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# Remembering the past: Mitotic bookmarking in a developing embryo

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## Abstract

During development, transcriptional properties of progenitor cells are stably propagated across multiple cellular divisions. Yet, at each division, chromatin faces structural constraints imposed by the important nuclear re-organization operating during mitosis. It is now clear that not all transcriptional regulators are ejected during mitosis, but rather that a subset of transcription factors, chromatin regulators and epigenetic histone marks are able to 'bookmark' specific loci, thereby providing a mitotic memory. Here we review mechanisms of mitotic bookmarking and discuss their impact on transcriptional dynamics in the context of multicellular developing embryos. We document recent discoveries and technological advances, and present current mathematical models of short-term transcriptional memory.

## Addresses

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## Keywords

Mitotic bookmarking, Epigenetics, Transcription factors, Development, Memory, Histone modifications, Mathematical modeling, Transcription.

## Introduction

During development of multicellular organisms, transcriptional programs must be faithfully transmitted during each cellular division to ensure cell fate maintenance. Two steps of the cell cycle, mitosis and replication exhibit particular topological constraints, hindering stable inheritance of transcriptional repertoires. However it is now well established that mitosis is not always accompanied by a total erasure of past chromatin states, from mother to daughter cells [1]. On the contrary, memory of active and repressed chromatin

states exists, both at the short-term between successive mitoses and in longer time scale through multiple generations [2].

In this review, we focus on recent advances in understanding how active states are transmitted through cellular divisions *in vivo*. We will discuss potential mechanisms and possible mathematical models of mitotic bookmarking and present their consequences in developing multicellular embryos.

Mechanisms of short-term memory have also been analyzed in the context of cultured cell lines and recent reviews summarize these findings [1,3]. Similarly, long-term memory of repressed states mediated by Polycomb family has been intensively reviewed and will not be discussed [4].

## Mitosis, a challenging chromatin environment?

During mitosis, genome undergoes a dramatic metamorphose, resulting in clear morphological features of mitotic cells. This chromatin landscape was long thought to represent a hostile environment, incompatible with transcription and transcription factor binding [5].

However, recent studies using sensitive techniques nuance this view. While chromatin folding and compartmentalization are wiped out during mitosis [3,6], local accessibility can be maintained. ATAC-Seq experiments on mitotic *Drosophila* embryos revealed that patterns of accessibility are largely maintained through mitosis for several regulatory elements [7]. One may think that this is peculiar to the early fly embryo, where divisions are particularly fast. However genome accessibility is also widely preserved during mitosis in murine erythroblast cells, yet with local loci specific modulations [8]. Mitotic chromatin landscape has not yet been examined in vertebrate embryos, probably because of the relatively rapid loss in synchrony of divisions, operating as early as the 4-cell stage in zebrafish embryos [9]. This technical issue should be bypassed by recent developments of single cell technologies. Indeed, single cell Hi-C experiments were performed in Embryonic Stem Cells (ESCs) throughout the cell cycle, thus revealing a much more dynamic picture of chromosome organization than previous bulk experiments with unsynchronized cells [10].

### Mitotic bookmarking and development

Transmission of chromatin states from mother to daughter cells can be achieved passively or *via* active mechanisms. Among known active supports of mitotic memory, stand three classes of regulators: epigenetic histone marks/chromatin regulators, general transcription factors (GTF) and sequence specific transcription factors (TF) (Figure 1).

The vast majority of experiments aiming to assess mitotic retention of these particular factors has been performed in cultured cells with more or less conflicting conclusions, partly due to the level of purity of the mitotic population, cross linking conditions and to the methods of detection (global decoration by imaging versus specific binding assessed by ChIP). In this section, rather than providing an exhaustive survey of potential mitotic bookmarkers [1], we focus on potential supports for transmission of active chromatin states in the context of multicellular developing embryos (Figure 2).

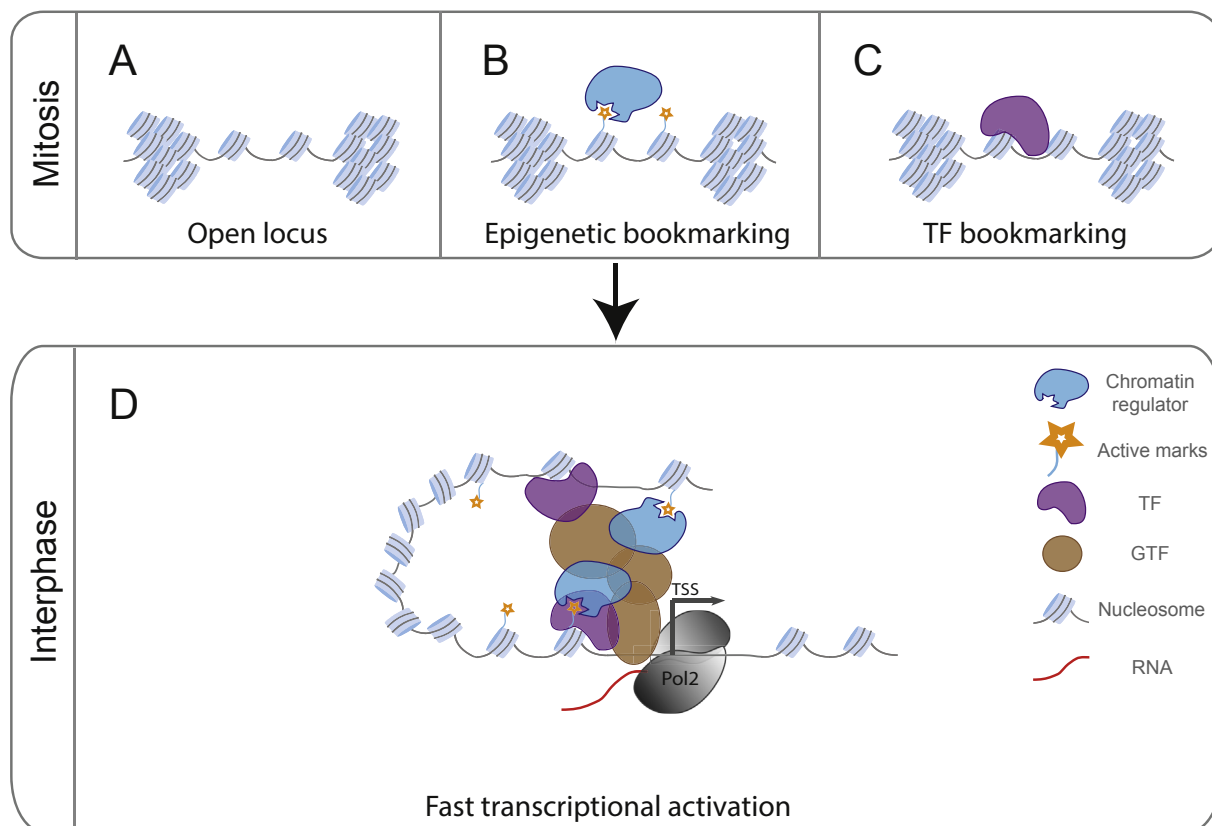
### Passive mechanisms of mitotic bookmarking

So far, clear evidences of passive supports of memory are lacking. In a recent work, by monitoring transcriptional activation in living early *Drosophila* embryos, it was shown that experiencing transcription prior to mitosis does not always lead to a rapid post-mitotic activation [11]. With a mesodermal enhancer, memory of active transcriptional states is unequivocally occurring [12], while with a dorsal ectoderm enhancer, memory is not detected [11].

However, given the widespread maintenance of chromatin accessibility during mitosis in the fly embryo, a passive mechanism is plausible (Figure 1).

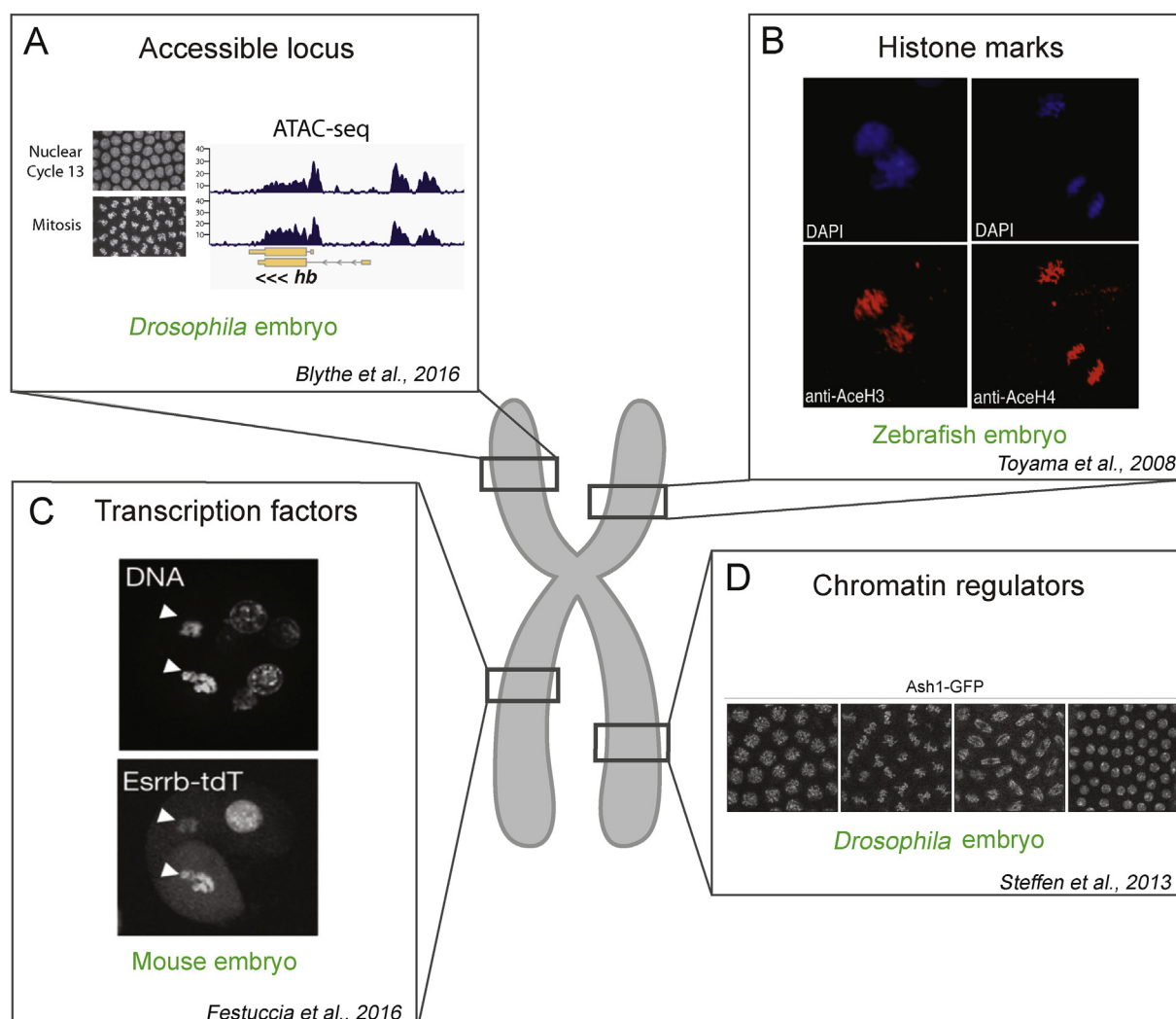
In interphase, gene expression seems to be partially regulated by local permissive or repressive environments, triggered by nuclear compartmentalization resulting from protein liquid–liquid demixing through phase separation [13] (e.g. HP1 in embryos [14]). The impact of phase separation during mitosis remains

Figure 1



Putative supports of memory of active genes/of transcriptional memory. Mutually non-exclusive mechanisms of mitotic bookmarking occurring at promoters and/or enhancers. A. Previously transcribing locus remains partially accessible during mitosis, thus facilitating post-mitotic re-activation. B. Histone marks and chromatin regulators (readers, writers) of an active chromatin state bind mitotic chromatin, thus 'bookmarking' particular loci for subsequent transcriptional activation. C. Transcription factors such as pioneer factors can associate to a subset of their targets during mitosis, consequently favoring their activation at mitotic exit. D. Through enhancer/promoter priming, all mechanisms illustrated in (A–C) lead to a rapid post-mitotic transcriptional activation.

Figure 2



Known mitotic chromatin landscapes. Potential supports of memory are illustrated by examples reported in embryos from different model organisms. A. Example of an accessible chromatin in mitotic *Drosophila* embryos, as shown by ATAC-seq experiments by Ref. [7]. B. Example of a histone modification mark, present in zebrafish embryonic dividing cells, revealed by fluorescent immunostaining (Dapi in blue, acetylated histone 3 and 4 in red) [23]. C. Representative example of mitotic chromosomal decoration by a transcription factor (Esrrb), in mouse embryo at morula stage [30]. D. Illustration of mitotic retention of a chromatin regulator, the histone methyltransferase Ash1 in living *Drosophila* embryos. Snapshot images from a time-lapse movie [20].

elusive, however mitotic structures can be assembled through phase separation in early *Caenorhabditis elegans* embryos [15]. Therefore, it is tempting to speculate that part of the mitotic maintenance of regulatory region accessibility could be due to local biochemical nuclear compartmentalization *via* phase separation. Whether this phenomenon stands as a passive or active support of mitotic memory is an open question.

### Epigenetic marks, their readers and writers

Owing to decades of genetic and biochemical studies, it is well established that antagonistic actions of Polycomb group proteins (PcG) and Trithorax group proteins (TrxG) allow for dynamic regulation of developmental

genes, yet with a cellular memory [4,16]. Epigenetic transmission of active transcriptional states is supported by conserved multifaceted TrxG complexes [4,16]. Among the best-characterized TrxG function is its histone methyltransferase activity leading to the trimethylation of lysine 4 of histone H3 tails (H3K4me3).

Whether H3K4me3 qualifies as an epigenetic mark during development is debated. Indeed, using a sensitive imaging technique (proximal ligation assay) in *Drosophila* embryos, H3K4me3 does not appear to be stable through replication [17], whereas it is clearly retained at discrete loci during mitosis [18]. In another model organism, *Xenopus* embryos, H3K4me3 has been

functionally associated to memory of active transcriptional states using nuclear transfer experiments [19]. Contrary to the myriad of available data concerning PcG complexes, little is known concerning the mechanisms of TrxG recruitment and particularly during mitosis *in vivo*. Generally speaking, we can distinguish two non-mutually exclusive mechanisms of mitotic epigenetic bookmarking: either through histone-modified tails (epigenetic mark), protected or not by their ‘reader’ enzymes or through their re-establishment after mitosis by chromatin ‘writer’ enzymes (Figure 1).

In *Drosophila* embryos, Ash1, a ‘writer’ member of TrxG, decorates mitotic chromosomes [20] (Figure 2). It is not known whether the mark deposited by this enzyme, H3K36me2 is also retained during mitosis. Interestingly, Ash1 enhances the recruitment of other histone methyltransferases like Trx and its mammalian homolog MLL [21], which in turn triggers H3K4 trimethylation at promoters [16,22].

Another mitotically retained member of TrxG family is the bromodomain-containing protein 4 (BRD4), shown to coat mitotic chromosomes of zebrafish embryos [23], even before zygotic transcriptional activation. Although with little validation in embryos, extensive work in mammalian cultured cells shows that BRD4 is a pleiotropic transcriptional and epigenetic regulator [24]. Interestingly H4K5ac, a mark recognized by BRD4 is detectable at particular promoters during mitosis in mammalian cells [25] as well as acetylated H4 in zebrafish embryos [23] and could thus be considered as a true ‘epigenetic’ bookmark (Figure 2).

#### Mitotic bookmarking by RNA Pol II machinery

Very few studies directly examined the localization of Pol II machinery during mitosis in embryos. The early fly embryo exhibits synchronous mitotic waves, whereby various steps of mitosis can be visualized in a single embryo. Using this ideal context, it was recently shown that active Pol II (Pol II-Ser5P) is present during prophase but is largely evicted at metaphase [7]. During mitosis, the clear majority of transcription ceases. However recent studies that employ sensitive techniques (for example pulse-labeling nascent RNA) nuance this statement and reveal low-level mitotic transcription in cell culture [26]. These paradigm-shifting findings would need to be confirmed in physiological conditions *in vivo*. However, they are in agreement with mitotic retention of GTF as the TATA binding protein, TBP, shown to bind mitotic chromosomes in ES [27] cells and to decorate chromatin in dividing mouse blastocysts [28].

#### Mitotic bookmarking by transcription factors

In developing embryos, very few sequence specific transcription factors have been shown to remain

associated to their targets during mitosis. However recent whole-genome profiling and live imaging revealed that several pluripotency TFs have the ability to bind mitotic chromosomes in cultured dividing ES cells [1,29]. This mitotic retention might also occur in mouse embryos but remains to be demonstrated. So far, among the few reported mitotically bound TF during development, stand Essrb (Figure 2) and Klf4 in dividing mouse blastomeres [30,31] and HNF1beta, retained during renal development in mice (Pontoglio lab, personal communication). Future investigations regarding TF retention during mitosis in embryos would require testing multiple fixation procedures and cross-validations with live imaging approaches. Indeed, formaldehyde fixation can lead to the artifactual displacement of some TFs from mitotic chromosomes [32,33].

Interestingly, the majority of the known mitotically retained TFs, for example some pluripotency factors (e.g. Oct4/Sox2/Klf4) function as pioneer factors [34]. Pioneer factors are a particular class of TFs, able to engage their target DNA at compacted nucleosomal regions, thereby fostering subsequent access to classical non-pioneer TF to their targets [35]. It is thus legitimate to ask whether pioneer factors might have intrinsic properties to bind chromatin during mitosis. However, little is known regarding the functional importance of pioneer factors for mitotic memory in developing multicellular organisms. Intriguingly, the pioneer factor Zelda, an essential activator of the early zygotic genome in *Drosophila* embryos, is not retained during mitosis and does not contribute to mitotic memory [11]. This could reflect that mitotic bookmarking is not a general feature of all pioneer factors, or alternatively, that Zelda is not a canonical pioneer factor.

#### Mitotic retention: stable versus dynamic bookmarking

Whole-genome kinetics of nucleosome turnover is much more dynamic than what conceptual ideas of stable epigenetic inheritance of chromatin states may provide. In cultured cells, histone modifications can be often erased and re-established several times during the cell cycle [36]. This finding may not be contradictory with mitotic bookmarking, when the binding properties of chromatin regulators are examined with advanced fluorescent microscopy methods (FCS, FRAP or SPT [37]).

In early *Drosophila* embryos, quantitative *in vivo* analysis of Ash1 dynamic properties demonstrate that this mitotically retained histone methyltransferase engages chromatin dynamically with estimated residence times in the order of seconds [20].

Similarly, in ES cells, pluripotency transcription factors bind mitotic chromosomes dynamically (e.g. residence time of Sox2 ~ 10 s) [1,29,38].



Of note, dynamic binding could be attributed to either sequence specific binding events, or to non sequence specific DNA binding [38]. The long-lived interactions, in the order of seconds generally reflect sequence specific bindings [29]. However, evidence for non-specific DNA binding during mitosis exists (e.g. FoxA) [39].

In order to clearly distinguish specific from non-specific binding events, one should ideally use mitotic Chip-seq experiments with quantitative imaging of DNA binding mutant versions of the TF assessed [37].

In sharp contrast to the dynamic mode of binding described earlier, some factors can bind to mitotic chromosomes in a stable manner. This is exemplified with TBP, which exhibits an average residence time in the order of minutes, in living dividing ES cells [27]. In conclusion, mitotic retention can occur through dynamic or stable binding, *via* sequence specific binding and non-specific binding.

### Consequences of mitotic binding on transcriptional dynamics

Mitotic retention does not directly bestow a bookmarking function. To firmly prove that a mitotically retained factor is involved in ‘memory’, one need to examine the consequences of its transient mitotic-specific depletion on dynamics of post-mitotic transcriptional activation.

These experiments are challenging and have thus far never been performed in embryos. However knockdown experiments of candidate bookmarking factors have been performed in synchronized mitotic cells [26,27,38] and in *Dictyostelium* [40]. Generally two types of outputs are assessed: whole-genome approaches to examine bulk transcriptional levels or live imaging following transcriptional reporters in single cells.

Mitotic-specific depletion of the hematopoietic transcription factor GATA1 in erythroid precursors delays the reactivation of key lineage specifying mitotically bookmarked genes [41]. Similarly, TBP mitotic transient depletion in ES cells delays the reactivation of the global ES cell transcriptional programme [27]. While displaying an overall genome-wide picture, these ensemble approaches lack temporal resolution. The MS2/MCP system allows to directly monitor actively transcribing loci (tagged with MS2 repeats) in living cells [42]. This technique not only measures the dynamics of transcriptional activation but also provides access to the lineage and thus to mitotic memory of active genes.

Using such approaches, memory of active genes has been first visualized in *Dictyostelium* [40], in mammalian cells [25] and more recently in *Drosophila* [12]. Using a stochastically expressed transgene, Ferraro *et al.* [12]

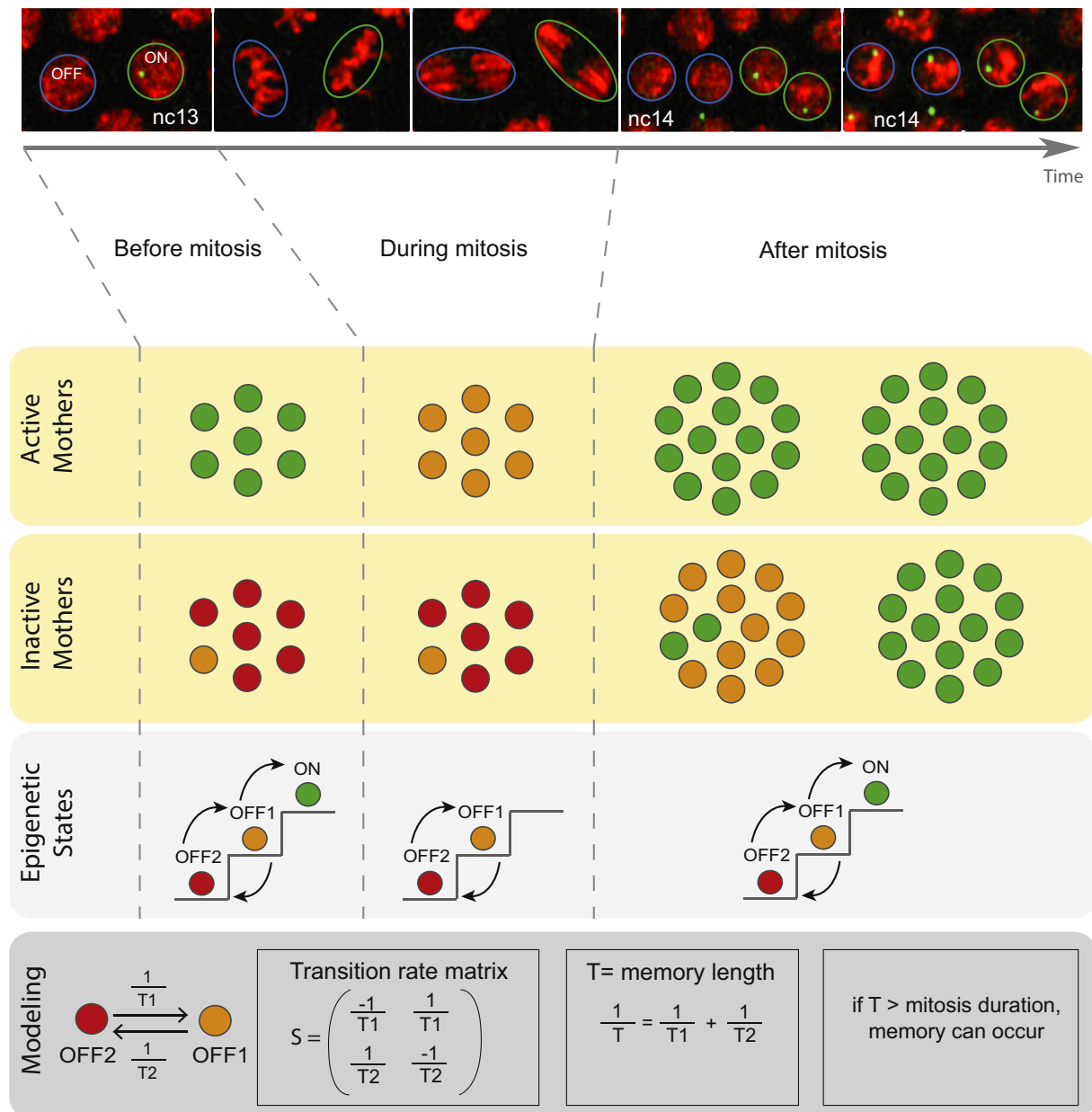
could distinguish transcriptionally active mother nuclei from their inactive neighbors. Quantitative analyses reveal that there is a higher probability for rapid reactivation after mitosis, when the mother was active. This bias corresponds to transcriptional mitotic memory. In the future, generalization of live imaging of transcription methods should allow for memory detection in other model organisms.

### Mathematical modeling of memory

A quantitative measure of mitotic memory is the difference in the timing of post-mitotic re-activation between descendants of active and inactive mothers. This difference can be explained by the fact that these two populations ‘travel’ through different states prior to activating transcription after mitosis. We can distinguish one active (ON) state and various inactive states (OFF), interpreted as more or less favorable chromatin landscapes [43]. The number of rate limiting kinetic steps leading from an inactive to an active state and *vice versa* can be higher than one, with lifetimes of inactive states ranging from one to several hundreds of minutes [43]. By stabilizing a competent yet inactive state (OFF1), mitotic bookmarking could prevent the decline of a mother cell to a less permissive state (OFF2), allowing a faster transcriptional recovery of the daughters (Figure 3).

A rather general class of mathematical models of memory can be based on Markov chains, used to explain transcriptional memory in *Drosophila* embryos [11] (Figure 3). Originally, Markov chains were used to describe memoryless processes. However, these models can acquire memory by an operation called “projection”. By projection, instead of following the dynamics of all the states of the system, one observes only the result of this “hidden” dynamics, namely the mRNA production. The projected dynamics exhibit memory in the sense that the future of the observed variable (mRNA production) depends not only on its present, but also on all past events that occurred prior to a time  $T$  ( $T$  is called memory length). This method, known as Mori-Zwanzig projection operator technique [44], has been recently applied to analyze propagation of signals in gene networks, as those occurring during vertebrate neural tube patterning [45]. In the model presented here (Figure 3), memory results from hidden states preservation, whereas memory length corresponds to the required time to forget differences between initial values of these states. For a finite-state, continuous-time Markov chain model, this time can be easily computed as the inverse of the smallest, non-zero eigenvalue of the transition rate matrix, in absolute value [46]. In the case of two inactive states, characterized by lifetimes  $T_1$  and  $T_2$ , respectively (Figure 3), memory length ( $T$ ) is the harmonic mean of the states lifetimes, which is long when both lifetimes are long. Thus, this model suggests that

Figure 3



Modeling memory of active states in an embryo. A. Snapshots of live imaging of an MS2-reporter transgene in a *Drosophila* embryo at nuclear cycle (nc) 13, during mitosis and at nc14. Nuclei are labeled with a Histone-RFP transgene and nascent MS2-mRNAs with MCP-GFP in green spots (Images from M. Lagha laboratory). B. Model of memory with two inactive states OFF1 (inactive but competent) and OFF2 (inactive and repressed). A third state ON is transcribing. The lifetimes of states OFF1, OFF2 are  $T_1$ ,  $T_2$ , and correspond to the transition time from OFF1 to OFF2, and from OFF2 to OFF1, respectively.  $T_3$  is the time to go from the state OFF1 to the active state. The transition rates  $1/T_1$ ,  $1/T_2$ ,  $1/T_3$  are reciprocals of the transition times. The memory length  $T$ , computed from the eigenvalues of the transition rate matrix, is  $T_1 T_2 / (T_1 + T_2)$ . Prior to mitosis, active mother nuclei (bookmarked) will be in state ON, whereas the population of inactive mothers will be distributed among states OFF1 and OFF2. During mitosis, the ON state is no longer accessible (transcription ceases). Therefore, previously active nuclei will be simply downgraded to state OFF1 while, provided that mitosis length is short compared to  $T$ , inactive nuclei will keep their states. After mitosis, daughters will be able to switch from OFF1 to ON or travel progressively from OFF2 to OFF1 to ON. The path to activation is shorter and consequently, the probability per unit of time to activate transcription after mitosis is increased for descendants of active mothers. Within a developmental pattern, this memory bias could favor the temporal coordination in gene activation (i.e. synchrony), with consequences in terms of cell fate.

efficient memory requires slow dynamics. Slow dynamics could in principle be favored by the presence of mitotic bookmarking, but this remains to be demonstrated.

### Consequences of memory during development

In the context of a developing multicellular embryo, mitotic memory can lead to a multitude of consequences, sometimes with opposite outcomes.

Inheritance of active transcriptional states ensures fidelity of transcriptional programs during their propagations through mitosis. Combined with other priming mechanisms during interphase, such as local chromatin opening at enhancers and promoter poisoning with paused polymerase, mitotic memory results in augmented transcriptional precision with less inter-cellular variability in levels of expression [47]. In sharp contrast, by allowing some cells to re-activate transcription faster than others, mitotic memory can enhance transcriptional noise [48]. From a theoretical point of view, memory can be seen as a ‘low pass filter’, buffering fast fluctuations, while allowing longer-lived fluctuations. Depending on memory length relative to mitosis duration and the architecture of the gene regulatory network (e.g. existence of feedback loops), mitotic memory can thus create precision and increase noise, but both are valuable during development [47]. For example, temporal precision in gene expression has been shown to be essential to gastrulation in the fast developing *Drosophila* embryo [49]. However, in slower developing embryos such as the mouse blastocyst, heterogeneity in gene expression has been proposed as advantageous since it allows for mis-patterning corrections [50].

Mitotic memory thus allows for a spectrum of consequences ranging from stability to flexibility and plasticity. However, for the particular developmental contexts of trans-differentiation or reprogramming, transcriptional states must be erased rather than memorized. Accordingly, demethylation of H3K4 facilitates reprogramming of *Xenopus* embryos [19] and mouse epiblast cells [22].

### Prospects

The vast majority of our knowledge regarding memory stems from studies performed in drug-synchronized cells in culture and has not yet been mirrored in multicellular developing embryos. This is mainly attributed to the dual technical challenge of profiling small number of cells/embryos and imaging living organisms.

With the recent technological advances in gene editing, signal amplification, microscopy combined to the possibility to probe the entire genome of limited material [51], developmental biology is embracing a new exciting era.

Synergizing quantitative biological data with synthetic biology frameworks [52] and mathematical modeling opens promising avenues for a better understanding of mechanisms and functional relevance of memory.

Recent combination of optogenetics with concomitant detection of transcriptional readouts in living embryos [53] should greatly facilitate testing the direct impact of candidate bookmarking factors on kinetics of transcriptional post-mitotic reactivation.

Decoding the role of mitotic bookmarking in developing model organisms endows two obvious consequences for human therapy. Indeed defective mitotic bookmarking has been associated to human pathological conditions, as exemplified by HNF1beta [54]. Moreover understanding how cells remember their past should greatly facilitate the design of reprogramming strategies for gene therapy.

### Conflict of interest statement

Nothing declared.

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We apologize to colleagues whose work could not be cited due to space constraints.

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