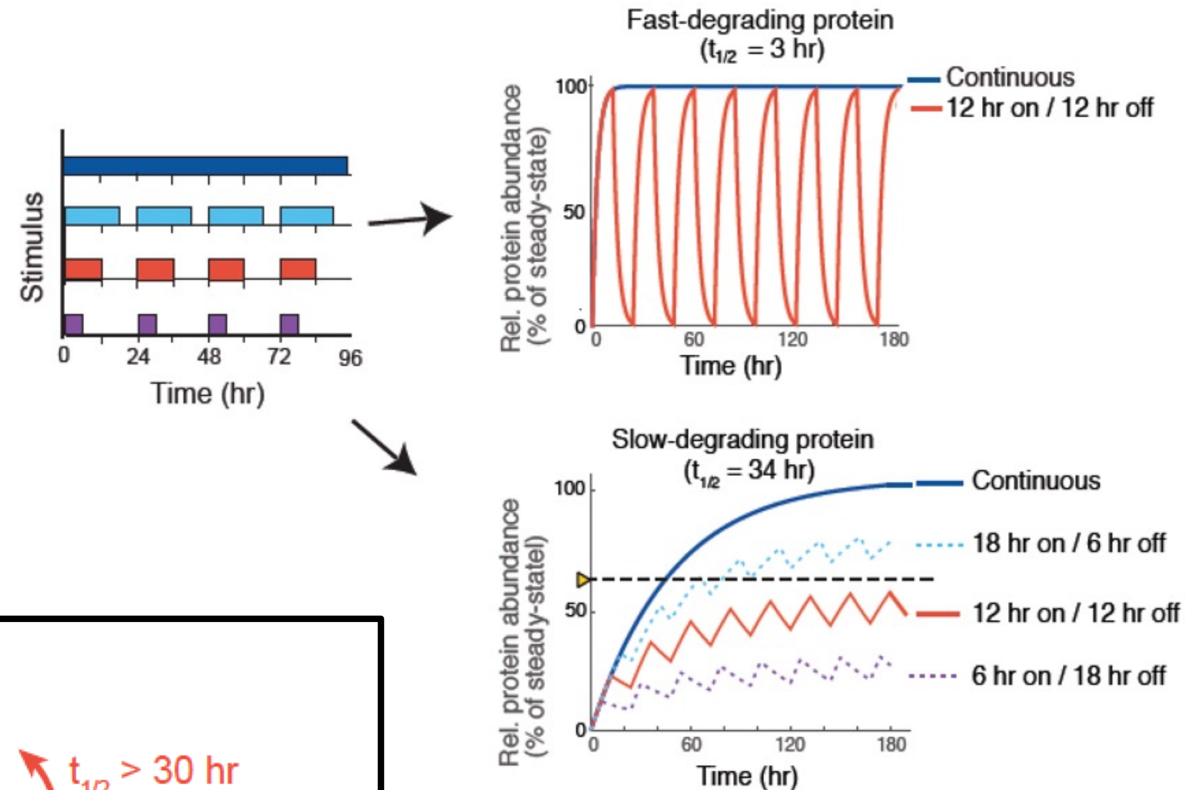
Ahrends,…,Teruel, *Science* 2014.

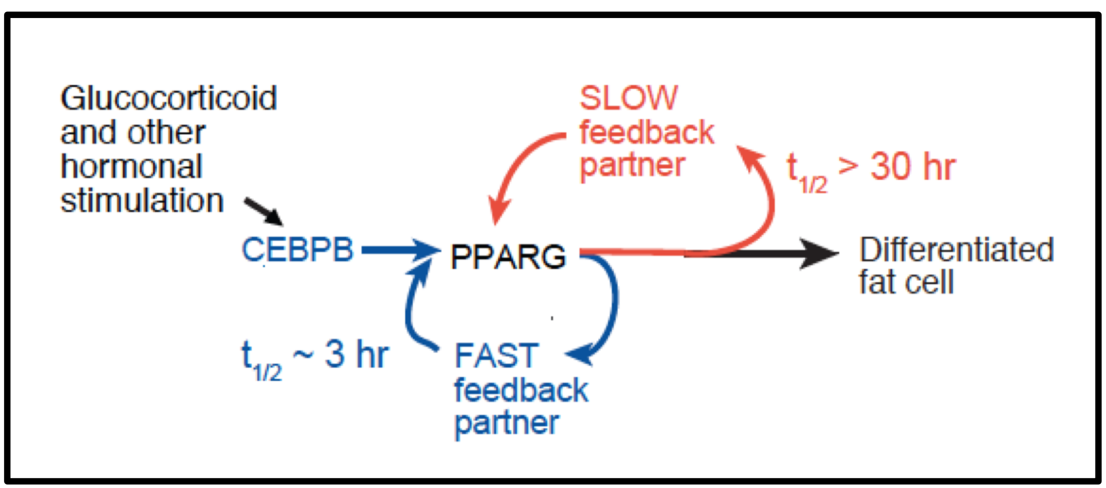
FABP4 is an example of a slow-degrading PPARG regulator that can mediate a slow increase in PPARG expression during adipogenesis



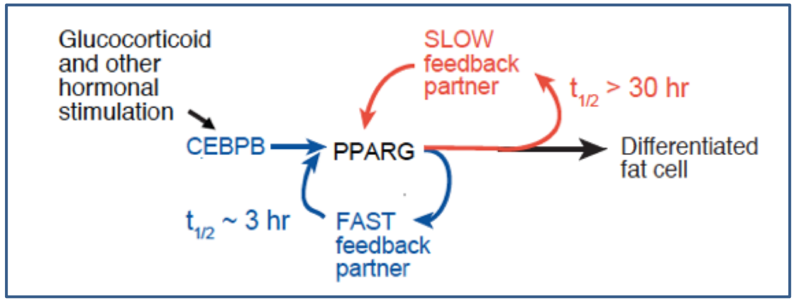
A slow feedback partner could both slow PPARG activation AND keep PPARG amplitude below threshold

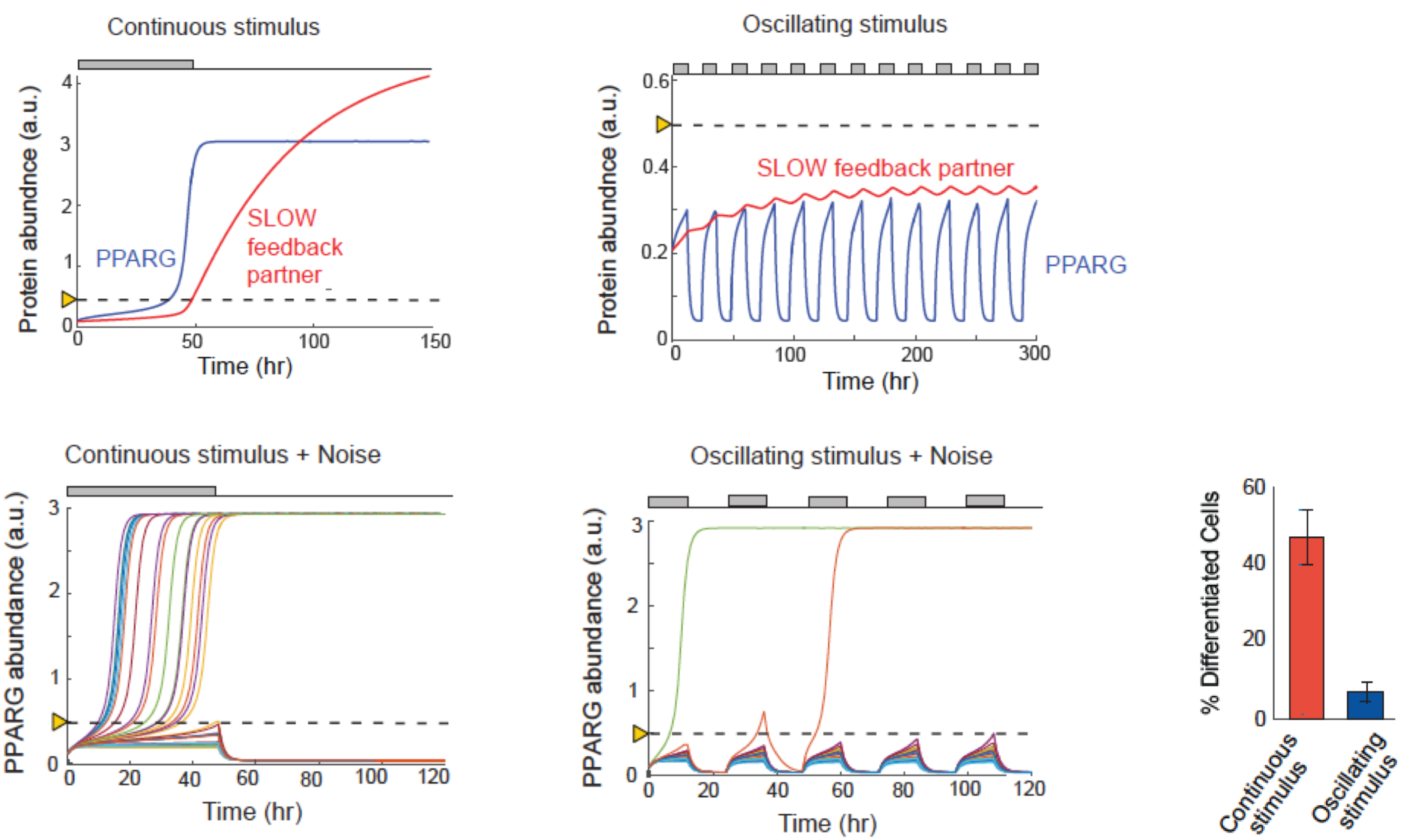




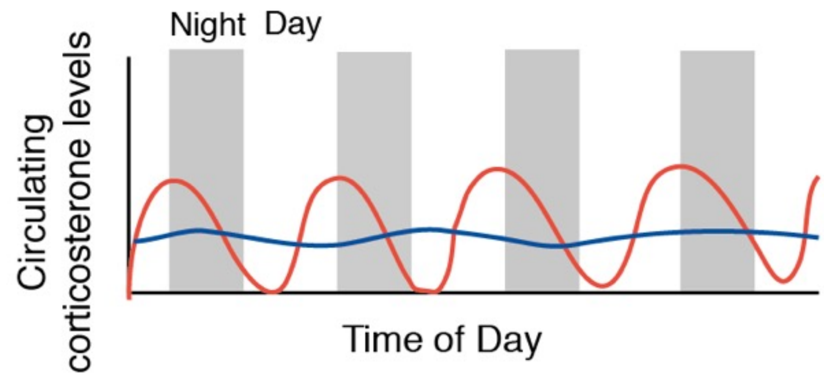


A signaling circuit with a fast and a slow positive feedback can trigger differentiation for continuous stimuli while rejecting daily oscillations

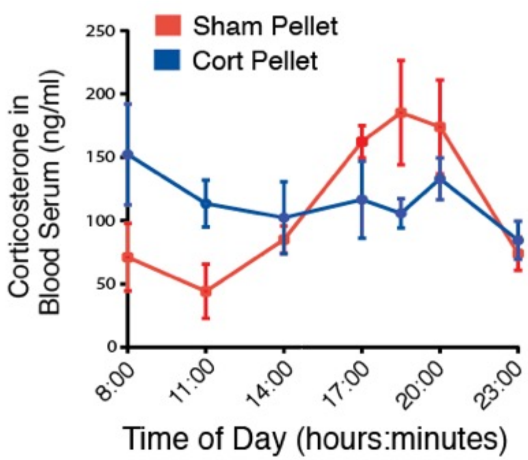


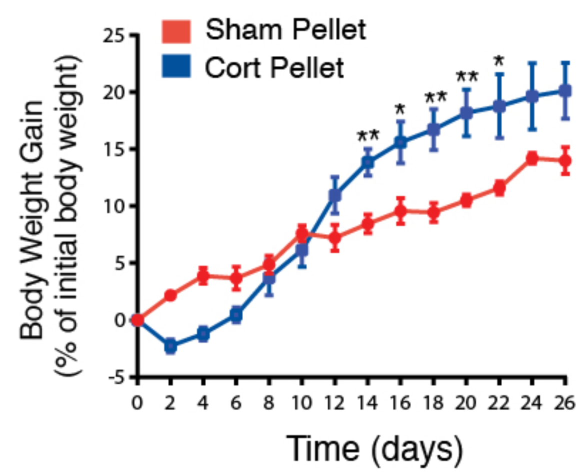


Flattening circadian glucocorticoid oscillations in mice resulted in significantly increased body weight

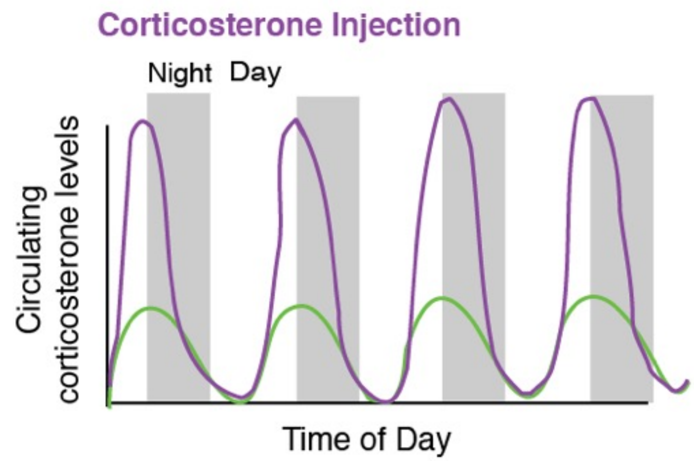


Implanted corticosterone wax pellets were used to continuously flatten circulating levels.

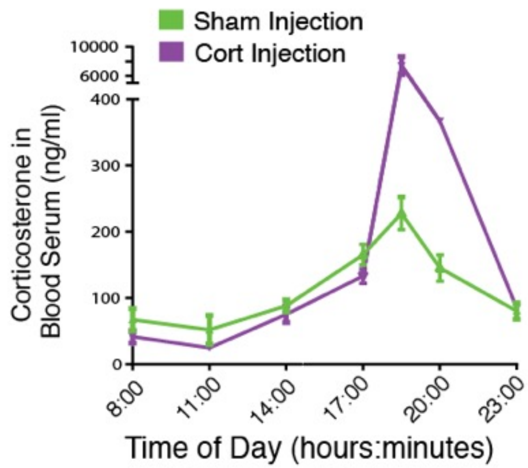


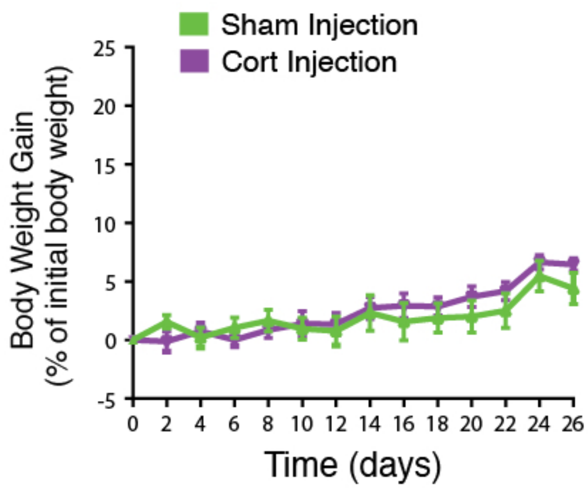


However, increasing glucocorticoid peak amplitudes even 40-fold had no effect on body weight!

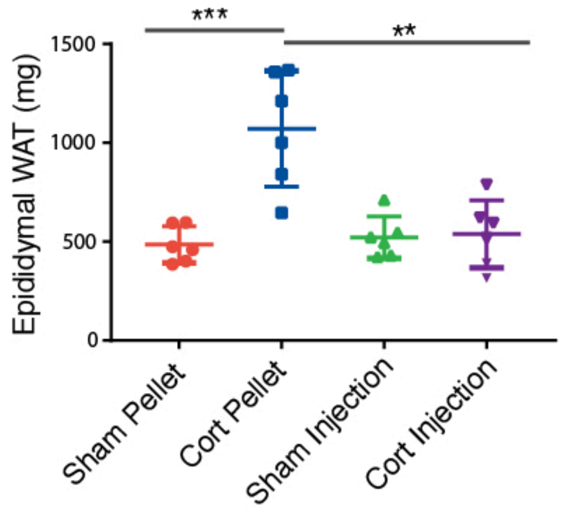


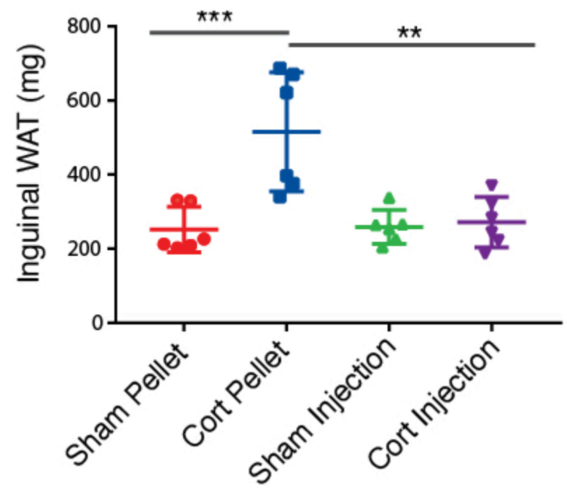
Corticosterone was injected daily at 5PM to increase daily peak levels

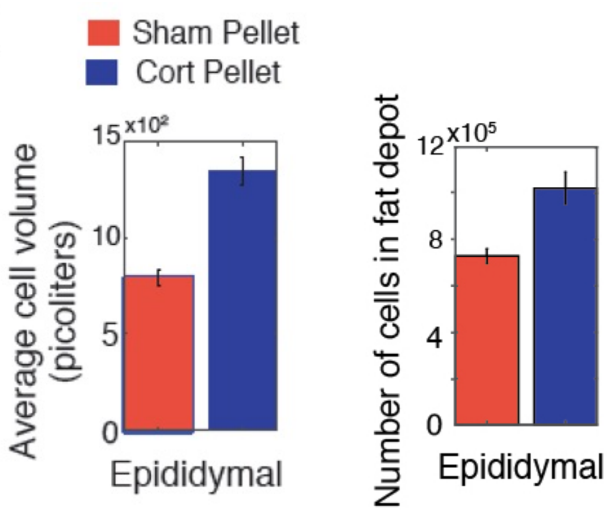




Fat mass doubled in mice when circadian glucocorticoid oscillations were flattened for 21 days

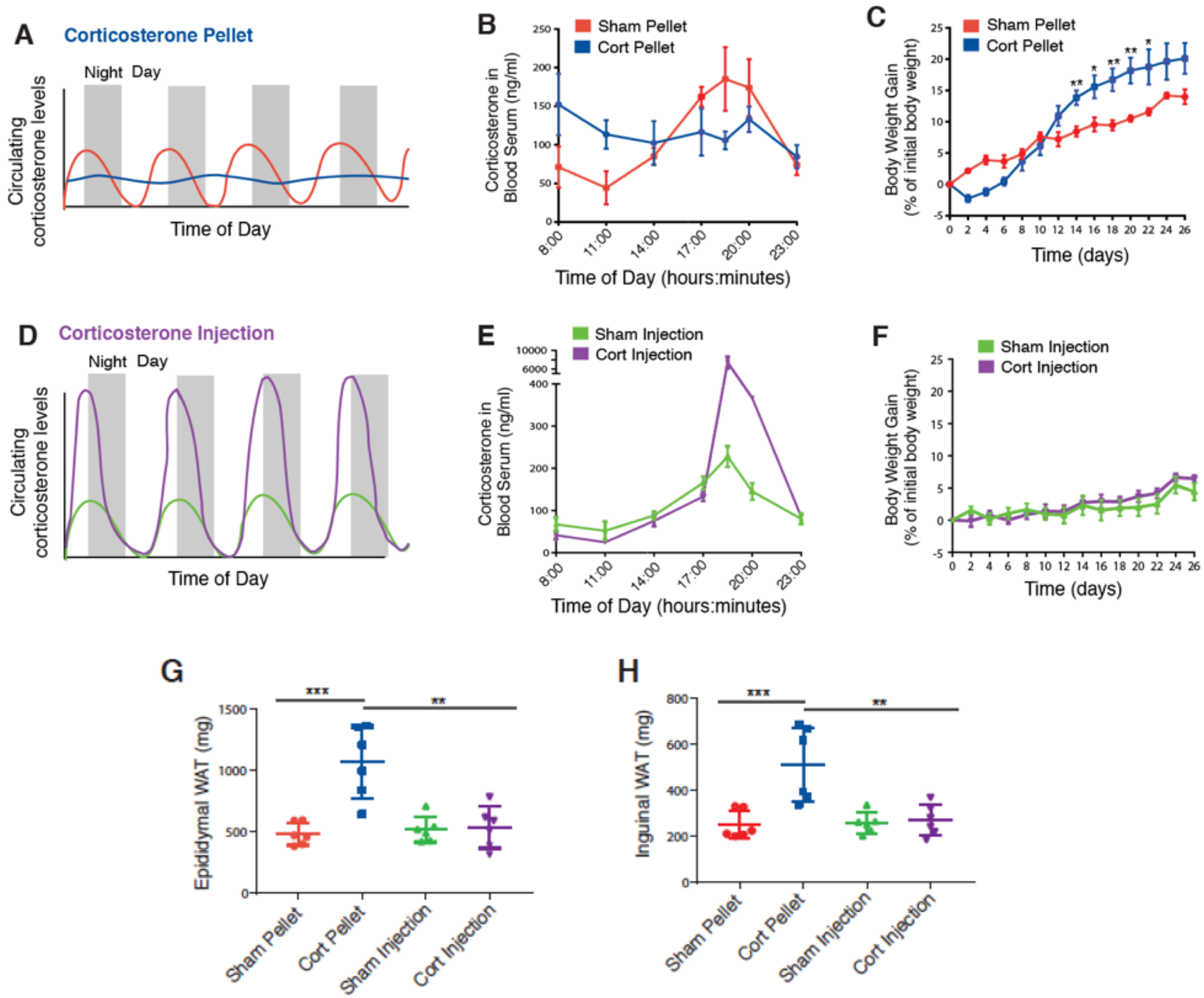




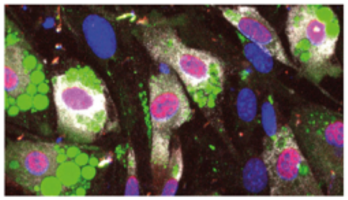


The increase in fat mass is due to both increased cell volume and adipogenesis

A general temporal control principle for cell differentiation, as well as a new therapeutic strategy to reduce fat mass?



Bahrami-Nejad,…,Teruel, *Cell Metabolism,* April 2018.



Precursor cells (blue) mature into adipose cells full of fat (green) with help from hormones, whose levels surge and fall over a day. Credit: Z. Bahrami-Nejad et al/Cell Metab.

PHYSIOLOGY · 06 APRIL 2018

Why fat piles on when the body's daily cycles are in disarray

Timing of hormone fluctuations influences fat cells' development.

Changes in the patterns of hormone production might cause weight gain when circadian rhythms are disrupted.

Hormones called glucocorticoids stimulate the production of mature fat cells. In humans, glucocorticoid levels naturally rise in the morning and fall in the evening, but stress can also elevate them.

To study how glucocorticoid levels relate to weight gain, Mary Teruel at Stanford University in California and her colleagues injected mice with glucocorticoids at varying times of day, but fed all mice the same amount of food. Mice given the hormone late in their wakeful phase gained weight. But mice injected just after they'd woken up — when their glucocorticoid levels were already naturally high — did not.

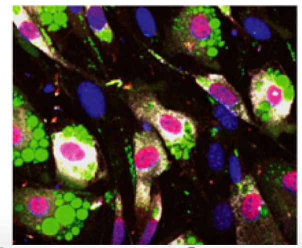
NIH RESEARCH MATTERS

April 17, 2018

Disrupting normal hormone cycle spurs fat cells

At a Glance

- In mice, interfering with the natural cycle of a certain hormone spurs development of more fat cells.
- This finding may help explain why stress and conditions associated with abnormal steroid hormone levels can cause obesity.

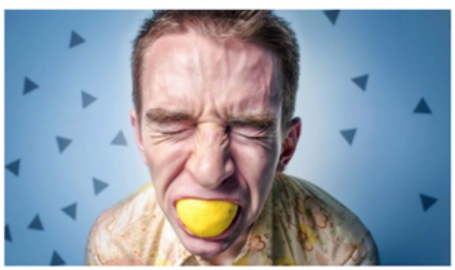


Your body has a natural, daily rhythm. Certain needs—to sleep, wake up, eat, and go to the bathroom every day—are patterned around a repeating 24-hour cycle. Hormones rise and fall at certain times of the day to prompt the body to do these things at the right time. When you go against these natural "circadian" rhythms—by forcing yourself to stay up too late, for example—your health may suffer. Disrupted circadian rhythms have been linked to obesity, sleep disorders, depression, and other health problems.

Previous studies have shown that steroid hormones known as glucocorticoid hormones, which drive adipocyte (fat cell) production from precursors, are secreted on a 12-hour cycle. Disrupting this cycle is linked to obesity. In addition to this natural daily rhythm, your body also secretes glucocorticoids during stressful situations. Some people have diseases, such as Cushing's

¿Por qué las personas aumentan de peso por el estrés ?

Nuevo estudio proporciona la primera comprensión molecular de por qué las personas aumentan de peso debido al estrés crónico



Nuevo estudio proporciona la primera comprensión molecular de por qué las personas aumentan de peso debido al estrés crónico. (Foto: Pixabay)

Redacción EC
04.04.2018 / 09:38 am

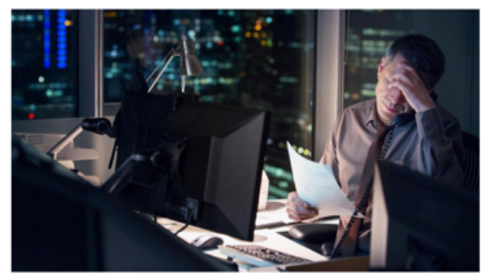
Investigadores de la Universidad de Stanford (California) determinaron que el control del ritmo de los glucocorticoides, comúnmente conocidos como las hormonas del **estrés**, reduce el **aumento de peso**, según un estudio publicado en la revista especializada Cell Metabolism.

Don't blame the ice cream: stressed-out cells are making you fat

Tom Whipple

April 4 2018, 12:01am,
The Times

Health



Stress causes the release of the hormone glucocorticoid, which is linked to weight gain
CHRIS RYAN/GETTY IMAGE

Being stressed doesn't just make you reach for the tub of ice cream, it also changes what your body does with that ice cream when you eat it.

The link between stress, sleeplessness and weight gain is long established. Now a study in the US has shown that part of this is due to effects at a cellular level. When hormones associated with stress are disrupted, it means more cells are converted to fat.

Mary Teruel, from Stanford University, began the research because of curiosity

Overall summary

- Fat cell differentiation involves a switch between two distinct populations of cells - undifferentiated and differentiated - and thus requires single cell approaches to understand.
- Control of low rates of differentiation requires noise in expression of regulators and multiple positive feedbacks
- Natural hormone signals oscillate. A striking characteristic of the fat cell differentiation system is that it filters out circadian glucocorticoid oscillations while equally strong continuous stimuli trigger differentiation.
- Circadian filtering requires fast and slow positive feedback to PPARG.
- Our results suggests a new therapeutic strategy to reduce fat mass by controlling timing of hormonal signaling.

Acknowledgements

<u>Teruel Lab</u>

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NIH RO1 DK24301044
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Stanford BioX Seed Grant
Stanford Diabetes Center Seed Grant