RING-finger-dependent manner [12]. Such discrepancies might be explained through experimental differences and will require further clarification. Nevertheless, the contribution of ubiquitylation to the realm of apoptosis remains intriguing. With respect to individual proteins, further assessment of the causal or consequential nature of their ubiquitylation is essential.

References

1 Shi, Y. (2002) Mechanisms of caspase activation and inhibition during apoptosis. *Mol. Cell* 9, 459–470
2 Salvesen, G.S. and Duckett, C.S. (2002) Apoptosis. IAP proteins: blocking the road to death's door. *Nat. Rev. Mol. Cell Biol.* 3, 401–410
3 Rodriguez, A. *et al.* (2002) Unrestrained caspase-dependent cell death caused by loss of Diap1 function requires the *Drosophila* Apaf-1 homolog, Dark. *EMBO J.* 21, 2189–2197
4 Wang, S.L. *et al.* (1999) The *Drosophila* caspase inhibitor DIAP1 is essential for cell survival and is negatively regulated by HID. *Cell* 98, 453–463
5 Goyal, L. *et al.* (2000) Induction of apoptosis by *Drosophila* reaper, hid and grim through inhibition of IAP function. *EMBO J.* 19, 589–597
6 Lisi, S. *et al.* (2000) Diverse domains of THREAD/DIAP1 are required to inhibit apoptosis induced by REAPER and HID in *Drosophila*. *Genetics* 154, 669–678
7 Crook, N.E. *et al.* (1993) An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J. Virol.* 67, 2168–2174
8 Bergmann, A. *et al.* (1998) Mechanisms and control of programmed cell death in invertebrates. *Oncogene* 17, 3215–3223
9 Yoo, S.J. *et al.* (2002) Apoptosis inducers Hid, Rpr and Grim negatively regulate levels of the caspase inhibitor DIAP1 by distinct mechanisms. *Nat. Cell Biol.* 4, 416–424
10 Hays, R. *et al.* (2002) Morgue mediates apoptosis in the *Drosophila* retina by promoting degradation of DIAP1. *Nat. Cell Biol.* 4, 425–431
11 Wing, J.P. *et al.* (2002) *Drosophila* morgue is a novel F box/ubiquitin conjugase domain protein important in grim-reaper mediated programmed cell death. *Nat. Cell Biol.* 4, 451–456
12 Ryoo, H.D. *et al.* (2002) Regulation of *Drosophila* IAP1 degradation and apoptosis by reaper and ubcD1. *Nat. Cell Biol.* 4, 432–438
13 Holley, C.L. *et al.* (2002) Reaper eliminates IAP protein through stimulated IAP degradation and generalized translational inhibition. *Nat. Cell Biol.* 4, 439–444
14 Wilson, R. *et al.* (2002) The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nat. Cell Biol.* 4, 445–450
15 Deng, L. *et al.* (2000) Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103, 351–361
16 Yang, Y. *et al.* (2000) Ubiquitin protein ligase activity of IAPs and their degradation in proteasomes in response to apoptotic stimuli. *Science* 288, 874–877
17 Suzuki, Y. *et al.* (2001) X-linked inhibitor of apoptosis protein (XIAP) inhibits caspase-3 and -7 in distinct modes. *J. Biol. Chem.* 276, 27058–27063
18 Huang, H. *et al.* (2000) The inhibitor of apoptosis, cIAP2, functions as a ubiquitin-protein ligase and promotes *in vitro* monoubiquitination of caspases 3 and 7. *J. Biol. Chem.* 275, 26661–26664
19 Miller, L.K. (1999) An exegesis of IAPs: salvation and surprises from BIR motifs. *Trends Cell Biol.* 9, 323–328
20 MacFarlane, M. *et al.* Proteasome-mediated degradation of Smac during apoptosis: XIAP promotes Smac ubiquitination *in vitro*. *J. Biol. Chem.* (in press)

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The nuclear pore complex and chromatin boundaries

Chi-Yun Pai and Victor G. Corces

The organization of the chromatin fiber within specific nuclear compartments and its arrangement into domains of higher-order structure are aspects of the regulation of gene expression that remain largely undefined. Boundary and insulator elements are likely to play important roles in establishing and maintaining the nuclear architecture required by eukaryotic cells to orchestrate the intricate array of events such as DNA replication, transcription and RNA processing. Recent results suggest a link between chromatin organization and nuclear transport and offer a glimpse of how nuclear organization might facilitate the integration of complex nuclear processes.

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Chromatin insulators and boundary elements are a class of DNA sequences defined operationally by two properties: they prevent enhancer–promoter interactions and they buffer transgenes from chromosomal position effects arising from genomic sequences adjacent to the transgene insertion site. Boundaries and insulators must play an important and conserved function in nuclear biology as they have now been found in a variety of organisms ranging from yeasts to human [1,2].

The observed effects of boundaries and insulators on transcription are merely the readout of the experimental approaches used to identify and define these sequences, and their effects on gene expression are likely a manifestation of their normal role in the biology of the cell. The two types of transcriptional properties characteristic of insulators – inhibition of enhancer–promoter interaction and buffering of chromosomal position effects – suggest very different alternative functions for these sequences in nuclear physiology. The fact that insulators can affect enhancer–promoter interactions can be viewed in the context of a role for these sequences in modulating enhancer function. In this view, insulators would be another regulatory sequence, in the same category as enhancers, silencers and promoter elements, whose primary function is the regulation of transcription. On the other hand, the fact that insulators can shield a transgene from position effects suggests that these sequences might separate euchromatic from heterochromatic regions and, in the process, set up chromatin domains that are permissive for transcription. In this view, the primary role of boundaries and insulators would be to compartmentalize the genome and organize the chromatin fiber within the nucleus. Such a role for insulators would bring these sequences into the same realm as matrix- and scaffold-attachment region (MAR/SAR) elements, sequences that are thought to be located at the base of chromatin loops and serve structural roles in chromatin organization [3]. Boundary elements could have an additional functional role in setting up chromatin domains permissive for transcription, and regulation of this function during cell differentiation could

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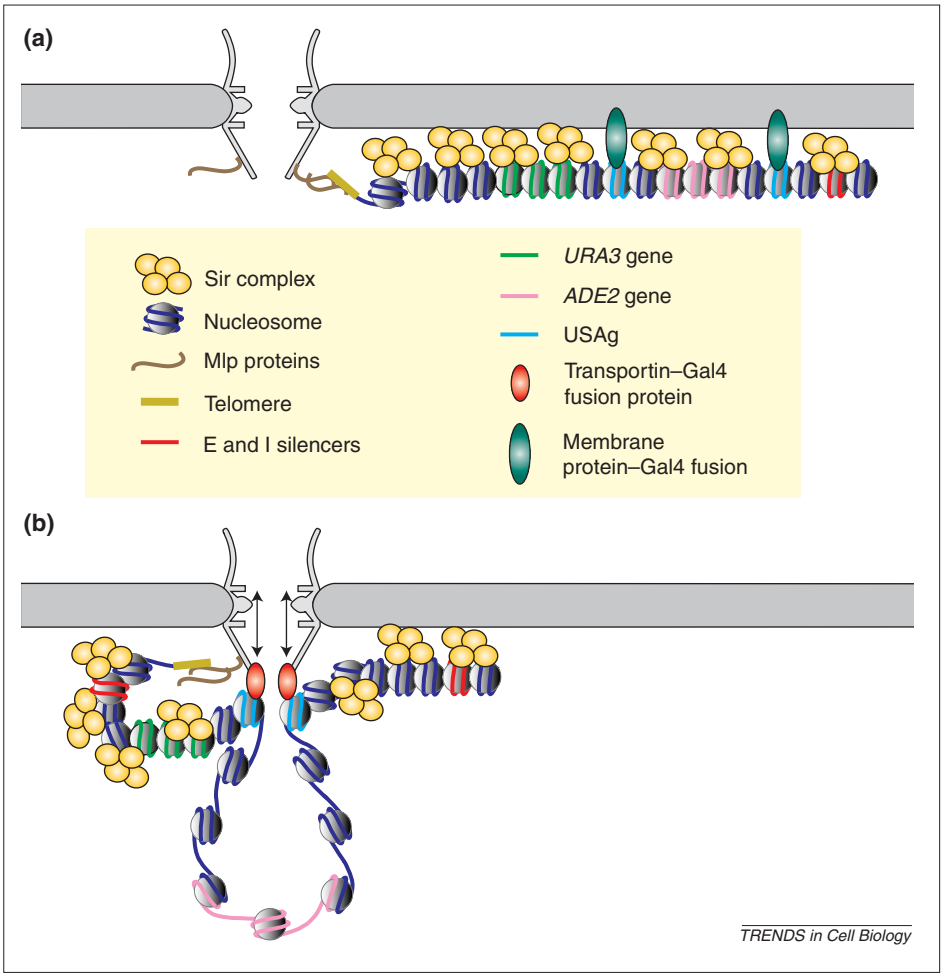

Fig. 1. Boundary activity and silencing in yeast. (a) The diagram shows part of the yeast nuclear membrane with a nuclear pore complex (NPC; gray). Sir-mediated silencing involves the recruitment of telomeres to the perinuclear compartment through interactions between the yKu complex and Mlp proteins at the NPC. In the absence of a functional boundary, silencing of the *ADE2* and *URA3* genes takes place. The same is true when membrane protein–Gal4 fusions are bound to the UASg (Gal4 DNA-binding sequence). (b) Establishment of a boundary through the binding of proteins with boundary activity, such as transportin–Gal4 fusion proteins, to the UASg is accomplished by interactions between transportins and the NPC. The boundary interferes with Sir-mediated silencing and results in a chromatin structure permissive for transcription. The *ADE2* gene is active, whereas the *URA3* gene is repressed.

account for broad aspects of gene regulation during development.

A connection between insulators and nuclear organization is reinforced by observations on the gypsy insulator of *Drosophila*. This insulator, originally identified in the gypsy retrotransposon, is also present at several-hundred sites in the fly genome. These sites come together at approximately 25 locations in the nuclear periphery, organizing the chromatin fiber into rosette structures. These rosettes are formed by chromatin loops, each presumably representing independent domains of chromatin organization and gene expression [4]. Recent results go further in showing a clear connection between the function of boundary and insulator elements and their ability to establish separate chromatin domains.

More interestingly, these results suggest that the boundary activity of a DNA sequence depends on its interaction with protein components of the nuclear pore complex (NPC). This nuclear structure, whose role in nuclear physiology has been thought to be mostly limited to the transport of proteins and RNAs between the nucleus and cytoplasm, might thus also play an important role in the regulation of gene expression.

The nuclear pore complex and boundary activity

In a recent paper, Ishii *et al.* [5] describe results from a clever boundary element-trap screen designed to unbiasly identify new proteins with boundary function. In this assay, two reporter genes, *ADE2* and *URA3*, were inserted between the E and I silencers of the yeast silent mating-type locus *HML*. *ADE2* is flanked by two Gal4-binding UASg elements, whereas *URA3* is not. Using a library of Gal4-tagged cDNAs, the authors were able to identify proteins with boundary activity. These Gal4-fusion proteins can establish a 'mini-domain' in the *ADE2* reporter that prevents heterochromatic silencing effects from the adjacent *HML* locus from spreading into the reporter gene. Under these conditions, *ADE2* can be transcriptionally activated, whereas the *URA3* reporter remains repressed (Fig. 1).

The screen yielded the unexpected finding of a relationship between boundary activity and proteins involved in nuclear transport or components of the NPC. The NPC serves as a gate to actively transport molecules in and out of the nucleus; it is a large protein complex with eight-fold rotational symmetry through the central axis of the pore, which forms a channel across the nuclear inner and outer membranes [6]. Genetic and biochemical studies have revealed most, if not all, of the NPC components, called nucleoporins (Nups). In addition to this large NPC structure, transport of cargo across the NPC requires adaptor proteins that shuttle between nucleus and cytoplasm. Many of these shuttle adaptors belong to the importin β superfamily, including the yeast transportins Cse1p, Los1p and Sxm1p [7]. Transportins bind to their cargos, such as mRNA to be exported or newly synthesized nuclear proteins to be imported; the energy and directionality of this process is provided by the monomeric GTPase Ran. Among the proteins found by Laemmli and colleagues to impart boundary activity are Gsp2p, a RanGTPase, and members of the importin β superfamily, including Srp1p, Cse1p, Los1p and Sxm1p. This finding suggests that NPCs might have functions other than to serve as transport gates, and that, by serving as anchorage sites for chromatin, NPCs might also be involved in the regulation of gene expression (Fig. 1). Interestingly, the boundary activity of transportins does not require their N-terminal RanGTP-binding domain, but entails the C-terminal region that mediates docking of the complex to the NPC. Furthermore, the boundary activity of transportins depends on the presence of the nucleoporin Nup2p, the major docking site for these transportins on the NPC basket. In addition, a Nup2p–Gal4 fusion protein also displays boundary activity in this assay [5].

Boundaries, silencing and nuclear compartments

There is some precedence for links between the NPC and chromatin regulation in yeast. Repression of transgenes adjacent to yeast telomeres and *HM* loci is accompanied by anchoring at perinuclear sites mediated by the yeast yKu70–yKu80 complex [8,9], which is a protein heterodimer involved in non-homologous DNA end-joining reactions as well as maintenance of proper telomere structure. This repression is caused by high local concentration of the Sir heterochromatic silencing proteins bound to telomeric sites. A search for yKu-binding proteins involved in nuclear envelope tethering resulted in the identification of Mlp1p and Mlp2p, which are extensions of the NPC [10]. Furthermore, tethering of a reporter gene to the nuclear envelope through a membrane-spanning protein results in silencing of the gene owing to the high local concentration of Sir proteins [11].

In the experiments carried out by Laemmli and coworkers, the same silencing mechanism is probably responsible for repression of the *ADE2* and *URA3* reporter genes in the absence of a functional boundary, and tethering to the NPC might specifically interfere with this silencing process. What is the mechanism whereby the boundary activity interferes with the propagation of the silencing activity from the adjacent *HML* E and I silencers? Since NPCs are located in the nuclear envelope, one might ask whether tethering to the nuclear periphery is sufficient for establishing boundary activity or whether the NPC plays a specific role in the process. Surprisingly, tethering of transgenes through Yif1–Gal4 and Yip1–Gal4 fusion proteins, which are proteins involved in ER-to-Golgi membrane transport that accumulate in the ER and the nuclear membrane and should target the reporter to the nuclear membrane [11], does not result in any boundary activity (Fig. 1) [5]. It thus appears as if only tethering to the NPC establishes a functional chromatin boundary and protects the reporter gene from heterochromatic repression.

Does tethering to the NPC always result in boundary activity? It has previously been shown that tethering of yeast telomeres to the nuclear envelope is mediated by binding of the telomeric chromatin to the NPC components Mlp1 and Mlp2, which in turn bind to Nup60 and Nup145 [12]. This NPC-mediated tethering, however, results in gene silencing instead of boundary activity, although in this case only one tethering site is present adjacent to the reporter gene. In addition, insulator activity mediated by transportins is Mlp1/Mlp2-independent [5], suggesting that simply tethering a gene to the NPC does not result in boundary activity and that NPC-mediated silencing follows a different biochemical path than that required to establish a boundary. The reason for this different behavior is unclear. One common feature of the transportins that establish boundary activity is that the interaction with their substrates is transient and highly dynamic. This feature is also a property of the nucleoporin Nup2p, which is the only nucleoporin that has a RanGTP-binding domain. Similarly to transportins, Nup2p shuttles back and forth between the nucleus and the cytoplasm to transport the Srp1/importin α in a RanGTP-dependent manner [13].

How does the NPC establish a boundary?

Previous results from *Drosophila* [4], as well as the new results from Laemmli and colleagues, suggest that tethering to a perinuclear compartment is required for the establishment of boundary activity. The yeast results actually suggest that the tethering site is quite specific as targeting to the NPC, but not the nuclear envelope, is required for the formation of a boundary. One possible explanation for the involvement of the NPC in boundary activity is that factors that suppress Sir-mediated silencing being imported into the nucleus through the NPC create an environment that antagonizes repression and allows transcription. This explanation is, however, at odds with the observation that Mlp-mediated NPC tethering does not result in boundary activity [5]. In addition, it is difficult to conceive that only factors that antagonize Sir-mediated silencing are preferentially transported or accumulate at the NPC. Finally, in the experimental set-up described by Ishii *et al.*, the local pools of these putative chromatin-opening factors should be very similar around the insulated *ADE2* and the repressed adjacent *URA3* genes.

An alternative explanation is that the interaction of the Gal4–transportin fusion proteins with the NPC creates a domain topologically isolated from the adjacent silenced chromatin. It has been shown previously that silencing at the *HML* locus causes the chromatin to assume a distinct topology resulting from an increase in negative supercoiling of the DNA [14]. It is possible that the establishment of a boundary through the interactions of transportins with the NPC interferes with the propagation of these changes in DNA topology. If this is the case, the nature of the interaction between proteins with boundary activity and the NPC must have special characteristics that allow the establishment of the boundary as Mlp1-mediated targeting does not result in this activity. Intuitively, one would expect that a very strong interaction would be required to interfere with the propagation of Sir-induced changes in DNA topology. Surprisingly, in this context is the observation that the proteins identified as conferring boundary activity, by the nature of their normal function in nuclear transport, only interact transiently with the NPC. Perhaps it is the very transient nature of this interaction that, by mechanisms that we do not yet understand, is at the heart of boundary function.

Beyond yeast: mechanisms of insulator function in higher eukaryotes

An important question elicited by the work of Laemmli and colleagues is whether the mechanism of boundary and insulator function involves tethering to NPCs in *Drosophila* and vertebrates too. A general role for the NPC in establishing chromatin boundaries would have interesting consequences as it would ensure that transcriptionally active genes would be located close to nuclear sites that facilitate export of the newly transcribed RNAs to the cytoplasm. While NPC-tethering represents a possible gene insulating mechanism, other mechanisms obviously coexist – as the insulator activity mediated by Gal4 fused to BEAF-32, a *Drosophila* insulator binding protein, is Nup2p independent [5]. Unlike yeast, the internal side of the nuclear envelope of higher-eukaryotic cells is surrounded by a filamentous meshwork that forms the nuclear lamina. This structure consists of nuclear lamins and a number of integral and peripheral membrane proteins, such as the lamin B receptor (LBR), lamina-associated polypeptide 1 (LAP1), LAP2, Tpr, emerin and barrier to autointegration factor (BAF). The nuclear lamina is part of the framework referred to as the nuclear matrix, which might

serve as a foundation on which many nuclear activities, such as RNA transcription, DNA replication and the establishment of higher-order chromatin structure, are organized. Some nuclear lamina proteins, such as lamin B, LAP2 and BAF, have been shown to bind to DNA [15], making this structure a potential docking site for chromatin and a good candidate to mediate silencing and boundary activities. In fact, MARs/SARs, which have been shown to mediate the anchoring of the chromatin fiber to the nuclear scaffold or nuclear matrix, also play roles in transcription and, in some cases, contain insulators or boundary elements [16]. These results, together with the observation of gypsy insulator sites that aggregate into multi-insulator clusters at the nuclear periphery of *Drosophila* cells, suggest a role for the nuclear lamina in boundary function in higher eukaryotes, although a role for the NPC in this process is also possible.

Concluding remarks

The results of Laemmli and colleagues give credence to the idea of an involvement of the perinuclear compartment, and specifically the NPC, in the establishment of chromatin boundaries and nuclear organization. The model is attractive because it integrates mechanisms of gene regulation with nuclear geography, making chromatin regions primed for transcription easily accessible to factors being imported into the nucleus as well as ensuring the efficient transport to the cytoplasm of newly synthesized RNAs. The attractiveness of this model should be nevertheless tempered by the realization that, although components of the NPC complex might display boundary activity in an artificial assay, it does not necessarily follow that NPC proteins normally play this role in nuclear function. The results are nevertheless enticing and should stimulate the field to further test this possibility and determine whether NPC components are also important for boundary function in other organisms.

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References

1 Gerasimova, T.I. and Corces, V.G. (2001) Chromatin insulators and boundaries: effects on transcription and nuclear organization. *Annu. Rev. Genet.* 35, 193–208
2 West, A.G. *et al.* (2002) Insulators: many functions, many mechanisms. *Genes Dev.* 16, 271–288
3 Hart, C.M. and Laemmli, U.K. (1998) Facilitation of chromatin dynamics by SARs. *Curr. Opin. Genet. Dev.* 8, 519–525
4 Gerasimova, T.I. *et al.* (2000) A chromatin insulator determines the nuclear localization of DNA. *Mol. Cell* 6, 1025–1035
5 Ishii, K. *et al.* (2002) Chromatin boundaries in budding yeast: the nuclear pore connection. *Cell* 109, 551–562
6 Gorlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* 15, 607–660
7 Chook, Y.M. and Blobel, G. (2001) Karyopherins and nuclear import. *Curr. Opin. Struct. Biol.* 11, 703–715
8 Hediger, F. and Gasser, S.M. (2002) Nuclear organization and silencing: putting things in their place. *Nat. Cell Biol.* 4, E53–55
9 Cockell, M. and Gasser, S.M. (1999) Nuclear compartments and gene regulation. *Curr. Opin. Genet. Dev.* 9, 199–205
10 Galy, V. *et al.* (2000) Nuclear pore complexes in the organization of silent telomeric chromatin. *Nature* 403, 108–112
11 Andrulis, E.D. *et al.* (1998) Perinuclear localization of chromatin facilitates transcriptional silencing. *Nature* 394, 592–595
12 Feuerbach, F. *et al.* (2002) Nuclear architecture and spatial positioning help establish transcriptional states of telomeres in yeast. *Nat. Cell Biol.* 4, 214–221
13 Solsbacher, J. *et al.* (2000) Nup2p, a yeast nucleoporin, functions in bidirectional transport of importin alpha. *Mol. Cell. Biol.* 20, 8468–8479
14 Bi, X. and Broach, J.R. (1997) DNA in transcriptionally silent chromatin assumes a distinct topology that is sensitive to cell cycle progression. *Mol. Cell. Biol.* 17, 7077–7087
15 Vlcek, S. *et al.* (2001) Nuclear envelope and nuclear matrix: interactions and dynamics. *Cell. Mol. Life Sci.* 58, 1758–1765
16 Kalos, M. and Fournier, R.E. (1995) Position-independent transgene expression mediated by boundary elements from the apolipoprotein B chromatin domain. *Mol. Cell. Biol.* 15, 198–207

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Meeting Report

Making new connections

Shelley Halpain

A Keystone symposium held in Taos, New Mexico, 21–26 March 2002 provided the setting for a pioneering gathering of cell biologists and neuroscientists. Under the guidance of organizers Tom Pollard, James Sabry and Carla Schatz, the meeting, entitled 'Cellular Motility and Signaling in the Wiring and Plasticity of Nervous Systems', brought together two groups of researchers that ordinarily rarely connect at scientific conferences. The goal of this new collegial interchange was to fuse expertise on cytoskeletal dynamics with emerging ideas in neuronal development.

At the meeting, many neuroscientists received their first in-depth exposure to recent advances in the area of actin filament regulation. T. Pollard (New Haven, USA) set the scene by describing the central role of the Arp2/3 complex in cell motility [1] and explained how new findings on the structure of these proteins are yielding insights into the mechanisms for formation of actin filament branches at the leading edge of motile cells. Regulation of the Arp2/3 complex by WASP-family proteins is a key step, and M. Rosen (Dallas, USA) continued the structural theme with NMR-based studies of WASP complexed with the GTPase Cdc42 that provide novel insights into WASP signaling.

A new angle on branching

Actin filaments at the leading edge are branched at rather precise 70° angles, and G. Borisy (Chicago, USA) suggested that this angle might provide the most efficient protrusive force for leading edge advance. He presented a 'Darwinian' model of protrusive motility, wherein a self-organizing assembly of actin filaments is

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