数理解析研究所講究録
第1853巻 2013年 172-179

Flowering gene regulation model and mechanism of local adaptation

Akiko Satake; *Graduate School of Environmental Sciences, Hokkaido University*, Sapporo, Japan
Yoh Iwasa; *Department of Biology, Faculty of Sciences, Kyushu University*, Fukuoka, Japan

***Abstract*—Biological memory, a sustained cellular response to a transient stimulus, has been found in many natural systems. The best example in plants is the winter memory by which plants can flower in favorable conditions in spring. For this winter memory, epigenetic regulation of *FLOWERING LOCUS C* (*FLC*), which acts as a floral repressor, plays a key role. Exposure to prolonged periods of cold results in the gradual suppression of *FLC*, which allows plants to measure the length of cold and to flower only after a sufficiently long winter. Here, we develop a model for chromatin modification, in which the dynamics of a single nucleosome are aggregated to on/off behavior of *FLC* expression at the cellular level and further integrated to a change of *FLC* expression at the whole-plant level. We propose cell-population coding of winter memory: each cell is described as a bistable system that shows heterogeneous timing of the transition from on to off in *FLC* expression under cold and measures the length of cold as the proportion of cells in the off state. This mechanism well explains robust *FLC* regulation and stable inheritance of winter memory after cell division in response to noisy signals.**

Keywords-component; Epigenetics; cell-to-cell variations; bistability; histone modification; flowering; stochastic process.

I. INTRODUCTION

In temperate climates, many plant species flower in spring. The floral transition in spring results from sensing seasonal changes in environmental signals and generating appropriate responses to the environment. Photoperiod and temperature are two of the main environmental signals used as cues of spring's arrival. In contrast to an accurate and predictable seasonal change in photoperiod, temperature fluctuates from day to day in a very noisy manner, and short-term trends in temperature are not foreseeable. Thus, a spring increase in photoperiod can be a reliable cue, but a change in temperature is not. As an alternative cue for the coming of spring, the plant has evolved to use the long-term seasonal trend in temperature, especially a prolonged period of cold. The process that promotes flowering after exposure to prolonged periods of winter cold is known as vernalization (reviewed in [1] and [2]). The plant's ability to respond to a prolonged winter season of cold but not to a short spell of cold prevents flowering prior to winter and permits flowering under the favorable conditions of spring because it effectively filters out daily variations and extracts a reliable long-term trend in temperature using a moving average. In the last 10–15 years, great progress has been made in understanding the molecular mechanisms controlling the vernalization response. For this winter memory, epigenetic regulation of *FLOWERING LOCUS C* (*FLC*), which acts as a floral repressor, plays a key role ([3] and [4]). Repression of *FLC* expression is triggered in response to prolonged cold exposure, resulting in flowering in spring.

Vernalization-mediated *FLC* repression is realized by changes in histone modification in *FLC* chromatin. A number of genes involved in the initiation and maintenance of *FLC* repression have

been isolated ([5] and [6]). Before vernalization, *FLC* chromatin is modified by active histone marks, such as methylation of histone H3 lysine 4 (H3K4). During vernalization, VERNALIZATION INSENSITIVE 3 (VIN3), a chromatin remodeling PHD finger protein, is induced ([2]). VIN3 binds to the chromatin of the *FLC* locus and initiates transformation from active to repressive chromatin modifications by interacting with members of Polycomb-group Repressive Complex 2 (PRC2) ([2], [7], and [8]), which enhances the deposition of one of the repressive histone marks, tri-methylation of lysine 27 of histone H3 (H3K27me3) ([9] and [10]). The chromatin mark H3K27me3 stimulates the recruitment of LHP1 ([11] and [12]), which, together with VRN1, assists in maintaining the stably silenced state of *FLC* ([13] and [14]) by enhancing trimethylation of H3K9, another repressive histone mark ([13]).

In *A. thaliana*, the repressive histone modifications generally persist, and cold-mediated *FLC* repression is stably maintained after plants resume growth under warm conditions ([3], [15] and [16] but see [17]). In contrast, in perennial herbs, *FLC* is repressed only transiently. In *A. alpina*, a perennial herb, the level of H3K27me3 decreases in the ortholog of *A. thaliana FLC* (called *PEP1*), and *PEP1* is reactivated after the plant returns to warm conditions ([18]). In another perennial herb, *A. halleri, FLC* expression increases gradually as temperature increases in spring under natural conditions ([19]). The recovery of *FLC* does not occur within a short time but takes several weeks. Thus, it is likely that these perennial plants also employ a winter memory, but the duration of the memory is significantly shorter than that in the annual *A. thaliana.*

In this paper, we mathematically formalize chromatin modification dynamics by reference to a computer simulation model studied by Dodd et al. (2007) [20] and address the following three questions from a perspective of stochastic theory. (1) In the robust response to a noisy signal, how can a plant measure a long-term cold exposure and respond only to continuous cold, but not to short spells of cold? (2) In winter memory, what is the mechanism for mitotically stable maintenance of *FLC* repression? (3) What causes the difference between annuals and perennials?

II. MODEL

We consider N units of nucleosomes located in a region of the *FLC* locus. Each nucleosome is in one of the three states: actively modified (A), unmodified (U), or repressively modified (R). Actively transcribed *FLC* chromatin is enriched with active histone marks such as histone H3 lysine 4 trimethylation, as well as acetylation of core histone tails of H3 and H4. Vernalization triggers a transition in nucleosomes from actively modified to unmodified by removing these active histone marks and further causes a transition from unmodified to repressively modified by adding repressive histone marks (Fig. 1). We assume that enzymes that are involved in transitions among the three nucleosome states are recruited to *FLC* chromatin sections by positive feedback. To be specific, actively or repressively modified nucleosomes are assumed to recruit enzymes that modify other nucleosomes in the same manner.

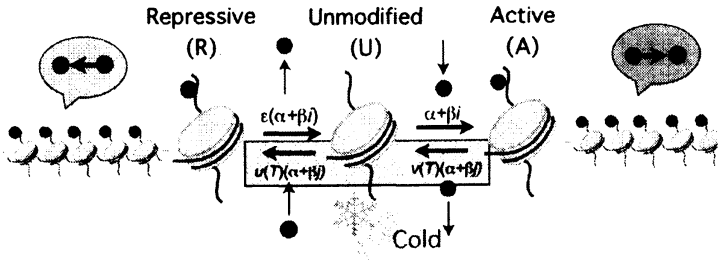
Let the number of actively modified, repressively modified, and unmodified nucleosomes as i, j, and N-i-j. The instantaneous rates of transition per nucleosome are defined in as follows:

(1) $r_{U\to A} = \alpha + \beta i$: This describes the transition rate from the unmodified state (U) to the actively modified state (A) due to the deposition of active histone marks. $r_{U\to A}$ increases as the number of actively modified units (i) increases because enzymes that deposit active histone marks are assumed to be recruited at a faster rate as the chromatin section becomes enriched with active

modifications. α is a rate constant independent of i. β is the strength at which histone-modifying enzymes are recruited to the *FLC* chromatin. We call β the strength of positive feedback.
(2) $r_{R\to U} = \varepsilon(\alpha + \beta i)$: This is the transition rate from the repressively modified state (R) to the unmodified state (U) due to the removal of repressive histone marks. $r_{R\to U}$ increases as i increases because of recruitment of histone-modifying enzymes. ε is a temperature-independent rate constant.
(3) $r_{U\to R} = u(T)(\alpha + \beta j)$: This is the transition rate from the unmodified state (U) to the repressively modified state (R) due to the deposition of repressive histone marks. $r_{U\to R}$ increases as the number of repressively modified units (j) increases because of recruitment of histone-modifying enzymes. $u(T)$ is a factor for temperature dependence, as explained below. T represents temperature.
(4) $r_{A\to U} = v(T)(\alpha + \beta j)$: This is the transition rate from the actively modified state (A) to the unmodified state (U) due to the removal of active histone marks. Similar to $r_{U\to R}$, $r_{A\to U}$ is an increasing function of j. $v(T)$ is a factor for temperature dependence, as explained below.

Exposure to cold triggers a series of histone modifications, ultimately resulting in the replacement of active histone marks by repressive ones. Thus, the transition from the active state (A) to the unmodified state (U) and that from the unmodified state (U) to the repressive state (R) would be influenced by temperature.

Figure 1. Schematic diagram of the model.



Because *VIN3*, which initiates the transition from the active to the repressive state, is expressed only under cold conditions ([3]), inequalities of $v_{cold} > v_{warm}$ and $u_{cold} > u_{warm}$ hold. Whether transition rates might revert back to original levels after cold remains an open question. Thus, we adopt a flexible assumption by considering that $v(T)$ and $u(T)$ are given by v'_{warm} and u'_{warm} after cold. In the model analysis, we first adopt a well-controlled temperature regime: the plant is placed under warm conditions for 12 weeks. Then, it is exposed to a cold temperature for 12 weeks, and finally it resumes growth under the original warm conditions. We also apply noisy temperature signals in nature, namely hourly temperatures in 1998 in Nishiwaki, central Honshu, Japan (35°00'N, 134°59.9'E), as external signals.
The model assumes that enzymes that deposit repressive (or active) histone marks are more likely to be recruited as the number of the repressively (or actively) modified nucleosomes

increases. This positive feedback gives rise to bistability, characterized by two stable equilibria: one with a high fraction and the other with a low fraction of repressive nucleosomes.

When ε and $v(T)$ are small, the stochastic process can be approximated by the deterministic dynamics plus random fluctuations. The deterministic dynamics for the fraction of repressively modified nucleosomes are given by:

$$\frac{dy}{dt} = f(y)/N,$$

where

$$f(y) = \frac{(1-y)v(T)(\alpha N + \beta N^2 y)u(T)(\alpha + \beta N y) - y\varepsilon(\alpha N + \beta N^2(1-y))(\alpha + \beta N(1-y))}{u(T)(\alpha + \beta N y) + \alpha + \beta N(1-y)}.$$

III. Result

When the positive feedback was weak (i.e., strength of enzyme recruitment, β, is small), the cell system became mono-stable in which each cell changed from the state with a high fraction of repressive nucleosomes ("high-R" state hereafter) to that with a low fraction of repressive nucleosomes ("low-R" state hereafter) in a noisy manner due to the shift from warm to cold conditions (Fig. 2a–c). In contrast, when the positive feedback was strong (i.e., β, is large), the cell system became bistable in which each cell responded to a temperature change in a digital manner by a rapid and (almost) noise-free transition from a high-R to a low-R state (Fig. 2d-f). Some cells switched from high-R to low-R states immediately after the change from warm to cold conditions (Fig. 2d), but others remained in a high-R state even after a change to cold conditions and eventually switched to a low-R state after a period of time that differed greatly among cells (Fig. 2e,f).

Asynchrony in timing of the transition among cells is the mechanism behind a slow repression (as well as a slow recovery) of *FLC*. We compared the level of *FLC* expression in a cell population under noisy temperature conditions. When the cell system is mono-stable, *FLC* expression changed up and down in response to short-term fluctuations of temperature. In contrast, when the cell system is bistable, *FLC* expression responded only to the long-term seasonal trend in temperature, resulting in a slow and smooth decline from fall to winter, and a slow increase from winter to spring. This trend is consistent with observation in a population of *A. harelli* ([19]).

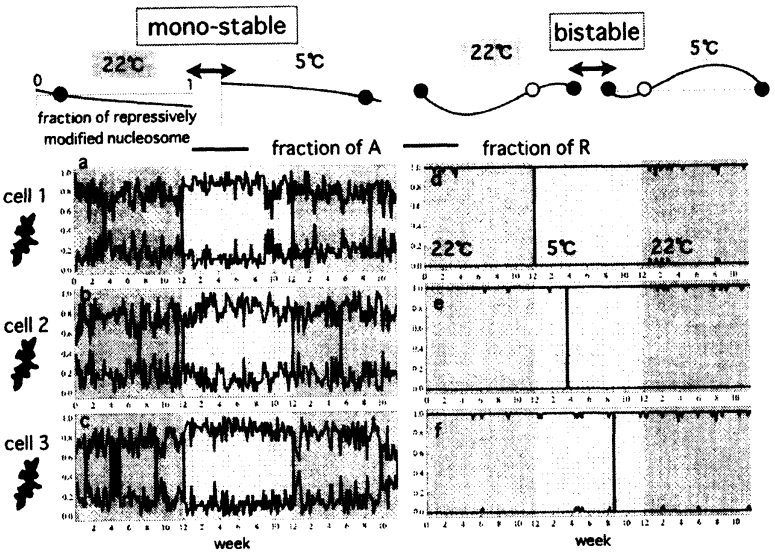


Figure 2. Response dynamics of *FLC* chromatin to temperature change at a cellular level. (a–c) Left panels show samples of time development of the fraction of active (red) and repressive (blue) nucleosomes when the positive feedback is weak. The strength of the positive feedback $\beta = 0.05$. (d-f) Right panels show comparable samples when the positive feedback is strong. Arrows indicate the timing of transition from the low-R to the high-R state. $\beta = 2.0$. Pink regions represent warm conditions, and blue regions are cold conditions. Number of nucleosomes is 20.

We can explicitly define the duration of winter memory at a cellular level by applying the concept of the mean first passage time in the theory of stochastic processes. Let $\bar{T}_{\text{activate}}(0,N)$ be the mean time until the cell reaches the fully active state after vernalization (i.e., $y = 0$), starting from the fully repressed state during vernalization (i.e., $y = 1$). In the fully active state, the number of actively modified nucleosomes is N, and that of repressively modified nucleosomes is 0, whereas the number of actively modified nucleosomes is 0, and that of repressively modified nucleosomes is N in the fully repressed state. We use $\bar{T}_{\text{activate}}(0,N)$ as an index of the duration of winter memory. $\bar{T}_{\text{activate}}(0,N)$ increases as the addition rate of repressive marks after vernalization (u'_{warm}) increases, implying that plants employ a long winter memory when the repressive marks are deposited rapidly. Winter memory can be longer than a year implying that the transition is virtually irreversible. $\bar{T}_{\text{activate}}(0,N)$ also increases with an increase of v'_{warm}/ε. v'_{warm}/ε represents the relative ratio of removal of active marks after vernalization (v'_{warm}) to that of repressive marks (ε). This result implies that the activity of components that initiate the vernalization response (e.g., VIN3) needs to be as great as that of demethyltransferase involved in the removal of repressive marks even after vernalization. Given that *VIN3* expression vanishes after the temperature rises, it is plausible to assume that v'_{warm} is as small as v_{warm}, which is the removal rate of active marks before vernalization. Thus, we demonstrate that stable maintenance of *FLC* repression after a return to warm conditions originates from rapid deposition of repressive marks after vernalization (i.e., large u'_{warm}).

Because the timing of the transition from silenced to activated state varies among cells, we here explore the duration of winter memory of the whole individual plant, which is represented by a population of cells. To do so, we formalize *FLC* expression dynamics in a cell population. When the cell system is bistable, each cell is in either a low-R or a high-R state, and the transition of a cell between the

two occurs at random times due to stochasticity (Fig. 2). Additionally, low-R and high-R states correspond to activated and silenced states, respectively, with respect to *FLC* transcription in a single cell. Thus, we formalized the dynamics of *FLC* expression as the population dynamics of activated cells in which *FLC* is actively transcribed. Let F_A be the fraction of activaed cells. A silenced cell reverts back to an activated cell at an instantaneous rate $p = 1/T_{\text{activate}}$. T_{activate} represents the waiting time until the transition from silenced to activated state as argued in the section 3.3.

In a similar manner, an activated cell changes to a silenced cell at an instantaneous rate $q = 1/T_{\text{repress}}$, where T_{repress} is the waiting time until the transition from an activated to silenced state. Using q and p, the temporal dynamics of F_A is given as:

$$\frac{dF_A}{dt} = p(1 - F_A) - qF_A. \qquad (1)$$

The solution of Eq. (6) is given as follows:

$$F_A(t) = \frac{p}{p+q}\left[1 - e^{-(p+q)t}\right] + F_A(0)e^{-(p+q)t}, \quad (2)$$

where $F_A(0)$ is the initial value of F_A. Because the population-averaged level of *FLC* expression (x_{FLC}) was assumed to be in proportion to the fraction of activated cells (F_A), slow repression (or recovery) of *FLC* predicted from computer simulations (Fig. 3d) should be given by Eq. (1). The *FLC* expression level converges to the equilibrium of $F_A^* = p/(p+q)$. Based on this equilibrium, we classified three phases: (1) silenced *FLC*, when F_A^* is smaller than 0.1; (2) activated *FLC*, when F_A^* is larger than 0.9; and (3) an intermediate *FLC*, when $0.1 \le F_A^* \le 0.9$. Note that the maximum level of *FLC* expression is normalized as 1.

Activated *FLC* is realized when either the removal of active marks or the addition of repressive marks occurs slowly (i.e., both $v(T)$ and $u(T)$ are small). An increase in $v(T)$ and $u(T)$ due to temperature changes from warm to cold drives a shift from activated to silenced *FLC*. When the plant is returned to warm conditions, v_{cold} would decline to an original level of v_{warm} because *VIN3* expression vanishes. When u_{cold} also returns to u_{warm} after a return to warm conditions, *FLC* expression is always reversible; *FLC* expression level increases exponentially and converges to the activated *FLC* level. To realize the stable maintenance of *FLC* repression after vernalization, u'_{warm} needs to stay at a level as high as u_{cold} even after the cold signal disappears. When u'_{warm} remains at a high level, the plant system retains a silenced *FLC* state, and winter memory is maintained over the life of the plant. These results suggest that differences between annuals and perennials can be explained by the different magnitude of u'_{warm}. Whether the cell system is mitotically active or not does not influence this argument.

IV. Discussion

We developed a stochastic model for chromatin modification in which the dynamics for the modification of a single nucleosome is aggregated to the on–off behavior of a cell and further

integrated to a change in *FLC* expression of a whole individual plant. Using the mathematical model, we addressed three questions: robust response to noisy temperature fluctuation, winter memory, and difference between annuals and perennials.

A robust response to noisy temperature fluctuations and winter memory coded as epigenetic memory can be realized through the bistability of cellular systems. The bistability is induced by positive feedback in chromatin modification, which can arise if nucleosomes carrying a specific modification recruit enzymes that catalyze similar modification of other nucleosomes. The importance of bistability for a robust response to perturbations and biological memory have been identified in many natural systems, such as a cell-fate decisions in *Xenopus* oocytes, synthetic toggle switch in *Escherichia coli*, and long-term synaptic depression in mammals. Our study added another example of the essential role of bistable cellular systems in realizing winter memory in plants.

Additionally, we found that cell-to-cell variation in on–off timing is effective in converting slow stochastic changes in individual cells to a gradual and steady response of the whole plant. We call this mechanism "cell-population coding of winter memory." In the stochastic model studied in this paper, the cell system is characterized by two stable equilibria of silenced state and activated state with respect to *FLC* expression. A shift in temperature from warm to cold conditions triggers a rapid transition from the activated to silenced state. This transition occurs stochastically, with timing differing greatly among cells, leading to a gradual increase in the fraction of silenced cells in a cell population. After some intervals, most of the cells eventually transform from the activated state to the silenced state, and the level of *FLC* expression of the whole tissue (including many cells) becomes sufficiently low. Analysis of the mean first passage time from activated to scilenced state showed that the mean time required for the full repression of *FLC* decreases as the deposition speed of repressive marks increases. This implies that the plant could respond to diverse intervals of cold exposure by adjusting the speed of deposition of repressive histone marks.

Calculation of the mean first passage time from a silenced to an activated state enabled us to measure the stability of *FLC* repression (i.e., the duration of winter memory). The duration of winter memory increased as the deposition of repressive histone marks became faster and duration time can be longer than 365 days in mitotically active cells. Based on these results and analysis of equilibrium levels of *FLC* expression at the cell population level, we conjecture that the deposition speed of repressive histone marks after cold can be a critical factor differentiating the behavior of annuals and perennials. In annuals, the deposition speed of repressive histone marks after vernalization should remain rapid as the level during vernalization, whereas in perennials, it should revert back to the level before vernalization after a return to warm conditions. This difference would discriminate between the stable maintenance of *FLC* repression in annuals and only transient repression of *FLC* in perennials.

Biological memory, defined as a sustained cellular response to a transient stimulus, has been intensively studied in many fields, including the cell cycle, cell fate, neurodegenerative diseases, and stem cell research. We believe that the mathematical framework developed here can be extended beyond the plant sciences to such diverse fields and can contribute to understanding the general design principles of slow responses of biological systems to internal and external signals.

ACKNOWLEDGMENT

This study was funded by PRESTO, Japan Science and Technology Agency

REFERENCES

[1] Dennis ES, Peacock WJ (2007) Epigenetic regulation of flowering. Curr Opin Plant Biol **10**: 520-527

[2] Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature **427**: 159–164

[3] Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell **11**: 949–956

[4] Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The *FLF* MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. Plant Cell **11**: 445–458

[5] He Y (2009) Control of the transition to flowering by chromatin modifications. Mol Plant **2**: 554-564

[6] Kim DH, Doyle MR, Sung S, Amasino RM (2009) Vernalization: winter and the timing of flowering in plants. Annu Rev Cell Div Biol **25**: 277-299

[7] Wood CC, Robertson M, Tanner G, Peacock WJ, Dennis ES, Helliwell CA (2006) The *Arabidopsis thaliana* vernalization response requires a polycomb-like protein complex that also includes VERNALIZATION INSENSITIVE 3. Proc Natl Acad Sci USA **103**: 14631-14636

[8] De Lucia F, Crevillen P, Jones AM, Greb T, Dean C (2008) A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. Proc Natl Acad Sci USA **105**: 16831-16836

[9] Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS, Zhang Y (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science **298**: 1039–43

[10] Schubert D, Clarenz O, Goodrich J (2005) Epigenetic control of plant development by Polycomb-group proteins. Curr Opin Plant Biol **8**: 553–61

[11] Turck F, Roudier F, Farrona S, Martin-Magniette ML, Guillaume E, Buisine N, Gagnot S, Martienssen RA, Coupland G, Colot V (2007) Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 Lysine 27. PLos Genet **3**: e86

[12] Zhang X, Germann S, Blus BJ, Khorasanizadeh S, Gaudin V, Jacobsen SE (2007) The Arabidopsis LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol **14**: 869-871

[13] Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C (2002) Multiple roles of *Arabidopsis*VRN1in vernalization and flowering time control. Science **297**: 243–46

[14] Mylne JS, Barrett L, Tessadori F, Mesnage S, Johnson L, Bernatavichute YV, Jacobsen SE, Fransz P, Dean C (2006) LHP1, the Arabidopsis homologue of HETEROCHROMATIN PROTEIN1, is required for epigenetic silencing of FLC. Proc Natl Acad Sci USA **103**: 5012-5017

[15] Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES (1999) The *FLF* MADS box gene: a repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. Plant Cell **11**: 445–458

[16] Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C (2004) Vernalization requires epigenetic silencing of *FLC* by histone methylation. Nature **427**: 164–167

[17] Shindo C, Lister C, Crevilenn P, Nordborg M, Dean C (2006) Variation in the epigenetic silencing of *FLC* contributes to natural variation in *Arabidopsis* vernalization response. Gene Dev **20**: 3079-3083

[18] Wang R, Farrona S, Vincent C, Joecker A, Schoof H, Turck F, Alonso-Blanco C, Coupland G, Albani MC (2009) *PEP1* regulates perennial flowering in *Arabis alpina*. Nature **459**: 423–427

[19] Aikawa S, Kobayachi MJ, Satake A, Shimizu KK, Kudoh H (2010) Robust control of the seasonal expression of the *Arabidopsis FLC* gene in a fluctuating environment. Proc Natl Acad Sci USA **107:** 11632-11637

[20] Dodd IB, Micheelsen MA, Sneppen K, Thon G (2007) Theoretical analysis of epigenetic cell memory by nucleosome modification. Cell **129:** 813-822