The condensed chromatin fiber: an allosteric chemo-mechanical machine for signal transduction and genome processing

2012 Phys. Biol. 9 013001

(http://iopscience.iop.org/1478-3975/9/1/013001)

IOP PUBLISHING PHYSICAL BIOLOGY

Phys. Biol. **9** (2012) 013001 (17pp) doi:10.1088/1478-3975/9/1/013001

PERSPECTIVE

The condensed chromatin fiber: an allosteric chemo-mechanical machine for signal transduction and genome processing

Annick Lesne[1,2], **Christophe Bécavin**[3] **and Jean–Marc Victor**[1]

[1] CNRS UMR 7600, Physique Théorique de la Matière Condensée, Université Pierre et Marie Curie, 4 place Jussieu, 75252 Paris Cedex 05, France
[2] Institut des Hautes Études Scientifiques, 35 route de Chartres, 91440 Bures-sur-Yvette, France
[3] Interactions Bactéries-Cellules INSERM U604 and INRA USC2020, Institut Pasteur, 25-28 rue du Docteur Roux, 75015 Paris, France

E-mail: lesne@lptmc.jussieu.fr, christophe.becavin@pasteur.fr and victor@lptmc.jussieu.fr

Received 29 July 2011
Accepted for publication 9 December 2011
Published 7 February 2012
Online at stacks.iop.org/PhysBio/9/013001

Abstract
Allostery is a key concept of molecular biology which refers to the control of an enzyme activity by an effector molecule binding the enzyme at another site rather than the active site (*allos* = other in Greek). We revisit here allostery in the context of chromatin and argue that allosteric principles underlie and explain the functional architecture required for spacetime coordination of gene expression at all scales from DNA to the whole chromosome. We further suggest that this functional architecture is provided by the chromatin fiber itself. The structural, mechanical and topological features of the chromatin fiber endow chromosomes with a tunable signal transduction from specific (or nonspecific) effectors to specific (or nonspecific) active sites. Mechanical constraints can travel along the fiber all the better since the fiber is more compact and regular, which speaks in favor of the actual existence of the (so-called 30 nm) chromatin fiber. Chromatin fiber allostery reconciles both the physical and biochemical approaches of chromatin. We illustrate this view with two supporting specific examples. Moreover, from a methodological point of view, we suggest that the notion of chromatin fiber allostery is particularly relevant for systemic approaches. Finally we discuss the evolutionary power of allostery in the context of chromatin and its relation to modularity.

1. Introduction

A major issue about transcriptional regulation in eukaryotes is to understand how signals (e.g. metabolic or hormonal signals) are turned into gene expression activation or silencing. Several challenging facts still ask for an explanation: some signals are not specific to a single gene; some factors, termed coregulators, do not directly bind a DNA sequence but rather modify histones by catalyzing post-translational modifications of their tails; regulatory events may take place downward the gene promoter; various delays are observed in the cell transcriptional response (Struhl 1999). The prokaryote 'operon' paradigm, where one signaling protein *locally* releases the specific inhibition of an associated gene so that transcription can take place, here dramatically fails. Gene regulation in eukaryotes is arguably not a local and one-to-one

process but rather a multi-factorial process unfolding in space and time in a controlled and coordinated way. Our argument to support this view is the role played by the chromatin fiber.

It is now acknowledged that transcriptional regulation in eukaryotes centrally involves chromatin (Widom 1998, Wolffe 1998, Felsenfeld and Groudine 2003, Bühler and Gasser 2009, Filion *et al* 2010). We argue, and demonstrate below, that it involves not only the biochemical features of the chromatin assembly but also, in an indissoluble way, the mechanical and topological properties of the 30 nm chromatin fiber (Lesne and Victor 2006). Actually, our functional arguments support the very existence of a chromatin fiber. Signaling events like factor binding or histone tail post-translational modifications, beyond having local consequences in terms of molecular recognition, can also be interpreted in terms of the chromatin conformational changes they trigger. Our claim is that these changes could control functional events, for instance, part of the transcription initiation, at distant and possibly multiple locations. In this regard, the chromatin fiber appears as a nanomachine capable to organize genomic processes in space and time, and to integrate more or less remote and delayed external events into an adapted transcriptional response. The consistency of the fiber features and functions along with its efficiency have presumably settled in the course of evolution, as a consequence of an unabated natural selection. This leads us to propose an extension of the notion of allostery and to describe the fiber and the embedded DNA as *allosteric entities* capable of signal transduction and processing.

Allostery refers to the propagation of a binding event with functional consequences at a distant site. The internal communication occurs through a global conformational change, termed an 'allosteric transition' in Monod *et al* (1965). This transition typically includes some structural modification at the active site switching its affinity to the ligand to a far higher value (Cui and Karplus 2008). Protein allostery has been extended to RNA with the discovery or design of allosteric ribozymes (Tang and Breaker 1997, Soukup *et al* 2001, Winkler and Breaker 2005). Despite the unbounded fruitfulness of the concept of allostery, it has never been applied to DNA nor chromatin fiber (Peracchi and Mozzarelli 2010). We claim that the conceptual framework relevant for allosteric proteins and ribozymes can be formulated and investigated in the context of DNA or even chromatin fiber.

The implementation and functional benefit of such a generalized allostery will be illustrated on two plausible scenarios. The first one describes how linker DNA (the stretch of DNA molecule between two adjacent nucleosomes) within a compact chromatin fiber is endowed with a conformational bistability, controlled by the acetylation status of the adjacent histone tails. This DNA conformational transition in turn controls the affinity of intercalating proteins for linker DNA and the cooperativity of their binding (Victor *et al* 2003). This scenario provides an example of the physical relays by which histone covalent modifications are capable to control protein binding at genomic sites. The second scenario describes how torsional constraints generated within heterochromatin by nascent RNA-polymerase activity triggers a sequential and cooperative conformational transition of the downward nucleosomes into a 'reversome' state, permissive to transcriptional elongation and forming a precursor allowing continued polymerase activity. Let us recall that the name 'reversome' (for *rever*se nucleo*some*) has been introduced in Bancaud *et al* (2007) for denoting a metastable particle containing the histone octamer, with DNA wrapped in a right-handed (positive) sense. This particle is obtained when extensive level of positive stress is placed on the nucleosome by a positive torque. The torque may be imposed either by magnetic tweezers (Bancaud *et al* 2007) or by an elongating RNA polymerase during transcription (Bécavin *et al* 2010). Both scenarios respectively parallel the Monod–Wyman–Changeux kinetic model of homotropic allostery (Monod *et al* 1965) and the induced-fit sequential allosteric Koshland–Némethy–Filmer scheme (Koshland *et al* 1966), both recalled in section 2.

Our extended notion of allostery underlines, and provides for, the need of an integrated functional and multilevel approach of eukaryote gene regulation. It raises a new challenge: accounting jointly for biochemical specificities of chromatin components and for mechanical and topological features of the chromatin fiber in the reconstruction and analysis of gene regulatory networks.

2. The notion of allostery

The notion of *allostery* introduced by Monod and Jacob (1961) refers to the control of an enzyme catalytic activity by an effector molecule binding the enzyme at another site (*allos* = other in greek) than the active site (Fersht 1985). It is related but sometimes confused with the notion of *cooperativity*, in part for historical reasons: one of the first kinetic models of allostery, proposed by Monod, Wyman and Changeux (Monod *et al* 1965), describes an instance of fully cooperative allostery, now termed *homotropic allostery*. It is based on the stabilization of a *concerted* conformational transition of all the enzyme identical subunits by the binding of the first ligand ('conformational capture'). The transition increases the subunit affinity for the (same) ligand and promotes its binding in a cooperative way (see figure 1). This scenario has been reinforced by the complete experimental investigation of a cooperative allosteric enzyme, hemoglobin, often taken as the paradigmatic example of allosteric enzymes (Perutz 1989, 1990). The competing model proposed by Koshland, Némethy and Filmer (Koshland *et al* 1966), relying on 'induced fit' and *sequential* conformational transitions of enzyme subunits, also accounts for some cooperativity. However, its kinetics and mechanistic explanation are different. The conjunction of allostery and cooperativity typically occurs in symmetric or tightly organized multi-subunits enzymes, in which the transition of one subunit into an active conformation enforces (or at least favors) the transition of other ones for steric and mechanical reasons.

Nevertheless, the relation between allostery and cooperativity is not at all a rule. In the case of *heterotropic*

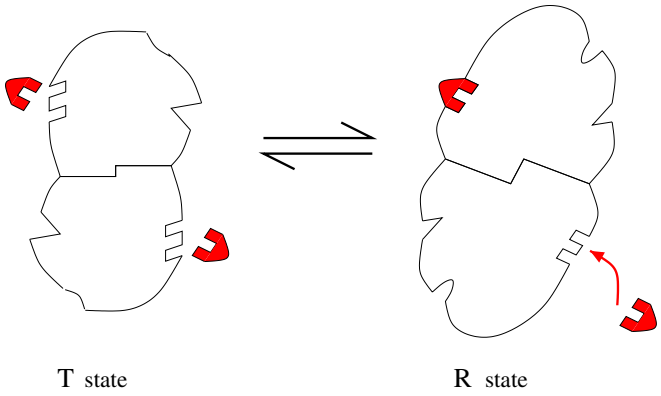


Figure 1. *Homotropic allostery*. The preferred isoform of the enzyme is the T-state, in which the affinity of each subunit for the substrate (in red/gray) is low. The binding of the first substrate molecule shifts the chemical equilibrium toward the R-state (conformational capture, Monod–Wyman–Changeux model (Monod *et al* 1965). Symmetry and mechanical rigidity of the enzyme ensure that all subunits (here two) experience *jointly* the transition T → R so that the net effect is a cooperativity of the substrate binding the enzyme. Namely the first binding event increases the rate of the other one(s). In the Koshland–Némethy–Filmer model (Kohsland *et al* 1966), the transition would be *sequential*, meaning that the transition T → R of one subunit triggers with a delay of the transition of the neighboring one. The first binding event thus progressively increases the rate of the other ones.

allostery, the effector molecule triggers some change at the distant active site. This change favors the binding of the substrate molecule (a different species), whose further transformations are in this way catalyzed by the enzyme (see figure 2). Allostery basically refers to the remote connection achieved by a chimeric enzyme between a signaling pathway ending at the enzyme effector site and a target pathway catalyzed by the enzyme at its active site. It is a perfect illustration of the *evolutionary tinkering* exposed by Jacob (1982) and the *gratuity* defended by Monod (1972). (Gratuity here refers to relationships that are not prescribed by physico-chemical laws or any similar necessity, e.g. steric constraints or kinetic features, but rather by chance (Monod 1972). It underlines that the existence of specific and adapted entities follows from several runs of natural selection among a randomly generated diversity.) Allostery indeed bridges two different pathways by the formation of a composite entity prone to adaptation and co-evolution. An allosteric mechanism, compared to a direct coupling, does not result from an immutable physico-chemical law but from the existence of a selection-tuned adaptor. As such, it has been called *the second secret of life* by Monod (the first one being the DNA double-helix). Moreover, achieving a causal relation between two pathways by means of an allosteric entity increases the functional robustness and adaptability of their relationship. Indeed, each part of the allosteric entity may adapt to a perturbation or, on longer time scales, adapt to the evolution of either the upward or downward pathways while preserving its bridging function (Lesne 2008). We here recover the benefit of modularity, a notion ubiquitously encountered in biological systems (Vespignani 2003, Wagner 2007).

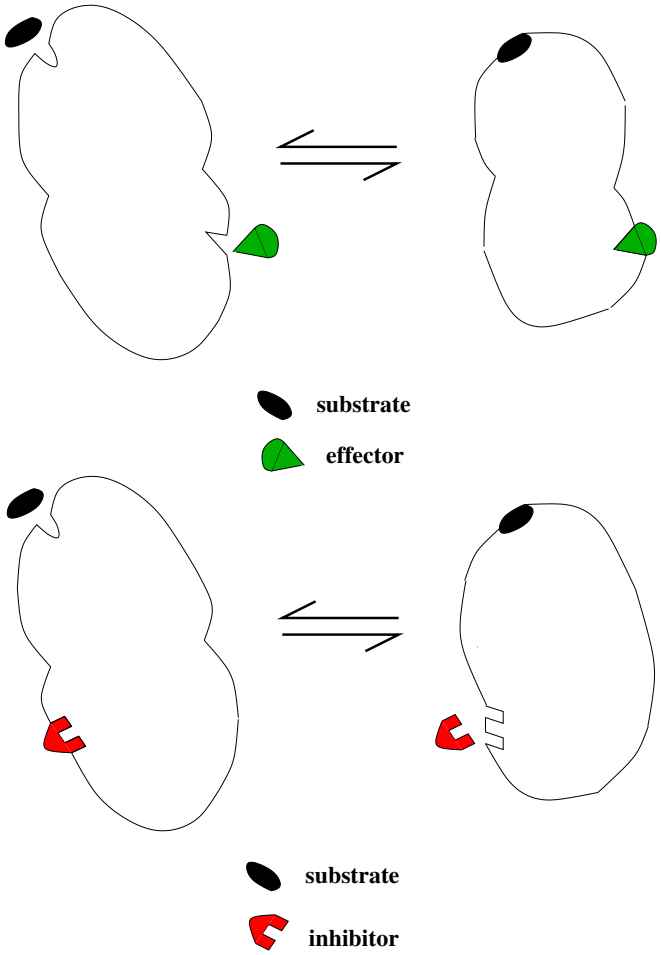


Figure 2. *Heterotropic allostery*. Top: binding of the effector (in green/gray) favors the binding of the substrate (in black) at the active site by capturing an isoform having a higher affinity for the substrate. Bottom: binding of the inhibitor (in red/gray) prevents substrate binding at the active site by capturing an isoform having a lower affinity for the substrate.

The evolutionary origin and adaptive enhancement of allosteric mechanisms have been thoroughly investigated for allosteric proteins (Peracchi and Mozzarelli 2010). It has been first evidenced using phylogenetic analyses of sequences of orthologous allosteric entities from different organisms (Georgelis *et al* 2009), typically showing residues co-conservation at the effector and active sites (Kannan *et al* 2007). A proof of principle that evolution can produce allosteric entities has been obtained with directed evolution experiments, in which random mutagenesis is supplemented with functional screening mimicking the effect of natural selection. Both allosteric ribozymes (Tang and Breaker 1997, Soukup *et al* 2001) and allosteric proteins (Mathonet *et al* 2006, Liang *et al* 2007) were thus generated or functionally improved. For instance, insertion of a pre-existing binding domain by gene recombination may produce protein switches (Guntas and Ostermeier 2004, Ostermeier 2005, Guntas *et al* 2005). Mutations in the residues along the allosteric pathway may hugely enhance the ligand-binding affinity of the active site (Jin *et al* 2006), supporting the evolutionary strengthening

of a preexisting structural connection between the effector site and the active site and its functional consequences.

We argue here that the notion of allostery is not restricted, by far, to enzymes and enzymatic catalysis. It is at work in two (often complementary) instances, easily recognized in allosteric enzymes but having a wider scope:

(i) when a composite object whose very existence couples different pathways or different subsystems, possibly of different natures. Its existence is the result of selection-driven co-evolution and adaptation with no prior (or only very weak) physico-chemical necessity. Acknowledged examples are tRNAs, providing the explicit association between an amino acid and one of the corresponding (anti)-codon, and *mechano-receptor neurons* translating a mechanical signal into an electric signal capable to trigger efferent neurons. If any, a weak physico-chemical affinity or relationship—although it does not play any role today—can be crucial in an evolutionary perspective since it explains how the connection between the two pathways occurred in the first place, long ago, and how the adaptor observed today (i.e. the allosteric entity) could have been designed to make the connection more persistent and tunable. An example is the slightly preferred affinity between an amino acid and its codons that is argued to have established the genetic code before the evolutionary design of tRNAs and aminoacyl-RNA transferases stabilized and quenched the correspondence (Thomas 1970, Demongeot 2007).

(ii) When some geometric constraints (symmetry, translocation, steric hindrance) or conservation of topological invariants (e.g. the linking number Lk in elastic filaments with fixed ends or closed) propagate the consequence of a given stimulus to another place. Allostery thus escapes the realm and scales of chemistry. Allosteric mechanisms rather operate at the level of macromolecules and macromolecular assemblies (or even cells) and rely in a central manner on the physical and topological properties of the system. Acknowledged examples are *symmetric multi-subunits enzymes* like hemoglobin, and *G-proteins* coupling extra- and intra-cellular signaling pathways across the cell membrane.

Most instances of signal transduction rely on allosteric objects, acting in a way similar to electric wire adaptors or voltage transformers. Reusing an old metaphor, a telegram written in English and sent from England to China by means of an allosteric channel would arrive translated in Chinese. (This metaphor has been proposed by Weismann to illustrate the impossibility of acquired trait inheritance that would be very like supposing that an English telegram to China is there received in the Chinese language (Weismann 1904).) We will here develop this claim in the context of transcriptional regulation where signals are any metabolic or hormone pathways observed to trigger a change in the gene expression profile of the cell, and the allosteric objects are the fiber itself and embedded DNA.

3. Epigenetic allostery in the condensed chromatin fiber

Let us now detail the extent to which our proposed extension of allostery is a notion pertinent to DNA and the chromatin fiber. Our approach substantiates and mechanistically explains the acknowledged fact that modulation of chromatin structure and dynamics plays a central role in all genomic processes (gene expression, replication, recombination, repair) (Felsenfeld and Groudine 2003). We will present the insights it gives on chromatin functional role of coordination, timing and remote control of transcriptional regulation (Lesne 2006, Lesne and Victor 2006).

3.1. Allosteric properties of DNA and the chromatin fiber

Chromatin fiber structure is determined in a bottom-up way by the assembly of DNA and histones and its local architectural features (Ben Haim *et al* 2001, 2002). Conversely it also exerts an essential top-down role in controlling the possible molecular processes occurring at the DNA level, by monitoring the accessibility of the interaction sites and most importantly by tuning their affinities for various factors through the mechanical constraints that the fiber imposes to DNA (Lesne and Victor 2006, Lavelle 2009, Lesne 2012).

The functional logic and benefits of this two-level causal loop and its concrete implementation can be captured by considering the chromatin fiber as an allosteric entity mediating a coupling between two distant, and physicochemically independent, genomic events. Accordingly, the chromatin fiber structure and its conformational changes orchestrate in space and time how the genomic sequences and their epigenetic typesetting are turned into specific switches, landmarks, recognition sites and checkpoints. Indeed, any event triggering a conformational change of the fiber will be indirectly coupled to all events whose occurrence and features (e.g. binding affinity and ensuing rates) are affected by this fiber change. Using the language of allostery, effectors are any signaling molecule (either a protein, a multivalent ion like a polyamine or any other ligand produced by the cell metabolism) or more generally, any change in surrounding conditions that is capable to elicit a conformational change of DNA or chromatin fiber, by which active sites are affected. Explicit examples of such effectors are enzymes that catalyze histone-tail covalent modifications or ATP-dependent remodeling factors that translocate the nucleosomes or change their structure. Effectors are not necessarily ATP-dependent. For example, they do not need ATP if they bind a structure previously prepared in a strained state and act as a switch that relaxes stored constraints.

Central to this extension of allostery is the notion of *chemo-mechanical coupling*. On the one hand, mechanical constraints experienced by macromolecules tune their chemical rates and affinities. Explicitly, an extra mechanical contribution is added to the free energy (i.e. $G = F - \text{force} \times \text{strain}$). On the other hand, out-of-equilibrium factor binding or enzymatic activity may generate, at the cost of ATP consumption, some mechanical constraints within

the macromolecules (Ingber 2003). A reversible allosteric modification achieves a transient regulation. An example is provided by HAT-catalyzed acetylation of histone tails (where HAT stands for histone acetyl-transferase) and HDAC-catalyzed deacetylation (where HDAC stands for histone deacetylase), where the effector site is the residue prone to acetylation and two different effector molecules are required for the direct and backward transformations.

Allosteric behavior in the context of epigenomics is intimately related to the structure of the chromatin fiber and its conformational dynamics. A first class of allosteric mechanisms is associated with the conservation of topological invariants, either at the DNA level or at the fiber level since both behave as flexible filaments. Note that topological constraints are an effective notion that is relevant at a supramolecular scale at which it makes sense to describe the entity (here DNA or the chromatin fiber) as a semi-flexible filament endowed with writhe, twist and linking number. At the molecular scale, there is only a complex balance between entropic and energetic contributions determining the possible isoforms. This fact reflects in the presence of both enthalpic (related to the secondary structure) and entropic (temperature-dependent, related to the tertiary structure) contributions to the twisting and bending persistence lengths of DNA (Marko and Siggia 1995). Similarly, at the next level, chromatin fiber elastic constants are expressed as a function of its local architecture and DNA persistence lengths (Ben Haim *et al* 2001). At the DNA level, tight organization of the fiber is essential in order that embedded DNA becomes sensitive to additional mechanical stresses and displays an allosteric behavior. Within condensed chromatin, an allosteric unit is formed by *each linker*, whose linking number is preserved by the anchoring of its ends onto fixed nucleosomes (the linking number measures the number of turns—of one end with respect to the other—that is stored within the filament (Crick 1976)). Mechanical constraints exerted by the condensed fiber onto DNA spread in the whole linker. The balance between elastic stresses generated by any binding event and DNA structural or conformational changes occurs at this linker level (Box 1). Another class of allosteric mechanisms is associated with geometric constraints, originating in the compactness and the symmetry of the condensed fiber structure. We recall that two main types of fiber structures have been proposed to date: a one-start helix in which consecutive nucleosomes are arranged as a solenoid, and a twisted two-start helix formed by a zigzag ribbon of nucleosomes. The solenoid model is no longer considered as a possible option due to the required energy for bending DNA in the corresponding structure. The two-start model is supported by the crystallographic study of the tetranucleosome (Schalch *et al* 2005) as well as by cross-linking experiments (Dorigo *et al* 2004) and quite recently by cryoelectron tomography of vitreous sections of erythrocyte nuclei (Scheffer *et al* 2011). More compact fiber structures have been proposed (Wong *et al* 2007), containing different numbers of starts (i.e. of helical piles of stacked nucleosomes). Examples of two-start and four-start structures are given in Box 2.

The geometric constraints imposed by such structures ensure a conformational rigidity of the fiber and embedded DNA that enforces the propagation of the effector event up to distant locations. For instance, the compact organization of the fiber generates steric hindrance within stacked nucleosome arrays and enforces concerted conformational changes, like a domino effect (Box 2). The central 'allosteric unit' is then formed by *each chromatin loop*, delineated by topological boundaries. These boundaries may follow from the presence of insulators or simply from defects in the regular structure of the fiber (Kim *et al* 2007, Bushey *et al* 2008, Ohlsson *et al* 2010, Botta *et al* 2010, Handoko *et al* 2011). They ensure the conservation of the linking number of the fiber stretch in the absence of topoisomerase activity. This conservation law couples any two events that occur within the stretch and modify the local contributions to the total linking number (Barbi *et al* 2005, Mozziconacci *et al* 2006). In fact, the fiber linking number is related to that of the embedded DNA according to $\mathsf{Lk}^{(fiber)} = \mathsf{Lk}^{(DNA)} - \mathsf{Lk}^0$ where Lk^0 is the linking number of the straight and relaxed DNA stretch (Barbi *et al* 2005). This relation expresses quantitatively the interplay between the DNA level and the fiber level. This is one of the reasons supporting the need to always consider in parallel the two levels. Indeed, any conformational transition of the nucleosome (and the ensuing modification of the DNA three-dimensional path around the histone core) and any local event twisting or bending DNA reflect in a modification of $\mathsf{Lk}^{(fiber)}$. Conversely, a conformational change of the fiber will affect the mechanical constraints experienced by the embedded DNA, hence all its transactions. The functional role in gene regulation of mechanical stresses, channeled by the architectural features of DNA and chromatin fiber, has also been underlined on experimental grounds by Kouzine *et al* (Kouzine *et al* 2008, Lavelle 2009).

3.2. *Cooperativity of the chromatin fiber allosteric behavior*

The conservation of the linking number of a chromatin loop not only elicits allosteric couplings at large scales but also enforces intra-fiber cooperativity. This cooperativity is expressed through concerted conformational transitions at the fiber scale (chromatin loops). In turn it brings about a (positive or negative) cooperativity between several events (e.g. factor binding or chemical modifications) occurring at distant places along the fiber. If the conformational transition occurs at the same time in the whole stretch, cooperativity follows a Monod–Wyman–Changeux-like scenario (Monod *et al* 1965). If in contrast the transition occurs sequentially, cooperativity follows a Koshland–Némethy–Filmer-like scenario (Koshland *et al* 1966). Hybrid scenarios sharing features of both (Horowitz 1995) are also possible since there is in fact a continuum between the two 'pure' scenarios (Goldbeter 1996, Danziger *et al* 2003). Some limitations on the cooperativity may arise from the limited rigidity, limited cohesion or limited symmetry of the chromatin fiber. A related observation has been made in the context of enzymatic catalysis (hemoglobin across various species) (Van Holde *et al* 2000). In huge assemblies of subunits, only a properly bound and mechanically constrained subset of subunits exhibit concerted conformational transitions that are at the origin

of a cooperative behavior. The experimental signature of this feature is a Hill index far lower than the total number of subunits. This Hill index gives a rough estimate of the size of the 'allosteric subset'. The equilibrium constant $L = [\mathrm{T}]_{eq}/[\mathrm{R}]_{eq}$ between the two states of a subunit (the states related by the allosteric transition T $\leftrightarrow$ R) has to be neither too low nor too strong in order to get a genuine cooperative effect. The subunits are quenched in the inactive T-state if L is too low whereas they switch independently toward the active R-state if L is too high, which both weaken the cooperativity. These constraints on cooperativity reflect in the bell-shaped and vanishing tails of the curve representing the Hill coefficient as a function of the equilibrium constant L (Goldbeter 1996). Cooperativity is also limited by a too large amount of noise that impairs the coordination between the subunit transitions, all the more since there are many subunits (typically here the base pairs or the nucleosomes). The maximal number $n_{\max}$ of subunits that can actually exhibit a cooperative behavior is bounded and the upper bound scales as $n_{\max} \sim 1/(\mathrm{noise})^2$. Obviously these general properties are of relevance in the chromatin context.

3.3. Transcriptional regulation and chromatin fiber allostery

Let us consider transcriptional regulation with this novel allosteric viewpoint. Transcription is a complex process that can be roughly separated in three phases: initiation, elongation and termination. They are all submitted to regulation, and in this way participate in gene expression regulation. Initiation involves a coordinated sequence of specific factor binding events, which leads to the assembly of the initiation complex at a gene promoter (Métivier *et al* 2003). We argue that initiation also requires some prior local and still unknown mechanism for loosening the very tight post-mitotic structure of the fiber and relaxing both compaction and topological constraints. This 'pre-initiation' stage, which allows the first *site-specific* binding event, is presumably less specific with respect to the DNA sequence. We suggest that it may be controlled at the chromatin fiber level as an allosteric mechanism relating some transcription signal to a fiber conformational change. This hypothesis agrees with the recent founding of a flexibility in the number of nucleosomes embedded in promoter sequences, in turn impacting on the local tightness of the chromatin fiber (Arneodo *et al* 2011).

Generally, transcription initiation—as well as the subsequent processes—relies on several types of information (Fox Keller 2009, Arneodo *et al* 2011), either genetic or epigenetic, structural or dynamics, and more or less spoiled with stochasticity. Namely, this information is contained

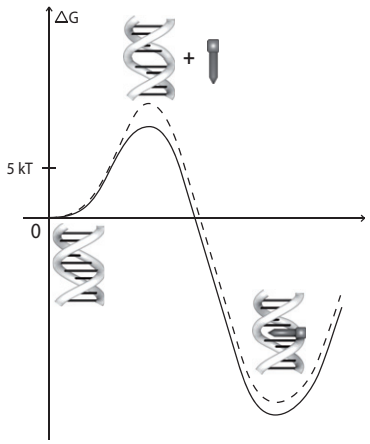
- in the genomic sequence (gene promoters, transcription factor binding sites);
- in bound proteins, either transcription factors binding DNA (for example TATA-box binding proteins) or coregulators binding DNA-bound proteins (histones or transcription factors);
- in histone chemical status that monitors the tightness of the chromatin fiber. In particular, histone-tail acetylation controls the anchoring of linker DNA onto the nucleosome, hence partly controls linker DNA-binding affinities (Victor *et al* 2003);
- in the chromatin conformation, condensed and topologically constrained or not;
- in the nuclear localization of the chromatin loop embedding the gene, e.g. near a nuclear pore (Cabal *et al* 2006);
- in the cell state, e.g. the phase of the cell cycle; and
- in the cell microenvironment, since a large fraction of changes in transcriptional activity are elicited by signals coming from outside the cell.

All these features act at different levels on RNA-polymerase binding and activity, either directly or in a way mediated by signaling pathways or physical constraints. In other words, the cell itself performs a multiscale integration of all these informations and inputs in order to achieve an adaptable, context-dependent regulation of gene transcription. As seen in sections 3.1 and 3.2, privileged means for this *in vivo* integration are provided by allosteric potentialities of the chromatin fiber. Indeed they allow us to coordinate distant events, of different natures, and to either focus or extend their range of influence. For example, the fiber may be essential in coordinating the binding of different chromatin-binding proteins (Grewal and Elgin 2007).

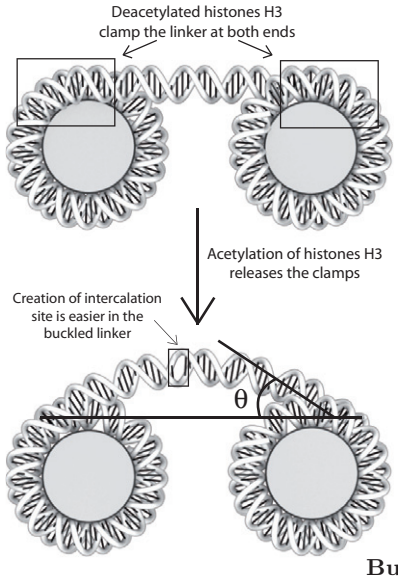
As an experimental support of our allosteric view on chromatin, let us mention the *in vivo* evidence that transcription factors can be bound to the promoter with no detectable transcription. The experiment involved specific genes, namely the α_1-antitrypsin gene and the HFN4α gene in mammalian cells, and chromatin immuno-precipitation was used to assess transcription factor binding (Svejstrup 2004). This observation suggests that some event, presumably a remodeling event of the fiber, is required to trigger transcription initiation. The arrival of general transcription factors is not enough. The fiber may here control not only the binding affinity of transcription factors but also the functional consequences of their binding. Even polymerase recruitment is not synonymous of active transcription, as shown by the recent observation of 'engaged polymerases' with no further activity (Core *et al* 2008). This is due to the presence of a nucleosome strongly positioned immediately after the transcription start site, thus hampering polymerase elongation activity (see the following subsection). These examples are possible instances of a nonspecific control, at the fiber level, of specific events, tagged by the prior binding of transcription factors at specific sites.

3.4. Two plausible allosteric scenarios within the condensed fiber

We will illustrate the importance of fiber allostery in transcription regulation with two scenarios, concerning respectively transcription initiation and transcription elongation:



Thermal activation of an intercalation site and its conformational capture by an intercalating protein. The dotted curve is obtained in end-constrained DNA and departs from the continuous curve obtained for free DNA (Chaires 2000).



Buckling instability of a linker DNA within chromatin fiber. Allostery here stems from this instability of the DNA constrained state. The allosteric entity is the linker, the subunits are the base pairs, the T-state corresponds to a straight linker and the R-state to a buckled linker (with the terminology of (Monod *et al* 1965)).

Box 1

(i) histone-tail controlled intercalation in linker DNA (see Box 1).

Within the condensed 30 nm chromatin fiber, nucleosomes are fixed and linkers are end-constrained. Thermally activated conformational fluctuation induces transient openings of an intercalation site. In turn, such an opening increases the mechanical constraints experienced by the linker containing this site and triggers linker buckling. This linker conformational change is captured when intercalation actually occurs. It then promotes further bindings. Indeed, we have shown in Victor *et al* (2003) that the binding energy cost $E(n)$ is linear with respect to the number n of intercalating proteins when the linker is buckled, whereas the binding energy cost $E_0(n)$ in the straight linker DNA is quadratic in n. Accordingly $E(n) \sim n \ll E_0(n) \sim n^2$ as n increases. This dependence means that linker DNA buckling promotes multiple bindings. (Strictly speaking, there is no cooperativity since $E(n) \sim nE(1)$. We should rather say that the 'anti-cooperativity' due to the quadratic dependence $E_(n) \sim n^2$ is suppressed.) Intercalation is not controlled by a restricted accessibility but by the modulation of DNA binding affinity for intercalating proteins following from the mechanical constraints experienced by the linker. Buckling cannot occur when the linker is tightly clamped onto the nucleosomes, which is the case when histone tails are deacetylated.

This scenario brings forward a Monod–Wyman–Changeux-like behavior of linker DNA with respect to protein intercalation. To make clear the parallel with the original scenario by Monod–Wyman–Changeux and its terminology (Monod *et al* 1965), the allosteric entity is here the linker, the subunits its base pairs, the T-state its straight conformation, the R-state its buckled conformation, and the effector an intercalating protein. This scenario is itself controlled in an allosteric way at the fiber level in two ways: (i) the decondensation of the chromatin fiber suppresses the bistability of the linker conformation (the linker is no longer constrained); (ii) the control of the buckling ability of a constrained linker DNA is itself an allosteric switch, triggered by acetylation of histone tails (or conversely deacetylation that quenches the straight linker conformation). We here see the articulation of two levels of allosteric behavior. Such *nested* allosteric regulation has been alluded on thermodynamical grounds in Robert *et al* (1987). It is close to the recent paradigm of two-component signal transduction, presented e.g. in Stock *et al* (2000), according which two transduction steps, and accordingly two adaptors, are involved between the initial triggering event and the target. This scenario may play an essential role in controlling the binding of TBP (TATA-box binding protein) that is a pre-requisite for transcription initiation (see figure 3). It is also relevant to HMGB proteins, which bind DNA with three intercalating residues while bending DNA and possibly enhancing DNA flexibility.

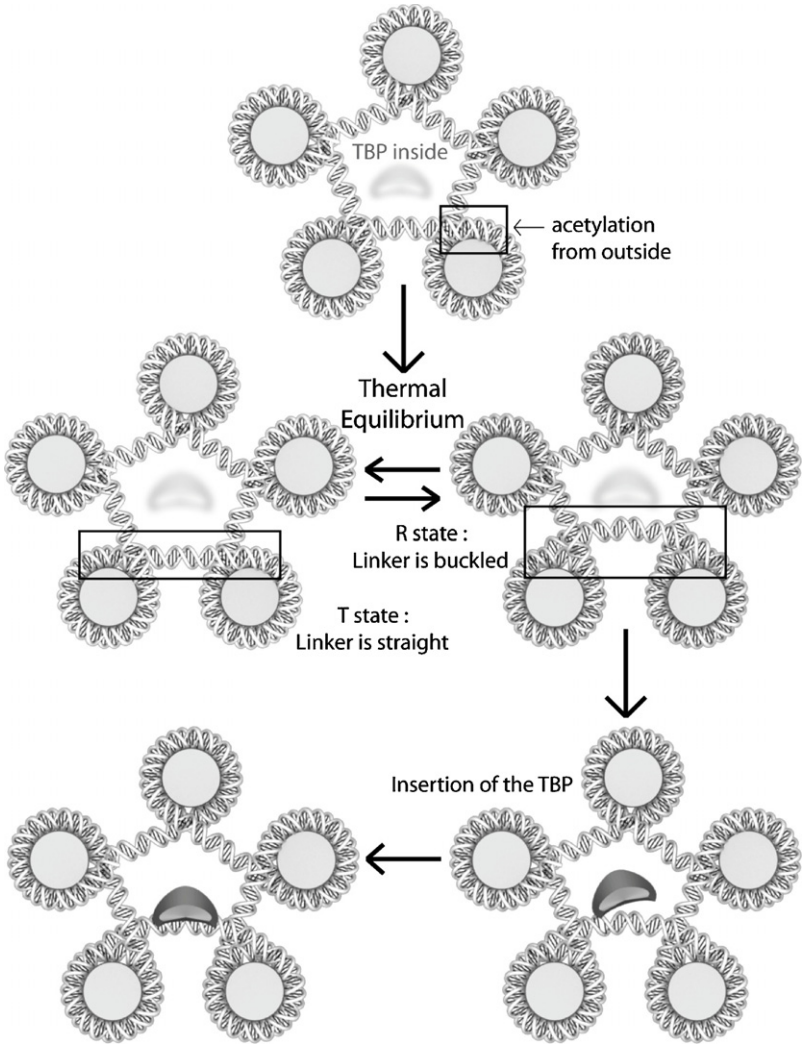


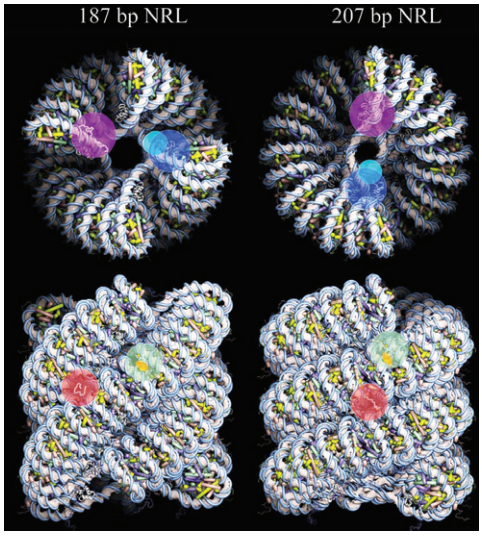
Figure 3. TBP binding and linker buckling instability. TBP binding strongly distorts DNA conformation, which generates elastic constraints within DNA when the linker containing the binding site has fixed ends. This figure illustrates how histone post-translational modifications control the binding of TBP by modifying the tightness of linker DNA anchoring onto nucleosomes within the condensed post-mitotic chromatin fiber. In the case when linker anchoring onto the nucleosomes is loose (acetylated histone tails), elastic constraints generated by the binding of the first subunit of TBP (a bis-intercalator) triggers the buckling of the linker. In turn, buckling makes the linker more prone (compared to a straight linker) to bind a second intercalating TBP subunit. In contrast, when the linker is clamped, buckling cannot occur. The cost associated with elastic constraints is quadratic with respect to the number of intercalations whereas it is linear in the buckled conformation. Binding of the second subunit, hence actual binding of TBP (a prerequisite for transcription initiation (Chen *et al* 2002)) is energetically prohibited.

Another example is given by SOX proteins, including the sex-determining SRY protein, which are *sequence-specific* DNA binding proteins that intercalate in the DNA minor groove.

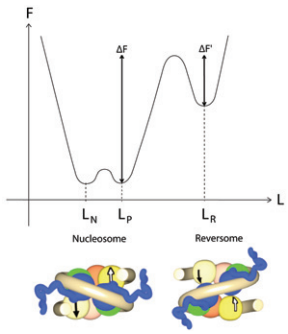
(ii) RNA-polymerase activity within heterochromatin (see Box 2).

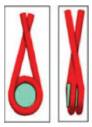
Elongation requires that RNA-polymerase encounters a specific conformation of the nucleosomes during its processing. Considering a RNA-polymerase engaged at some promoter site (Core *et al* 2008), we have shown in Bécavin *et al* (2010) that such a transcription-prone state of the nucleosomes ('reversome' state) can be triggered by the very RNA-polymerase initial activity and the torsional constraints it generates within a condensed chromatin loop. Steric constraints between stacked nucleosomes in the condensed fiber (*n*-start structure (Wong *et al* 2007), see Box 2) impose that the relaxation of the torsional constraints through nucleosome/reversome transitions occurs sequentially. These sequential transitions gradually turn the downward fiber stretch into a state fully permissive to RNA-polymerase processivity, in a kind of 'domino effect'. Dynamic consistency of the process—a product of co-evolution—brings a lot of information. In particular, RNA-polymerase velocity enforces the precursor formation and spreading, which in turn supports RNA-polymerase functional activity.

The allosteric mechanism at work in this scenario is reminiscent of the sequential induced-fit model of Koshland–Némethy–Filmer (Koshland *et al* 1966). Additional complexity here comes from the fact that the effector of the allosteric mechanism is also the recipient of its effect. The logical structure is that of an 'allosteric feedback loop'.

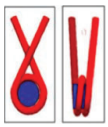


Alternative all-atom models (right: two-start structure, left: 4-start structure) of a compact chromatin fiber including linker histone H1. The close and regular nucleosome stacking along each start prevents nucleosomes to switch to reversomes, thus enforcing a concerted sequential transition. *Courtesy of Julien Mozziconacci.*





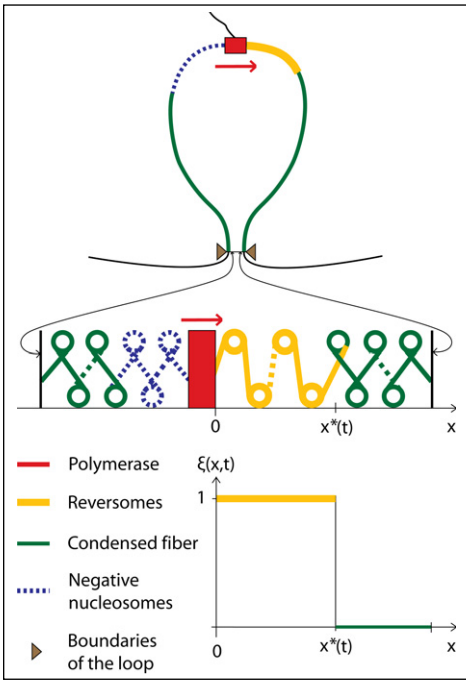
positively crossed



negatively crossed

Free-energy landscape for the nucleosome conformation. The reaction coordinate (abscissa L) is the linking number of nucleosomal DNA. This choice is relevant for investigating the landscape changes when a torque is applied to the DNA (light grey). The two main states of the nucleosome are (left) standard conformation, corresponding to two substates N and P according to the relative positions of the linkers (negative or positive crossing, details shown in the inset) and (right) reverse conformation (reversome), in which the histone core is partially unfolded and the nucleosomal DNA adopts a right-handed path around the histone core. Reversome is assumed to be the activated state permissive to RNAP progression through a nucleosome.

Box 2



RNA-polymerase progression within a chromatin loop: illustration of the domino effect. The loop is delineated by topological boundaries (triangles at the bottom of the loop). Torsional constraints exerted by the RNAP (red/dark grey rectangle) are trapped within the chromatin loop, and trigger the sequential transition of nucleosomes (thin green/gray line) downstream of the polymerase into reversomes (bold yellow/light grey line). The reversome density profile along the chromatin fiber (arclength x) is given below: in the bold yellow region $[0, x^*]$, the reversome density $\xi(x,t)$ equals 1. The wavefront is located at x^* and propagates downstream about 10 times faster than the RNAP progression. In the RNAP wake, the nucleosomes turn to the negative state (dashed blue line) to ensure the conservation of the total linking number of the loop.

We have suggested that this scenario accounts for the small amount of transcription observed in heterochromatin, which is necessary to recruit the silencing machinery. Striking experimental results recently evidenced that transcription indeed occurs within condensed chromatin fiber, right after the mitosis, and is required to trigger siRNA-induced silencing (Noma *et al* 2004, Grewal and Elgin 2007). Transcription has here a 'signaling role' rather than a 'productive role', so that its

rate remains quite low. However, this transcriptional activity shows that RNA polymerase can proceed through condensed chromatin fiber. Since it is followed by the formation of heterochromatin, we could expect that such a RNA polymerase activity is not accompanied with major decondensation of the post-mitotic fiber. Both facts provide a strong experimental support to the scenario proposed in Box 2.

In both cases (i) and (ii), allosteric behavior strongly relies on the fiber suitable compactness and organization. It fails in a loose fiber or tightly condensed fiber, that is, in a too loose or too rigid structure. In particular, linker DNA exhibits no buckling instability in decondensed chromatin. Steric constraints between stacked nucleosomes are essential to get sequential nucleosome/reversome transitions, ensuring that RNA-polymerase always faces a reversome during its progression. By tuning constraints experienced by its embedded parts hence their rigidity, chromatin fiber superstructure controls their allosteric potentialities. In the decondensed fiber, another mechanism, namely nucleosome disassembly, presumably occurs (Boeger *et al* 2008). This mechanism accommodates higher transcription rates.

The two scenarios presented here are presently out of reach of a direct experimental check but supported by the qualitative consistency of their assumptions and predictions with the observed facts.

Notably, Kouzine *et al* have shown *in vivo* that RNA-polymerase activity generates dynamic supercoiling (positive supercoiling downward the active RNA-polymerase and negative supercoiling upward). This supercoiling increases when the transcriptional activity increases, and it is capable to promote DNA structural transitions at stress-sensitive sites (Kouzine *et al* 2008). However, Kouzine *et al* only considered a structural effect at the DNA level, namely the modification of the recognition site due to supercoiling. The same limitation is encountered in the recent review (Levens and Benham 2011). In contrast, we consider either the change in the affinity of DNA-binding proteins for DNA (Victor *et al* 2003, Box 1) or the change in the energy landscape ruling nucleosome conformations (Bécavin *et al* 2010, Box 2). Both originate from an additional mechanical term in the free energy, which is proportional to the change in the fiber-linking number induced by DNA supercoiling.

4. Functional consequences of generalized allostery

Beyond the structural aspects of chromatin fiber allostery, we now explore some of its functional aspects. In short, fiber allostery covers any situation where an effector event controls a process at another location. It generally stems from an instability of a constrained chromatin fiber conformation. We argue below that it has strong implications in terms of localization and specificity of genomic functional processes. Conversely, we suggest that functional consequences of fiber allostery strongly support the very existence of the 30 nm chromatin fiber (still debated due to the lack of direct experimental observation). This puts the structure–function relationship of the chromatin fiber back in an evolution perspective. Fiber allostery also provides a useful guideline for the (necessary) systemic approach of genome functions.

4.1. Localization

An important feature of chromatin fiber allosteric behavior is the versatile correspondence between the stimulus range (localized vs extended) and that of the response. Four situations can be encountered:

(i) *stimulus and response are both localized*: a local event triggers a localized response at a distance (one would not speak of allostery if the response were co-localized with the stimulus). Examples are numerous. Let us mention acetylation of histone tails in a nucleosome favoring a partial unwrapping of nucleosomal DNA, hence site exposure and specific binding in the exposed stretch (Anderson *et al* 2001). Another example is cofactor binding at an enhancer site, which triggers transcription initiation at the gene promoter (Harnish *et al* 1998);

(ii) *stimulus and response are both extended*: for instance, an ionic change and its conformational consequences on both the DNA and the fiber. Another example is again acetylation of histone tails—but now over several nucleosomes—which induces a global decondensation of the fiber (Tóth *et al* 2004);

(iii) we suggest that it may happen that *a collective activation produces a localized event* (in general due to biological specificity and structural heterogeneity). Allosteric effect here typically proceeds by alleviating a nonspecific barrier, which promotes the occurrence of specific events. The effector can be any event inducing a global conformational change of the fiber that modulates the rate of local specific activities. For instance modulation of mechanical constraints experienced by the DNA promotes transcription factor binding DNA at a specific site (see Box 1). An example of collective activation is provided by the torsional constraints generated within a chromatin fiber loop by an active polymerase (see Box 2), which can induce at a distance, the activation of a cryptic gene;

(iv) *a local event is amplified and propagated by the fiber and triggers a large-scale response*. An example is given by a molecular motor (RNA-polymerase) generating torsional constraints within DNA, which may induce a conformational change of linker DNA (Box 1) or spread into a whole chromatin loop (Box 2).

4.2. Specificity

A similar diversity occurs as regards the respective specificity of the signal and the response. They can be both specific, both nonspecific or hybrid (a specific stimulus triggering nonspecific response, or a nonspecific activation triggering a specific change). This discussion seems to overlap that of the previous subsection; however, we suggest that localization and specificity only accidentally coincide and that it may be operational to distinguish between the two concepts. Speaking of a *signal*, rather than simply of an 'interaction', makes sense when the response is not proportional to the input but is rather an all-or-none output. This means that the signal is not an energy or matter provider, but the provider of an enzyme or some limiting factor required to trigger the output.

The signal is said to carry *information* (and then could be termed a *message*) when the associated input/output relation has a somehow gratuitous nature. In other words, the relation does not follow from inescapable '*ab initio*' physico-chemical constraints relating the input and the output but from the evolutionary design of adapted intermediary steps. What is termed 'signal specificity' (or 'effector specificity' in the language of allostery) covers several instances:

- sequence specificity (when the signal is a DNA-binding protein);
- amino-acid specificity (when the signal is relayed by histone-tail modifications)
- cofactor specificity (in the assembly of complexes);
- conformational specificity (e.g. recognition of DNA crossings by topoisomerase II).

The response specificity has thus to be investigated at the proper level. When the function lies in a DNA–protein interaction, response specificity can be appreciated in terms of the relief of the energy landscape at the DNA level and the way it is modulated by the signal (Chaires 2000). Indeed, such a landscape describes the energy barriers to be passed through for various binding events and conformational changes to occur (Gammaitoni *et al* 1990). A typical allosteric effect is to lower the energy barrier at the active site and promote the occurrence of binding events or, in contrast, to strengthen the energy barrier at the active site and select more stringently the events occurring at this site.

4.3. Signal transduction and information processing

Allosteric behavior is ubiquitous in *signal transduction*. An input, typically a signaling pathway ending in the binding of a signaling molecule, triggers an event of a different nature or belonging to a different pathway, at a different place (e.g. on the other side of a membrane, or in the present case of chromatin fiber, at a distant location along the genome). Signal transduction is nothing but an instance of information transfer and processing. For example, supercoiling conveys information along DNA over large distances (Kouzine *et al* 2008).

The relationship between the incoming signal and the induced response (for instance the tuning of some chemical reaction or some genomic process) is ensured by the very existence of an allosteric entity that relates the signal sensor and the active element emulating the response. Robust and fine-tuned adaptation of causal relations is promoted in living systems by the ubiquitous involvement of *transducers*. Also termed *adaptors* (the original name cast by Crick for tRNAs.), transducers are hybrid components matching different parts of the system of possibly different natures and logics. In order to switch an adapted interaction between parts A and B, mediated by a transducer $\mathcal{T}_{AB}$, into an adapted interaction between parts A and C, it is enough to change $\mathcal{T}_{AB}$ into a novel transducer $\mathcal{T}_{AC}$ whose A-side remains unaffected as regards its interaction site with A. Such transducers, including all kinds of interfaces, endow with a great flexibility the architecture of living systems. They make relevant both the notion of structural module (the parts) and the notion of functional module (the pathway coordinated by the proper sequence of transducers), reinforcing the importance of modularity in living systems (Vespignani 2003, Wagner 2007).

The event occurring at the effector site of an allosteric entity may be itself an allosteric process. Such an instance, which is the rule in sensory signal transduction, is known as a '*two-component system*' (Stock *et al* 2000). As indicated by the name, it involves two allosteric entities A and B. The first one, A, acts as a transducer that translates the signal into another signal that B 'understands', namely, that is capable to modify the effector site of B. The second one, B, is an allosteric entity that is triggered by the translated signal and turns this signal into action. This system achieves information transfer, translation and processing with the advantage of exhibiting more checkpoints at which the whole process can be proofread, controlled and adapted. In the scenario sketched in Box 1, the two components are histone tails and the DNA linker. The initial signal is brought by a coregulator that catalyzes a covalent modification (here acetylation) of the H3 histone tail at an effector site (the residue experiencing the modification). A first allosteric behavior arises by which this covalent modification loosens the anchoring of linker DNA on the nucleosome and endows it with the ability to switch between straight and buckled conformations with different protein-binding landscapes. This bistability gives rise to another allosteric behavior, according to which the intercalation of a protein captures the buckled conformation (the analog of an R-state), which favors additional bindings whereas the T-state-like straight conformation accommodates at most one intercalation.

4.4. Chemo-mechanical coupling

Chromatin fiber allostery is an example of the biological importance of *chemo-mechanical coupling*, far less acknowledged than molecular motors (Lipowsky and Liepelt 2008) and operating at a higher scale and organization level. Chemo-mechanical coupling has here a dual aspect, referring to (i) the modulation of chemical rates and binding constants by mechanical stresses experienced by the substrate (typically DNA) and (ii) the generation of mechanical constraints as a consequence of chemical reactions (e.g. histone tail post-translational modifications) and binding event (e.g. DNA-binding intercalating proteins). The latter aspect also includes the remodeling factor activity required to package chromatin into a compact ordered conformation, in which allosteric behavior can manifest itself. We meet here the remarkable fact that in polymer chemistry and biological systems, strains can be imposed on macromolecules without exerting any mechanical force, merely following from the chemical binding of an additional factor or the chemical modification of an ingredient. Strains can henceforth be converted into mechanical work or back into chemical energy at another site. Chromatin fiber through its allosteric behavior conveys energy over long distances. Chemo-mechanical coupling is at work within chromatin in several instances:

- torsional constraints generated within an end-fixed linker DNA by protein intercalation (Victor *et al* 2003) and

DNA mechanical constraints relaxation by histone-tail acetylation (Box 1, Lesne and Victor 2006). This mechanism is in particular implemented in TBP (TATA-box binding protein) binding and function (figure 3) (Chen *et al* 2002);
- torsional constraints generated within the chromatin fiber by RNA-polymerase activity and local conformational changes elicited at the level of nucleosomes by those torsional constraints within a condensed and tightly organized chromatin loop (Box 2) (Bécavin *et al* 2010);
- topologically-driven decondensation of a chromatin loop (between two anchored boundaries) triggered by a local conformational change in the nucleosomes (Barbi *et al* 2005, Mozziconacci *et al* 2006).

4.5. Chromatin fiber allostery as an evolutionary agent

We already mentioned that the very existence of allosteric entities is a result of evolution. They have been selected according to their ability to establish a functional and explicit connection between otherwise uncoupled pathways or processes. Allosteric connections are more flexible and more robust than a direct physico-chemical relationship. In the case where some change occurs in one of the pathways, it is enough to modify one-half of the entity (its interface with the pathway) to preserve the articulation between the actual pair of pathways. Hence, it is enough to have a co-evolution of a pathway and the associated sites on allosteric entities to maintain all its connections with other pathways and processes, with no need for a modification of the latter. We here recover the evolutionary importance and benefit of any more general modularity (Vespignani 2003, Wagner 2007). In the present context of epigenomics, mutations that modify the chromatin structure (e.g. mutations in the histone genes or addition of repeats (in linker DNA) that creates a defect) may strongly affect the processes controlled by the precise architecture of this stretch of fiber. One may wonder whether such a mechanism could explain the acknowledged fact that triplet extension is related to the development of Huntington chorea. Chromatin fiber allostery explains how minor genotype changes may have major phenotypic consequences. This ensemble of mechanisms appears as an evolutionary actor in promoting phenotype variability on which selection could act. The relation between allostery and evolution goes far beyond the adaptive origin of allosteric entities. Indeed, a major consequence of allostery is to considerably extend the set of possible relationships with almost no physicochemical limitations on the pairs of related pathways, among which natural selection will *a posteriori* amplify and retain the most efficient and beneficial ones. It also enlarges the spatial range and time scale of the interactions, allowing long-range communications and extended coordination essential to a consistent functioning of the cell. Allostery thus appears as a much efficient generator of diversity. This evolutionary power of allostery meets the notion of 'evolutionary tinkering' introduced by Jacob (1982) insofar as tinkering chimeric entities generates novel bridges between previously independent pathways or processes. Chromatin fiber allostery and chemo-mechanical coupling along the fiber thus provide a way to coordinate molecular evolution at a wide genomic scale.

5. Discussion

5.1. Further extensions of allostery and open issues

At least four aspects are present in allostery:

(i) structural determinants resulting in concerted or sequential conformational changes;
(ii) their kinetic consequences, mainly cooperativity and Hill kinetics;
(iii) the joint specificity and robustness of allosteric interactions;
(iv) evolutionary consequences in terms of adaptability of these interactions.

We have seen that geometrical constraints, e.g. steric hindrance and symmetries, play a central role in mediating allosteric connection between the effector and the active sites. Among these geometrical constraints, *chirality* is a much important one. Chirality is present at several levels (histone tails, DNA molecule, DNA path onto the nucleosome, 30 nm chromatin fiber, higher levels of organization). It is possibly modified by conformational changes (e.g. transition of nucleosomes into reversomes) and mechanical constraints (e.g. DNA supercoiling). However, its role in gene regulation is most often ignored. Investigating the epigenetic role of chirality within our allosteric viewpoint would be of great relevance. For instance, a challenging question is the role, if any, of the left-handed wrapping of DNA onto the nucleosome (Bancaud *et al* 2007).

At this point, our analysis has brought forward the structural, mechanical and topological ingredients involved in chromatin fiber allostery. However, a full functional understanding requires to describe not only the possible conformational transitions of the fiber and the mechanisms by which they are triggered, but also their kinetics. In this way, one would obtain the epigenomic analog of the kinetic allosteric models developed for enzymatic catalysis (Monod *et al* 1965, Koshland *et al* 1966) and give account of transcription rates and the time course of transcriptional response to stimuli. In the same spirit, cooperativity in the context of enzymatic catalysis is defined as a kinetic feature related to the Hill expression for the reaction rate and associated Hill exponent (Hill 1910, Fersht 1985), rather than as a structural feature. Structural cooperativity is at the origin, but not directly equivalent, to the kinetic signature (Horowitz 1995). The relationship between structural and kinetic manifestations of cooperativity have to be worked out again in the context considered here, either at the DNA level or at the fiber level. The main difficulty in tackling this open question is to get the required quantitative knowledge about the elementary events. At the present time, the two scenarios presented in Box 1 and Box 2 are out of reach of a direct experimental check. Their formal analogy with

Monod–Wyman–Changeux model and Koshland–Némethy–Filmer model, respectively, is waiting for kinetic data. Moreover, the *in vivo* fiber structure, let alone its dynamics, is not well established. Presumably chromatin fiber displays polymorphism (Wong *et al* 2007): several structures may coexist in a context-dependent and still unknown manner, which also hampers quantitative accounts about allosteric regulation. Meanwhile, another direction would be to develop allosteric models and to confront qualitatively their kinetic predictions with time-resolved transcriptome data. The allosteric viewpoint on chromatin fiber would provide valuable constraints in the inverse problem of interpreting kinetic transcriptome data and reconstructing transcriptional regulatory networks (Berg 2008).

The idea that evolution and adaptation induced by natural selection are the basic forces explaining the existence of allosteric objects also has methodological consequences. Following a functional guideline, i.e. aiming at accounting for the optimal achievement of the biological *functions*, will thus be the best, if not the only way to unravel allosteric mechanisms. Far more than a formal and technically fruitful analogy, the extension of the standard notion of allostery underlines the importance of accounting for evolution in considering biological processes observed today. Major clues for a systemic approach of genomic and epigenetic processes are provided by the functional benefits of allosteric mechanisms, namely remote control and 'gratuitous' coupling between signals and their downward consequences, synchronization and concerted responses at a supramolecular scale, tuning of the sensitivity of the response to a given signal or event, possibility of triggering global changes by a localized event, or conversely to control a single localized event by a global conformational change. It would be interesting to look for evolutionary arguments supporting fiber allostery, for instance, to investigate whether specific covariant modifications of histone tails or the presence of histone variants correlate with specific DNA sequences or repeat lengths so as to produce chromatin fiber structures with optimized efficiency for signal transduction and genome processing. We moreover suggest that phylogenetic analyses similar to those performed to delineate the evolutionary origin of allosteric proteins and ribozymes (see section 2) would be worth to be conducted for DNA and chromatin. Furthermore, it should be possible to design directed evolution experiments to enhance allosteric effects, e.g. improving allosteric pathways along DNA or chromatin fiber and the functional consequences at active sites (typically specific binding sites) of DNA allosteric transitions. Either direction would open a novel field of investigations leading to better understand how topological features and mechanical properties of DNA and chromatin fiber may participate to the genomic processes and their regulation.

5.2. Chemical versus physical reading of epigenetic modifications

The above examples and discussion show the dual functional impact of histone-tail covalent modifications. They may have local chemical consequences in terms of molecular recognition (the currently invoked activity of histone tail post-translational modifications (Grant 2001)) or control chromatin fiber allosteric behavior as described here. The first kind of consequences follows from a local process, at the residue level, that could be termed a 'biochemical reading' of histone tail post-translational modifications. It can be enough to have one modification to change drastically the binding affinity of a cofactor with the histone tail and switch its behavior, or the outcome of its competition for binding with other factors. For example, a given HAT catalyses the acetylation of one specific residue of one specific histone that yields a recognition site for a cofactor (Turner 2000, Jenuwein and Allis 2001, Eberharter and Becker 2002). The alternative consequences of histone-tail modifications, involving what could be termed a 'physical reading', occur presumably at a larger scale, in a nonlocal and nonspecific way. For example, acetylation of a single nucleosome relaxes constraints on linker DNA and modifies its binding landscape (see Box 1). Acetylation of several nucleosomes participates in the decondensation of the fiber (Tóth *et al* 2004). One may wonder whether such a mechanism provides a model for the catastrophic decondensation following H4-K16 acetylation (Shogren–Knaak *et al* 2006).

In this way, a nonspecific barrier is alleviated which lets specific events occur. According to the context (e.g. surrounding cofactors), the same histone covalent modification can have both kinds of consequences. For instance, methylation of H3 lysines either generates a binding site, involving chemical recognition of the methylated residue (Briggs *et al* 2002), or tunes DNA-binding affinity by relaxing mechanical constraints onto DNA and modifying its binding energy landscape. Moreover, histone modifications participate, although in a general fashion, in the relationship between chromatin structure, gene regulation and metabolism. A promising field of study is to investigate how environmental and metabolic factors influence the activity of chromatin modifying enzymes, which in turn contributes to transcriptional regulation via fiber allosteric properties. Functional effects depend on the precise *combination* of histone N-tail modifications, suggesting that they form a code (Turner 2000, Jenuwein and Allis 2001). We will consider this highly debated and open issue within our allosteric perspective on the chromatin fiber function in the next subsection.

5.3. Fiber allostery, chromatin code and gene regulatory networks

Chromatin fiber allostery achieves long-term and long-range coordination of gene expression. It accounts for the joint tuning of binding affinities of several factors, leading to cooperativity and coordinated regulation. It underlies in an essential way gene regulatory networks and offers a guideline to unravel their concrete implementation and functioning. Fiber allostery considerably enlarges the range of transcription factors, hence centrally participates in the connectivity of these networks. It may achieve global cis-regulation (at the level of a chromatin loop or more) by promoting other binding

events and activating promoters in *cis*. Physical features of the chromatin fiber may control the very wiring of these networks, making their topology highly plastic and context-dependent, and increasing the number of ways of controlling or modulating their functional dynamics.

Chromatin fiber allostery offers an efficient and relatively low-cost means to achieve robust information transfer between distant genomic sites. This transfer is fast since it amounts to a propagation of constraints, whereas a mechanism where the information is transferred through a transport of matter would be slow. We have seen in Box 2 an illustration of this time-scale difference: the reversome front propagates 10–20 times faster than the RNA-polymerase. Like any translocation-like mechanism, this transfer centrally relies on a *specific architecture* ensuring the relay of the excitation or constraint from a place to the neighboring one. We suggest that any fast long-range action requires a similar generalized allosteric mechanism.

The chromatin fiber functional role is not limited to relay signals. This role is also to emulate the proper response, as regards gene activation or silencing, to changes in the surrounding conditions or progression along the cell cycle. This is achieved in the allosteric fiber by the adapted articulation between the activity of biochemical actors and structural, conformational, mechanical and topological features of the fiber (we term 'structural' the local features of the assembly, 'conformational' the global three-dimensional features of the bead-on-string folding into a 30 nm fiber; 'mechanical' the features involving kinematics and elastic properties, and 'topological' the consequences of chirality and linking number conservation). Fiber allostery reconciles the physical approach of chromatin, seen as an organized mechanical substrate, with that based on chromatin biochemistry, namely specific recognition controlling specific reactions, assemblies or pathways. These two approaches are usually disjoint. In the biochemical approach, neither the dynamics (out-of-equilibrium aspects, self-organization) nor mechanical constraints are taken into account. It rather provides a sequential and linear scenario with specific causal determinants. This approach led to major discoveries, like SWI factors and remodeling, RNA interference transcriptional and post-transcriptional regulation. However, it obviously misses some key features, like long-range interactions, regulatory networks, mechanical constraints, actual interaction mechanisms or stochasticity inherent to elementary reactions. On the other hand, the physical approach is centered on the fiber structure and conformation dynamics. However, it misses specificity and molecular recognition. It is well known that physical features (e.g. electrostatics or conformational changes) affect biochemical reactions. Conversely, advances in understanding molecular motors evidenced that biochemical reactions are capable to produce physical effects like forces and torques. Physical features and biochemical reactions or binding are in constant interplay, each modulating and regulating the others in a reciprocal relationship. The benefit of this interplay is to endow specific but locally acting factors with the possibility of having long-range consequences, extended in space and time thanks to the mediation of the chromatin fiber. Conversely, fiber influence on lower-level processes can be explained in terms of specific triggering and tuning of elementary biochemical processes.

Chromatin fiber allostery supports and substantiates the proposal of a *chromatin code* at work in transcriptional regulation (Turner 2000, 2007, Benecke 2006). We have shown how signal-induced conformational changes of the fiber can in turn promote other binding events that become encoded in the incoming signal. The functionally required correlation between the signal and some secondary, possibly remote binding event is turned into a causal relationship thanks to the mediation of the chromatin fiber. We suggest that the decoding entity is here the chromatin fiber itself and its allosteric transitions (Lesne 2006). Chromatin fiber ensures the relay between the codeword (here the pattern of chromatin features, e.g. histone post-translational modifications) and its concrete meaning (here the expression profile). They have co-evolved so as to relate almost deterministically a genomic patterning to an expression profile, in a similar way as a tRNA bridges a codon and an amino acid. However, in contrast to the context-free genetic code, the grammar of the chromatin code is *context dependent* and decoding is performed at the level of a chromatin loop. For instance, the connection between a signaling event (binding of some factor) and the change in the binding affinity at another site will be present only in the compact chromatin fiber, and not in a more decondensed conformation. The input/output relation is established in a context-dependent way since the conformational change of the fiber is itself conditioned by its surroundings. This latter remark means that a given signal can trigger various downstream events (or none) according to the fiber structure and its possible conformational changes. The signal is 'understood' by the cell in a context-dependent way, or even not understood at all and thus having no consequences.

6. Conclusion

The relevance and fruitfulness of extending the notion of allostery to DNA and chromatin show that allosteric mechanisms are by no means restricted to enzymatic catalysis. In contrast, allostery is a ubiquitous notion, central to biological functions, and it appears as one of the specificity of living systems and their regulation. We argue that allosteric principles underlie and explain the functional architecture required for spacetime coordination, at supramolecular scales, of a wide range of regulatory processes, in particular genomic processes. Fiber allostery enables single localized events to induce collective activation, for instance, localized intercalation inducing buckling of the whole linker, localized RNA-polymerase inducing torsional constraints within a chromatin loop. Conversely, collective events may converge in a single localized effect, for instance, torsional constraints inducing a specific nucleosome transition due to steric hindrance. Another benefit of allosteric coupling is the possibility of specific activation of a nonspecific change, when the effector site is specific whereas the active site is not. For instance, each histone-tail post-translational modification is catalyzed by a specific enzyme and induces an overall

loosening of the fiber. Conversely, nonspecific activation of a specific event is also possible, when the active site is specific whereas the effector site is not. For instance, loosening of the fiber controls the binding of a specific factor at a specific site. We underline that the functional impact of the fiber structure does not lie only in the control of DNA accessibility and compaction. The part of accessibility impairment/recovery in the regulation of genomic processes should not be overestimated. Indeed, several experiments investigating the *in vivo* mobility of synthetic molecules (with no activity) have shown that quite large proteins can penetrate and travel within chromatin fiber (Phair *et al* 2004). We argue that the main control of transcriptional regulation achieved by the chromatin fiber is done at the level of transcription factor *binding affinities* and their tuning via the physical constraints exerted by the fiber on the DNA stretch containing the binding site. It is not enough that a transcription factor reaches its binding site for *actually* binding DNA.

Allosteric regulation can also be associated with delayed couplings and temporal regulation. For instance, a cycle of acetylation/deacetylation of histone tails may monitor a chromatin fiber breathing, if any, with a period controlled by the kinetic rates of histone modifications (Benecke 2003). This period prescribes an intrinsic time scale for related events. Let us also mention coordination, synchronization, remote control and checkpoints that can be achieved through allosteric mechanisms at the level of a chromatin loop. Remarkably, fiber allostery articulates processes of different natures, of different pathways, at different scales. In summary, the chromatin fiber can be seen as a *nano-machine* insofar as it transmits strains and more generally responses to stresses and signals from one part to another in a way achieving regulatory functions.

Moreover, from a methodological perspective, fiber allostery offers a guideline for unraveling mechanisms by which biochemical actors, although acting locally, are central in the control of genetic and epigenetic processes that are extended in space and time. It proposes a flexible mechanism accounting for how signals (entering the nucleus or internal to the nucleus) are turned into operational gene regulatory events. It gives clues to solve the main challenges of a systemic approach bridging chromatin fiber biochemistry, functional structure and conformational dynamics into a chromatin code that rules transcriptional regulatory networks.

Acknowledgments

Christophe Bécavin ackowledges the Institut des Hautes Études Scientifiques (IHÉS), Génopole, the Centre National de la Recherche Scientifique (CNRS) and the Agence Nationale pour la Recherche contre le Sida (ANRS) for financial support.

References

Anderson J D, Lowary P T and Widom J 2001 Effect of histone acetylation on the equilibrium accessibility of nucleosomal DNA target sites *J. Mol. Biol.* **307** 977–85

Arneodo A, Vaillant C, Audit B, Argoul F, D'Aubenton-Carafa Y and Thermes C 2011 Multi-scale coding of genomic information: from DNA sequence to genome structure and function *Phys. Rep.* **498** 45–188

Bancaud A *et al* 2007 Nucleosome chiral transition under positive torsional stress in single chromatin fibers *Mol. Cell.* **27** 135–47

Barbi M, Mozziconacci J and Victor J M 2005 How the 30 nm chromatin fiber deals with topological constraints *Phys. Rev.* E **71** 031910

Bécavin C, Victor J M and Lesne A 2010 When steric hindrance facilitates processivity: polymerase activity within chromatin *Biophys. J.* **98** 824–33

Benecke A 2003 Genomic plasticity in information processing by transcription coregulators *Complexus* **1** 65–76

Benecke A 2006 Chromatin code, local non-equilibrium dynamics and the emergence of transcription regulatory programs *Eur. Phys. J.* E **19** 379–84

Ben Haim E, Lesne A and Victor JM 2001 Chromatin: a tunable spring at work inside chromosomes *Phys. Rev.* E **64** 051921

Ben Haim E, Lesne A and Victor J M 2002 Adaptive elastic properties of chromatin fiber *Physica* A **314** 592–9

Berg J 2008 Dynamics of gene expression and the regulatory inference problem *Europhys. Lett.* **82** 28010

Boeger H, Griesenbeck J and Kornberg R D 2008 Nucleosome retention and the stochastic nature of promoter chromatin remodeling for transcription *Cell* **133** 716–26

Botta M, Haider S, Leung IX, Lio P and Mozziconacci J 2010 Intra- and inter-chromosomal interactions correlate with CTCF binding genome wide *Mol. Syst. Biol.* **6** 426

Briggs S D, Xiao T, Sun Z W, Caldwell J A, Shabanowitz J, Hunt D F, Allis C D and Strahl B D 2002 Gene silencing: trans-histone regulatory pathway in chromatin *Nature* **418** 498

Bühler M and Gasser S M 2009 Silent chomatin at the middle and ends: lessons from yeasts *EMBO J.* **28** 2149–61

Bushey A M, Dorman E R and Corces V G 2008 Chromatin insulators: regulatory mechanisms and epigenetic inheritance *Mol. Cell.* **32** 1–9

Cabal G G *et al* 2006 Molecular analysis of SAGA mediated nuclear pore gene gating activation in yeast *Nature* **441** 770–3

Chaires J B 2000 Energetics of intercalation reactions *Biochemistry* **39** 8439–47

Chen D, Hinkley C S, Henry R W and Hang S 2002 TBP dynamics in living human cells: constitutive association of TBP with mitotic chromosomes *Mol. Biol. Cell.* **13** 276–84

Core L J, Waterfall J J and Lis J T 2008 Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters *Science* **322** 1845–8

Crick F H C 1976 Linking numbers and nucleosomes *Proc. Natl Acad. Sci. USA* **73** 2639–43

Cui Q and Karplus M 2008 Allostery and cooperativity revisited *Protein Sci.* **17** 1295–307

Danziger O, Rivenzon-Segal D, Wolf S G and Horowitz A 2003 Conversion of the allosteric transition of GroEL from concerted to sequential by the single mutation Asp-155 → Ala *Proc. Natl Acad. Sci. USA* **100** 13797–802

Demongeot J 2007 Primitive genome and RNA relics *IEEE EMBS '07: IEEE Proc. (Piscataway)* pp 6338–42

Dorigo B, Schalch T, Kulangara A, Duda S, Schroeder R R and Richmond T J 2004 Nucleosome arrays reveal the two-start organization of the chromatin fiber *Science* **306** 1571–3

Eberharter A and Becker PB 2002 Histone acetylation: a switch between repressive and permissive chromatin *EMBO Rep.* **3** 224–9

Felsenfeld G and Groudine M 2003 Controlling the double helix *Nature* **421** 448–53

Fersht A 1985 *Enzyme Structure and Mechanisms* 2nd edn (New York: Freeman)

Filion G J *et al* 2010 Systematic protein location mapping reveals five principal chromatin types in Drosophila cells *Cell* **143** 212–24
Filion G J *et al* 2011 *Cell* **145** 160 (erratum)
Fox Keller E 2009 Rethinking the meaning of biological information *Biol. Theory* **4** 159–66
Gammaitoni L, Hänggi P, Jung P and Marchesoni F 1990 Reaction rate theory: fifty years after Kramers *Rev. Mod. Phys.* **62** 251–42
Georgelis N, Shaw J R and Hannah L C 2009 Phylogenetic analysis of ADP-glucose pyrophosphorylase subunits reveals a role of subunit interfaces in the allosteric properties of the enzyme *Plant Physiol.* **151** 66–77
Goldbeter A 1996 *Biochemical Oscillations and Cellular Rythms. The Molecular Bases of Periodic and Chaotic Behaviour* (Cambridge: Cambridge University Press)
Grant P A 2001 A tale of histone modifications *Genome Biol.* **2** reviews0003
Grewal S I S and Elgin S C R 2007 Transcription and RNA interference in the formation of heterochromatin *Nature* **447** 401–6
Guntas G and Ostermeier M 2004 Creation of an allosteric enzyme by domain insertion *J. Mol. Biol.* **336** 263–73
Guntas G, Mansell T J, Kim J R and Ostermeier M 2005 Directed evolution of protein switches and their application to the creation of ligand-binding proteins *Proc. Natl Acad. Sci. USA* **102** 11224–9
Handoko L *et al* 2011 CTCF-mediated functional chromatin interactome in pluripotent cells *Nat. Genet.* **43** 630–8
Harnish D C, Evans M J, Scicchitano M S, Baht R A and Karathanasis SK 1998 Estrogen regulation of the alipoprotein AI gene promoter by transcription cofactor sharing *J. Biol. Chem.* **273** 9270–8
Hill A V 1910 The possible effects of the aggregation oh the molecules on haemoglobin on its dissociation *J. Physiol. (Lond)* **40** 4–7
Horowitz A 1995 The relation between cooperativity in ligand binding and intra-molecular cooperativity in allosteric proteins *Proc. R Soc.* B **259** 85–7
Ingber D E 2003 Tensegrity: I. Cell structure and hierarchical systems biology *J. Cell. Sci.* **116** 1157–73
Jacob F 1982 *The Possible and the Actual* (Seattle, WA: University of Washington Press)
Jenuwein T and Allis C D 2001 Translating the histone code *Science* **293** 1074
Jin M, Song G, Kim Y S, Satrof N, Shimaoka M, Wittrup D and Springer T A 2006 Directed evolution to probe protein allostery and integrin I domains of 200,000 fold higher affinity *Proc. Natl Acad. Sci. USA* **103** 5758–63
Kannan N, Wu J, Anand G S, Yooseph S, Neuwald A F, Venter J C and Taylor S S 2007 Evolution of allostery in the cyclic nucleotide binding module *Genome Biol.* **8** R264
Kim T H, Abdullaev Z K, Smith A D, Ching K A, Loukinov D I, Green R D, Zhang M Q, Lobanenkov V V and Ren B 2007 Analysis of the vertebrate insulator protein CTCF-binding sites in the human genome *Cell* **128** 1231–45
Koshland D E Jr, Némethy G and Filmer D 1966 Comparison of experimental binding data and theoretical models in proteins containing subunits *Biochemistry* **5** 365–85
Kouzine F, Sanford S, Elisha-Feil Z and Levens D 2008 The functional response of upstream DNA to dynamic supercoiling *in vivo Nat. Struct. Mol. Biol.* **15** 146–54
Lavelle C 2009 Forces and torques in the nucleus: chromatin under mechanical constraints *Biochem. Cell. Biol.* **87** 307–22
Lesne A 2006 The chromatin regulatory code: beyond an histone code *Eur. Phys. J.* E **19** 375–7
Lesne A 2008 Robustness: confronting lessons from physics and biology *Biol. Rev.* **83** 509–32
Lesne A 2012 Multiscale analysis of biological functions *Acta. Biotheoretica* (at press) preprint IHES P/11/10
Lesne A and Victor J M 2006 Chromatin fiber functional organization: some plausible models *Eur. Phys. J.* E **19** 279–90
Levens D and Benham C J 2011 DNA stress and strain, *in silico*, *in vitro* and *in vivo Phys. Biol.* **8** 035011
Liang J, Kim J R, Boock J T, Mansell T J and Ostermeier M 2007 Ligand biding and allostery can emerge simultaneously *Protein Sci.* **16** 929–37
Lipowsky R and Liepelt S 2008 Chemo-mechanical coupling of molecular motors: thermodynamics, network representations, and balance conditions *J. Stat. Phys.* **130** 39–67
Marko J F and Siggia E D 1995 Stretching DNA *Macromolecules* **28** 8759–70
Mathonet P, Barrios H, Soumillion P and Fastrez J 2006 Selection of allosteric betalactamase mutants featuring an activity regulation by transition metal ions *Protein Sci.* **15** 2335–43
Métivier R, Penot G, Hübner M R, Reid G, Brand H, Kos M and Gannon F 2003 Estrogen receptor-α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter *Cell* **115** 751–63
Monod J 1972 *Chance and Necessity. An Essay on the Natural Philosophy of Modern Biology* (London: Collins)
Monod J and Jacob F 1961 General conclusions: telenomic mechanisms in cellular metabolism, growth, and differentiation *Cold Spring Harbor Symp. Quant. Biol.* vol 26 pp 389–401
Monod J, Wyman J and Changeux J P 1965 On the nature of allosteric transitions : a plausible model *J. Mol. Biol.* **12** 88–118
Mozziconacci J, Lavelle C, Barbi M, Lesne A and Victor J M 2006 A physical model for the condensation and decondensation of eukaryotic chromosomes *FEBS Lett.* **580** 368–72
Noma K, Sugiyama T, Cam H, Verdet A, Zofall M, Jia S, Moazed D and Grewal SIS 2004 RITS acts in *cis* to promote RNA-interference mediated transcriptional and post-transcriptional silencing *Nat. Genet.* **36** 1174–80
Ohlsson R, Lobanenkov V and Klenova E 2010 Does CTCF mediate between nuclear organisation and gene expression? *Bioessays* **32** 37–50
Ostermeier M 2005 Engineering allosteric protein switches by domain insertion *Protein Eng. Des. Sel.* **18** 359–64
Peracchi A and Mozzarelli A 2011 Exploring and exploiting allostery: models, evolution and drug targeting *Biochim. Biophys. Acta* **1814** 922–33
Perutz MF 1989 Mechanisms of cooperativity and allosteric regulation in proteins *Q. Rev. Biophys.* **22** 139–6
Perutz M F 1990 *Mechanisms of Cooperativity and Allosteric Regulation in Proteins* (Cambridge: Cambridge University Press)
Phair R D, Scaffidi P, Elbi C, Vecerova J, Dey A, Ozato K, Brown DT, Hager G, Bustin M and Misteli T 2004 Global nature of dynamic protein–chromatin interactions *in vivo*: three-dimensional genome scanning and dynamic interaction networks of chromatin proteins *Mol. Cell. Biol.* **24** 6393–402
Robert C H, Decker H, Richey B, Gill S J and Wyman J 1987 Nesting: hierarchies of allosteric interactions *Proc. Natl Acad. Sci. USA* **84** 1891–5
Schalch T, Duda S, Sargent D F and Richmond T J 2005 X-ray structure of a tetranucleosome and its implications for the chromatin fibre *Nature* **436** 138–41
Scheffer M P, Eltsov M and Frangakis A S 2011 Evidence for short-range helical order in the 30-nm chromatin fibers of erythrocyte nuclei *Proc. Natl Acad. Sci. USA* **108** 16992–7
Shogren-Knaak M, Ishii H, Sun J M, Pazin M J, Davie J R and Peterson C L 2006 Histone H4-K16 acetylation controls chromatin structure and protein interactions *Science* **311** 844–7

Soukup G A, DeRose E C, Koizumi M and Breaker R R 2001 Generating new ligand-binding RNAs by affinity maturation and disintegration of allosteric ribozymes *RNA* **7** 524–36
Stock A M, Robinson V L and Goudreau P N 2000 Two-component signal transduction *Annu. Rev. Biochem.* **69** 183–15
Struhl K 1999 Fundamental different logic of gene regulation in eukaryotes and prokaryotes *Cell* **98** 1–4
Svejstrup J Q 2004 The RNA polymerase II transcription cycle: cycling through chromatin *Biochim. Biophys. Acta.* **1677** 64–73
Tang J and Breaker R R 1997 Rational design of allosteric ribozymes *Chem. Biol.* **4** 453–9
Thomas B R 1970 The origin of the genetic code *Biochem. Biophys. Res. Commun.* **40** 1289–96
Tóth K F, Knoch T A, Wachsmuth M, Frank-Stöhr M, Stöhr M, Bacher CP, Müller G and Rippe K 2004 Trichostatin A-induced histone acetylation causes decondensation of interphase chromatin *J. Cell. Sci.* **117** 4277–87
Turner B M 2000 Histone acetylation and an epigenetic code *Bioessays* **22** 836–45
Turner B M 2007 Defining an epigenetic code *Nat. Cell. Biol.* **9** 2–6
Van Holde K E, Miller K I and Van Olden E 2000 Allostery in very large molecular assemblies *Biophys. Chem.* **86** 165–72
Vespignani A 2003 Evolution thinks modular *Nat. Genet.* **35** 118–9
Victor JM, Ben-Haïm E and Lesne A 2002 Intercalation and buckling instability of DNA linker within locked chromatin fiber *Phys. Rev.* E **66** 060901
Wagner A 2007 *Robustness and Evolvability in Living Systems* (Princeton, NJ: Princeton University Press)
Weismann A 1904 *The Evolution Theory* (translated by J A and M R Thomson) (London: Edward Arnold)
Widom J 1998 Structure, dynamics and function of chromatin *in vitro Annu. Rev. Biophys. Biomol. Struct.* **27** 285–327
Winkler W C and Breaker R R 2005 Regulation of bacterial gene expression by riboswitches *Annu. Rev. Microbiol.* **59** 487–517
Wolffe A P 1998 *Chromatin: Structure and Function* (New York: Academic)
Wong H, Victor J M and Mozziconacci J 2007 An all-atom model of the chromatin fiber containing linker histones reveals a versatile structure tuned by nucleosomal repeat length *PLoS One* **2** e877