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Systems biology meets chromatin function: a report on the Fourth Elmau Conference on Nuclear Organization

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Abstract

The Fourth Elmau Conference on Nuclear Organization (information, abstracts, and list with addresses of speakers at http://www.nuclearchitecture.com/) took place in Gosau, Austria, between 12 and 15 October 2006. The workshop was organized by Dean Jackson, Roel van Driel, Hans Lipps and Hans Westerhoff, and was sponsored by ABCAM, Boehringer, EMBO, and VWR. It was mainly divided into two topics: dynamic analysis of gene activation and expression, and structure and dynamics of chromatin fibres, nuclear space and epigenetics. A particular emphasis was given this time to systems biology approaches, which drove the 40 participants to extensive discussions and highly interdisciplinary scientific exchanges. Some of the concepts discussed are presented here.

Scope of the meeting

Since genome sequencing has identified the global set of human genes, the challenge now lies in the elucidation of gene function, and how gene functions are integrated to control cellular processes in time and space. In eukaryotic cells DNA is associated with histones and other proteins to make chromatin, the functional template for every DNA transaction event such as transcription, replication, recombination and repair. While the fundamental role of chromatin structure and dynamics in the control of these processes is now well acknowledged, it is becoming increasingly clear that the mechanistic understanding of biological functions at the molecular level will require the integration of complex molecular networks highly dynamic in time and space. Chromatin function and epigenetics are fields that are just beginning to be thought in terms of interaction networks.

After Elmau 2000, initiating the Elmau Conferences on Nuclear Organization, Elmau 2002 focused on gene regulation and nuclear structure (Jackson 2002) and Elmau 2004 dealt with ways to drive basic science to application (Herrmann & Fackelmayer 2005, Jackson 2005). This Elmau session (held in Gosau, Austria, due to a fire which destroyed Schloss Elmau in 2005) was focused on systems biology. Entitled 'Systems Biology meets Chromatin Function', it was an attempt to bring together scientists with expertise in chromatin biology and quantitative modelling. This

report gives an overview of concepts discussed during the workshop, and we hope it will lead to further collaborations between people in the two fields.

Systems biology in the nucleus: gradually leaving the traditional view of gene expression

The traditional view of gene expression considers how chromatin structure and transcription factors contribute to the control of RNA synthesis. However, genes do not operate in isolation. They are not only locally and specifically regulated, but are also components of network interactions that orchestrate the management of our genetic information. In recent years many reviews have regularly emphasized this need for a new paradigm integrating nuclear architecture and genome function (Berezney & Wei 1998, Dundr & Misteli 2001, O'Brien *et al*. 2003, Zaidi *et al*. 2005). Remarkably, while systems biology is being applied extensively to metabolic pathways and signalling networks, only a few attempts have been made to address chromatin function and epigenetic aspects (Benecke 2006).

A collaborative effort between groups in molecular cell biology, biophysics and computer modelling is needed in order to reveal novel insights into nuclear structure and DNA metabolism, and establish what is actually required to approach the so-called 'systems biology of chromatin function'. This should drive us towards understanding biological functions as they really occur in the context of biological systems. Some steps in this direction are discussed in this meeting review.

How to use models to understand biological interactions

Techniques such as microarrays are invaluable to obtain insight into gene networks, but lead to tremendous data sets and multiple cross-regulation levels which are difficult to understand. In a plenary lecture, **Hans Meinhardt** (Tübingen, Germany) approached the problem from the other side by asking what is the minimum molecular mechanism that would provide an explanation for the dynamic features observed. Using developmental pathways as examples, he showed that understandable interactions are able to describe elementary steps in surprising detail.

Following Meinhardt's talk, **Alexandre Blais** (New York, USA) proposed a different approach to understanding networks, using muscle cell differentiation as an example. He used expression profiling, ChIP-on-chip experiments, and computational analysis of transcription factor binding sites, to arrive at a large network of transcriptional interactions that regulate the differentiation process.

The juxtaposition of these two talks initiated a debate on how the data can be best understood: should we use large all-inclusive models, or small and understandable models?

Large models reveal complex behaviour in interconnected networks

One area where large models have provided deep biological insights is in the analysis of metabolism. Pioneering work presented by **David Fell** (Oxford, UK) showed how analysis of flow in metabolic networks, usually known as control analysis (Covert *et al*. 2004), can yield some surprising results. One basic question that can be asked in the metabolic network is the following: say you inhibit enzyme X, a rate-limiting enzyme which catalyses the reaction of A to a final product B, by 50%. Will the concentration of B drop 50%? Surprisingly, the most likely answer is no. This is because as the level of A builds up due to lack of enzyme X, A will start to be available for other reactions in the interconnected network, which will eventually produce B. Alternatively, or in addition, lower negative feedback from B will cause X levels to rise up again. Thus, though enzyme X is important in the normal course of metabolism, the overall network is not very sensitive to a change in X. Change at one point in an interconnected network can redirect the flux elsewhere or correct for the change, making such networks robust to changes. The understanding of flux dynamics in the metabolic network can be eventually used to control the flux. For example, maximize the production of a metabolic product in bacteria that has pharmaceutical or other uses.

Edda Klipp (Berlin, Germany) and **Hans Westerhoff** (Manchester, UK) provided a fascinating extension of control analysis to non-metabolic networks, such as osmotic regulation in yeast and MAPK signalling in mammalian cells. The models were comprehensive for the signalling modules involved,

and showed that control of the process was dispersed among multiple components. However, a substantial part of the regulatory control still resided in several key elements, such as Hog1 in the control of osmolarity, and Raf in MAPK signalling. This analysis may also argue for cautious interpretation of results from knocking down, knocking out, or over-expressing a gene. The outcome of such perturbations may underrate the importance of the perturbed gene, since the signal may flow along alternate paths in the interconnected network. Presenting a personal vision of how we may begin to combine different aspects of nuclear and chromatin organization and structure into a model, **Roel van Driel** (Amsterdam, Netherlands) proposed that global models tightly linked with experiments in a cycle of prediction, experimental verification, model improvement, and back to prediction, could be used to create a type of *in-silico* nucleus.

Different ways to oscillate

One of the surprising discoveries resulting from quantification of protein dynamics, especially at the single-cell level, is that not only can protein levels go up and down in time, and around in space, but that these changes can be oscillatory. **Mike White** (Liverpool, UK), one of the first to observe such oscillations, talked about their origin in the NF-kappaB signalling system. The NF-kappaB oscillations are formed by negative feedback control: NF-kappaB *trans*-activates its target genes, among whom is its own inhibitor, IkappaBα. IkappaBα inhibits NF-kappaB, which in turn reduces the levels of IkappaBα after a transcriptional delay, which in turn increases NF-kappaB activity again, and so on. The transcriptional delay makes the tuning slow but apparently possible: in each cycle NF-kappaB activity rises and drops less, a phenomenon known as damped oscillations.

But the NF-kappaB type of oscillator design is not what the cell evolved to coordinate arguably its most important process, the cell cycle. The design of the cell cycle oscillator was discussed by **Bela Novak** (Budapest, Hungary). The idea behind the proposed design was simple: each transition involves a one-directional irreversible step. Each step is made irreversible by a network property, such as a positive feedback loop. The analogy is a set of interconnected rooms around the circumference of a circular building. Each room has two entrances, but only the one on the right can be opened from that room. Someone walking around the rooms would be forced to go right each time, until he or she reaches the starting point.

The effects of chance and memory on cellular protein levels

Perhaps one factor influencing network structure such as the one proposed for the cell cycle oscillator, is the need to be robust to the random fluctuations of cellular components. Random fluctuations in protein levels within a single living cell have only very recently been measured in mammalian cells, using techniques of time-lapse microscopy and tagging of endogenous proteins in living cells (Sigal *et al*. 2006). **Marek Kimmel** (Houston, USA) and **Alex Sigal** (Rehovot, Israel) discussed modelling and implications of noise in protein levels. Kimmel proposed that network interactions are not deterministic, but have probabilities associated with them. When combined with all-or-none responses, this may lead to phenotypic heterogeneity, where some cells may be in one state and other cells in the second state, even though the signal they received was identical. Sigal discussed experiments which showed that differences between individual cells tended to persist for several cell generations. Thus, most cells 'remembered' their protein level rank within the population for that amount of time. In addition, proteins from the same system (the ribosome system was presented) tended to be correlated in their fluctuations. What this may mean is that cells can be different from each other in the activities of whole networks for a length of time sufficient to influence processes such as cell death and differentiation. Both talks discussed the possibility that noise may turn out to be important in determining cell fate in some situations. However, at this point, this is still speculative for mammalian cells, though evidence for such an effect has been found in bacteria (Balaban *et al*. 2004).

Multilevel regulation of chromatin structure and dynamics

Numerous protein complexes are dedicated to the regulation of chromatin structure and dynamics. Among them, post-translational and remodelling

factors are the most studied since they have been shown to have profound impact both on the dynamic of the chromatin fibre and on gene expression. However, the way they act is still obscure. Another important aspect is the multilevel control of gene expression through chromatin structure, both at the cluster level (through a permissive or non-permissive environment) and at the gene level (gene ON/OFF through promoter accessibility).

George Reid (Heidelberg, Germany) showed that initiation of transcription progresses through a series of ordered events in which histone modifications are cyclically and cooperatively regulated. Going further, he reported that DNA methylation also cycles during gene transcription. Namely, Reid showed that cycling occurred in methylation of the palendromic dinucleotide CpG, generally known to achieve silencing of transcription in vertebrates. Using drugs such as anthracyclin or doxorubicin, which intercalate into DNA and prevent transcription, Reid more precisely showed that cyclical methylation of CpG dinucleotide is associated with the transcriptional start site of at least 10 promoters and proposed that, analogous to the concept of the histone code, cyclical changes in methylation status of promoters may transiently allow for transcription to occur and may link promoter inactivity to epigenetic silencing that subsequently persists through cellular division.

In a second talk addressing the correlation between DNA methylation and gene expression, **Astrid Visser** (Leiden, The Netherlands) pointed out the fact that most techniques detecting epigenetic markers such as DNA methylation and histone modifications are hampered by the fact that they pool a large number of cells (hence masking cell-to-cell and inter-allele variability) and also largely exclude repetitive DNA elements (although they can serve as initiation sites for DNA methylation). For these reasons, Visser designed a new approach that will allow the analysis of epigenetic information of individual alleles. This approach is currently being tested to measure the DNA methylation level of a genomic region involved in facioscapulo-humeral dystrophy (FSHD).

Regarding histone modifications and control of transcription, **Jane Mellor** (Oxford, UK) showed that each phase of transcription is defined by combinatorial and dynamic lysine methylation and acetylation. Mellor more generally discussed the recruitment of the transcription regulators and the transcription machinery to promoter chromatin and tried to decipher the DNA genetic code and histone epigenetic code that coordinate the recruitment of these factors. Also dealing with epigenetic aspects of active/inactive chromatin, **Francis Stewart** (Dresden, Germany) showed that transcriptionally active states of chromatin can be epigenetically maintained through histone lysine methylation, mainly at H3 K4 (which precludes H3 K9 methylation, a marker of constitutive heterochromatin, while H3 K27 methylation marks facultative heterochromatin). The functional opposition between H3 K4 and K27 methylation was further supported by linkage to the antagonism between Polycomb- and trithorax-Group (PcG and trxG) protein action (Klymenko & Muller 2004). The association of trxG and PcG action with H3 K4 and H3 K27 methylation respectively provides a molecular explanation for this antagonism.

Jorg Langowski (Heidelberg, Germany) presented some of the results he obtained combining different biophysical techniques to study chromatin, such as fluorescence correlation spectroscopy (FCS), fluorescence resonance energy transfer (FRET) and atomic force microscopy (AFM). First, he investigated by FCS spatial variations in protein diffusion behaviour in HeLa cells expressing various fluorescent proteins, and showed that, although diffusion is lower in the nucleus than in the cytoplasm, most of the interphasic chromatin is accessible to medium-size protein complexes. Regarding the precise interphasic nucleosome concentration, he measured a mean value of 140 μM, locally amounting up to 250 μM, suggesting that a condensation-controlled regulation of sites accessibility takes place at length scales below 200 nM. Then he reported some FRET and AFM measurements done on mononucleosome systems, and showed that incorporation of linker histone H1 as well as increasing salt concentration decreased the distance between the linker DNA ends while acetylation of histone tails has an opposite effect, increasing the distance and thus 'opening' the nucleosome. Remarkably, however, selective acetylation of histone H4 compacts the structure.

Shifting to the chromatin fibre scale, **Daniela Rhodes** (Cambridge, UK) showed cryomicroscopy images which represent new data into the unravelling of the so-called '30 nm' fibre whose structure remains surprisingly controversial, despite considerable efforts during the past 30 years. However, knowledge of this structure is essential for under-

standing how nuclear processes such as DNA transcription, replication or repair are mechanistically regulated on their physiological template. Using DNA tandem arrays of nucleosome-positioning sequences, Rhodes and co-workers obtained highly regular model fibres in which nucleosomes are precisely spaced and have native-like composition of both the histone octamer and linker histone. The resulting arrays are suitable for structural analysis by single-particle electron cryomicroscopy and give new insight into the 30-nm fibre, along with the role of linker histone and histone octamer N-terminal tails in chromatin fibre folding. In particular, Rhodes proposed a model in which tight nucleosome packing (11 nucleosomes/11 nm in a 33 nm diameter fibre) is achieved through inter-digitations of nucleosomes from adjacent helical gyres.

Stressing the necessity to link the different scales already discussed in previous talks, i.e. DNA, nucleosome, chromatin fibre, chromatin loops, **Christophe Lavelle** (Villejuif, France) claimed that DNA topology, nucleosome polymorphism and chromatin dynamics are indeed interconnected and jointly participate in a cooperative manner to control transcription. He presented various experimental and theoretical results showing (a) that relaxation data on mononucleosome reconstituted on DNA minicircles were consistent with Langowski FRET studies as for the opening of nucleosome upon acetylation of histone tails; (b) how this nucleosome structural change could influence chromatin structure in a processive manner, leading to decondensation and outlooping of whole chromatin domains to allow transcription; and (c) how single-molecule micromanipulation of reconstituted chromatin fibre could help to obtain insight into the process of transcription elongation through a chromatin template.

Genes are not arranged randomly

It is now well acknowledged that interphase chromosomes occupy discrete, mutually exclusive territories exhibiting non-random distribution, constrained movement and limited intermingling at their boundaries (Cremer *et al*. 2006). Several levels of organization coexist at different scales: first, although mammalian chromosomes do not occupy precise locations, small ones as well as gene-dense ones tend to occupy the nuclear interior, leading to a kind of radial organization (Cremer *et al*. 2001, Bolzer *et al*. 2005); then, chromosomes themselves globally evolved with gene-rich and gene-poor regions called RIDGEs (regions of increased gene expression) and ANTIRIDGEs respectively (Caron *et al*. 2001, Versteeg *et al*. 2003); finally, local chromatin structure in these regions often dramatically influences the way genes are interacting. In that sense chromosome position and fine organization during interphase has a high impact on gene regulation, and one of the big challenges in nuclear architecture today is to build models that encompass the multiscale levels of chromatin organization in a continuous interconnected description (Lavelle & Benecke 2006).

Looking precisely into three-dimensional (3D) folding of the chromosomal fibre in the human interphasic nucleus, **Julio Mateos-Langerak** (Amsterdam, The Netherlands) showed that, despite a considerable cell-to-cell variation, subchromosomal domains that are rich in highly expressed genes (RIDGEs) are more decondensed, have a more irregular shape and are located more towards the nuclear interior, compared to clusters of low-expressed genes (ANTIRIDGEs). Mateos-Langerak also reported systematic measurements of the quantitative relationship between physical and genomic distances in the genome which confirmed the above-mentioned differences in folding between gene-rich and gene-poor domains. These results clearly unveil folding principles of the human genome and open the way to connect DNA sequence, i.e. 1D genome organization and the highly heterogeneous human transcriptome map, to the multiscale 3D folding of the chromatin fibre in the nucleus.

Addressing higher-order chromatin modifications, **Denise Sheer** (London, UK) showed that transcriptional activation of the human major histocompatibility complex (MHC) is preceded by massive chromatin remodelling of the entire locus that manifests as a giant chromatin loop extending from the chromosome territory. She presented images in which the MHC class II region (850 kbp) extends up to 5 μm out of chromosome 6 territory within minutes after induction. To investigate the signalling cascade leading to the transcriptional activation of MHC genes, Sheer integrated FISH analysis to visualize chromatin architecture with ChIP analysis of recruitment of transcription machinery. Interestingly, a role for histone acetylation in this process is indicated from increased acetylation at histones H3 and H4 across the MHC, and the induction of higher chro-

matin remodelling by the histone deacetylase inhibitor sodium butyrate. These observations are consistent with the chromonema model of chromosome structure in which the 30-nm fibre is compacted into a 100–130 nm-wide fibre from which locally decondensed chromatin extends as fibres of up to 2–3 μm in length at transcribed sites (Tumbar *et al.* 1999), and such drastic chromatin decondensation coupled with histone acetylation also suits the multiscale model of chromatin dynamics presented by Lavelle, in which modifications at the nucleosome level have immediate repercussions through a processive fashion up to the chromonema fibre and chromosome loop level. The association of MHC with promyelotic leukaemia (PML) bodies was also discussed, since these nuclear multiprotein domains have been suggested to function in transcription by forming in nuclear compartments of high transcriptional activity.

Using real-time video-microscopy study, **Susan Gasser** (Basel, Switzerland) analysed the dynamics of chromosomal domains in yeast interphase nuclei by tagging different types of chromosomal regions with a GFP-lac repressor fusion. She showed that internal transcribed regions are highly mobile, frequently moving more than 0.5 μm in 10 s in an energy- and nutrition-dependent manner. Remarkably, such local motion also happens to a similar extent in mammalian cells (Chubb *et al.* 2002), despite the difference in size and organization of the nucleus, which means chromatin dynamics in these cell occurs within the scale of a chromosome territory while it may cover the whole nucleus extent in yeast. However, both types of cells share the fact that attachment of chromatin to immobile nuclear substructures restricts its dynamics, as Gasser showed with telomeres, whose preferential localization to nuclear periphery constrains the extent of their movements. Interestingly, constraint on the movement of active loci seems to be imposed only by the chromatin fibre itself, since excising a circle from the transcriptionally active LYS2 locus makes it move without detectable constraints throughout the nucleoplasm. Gasser also showed that relocalization to the nuclear pore of a gene such as the subtelomeric HXK1 occurs upon induction, and concluded that nuclear position has an active role in determining optimal gene expression levels. Finally, since the understanding of chromatin dynamics in the nucleus is related to the knowledge of chromatin folding, Gasser presented data from *in-situ* hybridization and live imaging techniques in budding yeast. The results suggested that interphasic chromatin exists in a compact higher-order conformation with a persistence length of ~170–220 nm and a mass density of 110–150 bp/nm, consistent with 7–10 nucleosomes per 11 nm in a 30 nm-like fibre structure. Now, although these figures are also rather consistent with the chromatin fibre model presented by Rhodes, one cannot exclude a different molecular organization of these fibres due in particular to the difference in linker histone stoichiometry (~1/20 nucleosomes in budding yeast vs 1/1 in Rhodes's *in-vitro* fibres) and linker length (165 bp/nucleosome vs 177–207 bp respectively). In a related talk, **Lutz Gehlen** (Basel, Switzerland) investigated both theoretically and experimentally the effects of telomere anchoring on telomere interactions. In these studies juxtaposition of chromosomal sites that are equidistant from the centromere appeared as the dominant characteristic that influences interaction, probably due to stabilization in interphase of the Rabl organization that occurs in late anaphase.

Nuclear organization and the nuclear envelope

Many complexes operate inside the nucleus, and one might wonder if particular nuclear environments are occupied by each, depending on their specific role. This was mainly discussed by **Dean Jackson** (Manchester, UK). Namely, it has been clear for many years that nuclei are highly compartmentalized and that the nuclear organization is a major epigenetic regulator of chromatin function. In particular, nuclear organization provides dedicated sites for major nuclear activities such as RNA synthesis ('transcription factories'), DNA replication ('replication factories') or DNA repair. Since clustered active complexes are constrained, chromatin must move, and a key epigenetic regulator is thus the mechanism which facilitates the mobility of chromatin so that active chromatin (transcribed gene or active replicon) is able to access the synthetic factories: this lies at the heart of our understanding of nuclear structure and function (Cook 2002, Chakalova *et al.* 2005). Whether an underlying filamentous network such as the cytoskeleton also exists in the nucleus, leading to a so-called nuclear skeleton (or matrix or scaffold) that would participate in the overall organization of chromosome territories and/or distribution of factories, is still controversial. Simple interactions between numerous components of the interchromatin compartments could indeed be

sufficient to give a kind of pervasive substructure on to which active compartments might assemble.

Peter Fraser (Cambridge, UK) showed that essentially all transcription of individual genes takes place in transcription factories that are relatively stable sub-nuclear compartments. Transcription of most 'active' genes is not continuous, but appears to go through transcription cycles or bursts, with transcription switching on and off in conjunction with gene movements into and out of factories. More precisely, Fraser showed that actively transcribed genes dynamically engage transcription factories in a non-random manner with genes as far as 60 megabases apart compelled to share the same factory. Specific subsets of co-expressed genes preferentially co-associate in factories, suggesting the existence of physical transcriptional networks.

With emerging roles in signalling and gene expression, the nuclear envelope (NE) clearly serves as more than just a physical barrier separating the nucleus and cytoplasm, and the many nuclear proteins that form lamin-dependent complexes have roles in chromatin organization, gene regulation and signal transduction (Gruenbaum *et al*. 2005, Prunuske & Ullman 2006). Arguing that the specific radial organization of chromosomes itself could depend on the nuclear boundary as a scaffold or position marker, **David Vaux** (Oxford, UK) studied the role of lamin B1 in NE stability, chromosome territories position and gene expression. Vaux had already shown that this protein and its post-translational modifications are essential for the integrity of the nuclear lamina. Comparison of wild-type murine fibroblasts with transgenic fibroblast lacking lamin B1 showed that lamin B1 is a determinant of chromosome territory position in the mouse, identified chromosome 18 as an example of tight peripheral NE association and confirmed that this anchoring plays a role in the expression of a subset of genes on this chromosome.

Using a different cellular system, **Maarten Fornerod** (Amsterdam, The Netherlands) analysed the complete left arm of *Drosophila* chromosome 2 (22 Mb) for lamin B (Lam) interaction at a 100 bp resolution using DamID. Lam binding clusters were encountered on every scale at every position along the chromosome arm in a fractal-like arrangement. Using wavelet decomposition analysis, Fornerod showed that all cluster sizes were over-represented compared to randomized chromosome arms, with particular over-expression of clusters ~300 kb, mainly located at telomeric and centromeric regions. Furthermore, a striking correlation at specific genomic scales between gene expression, replication timing, active histone modifications and gene density indicates that the *Drosophila* genome is structured in a multifractal manner during interphase.

In a talk devoted to the anchoring of telomeres to the nuclear periphery, **Frédérique Magdinier** (Lyon, France) addressed the involvement of the subtelomeric element D4Z4 repeat in facio-scapulo-humeral dystrophy (FSHD). Since the most popular hypothesis for this disease is the involvement of telomeric position effect (TPE), they decided to test the role of the D4Z4 element on genome organization and regulation of telomere silencing. Magdinier showed that D4Z4 is an insulator element protecting from TPE. More precisely, D4Z4 is able to displace a telomere from the interior of the nucleus to the periphery and is furthermore able to interact with the A-type lamins, suggesting that the anchoring of this insulator element to a fixed structure might participate in insulation. Also dealing with the nuclear periphery, **Vered Raz** (Leiden, The Netherlands) combined molecular and cell biology approaches together with quantitative image processing to generate a three-dimensional reconstitution of the spatial organization of the nuclear lamina, telomeres, centromeres and PML bodies. In particular she showed that, upon activation of the caspase-8 pathway in human mesenchymal stem cells, lamina organization is changed, followed by specific changes in the localization of centromeres and telomeres, while that of PML bodies remains unchanged. Since similar changes were found in senescent cells, Raz suggested that these changes could indicate the vitality of the cell.

Finally, in the last talk of the meeting and also the single one addressing DNA replication, **Aloys Schepers** (Munich, Germany) presented the identification of new human origins of DNA replication by an origin-trapping assay, and showed that replication origins must be re-established during early stages of cell division cycle and that the origin recognition complex (ORC) participates in this process.

Modelling chromatin: the bare essentials?

The chromatin section of the workshop showed that regulation at the chromatin level is the type

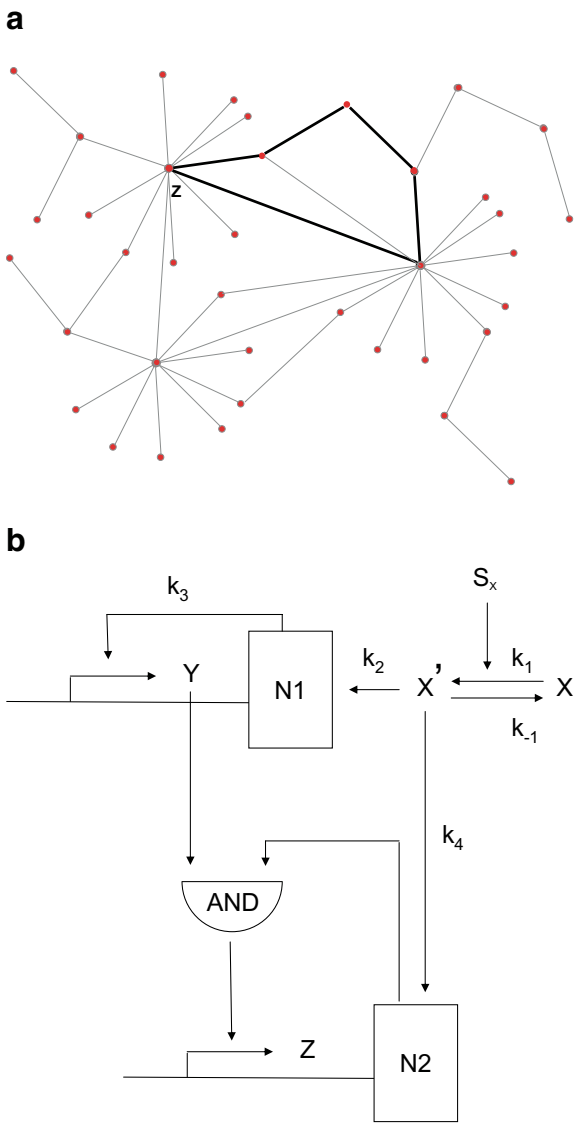

Figure 1. Modelling the bare essentials. Biological networks that perform a given function tend to have complicated connectivity of the type shown in (**a**). Each point (node) denotes a gene, and each line (edge) denotes that two genes are connected in the network. In this theoretical example the black lines denote the key interactions and grey lines weak second-order interactions in the regulation of the gene marked Z. Simplifying the network in (**a**) by disregarding the weak second-order interactions and knowing more about the key interactions gives rise to the model shown in (**b**). X, Y, and Z are genes, with X′ representing the active form of X. The signal Sx mediates X activation. N1 and N2 are elements on the DNA which permit the transcription of Y and Z respectively in this theoretical example. 'AND' denotes that inputs from both gene Y and N2 are needed for Z transcription. Note that, if we assume a separation of time-scales – N1 and N2 activation reaches steady state quickly after Sx, while Y takes longer to build up due to the transcriptional delay – network dynamics can be quickly grasped. The network would not allow Z transcription with short Sx pulses because, by the time Y builds up, N2 would already be inactivated. The exception might be an oscillation, with a period similar to the transcriptional delay.

of complicated biological system, with a network of components that are dynamic in both space and time, which system biology may help understand (Alon 2006). This type of understanding uses mathematical modelling of the interactions between network components. During the workshop there was a lively discussion that touched at the heart of this: how to use current biological data to

model information storage and retrieval from the chromatin.

How much information should be included in the model? After all, genes are similar to people in connectivity: eventually, everyone is somehow connected to everyone else. One option is to include as much as possible, which has been done in metabolic networks. This enables following flow through the network, which can be more complex than intuition, or a simplified model, may suggest. The disadvantage of models on this scale, unless they are built in a very modular way, is that they require huge amounts of accurate information, and may be hard or impossible for the human mind to grasp.

The other type of models are what can be thought of as 'bikini' models, covering the bare essentials. These models can be used to predict gene or protein behaviour, or to explain the behaviour in the simplest possible way (Meinhardt 2004). To make progress using such models, one needs a description of systems-level parameters. The starting point may be a map of how the different genes or proteins are connected (Figure 1a). However, this is not sufficient, and such a network must include the signs (activating or inhibitory) of the interactions, the rate or binding constants, how multiple inputs are integrated and, above all, it must be simplified to exclude weak interactions that may have only second order effects on the outcome (Figure 1a, grey lines). This kind of representation can then be used to set up a system of interrelated differential equations, which can then be solved numerically to yield, for example, the outcome of the interactions as reflected in the amount of final gene product under different conditions and at different times (Figure 1b). The simplification step is essential, since a model that does not include hundreds of equations and parameters may give more insight into what a network may be doing. An example of a simple model was presented by Fraser at the workshop, and tried to explain why co-regulated genes end up transcribed at the same transcription factories. It proposed that, once a transcription factor and its target gene co-localized to one factory, the transcription factor would literally stick around the factory for some time, capturing other co-regulated genes. This is not a particularly global description of cellular processes, but it gives nice insight into what may be going on in the process studied.

What are the data available for such an analysis? The connections between genes, and their signs, are generally available. The rate or binding constants for each reaction are harder to come by. How different inputs are integrated to regulate a given gene (the gate) is rarely known. The roots of this may be traced to how measurements were done in molecular biology, at least until recently. The assays are generally static, and yield qualitative but not quantitative information. Lately there has been a growing trend to quantify gene and protein dynamics in living cells (Heun *et al.* 2001, Setty *et al.* 2003, Wachsmuth *et al.* 2003, Sigal *et al.* 2006). This is very useful since, while there are other ways to obtain the constants or gates, this kind of behaviour allows a reverse engineering approach: finding the best-fitting constants and gates. It even allows finding gene modules such as feedforward and feedback loops, which have a typical response signature in either space or time (Milo *et al.* 2002, Nelson *et al.* 2004).

The bottom line for chromatin research is that systems biology may become a powerful tool to understand the outcomes of dynamic multicomponent interactions. Does this mean that researchers should leave everything and rush out to take mathematics and computer courses? Probably not. Collaborators with a mathematical and computational background can usually be found if the problem is interesting, and an intuitive understanding of the computational processes involved is generally sufficient. However, it should be realized that the models are as good as the data used to create them, and the kinds of experiments done should be designed to give the type of quantitative data that can be used for a systems biology approach.

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Supplementary Material

Most references of participants' own work have not been included in the text, due to space restrictions.

These references, comprising some of the speakers' recently published papers discussed during the workshop, are available online as supplementary material*.

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(*See also additional reference in Electronic Supplementary Material online*)

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***Electronic Supplementary Material**

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