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Inhibiting eukaryotic transcription

Which compound to choose? How to evaluate its activity?

Olivier Bensaude
IBENS; UMR CNRS 8197; UA INSERM 1024; Paris, France

This review first discusses ways in which we can evaluate transcription inhibition, describe changes in nuclear structure due to transcription inhibition, and report on genes that are paradoxically stimulated by transcription inhibition. Next, it summarizes the characteristics and mechanisms of commonly used inhibitors: α-amanitin is highly selective for RNAP II and RNAP III but its action is slow, actinomycin D is fast but its selectivity is poor, CDK9 inhibitors such as DRB and flavopiridol are fast and reversible but many genes escape transcription inhibition. New compounds, such as triptolide, are fast and selective and able to completely arrest transcription by triggering rapid degradation of RNAP II.

Key words: RNA polymerase, transcription, amanitin, actinomycin, DRB, flavopiridol, triptolide, CDK9, TFIIH, Rpb1

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*Correspondence to: Olivier Bensaude;
Email: bensaude@biologie.ens.fr

Introduction

Many anti-cancer drugs inhibit transcription and most transcription inhibitors have useful pharmacological properties. Many experiments require inhibition of transcription. In yeast, thermosensitive mutations in RNA polymerase (RNAP) subunits provide precious tools. A thermosensitive mutation has been characterized in mammalian RNAP II largest subunit, Rpb1.[1] However, transcription is maintained for at least one day at non-permissive temperature, which is a major inconvenient. Therefore, "chemical genetics" or "chemical biology" is usually preferred. Among the various drugs available to inhibit transcription, how to choose which one to use? Each has its advantages and drawbacks (**Table 1**). Selectivity, efficiency, rapidity of action and reversibility are key issues. How can we detect if a new compound inhibits transcription? This review will deal first with the general questions of how to evaluate transcription inhibition, describe changes in nuclear structure due to transcription inhibition, and report on genes that are paradoxically stimulated by transcription inhibition. Next, we will focus on widely used compounds (α-amanitin, actinomycin D, DRB, flavopiridol) and triptolide, a new compound that looks very promising.

Evaluating Transcription Inhibition

How can we determine if a compound inhibits transcription? Quantification of ^{3}H-uridine incorporation into RNA is the oldest method. ^{3}H-uridine permeates rapidly into cells, is metabolized and incorporated into nascent RNA transcripts. ^{3}H-RNA accumulation results from competing RNA synthesis and degradation. Thus, short labeling time (a fraction of an hour for mammalian cells) is recommended to favor synthesis over degradation. However, this method does not distinguish between polymerases. It gives a maximal weight to RNAP I activity as rRNAs represent 60–70% of total transcript mass.

Investigating RNA levels by northern blot, RT-Q-PCR, gene arrays on DNA chips or massive sequencing provides information on specific transcripts. One may follow the effect of drugs on short-lived RNAs using RNAs with long half-lives such as actin or rRNA as reference controls. However, one should keep in mind that inhibition of transcription may enhance the stability of some mRNAs, such as those of DNA damage-inducible (gadd) genes,

Table 1. Overview of widely used inhibitors of transcription

	Actinomycin D	α-amanitin	DRB	Flavopiridol	Triptolide
Concentration	>0.01 μgml^{-1} for class I genes >1 μgml^{-1} for class II genes	>2 μgml^{-1}	100 μM	>0.5 μM	1 μM
Stock solution in	DMSO	aqueous	DMSO	aqueous	DMSO
Target	DNA intercalation	RNAPII >> RNAPIII	CDK9 in P-TEFb	CDK9 in P-TEFb	XPB in TFIIH
	RNA polymerase elongation inhibited	RNA synthesis inhibited	RNAP II elongation inhibited rRNA processing impaired	RNAP II elongation inhibited, rRNA processing impaired	RNAP I and RNAP II initiation inhibited
Target Selectivity	GC-rich DNA sequences	RNAPII & RNAPIII only known targets	Other kinases inhibited	Other kinases inhibited	Other potential target: Polycystin-2 calcium channel
Inhibition Selectivity	Class I >> Class II >> Class III transcription	Class II >> Class III transcription	Class II transcription Class I processing	Class II transcription, Class I processing	Class II & Class I transcription
Consequence on RNA polymerase II	CTD hyperphosphorylation	RNAPII degradation	CTD serine 2 dephosphorylation	CTD serine 2 dephosphorylation	Proteasome-dependent RNAPII degradation
Reversibility	Weak	No	Yes	?	No
Rate	Fast, minutes	Slow, hours	Fast, minutes	Fast, minutes	Fast, minutes

and lead to their accumulation when inhibitors are employed at moderate concentrations.[2-5] But unstable RNAs such as c-fos do not seem to be affected.[6]

Fluorescence in situ hybridization (FISH) is a direct way to observe transcription of specific genes.[7] Although single RNA molecules might be detected and counted, this method is quite tricky to set up.[8] An easier, though time-consuming, alternative is chromatin immunoprecipitation (DNA ChIP) using anti-polymerase antibodies (the Rpb3 subunit or the N-terminal domain of Rpb1 are recommended). The distribution of RNAP molecules on a gene determined by Q-PCR roughly reflects its transcription. However, choosing adequate controls to evaluate immunoprecipitation efficiencies in different samples is a major difficulty.

The easiest quantitative procedure is definitely to monitor an inducible reporter gene such as luciferase. Tetracycline-inducible promoters are particularly convenient as they respond strongly and very quickly, within a few hours, but they require the use of genetically engineered cell lines.[9,10]

Transcription of a Subset of Genes is Enhanced Upon Global Transcription Inhibition

Due to feedback loops, enhanced transcription of a small set of genes occurs during global transcription inhibition. As a first example, transcription driven by the HIV-LTR is enhanced by amanitin and actinomycin.[11,12] The "silent" HIV-LTR drives an efficient transcription initiation that aborts after 60–80 nucleotides because P-TEFb recruitment to the promoter is deficient and cannot oppose the NELFs' to promote a productive elongation of transcription. Amanitin and actinomycin treatments enhance P-TEFb activity and release the block to elongation of transcription. This effect might be consequence of a feedback loop regulation leading to P-TEFb hyperactivation.[13,14] Upon transcription arrest, heterogeneous nuclear ribonucleoproteins (hnRNPs) that chaperone the nascent transcript are released.[15] Some of them (hnRNP A, K, Q and R types) then trap 7SK RNA that is no more available to bind the HEXIM1 protein and inactivate P-TEFb.[16-18]

A general transcription inhibition results in p53 accumulation, which activates transcription of p53 target genes, such as p21^{CIP} and Hdm2,[19-21] and promotes p53 translocation into mitochondria leading to apoptosis.[22] Following treatment with flavopiridol, DRB, amanitin or actinomycin, proteins such as p53 accumulate because of a feedback loop involving enhanced synthesis[23] and protein stability.[24-27] Inhibition of rRNA synthesis or defects in rRNA processing function as triggers of that process.[20,28] An arrest in ribosome assembly releases ribosomal protein subunits such as RPL26. These trap the Hdm2 (human) or mdm2 (murine) E3 ubiquitin ligases. Competition with p53 binding to Hdm2/mdm2, thus prevents its degradation.[29,30] Furthermore, the available RPL26 activates p53 mRNA translation. Indeed, efficient translation of p53 mRNA relies upon binding of RPL26 to a cap-independent and poly(A)-independent interaction between its 5' and 3' UTR.[31,32]

Changes in Extractability of Nuclear Components

Transcription inhibition is accompanied by notable changes in biochemical properties of nuclear proteins such as histones and hnRNPs. Histone H2B ubiquitination and histone H1b phosphorylation decrease in cells treated with either actinomycin D or DRB.[33,34] hnRNPs that chaperone pre-messenger RNA are easier to extract from nuclei of cells treated with inhibitors of class II gene transcription.[18,35,36] In contrast, efficient extraction of the positive transcription elongation factor (P-TEFb) subunits (CDK9 and/or Cyclin Ts) from nuclear material is harder and requires an increase in the ionic strength of the extraction buffer.[37]

Changes in P-TEFb and hnRNP properties are linked by a feedback loop involving 7SK snRNA.[13,14]

Changes in Nuclear Structure

Transcription inhibition results in major changes in nuclear structures. The nucleolus is reorganized upon transcription inhibition. Low concentrations of actinomycin D, which primarily inhibit RNAP I (i.e., rRNA transcription), result in segregation of the fibrillar center, the dense fibrillar center and the granular components of the nucleolus.[38] Blockage of rRNA transcription (by oxaliplatin, doxorubicin, mitoxantrone or methotrexate) or early rRNA processing steps (by camptothecin or CDK9 inhibitors such as flavopiridol or roscovitine) cause nucleolar disintegration, whereas blockage of late rRNA processing steps (by 5-fluorouracil, MG132 or homoharringtonine) leave nucleoli intact.[39] Conditions that inhibit RNAP II (5 μg/ml actinomycin D, DRB or amanitin) result in aggregation of several proteins from the nucleoplasm into nucleolar caps (for an extensive description on actinomycin effects on nucleolar caps see ref. 40). Two distinct caps are distinguished, the dark nucleolar caps (DNC) and the light nucleolar caps (LNC). Fibrillarin might be used as a marker of LNCs. The PTB-associated splicing factor (PSF) is mostly nucleoplasmic. Its assembly into DNCs is spectacular and might be used as a practical indicator of transcription inhibition. RNAP III inhibition with high amanitin concentrations promotes the reorganization of a perinucleolar compartment (PNC) distinct from the LNC and the PNCs.[41] This structure is enriched in polypyrimidine track binding protein (PTB) and several class III RNAs. Following RNAP III inhibition, the PNC is fragmented into a dotted structure forming a rosette with a hollow structure.

The splicing snRNPs usually colocalize with p80 coilin in coiled bodies; but, in cells treated with inhibitors, coilin clusters around the nucleolus in LNCs and snRNPs aggregate in distinct nucleoplasmic speckles.[42] U1 snRNP undergoes the most spectacular re-localization; it becomes clustered around the nucleoli in the DNCs.

All these changes are readily observable under the microscope and might serve as reliable indicators of transcription inhibition. Assembly of PSF into the DNCs is likely the most reliable indication of transcription inhibition.

α-Amanitin–Inhibiting RNA Polymerases II and III

α-Amanitin is a cyclic peptide isolated from Amanita mushrooms and responsible for their extreme toxicity. Amanitin binds with high specificity and high affinity (K_i = 3–4 nM) near the catalytic active site of RNAP II.[43] It traps a conformation of the enzyme that prevents nucleotide incorporation and translocation of the transcript.[44,45] RNAP II is the most sensitive polymerase.[46,47] RNAP III is a hundred-fold less sensitive than RNAP II. However, reduced expression of a number of class III genes in the presence of amanitin might be attributed to their regulation by RNAP II.[48,49] RNAP I is insensitive to amanitin.

α-amanitin is active on living plant, nematode, insect and mammalian cells. Yeast cells are insensitive to amanitin because of deficient uptake of the drug; nevertheless, the *S. cerevisiae* enzyme is highly susceptible. The organic anion-transporting polypeptide (OATP3) has been identified as the amanitin uptake transporter in human hepatic cells.[50] Amanitin uptake is slow (several hours). It is unusual to observe short-term effects. It must be used at much higher concentrations with living cells (at least 2 μg/ml) than in vitro. Methyl-amanitin oleate, a chemically modified derivative, permeates better and can be used at lower concentrations (0.010–0.1 μg/ml with human cells).

Amanitin is an irreversible inhibitor because it triggers degradation of Rpb1, the largest RNAP II subunit.[51] α-amanitin promotes polyubiquitination of Rpb1 in a nuclear extract prepared from cells arrested in S-phase.[52,53] However, the protease pathway has not been identified in living cells yet.

Several mutations in Rpb1 have been isolated that confer resistance to α-amanitin.[54,55] Despite mildly affecting the enzyme performance, amanitin-resistant alleles of Rpb1 can be used as efficient and convenient selection genes to obtain stable cell transformants. They are particularly convenient to obtain cells expressing tagged Rpb1 subunits that replace the endogenous one targeted to degradation by α-amanitin. The low concentration to be used (2 μg/ml with human cells) makes it a relatively inexpensive option.

Triptolide–Inhibiting TFIIH at Transcription Initiation

Triptolide is a diterpene triepoxide extracted from the plant *Tripterygium wilfordii*, used in Chinese traditional medicine. It has multiple interesting pharmacological properties including anti-inflammatory, immune modulation, anti-proliferative and pro-apoptotic activities (reviewed in ref. 56). Triptolide inhibits transcription at submicromolar concentrations.[57] Most of its proposed pharmacological effects relate to decreased gene expression[56] and might thus be attributed to inhibition of transcription. Triptolide binds to the XPB subunit of TFIIH.[58] The ATP-dependent helicase activity of XPB is required for the first step in transcription to open the double-strand DNA and create a "transcription bubble". Triptolide inhibits the ATPase activity of XPB, thus it prevents the formation of the "transcription bubble" and hence initiation of transcription. Triptolide treatment is irreversible as it binds covalently to XPB and induces a fast proteasome-dependent degradation of RNAPII.[58,59] Furthermore, class I gene transcription also relies on TFIIH and is inhibited by triptolide. This inhibitor is active at very low concentrations (IC_{50} = 109 nM for inhibition of RNA synthesis in HeLa cells). Less than an hour is required to inhibit transcription in HeLa cells at 10 μM concentration (Nguyen VT and Bensaude O, unpublished data). It is highly specific although it might also bind polycystin-2, a calcium channel.[60] Triptolide dissolves poorly in water, but promising water-soluble derivatives have been developed.[56] Because of its selectivity and its very rapid action, we expect the use of triptolide or its water-soluble derivatives to be generalized.

DRB and Flavopiridol—Kinase Inhibitors Preventing Entry into Transcription Elongation

CDK9 is required for efficient class I and class II gene expression. Transcription is a multistep process, RNAPs bind to DNA, the dsDNA is opened to form a so-called "transcription bubble" and transcription initiates. For most class II genes, negative elongation factors (NELFs) and Spt5, also known as DRB Sensitivity Inducing Factor (DSIF), provoke RNAP II pausing shortly after initiation and prevent a productive elongation of transcription.[61] Phosphorylation of these factors by Positive Transcription Elongation Factor (P-TEFb) is required to overcome the obstacle. CDK9 is the kinase subunit of P-TEFb. Hence, any CDK9 inhibitor will prevent productive transcription of most genes. Overall, Serine 2 phosphorylation in the heptapeptide CTD repeats of RNAP II decreases readily in cells exposed to CDK9 inhibitors.[64,65] Serine 2 phosphorylation of the CTD is required for pre-mRNA processing (splicing, termination and polyadenylation). Thus, splicing becomes impaired.[66] Transcription of short intron-less histone and <u> snRNA encoding genes is not affected by CDK9 inhibition.[62,63] Instead, 3' end processing of histone or U snRNA encoding genes is impaired.[62,63] CDK9 inhibitors also affect an early step in rRNA (class I gene) processing, thereby impairing ribosome biogenesis.[39]

Many compounds with potential pharmacological applications inhibit CDK9. Several CDK9 inhibitors are currently under clinical trial in chemotherapy,[67] in particular against chronic lymphocytic leukemia.[68] Some of these CDK9 inhibitors are occasionally used as inhibitors of transcription (e.g., roscovitine—also known as selicilib[37]—the isoquinoline sulfonamide (**H-8**),[69] and SNS-302).[70] However, DRB and flavopiridol are the most popular for molecular/cellular biology use (see below).

All CDK9 inhibitors compete with ATP for the kinase active site. Given its conservation, the selectivity of protein kinase inhibitors is a major issue that has been largely discussed by Knight and Shokat.[71] Comparison between inhibition efficiencies of various inhibitors on a panel of kinases may be found in reference 72–74. Many old studies used DRB as a casein kinase 2 inhibitor or H-8 as a PKA inhibitor, and improper conclusions were often drawn. These compounds efficiently target CDK9 and thus act as general transcription inhibitors like DRB or flavopiridol. New screenings have been designed to provide more selective inhibitors that might become useful pharmacological agents.[75]

One should mention the exquisitely selective "chemical genetic" method developed by Shokat and coworkers.[76] The ATP binding site of the kinase to be investigated is enlarged by replacement of a voluminous aminoacid residue selected from crystallographic data. The modified kinase becomes highly susceptible to naphtyl-ATP, which is too large to enter the ATP binding site and inhibit any natural kinase. This method has been successfully used with transcription CDKs.[77] However, it requires a gene replacement strategy that is relatively easy to set up in simple organisms such as yeast but quite difficult in mammals.

DRB (5,6-Dichloro-1-beta-Ribofuranosyl Benzimidazole) has been widely used as a transcription inhibitor. This compound was initially reported to inhibit nuclear heterogeneous RNA (hnRNA) synthesis.[78,79] It quickly appeared to cause "premature" chain termination. CDK9 was identified as its major target.[80] However, it also inhibits CDK7, the kinase subunit of TFIIH, with 3-fold lower efficiency.[72] The above-mentioned "DSIF" or DRB Sensitivity Inducing Factor refers to DRB. The crystal structure of CDK9 complexed with DRB has been resolved.[81] The chlorine atoms form halogen bonds with a hinge region characteristic of CDK9 near the ATP binding site.

DRB has to be used at concentrations close to its maximal solubility (100 μM). Mother solutions in DMSO and fast homogenization in warm media are recommended. RNAPII transcription arrests within minutes following addition of DRB to culture medium. Efficient transcription resumes within minutes when the medium is replaced by fresh medium to remove the drug. This property has recently been used to measure transcription rates.[82]

Despite the selectivity issue and limitations due to its poor solubility, DRB remains a very popular inhibitor. Its major advantages are common usage, rapidity of action and reversibility.

Flavopiridol is now becoming popular because it is the most efficient known CDK9 inhibitor.[83] Its inhibition constant (K_i = 3 nM) is several orders of magnitude lower than the ATP binding constant (K_m = 36 μM). The crystal structure of CDK9 complexed with flavopiridol has been solved.[84] It is buried into the ATP binding site after inducing a structural change in the kinase. However, it also inhibits rather efficiently (10-fold less) the cell cycle CDK1 and CDK4[74] as well as CDK8, the mediator kinase.[85] Its major advantages reside in its water solubility and its efficiency at submicromolar concentrations (IC_{50} = 100–300 nM) on living cells.

Actinomycin D—DNA Intercalators Blocking the Progression of RNA Polymerases

Many DNA intercalators inhibit transcription. But not all do so. For example, Hoechst 33,342 intercalates into nuclear DNA of living cells but does not affect its transcription significantly.[6] Ethidium bromide affects mitochondrial but not nuclear transcription.[86]

Actinomycin D or Dactinomycin is likely the most popular inhibitor of transcription. It comprises two cyclic peptides linked together by a phenoxazine derivative. It is isolated from Streptomyces bacteria. Actinomycin D is also one of the older chemotherapy drugs, commonly used to treat gestational trophoblastic cancer, testis cancer, Wilm's tumor, rhabdomyosacoma and Ewing's sarcoma. Transcription by all three eukaryotic polymerases is affected. Yet, class I gene transcription is by far the most sensitive (0.05 μg/ml) followed by class II gene transcription (0.5 μg/ml) and class III (around 5 μg/ml). The length of the transcription unit and its DNA sequence composition are determinant.[87] Actinomycin preferentially intercalates into GC rich sequences and stabilizes topoisomerase-I DNA covalent complexes that prevent RNA polymerase progression.[88]

Actinomycin D generates double-strand breaks in DNA and induces the synthesis of γ-histone H2AX molecules that accumulate into foci.[89] Transcription is slightly recovered when actinomycin D is removed from the culture medium but several hours are required.[90,91] Actinomycin promotes the accumulation of CTD phosphorylated RNAPII[11] likely due to an enhanced P-TEFb activity.[92]

We will terminate by mentioning two compounds with interesting properties. Cisplatin is a widely used anti-cancer drug. It cross-links DNA thereby blocking the progression of RNA polymerase molecules.[93] Yet, the cisplatin/DNA adducts, and hence the block, can be removed by the nucleotide excision repair machinery. Trabectedin or Yondalis (ET-743) is developed as a new anti-cancer drug that has been isolated from the marine tunicate *Ecteinascidia turbinata*. It intercalates into DNA and promotes a fast proteasome-dependent degradation of RNAP II.[94]

Concluding Remarks

To conclude, the classical inhibitors of transcription have advantages and drawbacks. Amanitin is highly selective for RNAP II and RNAP III but slow, actinomycin D is fast but its selectivity is poor, CDK9 inhibitors are fast and reversible but many genes escape transcription inhibition. New inhibitors such as triptolide are fast, selective and completely arrest transcription as they trigger rapid degradation of RNAP II.

The physiological effects of transcription inhibitors have been described as the "transcriptional stress response".[26] They occur after treatments such as irradiation that lead to DNA lesions. The stress response may have interesting pharmacological consequences. Friend virus-transformed murine erythroleukemia (MEL) cells differentiate when exposed to mild concentrations of actinomycin D[95] or DRB.[96] Flavopiridol attenuates leukocyte-endothelial cell interaction.[85] More generally, an arrest in transcription will lead to cell death, often through apoptosis.

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References

1. Sugaya K, Sasanuma S, Cook PR, Mita K. A mutation in the largest (catalytic) subunit of RNA polymerase II and its relation to the arrest of the cell cycle in G(1) phase. Gene 2001; 274:77-81.
2. Jackman J, Alamo IJ, Fornace AJJ. Genotoxic stress confers preferential and coordinate messenger RNA stability on the five gadd genes. Cancer Res 1994; 54:5656-62.
3. Lam LT, Pickeral OK, Peng AC, Rosenwald A, Hurt EM, Giltnane JM, et al. Genomic-scale measurement of mRNA turnover and the mechanisms of action of the anti-cancer drug flavopiridol. Genome Biol 2001; 2:41.
4. Lü X, Burgan WE, Cerra MA, Chuang EY, Tsai MH, Tofilon PJ, et al. Transcriptional signature of flavopiridol-induced tumor cell death. Mol Cancer Ther 2004; 3:861-72.
5. Chen Q, Cao M, Xiang WL, Sun Q, Zhang J, Hou RT, et al. Study on genes with altered expression in alpha-amanitin poisoned mice and evaluation on antagonistic effects of traditional Chinese medicines against toxicity of alpha-amanitin. Acta Biol Hung 2009; 60:281-91.
6. White CM, Heidenreich O, Nordheim A, Beerman TA. Evaluation of the effectiveness of DNA-binding drugs to inhibit transcription using the c-fos serum response element as a target. Biochemistry 2000; 39:12262-73.
7. Levsky JM, Singer RH. Fluorescence in situ hybridization: past, present and future. J Cell Sci 2003; 116:2833-8.
8. Femino AM, Fay FS, Fogarty K, Singer RH. Visualization of single RNA transcripts in situ. Science 1998; 280:585-90.
9. Gossen M, Bonin AL, Freundlieb S, Bujard H. Inducible gene expression systems for higher eukaryotic cells. Curr Opin Biotechnol 1994; 5:516-20.
10. Loew R, Heinz N, Hampf M, Bujard H, Gossen M. Improved Tet-responsive promoters with minimized background expression. BMC Biotechnol 2010; 10:81.
11. Cassé C, Giannoni F, Nguyen VT, Dubois MF, Bensaude O. The transcriptional inhibitors, actinomycin D and α-amanitin, activate the HIV-1 promoter and favor phosphorylation of the RNA polymerase II C-terminal domain. J Biol Chem 1999; 274:16097-106.
12. Imamichi T, Conrads TP, Zhou M, Liu X, Adelsberger JW, Veenstra TD, et al. A transcription inhibitor, actinomycin D, enhances HIV-1 replication through an interleukin-6-dependent pathway. J Acquir Defic Syndr 2005; 40:388-97.
13. Zhou Q, Yik JH. The Yin and Yang of P-TEFb regulation: implications for human immunodeficiency virus gene expression and global control of cell growth and differentiation. Microbiol Mol Biol Rev 2006; 70:646-59.
14. Barrandon C, Spiluttini B, Bensaude O. Non-coding RNAs regulating the transcriptional machinery. Biol Cell 2008; 100:83-95.
15. Dreyfuss G, Kim VN, Kataoka N. Messenger-RNA-binding proteins and the messages they carry. Nat Rev Mol Cell Biol 2002; 3:195-205.
16. Hogg JR, Collins K. RNA-based affinity purification reveals 7SK RNPs with distinct composition and regulation. RNA 2007; 13:868-80.
17. Van Herreweghe E, Egloff S, Goiffon I, Jady BE, Froment C, Monsarrat B, et al. Dynamic remodelling of human 7SK snRNP controls the nuclear level of active P-TEFb. EMBO J 2007; 26:3570-80.
18. Barrandon C, Bonnet F, Nguyen VT, Labas V, Bensaude O. The transcription-dependent dissociation of P-TEFb.HEXIM1.7SK RNA relies upon formation of hnRNP.7SK RNA complexes. Mol Cell Biol 2007; 27:6996-7006.
19. Gomes NP, Bjerke G, Llorente B, Szostek SA, Emerson BM, Espinosa JM. Gene-specific requirement for P-TEFb activity and RNA polymerase II phosphorylation within the p53 transcriptional program. Genes Dev 2006; 20:601-12.
20. Choong ML, Yang H, Lee MA, Lane DP. Specific activation of the p53 pathway by low dose actinomycin D: a new route to p53 based cyclotherapy. Cell Cycle 2009; 8:2810-8.
21. Beckerman R, Prives C. Transcriptional regulation by p53. Cold Spring Harb Perspect Biol 2011; 2:935.
22. Galluzzi L, Morselli E, Kepp O, Vitale I, Pinti M, Kroemer G. Mitochondrial liaisons of p53. Antioxid Redox Signal 2011; In press.
23. Mosner J, Mummenbrauer T, Bauer C, Sczakiel G, Grosse F, Deppert W. Negative feedback regulation of wild-type p53 biosynthesis. EMBO J 1995; 14:4442-9.
24. An WG, Chuman Y, Fojo T, Blagosklonny MV. Inhibitors of transcription, proteasome inhibitors and DNA-damaging drugs differentially affect feedback of p53 degradation. Exp Cell Res 1998; 244:54-60.
25. Demidenko ZN, Blagosklonny MV. Flavopiridol induces p53 via initial inhibition of Mdm2 and p21 and, independently of p53, sensitizes apoptosis-reluctant cells to tumor necrosis factor. Cancer Res 2004; 64:3653-60.
26. Ljungman M. The transcription stress response. Cell Cycle 2007; 6:2252-7.
27. Radhakrishnan SK, Bhat UG, Halasi M, Gartel AL. P-TEFb inhibitors interfere with activation of p53 by DNA-damaging agents. Oncogene 2008; 27:1306-9.
28. Hölzel M, Orban M, Hochstatter J, Rohrmoser M, Harasim T, Malamoussi A, et al. Defects in 18 S or 28 S rRNA processing activate the p53 pathway. J Biol Chem 2010; 285:6364-70.
29. Vousden KH, Prives C. Blinded by the light: the growing complexity of p53. Cell 2009; 137:413-31.
30. Zhang Y, Wang J, Yuan Y, Zhang W, Guan W, Wu Z, et al. Negative regulation of HDM2 to attenuate p53 degradation by ribosomal protein L26. Nucleic Acids Res 2010; 38:6544-54.
31. Takagi M, Absalon MJ, McLure KG, Kastan MB. Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. Cell 2005; 123:49-63.
32. Chen J, Kastan MB. 5'-3'-UTR interactions regulate p53 mRNA translation and provide a target for modulating p53 induction after DNA damage. Genes Dev 2010; 24:2146.
33. Davie JR, Murphy LC. Level of ubiquitinated histone H2B in chromatin is coupled to ongoing transcription. Biochemistry 1990; 29:4752-7.
34. Chadee DN, David Allis C, Wright JA, Davie JR. Histone H1b phosphorylation is dependent upon ongoing transcription and replication in normal and ras-transformed mouse fibroblasts. J Biol Chem 1997; 272:8113-6.
35. Piñol-Roma S, Dreyfuss G. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature 1992; 355:730-2.
36. Christian KJ, Lang MA, Raffalli-Mathieu F. Interaction of heterogeneous nuclear ribonucleoprotein C1/C2 with a novel cis-regulatory element within p53 mRNA as a response to cytostatic drug treatment. Mol Pharmacol 2008; 73:1558-67.
37. Biglione S, Byers SA, Price JP, Nguyen VT, Bensaude O, Price DH, et al. Inhibition of HIV-1 replication by P-TEFb inhibitors DRB, seliciclib and flavopiridol correlates with release of free P-TEFb from the large, inactive form of the complex. Retrovirology 2007; 4:47.

38. Boulon S, Westman BJ, Hutten S, Boisvert FM, Lamond AI. The nucleolus under stress. Mol Cell 2010; 40:216-27.
39. Burger K, Mühl B, Harasim T, Rohrmoser M, Malamoussi A, Orban M, et al. Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. J Biol Chem 2010; 285:12416-25.
40. Shav-Tal Y, Blechman J, Darzacq X, Montagna C, Dye BT, Patton JG, et al. Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition. Mol Biol Cell 2005; 16:2395-413.
41. Pollock C, Huang S. The perinucleolar compartment. J Cell Biochem 2009; 107:189-93.
42. Carmo-Fonseca M, Pepperkok R, Carvalho MT, Lamond AI. Transcription-dependent colocalization of the U1, U2, U4/U6 and U5 snRNPs in coiled bodies. J Cell Biol 1992; 117:1-14.
43. Bushnell DA, Cramer P, Kornberg RD. RNA polymerase II cocrystal at 2.8 A resolution. Proc Natl Acad Sci USA 2002; 99:1218-22.
44. Kaplan CD, Larsson KM, Kornberg RD. The RNA polymerase II trigger loop functions in substrate selection and is directly targeted by alpha-amanitin. Mol Cell 2008; 30:547-56.
45. Brueckner F, Cramer P. Structural basis of transcription inhibition by alpha-amanitin and implications for RNA polymerase II translocation. Nat Struct Mol Biol 2008; 15:811-8.
46. Kedinger C, Nuret P, Chambon P. Structural evidence for two alpha-amanitin sensitive RNA polymerases in calf thymus. FEBS Lett 1971; 15:169-74.
47. Weinmann R, Raskas HJ, Roeder RG. Role of DNA-dependent RNA polymerases II and III in transcription of the adenovirus genome late in productive infection. Proc Natl Acad Sci USA 1974; 71:3426-39.
48. Listerman I, Bledau AS, Grishina I, Neugebauer KM. Extragenic accumulation of RNA polymerase II enhances transcription by RNA polymerase III. PLoS Genet 2007; 3:212.
49. Raha D, Wang Z, Moqtaderi Z, Wu L, Zhong G, Gerstein M, et al. Close association of RNA polymerase II and many transcription factors with Pol III genes. Proc Natl Acad Sci USA 2010; 107:3639-44.
50. Letschert K, Faulstich H, Keller D, Keppler D. Molecular characterization and inhibition of amanitin uptake into human hepatocytes. Toxicol Sci 2006; 91:140-9.
51. Nguyen VT, Giannoni F, Dubois MF, Seo SJ, Vigneron M, Kédinger C, et al. In vivo degradation of RNA polymerase II largest subunit triggered by α-amanitin. Nucleic Acids Res 1996; 24:2924-9.
52. Lee KB, Sharp PA. Transcription-dependent polyubiquitination of RNA polymerase II requires lysine 63 of ubiquitin. Biochemistry 2004; 43:15223-9.
53. Jung Y, Lippard SJ. RNA polymerase II blockage by Cisplatin-damaged DNA. Stability and polyubiquitylation of stalled polymerase. J Biol Chem 2006; 281:1361-70.
54. Bartolomei MS, Corden JL. Clustered alpha-amanitin resistance mutations in mouse. Mol Gen Genet 1995; 246:778-82.
55. Chen Y, Chafin D, Price DH, Greenleaf A. Drosophila RNA polymerase II mutants that affect transcription elongation. J Biol Chem 1996; 271:5993-9.
56. Liu Q. Triptolide and its expanding multiple pharmacological functions. Int Immunopharmacol 2011; 11:377-83.
57. Leuenroth SJ, Crews CM. Triptolide-induced transcriptional arrest is associated with changes in nuclear substructure. Cancer Res 2008; 68:5257-66.
58. Titov DV, Gilman B, He QL, Bhat S, Low WK, Dang Y, et al. XPB, a subunit of TFIIH, is a target of the natural product triptolide. Nat Chem Biol 2011; 7:182-8.
59. Vispé S, DeVries L, Créancier L, Besse J, Bréand S, Hobson DJ, et al. Triptolide is an inhibitor of RNA polymerase I and II-dependent transcription leading predominantly to downregulation of short-lived mRNA. Mol Cancer Ther 2009; 8:2780-90.
60. Leuenroth SJ, Okuhara D, Shotwell JD, Markowitz GS, Yu Z, Somlo S, et al. Triptolide is a traditional Chinese medicine-derived inhibitor of polycystic kidney disease. Proc Natl Acad Sci USA 2007; 104:4389-94.
61. Nechaev S, Adelman K. Pol II waiting in the starting gates: Regulating the transition from transcription initiation into productive elongation. Biochim Biophys Acta 2011; 1809:34-45.
62. Medlin J, Scurry A, Taylor A, Zhang F, Peterlin BM, Murphy S. P-TEFb is not an essential elongation factor for the intronless human U2 snRNA and histone H2b genes. EMBO J 2005; 24:4154-65.
63. Pirngruber J, Shchebet A, Schreiber L, Shema E, Minsky N, Chapman RD, et al. CDK9 directs H2B monoubiquitination and controls replication-dependent histone mRNA 3'-end processing. EMBO Rep 2009; 10:894-900.
64. Dubois MF, Bellier S, Seo SJ, Bensaude O. Phosphorylation of the RNA polymerase II largest subunit during heat-shock and inhibition of transcription in HeLa cells. J Cell Physiol 1994; 158:417-26.
65. Lavoie SB, Albert AL, Handa H, Vincent M, Bensaude O. The peptidyl-prolyl isomerase Pin1 interacts with hSpt5 phosphorylated by Cdk9. J Mol Biol 2001; 312:675-85.
66. Ip JY, Schmidt D, Pan Q, Ramani AK, Fraser AG, Odom DT, et al. Global impact of RNA polymerase II elongation inhibition on alternative splicing regulation. Genome Res 2011; 21:390-401.
67. Wang LM, Ren D. Flavopiridol, the first cyclin-dependent kinase inhibitor: recent advances in combination chemotherapy. Mini Rev Med Chem 2010; 10:1058-70.
68. Abou-Nassar K, Brown J. Novel agents for the treatment of chronic lymphocytic leukemia. Clin Adv Hematol Oncol 2010; 8:886-95.
69. Marshall NF, Peng J, Xie Z, Price DH. Control of RNA polymerase II elongation potential by a novel carboxyl-terminal domain kinase. J Biol Chem 1996; 271:27176-83.
70. Chen R, Wierda WG, Chubb S, Hawtin RE, Fox JA, Keating MJ, et al. Mechanism of action of SNS-032, a novel cyclin-dependent kinase inhibitor, in chronic lymphocytic leukemia. Blood 2009; 113:4637-45.
71. Knight ZA, Shokat KM. Features of Selective Kinase Inhibitors. Chem Biol 2005; 12:621-37.
72. Mancebo HS, Lee G, Flygare J, Tomassini J, Luu P, Zhu Y, et al. P-TEFb kinase is required for HIV Tat transcriptional activation in vivo and in vitro. Genes Dev 1997; 11:2633-44.
73. Chao SH, Price DH. Flavopiridol inactivates P-TEFb and blocks most RNA polymerase II transcription in Vivo. J Biol Chem 2001; 276:31793-9.
74. Conroy A, Stockett DE, Walker D, Arkin MR, Hoch U, Fox JA, et al. SNS-032 is a potent and selective CDK 2, 7 and 9 inhibitor that drives target modulation in patient samples. Cancer Chemother Pharmacol 2009; 64:723-32.
75. Wang S, Griffiths G, Midgley CA, Barnett AL, Cooper M, Grabarek J, et al. Discovery and characterization of 2-anilino-4-(thiazol-5-yl)pyrimidine transcriptional CDK inhibitors as anticancer agents. Chem Biol 2010; 17:1111-21.
76. Bishop AC, Buzko O, Shokat KM. Magic bullets for protein kinases. Trends Cell Biol 2001; 11:167-72.
77. Tietjen JR, Zhang DW, Rodríguez-Molina JB, White BE, Akhtar MS, Heidemann M, et al. Chemical-genomic dissection of the CTD code. Nat Struct Mol Biol 2010; 17:1154-61.
78. Sehgal PB, Derman E, Molloy GR, Tamm I, Darnell JE. 5,6-Dichloro-1-ß-D-ribofuranosylbenzimidazole inhibits initiation of nuclear heterogeneous RNA chains in HeLa cells. Science 1976; 194:431-3.
79. Fraser NW, Sehgal PB, Darnell JE. DRB-induced premature termination of late adenovirus transcription. Nature 1978; 272:590-3.
80. Zhu Y, Pe'ery T, Peng J, Ramanathan Y, Marshall N, Marshall T, et al. Transcription elongation factor P-TEFb is required for HIV-1 Tat transactivation in vitro. Genes Dev 1997; 11:2622-32.
81. Baumli S, Endicott JA, Johnson L. Halogen bonds form the basis for selective P-TEFb inhibition by DRB. Chem Biol 2010; 17:931-6.
82. Singh J, Padgett RA. Rates of in situ transcription and splicing in large human genes. Nat Struct Mol Biol 2009; 16:1128-33.
83. Chao SC, Fujinaga K, Marion JE, Taube R, Sausville EA, Senderowicz AM, et al. Flavopiridol inhibits P-TEFb and blocks HIV-1 replication. J Biol Chem 2000; 275:28345-8.
84. Baumli S, Lolli G, Lowe ED, Troiani SLR, Bullock AN, et al. The structure of P-TEFb (CDK9/cyclin T1), its complex with flavopiridol and regulation by phosphorylation. EMBO J 2008; 27:1907-18.
85. Schmerwitz UK, Sass G, Khandoga AG, Joore J, Mayer BA, Berberich N, et al. Flavopiridol protects against inflammation by attenuating leukocyte-endothelial interaction via inhibition of cyclin-dependent kinase 9. Arterioscler Thromb Vasc Biol 2011; 31:280-8.
86. Zylber E, Vesco C, Penman S. Selective inhibition of the synthesis of mitochondria-associated RNA by ethidium bromide. J Mol Biol 1969; 44:195-204.
87. Perry RP, Kelley DE. Inhibition of RNA synthesis by actinomycin D: characteristc dose-response of different RNA species. J Cell Physiol 1970; 76:127-40.
88. Trask DK, Muller MT. Stabilization of type I topoisomerase-DNA covalent complexes by actinomycin D. Proc Natl Acad Sci USA 1988; 85:1417-21.
89. Mischo HE, Hemmerich P, Grosse F, Zhang S. Actinomycin D induces histone gamma-H2AX foci and complex formation of gamma-H2AX with Ku70 and nuclear DNA helicase II. J Biol Chem 2005; 280:9586-94.
90. Schluederberg A, Hendel RC, Chavanich S. Actinomycin D: renewed RNA synthesis after removal from mammalian cells. Science 1971; 172:577-9.
91. Hadjiolova KV, Hadjiolov AA, Bachellerie JP. Actinomycin D stimulates the transcription of rRNA minigenes transfected into mouse cells. Eur J Biochem 1995; 228:605-15.
92. Nguyen VT, Kiss T, Michels AA, Bensaude O. 7SK snRNA binds to and inhibits the activity of Cdk9/cyclin T complexes. Nature 2001; 414:322-5.
93. Ang WH, Myint M, Lippard SJ. Transcription inhibition by platinum-DNA cross-links in live mammalian cells. J Am Chem Soc 2010; 132:7429-35.
94. Aune GJ, Takagi K, Sordet O, Guirouilh-Barbat J, Antony S, Bohr VA, et al. Von Hippel-Lindau-coupled and transcription-coupled nucleotide excision repair-dependent degradation of RNA polymerase II in response to trabectedin. Clin Cancer Res 2008; 14:6449-55.
95. Terada TM, Epner E, Nudel U, Salmon J, Fibach E, Rifkind RA, et al. Induction of murine erythroleukemia differentiation by actinomycin D. Proc Natl Acad Sci USA 1978; 75:2795-9.
96. Hensold J, Barth D, Stratton CA. RNA polymerase II inhibitor, 5,6-dichloro-1-ß-D-Ribofuranosylbenzimidazole (DRB) causes erythroleukemic differentiation and transcriptional activation of erythroid genes. J Cell Physiol 1996; 168:105-13.