

Review

Insights into the Relationship between Nucleolar Stress and the NF- κ B PathwayJingyu Chen¹ and Lesley A. Stark^{1,2,*} 

The nuclear organelle the nucleolus and the transcription factor nuclear factor of κ -light-chain-enhancer of activated B cells (NF- κ B) are both central to the control of cellular homeostasis, dysregulated in common diseases and implicated in the ageing process. Until recently, it was believed that they acted independently to regulate homeostasis in health and disease. However, there is an emerging body of evidence suggesting that nucleoli and NF- κ B signalling converge at multiple levels. Here we will review current understanding of this crosstalk. We will discuss activation of the NF- κ B pathway by nucleolar stress and induction of apoptosis by nucleolar sequestration of NF- κ B/RelA. We will also discuss the role of TIF-IA, COMMD1, and nucleophosmin, which are key players in this crosstalk, and the therapeutic relevance, particularly with respect to the antitumour effects of aspirin.

Nucleoli–NF- κ B–Nucleoli: An Emerging Signalling Network

There are many parallels between nucleoli and the nuclear factor of κ -light-chain-enhancer of activated B cells (NF- κ B) transcription factor. For example, both are stimulated by the same plethora of stresses (Table 1), both regulate the same downstream physiological processes, both are dysregulated in a variety of common diseases which contributes to disease aetiology, and both are implicated in senescence and ageing [1–6]. Until recently, it was believed that these factors act independently to regulate cellular homeostasis in health, ageing, and disease. However, there is a growing body of evidence to suggest that nucleolar stress activates the NF- κ B pathway, and that nucleolar sequestration of NF- κ B components is a critical apoptotic regulator. Here we will review the various levels of convergence between NF- κ B and the nucleolus. We will also discuss the relevance of nucleoli–NF- κ B crosstalk to the antitumour mechanisms of aspirin and the potential relevance to senescence and ageing.

The Multifunctional Nucleolus

The nucleolus is a highly dynamic, multifunctional, subnuclear organelle [3,4,7]. Its most established role is as the hub of ribosome biogenesis, the process by which rRNA is transcribed, cleaved, and assembled into ribosomes (Figure 1). This process is the most energy consuming in the cell and as such, is tightly linked to metabolic and proliferative activity. The starting point, and arguably the rate-limiting step, is transcription of ribosomal DNA (rDNA) by the RNA polymerase I (PolI) preinitiation complex (PIC). This complex includes upstream binding factor (UBF), which acts as derepressor and coactivator; SL1 proteins, which confer promotor specificity; and TIF-IA (gene name *RRN3*), which is essential as it tethers PolI to the rDNA promoter [8]. The output of rDNA transcription is the 47S preribosomal RNA transcript, which is cotranscriptionally matured through nucleolytic processing and assembly with ribosomal proteins and cofactors, to eventually generate the 40S and 60S ribosomal subunits [9].

In addition to its traditional role in ribosome biogenesis, the nucleolus plays a critical role in processes such as signal recognition particle assembly [10], pre-tRNA maturation [11], telomerase assembly [12], ribonucleoprotein biogenesis [13], and even organization of the epigenome

Highlights

Nucleoli play a critical role in maintaining cellular homeostasis in health, ageing, and disease, as does the NF- κ B transcription factor pathway. Emerging data suggest that interplay between these two cellular factors regulates cell growth and death.

An atypical nucleolar stress response pathway, characterized by degradation of the PolI complex component, TIF-IA, and nucleolar enlargement, lies upstream of NF- κ B pathway activation.

Components of the NF- κ B complex accumulate in nucleoli in response to stress. The presence of RelA in nucleoli triggers cytoplasmic relocation of nucleophosmin which mediates apoptosis by transporting BAX to mitochondria.

The chemopreventive agent aspirin triggers TIF-IA–NF- κ B signalling in human colorectal tumours, which is important for its antitumour activity.

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Table 1. Stress Stimuli Disrupt Nucleoli and Activate NF- κ B^a

Stress type	Stimulus	Activation of NF- κ B	Refs	Nucleolar disruption	Refs
DNA damage/genotoxic stress	UV-C	Yes	[109,110]	Yes	[63,111–113]
	Gamma irradiation	Yes		Yes	
	Camptothecin	Yes	[109,114]	Yes	
	Bleomycin	Yes		Yes	
Oxidative stress	Reactive oxygen intermediate	Yes	[115]	Yes	[116,117]
	Hypoxia	Yes	[118]	Yes	
Chemotherapeutic/chemopreventive agents	Doxorubicin	Yes	[119]	Yes	[120]
	Etoposide	Yes	[114]		[112]
	Aspirin/nonsteroidal anti-inflammatory drugs	Yes	[81,82]	Yes	[1]
Nutrient stress	Serum starvation	Yes	[81]	Yes	[54]
Oncogenic stress	Ras/BCR-ABL	Yes	[2]	Yes	[121]

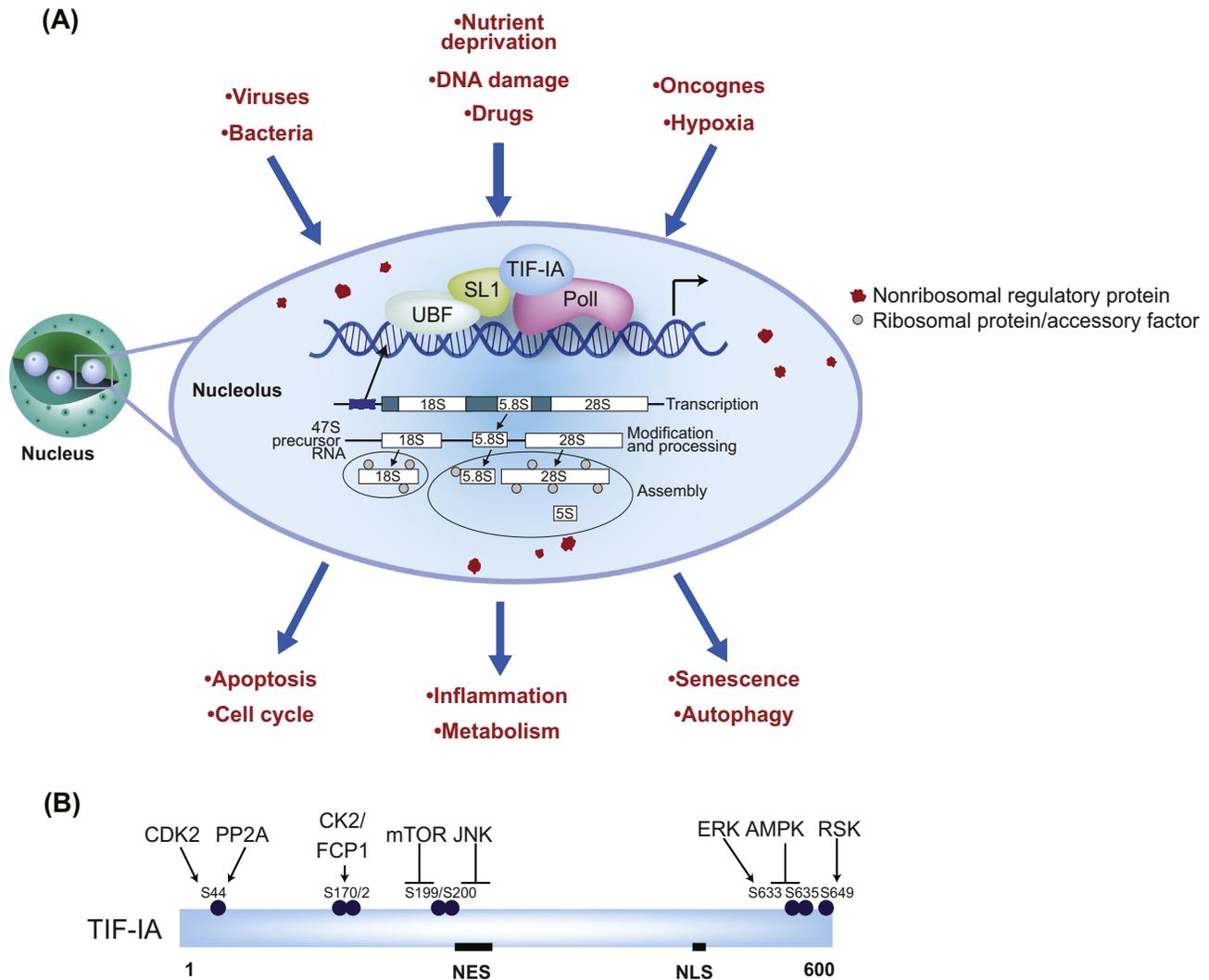
^aAdapted from [1].

[14,15]. Another key role for nucleoli is in the coordination of the cellular stress response (Figure 1) [3,16]. If cellular homeostasis is disrupted, for example, by nutrient deprivation, exposure to cytotoxic agents, viral infection, or oncogene inactivation, PolI-driven transcription is rapidly downregulated and a cascade of signalling events is triggered that influences cell physiology. This process is broadly termed nucleolar stress and can take many forms, dependent on cell context and the nature of the insult [14,17]. Outcomes associated with nucleolar stress include differentiation, cell cycle arrest, autophagy, DNA repair, senescence, and apoptosis [4,16,18,19].

The paradigm of nucleolar stress response is inhibition of rDNA transcription leading to stabilization of p53 [20,21]. While this is undoubtedly an important mechanism by which the organelle regulates cellular homeostasis, there are multiple lines of evidence indicating nucleoli can control apoptosis, senescence, and autophagy in a p53-independent manner [19,22–24]. New research has revealed activation of the NF- κ B pathway as an alternative key mechanism by which nucleolar stress regulates cell growth and death.

Nucleolar Stress and Activation of the NF- κ B Pathway

NF- κ B is a family of highly conserved, inducible transcription factors that play a critical role not only in innate and adaptive immunity, but also in stress response and the maintenance of cellular homeostasis [2,25]. The family comprise five members, namely, RelA (p65), RelB, c-Rel, p105/p50 (NF- κ B1), and p100/p52 (NF- κ B2). All members have a Rel homology domain which allows dimerization, translocation to the nucleus, and DNA binding, while only RelA, RelB, and C-Rel have the ability to drive transcription. The most abundant form of NF- κ B, and the primary mediator of NF- κ B-dependent stress response, is the RelA:P50 heterodimer. In resting cells, this complex is held in the cytoplasm by the inhibitor of NF- κ B (I κ B) protein, I κ B α [26,27]. When the cell is exposed to a wide array of stimuli, for example, inflammatory cytokines, bacterial pathogens, cytotoxic agents, DNA damaging agents, nutrient deprivation, hypoxia, and physical insult, I κ B α is phosphorylated and targeted for degradation by the 26S proteasome. This un masks the nuclear localization signal on RelA, allowing NF- κ B dimers to translocate to the nucleus and influence expression of over 150 target genes [28]. These genes control a broad range of physiological processes including proliferation, differentiation, senescence, cell cycle progression, and apoptosis.



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Figure 1. The Multifunctional Nucleolus. (A) Schematic demonstrating the nucleolus as the hub of ribosome biogenesis and as a critical regulator of cellular homeostasis. Ribosome biogenesis starts with transcription of the 47S pre-rRNA transcript by the Poll preinitiation complex (inset). The 47S transcript is cleaved and packaged to eventually generate the 40S and 60S ribosomal subunits. The function of the nucleolus is altered by a plethora of environmental and oncogenic stresses. This change in nucleolar function alters numerous cellular processes so that the cell can recover, or if the damage is too great, undergo cell death. There are thousands of regulatory proteins within nucleoli that have roles beyond ribosome biogenesis. It is believed to be the dynamic flux of these proteins to the nucleoplasm/cytoplasm following nucleolar stress that stimulates downstream signalling networks. (B) TIF-IA is targeted by multiple kinases and phosphatases dependent on environmental conditions, which controls the transcriptional output of the preinitiation complex and nucleolar function. Abbreviations: AMPK, AMP-activated protein kinase; CDK, cyclin-dependent kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; mTOR, mammalian target of rapamycin; NES, nuclear export signal. NLS, nuclear localization signal; Poll, RNA polymerase I; RSK, ribosomal s6 kinase; UBF, upstream binding factor.

A number of upstream pathways have been described that lead to degradation of I κ B. The most established of these is the canonical pathway. This is induced by classic NF- κ B stimuli [such as tumour necrosis factor alpha (TNF α)] and is characterized by rapid phosphorylation of I κ B α by the inhibitor of κ B kinase (IKK) complex [27]. By contrast, stress stimuli generally induce I κ B phosphorylation/degradation with a delayed and slow kinetic. Specific mechanisms have been proposed for this delayed response [29–33]. However, how multiple heterogeneous stresses converge on the NF- κ B pathway has been far from clear. Intuitively, given the parallels between

stresses that disrupt ribosome biogenesis and those that activate NF- κ B (Table 1), nucleolar disruption could provide a unifying mechanism. Indeed, a number of nucleolar proteins are known to regulate NF- κ B signalling. Furthermore, an atypical form of nucleolar stress has recently been shown to lie upstream of NF- κ B pathway activation.

Nucleolar Proteins Regulate NF- κ B Signalling

The nucleolar proteome contains over 4500 proteins that shuttle dynamically between this and other cellular compartments, depending on cell context [34]. Several studies have shown the importance of such nucleolar proteins in the regulation of NF- κ B signalling, especially in response to stress. For example, casein kinase type 2 (CK2), which is bound to TIF-IA in the Poll complex [35], has previously been shown to phosphorylate I κ B α in response to UV-C, potentiating NF- κ B pathway activation [30,36]. Similarly, phosphorylation of eIF2 α in response to endoplasmic reticulum stress can both inhibit Poll activity [37] and activate NF- κ B [31,32]. The nucleolar protein p14^{ARF}, which sequesters MDM2 in the nucleolus to regulate p53 stability [38], interacts with RelA and inhibits NF- κ B-driven transcription [39]. NIK (NF- κ B-inducing kinase), which acts upstream of the IKK complex to stimulate NF- κ B signalling [40], is a nucleolar shuttling protein [41], as is NF- κ B repressing factor (NKRF), which modulates rRNA processing in response to heat stress and represses NF- κ B-driven transcription [42,43]. Finally, ribosomal proteins themselves, when in ribosome-free form, can regulate NF- κ B signalling. The ribosomal protein L3 prevents the degradation of I κ B upon 5-fluorouracil treatment, thus repressing NF- κ B activity [44,45], while S3 promotes activity by interacting with NF- κ B complexes in the nucleus [46]. Hence, there are a number of signalling pathways by which stress-mediated nucleolar disruption could alter NF- κ B nuclear translocation and transcriptional activity.

Specific Nucleolar Disruption and Activation of the NF- κ B Pathway

The first direct evidence that disruption of nucleolar homeostasis may activate NF- κ B signalling came from studies showing that depletion of the multifunctional nucleolar protein, nucleophosmin (NPM, B23), induces degradation of I κ B and nuclear translocation of RelA in colon cancer cells [47]. Depletion of the small nucleolar RNA host gene 15 (*SNHG15*) has also been shown to influence NF- κ B signalling in a renal cell carcinoma model [48]. More recently, small interfering (si)RNA depletion of the Poll complex components UBF, TIF-IA, or RPA194 (a component of Poll) was shown to induce degradation of I κ B α , S536 phosphorylation of RelA (a marker for activation), nuclear translocation of RelA, increased NF- κ B transcriptional activity, and increased transcription of NF- κ B target genes in multiple cell types [1]. Interestingly, this effect was not mimicked by actinomycin D, CX5461, or BMH-21 which block Poll transcription initiation (CX5461 and BMH-21) and elongation (Actinomycin D) [49,50]. Hence, unlike p53 nucleolar stress response, the link between nucleolar disruption and NF- κ B activation is independent of rDNA transcription [21].

TIF-IA–NF- κ B Nucleolar Stress Response

Recent studies, aimed at further exploration of nucleoli–NF- κ B crosstalk, uncovered a novel pathway by which nucleolar function is altered by stress and revealed this atypical nucleolar stress pathway lies upstream of NF- κ B signalling [1]. This new pathway is centred around the PIC component TIF-IA.

TIF-IA is the master regulator of Poll-driven transcription [35,51]. Not only is it essential for anchoring Poll to the rDNA promoter, it is also the component of the PIC that transduces environmental signals to the Poll transcriptional machinery. Upon exposure of cells to exogenous stresses, TIF-IA is targeted by a complex network of kinases and phosphatases including mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), extracellular signal-regulated kinase (ERK), and protein phosphatase 2A (PP2A), which activate or inactivate the protein to fine-tune the transcriptional output [51–54]. The consequences of TIF-IA inactivation are context

dependent but in general, its loss leads to cell cycle arrest and apoptosis, confirming the importance of this protein as a critical regulator of cellular homeostasis [55–57].

Multiple stress stimuli of NF- κ B, including aspirin, UV-C, and the second messenger ceramide, not only alter the phosphorylation status of TIF-IA, but also induce degradation of the protein [1]. This effect is not observed in response to TNF α or the DNA damaging agent camptothecin, indicating specificity. The mechanism underlying stress-mediated TIF-IA degradation is complex as it involves both proteasomal and lysosomal pathways. It is independent of MDM2, the E3 ligase reported to be responsible for basal TIF-IA turnover [58], suggesting it is distinct. Indeed, stress-mediated TIF-IA degradation is dependent on dephosphorylation of TIF-IA at serine 44 (S44) and the Poll complex-associated factors UBF and p14ARF (Figure 2, Key Figure).

Cyclin-dependent kinase 4 (CDK4) is a key cell cycle protein that is inhibited in response to many stress stimuli [59]. It is also known to phosphorylate UBF to regulate rDNA transcription [60]. It was found that small-molecule inhibitors of CDK4 induce TIF-IA degradation by an identical mechanism to that utilized by stress agents, that is, dependent on UBF, P14ARF, and dephosphorylation of TIF-IA at S44 [1]. Based on these data, it was concluded that stress-mediated CDK4 inhibition lies upstream of TIF-IA degradation (Figure 2).

On exploration of the downstream consequences of TIF-IA degradation, it was noted that this precedes degradation of I κ B α and nuclear translocation of RelA/NF- κ B, suggesting a potential link. Indeed, blocking degradation of TIF-IA, using specific siRNAs and dominant negative UBF and TIF-IA mutants, blocked the effects of specific stresses and CDK4 inhibitors on the NF- κ B pathway (Figure 2) [1]. These data revealed a novel TIF-IA–NF- κ B nucleolar stress axis.

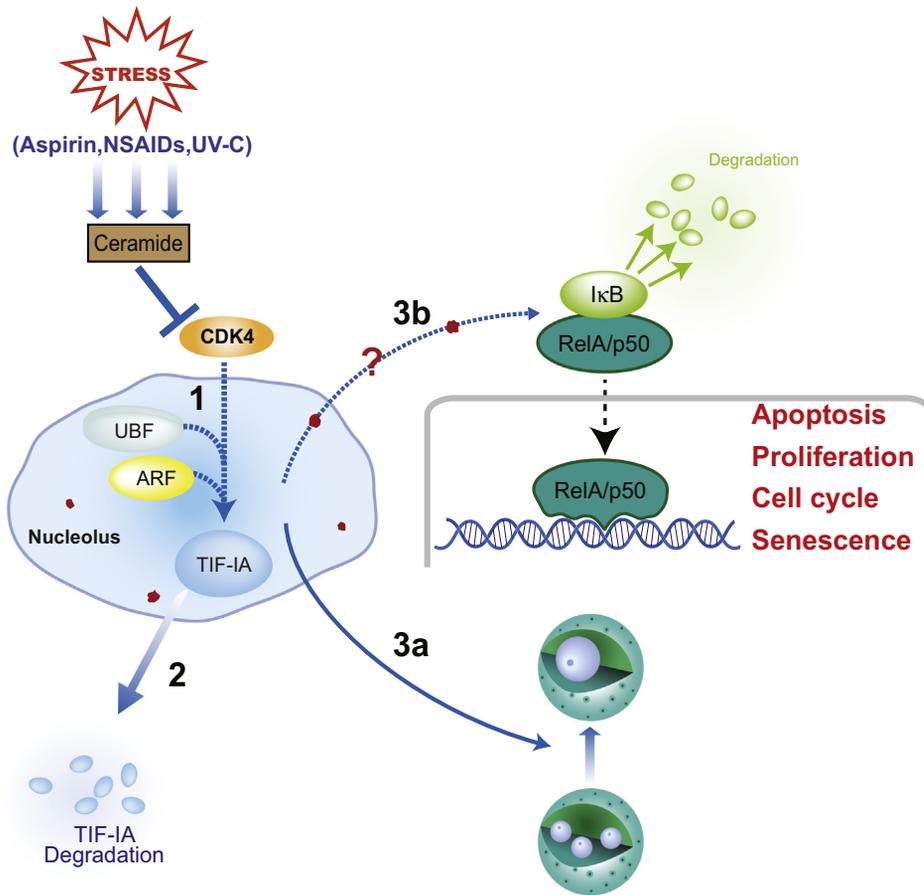
This novel nucleolar stress axis has now been observed in multiple mammalian cell types and in human colorectal tumours treated *ex vivo* with the chemopreventive agent aspirin, suggesting broad and *in vivo* relevance (see later) [1]. Although the signalling networks that link TIF-IA degradation to NF- κ B pathway activation are not yet clear, it has been proposed that release of NF- κ B regulatory proteins from the nucleolus to the nucleoplasm/cytoplasm upon TIF-IA degradation is responsible. Further research in this area will help us fully understand how both nucleoli and NF- κ B regulate cellular homeostasis under normal and stress conditions.

TIF-IA, Nucleolar Enlargement, and Activation of the NF- κ B Pathway

The nucleolus is a tripartite structure having three morphologically distinct subcompartments: the fibrillar centre (FC), the dense fibrillar component (DFC), and the granular component (GC) [14,61]. This structure is maintained by transcription of active rDNA and by liquid–liquid phase separation of nucleolar components. It is highly dynamic and can dramatically change in size and architecture depending on cellular environment [62,63]. Under conditions of stress, or if rDNA transcription is inhibited, the FC and the DFC migrate to the periphery of the organelle and form nucleolar caps in a process known as nucleolar segregation.

In general, nucleolar segregation is associated with a reduction in nucleolar size and phosphorylation-mediated inactivation of TIF-IA has been implicated in this effect [51,54,56]. However, in contrast to phosphorylation-mediated inactivation, degradation of TIF-IA causes a striking increase in nucleolar size, alongside inhibition of rDNA transcription and segregation of nucleolar components (Figure 3) [1]. The mechanism underlying the connection between TIF-IA loss and increased nucleolar size is currently unclear. However, an increase in nucleolar volume, associated with altered rRNA transcription and nucleolar architecture, has also been observed in response to the proteasome inhibitor MG132 [64] and the NEDD8 inhibitor MLN4924 [65]. These

Key Figure

TIF-IA–NF- κ B Nucleolar Stress Pathway.

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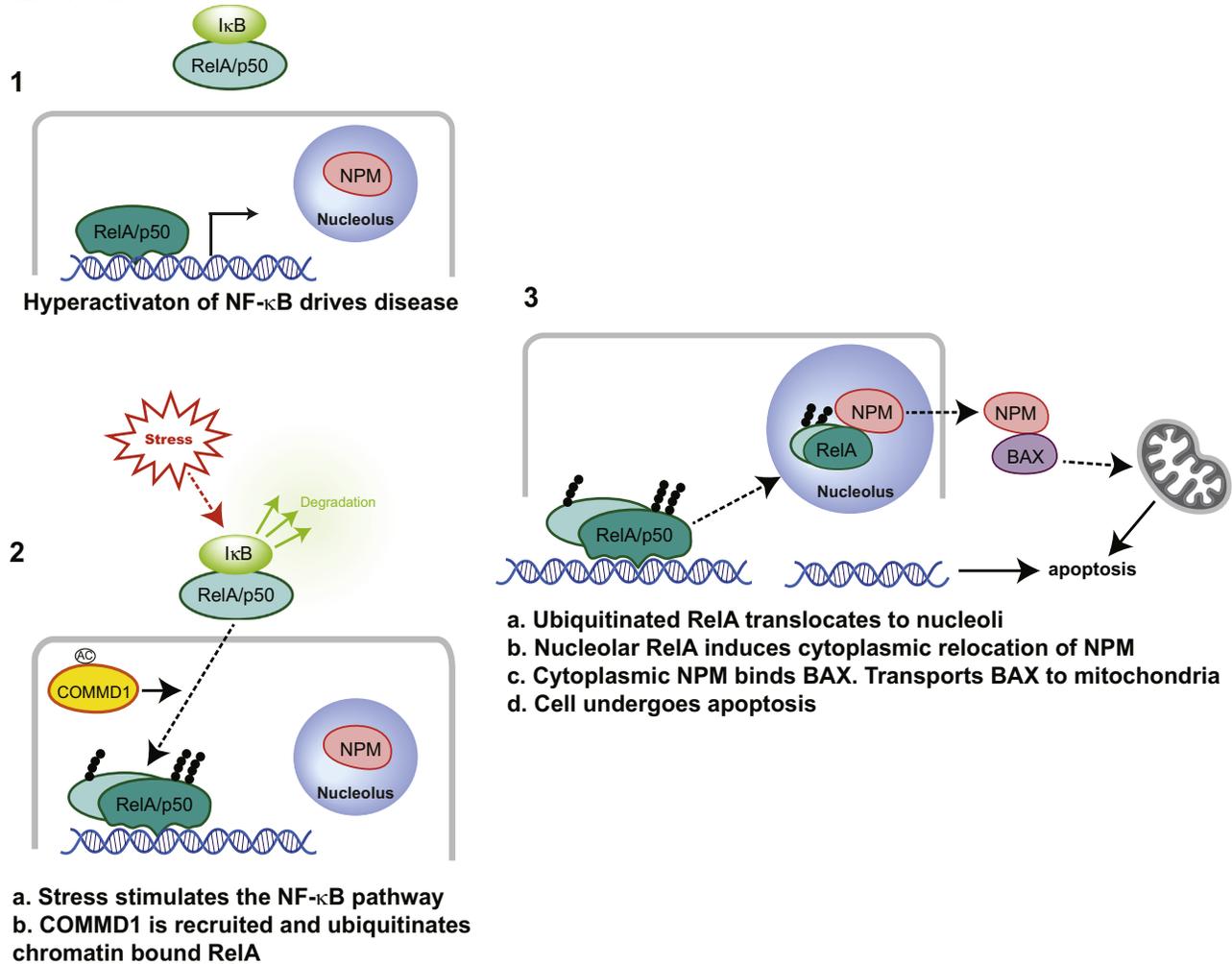
Figure 2. (1 and 2) When cells are exposed to specific stimuli, a cascade of events is triggered that leads to degradation of TIF-IA in a manner dependent on inhibition of CDK4, recruitment of the nucleolar protein p14ARF, and the Poll complex component UBF. This TIF-IA degradation has two consequences: (3a) a reduction in nucleolar number/increase in nucleolar size and (3b) degradation of I κ B α . I κ B α degradation allows NF- κ B to translocate to the nucleus and regulate expression of target genes that control cellular processes such as apoptosis, proliferation, differentiation, and senescence. We speculate that disruption of the Poll complex causes specific NF- κ B regulatory proteins (red spots) to translocate to the cytoplasm to activate NF- κ B signalling. These factors remain unknown. Abbreviations: ARF, p14ARF; CDK, cyclin-dependent kinase; I κ B, inhibitor of NF- κ B; NF- κ B, nuclear factor of κ -light-chain-enhancer of activated B cells; NSAID, nonsteroidal anti-inflammatory drug; UBF, upstream binding factor.

data question the long-standing paradigm that nucleolar size is linked to the rate of rDNA transcription. They also reveal a connection between TIF-IA, enlarged nucleoli, and activation of the NF- κ B pathway.

Nucleolar Size and NF- κ B in Senescence and Ageing

One other scenario where enlargement of nucleoli is associated with activation of the NF- κ B pathway is in cellular senescence: a state of permanent cell cycle arrest that plays an important role in

Cancer cell



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Figure 3. Nucleolar Sequestration of RelA Triggers a Nucleophosmin (NPM) Dependent, Nucleolar Apoptotic Pathway. (1) In diseased cells there is a cytoplasmic, inducible pool of NF-κB and a nuclear pool that drives transcription of antiapoptotic, progrowth, and proinflammatory genes. (2) Exposure to specific stress stimuli causes degradation of IκB and nuclear translocation of NF-κB complexes. It also causes acetylation of COMMD1, which is recruited by induced NF-κB and facilitates ubiquitination of chromatin-bound RelA. (3) This ubiquitination causes nucleolar translocation of the protein and reduced NF-κB-driven transcription. The nucleolar presence of RelA triggers cytoplasmic relocation of NPM. Here it binds BAX and transports BAX to the mitochondria to mediate apoptosis. Abbreviations: IκB, inhibitor of NF-κB; NF-κB, nuclear factor of κ-light-chain-enhancer of activated B cells.

development, tissue remodelling, cancer, and ageing [66]. A striking characteristic of senescence is a large increase in nucleolar size and a reduction in nucleolar number [67]. Another striking characteristic is increased NF-κB activity, which induces transcription of the inflammatory factors that constitute the senescence-associated secretory phenotype (SASP) [68]. SASP is essential for senescence as it reinforces cell cycle arrest, leads to paracrine senescence, and promotes senescence surveillance [68]. Although SASP induction occurs in parallel with increased nucleolar size and reduced number, it is currently unknown whether, in this context, nucleolar enlargement and NF-κB pathway activation are linked. However, recent studies suggest that this may be a possibility [67]. It has been shown that overexpression of TIF-1A in IMR90 primary human fibroblasts not only caused an increase in nucleolar size, but also induced SASP factors in an NF-κB-dependent manner. Nucleolar enlargement has recently been identified as a hallmark of

ageing tissue [69,70], as is chronic activation of the NF- κ B pathway [5]. It is now of considerable interest to determine whether these events are linked in ageing and senescence and whether TIF-1A plays a role in this process.

Nucleolar Sequestration of NF- κ B Proteins Regulates Cell Growth and Death

Cellular stress causes not only a dynamic flux of regulatory proteins out of nucleoli, but also sequestration of such proteins into the organelle [71–73]. This nucleolar sequestration has emerged as an important mechanism for maintaining cellular homeostasis. It can regulate gene expression, impact nuclear structure, modulate specific apoptotic pathways, and influence autophagy. It is particularly prevalent under conditions of proteotoxic stress (e.g., proteasome inhibition) when multiple proteins (e.g., p53, LC3II, sumoylated, and ubiquitinated proteins) translocate into the organelle and accumulate in structures termed nucleolar aggresomes or cavities [74–77].

Although the main switch in NF- κ B activation is cytoplasmic release from I κ B, once in the nucleus, NF- κ B proteins are modulated by a plethora of coactivators, repressors, and post-transcriptional modifications [78]. These nuclear regulatory pathways are important because they determine the genes that are activated or repressed by a specific signal and hence, the downstream consequences on cell physiology. A growing body of evidence suggests that nucleolar sequestration of NF- κ B proteins is one such nuclear regulatory mechanism. For example, the anti-TNF therapy, infliximab, induces ‘massive’ nucleolar localization of p50 in the hippocampus of rats with a portacaval shunt [79]. This nucleolar localization is associated with a decrease in transcription of NF- κ B target genes and a reduction in neuroinflammation. p50 has also been shown to be sequestered in nucleoli in a gastric cancer cell line [80]. The most striking evidence that nucleolar sequestration of NF- κ B proteins regulates NF- κ B activity comes from work on RelA. A large body of evidence indicates RelA is sequestered in the nucleolus in response to stress. Furthermore, it has been shown that once in nucleoli, RelA triggers a cascade of events that actively promotes apoptosis.

Nucleolar Sequestration of RelA

When investigating nuclear regulation of NF- κ B activity, it was noted that RelA is differentially distributed within nuclei dependent on the stimulus [81]. In response to classic stimuli such as TNF, the protein is distributed within the nucleoplasm, excluded from nucleoli (Figure 3). By contrast, it is localized to nucleoli in response to stress stimuli such as aspirin, UV-C radiation, and serum starvation [81]. A nucleolar localization signal (NoLS) was identified at the N terminal of RelA and, using a dominant negative mutant deleted for this motif, it was shown that nucleolar sequestration of RelA is causally involved in repression of basal NF- κ B-driven transcription and the induction of apoptosis.

Since these original observations, nucleolar sequestration of RelA has been observed in a number of other models. The nonsteroidal anti-inflammatory drugs sulindac, sulindac sulfone, and indomethacin have been shown to induce nucleolar translocation of RelA in colon cancer cell lines [82]. This translocation was dependent on the NoLS and was causally involved in the apoptotic effects of the agents. Similar results have been shown for a small-molecule CDK4 inhibitor [59,83] and the proteasome inhibitors MG132 and lactacystin [84]. The antitumour agent 2-methoxyestradiol (a naturally occurring derivative of estradiol) [85], the potent Trk inhibitor and antitumour agent K252a [86], and expression of the homeobox transcription factor Hox-A5 [87] have also been shown to induce nucleolar translocation of RelA associated with the induction of apoptosis.

It should be noted that most (although not all) of these studies have been carried out in colon cancer cell lines and others have failed to observe this phenomenon in other cell types exposed to

similar stresses. Hence, it is likely that nucleolar translocation of RelA is context dependent. Understanding the molecular signals that regulate the nuclear distribution of RelA would be highly advantageous as it may allow the protein to be targeted to nucleoli in medical conditions driven by hyperactivation of the NF- κ B pathway (discussed later). These signals remain unclear. However, the multifunctional protein COMMD1 is emerging as an important determinant [84,88]. It was shown that COMMD1 levels increase in response to specific stresses, that the protein facilitates the ubiquitination of RelA, and that RelA ubiquitination is essential for nucleolar translocation of the protein [84]. It has subsequently been shown that TNF and the stress agent aspirin have differential effects on the acetylation status of COMMD1, which could explain the differential distribution of RelA in response to these stimuli [88]. Interestingly, aspirin-mediated nucleoplasmic to nucleolar translocation of RelA is blocked in the presence of the transcriptional inhibitor, Actinomycin D [81]. Since transcription of COMMD1 does not change in response to aspirin [88], these data would suggest that additional factors may be involved.

RelA–Nucleophosmin Signalling

On exploring the mechanisms by which nucleolar RelA mediates apoptosis, a role for the multifunctional phosphoprotein nucleophosmin (NPM, B23) was uncovered (Figure 3, Step 3). This protein is predominantly nucleolar and plays a critical role in ribosome biogenesis [89]. However, it can also be found in both the nucleoplasm and the cytoplasm and is known to regulate multiple signalling networks [90]. In terms of NF- κ B, NPM has been shown to act as a coactivator at specific genes sites and to be involved in activation of the NF- κ B pathway in response to specific stimuli [91–94].

It was originally presumed that nucleolar RelA causes apoptosis because the protein is sequestered away from promoters of antiapoptotic genes (Figure 3). However, using a nucleolar targeting construct (RelA fused to the NoLS of the HIV-rev protein) it was shown that specifically targeting RelA to this compartment induces apoptosis [95]. It is now known that once in the nucleolus, RelA causes NPM to relocate to the cytoplasm, bind BAX, and then transport BAX to the mitochondria to initiate apoptosis [95–98].

Therapeutic Relevance of Nucleoli–NF- κ B Crosstalk: A Role in the Antitumour Activity of Aspirin

Aberrant nucleolar activity is causally associated with progression of many common diseases [4,99,100]. For example, hyperactivation induced by oncogenes and tumour suppressors contributes to cancer progression by allowing rapid protein synthesis and through dysregulation of nucleolar stress pathways [16,49]. Numerous studies implicate impairment of rDNA transcription in the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's disease while structural changes to nucleoli are evident in ischemic heart disease [47,100,101]. Nucleolar size and number also significantly correlate with longevity in a number of animal models [69,70]. Indeed, it now appears that proper dynamic control of nucleolar activity is crucial for maintaining tissue homeostasis and health. Similarly, aberrant NF- κ B activity is a common event in cancer, neurodegenerative disorders, and ageing and contributes to the aetiology of these conditions by activating progrowth/antiapoptotic genes, promoting a chronic inflammatory state and inducing senescence [2,5,102,103]. Further investigations into the links between nucleoli and NF- κ B may allow the development of therapeutics that target both pathways simultaneously. One agent that has already been shown to act through nucleolar–NF- κ B crosstalk is aspirin.

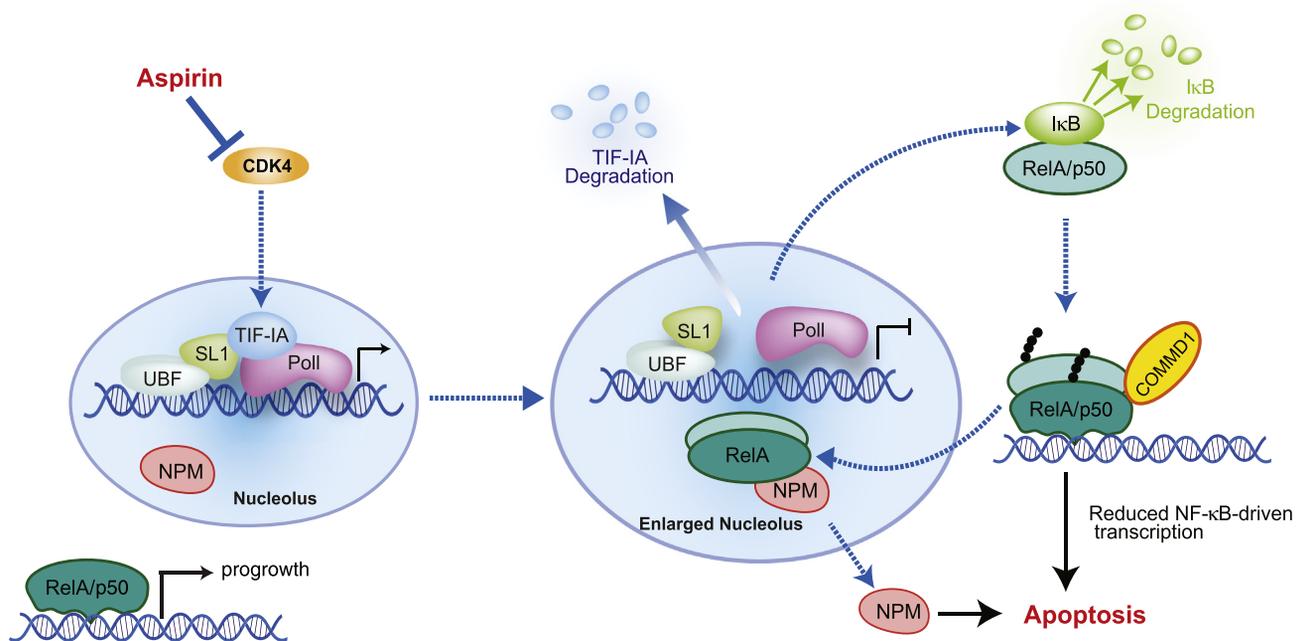
Overwhelming evidence indicates that aspirin and related agents have considerable antitumour activity and the potential to prevent colorectal and other cancers [104,105]. Epidemiological and experimental evidence also suggests that aspirin use prevents against neurodegenerative

disorders such as Alzheimer's disease [106]. However, aspirin cannot be recommended for prevention purposes due to its side effect profile.

In experiments aimed at understanding the mechanism of action of aspirin against colorectal cancer, it was found that the agent causes degradation of TIF-IA and inhibition of rDNA transcription [1]. Furthermore, it was shown that this degradation is causally linked to stimulation of the NF- κ B pathway and consequently, sequestration of RelA to nucleoli, repression of NF- κ B activity, and apoptosis (Figure 4) [81,95,107].

A link between TIF-IA degradation and NF- κ B signalling was observed in multiple colon cancer cells lines, in cell lines derived from human premalignant intestinal lesions, and in four of seven human tumours treated *ex vivo* with low doses of the agent, suggesting pharmacological relevance [1]. These data reveal a novel and exciting mechanism of action of aspirin that warrants further investigation not only in cancer models, but also in models of other diseases associated with dysregulation of NF- κ B and nucleolar pathways.

In addition to aspirin, a small-molecule CDK4 inhibitor has been shown to induce degradation of TIF-IA, which is causally linked to stimulation of the NF- κ B pathway and the induction of apoptosis (Figure 2) [1,83]. These data reveal another class of agents that simultaneously inhibit rDNA transcription and NF- κ B activity. Responses to aspirin are generally restricted to colon cancer cells [108]. By contrast, CDK4 inhibition caused TIF-IA-dependent NF- κ B pathway stimulation in multiple cell types, suggesting crosstalk between these pathways can be broadly targeted [1].



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Figure 4. Nucleoli-NF- κ B Crosstalk in Aspirin Response. High levels of Poll- and NF- κ B-driven transcription drive colon cancer progression. Exposure to the antitumour agent aspirin causes TIF-IA degradation, which inhibits rDNA transcription and stimulates cytoplasmic to nuclear translocation of RelA. This induced NF- κ B recruits factors to chromatin-bound NF- κ B which target RelA to the nucleolus. Nucleolar sequestration of RelA causes apoptosis of diseased cells by switching off NF- κ B activity and triggering relocation of nucleophosmin (NPM) from nucleoli. Abbreviations: CDK, cyclin-dependent kinase; I κ B, inhibitor of NF- κ B; NF- κ B, nuclear factor of κ -light-chain-enhancer of activated B cells; Poll, RNA polymerase I; UBF, upstream binding factor.

Concluding Remarks and Future Directions

In the past two decades, the nucleolus has emerged as a multifunctional organelle, regulating processes that go well beyond its traditional role in ribosome biogenesis. One of its more recently identified functions is as a central coordinator of stress response. Although it remains unclear how the organelle choreographs specific cellular outcomes following exposure to specific stresses, there is an intriguing and growing body of evidence to suggest that links between nucleoli and the NF- κ B pathway play a key role. However, this field is still in its infancy. Evidence for a link between TIF-IA, nucleolar size, and NF- κ B pathway activation is compelling, but further research is essential to understand the molecular signals that drive the relationship, the cellular contexts in which it is evident, and whether it plays a role in senescence and ageing. Similarly, it is important to understand the pathways responsible for nucleolar sequestration of NF- κ B and other proteins so that these can be exploited for therapeutic purpose. Given the number of common ageing disorders associated with dysfunction of both nucleoli and NF- κ B, we speculate that crosstalk between these pathways is a contributing factor. In the future, it will be fascinating to molecularly deconstruct the nucleolar–NF- κ B signalling network to understand exactly how this network is regulated in normal cells, and whether dysfunction contributes to disease (see Outstanding Questions).

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Outstanding Questions

What are the signalling networks that link degradation of TIF-IA to increased nucleolar size and activation of the NF- κ B pathway? Is NF- κ B pathway activation a consequence of changes in nucleolar number/structure? What are the biological contexts in which this signalling network is relevant? Does it play a role in ageing and senescence? Understanding the molecular links between PolI complex disruption and NF- κ B pathway activation could open exciting new opportunities to intervene therapeutically in pathologies associated with dysfunction of both nucleoli and NF- κ B.

How is NF- κ B modulated by acetylated COMMD1 to allow nucleolar translocation? What are the carriers that transport ubiquitinated RelA and other proteins to nucleoli under conditions of proteotoxic stress? What does RelA do in nucleoli to trigger cytoplasmic release of nucleophosmin? Defining the mechanisms that regulate the nuclear distribution of RelA may allow the development of agents that target it to this cellular compartment to switch off aberrant NF- κ B activity.

Can TIF-IA degradation and/or nucleolar size be used as a marker for aspirin response in colorectal cancer? Given the side effects of aspirin, defining patients with premalignant or early stage cancers that would respond to the agent would reap great rewards as it would allow it to be targeted to a population where the benefits outweigh the risks.

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