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A dynamic actin-dependent nucleoskeleton and cell identity

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Actin is an essential regulator of cellular functions. In the eukaryotic cell nucleus, actin regulates chromatin as a *bona fide* component of chromatin remodelling complexes, it associates with nuclear RNA polymerases to regulate transcription and is involved in co-transcriptional assembly of nascent RNAs into ribonucleoprotein complexes. Actin dynamics are, therefore, emerging as a major regulatory factor affecting diverse cellular processes. Importantly, the involvement of actin dynamics in nuclear functions is redefining the concept of nucleoskeleton from a rigid scaffold to a dynamic entity that is likely linked to the three-dimensional organization of the nuclear genome. In this review, we discuss how nuclear actin, by regulating chromatin structure through phase separation may contribute to the architecture of the nuclear genome during cell differentiation and facilitate the expression of specific gene programs. We focus specifically on mitochondrial genes and how their dysregulation in the absence of actin raises important questions about the role of cytoskeletal proteins in regulating chromatin structure. The discovery of a novel pool of mitochondrial actin that serves as 'mitoskeleton' to facilitate organization of mtDNA supports a general role for actin in genome architecture and a possible function of distinct actin pools in the communication between nucleus and mitochondria.

Keywords: chromatin and transcription regulation; development and differentiation; genome organization and integrity; mitochondria; nuclear actin.

Abbreviations: ACTL6A, actin-like protein 6A; ARP, actin-related protein; BAP, Brahma-associated protein; BMP, bone morphogenetic protein; CEBP, CCAAT/enhancer-binding protein; ChIP-seq, chromatin immunoprecipitations combined with deep sequencing; COX, cytochrome oxidase; CTD, C-terminal domain; dpc, days post-coitum; Exp6, exportin 6; HAS, helicase-SANT-associated; hnRNPs, heterogeneous nuclear ribonucleoproteins; iPSCs, induced pluripotent stem cells; KO, knockout; MEFs, mouse embryonic fibroblasts; MMP, mitochondrial membrane potential; MSCs, mesenchymal stem cells; mtDNA, mitochondrial DNA; NES, nuclear export signal; NM1, nuclear myosin I; OXPHOS, oxidative phosphorylation; PPAR-γ, peroxisome proliferator-activated receptor-γ; RNPs, ribonucleoprotein complexes; SAF-A, Scaffold Attachment Factor A; S/MAR, scaffold/matrix attachment regions; SWI/SNF, SWItch/Sucrose Non-Fermentable; TADs, topologically associating domains; TSS, transcription start site; YAP, Yes-associated protein

During differentiation dividing cells undergo alterations in their functional and phenotypic profiles to acquire a new identity. This is achieved through highly regulated changes in the expression of sets of genes with cellular differentiation even being defined in terms of the theory of variable gene activity (*1–3*). This theory suggests that an appropriately selected group of genes constituting one or more gene programs promotes differentiation and leads to specialized cell types. Transcriptional regulation lies at the core of cellular identity and transcriptional activators which act on specific target genes to induce transcriptional networks define the structural and functional properties of gene control programs. Transcriptional reprogramming, therefore, refers to the process by which mature and specialized cells can be reverted to induced pluripotent stem cells (iPSCs) or other specialized cell types (*4*). The different regulatory layers that control these transitions are beginning to be dissected in reprogramming experiments which have highlighted the importance of master regulators, genes at the top of the regulation hierarchy, particularly related to cell fate and differentiation. These genes are under the control of key signalling pathways, such as TGF-β signalling, that often synergize with the actin cytoskeleton (*5*). Well-characterized examples are the MRTF-SRF axis that is regulated by actin dynamics and controls myofibroblastic differentiation (*6*, *7*), mTOR signalling that regulates mechanically induced cytoskeletal reorganization and lineage selection in mesenchymal stem cells (MSCs; *8*), the Wnt pathway that works as major homeostatic signalling cascade in development and stem cell homeostasis (*9–11*) and the bone morphogenetic protein (BMP) pathway that regulates remodelling of the actin cytoskeleton and is

required for chondrogenesis and osteogenesis as well as adipogenesis (*12*, *13*). BMPs target MSCs to trigger activation of transcription factors such as the peroxisome proliferator-activated receptor-γ (PPAR-y) and Runx2 in adipogenic and osteogenic specification (*13*). Sox9, a key transcription factor of chondrogenesis, is activated through the BMP pathway and a CCAAT box in the proximal promoter (*14*). Aberrant activation of these homeostatic signalling pathways is a hallmark of various types of cancers. For instance, recent work has established that the forkhead transcription factor FoxB2 that regulates Wnt7-dependent development of the endocrine system is aberrantly expressed and leads to abnormal activation of Wnt signalling compatible with prostate cancer (*15*).

The behaviour of gene programs depends not only on the expression of master regulatory genes but also on their synergy with chromatin remodelling complexes and epigenomic modifiers (*16–18*). This is especially important in the context of differentiation and disease (*19*, *20*) where cis-regulatory, synergistic molecular events between epigenetic and transcription factors is referred to as transcriptional cooperation. Crosstalk between transcription activators and chromatin regulators is also the basis of metabolic changes that occur during differentiation when, for instance, mitochondrial dynamics and morphology undergo drastic alterations (*21*). At the molecular level, alterations from elongated mitochondria in differentiated cells to spherical-shaped mitochondria during the formation of iPSCs are accompanied by changes in energy metabolism essential during the reprogramming process (*21*). Changes in mitochondrial morphology occur concomitantly with changes in their metabolic profiles that switch from oxidative phosphorylation (OXPHOS) to glycolysis (*21*), presumably by tightly regulating mitochondrial gene expression.

Given the complexity of differentiation and its hierarchical nature, understanding how transcriptional networks are established and regulated at multiple layers provides an opportunity to obtain temporal frameworks of differentiation. In addition, it is becoming increasingly relevant to establish how transcriptional activators selectively synergize with chromatin regulators during differentiation and how these mechanisms are integrated within the three-dimensional (3D) hierarchical organization of the genome into compartments and topologically associating domains (TADs) (*22*). It is beginning to become apparent that dynamic changes in the 3D organization of the genome occur in a controlled manner throughout differentiation (*23*) when transcriptional networks must be established and maintained for sustained expression of gene programs.

The precise mechanisms that co-regulate transcription, chromatin regulation and 3D genome organization during cell differentiation are yet to be fully understood. However, recent work suggests that cytoskeletal proteins like actin may play an important role in coordinating these processes. Signalling pathways that regulate differentiation, for instance, also impact the cytoplasmic actin pool which, in turn, induces changes in the localization of key transcription factors and promote chromosome intermingling (*24–27*). Similarly, changes in nuclear actin levels also impact key chromatin remodelling complexes which are known to affect the expression of genes involved in neurogenesis, osteogenesis and adipogenesis. In this review, we, therefore, focus on the different roles of nuclear actin in reshaping the genome via regulation of transcription and chromatin organization.

Actin in the Nucleus From Past to Present Evidence

Actin is one of the most highly conserved and abundant proteins in eukaryotic cells. For many decades, the presence of actin inside the nucleus remained controversial and it was primarily treated as a cytoplasmic protein involved in regulating cell shape, cell motility and cytoskeletal organization (*28*, *29*). Although some early studies suggested the presence of a nuclear actin pool (*30–33*), this idea was only recently accepted concomitant with the development of new experimental tools and models. Using a custom-made anti-actin antibody actin was detected on active transcription sites both on isolated polytene chromosomes and *in vivo* and this association was found to be required for RNA polymerase II transcription in living cells (*34*, *35*). Part of the scepticism on the existence of nuclear actin arose from the lack of visible actin filaments within the nucleus of most cell types and was only resolved with the introduction of advanced visualization methods utilizing the 17 amino acid long peptide lifeact (*36*) or the actin-staining chromobody nAC-GFP-NLS (*37*). These methods have not only demonstrated the presence of actin in the cell nucleus but have also confirmed its ability to polymerize in the nucleus by detecting the presence of thin nuclear actin filaments. Of the three known actin isoforms, skeletal muscle or α-actin and non-muscle β- and γ-actin, only β-actin has been convincingly shown to be in the cell nucleus. Actin is actively imported into the nucleus through the importin/Ran system in complex with the NLS-containing cofilin and disruption of this mechanism is lethal for embryonic development (*38–40*). Similarly, actin is actively exported from the nucleus via exportin 6 (Exp6) (*40*). Nuclear export is enhanced by the small actin binding protein profilin that contains a nuclear export signal (NES) and is also known to suppress nuclear actin polymerization (*40*). Actin polymerization in the nucleus has been observed by several labs in different contexts (*41–44*) and has been shown to be regulated through a cohort of G-actin and F-actin binding proteins (*45*, *46*) in a way that is similar to the regulation of cytoplasmic actin polymerization. Dynamic nuclear actin polymerization seems to be required for both nuclear architecture and nuclear function. Indeed, results from chromatin immunoprecipitations combined with deep sequencing (ChIP-seq) have demonstrated that actin associates with both mouse and *Drosophila* genomes (*47*, *48*), although this association is likely to be mediated by other nuclear factors. Expression of actin mutants with dysregulated polymerization

functions have shown that regulated actin polymerization is necessary for transcription (*49*). In line with this observation, regulators of actin polymerization are directly involved in transcription. The G-actin binding proteins cofilin and profilin, required for actin dynamics, localize to nuclei of mammalian and insect cells, associate with active genes as revealed by immunohistochemistry and chromatin immunoprecipitation (ChIP) experiments and function in transcription elongation (*50*, *51*). Similarly, ARP2/3 and N-WASP, required for actin polymerization and branching, are also involved in transcription (*52*, *53*) and the Gurdon lab has demonstrated that WAVE-dependent nuclear actin polymerization is required during transcriptional reprogramming (*54*). Interestingly, the pool of transcription-competent actin also seems to perform its transcriptional role in complex with certain myosin species (*55*).

Although it has been shown that actin works together with all three eukaryotic nuclear RNA polymerases (*56*, *57*) while also being a component of several chromatin remodelling complexes (*58*, *59*), how these molecular functions synergize is not clear at this stage. In the next sections, we discuss emerging evidence demonstrating the role of nuclear actin in transcriptional and chromatin regulation and how these roles converge to promote mitochondrial function during cell differentiation.

Actin in Transcription

Initial evidence for actin's involvement in transcription came in 1984 when actin was found in complex with RNA polymerase II isolated from HeLa cells lysates while the G-actin binding protein gelsolin was detected on the lampbrush chromosomes in the amphibian oocytes (*60*, *61*). Later, studies in the dipteran *Chironomus tentans* revealed a functional association of actin with active transcription sites and a direct involvement of actin in transcription (*34*, *35*). Biochemical studies further revealed that actin is in complex with eukaryotic RNA polymerases (*56*, *57*, *62–65*). Actin binds to the largest RNA Pol I subunit (*62*), to the Pol II C-terminal domain (CTD) as well as to common subunits Rbp6 and Rbp8 found in Pol I, Pol II and Pol III (*66–68*), altogether supporting possible roles of actin at different stages of the transcriptional process. The function of actin in transcription is also closely associated with nuclear myosin I (NM1), first discovered and best described among the currently known nuclear myosin species (*55*, *69*), with well-characterized functions in Pol I and Pol II transcription (*49*, *62*, *64*, *66*, *69–72*). In Pol I transcription, NM1 is associated with the transcription factor TIF-IA, Pol I and actin to form the pre-initiation complex at the gene promoter (*64*). To activate transcription, NM1 functions as a molecular switch between actin and the chromatin remodeller B-WICH (*70*, *71*, *73–75*). Bound to the chromatin through its CTD (*70*), NM1 either interacts with polymerase-bound actin or with the ATPase SNF2H. SNF2H is a component of the WICH chromatin remodelling complex responsible for nucleosome repositioning and for anchoring of histone acetyl transferase PCAF and histone methyl transferase Set1. Once recruited to the transcription start site (TSS), PCAF and Set1 acetylate and methylate histone H3 allowing the polymerase machinery to proceed across the gene (*47*, *70*, *73*, *74*). Consistent with a possible role for NM1 and actin in regulating the WICH complex, in Pol II transcription, NM1 was found to be responsible for SNF2H-dependent chromatin remodelling as well as PCAF- and Set1-dependent histone modifications needed for transcription activation (*72*, *76*).

During transcriptional elongation, co-transcriptional assembly of nascent transcripts into ribonucleoprotein complexes (RNPs) is also an actin-dependent process that actin seems to perform in complex with a subset of heterogeneous nuclear ribonucleoproteins (hnRNPs). In *C.tentans*, actin binds to the hnRNP A1-like hrp36 and accompanies the nascent transcript from gene to polysomes (*34*). Co-transcriptional actin association with the *C.tentans* hrp65, homologue to the mammalian co-activator PSF, is required for elongation of nascent RNA (*35*). Similarly, in mammals, actin co-transcriptionally binds to the A/B-type hnRNP CBF-A/hnrnpab and accompanies RNA during nucleocytoplasmic transport (*77–80*). Further, in complex with hnRNP U/SAF-A (Scaffold Attachment Factor A) actin binds to the hyperphosphorylated Pol II CTD to facilitate PCAF recruitment and PCAF-dependent histone H3 acetylation for elongation of nascent RNA (*67*, *77*, *81*).

Although it is unclear how these numerous actin interactions are coordinated, depending on the binding partner and, possibly, polymerization state, they suggest that actin regulates multiple stages of gene expression ranging from formation of pre-initiation complexes and transcription elongation to formation and stabilization of RNPs for nuclear export and trafficking.

Chromatin Regulation, 3D Genome Organization, Nucleoskeleton and the Role of Nuclear Actin

Upstream of actin's transcriptional functions, its involvement in chromatin regulation as a subunit of ATP-dependent chromatin remodelling complexes is now well-established (*58*, *59*). Depending on the ATPase subunit, mammalian chromatin remodellers can be divided into four groups—SWI/SNF, ISWI, CHD and INO80 (*82*) and actin and myosin have been shown to play critical roles in regulating several of them (*55*, *76*, *83*). For example, the INO80 chromatin remodelling complex is involved in inositol metabolism as a transcriptional co-activator, making it essential for DNA repair, replication and cell cycle control (*84*, *85*). Similarly, in the yeast INO80 complex, actin interacts with Arp4 and Arp8, binding to histones and extra-nucleosomal/linker DNA to function as an allosteric sensor that regulates INO80-dependent nucleosome spacing (*86–88*).

Actin is also a component of the mammalian chromatin remodelling complex BAF (BRG1- or BRM-associated factors) (*58*, *89*, *90*). This complex is

homologous to the *Drosophila* BAP (Brahma-associated protein) complex (*91*) and belongs to the SWI/SNF (SWItch/Sucrose Non-Fermentable) family of chromatin remodellers which contain at least 15 subunits including the ATPase Brg1 (*89*). The BAF complex consists of three functional modules which form a spanner head-shaped structure surrounding the nucleosome. The Brg1 subunit forms the backbone with its ATPase domain grasping the nucleosome and partially wrapping around nucleosomal DNA. In the same complex, the helicase-SANT-associated (HSA) domain is a part of the ARP (actin-related protein) module and the pre-HSA domain is anchored into the base module packed against the bottom surface of the nucleosome. The ARP module contains a heterodimer of actin-like protein 6A (ACTL6A) and β-actin. Its function is to bridge and stabilize the ATPase and base modules around the nucleosome (Fig. 1A), a function critical for BAF1 association with the chromatin (*92*). Indeed, results from ChIP-seq analysis with anti-Brg1 antibodies show that β-actin deletion leads to dissociation of Brg1 from DNA (see Fig. 1B), affecting both transcription and heterochromatin segregation at the nuclear lamina (*83*). These observations support a model where a β-actin-dependent nucleoskeleton could impact chromatin structure by regulating Brg1 activity (*93*).

Interestingly, the presence of β-actin in chromatin remodelling complexes like BAF also gives rise to the intriguing possibility that changes in actin levels could influence 3D genome organization. Recent work utilizing chromosome conformation capture techniques such as 3C and HiC have revealed that the eukaryotic chromosomes are organized into a multi-layered hierarchical structure with chromosomal territories being divided into large scale transcriptionally active and repressed compartments (A and B compartments), and further giving rise to TADs and chromatin loops (*94*, *95*). It has been shown that architectural proteins like CTCF play a critical role in regulating TADs and loops (*95*) while chromatin remodellers such as BAF, polycomb complexes and HP1α contribute to compartment-level organization via phase separation-based mechanisms (*96*, *97*). While a direct role for β-actin in regulating 3D genome organization is yet to be elucidated, several recent studies hint at such a

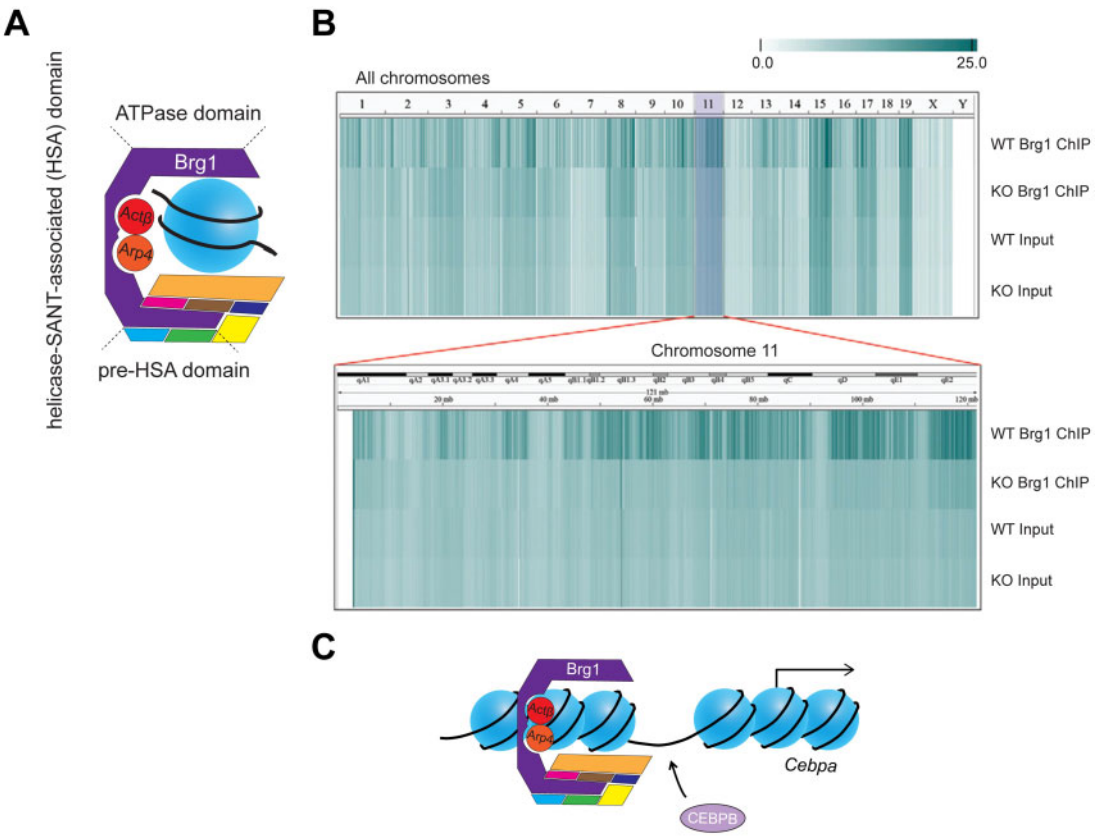

Fig. 1. Actin-dependent structure and function of the BAF1 complex. (**A**) BAF complex wrapping around the nucleosome consists of several proteins which are interconnected by BRG1 which forms backbone for the whole complex and possess ATPase activity and actin-ARP binding sites. Model based on He *et al.* (*92*). (**B**) Heat map of normalized reads density Reads Per Kilobase of transcript, per Million mapped reads (RPKM) obtained from Brg1 ChIP-seq across the whole genome between WT and KO cells. Input was shown as the background. Top: RPKM levels on all chromosomes; bottom: RPKM levels on chromosome 11. Scale bar, RPKM range (adapted from Ref. *83*). (**C**) A speculative model describing actin-dependent recruitment of Brg1 upstream the TSS of the Cebpa genes. This promotes an open chromatin configuration at specific CEBPB binding sites, allows for CEBPB binding and promotes CEBPA gene activation and adipogenic differentiation (*129*).

function. It has been shown, for instance, that β-actin knockout (KO) cells exhibit dramatic alterations in the transcriptional landscape coupled with epigenetic changes such as loss of BRG1 chromatin association and accumulation of H3K9Me3/HP1α-positive heterochromatin (*83*). It has also been shown that nuclear actin levels can influence RNA polymerase II clustering (*98*). Since the formation of heterochromatic B compartments is thought to be mediated by HP1α and H3K9Me3-induced phase separation, while euchromatic A compartments are proposed to arise from multivalent interactions between RNA polymerase, transcription factors and chromatin regulators (*97*), the ability of nuclear actin to influence both these processes places it in an ideal position to potentially regulate the structure of both A and B compartments.

Similarly, β-actin-dependent loss of BRG1 chromatin binding can potentially lead to changes in both compartment and TAD level genome organization. Brg1 has been shown to play a role in activation of distal enhancers by regulating long-range chromatin interactions and in the regulation of TAD boundaries (*99–101*). Furthermore, the BRG1 containing BAF complex is known to directly antagonize chromatin binding of polycomb group proteins during development (*102*). Any changes in BRG1 occupancy are, therefore, expected to induce corresponding changes in the activity of polycomb group proteins and PRC2 mediated epigenetic marks such as H3K27me3. As facultative heterochromatin features like H3K27me3, PRC1 and PRC2 are known to induce phase separation and aid in clustering of nucleosomes, polycomb-bound chromatin (*96*, *97*) and regulate long-range chromatin interactions (*103*), they constitute another mechanism by which β-actin could affect 3D genome structure at the level of compartments.

Another potential pathway through which actin levels may impact global genome architecture is via actin's interaction with hnRNPs. The recent finding that actin levels can influence clustering of transcription foci (*98*) has led to the hypothesis that such clustering could be facilitated by the actin-hnRNP U/SAF-A complex binding to the hyperphosphorylated Pol II CTD (*67*). Interestingly, hnRNP U/SAF-A is not only known to play a role in compartment-level 3D genome organization (*104*) but also acts as an attachment factor linking specific DNA elements and scaffold/matrix attachment regions (S/MAR) to the nuclear matrix (*105*). Furthermore, consistent with the role of phase separation in compartment-level organization, purified hnRNP U can bind DNA to form higher-ordered nucleic-acid-protein complexes *in vitro* (*106*). This is particularly interesting also in view of the recent observation that cross-linked actin filaments can form liquid droplets (*107*) and nuclear phosphoinositides (PI4,5P2) synergize with the actin-NM1 complex to form active Pol II (*108*). Uncovering the relationship between actin-dependent chromatin remodellers such as BAF, the actin-hnRNP U/SAF-A complex and the synergy with both NM1 and Pol II can, therefore, shed light on the idea of a dynamic nucleoskeleton that simultaneously influences gene expression, local chromatin organization and 3D genome architecture.

Actin in Transcriptional Reprogramming During Cell Differentiation

Compatible with its role in chromatin and transcription regulation, several studies have recently highlighted the critical involvement of nuclear actin dynamics in transcriptional reprogramming during differentiation (*109*). As mentioned earlier, nuclear or transcriptional reprogramming is the process by which the identity of specialized cells may be changed to the embryonic-like pluripotent state or other cell types (*4*).

Using isolated nuclei (germinal vesicles) from *Xenopus* oocytes, the Gurdon lab highlighted the importance of nuclear actin in transcriptional reprogramming. Oocyte nuclei are particularly suited for these experiments since they are transcriptionally active and have high levels of nuclear actin. Due to their size, they can be easily imaged to study actin polymerization and, above all, they are free from cytoplasmic actin contamination (*110–112*). Using this model, Miyamoto *et al*. reported that upon depletion of the actin binding protein Wave1 (WASF1), known to regulate actin polymerization, expression of the pluripotency gene Oct4 is repressed (*112*, *113*). Similarly, in mammalian cells, overexpression of actin leads to activation of Oct4, possibly through recruitment of β-catenin to the Oct4 gene which is dependent on polymeric actin (*114*, *115*). This needs to be further investigated but it is possible that the underlying mechanism is BAF-dependent and actin polymerization may be important for its recruitment. In fact, although in the BAF complex actin is primarily in a monomeric state and forms a heterodimer with Arp4 to regulate Brg1 function (*116*), it retains the ability to polymerize (*90*). The BAF complex with its ATPase Brg1 has been shown not only to regulate expression of Oct4 (*117–119*) but also to facilitate its binding to its DNA targets together with other pluripotency transcription factors such as Sox2 and Nanog (*117*, *118*, *120*). Moreover, Brg1 overexpression enhances the efficiency of iPSCs production from mouse embryonic or human adult fibroblasts (*121*). These considerations suggest that actin has multiple roles during transcriptional reprogramming depending on its polymeric state and/or binding partners. Polymeric actin may help to recruit activators and remove repressors, including repressive epigenetic marks, from pluripotency genes such as Oct4 that otherwise remain occluded, allowing actin-dependent remodelling complexes such as BAF to change the local chromatin landscape to a configuration compatible with actin-dependent Pol II transcription (*112*). Thus, nuclear actin, therefore, seems to be an important player for the establishment of pluripotency and, in fact, transcriptional profiling of β-actin KO MEFs by RNA-seq shows differential expression of multipotency and pluripotency genes involved in cell fate and stemness (see Fig. 2).

It is, therefore, not surprising that the amount of nuclear actin and/or the ratio between cytoplasmic and nuclear pools of actin is important during differentiation. In *Xenopus* oocytes, for example, high

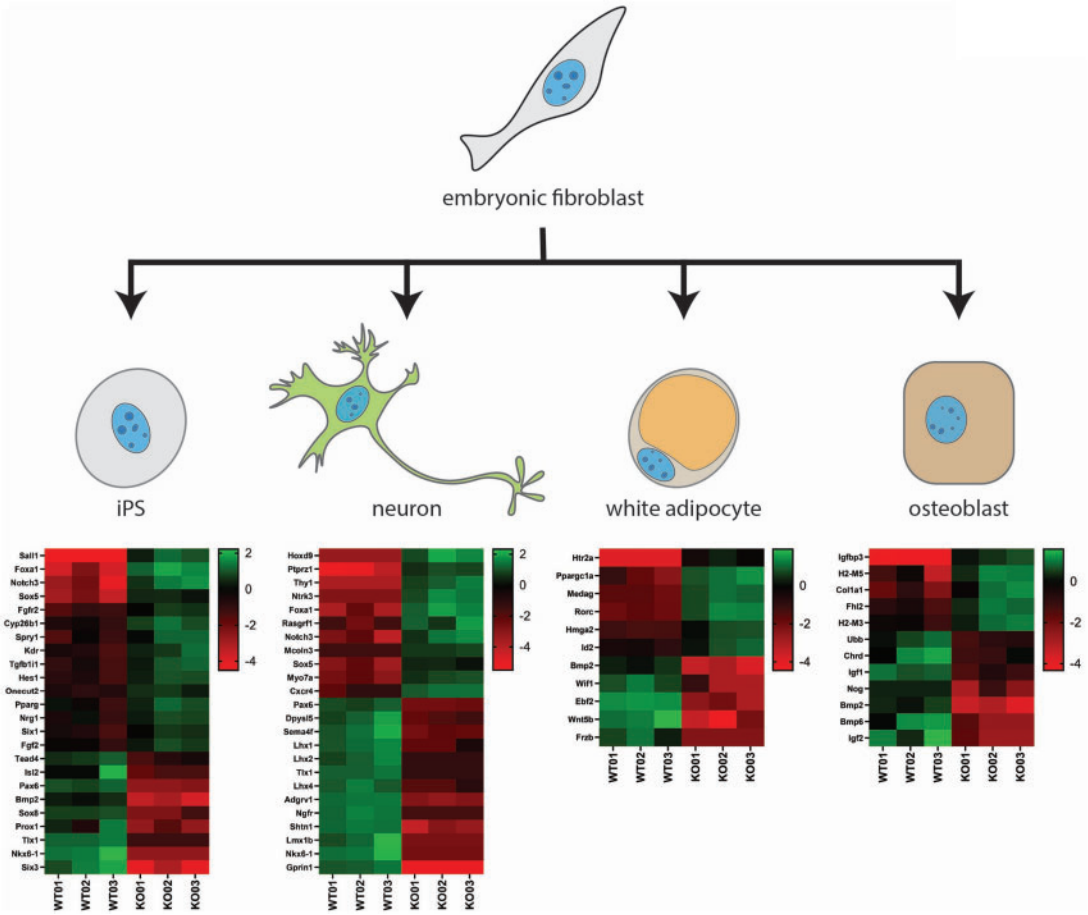

Fig. 2. β-Actin affects reprogramming and differentiation into different cell types. Genes were selected based on the gene ontology analysis of differentially expressed genes between wild-type MEFs and β-actin KO MEFs obtained by transcriptional profiling (see Ref. *83*). GO terms 'cell fate commitment', 'neuron differentiation', 'adipose tissue development' and 'positive regulation of fat cell differentiation' and 'osteoblast differentiation' were used for selection of most differentially expressed genes related to differentiation into iPSCs, neurons, adipocytes and osteoblasts, respectively.

nuclear actin levels are necessary during oogenesis. After maturation and fertilization, nuclear actin levels are decreased by Exp6 overexpression which leads to increased nuclear actin export to the cytoplasm (*110*). Similarly, in *Drosophila*, Exp6 silencing negatively regulates oogenesis by dysregulation of the cytoplasmic/nuclear actin ratio (*38*). Recently, it has been shown that mouse fertilized embryos require a certain amount of nuclear actin for subsequent development (*122*). Increased nuclear actin levels are also needed for transcriptional regulation during macrophage differentiation (*123*). A remarkable example of such regulation was also reported by Le *et al.* (*124*) who showed that upon mechanical stress, the protein Emerin is translocated from inner to outer nuclear membranes where it promotes rapid actin polymerization with non-muscle myosin IIA. This leads to loss of lamina-associated heterochromatin, subsequently bound by PRC2 to catalyze dimethylation and trimethylation of histone H3 on Lys27 (H3K27me3). The insufficient amount of free nuclear actin leads to Pol II stalling on genes which are recognized by PRC2 and silenced by means of H3K27 trimethylation (*124*). In MSCs, accumulation of actin in the nucleus leads to differentiation to osteogenic and, to a lesser extent, adipogenic lineages by sequestering the inhibitory protein YAP (Yes-associated protein, a mediator of Src/Yes signalling). There is an evidence that nuclear actin binds to YAP promoting its translocation to the cytoplasm while releasing the transcription factor Runx2, master regulator of osteogenesis, for activation of specific sets of osteogenic genes (*125*).

Further support for a role of β-actin in development and differentiation came from recent studies reporting that a β-actin KO mouse is embryonic lethal at 8–10.5 dpc (days post-coitum), a stage immediately prior to the onset of key developmental pathways (*126*, *127*), including neurogenesis, adipogenesis and osteogenesis. Subsequent studies also generated stable mouse embryonic fibroblasts (MEFs) from a 10.5 dpc embryonic lethal β-actin KO mouse (*127*). These cells exhibit overexpression of actin isoforms other than β-actin, including α-smooth muscle actin (α-SMA) and γ-actin which compensate cytoplasmic but not nuclear functions as suggested by their transcriptionally reprogrammed state (*83*, *127*). Results from RNA-seq analyses performed on β-actin KO MEFs as well as wild-type and heterozygous condition with only one disrupted β-

actin allele demonstrated that loss of β-actin induces a general transcriptional reprogramming with both activation and repression of gene programs related to cell fate and differentiation (see Fig. 2). Phenotypically, β-actin KO MEFs exhibit a degree of cytoskeletal reorganization and enhanced fibrogenic and angiogenic features (*83*). Evidence that β-actin depletion impairs Brg1 deposition across the mouse genome (see Fig. 1B) suggests that in the absence of β-actin there are major chromatin rearrangements that most likely affect transcription (*93*). Follow up experiments focussing on direct reprogramming of β-actin KO MEFs to neurons, adipocytes and osteocytes showed a direct role of β-actin in transcriptional reprogramming during neurogenesis (*128*), adipogenesis (*129*) and osteogenesis (*130*). In the case of neuronal reprogramming, β-actin deletion led to accumulation of the heterochromatin mark H3K9me3 and loss of Brg1 at TSSs of Zic and Irx genes (*128*), which are among the earliest pro-neuronal and neuronal transcription factors responding to neural-inducing signals and promoting pro-neuronal gene expression (*131*, *132*). Considering that Brg1 function depends on the nuclear actin pool, these results really point towards the possibility that nuclear actin regulates neuronal development and differentiation as a part of the BAF chromatin remodelling complex, whose role in neurogenesis has been previously described (*133–135*). Similarly, upon direct reprogramming of β-actin KO MEFs to adipocytes (*129*), we recently reported that actin-dependent Brg1 remodelling is needed for activation of the pioneer transcription factor CEBPA (CCAAT/enhancer-binding protein alpha), responsible for differentiation of preadipocytes into white adipocytes (*136*). Adipocyte differentiation requires a signalling network and transcription factors including PPAR-γ, members of the CCAAT/enhancer-binding protein (CEBP) family and the BAF complex. PPAR-γ works as master regulator of adipogenesis whereas CEBPA functions as a pioneer factor in adipocyte sub-type specificity, promoting differentiation to white adipocytes over brown adipocytes. It turns out that loss of β-actin impairs Brg1-deposition at one of the CEBPB binding sites upstream of the CEBPA gene. Since CEBPA activation requires CEBPB binding, loss of BRG1 and chromatin accessibility at the CEBPB binding site leads to dysregulation of adipogenic differentiation (*129*). Remarkably, this phenotype is rescued by reintroduction of NLS-tagged β-actin in the nucleus of β-actin KO MEFs (*129*), supporting the idea that by regulating chromatin and transcriptional reprogramming nuclear actin can act as a key regulator of cell differentiation (Fig. 1C). In yet another differentiation model, β-actin deletion impairs expression of master regulators of osteogenesis along with nuclear-encoded mitochondrial genes, displaying a hypermineralization phenotype compatible with dysregulated mitochondrial function (*130*).

In summary, by performing key functions in chromatin and transcriptional regulation to control expression of gene programs, the pool of nuclear actin is emerging as an essential factor for cell differentiation.

Mitochondrial Genes: a Paradigm to Study Actin-Dependent Gene Expression Regulation and Genome Organization in Differentiating Cells

Mitochondria play a central role in cell differentiation and, in this context, regulation of mitochondrial genes is particularly important. During differentiation, mitochondria not only change their morphology, but also their metabolic profiles from OXPHOS to glycolysis through rapid alterations in gene expression (*21*). Morphological changes that occur during mitochondrial biogenesis and accompany differentiation are known to be regulated by the cytoplasmic actin pool that is specifically required during fission of elongated mitochondria into round-shaped mitochondria (*137*, *138*). Interestingly, the nuclear actin pool and a recently described pool of mitochondrial actin seem to be necessary for expression of nuclear- and mitochondria-encoded mitochondrial genes, respectively (*139*, *140*), with a potential impact on cell differentiation.

In mammals, 1.5 billion years of coevolution of nuclear and mitochondrial genomes have led to the reduction of the mitochondrial genome and transfer of the majority of mitochondrial genes to the cell nucleus leaving only 37 mitochondrial-encoded genes. Among these genes, 13 encode structural components of the OXPHOS system, 2 genes encode mitochondrial rRNAs and 22 genes encode mitochondrial tRNAs (*141*). On the other hand, ∼1,500 nuclear-encoded genes have been suggested to have a role in mitochondrial function with 250–300 directly regulating mitochondrial gene expression (*142*) and several studies have reported on the importance of nuclear-encoded gene mutations in human mitochondrial diseases (*143*, *144*). Most mitochondrial functions exclusively depend on nuclear-encoded genes and their regulation. OXPHOS, however, is an exception since it requires both nuclear- and mitochondrial-encoded OXPHOS genes and a tight coordination of their expression to prevent accumulation of unaffiliated subunits of the different complexes involved in electron transfer at the mitochondrial membrane (*142*). OXPHOS complexes responsible for cellular bioenergetics are formed by the direct interactions of 13 mitochondrial-encoded and ∼80 nuclear-encoded genes in most animals. While expression of nuclear-encoded OXPHOS genes relies mostly on the regulation of their transcription or translation, expression of mitochondrial-encoded OXPHOS genes also depends on mitochondrial DNA (mtDNA) replication, mtDNA segregation and copy number (*145–149*). How coordinated expression of nuclear- and mitochondrial-encoded OXPHOS genes is achieved remains to be understood but recent evidence suggests that it is possibly executed through a crosstalk between nuclear and mitochondrial β-actin pools.

Actin may control expression of OXPHOS genes through its ability to regulate nuclear and mitochondrial genomes (*140*). Nuclear-encoded OXPHOS genes

are organized in clusters (*150*) and it is possible that organisms have developed genomic features that facilitate association into hubs that are co-transcriptionally regulated, contributing to stoichiometric OXPHOS subunits production and, therefore, biochemical efficiency of OXPHOS complexes. In support of this hypothesis, studies using chromosome conformation capture techniques demonstrated that all 10 nuclear subunits of cytochrome oxidase (COX) encoded on nine different chromosomes as well as Tfam, Tfb1m and Tfb2m genes, located on three chromosomes and vital for the transcription of the three mitochondria-encoded COX subunit genes, all reside in the same intranuclear hub in neuronal nuclei (*151*). Given the emerging role of β-actin in 3D genome organization and recent evidence that nuclear β-actin is required for pol II transcription-dependent formation of transcription hubs (*98*), it can be speculated that nuclear OXPHOS gene activities may be regulated by nuclear β-actin by precisely maintaining a genomic architecture compatible with transcription. Similar considerations apply to the mitochondrial β-actin pool that seems to have a critical role in establishing an actin-based 'mitoskeleton' required for correct segregation of nucleoids, mtDNA transcription and mitochondrial membrane potential (MMP; *128*). Although these models require further investigations, actin-dependent regulation of OXPHOS gene expression does impact differentiation. In adipogenesis, this is particularly important because alterations in mitochondrial function affect formation of white and brown adipose tissue and can lead to disease (*131*). Similarly, in osteogenesis mitochondria play a critical role in the mineralization of the extracellular matrix as calcium-phosphate granules are produced in mitochondria before their export to the extracellular space (*152*, *153*). During osteogenic reprogramming β-actin depletion leads to hypermineralization and this phenotype is a direct consequence of abnormal upregulation of nuclear-encoded mitochondrial genes, including OXPHOS genes (*130*). This upregulation may result from a compensatory effect against defects in maintaining MMP in the absence of nuclear β-actin prior to differentiation, altered expression of nuclear and mitochondrial-encoded OXPHOS genes or from a general impairment of mtDNA transcription (*128*). Future work will address if this specific phenotype is due to β-actin-dependent alterations in chromatin regulation and genomic architecture.

The discovery of distinct nuclear and mitochondrial β-actin pools also provides potential insights into a novel role of actin in the communication between nucleus and mitochondria through regulation of their respective genomes. Anterograde (from nucleus to mitochondria) and retrograde (from mitochondria to nucleus) signalling is a requirement for numerous cellular functions and we speculate that it may be achieved by targeting actin pools in the cell nucleus and in mitochondria. While the aim of nuclear-to-mitochondria anterograde signals is to achieve fine-tuned mitochondrial bioenergetics and functional population of mitochondria, through retrograde signalling mitochondria constantly send molecular signals to the nucleus, reflecting metabolic status and mitochondrial activity, needed to maintain cellular homeostasis (*154*). Candidates for co-regulating the dynamics of nuclear and mitochondrial actin pools are a cohort of signalling molecules such as nutrients, Wnts and BMPs that are all known to target the actin cytoskeleton and by doing so may play a fundamental role in this bidirectional communication. Through signalling there may be coordinated mechanisms required to regulate the state of actin polymerization in both nuclear and mitochondrial compartments to maintain OXPHOS expression (see Fig. 3). Understanding the crosstalk between nuclear and mitochondrial β-actin pools during differentiation, therefore, becomes a primary goal for future investigations.

Concluding Remarks

Cytoskeletal proteins have emerged as key regulators of nuclear functions, many of which have been dissected in the past years. It is now well accepted that nuclear actin is involved in chromatin and transcription regulation and that these molecular functions are performed with an array of nuclear factors, several myosin species and regulators of actin polymerization. In fact, >30 proteins affecting actin polymerization have been found in the nucleus (*46*), including proteins that have been historically considered as components of the nucleoskeleton (*45*). This allows for an endless variability of potential actin states required for nuclear functions redefining the very concept of nucleoskeleton from a rigid nuclear scaffold reminiscent of the nuclear matrix to an interconnected dynamic network of states and processes containing actin and actin-bound machineries including chromatin, protein complexes and nucleic acids that together play a role in the architectural features of the genome. In fact, the involvement of cytoskeletal proteins in the architecture of the mammalian genome is not surprising considering that in bacteria actin-like proteins are involved in chromosome segregation (*155*, *156*) and this seems to be the case also in mitochondria where a pool of β-actin is involved in mtDNA segregation (*140*).

In the specific case of the mammalian genome, the emerging role of actin in 3D genome organization has opened up many questions. There is evidence that nuclear actin polymerization and myosin are required for movement of heterochromatin in response to DNA damage (*157*, *158*) and in long-range movement of chromatin loops upon transcription activation (*159*, *160*). Therefore, how does rapid actin polymerization and depolymerization affect genome architecture? Although at the moment there is no clear answer to this, the requirement and engagement of regulated actin polymerization and depolymerization (*41*) may depend on the identity and size of individual nuclei with different architectural requirements to ensure expression of certain gene programs over others. A scenario that has not been investigated in depth is the possibility that regulated actin polymerization is fundamental for recruitment of chromatin remodellers

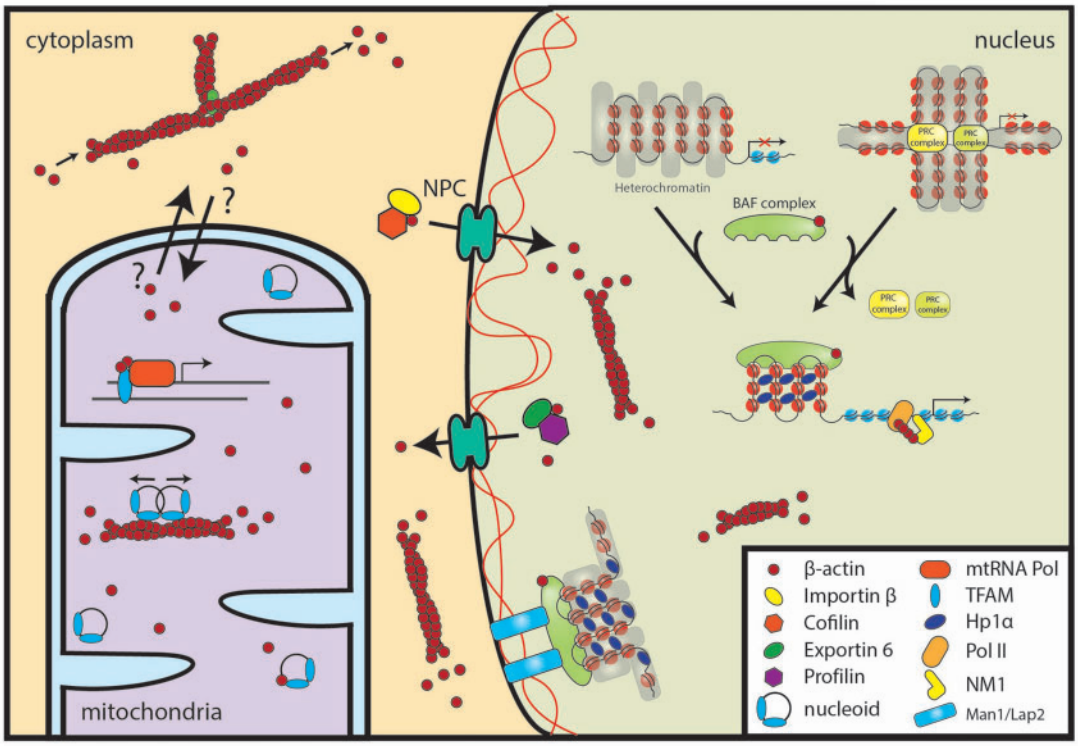

Fig. 3. Actin communication and regulation across organelles. Actin has been shown to be localized in the cytoplasm, nucleus and mitochondria. Nuclear import and export are achieved through chaperone complexes containing importin β and cofilin (for import) and Exp6 and profilin (for export). Actin shuttles to the mitochondria but the mechanism of transport is not known. In the nucleus, actin is part of several remodelling complexes, such as BAF as well as part of transcription machinery. Depending on the complex composition, actin can activate or repress different sets of genes. In mitochondria, actin is needed for segregation of mtDNA during replication and expression of nuclear-encoded OXPHOS genes.

(*112*) and whether this eventually affects what gene programs are activated or repressed by actin-dependent changes in 3D genome organization at compartment level. Furthermore, do these mechanisms reflect the role of actin polymerization in heterochromatin segregation in response to DNA damage or external stimuli? Having placed nuclear actin in the road map of genome biology we are now left with many open questions. In the context of nucleoskeleton and stabilization of long-range chromatin interactions and loops, the synergy between actin and hnRNP U/SAF-A represents an intriguing pathway that may contribute to activation of gene programs during differentiation.

In contrast with nuclear actin, we know much less about the mitochondrial actin pool and its dynamic properties. In addition, how actin is transported into mitochondria is not known. A way to address nuclear and mitochondrial actin involvement in differentiation, given the difficulty to generate β-actin KO mouse models, is to establish protocols for direct reprogramming of embryonic fibroblasts where the β-actin alleles are not functional. Some of these models for neurogenesis, adipogenesis and osteogenesis proved useful to get initial mechanistic insights on how loss of a nuclear pool of β-actin generally affects differentiation. In combination with advanced genomics including ATAC-seq and HiC-seq to study changes in chromatin accessibility and in the 3D organization of the genome, these differentiation models, including generation of iPSCs, have potential to uncover how gene programs are temporally established during acquisition of cellular identity. Due to their nuclear organization in the form of clusters, mitochondrial genes are particularly interesting models and detailed analysis of how expression of mitochondrial genes is affected upon β-actin depletion using imaging-based high content phenotypic profiling and next generation sequencing promises interesting avenues for future research. The multifunctional nature of the different β-actin pools in cytoplasm, nucleus and mitochondria poses, however, a significant roadblock, and necessitates the identification of novel tools for visualization of individual actin pools in a polymerized or depolymerized form. Superresolution microscopy and CryoEM techniques for structural cell biology may facilitate ultrastructural analysis of actin filament networks particularly in the nucleus and mitochondria while providing a basis to understand how communication between nuclear genome and mitochondrial genome is pursued in the context of cell differentiation and may be dysregulated in metabolic diseases categorized under a general umbrella of 'nuclear and mitochondrial actinopathies'.

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Conflict of Interest
None declared.

References

1. Mar, J.C., Matigian, N.A., Mackay-Sim, A., Mellick, G.D., Sue, C.M., Silburn, P.A., McGrath, J.J., Quackenbush, J., and Wells, C.A. (2011) Variance of gene expression identifies altered network constraints in neurological disease. *PLoS Genet*. **7**, e1002207
2. Chalancon, G., Ravarani, C.N.J., Balaji, S., Martinez-Arias, A., Aravind, L., Jothi, R., and Babu, M.M. (2012) Interplay between gene expression noise and regulatory network architecture. *Trends Genet*. **28**, 221–232
3. Mason, E.A., Mar, J.C., Laslett, A.L., Pera, M.F., Quackenbush, J., Wolvetang, E., and Wells, C.A. (2014) Gene expression variability as a unifying element of the pluripotency network. *Stem Cell Rep*. **3**, 365–377
4. Halley-Stott, R.P., Pasque, V., and Gurdon, J.B. (2013) Nuclear reprogramming. *Development* **140**, 2468–2471
5. Moustakas, A. and Heldin, C.H. (2008) Dynamic control of TGF-β signaling and its links to the cytoskeleton. *FEBS Lett*. **582**, 2051–2065
6. Velasquez, L.S., Sutherland, L.B., Liu, Z., Grinnell, F., Kamm, K.E., Schneider, J.W., Olson, E.N., and Small, E.M. (2013) Activation of MRTF-A–dependent gene expression with a small molecule promotes myofibroblast differentiation and wound healing. *Proc. Natl. Acad. Sci. USA* **110**, 16850–16855
7. Werner, S., Lützkendorf, J., Müller, T., Müller, L.P., and Posern, G. (2019) MRTF-A controls myofibroblastic differentiation of human multipotent stromal cells and their tumour-supporting function in xenograft models. *Sci. Rep*. **9**, 11725
8. Sen, B., Xie, Z., Case, N., Thompson, W.R., Uzer, G., Styner, M., and Rubin, J. (2014) mTORC2 regulates mechanically induced cytoskeletal reorganization and lineage selection in marrow derived mesenchymal stem cells. *J. Bone Miner. Res*. **29**, 78–89
9. Akiyama, T. and Kawasaki, Y. (2006) Wnt signaling and the actin cytoskeleton. *Oncogene* **25**, 7538–7544
10. Nusse, R. and Clevers, H. (2017) Wnt/β-Catenin signaling, disease, and emerging therapeutic modalities. *Cell* **169**, 985–999
11. MacDonald, B.T., Tamai, K., and He, X. (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev. Cell* **17**, 9–26
12. Gamell, C., Osses, N., Bartrons, R., Rückle, T., Camps, M., Rosa, J.L., and Ventura, F. (2008) BMP2 induction of actin cytoskeleton reorganization and cell migration requires PI3-kinase and Cdc42 activity. *J. Cell Sci*. **121**, 3960–3970
13. Kang, Q., Song, W.-X., Luo, Q., Tang, N., Luo, J., Luo, X., Chen, J., Bi, Y., He, B.-C., Park, J.K., Jiang, W., Tang, Y., Huang, J., Su, X., Zhu, G.-H., He, Y., Yin, H., Hu, Z., Wang, Y., Chen, L., Zuo, G.-W., Pan, X., Shen, J., Vokes, T., Reid, R.R., Haydon, R.C., Luu, H.H., and He, T.-C. (2009) A comprehensive analysis of the dual roles of BMPs in regulating adipogenic and osteogenic differentiation of mesenchymal progenitor. *Cells Stem Cells Dev*. **18**, 545–558
14. Pan, Q., Yu, Y., Chen, Q., Li, C., Wu, H., Wan, Y., Ma, J., and Sun, F. (2008) Sox9, a key transcription factor of bone morphogenetic protein-2-induced chondrogenesis, is activated through BMP pathway and a CCAAT box in the proximal promoter. *J. Cell. Physiol*. **217**, 228–241
15. Moparthi, L., Pizzolato, G., and Koch, S. (2019) Wnt activator FOXB2 drives the neuroendocrine differentiation of prostate cancer. *Proc. Natl. Acad. Sci. USA* **116**, 22189–22195
16. Chen, T. and Dent, S.Y. (2014) Chromatin modifiers and remodellers: regulators of cellular differentiation. *Nat. Rev. Genet*. **15**, 93–106
17. Hota, S.K. and Bruneau, B.G. (2016) ATP-dependent chromatin remodeling during mammalian development. *Development* **143**, 2882–2897
18. Filipp, F.V. (2017) Crosstalk between epigenetics and metabolism-Yin and Yang of histone demethylases and methyltransferases in cancer. *Brief Funct. Genom*. **16**, 320–325
19. Wilson, S., Fan, L., Sahgal, N., Qi, J., and Filipp, F.V. (2017) The histone demethylase KDM3A regulates the transcriptional program of the androgen receptor in prostate cancer cells. *Oncotarget* **8**, 30328–30343
20. Filipp, F.V. (2019) Epioncogenes in cancer—identification of epigenomic and transcriptomic cooperation networks by multi-omics integration of ChIP-Seq and RNA-Seq data. *Syst. Biol. Methods Mol. Biol*. **1800**, 101–121
21. Seo, B.J., Yoon, S.H., and Do, J.T. (2018) Mitochondrial dynamics in stem cells and differentiation. *Int. J. Mol. Sci*. **19**, 3893
22. Bonev, B. and Cavalli, G. (2016) Organization and function of the 3D genome. *Nat. Rev. Genet*. **17**, 661–678
23. Zheng, H. and Xie, W. (2019) The role of 3D genome organization in development and cell differentiation. *Nat. Rev. Mol. Cell Biol*. **20**, 535–550
24. Miralles, F., Posern, G., Zaromytidou, A.I., and Treisman, R. (2003) Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113**, 329–342
25. Vartiainen, M.K., Guettler, S., Larijani, B., and Treisman, R. (2007) Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL. *Science* **316**, 1749–1752
26. Vartiainen, M.K. (2008) Nuclear actin dynamics—from form to function. *FEBS Lett*. **582**, 2033–2040
27. Uhler, C. and Shivashankar, G.V. (2017) Chromosome intermingling: mechanical hotspots for genome regulation. *Trends Cell Biol*. **27**, 810–819
28. Perrin, B.J. and Ervasti, J.M. (2010) The actin gene family: function follows isoform. *Cytoskeleton* **67**, 630–634
29. Davidson, A.J. and Wood, W. (2016) Unravelling the actin cytoskeleton: a new competitive edge? *Trends Cell Biol*. **26**, 569–576
30. Clark, T.G. and Merriam, R.W. (1977) Diffusible and bound actin nuclei of *Xenopus laevis* oocytes. *Cell* **12**, 883–891
31. Clark, T.G. and Rosenbaum, J.L. (1979) An actin filament matrix in hand-isolated nuclei of *X. laevis* oocytes. *Cell* **18**, 1101–1108
32. Lane, N.J. (1969) Intranuclear fibrillar bodies in actinomycin D-treated oocytes. *J. Cell Biol*. **40**, 286–291
33. Pederson, T. and Aebi, U. (2002) Actin in the nucleus: what form and what for? *J. Struct. Biol*. **140**, 3–9
34. Percipalle, P., Zhao, J., Pope, B., Weeds, A., Lindberg, U., and Daneholt, B. (2001) Actin bound

to the heterogeneous nuclear ribonucleoprotein hrp36 is associated with Balbiani ring mRNA from the gene to polysomes. *J. Cell Biol.* **153**, 229–236
35. Percipalle, P., Fomproix, N., Kylberg, K., Miralles, F., Bjorkroth, B., Daneholt, B., and Visa, N. (2003) An actin-ribonucleoprotein interaction is involved in transcription by RNA polymerase II. *Proc. Natl. Acad. Sci. USA* **100**, 6475–6480
36. Riedl, J., Crevenna, A.H., Kessenbrock, K., Yu, J.H., Neukirchen, D., Bista, M., Bradke, F., Jenne, D., Holak, T.A., Werb, Z., Sixt, M., and Wedlich-Soldner, R. (2008) Lifeact: a versatile marker to visualize F-actin. *Nat. Methods* **5**, 605–607
37. Plessner, M., Melak, M., Chinchilla, P., Baarlink, C., and Grosse, R. (2015) Nuclear F-actin formation and reorganization upon cell spreading. *J. Biol. Chem.* **290**, 11209–11216
38. Dopie, J., Skarp, K.P., Rajakylä, E.K., Tanhuanpää, K., and Vartiainen, M.K. (2012) Active maintenance of nuclear actin by importin 9 supports transcription. *Proc. Natl. Acad. Sci. USA* **109**, E544–E552
39. Kelpsch, D.J. and Tootle, T.L. (2018) Nuclear actin: from discovery to function. *Anat. Rec.* **301**, 1999–2013
40. Stüven, T., Hartmann, E., and Görlich, D. (2003) Exportin 6: a novel nuclear export receptor that is specific for profilin.actin complexes. *EMBO J.* **22**, 5928–5940
41. Gieni, R.S. and Hendzel, M.J. (2009) Actin dynamics and functions in the interphase nucleus: moving toward an understanding of nuclear polymeric actin. *Biochem. Cell Biol.* **87**, 283–306
42. Hendzel, M.J. (2014) The F-act's of nuclear actin. *Curr. Opin. Cell Biol.* **28**, 84–89
43. Grosse, R. and Vartiainen, M.K. (2013) To be or not to be assembled: progressing into nuclear actin filaments. *Nat. Rev. Mol. Cell Biol.* **14**, 693–697
44. Caridi, C.P., Plessner, M., Grosse, R., and Chiolo, I. (2019) Nuclear actin filaments in DNA repair dynamics. *Nat. Cell Biol.* **21**, 1068–1077
45. Simon, D.N. and Wilson, K.L. (2011) The nucleoskeleton as a genome-associated dynamic 'network of networks'. *Nat. Rev. Mol. Cell Biol.* **12**, 695–708
46. Kristo, I., Bajusz, I., Bajusz, C., Borkuti, P., and Vilmos, P. (2016) Actin, actin-binding proteins, and actin-related proteins in the nucleus. *Histochem. Cell Biol.* **145**, 373–388
47. Almuzzaini, B., Sarshad, A.A., Rahmanto, A.S., Hansson, M.L., Von Euler, A., Sangfelt, O., Visa, N., Farrants, A.O., and Percipalle, P. (2016) In beta-actin knockouts, epigenetic reprogramming and rDNA transcription inactivation lead to growth and proliferation defects. *FASEB J.* **30**, 2860–2873
48. Sokolova, M., Moore, H.M., Prajapati, B., Dopie, J., Meriläinen, L., Honkanen, M., Matos, R.C., Poukkula, M., Hietakangas, V., and Vartiainen, M.K. (2018) Nuclear actin is required for transcription during *Drosophila* oogenesis. *iScience* **9**, 63–70
49. Ye, J., Zhao, J., Hoffmann-Rohrer, U., and Grummt, I. (2008) Nuclear myosin I acts in concert with polymeric actin to drive RNA polymerase I transcription. *Gene Dev.* **22**, 322–330
50. Obrdlik, A. and Percipalle, P. (2011) The F-actin severing protein cofilin-1 is required for RNA polymerase II transcription elongation. *Nucleus* **2**, 72–79
51. Söderberg, E., Hessle, V., von Euler, A., and Visa, N. (2012) Profilin is associated with transcriptionally active genes. *Nucleus* **3**, 290–299
52. Sadhukhan, S., Sarkar, K., Taylor, M., Candotti, F., and Vyas, Y.M. (2014) Nuclear role of WASp in gene transcription is uncoupled from its ARP2/3-dependent cytoplasmic role in actin polymerization. *J. Immunol.* **193**, 150–160
53. Yoo, Y., Wu, X., and Guan, J.L. (2007) A novel role of the actin-nucleating Arp2/3 complex in the regulation of RNA polymerase II-dependent transcription. *J. Biol. Chem.* **282**, 7616–7623
54. Miyamoto, K., Teperek, M., Yusa, K., Allen, G.E., Bradshaw, C.R., and Gurdon, J.B. (2013) Nuclear Wave1 is required for reprogramming transcription in oocytes and for normal development. *Science* **341**, 1002–1005
55. Venit, T., Mahmood, S.R., Endara-Coll, M., and Percipalle, P. (2020) Nuclear actin and myosin in chromatin regulation and maintenance of genome integrity. *Int. Rev. Cell Mol. Biol.* **355**, 67–108
56. de Lanerolle, P. and Serebryannyy, L. (2011) Nuclear actin and myosins: life without filaments. *Nat. Cell Biol.* **13**, 1282–1298
57. Percipalle, P. (2013) Co-transcriptional nuclear actin dynamics. *Nucleus* **4**, 43–52
58. Olave, I.A., Reck-Peterson, S.L., and Crabtree, G.R. (2002) Nuclear actin and actin-related proteins in chromatin remodelling. *Annu. Rev. Biochem.* **71**, 755–781
59. Klages-Mundt, N.L., Kumar, A., Zhang, Y., Kapoor, P., and Shen, X. (2018) The nature of actin-family proteins in chromatin-modifying complexes. *Front. Genet.* **9**, 398
60. Egly, J.M., Miyamoto, N.G., Moncollin, V., and Chambon, P. (1984) Is actin a transcription initiation factor for RNA polymerase B? *EMBO J.* **3**, 2363–2371
61. Scheer, U., Hinssen, H., Franke, W.W., and Jockusch, B.M. (1984) Microinjection of actin-binding proteins and actin antibodies demonstrates involvement of nuclear actin in transcription of lampbrush chromosomes. *Cell* **39**, 111–122
62. Fomproix, N. and Percipalle, P. (2004) An actin-myosin complex on actively transcribing genes. *Exp. Cell Res.* **294**, 140–148
63. Grummt, I. (2006) Actin and myosin as transcription factors. *Curr. Opin. Genet. Dev.* **16**, 191–196
64. Philimonenko, V.V., Zhao, J., Iben, S., Dingova, H., Kysela, K., Kahle, M., Zentgraf, H., Hofmann, W.A., de Lanerolle, P., Hozak, P., and Grummt, I. (2004) Nuclear actin and myosin I are required for RNA polymerase I transcription. *Nat. Cell Biol.* **6**, 1165–1172
65. Visa, N. and Percipalle, P. (2010) Nuclear functions of actin. *Cold Spring Harb. Perspect. Biol.* **2**, a000620
66. Hofmann, W.A., Stojiljkovic, L., Fuchsova, B., Vargas, G.M., Mavrommatis, E., Philimonenko, V., Kysela, K., Goodrich, J.A., Lessard, J.L., Hope, T.J., Hozak, P., and de Lanerolle, P. (2004) Actin is part of pre-initiation complexes and is necessary for transcription by RNA polymerase II. *Nat. Cell Biol.* **6**, 1094–1101
67. Kukalev, A., Nord, Y., Palmberg, C., Bergman, T., and Percipalle, P. (2005) Actin and hnRNP U cooperate for productive transcription by RNA polymerase II. *Nat. Struct. Mol. Biol.* **12**, 238–244
68. Hu, P., Wu, S., and Hernandez, N. (2004) A role for beta-actin in RNA polymerase III transcription. *Genes Dev.* **18**, 3010–3015
69. Pestic-Dragovich, L., Stojiljkovic, L., Philimonenko, A.A., Nowak, G., Ke, Y., Settlage, R.E.,

Shabanowitz, J., Hunt, D.F., Hozak, P., and de Lanerolle, P. (2000) A myosin I isoform in the nucleus. *Science* **290**, 337–341
70. Sarshad, A., Sadeghifar, F., Louvet, E., Mori, R., Böhm, S., Al-Muzzaini, B., Vintermist, A., Fomproix, N., Östlund, A.-K., and Percipalle, P. (2013) Nuclear myosin 1c facilitates the chromatin modifications required to activate rRNA gene transcription and cell cycle progression. *PLoS Genet*. **9**, e1003397
71. Sarshad, A.A., Corcoran, M., Al-Muzzaini, B., Borgonovo-Brandter, L., Von Euler, A., Lamont, D., Visa, N., and Percipalle, P. (2014) Glycogen synthase kinase (GSK) 3β phosphorylates and protects nuclear myosin 1c from proteasome-mediated degradation to activate rDNA transcription in early G1 cells. *PLoS Genet*. **10**, e1004390
72. Almuzzaini, B., Sarshad, A.A., Farrants, A.-K.Ö., and Percipalle, P. (2015) Nuclear myosin 1 contributes to a chromatin landscape compatible with RNA polymerase II transcription activation. *BMC Biol*. **13**, 35
73. Percipalle, P. and Farrants, A.-K. (2006) Chromatin remodelling and transcription: be-WICHed by nuclear myosin 1. *Curr. Opin. Cell Biol*. **18**, 267–274
74. Percipalle, P., Fomproix, N., Cavellan, E., Voit, R., Reimer, G., Kruger, T., Thyberg, J., Scheer, U., Grummt, I., and Farrants, A.K. (2006) The chromatin remodelling complex WSTF-SNF2h interacts with nuclear myosin 1 and has a role in RNA polymerase I transcription. *EMBO Rep*. **7**, 525–530
75. Sarshad, A.A. and Percipalle, P. (2014) New insight into role of myosin motors for activation of RNA polymerases. *Int. Rev. Cell Mol. Biol*. **311**, 183–230
76. Venit, T., Semesta, K., Farrukh, S., Endara-Coll, M., Havalda, R., Hozak, P., and Percipalle, P. (2020) Nuclear myosin 1 activates p21 gene transcription in response to DNA damage through a chromatin-based mechanism. *Commun. Biol*. **3**, 115
77. Percipalle, P., Jonsson, A., Nashchekin, D., Karlsson, C., Bergman, T., Guialis, A., and Daneholt, B. (2002) Nuclear actin is associated with a specific subset of hnRNP A/B-type proteins. *Nucleic Acids Res*. **30**, 1725–1734
78. Raju, C.S., Göritz, C., Nord, Y., Hermanson, O., López-Iglesias, C., Visa, N., Castelo-Branco, G., and Percipalle, P. (2008) In cultured oligodendrocytes the A/B-type hnRNP CBF-A accompanies MBP mRNA bound to mRNA trafficking sequences. *Mol. Biol. Cell* **19**, 3008–3019
79. Raju, C.S., Fukuda, N., López-Iglesias, C., Göritz, C., Visa, N., and Percipalle, P. (2011) In neurons, activity-dependent association of dendritically transported mRNA transcripts with the transacting factor CBF-A is mediated by A2RE/RTS elements. *Mol. Biol. Cell* **22**, 1864–1877
80. Fukuda, N., Fukuda, T., Sinnamon, J., Hernandez-Hernandez, A., Izadi, M., Raju, C.S., Czaplinski, K., and Percipalle, P. (2013) The transacting factor CBF-A/Hnrnpab binds to the A2RE/RTS element of protamine 2 mRNA and contributes to its translational regulation during mouse spermatogenesis. *PLoS Genet*. **9**, e1003858
81. Obrdlik, A., Kukalev, A., Louvet, E., Farrants, A.-K., Caputo, L., and Percipalle, P. (2008) The histone acetyltransferase PCAF associates with actin and hnRNP U for RNA polymerase II transcription. *Mol. Cell. Biol*. **28**, 6342–6357
82. Vignali, M., Hassan, A.H., Neely, K.E., and Workman, J.L. (2000) ATP-dependent chromatin-remodeling complexes. *Mol. Cell. Biol*. **20**, 1899–1910
83. Xie, X., Almuzzaini, B., Drou, N., Kremb, S., Yousif, A., Farrants, A.O., Gunsalus, K., and Percipalle, P. (2018) β-Actin-dependent global chromatin organization and gene expression programs control cellular identity. *FASEB J*. **32**, 1296–1314
84. Kapoor, P., Chen, M., Winkler, D.D., Luger, K., and Shen, X. (2013) Evidence for monomeric actin function in INO80 chromatin remodeling. *Nat. Struct. Mol. Biol*. **20**, 426–432
85. Shen, X., Mizuguchi, G., Hamiche, A., and Wu, C. (2000) A chromatin remodelling complex involved in transcription and DNA processing. *Nature* **406**, 541–544
86. Brahma, S., Ngubo, M., Paul, S., Udugama, M., and Bartholomew, B. (2018) The Arp8 and Arp4 module acts as a DNA sensor controlling INO80 chromatin remodeling. *Nat. Commun*. **9**, 3309
87. Choi, K., Park, C., Lee, J., Oh, M., Noh, B., and Lee, I. (2007) Arabidopsis homologs of components of the SWR1 complex regulate flowering and plant development. *Development* **134**, 1931–1941
88. Harata, M., Oma, Y., Mizuno, S., Jiang, Y.W., Stillman, D.J., and Wintersberger, U. (1999) The nuclear actin-related protein of *Saccharomyces cerevisiae*, Act3p/Arp4, interacts with core histones. *Mol. Biol. Cell* **10**, 2595–2605
89. Zhao, K., Wang, W., Rando, O.J., Xue, Y., Swiderek, K., Kuo, A., and Crabtree, G.R. (1998) Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **95**, 625–636
90. Rando, O.J., Zhao, K., Janmey, P., and Crabtree, G.R. (2002) Phospatidylinositol-dependent actin filament binding by the SWI/SNF-like BAF chromatin remodelling complex. *Proc. Natl. Acad. Sci. USA* **99**, 2824–2829
91. Papoulas, O., Beek, S.J., Moseley, S.L., McCallum, C.M., Sarte, M., Shearn, A., and Tamkun, J.W. (1998) The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. *Development* **125**, 3955–3966
92. He, S., Wu, Z., Tian, Y., Yu, Z., Yu, J., Wang, X., Li, J., Liu, B., and Xu, Y. (2020) Structure of nucleosome-bound human BAF complex. *Science* **367**, 875–881
93. Xie, X. and Percipalle, P. (2018) An actin-based nucleoskeleton involved in gene regulation and genome organization. *Biochem. Biophys. Res. Commun*. **506**, 378–386
94. Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., Sandstrom, R., Bernstein, B., Bender, M.A., Groudine, M., Gnirke, A., Stamatoyannopoulos, J., Mirny, L.A., Lander, E.S., and Dekker, J. (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289–293
95. Rao, S.S., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., Robinson, J.T., Sanborn, A.L., Machol, I., Omer, A.D., Lander, E.S., and Aiden, E.L. (2014) A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**, 1665–1680
96. Illingworth, R.S. (2019) Chromatin folding and nuclear architecture: PRC1 function in 3D. *Curr. Opin. Genet. Dev*. **55**, 82–90

97. Stam, M., Tark-Dame, M., and Fransz, P. (2019) 3D genome organization: a role for phase separation and loop extrusion? *Curr. Opin. Plant Biol.* **48**, 36–46
98. Wei, M., Fan, X., Ding, M., Li, R., Shao, S., Hou, Y., Meng, S., Tang, F., Li, C., and Sun, Y. (2020) Nuclear actin regulates inducible transcription by enhancing RNA polymerase II clustering. *Sci. Adv.* **6**, eaay6515
99. Alexander, J.M., Hota, S.K., He, D., Thomas, S., Ho, L., Pennacchio, L.A., and Bruneau, B.G. (2015) Brg1 modulates enhancer activation in mesoderm lineage commitment. *Development* **142**, 1418–1430
100. Barutcu, A.R., Lajoie, B.R., Fritz, A.J., McCord, R.P., Nickerson, J.A., van Wijnen, A.J., Lian, J.B., Stein, J.L., Dekker, J., Stein, G.S., and Imbalzano, A.N. (2016) SMARCA4 regulates gene expression and higher-order chromatin structure in proliferating mammary epithelial cells. *Genome Res.* **26**, 1188–1201
101. Wang, X., Lee, R.S., Alver, B.H., Haswell, J.R., Wang, S., Mieczkowski, J., Drier, Y., Gillespie, S.M., Archer, T.C., Wu, J.N., Tzvetkov, E.P., Troisi, E.C., Pomeroy, S.L., Biegel, J.A., Tolstorukov, M.Y., Bernstein, B.E., Park, P.J., and Roberts, C.W.M. (2017) SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation. *Nat. Genet.* **49**, 289–295
102. Kadoch, C., Williams, R.T., Calarco, J.P., Miller, E.L., Weber, C.M., Braun, S.M., Pulice, J.L., Chory, E.J., and Crabtree, G.R. (2017) Dynamics of BAF-Polycomb complex opposition on heterochromatin in normal and oncogenic states. *Nat. Genet.* **49**, 213–222
103. Bantignies, F., Roure, V., Comet, I., Leblanc, B., Schuettengruber, B., Bonnet, J., Tixier, V., Mas, A., and Cavalli, G. (2011) Polycomb-dependent regulatory contacts between distant Hox Loci in *Drosophila*. *Cell* **144**, 214–226
104. Fan, H., Lv, P., Huo, X., Wu, J., Wang, Q., Cheng, L., Liu, Y., Tang, Q.Q., Zhang, L., Zhang, F., Zheng, X., Wu, H., and Wen, B. (2018) The nuclear matrix protein HNRNPU maintains 3D genome architecture globally in mouse hepatocytes. *Genome Res.* **28**, 192–202
105. Gohring, F. and Fackelmayer, F.O. (1997) The scaffold/matrix attachment region binding protein hnRNP-U (SAF-A) is directly bound to chromosomal DNA in vivo: a chemical cross-linking study. *Biochemistry* **36**, 8276–8283
106. Fackelmayer, F.O., Dahm, K., Renz, A., Ramsperger, U., and Richter, A. (1994) Nucleic-acid-binding properties of hnRNP-U/SAF-A, a nuclear-matrix protein which binds DNA and RNA in vivo and in vitro. *Eur. J. Biochem.* **221**, 749–757
107. Weirich, K.L., Banerjee, S., Dasbiswas, K., Witten, T.A., Vaikuntanathan, S., and Gardel, M.L. (2017) Liquid behavior of cross-linked actin bundles. *Proc. Natl. Acad. Sci. USA* **114**, 2131–2136
108. Sztacho, M., Sobol, M., Balaban, C., Escudeiro Lopes, S.E., and Hozák, P. (2019) Nuclear phosphoinositides and phase separation: important players in nuclear compartmentalization. *Adv. Biol. Regul.* **71**, 111–117
109. Xie, X., Mahmood, S.R., Gjorgjieva, T., and Percipalle, P. (2020) Emerging roles of cytoskeletal proteins in regulating gene expression and genome organization during differentiation. *Nucleus* **11**, 53–65
110. Bohnsack, M.T., Stuven, T., Kuhn, C., Cordes, V.C., and Gorlich, D. (2006) A selective block of nuclear actin export stabilizes the giant nuclei of *Xenopus* oocytes. *Nat. Cell Biol.* **8**, 257–263
111. Gall, J.G. and Wu, Z. (2010) Examining the contents of isolated *Xenopus* germinal vesicles. *Methods* **51**, 45–51
112. Miyamoto, K. and Gurdon, J.B. (2013) Transcriptional regulation and nuclear reprogramming: roles of nuclear actin and actin-binding proteins. *Cell. Mol. Life Sci.* **70**, 3289–33302
113. Miyamoto, K., Pasque, V., Jullien, J., and Gurdon, J.B. (2011) Nuclear actin polymerization is required for transcriptional reprogramming of Oct4 by oocytes. *Genes Dev.* **25**, 946–958
114. Yamazaki, S., Yamamoto, K., Tokunaga, M., Sakata-Sogawa, K., and Harata, M. (2015) Nuclear actin activates human transcription factor genes including the OCT4 gene. *Biosci. Biotechnol. Biochem.* **79**, 242–246
115. Yamazaki, S., Yamamoto, K., de Lanerolle, P., and Harata, M. (2016) Nuclear F-actin enhances the transcriptional activity of beta-catenin by increasing its nuclear localization and binding to chromatin. *Histochem. Cell Biol.* **145**, 389–399
116. Nishimoto, N., Watanabe, M., Watanabe, S., Sugimoto, N., Yugawa, T., Ikura, T., Koiwai, O., Kiyono, T., and Fujita, M. (2012) Heterocomplex formation by Arp4 and beta-actin is involved in the integrity of the Brg1 chromatin remodeling complex. *J. Cell Sci.* **125**, 3870–3882
117. Ho, L., Ronan, J.L., Wu, J., Staahl, B.T., Chen, L., Kuo, A., Lessard, J., Nesvizhskii, A.I., Ranish, J., and Crabtree, G.R. (2009) An embryonic stem cell chromatin remodeling complex, esBAF is essential for embryonic stem cell self-renewal and pluripotency. *Proc. Natl. Acad. Sci. USA* **106**, 5181–5186
118. Singhal, N., Esch, D., Stehling, M., and Scholer, H.R. (2014) BRG1 is required to maintain pluripotency of murine embryonic stem cells. *Biores. Open Access* **3**, 1–8
119. King, H.W. and Klose, R.J. (2017) The pioneer factor OCT4 requires the chromatin remodeller BRG1 to support gene regulatory element function in mouse embryonic stem cells. *eLife* **6**, e22631
120. Miller, E.L., Hargreaves, D.C., Kadoch, C., Chang, C.Y., Calarco, J.P., Hodges, C., Buenrostro, J.D., Cui, K., Greenleaf, W.J., Zhao, K., and Crabtree, G.R. (2017) TOP2 synergizes with BAF chromatin remodeling for both resolution and formation of facultative heterochromatin. *Nat. Struct. Mol. Biol.* **24**, 344–352
121. Singhal, N., Graumann, J., Wu, G., Araúzo-Bravo, M.J., Han, D.W., Greber, B., Gentile, L., Mann, M., and Schöler, H.R. (2010) Chromatin-remodeling components of the BAF complex facilitate reprogramming. *Cell* **141**, 943–955
122. Okuno, T., Li, W.Y., Hatano, Y., Takasu, A., Sakamoto, Y., Yamamoto, M., Ikeda, Z., Shindo, T., Plessner, M., Morita, K., Matsumoto, K., Yamagata, K., Grosse, R., and Miyamoto, K. (2020) Zygotic nuclear F-actin safeguards embryonic development. *Cell Rep.* **31**, 107824
123. Xu, Y.Z., Thuraisingam, T., Morais, D.A., Rola-Pleszczynski, M., and Radzioch, D. (2010) Nuclear translocation of beta-actin is involved in transcriptional regulation during macrophage differentiation of HL-60 cells. *Mol. Biol. Cell* **21**, 811–820
124. Le, H.Q., Ghatak, S., Yeung, C.Y., Tellkamp, F., Gunschmann, C., Dieterich, C., Yeroslaviz, A., Habermann, B., Pombo, A., Niessen, C.M., and Wickstrom, S.A. (2016) Mechanical regulation of transcription controls Polycomb-mediated gene silencing during lineage commitment. *Nat. Cell Biol.* **18**, 864–875

125. Sen, B., Xie, Z., Uzer, G., Thompson, W.R., Styner, M., Wu, X., and Rubin, J. (2015) Intranuclear actin regulates osteogenesis. *Stem Cells* **33**, 3065–3076
126. Shmerling, D., Danzer, C.P., Mao, X., Boisclair, J., Haffner, M., Lemaistre, M., Schuler, V., Kaeslin, E., Korn, R., Burki, K., Ledermann, B., Kinzel, B., and Muller, M. (2005) Strong and ubiquitous expression of transgenes targeted into the beta-actin locus by Cre/lox cassette replacement. *Genesis* **42**, 229–235
127. Tondeleir, D., Lambrechts, A., Muller, M., Jonckheere, V., Doll, T., Vandamme, D., Bakkali, K., Waterschoot, D., Lemaistre, M., Debeir, O., Decaestecker, C., Hinz, B., Staes, A., Timmerman, E., Colaert, N., Gevaert, K., Vandekerckhove, J., and Ampe, C. (2012) Cells lacking beta-actin are genetically reprogrammed and maintain conditional migratory capacity. *Mol. Cell. Proteomics* **11**, 255–271
128. Xie, X., Jankauskas, R., Mazari, A.M.A., Drou, N., and Percipalle, P. (2018) β-Actin regulates a heterochromatin landscape essential for optimal induction of neuronal programs during direct reprograming. *PLoS Genet*. **14**, e1007846
129. Al-Sayegh, M.A., Mahmood, S.R., Abul Khair, S.B., Xie, X., El Gindi, M., Kim, T., Almansoori, A., and Percipalle, P. (2020) β-actin contributes to an open chromatin for activation of the adipogenic pioneer factor CEBPA during transcriptional reprograming. *Mol. Biol. Cell* **31**, 2495–2629
130. Gjorgjieva, T., Xie, X., Commins, P., Pasricha, R., Mahmood, S.R., Gunsalus, K.C., Naumov, P., and Percipalle, P. (2020) Loss of β-actin leads to accelerated mineralization and dysregulation of osteoblast-differentiation genes during osteogenic reprogramming. *Adv. Sci.* **2020**, 2002261
131. Lee, J.H., Park, A., Oh, K.-J., Lee, S.C., Kim, W.K., and Bae, K.-H. (2019) The role of adipose tissue mitochondria: regulation of mitochondrial function for the treatment of metabolic diseases. *Int. J. Mol. Sci.* **20**, 4924
132. Rogers, C.D., Moody, S.A., and Casey, E.S. (2009) Neural induction and factors that stabilize a neural fate. *Birth Defect. Res. C* **87**, 249–262
133. Matsumoto, S., Banine, F., Struve, J., Xing, R., Adams, C., Liu, Y., Metzger, D., Chambon, P., Rao, M.S., and Sherman, L.S. (2006) Brg1 is required for murine neural stem cell maintenance and gliogenesis. *Dev. Biol.* **289**, 372–383
134. Seo, S., Richardson, G.A., and Kroll, K.L. (2004) The SWI/SNF chromatin remodeling protein Brg1 is required for vertebrate neurogenesis and mediates transactivation of Ngn and NeuroD. *Development* **132**, 105–115
135. Zhang, Z., Cao, M., Chang, C.W., Wang, C., Shi, X., Zhan, X., Birnbaum, S.G., Bezprozvanny, I., Huber, K.M., and Wu, J.I. (2016) Autism-associated chromatin regulator Brg1/SmarcA4 is required for synapse development and myocyte enhancer factor 2-mediated synapse remodeling. *Mol. Cell. Biol.* **36**, 70–83
136. Linhart, H.G., Ishimura-Oka, K., DeMayo, F., Kibe, T., Repka, D., Poindexter, B., Bick, R.J., and Darlington, G.J. (2001) C/EBPalpha is required for differentiation of white, but not brown, adipose tissue. *Proc. Natl. Acad. Sci. USA* **98**, 12532–12537
137. Boldogh, I.R. and Pon, L.A. (2006) Interactions of mitochondria with the actin cytoskeleton. *Biochim. Biophys. Acta* **1763**, 450–462
138. Senning, E.N. and Marcus, A.H. (2010) Actin polymerization driven mitochondrial transport in mating *S. cerevisiae*. *Proc. Natl. Acad. Sci. USA* **107**, 721–725
139. Reyes, A., He, J., Mao, C.C., Bailey, L.J., Di Re, M., Sembongi, H., Kazak, L., Dzionek, K., Holmes, J.B., Cluett, T.J., Harbour, M.E., Fearnley, I.M., Crouch, R.J., Conti, M.A., Adelstein, R.S., Walker, J.E., and Holt, I.J. (2011) Actin and myosin contribute to mammalian mitochondrial DNA maintenance. *Nucleic Acids Res.* **39**, 5098–5108
140. Xie, X., Venit, T., Drou, N., and Percipalle, P. (2018d) In mitochondria β-actin regulates mtDNA transcription and is required for mitochondrial quality control. *iScience* **3**, 226–237
141. Calvo, S.E., Clauser, K.R., and Mootha, V.K. (2016) MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res.* **44**, D1251–D1257
142. Pearce, S.F., Rebelo-Guiomar, P., D'Souza, A.R., Powell, C.A., Van Haute, L., and Minczuk, M. (2017) Regulation of mammalian mitochondrial gene expression: recent advances. *Trends Biochem. Sci.* **42**, 625–639
143. Boczonadi, V. and Horvath, R. (2014) Mitochondria: impaired mitochondrial translation in human disease. *Int. J. Biochem. Cell Biol.* **48**, 77–84
144. Van Haute, L., Pearce, S.F., Powell, C.A., D'Souza, A.R., Nicholls, T.J., and Minczuk, M. (2015) Mitochondrial transcript maturation and its disorders. *J. Inherit. Metab. Dis.* **38**, 655–680
145. Ali, A.T., Boehme, L., Carbajosa, G., Seitan, V.C., Small, K.S., and Hodgkinson, A. (2019) Nuclear genetic regulation of the human mitochondrial transcriptome. *eLife* **8**, e41927
146. Herbers, E., Kekalainen, N.J., Hangas, A., Pohjoismaki, J.L., and Goffart, S. (2019) Tissue specific differences in mitochondrial DNA maintenance and expression. *Mitochondrion* **44**, 85–92
147. Jokinen, R., Marttinen, P., Sandell, H.K., Manninen, T., Teerenhovi, H., Wai, T., Teoli, D., Loredo-Osti, J.C., Shoubridge, E.A., and Battersby, B.J. (2010) Gimap3 regulates tissue-specific mitochondrial DNA segregation. *PLoS Genet*. **6**, e1001161
148. Mootha, V.K., Bunkenborg, J., Olsen, J.V., Hjerrild, M., Wisniewski, J.R., Stahl, E., Bolouri, M.S., Ray, H.N., Sihag, S., Kamal, M., Patterson, N., Lander, E.S., and Mann, M. (2003) Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* **115**, 629–640
149. Wachsmuth, M., Hubner, A., Li, M., Madea, B., and Stoneking, M. (2016) Age-related and heteroplasmy-related variation in human mtDNA copy number. *PLoS Genet*. **12**, e1005939
150. De Grassi, A., Lanave, C., and Saccone, C. (2008) Genome duplication and gene-family evolution: the case of three OXPHOS gene families. *Gene* **421**, 1–6
151. Dhar, S.S., Ongwijitwat, S., and Wong-Riley, M.T. (2009) Chromosome conformation capture of all 13 genomic Loci in the transcriptional regulation of the multisubunit bigenomic cytochrome C oxidase in neurons. *J. Biol. Chem.* **284**, 18644–18650
152. Boonrungsiman, S., Gentleman, E., Carzaniga, R., Evans, N.D., McComb, D.W., Porter, A.E., and Stevens, M.M. (2012) The role of intracellular calcium phosphate in osteoblast-mediated bone apatite formation. *Proc. Natl. Acad. Sci. USA* **109**, 14170–14175
153. Pei, D.D., Sun, J.L., Zhu, C.H., Tian, F.C., Jiao, K., Anderson, M.R., Yiu, C., Huang, C., Jin, C.X., Bergeron, B.E., Chen, J.H., Tay, F.R., and Niu, L.N. (2018) Contribution of mitophagy to cell-mediated

mineralization: revisiting a 50-year-old conundrum. *Adv. Sci.* **5**, 1800873
154. Roman, B.S., Steenbergen, C., and Das, S. (2019) The secret messages between mitochondria and nucleus in muscle cell biology. *Arch. Biochem. Biophys.* **666**, 52–62
155. Gitai, Z., Dye, N.A., Reisenauer, A., Wachi, M., and Shapiro, L. (2005) MreB actin-mediated segregation of a specific region of a bacterial chromosome. *Cell* **120**, 329–341
156. Kruse, T. and Gerdes, K. (2005) Bacterial DNA segregation by the actin-like MreB protein. *Trends Cell Biol.* **15**, 343–345
157. Caridi, C.P., D'Agostino, C., Ryu, T., Zapotoczny, G., Delabaere, L., Li, X., Khodaverdian, V.Y., Amaral, N., Lin, E., Rau, A.R., and Chiolo, I. (2018) Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. *Nature* **559**, 54–60
158. Schrank, B.R., Aparicio, T., Li, Y., Chang, W., Chait, B.T., Gundersen, G.G., Gottesman, M.E., and Gautier, J. (2018) Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. *Nature* **559**, 61–66
159. Dundr, M., Ospina, J.K., Sung, M.H., John, S., Upender, M., Ried, T., Hager, G.L., and Matera, A.G. (2007) Actin-dependent intranuclear repositioning of an active gene locus in vivo. *J. Cell Biol.* **179**, 1095–1103
160. Wang, A., Kolhe, J.A., Gioacchini, N., Baade, I., Brieher, W.M., Peterson, C.L., and Freeman, B.C. (2020) Mechanism of long-range chromosome motion triggered by gene activation. *Dev. Cell* **52**, 309320.e5