

Review

Kaspar Burger and Dirk Eick*

Functional ribosome biogenesis is a prerequisite for p53 destabilization: impact of chemotherapy on nucleolar functions and RNA metabolism

Abstract: The production and processing of ribosomal RNA is a complex and well-coordinated nucleolar process for ribosome biogenesis. Progress in understanding nucleolar structure and function has led to the unexpected discovery of the nucleolus as a highly sensitive sensor of cellular stress and an important regulator of the tumor suppressor p53. Inhibition of ribosomal RNA metabolism has been shown to activate a signaling pathway for p53 induction. This review elucidates the potential of classical and recently developed chemotherapeutic drugs to stabilize p53 by inhibiting nucleolar functions.

Keywords: chemotherapy; kinase inhibitor; ribosome biogenesis; rRNA processing, tumor suppressor p53.

***Corresponding author: Dirk Eick,** Department of Molecular Epigenetics, Helmholtz Zentrum München and Center for Integrated Protein Science Munich (CIPSM), Marchioninistrasse 25, 81377 Munich, Germany, e-mail: eick@helmholtz-muenchen.de
Kaspar Burger: Department of Molecular Epigenetics, Helmholtz Zentrum München and Center for Integrated Protein Science Munich (CIPSM), Marchioninistrasse 25, 81377 Munich, Germany

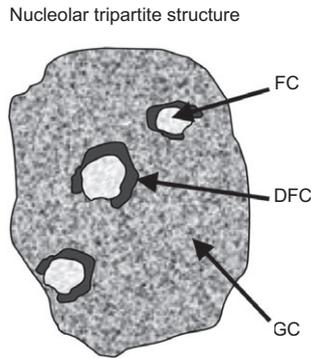
Ribosome biogenesis takes place in the nucleolus

Ribosomes in eukaryotes are synthesized and assembled in a specialized nuclear organelle, the nucleolus (Brown and Gurdon, 1964; Miller and Beatty, 1969). In human cells, nucleoli form around nucleolar organizer regions (NORs) on acrocentric chromosomes 13, 14, 15, 21, and 22 as membrane-free sub-cellular structures. In total, NORs contain about 400 tandemly repeated ribosomal (r)DNA genes coding for ribosomal (r)RNA (Perry, 1962; Ritossa and Spiegelman, 1965). Ribosome biogenesis emerged as one of the most complex and controlled cellular processes that consumes up to 80% of the cellular energy in proliferating cells (Thomas, 2000; Prieto and McStay, 2008). Mammalian

nucleoli display a tripartite structure (Figure 1) consisting of the fibrillar centre (FC), the dense fibrillar component (DFC), and the granular component (GC). Distinct steps in ribosome biogenesis can be allocated to one of the three components of the tripartite structure. Transcription of the primary 47S rRNA by RNA polymerase (RNAP) I takes place at the interface of FC and DFC and requires several essential transcription factors: transcription initiation factor 1A (TIF-1A), the selectivity factor 1 (SL1) complex, upstream binding factor (UBF), and transcription termination factor 1 (TTF-1). These factors regulate start site selection, recruitment of RNAPI to and escape from the promoter (Schneider, 2012). The first processing steps of the 47S rRNA precursor occur in the pre-90S ribosome and involve a cleavage cascade and stepwise production of intermediate forms of rRNAs, which finally mature to 18S, 5.8S, and 28S rRNA (Hadjiolova et al., 1993) (Figure 2). Processing of rRNA is orchestrated by the interplay of >200 factors, which catalyse snoRNA-mediated rRNA modification, and cleavage and trimming of rRNA by endo- and exonucleases, respectively. Finally, 40S and 60S ribosomal subunits are exported to the cytoplasm to translate messenger (m)RNA. The ribosome maturation process is described in more detail in Figure 2. Recent findings suggest that ribosomes are more than just machines that translate mRNA. Protein composition of ribosomes and/or mutations in ribosomal proteins can alter gene regulation and tissue development. For example, a mutation in ribosomal protein Rpl38 reduces the translational efficacy for Hox factors in a tissue-specific manner (Kondrashov et al., 2011). Great heterogeneity in ribosomal protein expression suggests the existence of ribosomes, which are specialized for translation of mRNAs in a tissue-specific manner (Xue and Barna, 2012).

Ribosome biogenesis in cancer cells

Ribosome biogenesis is intimately connected to cell growth and proliferation and up- or down-regulated



FC: Fibrillar centre

DFC: Dense fibrillar component

GC: Granular component

Figure 1 Scheme of nucleolar structures. Nucleoli comprise a tripartite structure including fibrillar centres (FC), dense fibrillar components (DFC), and granular components (GC). Analysis of protein and RNA compositions show that distinct steps of ribosome biogenesis can be allocated to distinct nucleolar components. rDNA transcription takes place at the interface between FC and DFC and produces large amounts of unprocessed rRNA precursors. Processing and modification of rRNA mainly takes place in DFC. Most ribosomal proteins are present in GC, the major site of ribosome subunit assembly.

dependent on growth factor stimulation or nutrient availability. Changes in ribosome biogenesis rates also probably have major implications for tumor development (Ruggero and Pandolfi, 2003; White, 2005). Activation of proto-oncogenes and inactivation of tumor suppressor genes strongly up-regulate transcription and processing rates for rRNAs (Drygin et al., 2010; Hannan et al., 2011). For example, the proto-oncogene *c-Myc*, the casein kinase II (CK II), the extracellular signal-regulated kinase (ERK), or the PI3K (phosphatidylinositol-3-kinase)-AKT-mTOR (mammalian target of rapamycin) axis promote transcription and/or processing of rRNA and are frequently activated in cancer cells (Boon et al., 2001; Schuhmacher et al., 2001; Schlosser et al., 2003). However, it is still unclear, whether up-regulation of ribosome biogenesis in cancer cells reflects the cause of tumorigenesis or the consequence of elevated growth and proliferation. Recent findings support the first theory.

Mutations in genes encoding ribosomal proteins have been described for various hematologic disorders with cancer predisposition (Narla and Ebert, 2010; van Riggelen et al., 2010). Ribosomal genes *RPL5* and *RPL11* are frequently mutated in T-cell lymphoblastic leukemia (De Keersmaecker et al., 2013). RNAPI transcription factors UBF and TIF-1A are already increased in pre-malignant cells, suggesting that up-regulation of ribosome

biogenesis may drive tumor formation and is not simply a consequence of tumor progression (Ruggero, 2012).

The nucleolus as sensor and integrator of cellular stress

Damage of DNA and interference with gene expression are key characteristics of mutagens such as viruses, irradiation, cytotoxic drugs, and other environmental hazards like heat shock or hypoxia. How can cells integrate such a variety of different stresses? Stabilization of the tumor suppressor p53 and its ability to induce cell cycle arrest, senescence, or apoptosis has been known for a long time to be central for the cellular stress response (Levine, 1997; Vousden and Prives, 2009). However, a comprehensive model of how to integrate various stresses and funnel them to p53 has been lacking. A hallmark study defined the nucleolus as universal stress integrator (Rubbi and Milner, 2003). Using micropore UV-irradiation, Rubbi and Milner could show that damaging of nucleolar, but not nucleoplasmatic DNA is necessary and sufficient to induce p53. Interestingly, cells can tolerate high amounts of nucleoplasmatic irradiation and DNA damage as long as lesions are excluded from rDNA. Further experiments showed that inhibition of the RNAPI transcription machinery by anti-UBF antibodies triggered p53 stabilization in the absence of DNA damage. The knockout of RNAPI transcription factor TIF-1A results in retarded development of embryos and increased rates of apoptosis (Yuan et al., 2005). TIF-1A knockout destroys the integrity of nucleoli, stabilizes p53 and induces apoptosis. Similar results were obtained after depletion of rRNA processing factors Utp18, Bop1, Pes1, WDR12 or Nucleostemin (Pestov et al., 2001; Holzel et al., 2005; Romanova et al., 2009; Holzel et al., 2010b). In agreement with these observations, p53 levels show cell cycle-specific changes. The levels of p53 are low during interphase, but enhanced in mitosis and early G1-phase as long as the nucleoli are disassembled (David-Pfeuty, 1999). Taken together, several lines of evidence indicate that the integrity of the nucleolus is a prerequisite for low levels of p53. The high liability of the nucleolus to stress elevates p53 levels.

But how does the nucleolus respond to stress mechanistically? The E3-ubiquitin ligase Hdm2 is the key enzyme that regulates p53 levels. Hdm2 continuously binds and ubiquitinates p53, thereby targeting p53 for proteasomal degradation (Stommel et al., 1999; Ljungman, 2000; Xirodimas et al., 2001). For its ubiquitination and degradation, p53 requires the transit through a functionally intact nucleolus

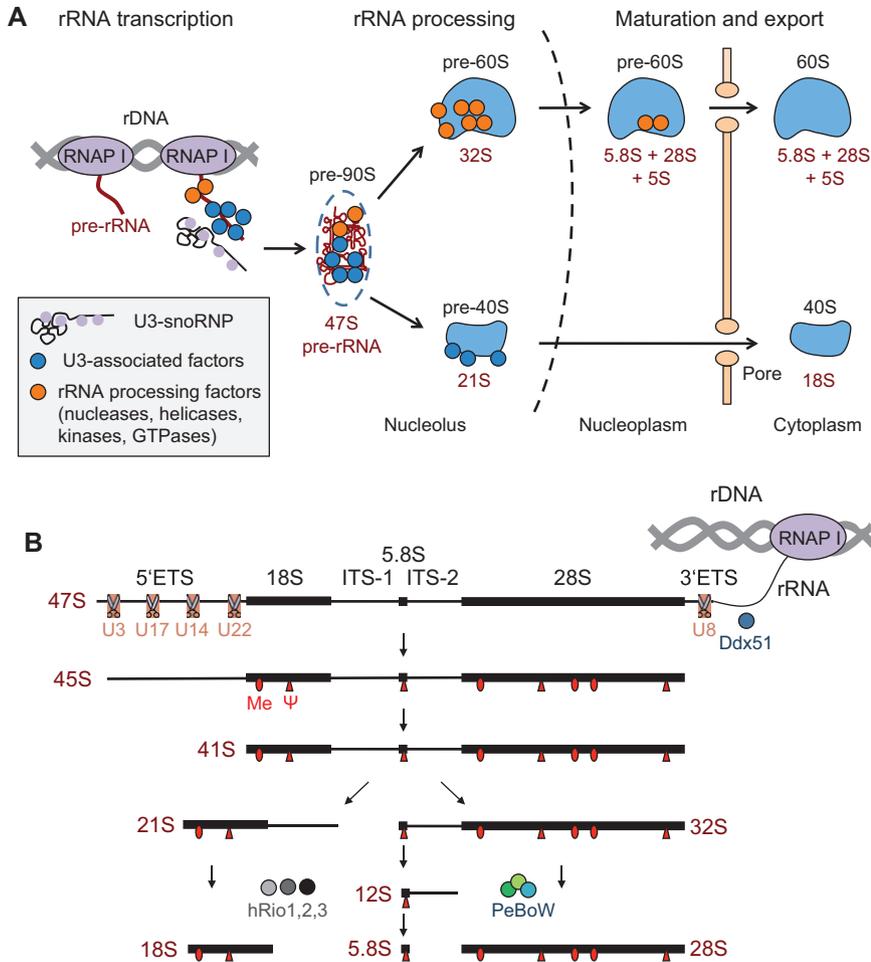


Figure 2 Mammalian ribosome biogenesis.

(A) Ribosome biogenesis consists of rRNA synthesis, rRNA processing and ribosome maturation. RNAPII transcribes a 14 kb-long, polycistronic 47S rRNA precursor that contains 18S, 5.8S, and 28S rRNAs. The fraction of rRNA within the transcriptome can be up to 90%, implying that transcriptionally active rDNA genes are heavily transcribed (Rabani et al., 2011). RNAPII transcription facilitates the synthesis of 79 ribosomal L- and S-proteins, a large cohort of small nucleolar (sno)RNAs, and >200 mRNAs encoding non-ribosomal nucleolar factors, including kinases, helicases, nucleases, and GTPases. In addition, 5S rRNA is produced by RNAPIII. The orchestrated activity of all three RNA polymerases ensures the correct transcription, processing, and modification of rRNA and its assembly into 40S and 60S ribosomal subunits that compose functional 80S ribosomes in the cytoplasm (Eichler and Craig, 1994; Boisvert et al., 2007). (B) Mammalian rRNA processing. After synthesis the 47S primary transcript is processed into mature 18S, 5.8S, and 28S rRNAs. Endo- and exonucleases cleave and trim external (ETS) and internal transcribed spacers (ITS) of 47S rRNA. Five critical snoRNAs are required for initial cleavages of the 47S rRNA precursor at the 5' end (snoRNAs U3, U14, U17, and U22) or 3' end (snoRNA U8), respectively. In addition, distinct sites of the 47S rRNA precursor are methylated at the 2' oxygen of the ribose (Me) and a subset of uridines is converted to pseudouridines (ψ). In total, several hundreds of modifications are introduced in rRNA, assisted by small nucleolar ribonucleoproteins (snoRNPs). Each snoRNP contains one modifying enzyme (fibrillarin methyltransferase for Me, dyskerin pseudouridine synthase for ψ), one snoRNA, and additional stabilizing proteins (Kiss, 2002). Mutations in the pseudouridine synthase dyskerin impair the correct formation of ψ modification patterns and reduce translational fidelity of ribosomes (Ruggiero et al., 2003). Extensive rRNA modification occurs at catalytically active rRNA regions within the decoding and peptidyl transferase centers of the ribosome (Bakin et al., 1994). Specific processing factors have been characterized in mammals. For example, the DEAD box helicase Ddx51 is crucial for processing of 47S rRNA 3' sequences (Srivastava et al., 2010). Ddx51 catalyzes the removal of snoRNA U8 from the unprocessed 3' end, thereby facilitating exosomal degradation of the 3'ETS sequence. The rRNA processing factors Pes1, Bop1, and WDR12 form a trimeric complex (PeBoW) and are required for 32S rRNA processing and large 60S subunit formation (Lapik et al., 2004; Holzel et al., 2005; Grimm et al., 2006; Holzel et al., 2007; Rohrmoser et al., 2007). Human (h)Rio kinase family members hRio1, hRio2, and hRio3 are required for the cytoplasmatic processing of 21S rRNA to mature 18S rRNA and 40S subunit maturation (Zemp et al., 2009; Baumas et al., 2012; Widmann et al., 2012).

(Boyd et al., 2011). Inhibition of rRNA transcription and processing triggers the translocation of various nucleolar proteins into the nucleoplasm, most prominently of the ribosomal proteins Rpl5, Rpl11, and Rpl23, which bind and inactivate Hdm2 and thereby stabilize p53 (Lohrum et al., 2003; Bhat et al., 2004; Dai and Lu, 2004; Dai et al., 2004; Hernandez-Verdun et al., 2010). It has recently been shown that Rpl5 and Rpl11 mutually stabilize each other and are continuously imported into nucleoli to colocalize with Hdm2 even after nucleolar disruption, suggesting that the main source of Rpl5 and Rpl11 that binds to Hdm2 is newly synthesized rather than pre-existing (Bursac et al., 2012). Interestingly, recent work identified Pict1 as a tethering factor for ribosomal proteins and as an important regulator of p53. In unstressed cells, Pict1 binds and tethers Rpl11 and other ribosomal proteins in the nucleolus, thereby sustaining Hdm2 activity. Pict1 levels decrease upon nucleolar stress. This results in the translocation of Rpl11 into the nucleoplasm and p53 stabilization (Sasaki et al., 2011). The nucleolar protein MYBBP1A is another important regulator of p53 levels. MYBBP1A is tethered to the nucleolus through nucleolar RNA. Upon nucleolar stress, MYBBP1A translocates to the nucleoplasm and facilitates p53 acetylation and stabilization by p300 acetyl transferase (Kuroda et al., 2011). Taken together, functional ribosome biogenesis requires an intact nucleolar structure and is a prerequisite for maintenance of low levels of p53.

The p53 response comes in different flavours

p53 is regarded as the guardian of the genome, which senses DNA damage by induction of G1-arrest or apoptosis (Carson and Lois, 1995; Levine, 1997; Vousden and Prives, 2009). The induction of p53 is a major aim in chemotherapy to trigger cell cycle arrest and/or apoptosis in tumor cells. Indeed, a strong nuclear accumulation of p53 can be detected upon treatment of cells with various DNA-damaging cancer therapy drugs (Fritsche et al., 1993). For a long time, DNA was regarded as the most important functional target of many classical, genotoxic drugs. For example, alkylating or DNA-intercalating drugs trigger a damage response involving kinases such as ataxia telangiectasia mutated kinase (ATM) or checkpoint kinase 1 (CHK1), which modify p53 at various positions. Activated p53 induces cell cycle inhibitors such as p21^{Cip} and p27^{Cip}, which negatively regulate Cdk2 activity and arrest cells in G1-phase of the cell cycle (Lane, 1992; Harper et al., 1995; Sakaguchi et al., 1998; Ljungman, 2000; Vogelstein et al., 2000).

However, more recent data suggest that different qualities of p53 response exist depending on the type of stress. Efeyan and colleagues found that oncogenic signaling, such as induction of the tumor suppressor p14^{ARF}, contributes to the bulk of tumor protective p53 function, whereas DNA damage by classical chemotherapy is less important for p53 function (Efeyan et al., 2006). In fact, the presence of p14^{ARF} has been found to be critical for the survival of mice treated with DNA-damaging agents, and tumors are associated more frequently with mutations in p14^{ARF} than mutations in kinases of the DNA-damaging pathway (Bartek and Lukas, 2003; Sharpless, 2005). p14^{ARF} inhibits Hdm2 and can induce p53 in analogy to the nucleolar stress response (Tao and Levine, 1999; Sherr and Weber, 2000). p53 serine-15 phosphorylation, a hallmark of DNA-damaging agents, is absent after inhibition of rRNA processing (Holzel et al., 2010a), and p14^{ARF} is not required for p53 stabilization after knockdown of rRNA processing factors (Holzel et al., 2005). This suggests an Hdm2-dependent, but DNA damage and p14^{ARF} independent pathway for p53 stabilization after inhibition of rRNA processing. It has also been shown that p53 levels are crucial for its activity, while p53 modifications rather fine-tune its activity (Blattner et al., 1999). Taken together, the quality of p53 induction by nucleolar stress differs significantly from p53 induction upon classical DNA-damaging chemotherapy. For cancer therapy, the nucleolar stress-mediated p53 response may be superior, because it induces p53 without damaging DNA. But which chemotherapeutic drugs inhibit ribosome biogenesis?

Genotoxic inhibitors of ribosome biogenesis

The increased size of nucleoli is a diagnostic marker for the proliferative state of cancer cells (Derenzini et al., 1998, 2000). Early studies utilized changes in nucleolar morphology as a marker to measure the responsiveness of tumor cells to classical chemotherapeutic drugs. For example, antibiotics such as doxorubicin, daunorubicin, mitomycin C or actinomycin D cause nucleolar segregation and translocation of the nucleolar protein nucleophosmin (NPM1) to the nucleoplasm (Merski et al., 1976; Daskal et al., 1978; Wassermann et al., 1986; Chan et al., 1987, 1988). Upon stress relief NPM1 relocates to the nucleolus (Chan et al., 1996). Nucleolar disruption usually is a consequence of inhibition of ribosome biogenesis. Various drugs inhibit ribosome biogenesis at the level of rRNA transcription albeit by different mechanisms.

Treatment with the anti-metabolite and dihydrofolate reductase inhibitor methotrexate disrupts nucleolar integrity and inhibits RNAPII transcription (Safa and Tseng, 1984; Greenhalgh and Parish, 1990; Thiry et al., 1997). Actinomycin D intercalates in GC-rich regions of rDNA, thereby specifically inhibiting rRNA elongation at low concentrations (Fetherston et al., 1984). The alkylating agent cyclophosphamide inhibits rRNA synthesis, reduces nucleoli in number and size, and causes nucleolar segregation in patients (Matejokova and Smetana, 1975; Kacerovska et al., 1981; Likovsky et al., 1993). Similarly, mitomycin C treatment inhibits RNAPII transcription by alkylating guanosines and inducing inter-strand cross-links in rDNA (Rey et al., 1993). Irinotecan/topotecan inhibits RNAPII transcription by trapping topoisomerase I to rDNA, which causes DNA strand breaks (Pondarre et al., 1997). The impact of the platin analog cis-diamminedichloroplatinum(II) (cisplatin) on ribosome biogenesis has been studied in detail (Berry et al., 1983; Leibbrandt et al., 1995). Cisplatin induces intrastrand DNA cross-links, inhibits RNAPII transcription, and causes a redistribution of nucleolar transcription factors. At low concentrations cisplatin specifically inhibits RNAPII, but not RNAPII transcription (Treiber et al., 1994; Jordan and Carmo-Fonseca, 1998). Cisplatin also triggers nucleolar accumulation of the small nuclear (sn)RNA biogenesis factor coilin. Cisplatin interacts with coilin and UBF, thereby inhibiting RNAPII initiation (Gilder et al., 2011). Finally, a comprehensive study of the inhibitory effect of 36 cytostatic drugs on rRNA synthesis and nucleolar integrity confirmed previous results and revealed that about one-third of the drugs inhibit ribosome biogenesis either at the level of rRNA transcription or rRNA processing (Burger et al., 2010). Interestingly, inhibition of rRNA transcription and early, but not late, rRNA processing is accompanied by nucleolar disruption.

Non-genotoxic inhibitors of ribosome biogenesis

Classical DNA-damaging chemotherapy can cause secondary tumors, especially in survivors of childhood cancer. Therefore, alternative drugs are of particular interest. If ribosome biogenesis is a critical target of chemotherapy and p53 control, drugs are desirable for inhibition of rRNA synthesis without genotoxic side effects. In this context, the antimetabolite 5-fluorouracil (5-FU), but not its analog 5-fluorocytosine, was identified as potent inhibitor of RNA metabolism in various studies. In an analogy to nucleolar

stress, 5-FU cytotoxicity is p53-dependent and relies on Hdm2 activity (Berger, 1977; Greenhalgh and Parish, 1990; Ghoshal and Jacob, 1994; Bunz et al., 1999). Interestingly, 5-FU was found to form stable adducts with dyskerin pseudouridine synthase. Depletion of dyskerin reduced 5-FU cytotoxicity (Hoskins and Butler, 2008). 5-FU probably confers cytotoxicity by the reduction of pseudouridylation of rRNA, which is a requirement for correct rRNA processing. In addition, incorporation of 5-FU into small nuclear (sn)RNA U2 prevents pseudouridylation and formation of functional snRNPs for pre-mRNA splicing (Zhao and Yu, 2007). These data suggest that 5-FU treatment strongly inhibits the metabolism of RNAs rather than interfering with the DNA metabolism.

Previous work from the Hernandez-Verdun and our laboratory showed that cyclin-dependent kinases (Cdks) regulate the formation and maintenance of the nucleolus. Specific Cdk-inhibitors like roscovitine, olomoucine, 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB) or flavopiridol selectively prevent the processing, but not the synthesis of 47S rRNA (Sirri et al., 2002; Schlosser et al., 2003; Burger et al., 2010) (Figure 3). Inhibition of 47S rRNA processing by DRB is accompanied by the formation of a perinucleolar necklace structure, which contains mislocated, unprocessed pre-rRNAs and rRNA processing factors, like fibrillarin that are disconnected from the rDNA transcription machinery (David-Pfeuty et al., 2001; Louvet

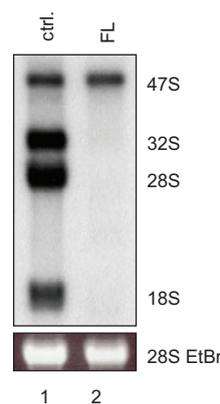


Figure 3 Cdk9-inhibitor flavopiridol blocks 47S rRNA processing. Efficient processing of the 47S rRNA primary transcript is dependent on Cdk9 activity. An autoradiograph of a [32 P]-ortho-phosphate *in vivo* metabolic labelling experiment in the presence of 0.1% DMSO (ctrl.) or 800 nM flavopiridol (FL) for 6 h. Treatment with the Cdk9-inhibitor FL strongly reduces pre-rRNA processing and stabilizes an unprocessed 47S rRNA precursor. Similar results were obtained after treatment with Cdk-inhibitors roscovitine, olomoucine, or 5,6-dichlorobenzimidazole 1- β -D-ribofuranoside (DRB) (Sirri et al., 2002; Schlosser et al., 2003; Burger et al., 2010). Ethidium bromide-stained 28S rRNA is used as loading control.

et al., 2005). Similarly, the translocation of the Cajal body marker coilin into necklace structures has been observed upon treatment with α -amanitin, a specific inhibitor of RNAPII transcription (Haaf and Ward, 1996). These data suggest that ongoing RNAPII transcription is crucial for the maintenance of nucleolar structure and function. Inhibition of RNAPII transcription by Cdk-inhibitors may be a promising approach for interference with ribosome biogenesis, induction of p53, and inhibition of cellular growth and proliferation. Flavopiridol is currently tested in clinical trials for therapy of relapsed chronic lymphocytic leukemia (CLL). Patients with a genetically high-risk CLL have been reported to be highly responsive to flavopiridol (Byrd et al., 2007; Lin et al., 2009; Phelps et al., 2009).

Given that all three RNA polymerases are required for ribosome biogenesis, inhibition of transcription is a feasible approach to trigger nucleolar stress. Recent work characterized two new small molecule inhibitors for RNAPI, CX-3543 and CX-5461, which were initially developed and characterized by Cyclene Pharmaceuticals, Inc. in collaboration with the laboratory of Ross Hannan (Melbourne). CX-3543 disrupts the interaction of the nucleolar processing factor nucleolin with rDNA G-quadruplex complexes in the nucleolus and induces apoptosis in cancer cells (Drygin et al., 2009). CX-5461 specifically blocks recruitment of the SL1 complex for RNAPI initiation and induces senescence and autophagy, but not apoptosis, in solid tumor cell lines (Drygin et al., 2011). Targeting of RNAPI transcription with CX-5461 selectively kills B-lymphoma cells and prolongs survival of tumor-bearing mice. The therapeutic efficacy could be correlated with nucleolar disruption and activation of p53-dependent apoptotic signaling (Bywater et al., 2012). A group of specific RNAPI inhibitors with therapeutic potential has very recently been described with the family of ellipticines, which also target the SL1 complex (Andrews et al., 2013). Thus, non-genotoxic inhibition of RNAPI transcription could be an important therapeutic strategy for cancer specific activation of p53 in cancer cells.

In addition to RNA polymerases, other enzymes and signaling pathways have been reported to be critical for rRNA metabolism. The deregulation of the PI3K-AKT-mTOR signaling pathway and/or the hyperactivation of c-Myc are frequently observed in human cancer. Together, PI3K-AKT-mTOR and c-Myc form a signaling network that up-regulates ribosome biogenesis at the level of rRNA transcription and processing, but also enhances translation initiation and protein synthesis. The allosteric AKT inhibitor MK-2206 was shown to antagonize RNAPI transcription in E μ -Myc induced B-cell lymphoma. MK-2206 treatment is associated with a rapid reduction of tumor

weight and delayed disease progression (Chan et al., 2011; Hannan et al., 2011). The inhibition of the mTORC1 complex, a downstream target of AKT kinase, by rapamycin or analogs thereof inhibits rRNA processing and induces degradation of unprocessed pre-rRNA. Rapamycin does not affect the overall nucleolar morphology, but induces the loss of the mTORC1 complex from nucleoli (Iadevaia et al., 2012). Together, these findings suggest that the PI3K/AKT/mTOR/c-Myc growth control network could be a therapeutic target in tumor cells with unrestrained ribosome biogenesis. Inhibitors of AKT activity and mTOR signaling are currently used in clinical trials (Engelman, 2009). For example, 35% of patients with relapsed/refractory Waldenström's macroglobulinemia (lymphoplasmatic lymphoma) are responsive to the AKT inhibitor perifosine (Ghobrial et al., 2010).

NPM1 is a multifunctional nucleolar protein with endoribonuclease activity that promotes rRNA processing during ribosome biogenesis. Deconjugation of SUMO2 modification from NPM1 by SENP3 protease is required for NPM1 function. Depletion of NPM1 or SENP3 protease blocks processing of the 32S rRNA intermediate into mature 28S rRNA and induces cell death (Itahana et al., 2003; Haindl et al., 2008; Lindstrom, 2011). NPM1 expression is frequently altered in tumors and drives leukemia initiation (Grisendi et al., 2006; Vassiliou et al., 2011). A mutant version of NPM1 in acute myeloid leukemia (AML), NPM1c, is found in >30% of AML patients and characterized by the gain of a nuclear export signal and localization to the cytoplasm. Interestingly, AML patients with NPM1c mutations are hypersensitive to chemotherapeutic drugs and show prolonged survival (Falini et al., 2009). Given that the nucleolus is a highly sensitive stress sensor, it is tempting to speculate that the hypersensitivity of AML cells with NPM1c mutation is caused by dysfunctional ribosome biogenesis. Two small molecule inhibitors have been described for NPM1. The compound NSC348884 binds to a hydrophobic pocket of NPM1 and inhibits its oligomerization. NSC348884 inhibits cell proliferation at an IC₅₀ of 1.7–4.0 μ M, induces p53, and synergizes with the cytotoxicity of doxorubicin in distinct cancer cell lines (Qi et al., 2008). The second compound avrainvillamide is an alkaloid and has been identified in an affinity screen with NPM1. Avrainvillamide stabilizes p53 and induces apoptosis. A screen with NPM1 cysteine/alanine substitution mutants identified cysteine residue 275 in NPM1 as critical for binding of avrainvillamide (Wulff et al., 2007). However, whether the growth inhibitory activity of NPM1-inhibitors is conferred by blocking of rRNA processing has not been studied yet.

The data discussed in this review suggest that the inhibition of ribosome biogenesis is a promising approach

for cancer therapy. Although the number of non-genotoxic drugs is currently limited, an increasing amount of small molecule inhibitors might help to broaden therapeutic options. In 2005, the nucleolar proteome with 692 proteins became available (Andersen et al., 2005). Interestingly, a large panel of the nucleolar factors is associated with cell cycle control, including kinases and phosphatases with potential regulatory function in ribosome biogenesis. Further research is required to elucidate the function of

nucleolar enzymes and to open doors for the development of novel therapeutic approaches.

Acknowledgements: The work of D.E. is supported by the Deutsche Forschungsgemeinschaft (SFB/TR5 and SFB684).

Received March 25, 2013; accepted April 26, 2013; previously published online May 1, 2013

References

- Andersen, J.S., Lam, Y.W., Leung, A.K., Ong, S.E., Lyon, C.E., Lamond, A.I., and Mann, M. (2005). Nucleolar proteome dynamics. *Nature* *433*, 77–83.
- Andrews, W.J., Panova, T., Normand, C., Gadad, O., Tikhonova, I.G., and Panov, K.I. (2013). Old drug, new target: ellipticines selectively inhibit RNA polymerase I transcription. *J. Biol. Chem.* *288*, 4567–4582.
- Bakin, A., Lane, B.G., and Ofengand, J. (1994). Clustering of pseudouridine residues around the peptidyltransferase center of yeast cytoplasmic and mitochondrial ribosomes. *Biochemistry* *33*, 13475–13483.
- Bartek, J. and Lukas, J. (2003). Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell* *3*, 421–429.
- Baumas, K., Soudet, J., Caizergues-Ferrer, M., Faubladier, M., Henry, Y., and Mougou, A. (2012). Human RioK3 is a novel component of cytoplasmic pre-40S pre-ribosomal particles. *RNA Biol.* *9*, 162–174.
- Berger, E. (1977). The ribosomes of *Drosophila*. Normal and defective ribosome biosynthesis in *Drosophila* cell cultures. *Mol. Gen. Genet.* *155*, 35–40.
- Berry, J.P., Galle, P., Viron, A., Kacerovska, H., and Macieira-Coelho, A. (1983). Preferential nucleolar localization of Cis-DDP in human fibroblasts. *Biomed. Pharmacother.* *37*, 125–129.
- Bhat, K.P., Itahana, K., Jin, A., and Zhang, Y. (2004). Essential role of ribosomal protein L11 in mediating growth inhibition-induced p53 activation. *EMBO J.* *23*, 2402–2412.
- Blattner, C., Tobiasch, E., Litfen, M., Rahmsdorf, H.J., and Herrlich, P. (1999). DNA damage induced p53 stabilization: no indication for an involvement of p53 phosphorylation. *Oncogene* *18*, 1723–1732.
- Boisvert, F.M., van Koningsbruggen, S., Navascues, J., and Lamond, A.I. (2007). The multifunctional nucleolus. *Nat. Rev. Mol. Cell Biol.* *8*, 574–585.
- Boon, K., Caron, H.N., van Asperen, R., Valentijn, L., Hermus, M.C., van Sluis, P., Roobeek, I., Weis, I., Voute, P.A., Schwab, M., et al. (2001). N-myc enhances the expression of a large set of genes functioning in ribosome biogenesis and protein synthesis. *EMBO J.* *20*, 1383–1393.
- Boyd, M.T., Vlatkovic, N., and Rubbi, C.P. (2011). The nucleolus directly regulates p53 export and degradation. *J. Cell Biol.* *194*, 689–703.
- Brown, D.D. and Gurdon, J.B. (1964). Absence of ribosomal Rna synthesis in the anucleolate mutant of *Xenopus Laevis*. *Proc. Natl. Acad. Sci. USA* *51*, 139–146.
- Bunz, F., Hwang, P.M., Torraine, C., Waldman, T., Zhang, Y., Dillehay, L., Williams, J., Lengauer, C., Kinzler, K.W., and Vogelstein, B. (1999). Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J. Clin. Invest.* *104*, 263–269.
- Burger, K., Muhl, B., Harasim, T., Rohmoser, M., Malamoussi, A., Orban, M., Kellner, M., Gruber-Eber, A., Kremmer, E., Holzel, M., et al. (2010). Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. *J. Biol. Chem.* *285*, 12416–12425.
- Bursac, S., Brdovcak, M.C., Pfannkuchen, M., Orsolich, I., Golomb, L., Zhu, Y., Katz, C., Daftuar, L., Grabusic, K., Vukelic, I., et al. (2012). Mutual protection of ribosomal proteins L5 and L11 from degradation is essential for p53 activation upon ribosomal biogenesis stress. *Proc. Natl. Acad. Sci. USA* *109*, 20467–20472.
- Byrd, J.C., Lin, T.S., Dalton, J.T., Wu, D., Phelps, M.A., Fischer, B., Moran, M., Blum, K.A., Rovin, B., Brooker-McEldowney, M., et al. (2007). Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. *Blood* *109*, 399–404.
- Bywater, M.J., Poortinga, G., Sanij, E., Hein, N., Peck, A., Cullinane, C., Wall, M., Cluse, L., Drygin, D., Anderes, K., et al. (2012). Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. *Cancer Cell* *22*, 51–65.
- Carson, D.A. and Lois, A. (1995). Cancer progression and p53. *Lancet* *346*, 1009–1011.
- Chan, J.C., Hannan, K.M., Riddell, K., Ng, P.Y., Peck, A., Lee, R.S., Hung, S., Astle, M.V., Bywater, M., Wall, M., et al. (2011). AKT promotes rRNA synthesis and cooperates with c-MYC to stimulate ribosome biogenesis in cancer. *Sci Signal* *4*, ra56.
- Chan, P.K., Aldrich, M.B., and Chakrabarty, S. (1988). Assessment of tumor cell sensitivity to mitomycin C by “B23 translocation” assay. *Cancer Lett.* *40*, 143–149.
- Chan, P.K., Aldrich, M.B., and Yung, B.Y. (1987). Nucleolar protein B23 translocation after doxorubicin treatment in murine tumor cells. *Cancer Res* *47*, 3798–3801.
- Chan, P.K., Qi, Y., Amley, J., and Koller, C.A. (1996). Quantitation of the nucleophosmin/B23-translocation using imaging analysis. *Cancer Lett.* *100*, 191–197.
- Dai, M.S. and Lu, H. (2004). Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. *J. Biol. Chem.* *279*, 44475–44482.

- Dai, M.S., Zeng, S.X., Jin, Y., Sun, X.X., David, L., and Lu, H. (2004). Ribosomal protein L23 activates p53 by inhibiting MDM2 function in response to ribosomal perturbation but not to translation inhibition. *Mol. Cell. Biol.* *24*, 7654–7668.
- Daskal, Y., Woodard, C., Crooke, S.T., and Busch, H. (1978). Comparative ultrastructural studies of nucleoli of tumor cells treated with adriamycin and the newer anthracyclines, carminomycin and marcellomycin. *Cancer Res.* *38*, 467–473.
- David-Pfeuty, T. (1999). Potent inhibitors of cyclin-dependent kinase 2 induce nuclear accumulation of wild-type p53 and nucleolar fragmentation in human untransfected and tumor-derived cells. *Oncogene* *18*, 7409–7422.
- David-Pfeuty, T., Nouvian-Dooghe, Y., Sirri, V., Roussel, P., and Hernandez-Verdun, D. (2001). Common and reversible regulation of wild-type p53 function and of ribosomal biogenesis by protein kinases in human cells. *Oncogene* *20*, 5951–5963.
- De Keersmaecker, K., Atak, Z.K., Li, N., Vicente, C., Patchett, S., Girardi, T., Gianfelici, V., Geerdens, E., Clappier, E., Porcu, M., et al. (2013). Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. *Nat. Genet.* *45*, 186–190.
- Derenzini, M., Trere, D., Pession, A., Govoni, M., Sirri, V., and Chieco, P. (2000). Nucleolar size indicates the rapidity of cell proliferation in cancer tissues. *J. Pathol.* *191*, 181–186.
- Derenzini, M., Trere, D., Pession, A., Montanaro, L., Sirri, V., and Ochs, R.L. (1998). Nucleolar function and size in cancer cells. *Am. J. Pathol.* *152*, 1291–1297.
- Drygin, D., Lin, A., Bliesath, J., Ho, C.B., O'Brien, S.E., Proffitt, C., Omori, M., Haddach, M., Schwaebe, M.K., Siddiqui-Jain, A., et al. (2011). Targeting RNA polymerase I with an oral small molecule CX-5461 inhibits ribosomal RNA synthesis and solid tumor growth. *Cancer Res.* *71*, 1418–1430.
- Drygin, D., Rice, W.G., and Grummt, I. (2010). The RNA polymerase I transcription machinery: an emerging target for the treatment of cancer. *Annu. Rev. Pharmacol. Toxicol.* *50*, 131–156.
- Drygin, D., Siddiqui-Jain, A., O'Brien, S., Schwaebe, M., Lin, A., Bliesath, J., Ho, C.B., Proffitt, C., Trent, K., Whitten, J.P., et al. (2009). Anticancer activity of CX-3543: a direct inhibitor of rRNA biogenesis. *Cancer Res.* *69*, 7653–7661.
- Efeyan, A., Garcia-Cao, I., Herranz, D., Velasco-Miguel, S., and Serrano, M. (2006). Tumour biology: policing of oncogene activity by p53. *Nature* *443*, 159.
- Eichler, D.C. and Craig, N. (1994). Processing of eukaryotic ribosomal RNA. *Prog. Nucleic Acid. Res. Mol. Biol.* *49*, 197–239.
- Engelman, J.A. (2009). Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat. Rev. Cancer* *9*, 550–562.
- Falini, B., Bolli, N., Liso, A., Martelli, M.P., Mannucci, R., Pileri, S., and Nicoletti, I. (2009). Altered nucleophosmin transport in acute myeloid leukaemia with mutated NPM1: molecular basis and clinical implications. *Leukemia* *23*, 1731–1743.
- Fetherston, J., Werner, E., and Patterson, R. (1984). Processing of the external transcribed spacer of murine rRNA and site of action of actinomycin D. *Nucleic Acids Res.* *12*, 7187–7198.
- Fritsche, M., Haessler, C., and Brandner, G. (1993). Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene* *8*, 307–318.
- Ghobrial, I.M., Gertz, M., Laplant, B., Camoriano, J., Hayman, S., Lacy, M., Chuma, S., Harris, B., Leduc, R., Rourke, M., et al. (2010). Phase II trial of the oral mammalian target of rapamycin inhibitor everolimus in relapsed or refractory Waldenstrom macroglobulinemia. *J. Clin. Oncol.* *28*, 1408–1414.
- Ghoshal, K. and Jacob, S.T. (1994). Specific inhibition of pre-ribosomal RNA processing in extracts from the lymphosarcoma cells treated with 5-fluorouracil. *Cancer Res.* *54*, 632–636.
- Gilder, A.S., Do, P.M., Carrero, Z.I., Cosman, A.M., Broome, H.J., Velma, V., Martinez, L.A., and Hebert, M.D. (2011). Coilin participates in the suppression of RNA polymerase I in response to cisplatin-induced DNA damage. *Mol. Biol. Cell* *22*, 1070–1079.
- Greenhalgh, D.A. and Parish, J.H. (1990). Effect of 5-fluorouracil combination therapy on RNA processing in human colonic carcinoma cells. *Br. J. Cancer* *61*, 415–419.
- Grimm, T., Holzel, M., Rohmoser, M., Harasim, T., Malamoussi, A., Gruber-Eber, A., Kremmer, E., and Eick, D. (2006). Dominant-negative Pes1 mutants inhibit ribosomal RNA processing and cell proliferation via incorporation into the PeBoW-complex. *Nucleic Acids Res.* *34*, 3030–3043.
- Grisendi, S., Mecucci, C., Falini, B., and Pandolfi, P.P. (2006). Nucleophosmin and cancer. *Nat. Rev. Cancer* *6*, 493–505.
- Haaf, T. and Ward, D.C. (1996). Inhibition of RNA polymerase II transcription causes chromatin decondensation, loss of nucleolar structure, and dispersion of chromosomal domains. *Exp. Cell. Res.* *224*, 163–173.
- Hadjiolova, K.V., Nicoloso, M., Mazan, S., Hadjiolov, A.A., and Bachelier, J.P. (1993). Alternative pre-rRNA processing pathways in human cells and their alteration by cycloheximide inhibition of protein synthesis. *Eur. J. Biochem.* *212*, 211–215.
- Haindl, M., Harasim, T., Eick, D., and Muller, S. (2008). The nucleolar SUMO-specific protease SENP3 reverses SUMO modification of nucleophosmin and is required for rRNA processing. *EMBO Rep.* *9*, 273–279.
- Hannan, K.M., Sanij, E., Hein, N., Hannan, R.D., and Pearson, R.B. (2011). Signaling to the ribosome in cancer – it is more than just mTORC1. *IUBMB Life* *63*, 79–85.
- Harper, J.W., Elledge, S.J., Keyomarsi, K., Dynlacht, B., Tsai, L.H., Zhang, P., Dobrowolski, S., Bai, C., Connell-Crowley, L., Swindell, E., et al. (1995). Inhibition of cyclin-dependent kinases by p21. *Mol. Biol. Cell.* *6*, 387–400.
- Hernandez-Verdun, D., Roussel, P., Thiry, M., Sirri, V., and Lafontaine, D.L. (2010). The nucleolus: structure/function relationship in RNA metabolism. *Wiley Interdiscip. Rev. RNA* *1*, 415–431.
- Holzel, M., Burger, K., Muhl, B., Orban, M., Kellner, M., and Eick, D. (2010a). The tumor suppressor p53 connects ribosome biogenesis to cell cycle control: a double-edged sword. *Oncotarget* *1*, 43–47.
- Holzel, M., Grimm, T., Rohmoser, M., Malamoussi, A., Harasim, T., Gruber-Eber, A., Kremmer, E., and Eick, D. (2007). The BRCT domain of mammalian Pes1 is crucial for nucleolar localization and rRNA processing. *Nucleic Acids Res.* *35*, 789–800.
- Holzel, M., Orban, M., Hochstatter, J., Rohmoser, M., Harasim, T., Malamoussi, A., Kremmer, E., Langst, G., and Eick, D. (2010b). Defects in 18 S or 28 S rRNA processing activate the p53 pathway. *J. Biol. Chem.* *285*, 6364–6370.

- Holzel, M., Rohrmoser, M., Schlee, M., Grimm, T., Harasim, T., Malamoussi, A., Gruber-Eber, A., Kremmer, E., Hiddemann, W., Bornkamm, G.W., et al. (2005). Mammalian WDR12 is a novel member of the Pes1-Bop1 complex and is required for ribosome biogenesis and cell proliferation. *J. Cell. Biol.* *170*, 367–378.
- Hoskins, J. and Butler, J.S. (2008). RNA-based 5-fluorouracil toxicity requires the pseudouridylation activity of Cbf5p. *Genetics* *179*, 323–330.
- Iadevaia, V., Zhang, Z., Jan, E., and Proud, C.G. (2012). mTOR signaling regulates the processing of pre-rRNA in human cells. *Nucleic Acids Res.* *40*, 2527–2539.
- Itahana, K., Bhat, K.P., Jin, A., Itahana, Y., Hawke, D., Kobayashi, R., and Zhang, Y. (2003). Tumor suppressor ARF degrades B23, a nucleolar protein involved in ribosome biogenesis and cell proliferation. *Mol. Cell.* *12*, 1151–1164.
- Jordan, P. and Carmo-Fonseca, M. (1998). Cisplatin inhibits synthesis of ribosomal RNA in vivo. *Nucleic Acids Res.* *26*, 2831–2836.
- Kacerovska, H., Likovsky, Z., and Smetana, K. (1981). Nucleolar silver stained granules in rat Yoshida sarcoma cells after RNA synthesis inhibition. *Neoplasma* *28*, 513–516.
- Kiss, T. (2002). Small nucleolar RNAs: an abundant group of noncoding RNAs with diverse cellular functions. *Cell* *109*, 145–148.
- Kondrashov, N., Pusic, A., Stumpf, C.R., Shimizu, K., Hsieh, A.C., Xue, S., Ishijima, J., Shiroishi, T., and Barna, M. (2011). Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell* *145*, 383–397.
- Kuroda, T., Murayama, A., Katagiri, N., Ohta, Y.M., Fujita, E., Masumoto, H., Ema, M., Takahashi, S., Kimura, K., and Yanagisawa, J. (2011). RNA content in the nucleolus alters p53 acetylation via MYBBP1A. *EMBO J.* *30*, 1054–1066.
- Lane, D.P. (1992). Cancer: p53, guardian of the genome. *Nature* *358*, 15–16.
- Lapik, Y.R., Fernandes, C.J., Lau, L.F., and Pestov, D.G. (2004). Physical and functional interaction between Pes1 and Bop1 in mammalian ribosome biogenesis. *Mol. Cell.* *15*, 17–29.
- Leibbrandt, M.E., Wolfgang, G.H., Metz, A.L., Ozobia, A.A., and Haskins, J.R. (1995). Critical subcellular targets of cisplatin and related platinum analogs in rat renal proximal tubule cells. *Kidney Int.* *48*, 761–770.
- Levine, A.J. (1997). p53, the cellular gatekeeper for growth and division. *Cell* *88*, 323–331.
- Likovsky, Z., Peterka, M., and Peterkova, R. (1993). Drug-induced changes of rRNA biosynthesis – a marker of toxic damage to embryonal cell population. *Funct. Dev. Morphol.* *3*, 3–9.
- Lin, T.S., Ruppert, A.S., Johnson, A.J., Fischer, B., Heerema, N.A., Andritsos, L.A., Blum, K.A., Flynn, J.M., Jones, J.A., Hu, W., et al. (2009). Phase II study of flavopiridol in relapsed chronic lymphocytic leukemia demonstrating high response rates in genetically high-risk disease. *J. Clin. Oncol.* *27*, 6012–6018.
- Lindstrom, M.S. (2011). NPM1/B23: a multifunctional chaperone in ribosome biogenesis and chromatin remodeling. *Biochem. Res. Int.* *2011*, 195209.
- Ljungman, M. (2000). Dial 9-1-1 for p53: mechanisms of p53 activation by cellular stress. *Neoplasia* *2*, 208–225.
- Lohrum, M.A., Ludwig, R.L., Kubbutat, M.H., Hanlon, M., and Vousden, K.H. (2003). Regulation of HDM2 activity by the ribosomal protein L11. *Cancer Cell* *3*, 577–587.
- Louvet, E., Junera, H.R., Le Panse, S., and Hernandez-Verdun, D. (2005). Dynamics and compartmentation of the nucleolar processing machinery. *Exp. Cell Res.* *304*, 457–470.
- Matejokova, E. and Smetana, K. (1975). Morphological changes in the nucleoli of peripheral-blood lymphocytes as prognostic criteria in the chemotherapy of malignant tumors. *Neoplasma* *22*, 303–312.
- Merski, J.A., Daskal, I., and Busch, H. (1976). Effects of adriamycin on ultrastructure of nucleoli in the heart and liver cells of the rat. *Cancer Res.* *36*, 1580–1584.
- Miller, O.L., Jr. and Beatty, B.R. (1969). Visualization of nucleolar genes. *Science* *164*, 955–957.
- Narla, A. and Ebert, B.L. (2010). Ribosomopathies: human disorders of ribosome dysfunction. *Blood* *115*, 3196–3205.
- Perry, R.P. (1962). The cellular sites of synthesis of ribosomal and 4S RNA. *Proc. Natl. Acad. Sci. USA* *48*, 2179–2186.
- Pestov, D.G., Strezoska, Z., and Lau, L.F. (2001). Evidence of p53-dependent cross-talk between ribosome biogenesis and the cell cycle: effects of nucleolar protein Bop1 on G(1)/S transition. *Mol. Cell. Biol.* *21*, 4246–4255.
- Phelps, M.A., Lin, T.S., Johnson, A.J., Hurh, E., Rozewski, D.M., Farley, K.L., Wu, D., Blum, K.A., Fischer, B., Mitchell, S.M., et al. (2009). Clinical response and pharmacokinetics from a phase 1 study of an active dosing schedule of flavopiridol in relapsed chronic lymphocytic leukemia. *Blood* *113*, 2637–2645.
- Pondarre, C., Strumberg, D., Fujimori, A., Torres-Leon, R., and Pommier, Y. (1997). In vivo sequencing of camptothecin-induced topoisomerase I cleavage sites in human colon carcinoma cells. *Nucleic Acids Res.* *25*, 4111–4116.
- Prieto, J.L. and McStay, B. (2008). Pseudo-NORs: a novel model for studying nucleoli. *Biochim. Biophys. Acta* *1783*, 2116–2123.
- Qi, W., Shakalya, K., Stejskal, A., Goldman, A., Beeck, S., Cooke, L., and Mahadevan, D. (2008). NSC348884, a nucleophosmin inhibitor disrupts oligomer formation and induces apoptosis in human cancer cells. *Oncogene* *27*, 4210–4220.
- Rabani, M., Levin, J.Z., Fan, L., Adiconis, X., Raychowdhury, R., Garber, M., Gnirke, A., Nusbaum, C., Hacohen, N., Friedman, N., et al. (2011). Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. *Nat. Biotechnol.* *29*, 436–442.
- Rey, J.P., Scott, R., and Muller, H. (1993). Induction and removal of interstrand crosslinks in the ribosomal RNA genes of lymphoblastoid cell lines from patients with Fanconi anemia. *Mutat. Res.* *289*, 171–180.
- Ritossa, F.M. and Spiegelman, S. (1965). Localization of Dna complementary to ribosomal RNA in the nucleolar organizer region of *Drosophila Melanogaster*. *Proc. Natl. Acad. Sci. USA* *53*, 737–745.
- Rohrmoser, M., Holzel, M., Grimm, T., Malamoussi, A., Harasim, T., Orban, M., Pfisterer, I., Gruber-Eber, A., Kremmer, E., and Eick, D. (2007). Interdependence of Pes1, Bop1, and WDR12 controls nucleolar localization and assembly of the PeBoW complex required for maturation of the 60S ribosomal subunit. *Mol. Cell. Biol.* *27*, 3682–3694.
- Romanova, L., Grand, A., Zhang, L., Rayner, S., Katoku-Kikyo, N., Kellner, S., and Kikyo, N. (2009). Critical role of nucleostemin in pre-rRNA processing. *J. Biol. Chem.* *284*, 4968–4977.
- Rubbi, C.P. and Milner, J. (2003). Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J.* *22*, 6068–6077.

- Ruggero, D. (2012). Revisiting the nucleolus: from marker to dynamic integrator of cancer signaling. *Sci. Signal.* 5, pe38.
- Ruggero, D., Grisendi, S., Piazza, F., Rego, E., Mari, F., Rao, P.H., Cordon-Cardo, C., and Pandolfi, P.P. (2003). Dyskeratosis congenita and cancer in mice deficient in ribosomal RNA modification. *Science* 299, 259–262.
- Ruggero, D. and Pandolfi, P.P. (2003). Does the ribosome translate cancer? *Nat. Rev. Cancer* 3, 179–192.
- Safa, A.R. and Tseng, M.T. (1984). Inhibition of protein synthesis and cell proliferation in cultured human breast cancer cells treated with mitoxantrone. *Cancer Lett.* 24, 317–326.
- Sakaguchi, K., Herrera, J.E., Saito, S., Miki, T., Bustin, M., Vassilev, A., Anderson, C.W., and Appella, E. (1998). DNA damage activates p53 through a phosphorylation-acetylation cascade. *Genes Dev.* 12, 2831–2841.
- Sasaki, M., Kawahara, K., Nishio, M., Mimori, K., Kogo, R., Hamada, K., Itoh, B., Wang, J., Komatsu, Y., Yang, Y.R., et al. (2011). Regulation of the MDM2-P53 pathway and tumor growth by PICT1 via nucleolar RPL11. *Nat. Med.* 17, 944–951.
- Schlosser, I., Holzel, M., Murnseer, M., Burtscher, H., Weidle, U.H., and Eick, D. (2003). A role for c-Myc in the regulation of ribosomal RNA processing. *Nucleic Acids Res.* 31, 6148–6156.
- Schneider, D.A. (2012). RNA polymerase I activity is regulated at multiple steps in the transcription cycle: recent insights into factors that influence transcription elongation. *Gene* 493, 176–184.
- Schuhmacher, M., Kohlhuber, F., Holzel, M., Kaiser, C., Burtscher, H., Jarsch, M., Bornkamm, G.W., Laux, G., Polack, A., Weidle, U.H., et al. (2001). The transcriptional program of a human B cell line in response to Myc. *Nucleic Acids Res.* 29, 397–406.
- Sharpless, N.E. (2005). INK4a/ARF: a multifunctional tumor suppressor locus. *Mutat. Res.* 576, 22–38.
- Sherr, C.J. and Weber, J.D. (2000). The ARF/p53 pathway. *Curr. Opin. Genet. Dev.* 10, 94–99.
- Sirri, V., Hernandez-Verdun, D., and Roussel, P. (2002). Cyclin-dependent kinases govern formation and maintenance of the nucleolus. *J. Cell. Biol.* 156, 969–981.
- Srivastava, L., Lapik, Y.R., Wang, M., and Pestov, D.G. (2010). Mammalian DEAD box protein Ddx51 acts in 3' end maturation of 28S rRNA by promoting the release of U8 snoRNA. *Mol. Cell Biol.* 30, 2947–2956.
- Stommel, J.M., Marchenko, N.D., Jimenez, G.S., Moll, U.M., Hope, T.J., and Wahl, G.M. (1999). A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. *EMBO J.* 18, 1660–1672.
- Tao, W. and Levine, A.J. (1999). P19(ARF) stabilizes p53 by blocking nucleo-cytoplasmic shuttling of Mdm2. *Proc. Natl. Acad. Sci. USA* 96, 6937–6941.
- Thiry, M., Jamison, J.M., Gilloteaux, J., Summers, J.L., and Goessens, G. (1997). Ultrastructural nucleolar alterations induced by an ametantrone/poly(A-U) complex. *Exp. Cell. Res.* 236, 275–284.
- Thomas, G. (2000). An encore for ribosome biogenesis in the control of cell proliferation. *Nat. Cell. Biol.* 2, E71–72.
- Treiber, D.K., Zhai, X., Jantzen, H.M., and Essigmann, J.M. (1994). Cisplatin-DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human upstream binding factor). *Proc. Natl. Acad. Sci. USA* 91, 5672–5676.
- van Riggelen, J., Yetil, A., and Felsher, D.W. (2010). MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat. Rev. Cancer* 10, 301–309.
- Vassiliou, G.S., Cooper, J.L., Rad, R., Li, J., Rice, S., Uren, A., Rad, L., Ellis, P., Andrews, R., Banerjee, R., et al. (2011). Mutant nucleophosmin and cooperating pathways drive leukemia initiation and progression in mice. *Nat. Genet.* 43, 470–475.
- Vogelstein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. *Nature* 408, 307–310.
- Vousden, K.H. and Prives, C. (2009). Blinded by the light: the growing complexity of p53. *Cell* 137, 413–431.
- Wassermann, K., Zwelling, L.A., Mullins, T.D., Silberman, L.E., Andersson, B.S., Bakic, M., Acton, E.M., and Newman, R.A. (1986). Effects of 3'-deamino-3'-(3-cyano-4-morpholinyl) doxorubicin and doxorubicin on the survival, DNA integrity, and nucleolar morphology of human leukemia cells in vitro. *Cancer Res.* 46, 4041–4046.
- White, R.J. (2005). RNA polymerases I and III, growth control and cancer. *Nat. Rev. Mol. Cell. Biol.* 6, 69–78.
- Widmann, B., Wandrey, F., Badertscher, L., Wyler, E., Pfannstiel, J., Zemp, I., and Kutay, U. (2012). The kinase activity of human Rio1 is required for final steps of cytoplasmic maturation of 40S subunits. *Mol. Biol. Cell.* 23, 22–35.
- Wulff, J.E., Siegrist, R., and Myers, A.G. (2007). The natural product avrainvillamide binds to the oncoprotein nucleophosmin. *J. Am. Chem. Soc.* 129, 14444–14451.
- Xirodimas, D.P., Stephen, C.W., and Lane, D.P. (2001). Cocompartmentalization of p53 and Mdm2 is a major determinant for Mdm2-mediated degradation of p53. *Exp. Cell. Res.* 270, 66–77.
- Xue, S. and Barna, M. (2012). Specialized ribosomes: a new frontier in gene regulation and organismal biology. *Nat. Rev. Mol. Cell Biol.* 13, 355–369.
- Yuan, X., Zhou, Y., Casanova, E., Chai, M., Kiss, E., Grone, H.J., Schutz, G., and Grummt, I. (2005). Genetic inactivation of the transcription factor TIF-IA leads to nucleolar disruption, cell cycle arrest, and p53-mediated apoptosis. *Mol. Cell.* 19, 77–87.
- Zemp, I., Wild, T., O'Donohue, M.F., Wandrey, F., Widmann, B., Gleizes, P.E., and Kutay, U. (2009). Distinct cytoplasmic maturation steps of 40S ribosomal subunit precursors require hRio2. *J. Cell. Biol.* 185, 1167–1180.
- Zhao, X. and Yu, Y.T. (2007). Incorporation of 5-fluorouracil into U2 snRNA blocks pseudouridylation and pre-mRNA splicing in vivo. *Nucleic Acids Res.* 35, 550–558.



Kaspar Burger studied at the Ludwig Maximilians University of Munich and University of Leeds, UK. In 2009 he received his Diploma in Biology and joined Dirk Eick's laboratory, working on the impact of chemotherapeutic drugs on human ribosome biogenesis. Kaspar Burger received his PhD in 2013 for the identification and characterization of Cdk9 as essential kinase for processing of ribosomal RNA. Currently he works as Postdoc in the laboratory of Dirk Eick.



Dirk Eick graduated in Genetics and received his PhD from the University Cologne 1983 with Prof. Walter Doerfler. From 1984–1988 he was Postdoc in the Institute of Virology, University Freiburg, with Prof. Georg Bornkamm. In Freiburg he discovered the proto-oncogene *c-myc* as the first metazoa gene that is regulated at the level of RNA elongation. In 1989 he moved to the Helmholtz Center Munich as group leader. Since 1991 he is member of the Faculty of Biology of the Ludwig-Maximilians University (LMU) Munich, and since 2006 member of the LMU excellence cluster CIPSM (Center of Integrated Protein Science). Since 2008 he is head of the Department for Molecular Epigenetics at the Helmholtz Center Munich. The work of his laboratory focuses on the connection of ribosome biogenesis and cell cycle control and on the modification and function of the carboxy-terminal domain (CTD) of RNA polymerase II in the control of gene expression.