A molecular dissection of nuclear function

Conference on The Dynamic Nucleus: Questions and Implications

David L. Spector¹ & Susan M. Gasser²⁺

¹Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA, and ²University of Geneva, Geneva, Switzerland

The meeting 'The Dynamic Nucleus: Questions and Implications' took place at Imperial College, London, UK, between June 27 and 29, 2002. It was sponsored by the MRC Clinical Sciences Centre in London and was organized by Niall Dillon and Ana Pombo.

EMBO reports 4, 18–23 (2003)
DOI: 10.1038/embor701

Rapid diffusion of nuclear factors

A large number of studies that monitor fluorescence recovery kinetics of green fluorescent protein (GFP) fusion proteins after photobleaching (FRAP; see Fig. 2) have taught us that nuclear factors are highly dynamic, whether they are transcription factors, polymerases, actin isoforms, linker histones or components of still-mysterious nuclear bodies. One might wonder whether these extremely rapid diffusion kinetics reflect a 'principle of nuclear organization' or a measure of nature's tendency towards entropy. Nevertheless, using this technique, G. Hager (Bethesda, Maryland, USA) showed that the glucocorticoid receptor (GR) exchanges rapidly at its promoter-binding site in the presence of an activating ligand, with a half-maximal time for recovery of merely 5 s. In contrast, RNA polymerase II (RNA pol II) exchanges relatively slowly—perhaps reflecting its engaged state—requiring 13 min for full fluorescence recovery. Hager's most recent studies show that the remodelling of promoter chromatin that is initiated by GR requires ATP-dependent nucleosome remodelling complexes, and that GR itself is displaced by the remodelling event. This suggests that the GR uses a hit-and-run mechanism, transiting to a promoter and recruiting the remodelling complex that will result in its own displacement, while opening the domain for the polymerase (Fletcher *et al.*, 2002). Consistently, the presence and activity of RNA pol II pass through a maximum shortly after stimulation with hormone. Intriguingly, promoter histones also become deacetylated during this period. Once the receptor is released, it apparently returns to the template many times during promoter activation. This hit-and-run model was corroborated with similar data for the aryl hydrocarbon receptor. The fact that nuclear hormone receptors tend to be found in foci raises questions about the spatial arrangement of active and coordinately regulated genes, an aspect that could be addressed in the future with chromatin-tagging techniques.

Similar studies by M. Mancini (Houston, Texas, USA) demonstrated analogous rapid protein-exchange kinetics for both the oestrogen receptor- α (Stenoien *et al.*, 2001) and for ataxin-1, a protein that contains stretches of polyglutamine (poly-Q) of variable lengths in spinocerebellar ataxia type 1 neurodegeneration. This aberrant form seems to accumulate in nuclear inclusions, which are a hallmark of many poly-Q diseases. Wild-type ataxin-1 was seen to equilibrate very rapidly between bleached and non-bleached regions of the nucleoplasm. The mutant ataxin within inclusions was also surprisingly mobile, although it showed varying levels of

Introduction

Over the course of two and a half days, hypotheses and results about the organization and function of the eukaryotic nucleus were discussed intensely in the congenial atmosphere of this meeting. The topics covered ranged from DNA repair to nuclear actin, from nucleolar assembly to the deposition of histone variants, and from technical aspects of fluorescence signal separation to the analysis of chromatin dynamics in living cells. Many nuclear domains have previously been identified (Fig. 1) and recent advances in live-cell imaging techniques have opened the door for real-time analyses of the diverse range of nuclear functions associated with these structures. Nearly every major nuclear process was discussed, yet the most novel ideas dealt with the dynamics of nuclear structures and events. New ways to monitor dynamics, paired with the fact that nuclear organization now attracts experts from a wide range of fields, left participants uniformly excited about the potential offered by the marriage of live fluorescence imaging and molecular genetics. A few highlights of the work presented at the meeting follow.

¹Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724, USA

²University of Geneva, Department of Molecular Biology, 30, Quai Ernest Ansermet, CH-1211 Geneva, Switzerland

⁺Corresponding author. Tel: +41 22 702 6127; Fax: +41 22 702 6868; E-mail: susan.gasser@molbio.unige.ch

Submitted 30 September 2002; accepted 5 November 2002

Fig. 1. | Diagram of a mammalian cell nucleus showing many of the nuclear domains that have been identified so far. Although each of these domains can be easily observed by light or electron microscopy approaches, remarkably, a large number of their protein constituents are highly dynamic. For a detailed overview of these compartments see Spector (2001).

immobilization in different cells. Immobility was shown to be correlated with high levels of ubiquitination and low levels of proteasomes, and could be further aggravated by proteasome inhibitors or heat shock. This suggests that ataxin inclusions are not mere aggregates but might be undergoing constant turnover by the nuclear protein degradation machinery. Future studies must elucidate whether or not ataxin-1 dynamics are functionally related to the disease state.

Locus control regions: establishing expression patterns

Transient mechanisms of gene activation are also thought to function at tissue-specific enhancers such as those of the β -globin locus control region (LCR; see Fig. 3), a system thoroughly studied by F. Grosveld (Rotterdam, The Netherlands) and P. Fraser (Cambridge, UK). Grosveld presented data indicating that activation of the α - or β -globin loci is stochastic in nature. The decision to activate is made before the actual induction of transcription, probably at the level of chromatin modification (Fig. 3). This choice is then clonally inherited. The fact that α -globin loci (located in a constitutively active chromatin domain) are generally activated a few hours before the β -globin loci (located in a closed chromatin structure in non-expressing cells) suggests that chromatin structure might regulate the decision. Because activation is an independent, stochastic event within each of the four loci (i.e. two α -globin and two β -globin loci), a cell can support either biallelic or monoallelic expression, without allelic

exclusion. Once the LCR has activated the β -globin locus, several genes can be expressed (as demonstrated by mRNA in the cytoplasm), although only one gene is transcribed at any given moment (as shown by analysis of the primary transcript in the nucleus). This suggests that there is a competitive mechanism that excludes other promoters when one is activated. However, this commitment to a promoter is temporary, because the LCR can switch between β -globin promoters. Thus, there are at least two levels of regulation involving the LCR, one in the choice of whether to activate the locus, and a second in the transcriptional activation of a specific gene.

On a more mechanistic level, P. Fraser has applied a novel 'FISH-trap' technique to the β -globin locus to capture or trap chromatin interactions and examine how the LCR acts over distance to stimulate the promoter of an activated gene. He asked whether activation is due to direct gene–LCR contact (looping; Fig. 3) or whether it reflects other processes controlled by, or propagated from, the LCR. His data convincingly indicate the former, because he recovers enhancer and gene sequences in close proximity in erythroid cells (Carter *et al.*, 2002). The question of whether the long-range contact is transient or semi-stably maintained remains open. The former would be consistent with the hit-and-run model for promoter activation derived from hormone receptor studies. Taken together, however, the data of Grosveld and Fraser suggest that nature has found an alternative way to regulate and coordinate tissue-specific promoters.

Fig. 2. | Diagram of a FRAP (fluorescence recovery after photobleaching) experiment. A cell expressing a nucleolar GFP fusion protein is imaged before the bleach; at zero time one nucleolus is bleached (dotted circle) and recovery to the bleached area from the nucleoplasmic pool of the protein is monitored over time (seconds to hours).

Heritable states of repression

Not all gene regulation is a question of transient states, and no example of this is more striking than the heritable repression of genes in differentiated cells that is correlated with a re-positioning of the gene to centromeric heterochromatin *in trans*. The latter has been elegantly demonstrated by studies of lymphocyte-specific genes by M. Merckenschlager and M. Fisher (London, UK). Recent data from this laboratory show that, contrary to common belief, the repression of tissue-specific genes is not invariably coupled with a late replicating state. Indeed, all lymphoid-specific genes studied were found to be replicated early in S phase whether they were silenced or not. Only the integration of a tissue-specific transgene directly into centric heterochromatin produced a change in its timing of replication. This shows, first of all, that transcriptional states are not necessarily the mechanism that determines when an origin fires. Second, it rules out the model that replication timing is an essential component of heritable gene repression. In contrast, Fisher reported that in G2 phase, silent tissue-specific genes remain paired with their replicated sister chromatid significantly longer than active genes, suggesting a functional relationship between this prolonged contact between homologues and a heritable repressed state. It remains to be seen whether this is a general principle in the propagation of silent chromatin.

S. Gasser's laboratory (Geneva, Switzerland) reported that transcriptionally silent chromatin in budding yeast is relatively immobile in interphase, lying fixed to the inner nuclear envelope, in contrast to the rapid dynamics of the gene when it is actively transcribed. Recent results show that chromatin that is silenced through the binding of silent information regulators can autonomously tether itself to elements of the nuclear envelope. This indicates that a silent locus can mediate its own subnuclear localization through interaction with structural nuclear elements. If subnuclear position determines the timing of origin firing, one might further conclude that the repressed state imposes late firing by determining the origin's subnuclear positioning.

With regard to the effects of mouse satellite DNA on the activity of nearby genes, N. Dillon (London, UK) was able to show convincingly that a $\lambda 5$ transgene can be efficiently transcribed and correctly regulated even when it is placed in centromeric heterochromatin, provided that a domain of 19 kilobases is integrated. When a smaller fragment was used, a variegated expression pattern was seen: 'on' in some cells, 'off' in others. Nevertheless, the gene can have a variable spatial position, being either at the edge or in the middle of a heterochromatic zone within the nucleus. The activation process involves several steps, including relocation to the edge of the heterochromatin zone, but the gene remains in contact with heterochromatin even when it is transcribed. Similar situations have been observed in yeast, where it was shown that perinuclear positions are not sufficient to confer transcriptional repression. At best, a position in or near heterochromatin confers a state that is permissive to silencing, should the competition for transcriptional activation be lost. Once again, we confront the idea that stochastic or competitive events can be dominant regulators of gene expression.

Chromatin unfolding and histone modification

A more systematic analysis of the recruitment events that occur as the herpes simplex viral transactivator VP16 activates a domain was presented by A. Belmont (Urbana, Illinois, USA). He made use of an amplified *lac* operator–dihydrofolate reductase construct that tags a large domain such that both chromatin dynamics and protein recruitment can be revealed (Fig. 4). Once the transcription factor is targeted, the opening of the compact silent domain (starting from an estimated compaction ratio of more than 12,000-fold) begins after ~20 min, and is fully open by 4–6 h. The decompaction is correlated with histone acetylation and the recruitment of the Brg1- and Brahma (Brm)-containing nucleosome remodelling complexes. Perhaps most surprising is the rapid arrival of the large PtdIns-3-OH kinase-like transformation/transcription domain-associated protein (TRRAP), ~10–20 min before the increase in histone acetylation. TRRAP forms a complex with either the histone acetylase GCN5 or Tip60, which are also found in the nucleosome remodelling

Fig. 3.1 The organization of the β -globin locus and proposed mechanisms of gene activation. In mouse and human cells globin genes are arranged sequentially (grey boxes), flanked by a locus control region (LCR) at the 5' extremity. In non-erythroid lineages the domain is inaccessible to nuclease. During sequential activation steps the domain first becomes generally sensitive to DNase before the activation of specific genes; after the binding of specific factors to the LCR, five hypersensitive sites (HSS) can be detected. In cells capable of expressing globin genes, preinitiation complexes form at multiple promoters. It is proposed that activation requires direct contact between as yet unspecified factors bound to the LCR and the preinitiation complex (CBP, TAFs and TBP) bound at the promoter concerned.

complexes SAGA and NuA4, respectively. Whereas GCN5, PCAF (p300/CBP-associated factor) and CBP (CREB-binding protein)/p300 recruitment coincides with the burst in histone acetylation and the arrival of the chromatin remodelling catalytic subunits Brg1 and Brm, Tip60 is not detected until much later. These results suggest that histone acetyltransferases and other remodelling components are recruited as separate subunits, or partial complexes, in the context of condensed chromatin. It is possible that different enhancer-promoter combinations will determine both the order of assembly for chromatin-modifying machines and whether they arrive as individual subunits.

Changing local histone patterns is a major theme in chromatin research and S. Henikoff (Seattle, Washington, USA) presented an intriguing study on the deposition of a variant histone H3 that, unlike most nucleosome assembly events, is fully uncoupled from replication (Ahmad & Henikoff, 2002). *Drosophila*, like most higher eukaryotes, has three distinct forms of histone H3: one is the normal core histone H3, the second is a closely related variant called histone H3.3, and a third has a long amino-terminal extension and localizes exclusively to centromeres.

Whereas the normal fly histone H3 is incorporated into nucleosomes exclusively in a replication-coupled assembly mechanism, Henikoff showed that histone H3.3 can be deposited throughout the cell cycle, using both replication-dependent and replication-independent mechanisms. H3.3 is associated exclusively with actively transcribed regions, being particularly enriched in active ribosomal DNA (rDNA) loci. The incorporation of the H3.3 variant is therefore proposed to correlate with transcription, and would provide a method of 'marking' a transcribed locus for preferential reactivation during the next cell cycle. Alternatively, it might tag sites for early replication or localization to a particular nuclear compartment. It will be important to establish whether the regulated localization of other histone variants is also due to different assembly mechanisms.

A new view of nuclear disassembly

Equally important to the coordination of nuclear functions during interphase are the processes involved in nuclear disassembly and reassembly during mitosis. Biochemical data have suggested that protein phosphorylation is central to the disassembly of the nuclear envelope (NE) during prophase of mitosis, and that dephosphorylation of these same proteins results in the NE's reassembly during late anaphase and early telophase. However, J. Ellenberg (Heidelberg, Germany) presented data demonstrating an additional level of regulation, on the basis of elegant studies in living cells. By micro-injection of a 500-kDa fluorescent dextran into the cytoplasm of mammalian fibroblasts, Ellenberg was able to show that NE breakdown begins with a very localized disruption, followed by complete breakdown. In early prophase, microtubules attach to the NE via minus-end-directed motors, creating the initial tension. In mid-prophase, folds appear in the NE and the lamina stretches, resulting in 'tearing' of the NE in late prophase and, finally, lamin depolymerization (Beaudouin *et al.*, 2002). These mechanical events seem to take place in coordination with changes in the phosphorylation status of numerous nuclear proteins. In the starfish oocyte, NE breakdown occurs independently of microtubules but in an otherwise similar two-step process. Again, this starts with a gradual disassembly of nuclear pores and is completed by a rapidly spreading fenestration of the nuclear membrane. The NE was observed to depolymerize locally at the animal pole, and fluorescently conjugated dextrans entered and spread in a wave ($1 \mu\text{m s}^{-1}$) across the nucleus. Interestingly, when fibroblast microtubules were depolymerized before prophase, the cells entered mitosis using the mechanism observed in starfish, although mitotic entry was delayed by 15 min, indicating that backup mechanisms exist even in microtubule-controlled systems.

Visualizing transcription

One of the first nuclear compartments to assemble after exit from mitosis is the nucleolus, the site of rDNA transcription and rRNA processing and packaging. D. Hernandez-Verdun (Paris, France) showed that cyclin-dependent kinase-1 (CDK1) activity represses rDNA transcription during mitosis. Although the inhibition of CDK1 induces a resumption of rDNA transcription and pre-nucleolar body formation, it is not sufficient to induce the proper recruitment of the rRNA-processing machinery, and consequently rRNAs are not cleaved and nucleoli are not assembled (Sirri *et al.*, 2002). Hernandez-Verdun concluded that another CDK is involved in this second process.

Studies from T. Misteli's laboratory (Bethesda, Maryland, USA) addressed the dynamics of the RNA polymerase I (RNA pol I) transcription machinery on clusters of rDNA genes in nucleolar fibrillar

centres. Using FRAP, Misteli was able to show that the recovery curves for different RNA pol I subunits and transcription factors are different, an indication that these proteins are not recruited into the nucleolus as a stable holoenzyme complex but as individual subunits. He went on to estimate that the transcription of one rDNA transcript as measured by FRAP in living cells takes roughly 140 s. On the basis of this calculation and previous work from the Miller laboratory indicating that about 100 RNA pol I molecules are present on a rDNA gene at any given time, he estimated that there is a 50% probability of a subunit's hitting the promoter once inside the nucleolus, and of those that hit, 10% are incorporated into complexes and participate in elongation. These results suggest that RNA pol I is assembled anew at each round of transcription from a steady stream of subunits through the nucleolus, by means of metastable intermediates.

Once the rRNA transcript has been processed, it is packaged into a pre-ribosomal particle within the nucleolus. T. Pederson's laboratory (Worcester, Massachusetts, USA) has examined questions relating to the transport of ribosomal subunits from the nucleolus to the cytoplasm. Using a series of caged fluorescein oligodeoxynucleotides complementary to several regions of 28S rRNA, Pederson and his colleague J. Politz were able to observe bright nucleolar signals after photolytic uncaging. A substantial portion of the signal proceeded to move outwards from the uncaging site, departing in all directions from the nucleolus, and filling the nucleus within ~30 s. There was no indication of preferred routes of traffic or selective transport to nuclear pores closest to the nucleoli. Moreover, the movement was unaffected by a decrease in temperature, which is indicative of a diffusion-driven process.

D. Spector's group (Cold Spring Harbor, New York, USA) reported on the development of a live cell system for the direct visualization of a stably integrated genetic locus, as well as its RNA and protein products. Using this system, they showed the dynamic transitions from the 'off' to the 'on' state, as well as monitoring the recruitment of proteins involved in gene expression. Using several colour variants of GFP, they were able to reveal nascent RNA at the transcription site within 5–8 min of induction of the transcription unit array.

In an intriguing new development, work from P. Hozak (Prague, Czech Republic) and P. DeLanerolle (Chicago, Illinois, USA), showed that nuclear myosin 1 β (NM1) co-localizes with RNA pol II *in vivo* and that this association is abrogated upon the inhibition of transcription. Supporting the possible involvement of myosin and actin in transcription, they went on to show that antibodies against actin or NM1 inhibit transcription *in vitro* and *in vivo*, whereas the addition of NM1 could stimulate elongation. To determine whether these associations were unique to RNA pol II, they went on to examine the RNA pol I machinery. They found that actin was associated with the fibrillar centres of transcriptionally inactive nucleoli, whereas NM1 was found within the transcriptionally active dense fibrillar component, as well as in the granular regions of nucleoli. Supporting these localization studies, actin co-immunoprecipitated with the RNA pol I holoenzyme and either anti-actin or anti-NM1 antibodies were found to inhibit RNA pol I transcription on naked DNA as well as on a preassembled chromatin template. On the basis of these data, it was suggested that NM1 might function to drive the polymerase during transcriptional elongation. M. Hendzel (Edmonton, Alberta, Canada) has pursued a similar theme showing that cells incubated with the actin-depolymerizing drug latrunculin B exhibit a 45–95% decrease in RNA pol I and pol II transcription, depending on the cell type examined.

Fig. 4. Mechanisms for tagging chromosomes with *lac* operators to monitor chromatin dynamics in living cells. Arrays of bacterial *lac* repressor-binding sites (*lac* operators; *lac op*) are cloned next to a selectable marker. After insertion of the linear fragment into the genome, and selection for the marker gene, the *lac* operator array is inserted singly (for example, in yeast) or as multimers (for example, in mammalian cells). Visualization of the chromosomal locus through the binding of a GFP–*lac*-repressor fusion can be combined with nuclear pore fluorescence (yeast image, left-hand side) or with background nuclear fluorescence to determine the boundaries of the nucleus (human fibroblast, right-hand side). Rapid time-lapse microscopy is used to monitor movement of the insert. (Images courtesy of F. Hediger (University of Geneva, Geneva, Switzerland) and J. Chubb (MRC, Edinburgh, UK).)

Subcompartments for repression, repair and replication

Polycomb group proteins have been characteristically reported to be associated with inactive chromatin. Interestingly, the R. van Driel laboratory (Amsterdam, The Netherlands) reported that these proteins are excluded from condensed chromatin in HeLa cells, and localize instead to the surrounding chromatin, called the perichromatin domain (Cmarko *et al.*, 2002). Because Polycomb proteins associate with silenced loci, this might indicate that heritably repressed genes are not segregated into the most highly compacted heterochromatic zones of the nucleus but remain in adjacent spaces. *In situ* hybridization assays can readily address this question.

J. Hoeijmakers (Rotterdam, The Netherlands) is interested in the dynamics and organization of nucleotide excision repair (NER), an important process that counters the effects of DNA damage. FRAP analysis of several proteins involved in NER indicated that these proteins are mobile and have different diffusion rates and nuclear distributions, arguing against the existence of a stable, preassembled NER 'holocomplex'. After induction of DNA damage, a fraction of NER proteins was shown to become transiently immobile in a dose- and time-dependent manner, corresponding with immobilization for a period of one repair event

lasting ~4 min. On the basis of these data, it was suggested that the assembly of NER factors into a functional complex occurs at the site of a lesion.

Similar ideas were presented by C. Cardoso (Berlin, Germany) and D. Zink (Munich, Germany) about the mobility and sequential assembly of replication enzymes during the temporally delayed activation of replication foci that occurs as S phase progresses. Although origins themselves might be pre-organized into compartments that have reproducible temporal patterns of origin firing, the replication machinery seems to assemble sequentially at foci in a manner that is correlated with the activation of DNA synthesis. As for repair complexes, this argues against a preformed 'holo-replisome' that sits primed for an activating signal.

Chromatin dynamics in real time

Several laboratories have turned from studying the dynamics of fluorescently tagged proteins to the analysis of chromatin movement in living cells through the use of lacOP/GFP-lac repressor-tagged DNA (Fig. 4). Whether the work was performed in budding yeast (Gasser laboratory), in flies (J. Vazquez and J. Sedat, San Francisco, California, USA) or in human cultured cells (W. Bickmore, Edinburgh, UK), a few general conclusions can be drawn. It is clear that there are two distinct types of chromatin motion. One encompasses very rapid and short-scale movements (0.2–0.5 μm) with virtually random moments, although they are constrained to relatively small zones of the nucleus. These movements are particularly obvious at open or active loci and in yeast are sensitive to ATP levels in the cell, although transcription elongation inhibitors do not quench the movement. Moreover, this motion is sensitive to neither nocodazole nor latrunculin A, suggesting that it is independent of microtubules and actin filaments. Remarkably, both in yeast and in human cells, transcriptionally silent domains, which are often found near the nuclear periphery, have significantly reduced radii of movement (Chubb *et al.*, 2002; Heun *et al.*, 2001).

A second, long-range movement of a tagged locus has been documented during the G2 phase of differentiating *Drosophila* sperm cells. Vazquez and Sedat have monitored the general displacement of a locus across the G2 nucleus, a movement that has a much slower diffusion constant but a distinct directionality (Vazquez *et al.*, 2001). Further genetic studies will be necessary to identify what provides the 'motor' for chromatin mobility, and whether the two types of movement are interdependent or coordinately regulated. The use of direct fluorescence to monitor chromatin movement is a relatively recent development, but one that promises to uncover new principles of nuclear dynamics. Certainly, to grasp the implications of these microscopic movements, there will be a need for advanced computer modelling of chromatin movement, as demonstrated by C. Cremer (Heidelberg, Germany).

Perspectives

The study of nuclear dynamics has assumed a momentum of its own, bringing together research topics and expertise from a wide variety of fields. The events of transcription, pre-mRNA splicing, DNA replication and repair are increasingly seen to depend on spatial aspects

of organization: factor concentration and diffusion constants are becoming relevant biological criteria for the analysis of nuclear events, and new techniques are needed to estimate these within the living cell. The advent of this real-time approach to nuclear functions has promoted an unprecedented interest in nuclear organization and has generated several new live imaging techniques. The meeting in London demonstrated this clearly, leaving its participants convinced that this field will continue to be a source of novel ideas and principles that will guide us to a complete molecular understanding of the maintenance and expression of the eukaryotic genome.

ACKNOWLEDGEMENTS

The meeting was sponsored by the Medical Research Council, UK, with financial support from the Wellcome Trust, and Carl Zeiss Ltd. We thank Jim Duffy for drawing Fig. 2, and the meeting's speakers for agreeing to the included citations. S.M.G. is supported by the Swiss National Science Foundation and D.L.S. by the National Institute of General Medical Sciences/NIH, USA.

REFERENCES

- Ahmad, K. & Henikoff, S. (2002) The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* **9**, 1191–2009.
- Beaudouin, J., Gerlich, D., Daigle, N., Eils, R. & Ellenberg, J. (2002) Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. *Cell* **108**, 83–96.
- Carter, D., Chakalova, L., Osborne, C.S., Dai, Y.-F. & Fraser, P. (2002) Long-range chromatin regulatory interactions *in vivo*. *Nature Genet.* (in the press).
- Chubb, J.R., Boyle, S., Perry, P. & Bickmore, W.A. (2002) Chromatin motion is constrained by association with nuclear compartments in human cells. *Curr. Biol.* **12**, 439–445.
- Cmarko, D., Otte A.P., Verschure, P.J., Van Driel, R. & Fakan, S. (2003) Polycomb group gene silencing proteins are concentrated in the perichromatin compartment of the mammalian nucleus. *J. Cell Sci.* (in the press).
- Fletcher, T.M., Xiao, N., Mautino, G., Baumann, C.T., Warren, B.S. & Hager, G.L. (2002) ATP-dependent mobilization of the glucocorticoid receptor during chromatin remodeling. *Mol. Cell Biol.* **22**, 3255–3263.
- Heun, P., Laroche, T., Shimada, K., Furrer, P. & Gasser, S.M. (2001) Chromosome dynamics in the yeast interphase nucleus. *Science* **294**, 2181–2186.
- Sirri, V., Hernandez-Verdun, D. & Roussel, P. (2002) Cyclin-dependent kinases govern formation and maintenance of the nucleolus. *J. Cell Biol.* **156**, 969–981.
- Spector, D.L. (2001) Nuclear domains. *J. Cell Sci.* **114**, 2891–2893.
- Stenoien, D.L., Mancini, M.G., Patel, L., Smith, C.L., O'Malley, B.W. & Mancini, M.A. (2001) FRAP reveals estrogen receptor- α mobility is ligand- and proteasome-dependent. *Nature Cell Biol.* **3**, 15–23.
- Vazquez, J., Belmont, A.S. & Sedat, J.W. (2001) Multiple regimes of constrained chromosome motion are regulated in the interphase *Drosophila* nucleus. *Curr. Biol.* **11**, 1227–1239.

David L. Spector

Susan M. Gasser