Nucleoporins redistribute inside the nucleus after cell cycle arrest induced by histone deacetylases inhibition

Miguel Pérez-Garrastachu, Jon Arluzea, Ricardo Andrade, Alejandro Díez-Torre, Marta Urtizberea, Margarita Silioa, and Juan Aréchaga

Laboratory of Stem Cells, Development & Cancer, Department of Cell Biology and Histology, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Biscay, Spain; High Resolution and Analytical Biomedical Microscopy Core Facility, SGiker, University of the Basque Country (UPV/EHU), Leioa, Biscay, Spain

ABSTRACT
Nucleoporins are the main components of the nuclear-pore complex (NPC) and were initially considered as mere structural elements embedded in the nuclear envelope, being responsible for nucleocytoplasmic transport. Nevertheless, several recent scientific reports have revealed that some nucleoporins participate in nuclear processes such as transcription, replication, DNA repair and chromosome segregation. Thus, the interaction of NPCs with chromatin could modulate the distribution of chromosome territories relying on the epigenetic state of DNA. In particular, the nuclear basket proteins Tpr and Nup153, and the FG-nucleoporin Nup98 seem to play key roles in all these novel functions. In this work, histone deacetylase inhibitors (HDACi) were used to induce a hyperacetylated state of chromatin and the behavior of the mentioned nucleoporins was studied. Our results show that, after HDACi treatment, Tpr, Nup153 and Nup98 are translocated from the nuclear pore toward the interior of the cell nucleus, accumulating as intranuclear nucleoporin clusters. These transitory structures are highly dynamic, and are mainly present in the population of cells arrested at the G0/G1 phase of the cell cycle. Our results indicate that the redistribution of these nucleoporins from the nuclear envelope to the nuclear interior may be implicated in the early events of cell cycle initialization, particularly during the G1 phase transition.

KEYWORDS
cell cycle; HDAC inhibitors; nuclear-basket; nuclear pore complex; nucleoporin; nucleoskeleton; Nup62; Nup98; Nup153; Tpr

Introduction
The cell nucleus is the most prominent organelle of eukaryotic cells. It is surrounded by the nuclear envelope (NE), a double lipid bilayer membrane that isolates it from the cytoplasm. The inner nuclear membrane of the NE is underlayed by a proteinaceous component called the nuclear lamina and is seeded in multiple places by nuclear pore complexes (NPCs), which are big structures comprised of several copies of ~30 different subunits of nucleoporins.

The functional organization of the nucleus is based on the distribution of chromatin in specific regions of the nucleus, referred to as chromosome territories. The molecular mechanisms underlying this structural arrangement are starting to be understood and 2 models have been proposed: a deterministic model and a self-organization system. In this regard, large genomic regions associated with the nuclear lamina would be responsible for the functional organization of the nucleus. Additionally, other non-chromatin components could be responsible for this function in association with the lamina, by forming the nucleoskeleton. Although the NPC was initially defined as a nuclear envelope anchored structure whose only function was to allow trafficking between the nucleus and the cytoplasm, several studies have already proposed that nucleoporins are related to chromatin organization and might be transitory components of the nucleoskeleton. However, it has not been until recent years that, thanks to modern genomic and proteomic tools, the role of nucle-
oporins in nuclear organization is starting to be settled.\textsuperscript{16-18}

Nucleoporins can be classified into 2 main groups; core nucleoporins which from the basic framework and peripheral nucleoporins. The nucleoporins Tpr, Nup153, Nup98, Nup62 and Nup50 interact with genomic components inside the nucleoplasm, as these nucleoporins have 2 dynamic populations: one which may be anchored to the NPC and another one shuttling between the NPC and the nuclear interior.\textsuperscript{19-24} Moreover, this interaction is associated with genes of active transcription and enriched in active histone marks related to transcriptional stimulation.\textsuperscript{25,26} Core nucleoporins seem to be less associated with chromatin, and this interaction is usually associated with the repression of transcription, as is the case of Nup93\textsuperscript{27} and Nup88\textsuperscript{24}. However, the interaction of specific nucleoporins with chromatin seems to depend on the cell type or the organism,\textsuperscript{28} like mammalian Nup93 and its yeast analog Nic96, which are associated with silenced and transcribed DNA, respectively.\textsuperscript{27,29} Likewise, Nup153 would bind actively transcribed genes in Drosophila\textsuperscript{30,26} while, in human stem cells, it would silence the transcription of developmental genes.\textsuperscript{31} All these apparent contradictions point to a complex regulation of nucleoporin movement through the nuclear interior, which would be related to their active role in the modulation of chromatin organization.

Histone deacetylase inhibitors (HDACi) are known to induce global histone acetylation by inhibiting class I and class II HDACs.\textsuperscript{32,33} There are multiple effects of HDAC inhibitors, as they can trigger cell cycle arrest and inhibition of proliferation, differentiation processes, autophagy, apoptosis, chromatin remodeling and genomic reorganization.\textsuperscript{33-37} Thus, HDACi are a proper tool to study the relationship between the acetyl epigenetic hallmark and nucleoporins. Previous studies have reported that Nup93 associates with actively transcribed regions at the NPC in response to histone hyperacetylation.\textsuperscript{27} As Nup93 is a member of the NPC core, we wondered what would happen with nucleoporins from the nuclear-pore basket in a similar scenario. To assess this question, we submitted human neuroblastoma cells and other human cell lines to global histone deacetylase inhibition, and analyzed the changes in the distribution pattern or in the dynamics of the basket-related nucleoporins Nup153, Tpr and the shuttling FG-nucleoporin Nup98. Our results show that, after exposure to HDACi, there is a redistribution of these nucleoporins to intranuclear clusters, which appears to be related to the blocking effect of HDACi-related drugs at the G0/G1 transition of the cell cycle.

**Results**

**Nucleoporins from the nuclear basket of the nuclear pore complex relocate to intranuclear clusters after HDACi treatment**

We observed by means of confocal microscopy that several protein components of the NPC basket relocate from the nuclear envelope to intranuclear aggregates after treatment with Trichostatin A (TSA), a potent HDACi. As shown in Fig. 1, the nucleoporins Nup98, Nup153 and Tpr exhibit a common nuclear envelope ring staining pattern in untreated neuroblastoma SMS-KCNR cells. These cells commonly exhibit one or a few cytoplasmic invaginations of the nuclear envelope, which project into the nucleus, and are commonly observed as nuclear dots in confocal microscopy images. However, we found that treatment with 100 nM TSA for 24 h caused a marked accumulation of all 3 nucleoporins in several clusters inside the nucleus, which we named “intranuclear nucleoporin clusters” (INCs). We performed the same experiments using other HDAC inhibitors, and found that both sodium butyrate (Butyrate) and suberoylanilide hydroxamic acid (SAHA) had a similar effect on the generation of INCs (Fig. 1A). Notably, the fluorescence intensity of all these nucleoporins at the nuclear envelope was lower in the nuclei containing INCs than in the nuclei where they were absent (Fig. 1B). This phenomenon is not an artifact of the immunostaining procedure, since it was also observed when the same experiments were performed using living cells stably expressing the fluorescent recombinant protein Nup153-GFP (Fig. 1C and supplementary video 1).

To exclude the possibility that this effect was specific to the SNS-KCNR cell line, we repeated the same experiments using different human cell lines. After 24 h treatment with HDACi, INCs could also be observed in cell lines of different origin such as breast carcinoma (MCF7/6), cervix carcinoma (HeLa), melanoma (A375) and tumor associated myofibroblasts (CT5.3 hTERT) (see Supplementary Fig. 1). Thus, the intranuclear accumulation of nuclear basket nucleoporins seems to occur...
in a wide variety of cells from tissues of different origin, following exposure to HDAC inhibitors.

*Intranuclear nucleoporin clusters are not associated with nuclear envelope invaginations*

As mentioned before, SMS-KCNR cell line nuclei have large nuclear envelope invaginations with NPCs spanning through the nuclear interior (Fig. 2). To exclude the possibility that the INCs were NPC accumulations in nuclear envelope foldings, we examined the coincidence of the concanavalin A lectin and nuclear basket nucleoporin labeling at both a microscopic and ultrastructural level using confocal microscopy and TEM, respectively. We did not find concanavalin A in the INCs where Tpr was accumulated (Fig. 2A). Similar results were obtained with Nup153 and Nup98 (data not shown). These double-labeling studies also allowed us to observe the differences in the pattern of staining between concanavalin A and nuclear basket nucleoporins. Thus, whereas lectin staining was continuous and located at the outer part of the nuclear envelope, Tpr staining exhibited the archetypal nucleoporin punctuate pattern, which was slightly located further toward the nuclear interior, as it is a nuclear basket component (Fig. 2A). Interestingly, double labeling of the nucleoporin Nup62 and Tpr revealed that the central plug nucleoporin Nup62 did not
redistribute to the INCs after TSA treatment (Fig. 2B). TEM images showed that, although chromatin was more condensed at patches inside the nuclei of TSA treated cells, there were not significant modifications at the nuclear envelope level (Fig. 2C and supplementary Fig. 5). These results indicate that after HDACi treatment, drastic nuclear envelope alterations do not occur and that INCs are not conditioned by nuclear envelope invaginations.

**Nup98, Nup153 and Tpr colocalize at intranuclear nucleoporin clusters**

After the observation that only some of the nuclear envelope components accumulated at the nuclear interior after HDACi treatment, we wondered if they all concentrated at the same intranuclear clusters. Double colocalization studies confirmed that the nucleoporins Nup98, Nup153 and Tpr redistributed from the nuclear side of the NPC to the same INCs (Fig. 3A). Additionally, super-resolution confocal microscopy allowed us to discriminate slight differences in the localization of Nup62, Nup153 and Tpr. Our experiments confirm previous studies\(^\text{38-40}\) that, although both Tpr and Nup153 appear at the nuclear basket, Tpr is located slightly deeper inside the nucleus than Nup153. However, no clear structure or specific distribution of nucleoporins could be distinguished at INCs (Fig. 3B). The known relative position of the nucleoporins Tpr, Nup153 and Nup62 at the NPC was also confirmed by super-resolution confocal microscopy (supplementary Fig. 2).

**Intranuclear nucleoporin clusters are present only in G0/G1 cell cycle arrested cells**

One evident observation was that INCs did not appear in all cultured cells after exposure to HDACi (Fig. 4A). In fact, they seemed to coexist with a second population of larger cells with bigger nuclei, in which INCs were absent. These large cells lacking the intranuclear clusters exhibited a brighter rim staining of the nuclear envelope when compared with the smaller nuclei containing INCs. As HDACi are known to induce cell cycle arrest, we wondered if the presence of these 2 cell populations could be related to their arrest in 2 different phases of the cell cycle.
To assess this question, we performed cell cycle analysis by flow cytometry and compared the results with systematic counting of nuclei containing INCs from confocal microscopy images accompanied with nuclear area measurement. First, we constructed a dose-response curve for TSA, sodium butyrate and SAHA (supplementary Fig. 3) and found that SAHA and TSA had a dual effect on the cell cycle, depending on the concentration used. At lower concentrations (100 nM TSA or 2 μM SAHA), most of the population was arrested in G0/G1 (87% and 67% of cell population, respectively). However, at higher concentrations (250 nM TSA and 4 μM SAHA), 2 different populations coexisted, with some cells arrested in G0/G1 (42% and 43% respectively) and another population

**Figure 3.** Colocalization experiments of Nup98, Nup153 and Tpr at the INCs of 100 nM TSA treated cells. (A) Deconvoluted images from structured illumination microscopy show that the 3 studied proteins colocalize at the INCs. Scale bar: 10 μm. (B) Super-resolution images of 100 nM TSA treated cells, double-labeled for Nup153 and Tpr show colocalization of both nucleoporins at INCs. At the nuclear envelope, the inner localization toward the nuclear interior of Tpr compared with the nuclear basket Nup153 can be detected (the region of interest exhibits an INC and a fragment of nuclear envelope). Scale bar: 4 μm. See supplementary Figure 2 for more information.
blocked at G2/M (57% and 52% respectively) (Fig. 4B and supplementary Figure 4).

Accordingly, we counted the number of nuclei containing INCs in immunofluorescence confocal microscopy images and found that the results correlated with those obtained by flow cytometry. Thus, after treatment at the lower concentration of 100 nM TSA, there was a much higher percentage of cells containing INCs (80%) than when cells were exposed to the higher concentration of 250 nM TSA, when only 34% of the cell population exhibited INCs. We noticed that the percentage of INC positive nuclei was similar to the population arrested at the G0/G1 phase. Moreover, there was also a clear relationship between the presence of INCs and the size of the nucleus, as the majority of the aggregates were only present in small nuclei and were absent from the large nuclei population (Fig. 4C). A similar increase in the number of INC containing nuclei was also observed at the lower concentration of SAHA, together with an increase in the proportion of large cells without nuclear clusters at a higher SAHA concentration (supplementary Fig. 4C).

Considering these 3 parameters as a whole, one can presume that the accumulation of nuclear basket nucleoporins in the nuclear interior seems to be related to the cell cycle arrest in G0/G1, which is elicited by a specific concentration of HDACi inhibitors.

To confirm that the presence of INCs is associated to the G0/G1 phase of the cell cycle, we performed an
additional experiment using the cell cycle phase marker Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI), which reveals the phase of the cell cycle by expressing 2 recombinant proteins, one encoding a GFP tagged protein which is only expressed during S, M and G2 phases, and a RFP tagged protein for the G1 phase (see Fig. 5 B and materials and methods). The experiments were performed by transfecting cells with FUCCI and treating them with SAHA at 2 \( \mu M \) and 4 \( \mu M \). As FUCCI shows fluorescence in green and red, we immunodetected Nup153 with a Cy5 coupled secondary antibody (far red) to unravel the presence of INCs.

We analyzed and classified the cells depending on 3 parameters: the frequency of cells with nuclei presenting INCs, their respective nuclear area, and their phase of the cell cycle revealed by FUCCI (Fig. 5). Thus, we observed 3 populations of cells:

A first group of cells with large nuclei, high levels of Nup153 at the NE and absence of INCs, which mostly expressed the green fluorescent tag (S, G2 and M). A second set of cells expressing red or both green and red cell cycle markers, in which the proportion of cells containing INCs inside their nuclei was variable. Interestingly, the third population comprised cells with small nuclei, which had INCs and did not express any of the cell cycle proteins, indicative that these cells were at early G1 phase. Moreover, the proportion of each population was dependent on the concentration of the drug. At low concentration (2 \( \mu M \) SAHA), there was a high number of cells with small nuclei with INCs which were at G1, and a scarce number of large cells without INCs at S/G2/M (Fig. 5). However, at a higher concentration (4 \( \mu M \) SAHA), the proportion of each population was inverted, with many large cells without INCs at S/G2/M (Fig. 5C). Taken together, these results claim that INCs appear in small nuclei arrested in G0/G1 phase, while cells in the G2 phase do not show INCs in their nuclei.

**Chromatin hyper-acetylation is needed for intranuclear nucleoporin cluster formation**

After finding a relationship between cell cycle arrest in G0/G1 and the presence of INCs, we questioned whether this effect was dependent on chromatin hyper-acetylation or a consequence of cell cycle blockage. We exposed cells to L-mimosine or mitomycin C, two well-known cell cycle disruptors with a mechanism of action different to that of HDACi. L-mimosine is an Iron/Zinc chelator which causes iron depletion leading to the activation of DNA double strand break and DNA damage response machinery, impeding DNA replication. Mitomycin-C binds DNA leading to its cross-linking and thus inhibiting DNA synthesis and function. As expected, we observed that after 24 h treatment with these compounds, both of them exerted a drastic cell cycle arrest (Fig. 6A). Interestingly, none of them showed intranuclear clusters for any of the studied nucleoporins (Fig. 6B). These results lead us to conclude that INC formation is not only related to G1 cell cycle arrest, but that it is also mediated by the chromatin hyper-acetylation status exerted by HDACi.

**HDACi treatment generates intranuclear nucleoporin accumulation but it does not increase its mRNA expression levels**

In our immunofluorescence experiments, we observed a very high intensity labeling of nucleoporins in INCs (Figs. 1B, 1C, 2B), which might be caused by the redistribution of existing nucleoporins or by their overexpression after transcription, leading to this intranuclear accumulation. Western blotting experiments revealed that, when cells were treated with TSA, the total amount of Tpr, Nup153 and Nup98 protein levels increased (Fig. 7A). However, levels of the central plug nucleoporin Nup62, which was not relocated to INCs after HDACi treatment (Fig. 2B), diminishes slightly after TSA treatment. The effect of TSA treatment on chromatin hyper-acetylation was observed by the vast increase of acetyl histone 3 (Fig. 7A). Neither the levels of tubulin nor the levels of transcription (RNA pol II CTDSer5P) or replication (PCNA) related proteins were altered by this HDACi.

After detecting an increase in the protein levels of Nup153, Tpr and Nup98 in TSA treated cultures, we performed quantitative RT-PCR to check if there was a substantial change at the mRNA level. Surprisingly, mRNA levels of all 3 nucleoporins did not increase, but rather, were slightly downregulated (Fig. 7B). These results show a contradictory effect of TSA, which appears to increase the level of nucleoporins, causing them to accumulate at nuclear foci, but also block their expression at the transcription level.

**Nucleoporin clusters are not associated with transcription or replication foci**

DNA transcription and replication take place in specialized areas inside the nucleus, known as...
Figure 5. Presence or absence of INCs in relation to the nuclear size and the phase of the cell cycle after treatment with HDACi. FUCCI transfected cells were treated with a low (2 \( \mu \text{M} \)) or high (4 \( \mu \text{M} \)) concentration of SAHA and immunostained for Nup153. The red construct (Cdt1-RFP) is expressed only in cells in the G0 and G1 phase of the cell cycle, whereas the green construct (Geminin-EGFP) is present during the S, G2 and M phases of the cell cycle. Colorless nuclei correspond to cells in early G1, which are starting to synthesize Cdt1-RFP. Yellow nuclei belong to cells at the G1/S transition, when Cdt1-RFP is starting to be degraded while Geminin-EGFP is already present.

(A) Representative field of FUCCI transfected cells after fixation and immunodetection of Nup153. Scale bar: 10 \( \mu \text{m} \). (B) Frequency histograms displaying the proportion of cells containing INCs in relation to their nuclear size or their phase of the cell cycle. (C) Percentage of cells at each phase of the cell cycle and presence or absence of INCs after exposure to a low or high concentration of SAHA. \( \Sigma \) refers to the combination of early G1, G0+G1 and G1/S FUCCI indicators.
Figure 6. (A) Cell cycle histograms obtained by flow cytometry showing the arresting effect of L-mimosine and mitomycin C. L-mimosine (400 μM) blocked 76% of cells at the G0/G1 phase while mitomycin-C (10 μg/mL) arrested them in the S phase (43.15%). Y-axis: cell frequency. X-axis: propidium iodide fluorescence intensity. (B) Immunolocalization of Nup98, Nup153 and Tpr by confocal microscopy showed that after treatment with both antimitotic drugs, none of the nucleoporins accumulated in aggregates at the nuclear interior.
transcription factories and replication foci. Both processes might be altered when HDACi are present, due to its chromatin decondensation properties and cell cycle arrest effect. As some nucleoporins are proposed to interact with active transcribed chromatin and assist DNA replication, we hypothesized that INCs might be enriched in markers of such nuclear structures. Thus, we performed double immuno-fluorescence experiments of Nup153 with PCNA or RNApol II CTDSer5P in control and TSA100 nM treated cells, in which most cells are arrested at G0/G1. As stated above, the amount of PCNA protein did not change as a result of TSA treatment (Fig. 7A). By confocal microscopy, PCNA label in TSA treated cells exhibited a diffuse pattern, typical for G0/G1 cells. As expected, Nup153 formed INCs, but they did not colocalize with any appreciable structure of PCNA. Similar results were obtained after double staining of Nup153 with RNApol II Ser5P. In both control and treated cells, transcription foci could be observed as small and intense dots, but we did not observe any colocalization with Nup153 INCs (Fig. 8B).

The mobility of Nup153 at the nuclear envelope and in INCs is reduced after treatment with HDACi

To assess if the dynamics of Nup153 was different in HDACi treated cells, we performed FRAP experiments in living cells stably expressing Nup153-GFP, both for control cells and after treatment with 100 nM TSA for 24. In control cells, only a region of the nuclear envelope was bleached (Fig. 9). In TSA treated cells, a region of the nuclear envelope (NE) was bleached and, right afterwards, an INC inside the very same cell was bleached, so that the mobility of Nup153 in both compartments could be compared. Inverting the order of bleaching (first an INC and then the NE) had no effect on the dynamics of Nup153 (data not shown). Our results show that Nup153 in control cells recovered rapidly after bleaching (Fig. 9B), as has been described previously. However, the recovery dynamics of Nup153 after TSA treatment was significantly slower than in control cells, taking longer to stabilize than in control cells. The reduction in the mobility of Nup153 was independent of the bleached region, as the recovery curves for INCs and NE were indistinguishable. These results
suggest that treatment with TSA induces a change in the mobility of Nup153 by its interaction with non-soluble components inside the nucleus.

Discussion

We have observed an intranuclear accumulation of nuclear pore basket related nucleoporins in intense and numerous foci (which we called INCs) when chromatin is hyperacetylated by the action of HDACi. To our knowledge, the only nucleoporin reported to form similar intranuclear bodies is Nup98, which composes the Gly-Leu-Phe-Gly (GLFG) repeat intranuclear bodies. The colocalization of the 3 studied nucleoporins in INCs is not surprising, because Tpr has been demonstrated to interact with Nup153\(^{52}\) and with Nup98.\(^{53}\) In a similar manner, Nup153 and Nup98 are both Phe and Gly (FG) repeat nucleoporins known to shuttle between the NPC and the nucleoplasm, mediating in common transcription processes,\(^{23}\) although their intranuclear dynamics differ significantly.\(^{21}\) The differences in Nup153 mobility observed by photobleaching experiments points to the existence of 2 populations of this nucleoporin, which would explain its differential localization.\(^{21}\) Tpr is also found inside the nucleus, where it forms long filaments generating

Figure 8. Double immunolocalization of Nup153-PCNA and Nup153-RNApol II Ser5P. PCNA accumulates in replication foci in S cells (arrows) or diffused in G1 cells (arrowhead), but it is not present at Nup153 intranuclear clusters after exposure to 100 nM TSA. Likewise, active RNA polymerase II (RNApol II Ser5P) is located in transcription factories in control cultures (arrows) and diffuses throughout the nucleus after 100 nM TSA. Scale bar: 10 μm.
heterochromatin exclusion zones. In Drosophila and yeast, Nup153, Nup98, the Tpr analog Megator, and the myosin-like proteins Mlp-1 and Mlp-2 are all associated with regions of active transcription, especially in genomic regions implicated in development and cell cycle. These findings suggest these 3 nucleoporins play an active role in DNA transcription and chromatin organization, apart from their role in nucleocytoplasmic transport. In this context, epigenetic hallmarks are key players of nucleoporin-chromatin interaction, but the molecular mechanisms by which this happens remain obscure, although there is evidence that chromatin remodeling factors such as CREB binding protein, HDAC1, SAGA and TREX-2 interact with nucleoporins, especially the ones with FG repeats. We have not been able to identify the molecular mechanisms by means of which the 3 studied nucleoporins form the reported INCs. However, HDAC inhibitors could be an experimental model to study epigenetic hallmarks mediating interactions between nucleoporins and chromatin.

Significantly, Nup62 does not appear in INCs. Nup62 is a protein of the central plug of NPCs and behaves differently from the other studied nucleoporins, as it has a much lower turn-over and less mobility capacities. However, this is not the case in Drosophila, where it has been reported to coexist inside the nucleoplasm with Nup50 and Nup98. Nup93, a nucleoporin which is not located at the nuclear pore basket, has been reported to remain at the NPC after HDAC inhibition by TSA. Regarding other core proteins such as the 107–160 complex (Y complex), it has recently been reported that it forms intranuclear bodies inside the nucleoplasm. This puzzle suggests that only some nucleoporins are accumulated in the nuclear interior and that this capacity is dependent, at least, on HDAC activity inhibition.

We have detected an interesting correlation between the presence of INCs and the state of the cell cycle, with intranuclear clusters only evident during the G1 phase. On the other hand, cells in G2 are larger and have a high accumulation of nucleoporin labeling restricted to the nuclear envelope. During G1, biogenesis of NPC components is taking place, a process regulated by cyclin-dependent kinases (CDKs) and inhibited by the CDK inhibitor roscovitine. It is feasible that, after HDACi treatment, NPC biogenesis may be inhibited and, therefore, NPC components would accumulate in the nuclear interior forming INCs. This hypothesis is supported by the fact that L-mimosine treatment, which does not affect cyclin-dependent kinase (Cdk) levels, does not induce the formation of INCs. HDAC inhibitors are reported to arrest cell cycle both at G1/S and G2/M, in agreement with our results. By using different concentrations of each drug, we have been able to alter the proportion of cells in each phase of the cell cycle, which has allowed us to study the dynamics of the intranuclear accumulation of NPC components during HDAC inhibition. Thus, we have observed that it is restricted to the G1 phase of the cell cycle. It has been reported that the HDACi blocking effect at G1/S takes place via CDK inhibition through overexpression of p21, but the G2/M mechanisms remain unidentified.

Figure 9. Quantitative FRAP analysis of Nup153-EGFP in stably transfected SMS-KCNR cells (A) Representative samples of control and 100 nM TSA treated cells are shown. In cells exposed to TSA, both the nuclear envelope (NE) and an Intranuclear Nup Cluster (INC) of the same cell were consecutively bleached. The bleached area is indicated by a rectangle. Scale bar: 10 μm. (B) Average normalized fluorescence signals with their corresponding standard deviations of control nuclear envelopes (n = 34) compared with the nuclear envelopes and INCs of 100 nM TSA treated cells (n = 32).
We believe that our experimental system could provide tools to analyze the relationship between HDACi, cell cycle and NPC dynamics at the molecular level.

Our Western blotting experiments indicate that the protein levels of Nup153, Nup98 and Tpr increase significantly after HDACi exposure. Surprisingly, we also found that the corresponding gene expression was slightly downregulated for all 3 nucleoporins. A possible explanation for this phenomenon is that HDACi treatment impedes nucleoporin degradation. Recent work has demonstrated that the SUMO degradation pathway is implicated in nucleoporin homeostasis through the activity of the SUMO-specific proteases SENP-1 and SENP-2.\textsuperscript{67} In the mentioned study, knockdown of SENP-1 and SENP-2 resulted in lower protein levels of Nup98 and Nup153, while Nup62 levels remained unaltered. Our Western blotting results show that the levels of Nup98, Nup153 and Tpr, which are present at INCs, are increased. On the other hand, Nup62, which is absent from these structures, remains constant. Taken together, these results raise the possibility that a nucleoporin degradation pathway may be inhibited via HDAC inhibition through SENP-1 SENP2 stimulation. Further experiments will determine the role of post-translational modification of these nucleoporins in relation to their intranuclear dynamics. In this context, it is known that Tpr is a sumoylation substrate.\textsuperscript{68} Along with ubiquitination and sumoylation, other post-translational modifications such as phosphorylation and glycosylation of NPC components are being investigated as regulators of nucleoporin functions.\textsuperscript{69-73}

Indeed, recent studies have demonstrated that S2094-Tpr phosphorylation regulates its location to the nucleolus,\textsuperscript{72} and ubiquitination of the yeast nuclear pore basket proteins Nup60 and Nup2 governs their dynamic association with the NPC.\textsuperscript{69} Thus, HDACi treatment may be able to alter post-translational modifications of Nup98, Nup153 and Tpr.

We have demonstrated here that INCs are not related to drastic alterations of the nuclear envelope structure. This aspect is important, as the overall nuclear shape is not greatly modified after HDAC inhibition. However, it has been reported that deregulation of Nup153 protein levels, by knockdown or overexpression, leads to nuclear shape alteration.\textsuperscript{73-75} Nup153 has 2 well-known populations:\textsuperscript{76} one is the NPC-anchored population and the other consists of these proteins shuttling between the NPC and nucleoplasm. In our immunolocalization experiments, it is evident that after HDACi treatment, Nup153 signal (and Tpr and Nup98 as well) was greatly reduced at the nuclear envelope but very intensified in the INCs. We propose that after HDAC inhibition, the population of Nup153 related to the NPC is moved toward the nuclear interior. As Tpr requires Nup153 for NPC anchoring,\textsuperscript{77} their interaction would bring along Tpr to the nucleoplasm. Nup98, as a \textit{bona fide} Nup153 and Tpr interacting protein, could also be driven to the INCs.\textsuperscript{53} Finally, Nup153, Tpr and Nup98 would be accumulated by inhibition of their degradation pathway. According to this proposed model, only accumulation of the NPC-bound Nup153 or its full depletion would induce nuclear envelope aberration. It has recently been demonstrated\textsuperscript{78} that after TSA treatment in immortalized mouse embryonic fibroblasts, internal Lamin A structures were surrounded by heterochromatin protein 1 clusters. Our results differ slightly from this observation, as we did not observe any alteration of nuclear envelope invagination, although it is possible that internal lamins were not associated with the nuclear envelope, forming part of a proposed higher structure called the nucleoskeleton\textsuperscript{11,79}.

By FRAP experiments, we have observed that the mobility of Nup153 when it is located at the NE or at INCs was similar after HDACi treatment. However, the dynamics of this nucleoporin in treated cells was significantly different than in control cells. Thus, Nup153 in control cells was more soluble than in treated cells, indicating that Nup153 might be interacting with nuclear components that do not diffuse freely throughout the nucleoplasm. Previous analysis of Nup153 dynamics has reported that this nucleoporin is more mobile than its NPC core partners.\textsuperscript{20} Moreover, the mobility of Nup98 and Nup107 is also different depending on their localization at the NPC or at intranuclear bodies. Thus, the intranuclear pool of Nup98 and Nup107 forming aggregates recovered quicker than its NPC bound counterparts, suggesting that the nuclear aggregates were in a quicker interchange with the nucleoplasmic soluble pool.\textsuperscript{22,62} Surprisingly, in our experiments, photobleaching of Nup153 located in INCs or at the NE recovered similarly, much slower than Nup153 in non treated cells. This phenomenon might be explained by the fact that, after TSA treatment, the nuclear envelope signal decreased, suggesting that the soluble fraction of
Nup153 trafficking from the NE though the nucleoplasm would transform into a static one inside the INC. The alteration in its behavior might be due to chromatin hyperacetylation or by a Nup153 posttranslational modification induced by HDACi, which would alter its mobility by its interaction with insoluble components or chromatin associated complexes. Future studies will allow us to decipher which are the epigenetic marks that exert such an influence in Nup153 dynamics.

Although the mechanisms which govern the nucleoplasmic pool of nucleoporins seem to be complex, we hypothesize that histone hyperacetylation caused by HDAC inhibitors alters the dynamic equilibrium of nucleoporins toward the chromatin interacting regions, enriched in acetylated epigenetic hallmarks, resulting in INC formation. The mechanism by which this is driven would implicate the recruitment of chromatin remodeling factors, such as CBP (CREB binding protein), or the accumulation of nucleoporins due to an inhibition of their degradation pathways accompanied by Cdk inhibition. The interesting fact that there is a relationship between the cell cycle state and the presence of intranuclear nucleoporin clusters adds new perspectives about the role of NPC components in cellular functions other than the established model for transport across the nuclear envelope.

**Materials and methods**

**Cell culture and HDAC inhibition**

SMS-KCNR, HeLa, MCF7/6, CT5.3hTERT and A375 cells were cultured in DMEM (Lonza BE12–733F) supplemented with 1%L-Glutamine (Sigma-Aldrich G7513), 1%Penicillin-Streptomycin (Sigma-Aldrich P0781) and 10%Fetal Bovine Serum (FBS) (HyClone SV30160.03) at 37°C and 5%CO2. All HDAC inhibitors (Trichostatin A: Sigma-Aldrich T1952, sodium butyrate: Sigma-Aldrich B5887 and suberoylanilide hydroxamic (SAHA, Vorinostat, Selleckchem S1047) were added to the culture medium at the appropriate concentration and mixed before addition to the cell culture. The HDAC inhibition was done during 24 hours.

**Cell cycle analysis by flow cytometry**

After HDACi treatment, cells were collected by trypsinization, fixed in EtOH 70% during 1 h at 4°C, washed with PBS and finally resuspended to a final concentration of 10^6 cells/mL. Cell suspension was incubated with propidium iodide (20 μg/mL) and RNase A (250 μg/mL) during 1 h at 4°C in the dark. Cells were analyzed in a Beckman Coulter Galios flow cytometer.

**Immunocytochemistry**

50,000 cells were seeded on 12 mm glass coverslips coated with gelatin during 1 hour in a P24 plate. After TSA treatment, cells were fixed in 4% paraformaldehyde (20 min room temperature), permeabilized with 0.2% Triton-X100 (10 min room temperature), blocked in 10% FBS (2 h room temperature) and incubated with the appropriate antibodies dissolved in 5% FBS overnight at 4°C. Secondary antibodies were incubated during 2 h at room temperature in the dark. Coverslips were mounted in Fluoromount G (Southern Biotech 0100–01) and sealed with nail polish. As indicated for each image, confocal microscopy was performed using the following equipment: Zeiss Axiobserver fluorescence microscope with structured illumination (Zeiss Apotome 2) and Leica TCS SP2 AOBS (for live and FRAP analysis of Nup153-GFP expressing cells). Super-resolution imaging was performed with a Zeiss LSM880 Airyscan (BioCruces Health Research Institute), an Olympus FV3000 OSR, a Leica TCS SP8 Hyvolution or a stimulated emission depletion microscope, Leica TCS STED CW SP8 (Achucarro Basque Center for Neuroscience). In all double labeling experiments, sequential acquisition was used to avoid signal crosstalk. Sampling was always set to fulfill Nyquist criterion and the best available optics (maximum numerical aperture and optical corrections) were used. Image processing and cell counting: fluorescence intensity profiles were constructed exporting raw fluorescence intensity data to a spreadsheet file. INC counting and nuclear area measurement was performed using ImageJ/FIJI software, applying the same threshold values in images taken under the same acquisition conditions.

**Antibodies**

Tpr 1:1000 (Abcam ab84516), Nup153 1:1000 (Abcam ab96462), Nup98 1:1000 (Sigma N1038), Nup62 1:100 (Transduction Laboratories N43620), Acety-L-Histone H3 1:2000 (Millipore 06–599), α-Tubulin 1:4000 (Sigma T5168), PCNA 1:600 (Chromotek 16D10), RNApol II CTDSer5P 1:1000 (Chromotek 3E8).
**Secondary antibodies**

- anti-mouse alexa555 1:2000 (Molecular Probes A31570), anti-rat alexa488 1:2000 (Molecular Probes A11006), anti-rabbit alexa488 1:2000 (Molecular Probes A21206), anti-mouse-Cy5 1:500 (Jackson ImmunoResearch 715-176-151), anti-mouse-HRP 1:4000 (Santa Cruz sc-2005), anti-rabbit-HRP 1:4000 (Santa Cruz sc-2030), anti-rat-HRP 1:4000

**Western blotting**

Twenty μg of whole cell lysate were loaded per lane of 10% and 7.5% PAGE and run in running buffer (25 mM Tris pH8.3; 192 mM glycine; 3.5 mM SDS). Proteins were transferred to nitrocellulose membranes in transfer buffer (25 mM Tris pH8.3; 192 mM glycine; 20% methanol; 3.5 mM SDS) during 4 h in a cold room at a constant current intensity of 600 mA and variable voltage (250V max) and power (600W max). Membrane blocking was done with 5% nonfat-dry milk dissolved in TBST (10 mM Tris-HCl pH8; 150 mM NaCl; 0.05% Tween20) or with 10% FBS dissolved in TBST for phosphorylated epitopes. Appropriate antibody incubation was done in blocking solution in a cold room with permanent agitation. Secondary antibodies were incubated in TBST during 2 h at room temperature in constant agitation, washed 3 times in TBST and developed using the Luminata Crescendo Western HRP Substrate (Millipore WBLUR0500). Western blotting images were taken in a Syngene G box imaging system.

**RT-PCR**

mRNA was extracted from cell cultures using the RNeasy Protect Mini Kit (Quiagen 74124) following the manufacturer instructions including the DNase I treatment step. 1 μg of mRNA was retrotranscribed using the iScript cDNA synthesis kit (BioRad 1708890). Power SYBR Green PCR Master Mix (Applied Biosystems 4367659) was mixed with 35 ng of cDNA and 500 nM of forward and reverse primers in a final volume of 15 μL. RT-PCR was carried on in an ABI Prism 7000 Sequence Detection System (Applied Biosystems). After checking that the dissociation curves had product specific peaks only, raw data was processed with the delta delta Ct method. Different calibrator genes were tested and the 2 most robust genes (PPIA and GAPDH) were selected using the normFinder plugin for spreadsheet files. Primers are listed in Supplementary table 1.

**Plasmids and stable transfection**

Full length Nup153-EGFP (pEGFP(C3)-Nup153 was a gift from Birthe Fahrenkrog (Addgene plasmid#64268). SMS-KCNR cells were transfected using Lipofectamine 3000 (L3000–008 Invitrogen) following manufacturer’s instructions. 48 h after transfection, cells were cultured in complete culture medium supplemented with G-418 (04727878001 Roche) at a concentration of 800 μg/mL. After 2 weeks of antibiotic selection, colonies expressing low amounts of plasmid were expanded using a limited dilution technique.

**Transmission electron microscopy**

After prefixation in glutaraldehyde 0.5%, cell cultures were scraped, pelleted and processed with a standard TEM protocol as described previously. Micrographs were obtained using an EM208S Philips TEM microscope.

**Live time lapse imaging**

Cells were seeded on 35 mm glass bottom dishes (Ibidi 81158). Control and HDACi treated cells were recorded in a Nikon Biostation IMq time lapse imaging system.

**Fluorescence recovery after photobleaching**

Live SMS-KCNR-Nup153EGFP cells were seeded on 35 mm imaging glass bottom dishes (Ibidi 81158) and were observed in a Leica TCS SP2 AOBS confocal microscope. FRAP experiments were done using the Leica imaging software assistant. FRAP data was analyzed using the Leica imaging software assistant and a calcl spreadsheet.

**Fluorescence ubiquitination cell cycle indicator (FUCCI) and nuclear size analysis**

50000 SMS-KCNR cells were seeded on 12 mm glass coverslips coated with gelatin during 1 hour in a P24 plate. When cells settled over the coverslips, 40 particles per cell of FUCCI (Molecular probes P36238) were added in a final volume of 500 μL of complete culture medium and incubated overnight. HDAC inhibitor was...
added and after 24 hours cells were processed for immunocytochemistry. Nuclear size calculations were made measuring the DAPI signal using ImageJ/FIJI software, applying the same threshold values in images taken under the same acquisition conditions.

**Abbreviations**

(CDK) cyclin-dependent kinase  
(FG) Phe and Gly  
(FRAP) fluorescence recovery after photobleaching  
(GLFG) Gly-Leu-Phe-Gly  
(HDACi) histone deacetylase inhibitor  
(INC) intranuclear nucleoporin cluster  
(NE) nuclear envelope  
(NPC) nuclear pore complex  
(STED) stimulated emission depletion  
(TEM) transmission electron microscopy  
(TSA) trichostatin A

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We appreciate assistance from Asier Ruiz and Laura Escobar in the acquisition of super resolution images with both STED and Hyvolution confocal microscopes. Critical reading and editorial assistance by Dr. David J. Fogarty and Mr. Juan Luis Vidaurrezaga were very much appreciated.

**Funding**

This work was supported by research grants from the Spanish Ministry of Economy and Competitiveness (SAF2012–39773), the regional Basque Government Research Groups Program (IT1125–16), University of the Basque Country Research Group Grant (GIU16/06) and the SAIOTEK Program (SOA11UN001) of the Basque Science, Technology & Innovation Network (SPRI) to Prof. J. Aréchaga. Miguel Pérez-Garrastachu has a PhD fellowship from the University of the Basque Country.

**ORCID**

Juan Aréchaga http://orcid.org/0000-0001-8356-3578

**References**

[1] Goldberg MW, Fiserova J, Huttenlauch I, Stick R. A new model for nuclear lamina organization. Biochem Soc Trans 2008; 36:1339-43; PMID:19021552; https://doi.org/10.1042/BST0361339

[2] Grossman E, Medalia O, Zwerger M. Functional architecture of the nuclear pore complex. Annu Rev Biophys 2012; 41:557-84; PMID:22577827; https://doi.org/10.1146/annurev-biophys-050111-102328

[3] Cronshaw JM, Krutchinsky AN, Zhang W, Chait BT, Matunis MJ. Proteomic analysis of the mammalian nuclear pore complex. J Cell Biol 2002; 158:915-27; PMID:12196509; https://doi.org/10.1083/jcb.200206106

[4] Cremer T, Cremer M. Chromosome territories. Cold Spring Harb Perspect Biol 2010; 2:a003889; PMID:20300217; https://doi.org/10.1010/cshperspect.a003889

[5] Branco MR, Pombo A. Intermingling of chromosome territories in interphase suggests role in translocations and transcription-dependent associations. PLoS Biol 2006; 4:780-8; https://doi.org/10.1371/journal.pbio.0040138

[6] Bickmore WA, van Steensel B. Genome Architecture: Domain Organization of Interphase Chromosomes. Cell 2013; 152:1270-84; PMID:23498936; https://doi.org/10.1016/j.cell.2013.02.001

[7] Finn EH, Misteli T, Shachar S, Beliveau BJ, Joyce EF, Apostolopoulos N, Yilmaz F, Fonseka CY, McCole RB, Chang Y, et al. Painting a Clearer Picture of Chromatin. Dev Cell 2016; 36:356-7; PMID:26906730; https://doi.org/10.1016/j.devcel.2016.02.002

[8] Misteli T. Beyond the sequence: cellular organization of genome function. Cell 2007; 128:787-800; PMID:17320514; https://doi.org/10.1016/j.cell.2007.01.028

[9] Simon DN, Wilson KL. The nucleoskeleton as a genome-associated dynamic “network of networks”. Nat Rev Mol Cell Biol 2011; 12:695-708; PMID:21971041; https://doi.org/10.1038/nrm3207

[10] Kind J, van Steensel B. Genome-nuclear lamina interactions and gene regulation. Curr Opin Cell Biol 2010; 22:320-5; PMID:20444586; https://doi.org/10.1016/j.ceb.2010.04.002

[11] Wente SR, Rout MP. The nuclear pore complex and nuclear transport. Cold Spring Harb Perspect Biol 2010; 2:a000562; PMID:20630994; https://doi.org/10.1010/cshperspect.a000562

[12] Blobel G. Gene gating: a hypothesis. Proc Natl Acad Sci U S A 1985; 82:8527-9; PMID:2866238; https://doi.org/10.1073/pnas.82.24.8527

[13] Arlucea J, Andrade R, Alonso R, Aréchaga J. The nuclear basket of the nuclear pore complex is part of a higher-order filamentous network that is related to chromatin. J Struct Biol 1998; 124:51-8; PMID:9931273; https://doi.org/10.1006/jsbi.1998.4054

[15] Chan JK, Park PC, De Boni U. Association of DNase sensitive chromatin domains with the nuclear periphery in 3T3 cells in vitro. Biochem Cell Biol 2000; 78:67-78; PMID:10874467; https://doi.org/10.1139/e09-074

[16] Sarma NJ, Willis K. The new nucleoporin: regulator of transcriptional repression and beyond. Nucleus 2012;
[17] Bukata L, Parker SL, D’Angelo MA. Nuclear pore complexes in the maintenance of genome integrity. Curr Opin Cell Biol 2013; 25:378-86; PMID:23567027; https://doi.org/10.1016/j.ceb.2013.03.002

[18] Ibarra A, Hetzer MW. Nuclear pore proteins and the control of genome functions. Genes Dev 2015; 29:337-49; PMID:25691464; https://doi.org/10.1101/gad.256945.114

[19] Daigle N, Beaudouin J, Hartnell L, Imreh G, Hallberg E, Lippincott-Schwartz J, Ellenberg J. Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. J Cell Biol 2001; 154:71-84; PMID:11448991; https://doi.org/10.1083/jcb.200101089

[20] Ellenberg J, Siggia ED, Moreira JE, Smith CL, Presley JF, Worman HJ, Lippincott-Schwartz J. Nuclear membrane dynamics and reassembly in living cells: Targeting of an inner nuclear membrane protein in interphase and mitosis. J Cell Biol 1997; 138:1193-206; PMID:9298976; https://doi.org/10.1083/jcb.138.6.1193

[21] Rabut G, Doye V, Ellenberg J. Mapping the dynamic organization of the nuclear pore complex inside single living cells. Nat Cell Biol 2004; 6:1114-21; PMID:15502822; https://doi.org/10.1038/ncb1184

[22] Grifﬁs ER, Altan N, Lippincott-Schwartz J, Powers MA. Nup98 is a mobile nucleoporin with transcription-dependent dynamics. Mol Biol Cell 2002; 13:1282-97; PMID:11950939; https://doi.org/10.1091/mbc.E03-10-0743

[23] Grifﬁs ER, Craige B, Dimanno C, Ullman KS, Powers MA. Distinct functional domains within nucleoporins Nup153 and Nup98 mediate transcription-dependent mobility. Mol Biol Cell 2004; 15:1991-2002; PMID:14718558; https://doi.org/10.1091/mbc.E03-10-0743

[24] Capelson M, Liang Y, Schulte R, Mair W, Wagner U, Hetzer MW. Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. Cell 2010; 140:372-83; PMID:20144761; https://doi.org/10.1016/j.cell.2009.12.054

[25] Kalverda B, Pickersgill H, Shloma V V, Fornerod M. Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleolus. Cell 2010; 140:360-71; PMID:20144760; https://doi.org/10.1016/j.cell.2010.01.011

[26] Vaquerizas JM, Suyama R, Kind J, Miura K, Luscombe NM, Akhtar A. Nuclear pore proteins Nup153 and megator deﬁne transcriptionally active regions in the Drosophila genome. PLoS Genet 2010; 6:e1000846; PMID:20174442; https://doi.org/10.1371/journal.pgen.1000846

[27] Brown CR, Kennedy CJ, Delmar VA, Forbes DJ, Silver PA. Global histone acetylation induces functional genomic reorganization at mammalian nuclear pore complexes. Genes Dev 2008; 22:627-39; PMID:18316479; https://doi.org/10.1101/gad.1632708

[28] Talamas JA, Capelson M. Nuclear envelope and genome interactions in cell fate. Front Genet 2015; 6:95; PMID:25852741; https://doi.org/10.3389/fgene.2015.00095

[29] Casolari JM, Brown CR, Komili S, West J, Hieronymus H, Silver PA. Genome-Wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. Cell 2004; 117:427-39; PMID:15137937; https://doi.org/10.1016/S0092-8674(04)00448-9

[30] Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, Schelder M, Vermeulen M, Buscaino A, Duncan K, Mueller J, et al. Nuclear pore components are involved in the transcriptional regulation of dosage compensation in Drosophila. Mol Cell 2006; 21:811-23; PMID:16543150; https://doi.org/10.1016/j.molcel.2006.02.007

[31] Jacinto F V, Benner C, Hetzer MW. The nucleoporin Nup153 regulates embryonic stem cell pluripotency through gene silencing. Genes Dev 2015; 29:1224-38; PMID:26080816; https://doi.org/10.1101/gad.260919.115

[32] Drummond DC, Noble CO, Kirpotin DB, Guo Z, Scott GK, Benz CC. Clinical development of histone deacetylase inhibitors as anticancer agents. Annu Rev Pharmacol Toxicol 2005; 45:495-528; PMID:15822187; https://doi.org/10.1146/annurev.pharmtox.45.120403.095825

[33] Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nat Rev Drug Discov 2014; 13:673-91; PMID:25131830; https://doi.org/10.1038/nrd4360

[34] Taddei A, Roche D, Bickmore WA, Almouzni G. The effects of histone deacetylase inhibitors on heterochromatin: implications for anticancer therapy? EMBO Rep 2005; 6:520-4; PMID:15940285; https://doi.org/10.1038/sj.embor.7400441

[35] Delcuve GP, Khan DH, Davie JR, Groth A, Rocha W, Verreau A, Almouzni G, Shabazzian M, Grunstein M, Tse C, et al. Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors. Clin Epigenetics 2012; 4:5; PMID:22414492; https://doi.org/10.1186/1868-7083-4-5

[36] Zink D, Amaral MD, Englmann A, Lang S, Clarke LA, Rudolph C, Alt F, Luther K, Braz C, Sadoni N, et al. Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. J Cell Biol 2004; 166:815-25; PMID:15364959; https://doi.org/10.1038/jcb.200404107

[37] Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, van Steensel B. Characterization of the Drosophila melanogaster genome at the nuclear lamina. Nat Genet 2006; 38:1005-14; PMID:16878134; https://doi.org/10.1038/ng1852

[38] Krull S, Thyberg J, Bjo B, Rackwitz H, Cordes VC. Nucleoporins as components of the nuclear pore complex core structure and tpr as the architectural element of the nuclear basket. Mol Biol Cell 2004; 15:4261-77

[39] Fahrenkrog B, Maco B, Fager AM, Köser J, Sauer U, Ullman KS, Aebi U. Domain-specific antibodies reveal multiple-site topology of Nup153 within the nuclear pore complex. J Struct Biol 2002; 140(1–3):254-67 page

[40] Duheron V, Chatel G, Sauder U, Oliveri V, Fahrenkrog B. Structural characterization of altered nucleoporin Nup153 expression in human cells by thin-section
electron microscopy. TL - 5. Nucleus 2014; 5 VN-re:601-12; https://doi.org/10.4161/19491034.2014.990853

[41] Tomura M, Sakaue-Sawano A, Mori Y, Takase-Utsugi M, Hata A, Ohtawa K, Kanagawa O, Miyawaki A. Contrast- ing Quiescent G0 Phase with Mitotic Cell Cycling in the Mouse Immune System. PLoS One 2013; 8:1-10; https://doi.org/10.1371/journal.pone.0073801

[42] Milhaliov I, Russev G, Anachkova B. Treatment of mammalian cells with mimosine generates DNA breaks. Mutat Res Repair 2000; 459:299-306; https://doi.org/10.1016/S0921-8770(00)00007-0

[43] Kubota S, Fukumoto Y, Ishibashi K, Soeda S, Kubota S, Yuki R, Nakayama Y, Aoyama K, Yamaguchi N, Yamaguchi N. Activation of the prereplication complex is blocked by mimosine through reactive oxygen species-activated ataxia telangiectasia mutated (ATM) protein without DNA damage. J Biol Chem 2014; 289:5730-46; PMID:24421316; https://doi.org/10.1074/jbc.M113.546655

[44] Deans AJ, West SC. DNA interstrand crosslink repair and cancer. Nat Rev Cancer 2011; 11:467-80; PMID:21701511; https://doi.org/10.1038/nrc3088

[45] Chakalova L, Fraser P. Organization of transcription factories. BMC Biophys 2013; 6:2; PMID:23394119; https://doi.org/10.1186/2046-1682-6-2

[46] Canals-Hamann AZ, das Neves RP, Reittie JE, Iborra FJ, Pombo A, Jackson DA, Cook PR. Active RNA polymerases are localized within discrete transcription factories in human nuclei. J Cell Sci 1996; 109(Pt 8):927-44; PMID:8779830

[47] Di Nunzio F, Fricke T, Miccio A, Valle-Casuso JC, Perez R, Miskimins R, Miskimins WK. Mimosine arrests cells in G1 by enhancing the levels of p27(Kip1). Exp Cell Res 2000; 254:64-71; PMID:10623466; https://doi.org/10.1006/excr.1999.4743

[48] Rieder D, Trajanoski Z, McNally JG. Transcription factor-mediated binding of Tpr to the Periphery of the nuclear pore complex. Mol Biol Cell 2003; 14:1923-40

[49] Fontoura BM, Dales S, Blobel G, Zhong H. The nucleoporin Nup98 associates with the intranuclear filamentous protein network of TPR. Proc Natl Acad Sci U S A 2001; 98:3208-13; PMID:11248057; https://doi.org/10.1073/pnas.061014698

[50] Chakalova L, Fraser P. Organization of transcription factories. Front Genet 2012; 3:221; PMID:22855394; https://doi.org/10.1128/MCB.19.1.764

[51] Zimowska G, Aris JP, Paddy MR. A Drosophila Tpr protein homolog is localized both in the extrachromosomal channel network and to nuclear pore complexes. J Cell Sci 1997; 110(Pt 8):927-44; PMID:9152019

[52] Vinerachandra V, Reja R, Pugh BF, Clausen T, Koster A. The human TREX-2 channel network and to nuclear pore complexes. J Cell Sci 2007; 120:1645-56; PMID:17681127; https://doi.org/10.1042/jcs.20061032

[53] Wang G, Miskimins R, Miskimins WK. Mimosine arrests cells in G1 by enhancing the levels of p27(Kip1). Exp Cell Res 2000; 254:64-71; PMID:10623466; https://doi.org/10.1006/excr.1999.4743
[65] Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene 2007; 26:5541-52; PMID:17694093; https://doi.org/10.1038/sj.onc.1210620

[66] Kim YB, Ki SW, Yoshida M, Horinouchi S. Mechanism of cell cycle arrest caused by histone deacetylase inhibitors in human carcinoma cells. J Antibiot (Tokyo) 2000; 53:1191-200; PMID:11132966; https://doi.org/10.7164/antibiotics.53.1191

[67] Chow KH, Elgort S, Dasso M, Powers MA, Ullman KS. The SUMO proteases SENP1 and SENP2 play a critical role in nucleoporin homeostasis and nuclear pore complex function. Mol Biol Cell 2014; 25:160-8; PMID:24196834; https://doi.org/10.1091/mbc.E13-05-0256

[68] Matafora V, D’Amato A, Mori S, Blasi F, Bachi A. Proteomics analysis of nucleolar SUMO-1 target proteins upon proteasome inhibition. Mol Cell Proteomics 2009; 8:2243-55; PMID:19596686; https://doi.org/10.1074/mcp.M900079-MCP200

[69] Niño CA, Guet D, Gay A, Brutus S, Jourquin F, Mendiratta S, Salamero J, Géli V, Dargemont C. Posttranslational marks control architectural and functional plasticity of the nuclear pore complex basket. J Cell Biol 2016; 212:167-80

[70] Li B, Kohler JJ. Glycosylation of the nuclear pore. Traffic 2014; 15:347-61; PMID:24423194; https://doi.org/10.1111/tra.12150

[71] Kosako H, Isamoto N. Phosphorylation of nucleoporins. Nucleus 2010; 1:309-13; PMID:21327077; https://doi.org/10.4161/nucl.1.1.11744

[72] Rajanala K, Sarkar A, Jhingan GD, Priyadarshini R, Jalan M, Sengupta S, Nandicoori VK. Phosphorylation of nucleoporin Tpr governs its differential localization and is required for its mitotic function. J Cell Sci 2014; 127:3505-20; PMID:24938596; https://doi.org/10.1242/jcs.149112

[73] Lussi YC, Shumaker DK, Shimi T, Fahrenkrog B. The nucleoporin Nup153 affects spindle checkpoint activity due to an association with Mad1. Nucleus 1:71-84; PMID:21327106; https://doi.org/10.4161/nucl.1.1.10244

[74] Zhou L, Panté N. The nucleoporin Nup153 maintains nuclear envelope architecture and is required for cell migration in tumor cells. FEBS Lett 2010; 584:3013-20; PMID:20561986; https://doi.org/10.1016/j.febslet.2010.05.038

[75] Bastos R, Lin A, Enarson M, Burke B. Targeting and function in mRNA export of nuclear pore complex protein Nup153. J Cell Biol 1996; 134:1141-56; PMID:8794857; https://doi.org/10.1083/jcb.134.5.1141

[76] Ball JR, Ullman KS. Versatility at the nuclear pore complex: lessons learned from the nucleoporin Nup153. Chromosoma 2005; 114:319-30; PMID:16133350; https://doi.org/10.1007/s00442-005-0019-3

[77] Hase ME, Cordes VC. Direct interaction with nup153 mediates binding of Tpr to the periphery of the nuclear pore complex. Mol Biol Cell 2003; 14:1923-40; PMID:12802065; https://doi.org/10.1091/mbc.E02-09-0620

[78] Legartova S, Stixová L, Laur O, Kozubek S, Sehnalová P, Bártová E. Nuclear structures surrounding internal lamin invaginations. J Cell Biochem 2013; 487:476-87

[79] Hozák P, Sasseville AM, Raymond Y, Cook PR. Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. J Cell Sci 1995; 108:635-44; PMID:7769007

[80] Duheron V, Chatel G, Sauder U, Oliveri V, Fahrenkrog B. Structural characterization of altered nucleoporin Nup153 expression in human cells by thin-section electron microscopy. Nucleus 2015; 5:601-12; https://doi.org/10.4161/19491034.2014.990853

[81] Andrade R, Arlucea J, Alonso R, Aréchaga J. Nucleoplasmin binds to nuclear pore filaments and accumulates in specific regions of the nucleolar cortex. Chromosoma 2001; 109:545-50; PMID:11305787; https://doi.org/10.1007/s004420000121