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POINT-OF-VIEW

FUBP/KH domain proteins in transcription: Back to the future

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ABSTRACT
Drosophila genetic studies demonstrate that cell and tissue growth regulation is a primary developmental function of P-element somatic inhibitor (Psi), the sole ortholog of FUBP family RNA/DNA-binding proteins. Psi achieves growth control through interaction with Mediator, observations that should put to rest controversy surrounding Pol II transcriptional functions for these KH domain proteins.

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Preface

Here I provide an historical perspective on the FUBP family of KH domain proteins in transcription, starting ~25 years ago with discovery of FUBP1s single stranded DNA (ssDNA) binding function, and implication in activating transcription of the *MYC* oncogene.[1-3] Despite the decades that have passed, the transcriptional function of the FUBP family remains somewhat controversial; most literature attributes RNA-binding functions, both as negative and positive regulators of mRNA splicing, mRNA stability, mRNA export and mRNA translation (reviewed in Ref. [4]). The dogma remains: RNA processing constitutes the primary role of FUBP proteins, while interaction with ssDNA to elicit transcriptional control is a lesser function. Moreover, as evidence toward understanding FUBP1 function has been gathered primarily using mammalian systems, where function can be obscured by redundancy between multiple family members, key physiologic roles have remained unclear. Recent *Drosophila* genetic studies revealed a major developmental function of the sole FUBP ortholog (Psi); interaction with the RNA Polymerase II (Pol II) Mediator complex, regulation of *MYC* expression and control of cell and tissue growth.[5]

FUSE—a nuclease-sensitive site in the *MYC* promoter modulates transcription

Even small changes in abundance of the MYC oncoprotein can significantly alter cell growth and proliferation,[6] hallmarks of cancer. The interest in *MYC* promoter regulation was fuelled by the observation that translocated *MYC* alleles in Burkitt's Lymphoma that contain either truncated or mutated exon 1.[7,8] Analysis of the endogenous *MYC* promoter in human leukemia cell lines revealed that induction of differentiation, and transcriptional downregulation of *MYC*, was associated with a block to Pol II elongation.[9] The exon 1 mutation prevents the Pol II block and leads to constitutive Pol II read-through and elevated *MYC*.[8,10-12]

The complexity of the *MYC* promoter and regulation by post-initiation release of paused Pol II reflects the multitude of signaling inputs converging on *MYC* transcription. As early studies found a correlation between *MYC* promoter sensitivity to nuclease cleavage and expression levels,[9,10,13] analysis of nuclease-sensitive elements was used as a means to understand integration of signaling inputs. Interestingly, of the multiple regions containing sequence-specific binding sites predicted for *MYC* regulators, only the region 1.5kb upstream of the P1 promoter lost binding activity, following induced differentiation and downregulation of *MYC* expression.[1] The term Far Upstream

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Sequence Element (FUSE) was coined for this region of binding activity, which was most abundant before the decrease in *MYC* associated with differentiation. FUSE serves a positive role in *MYC* transcription as deletion significantly reduces *MYC*-reporter activity, but insertion of multiple copies of FUSE upstream, in isolation, cannot stimulate the *MYC* promoter.[1] Thus, FUSE does not behave as a traditional enhancer, but constitutes a non-canonical mechanism for transcriptional control.

FUBP1—discovery based on function

Oligonucleotide affinity chromatography, from proliferating/undifferentiated cell extracts, using the A–T rich double stranded FUSE identified the Fuse Binding Protein (initially FBP and recently renamed FUBP1). Electrophoretic mobility-shift assay (EMSA) with oligonucleotide probes only detected FUBP1 on the noncoding strand; no significant complex formation was observed with annealed double-stranded FUSE, for the coding strand, nor using non-FUSE sequence single-stranded oligonucleotide probes.[2] FUBP1 binding was dramatically decreased following induction of differentiation and downregulation of *MYC* in leukemia cell lines, consistent with preferential interaction with the active *MYC* promoter. These observations were difficult to navigate as initial analysis of the FUBP1 protein sequence[2] revealed no significant homology with known DNA-binding motifs, but identified the "FBP repeat" (30-residue sheet-turn-helix repeats). These repeat domains displayed homology with KH domains found in heterogeneous nuclear ribonucleoprotein complex (hnRNP) protein K,[14] which was also found upstream of the *MYC* P1 promoter on single stranded CT elements.[15] Deletion analysis revealed that, although there were four KH domains, only two were required for FUBP1 binding to FUSE. It was, therefore, hypothesized that FUBP1 might bind two DNA sites to enable promoter looping between the FUSE and downstream Pol II regulatory factors, which was indeed confirmed in future studies (discussed below).

At the time of these studies, the dogma was that transcription factors anchored to B-form *cis* elements in promoters, via a sequence-specific double stranded DNA-binding domain, while their effector domain modified Pol II activity through protein–protein interactions with the transcriptional machinery. In contrast, the molecular architecture of FUBP1 was certainly unusual, being comprised of an array of KH domain ssDNA binding motifs in quadruplicate and an N-terminal activation domain. These revelatory observations suggested that, in addition to conventional dsDNA-binding transcriptional regulators, activity of single-strand nucleic acid binding proteins could drive Pol II activity. The conceptual challenges arising when non-conventional functions are attributed to a given protein were highlighted by the skepticism in the transcription field toward FUBP1-dependent mechanisms for transcriptional control.

FUBP1—a non-conventional transcriptional regulator

At all promoters, melting of duplex DNA is obligatory for Pol II entry and transcriptional initiation. DNase sensitivity of the *MYC* promoter correlates with transcription; increased *MYC* expression will result in region-specific destabilization of B-DNA in torsionally strained regions of the active *MYC* promoter.[16] As FUBP1 binds single stranded FUSE, the formation of the FUBP1–DNA complex naturally requires prior unwinding of the DNA helix, provided by the forward movement of Pol II in the active *MYC* promoter that will generate the energy required for FUSE melting and FUBP1 binding. The studies in the mid-80s revealed broad regions of specific SI nuclease (single-strand nucleic acid) sensitivity in chromatin upstream of the human *MYC* gene,[9,10,13] but these sites had not been mapped with sufficient accuracy to relate them directly to the FUSE. Potassium permanganate reacts preferentially with thymine in ssDNA, thus enabling conformation to be determined with single-base resolution. Permanganate mapping of the *MYC* coding strand revealed hyperreactive thymidine residues in FUSE, consistent with an open single strand extending from FUSE toward P1.[16] In contrast, the noncoding strand was predominantly hyporeactive, particularly protected were nucleotides in the DNA segment preferentially bound by FUBP1 *in vitro*, i.e. consistent with FUBP1 binding the single stranded noncoding strand of FUSE *in vivo*.[2]

As the A–T-rich FUSE is contained within a region of helical instability, strand separation associated with melting and subsequent FUBP1 binding were

predicted to drive supercoiling and generate torsional energy within adjacent dsDNA strands.[16] *In vitro*, FUBP1 can force double strand separation of the FUSE contained in supercoiled, not relaxed, double stranded plasmid DNA, driving further opening at distances over 2.8kb.[3] As conversion of negatively supercoiled plasmid to the relaxed form using Topoisomerase I (topo-I) abolished all strand melting, the helical stabilization effect on FUSE in supercoiled DNA is a result of non-B-DNA induced by FUBP1 at the more distant sites. Bringing these data together, promoter activity can, therefore, drive FUSE opening and enable interaction with FUBP1, while the associated increase in Pol II transcription will permit torsionally stressed, supercoiled DNA surrounding FUSE to further drive DNA strand separation and facilitate maximal *MYC* transcription. Thus, energy generated from the torsional stress and supercoiling associated with Pol II movement can be harnessed as a productive force in transcription. In context of chromatin-associated FUSE, it had been speculated that because non-B-DNA is incompatible with nucleosome binding, FUBP1 binding *in vivo* might require nucleosome-free regions.[23] Nuclease sensitivity assays have shown that when *MYC* transcription is off, there is a nucleosome over FUSE that becomes mobilized after promoter activation.[16] It has been speculated that negative supercoiling propagated upstream might favor nucleosome binding, while positive supercoiling from a divergent promoter could also force the nucleosome off, but fundamentally we do not know how this occurs.

Twenty years ago, these concepts were rather radical. To propose that transcriptional regulatory proteins engaged with specific ssDNA structures in promoters, rather than with double stranded sequences, was controversial enough. To posit that DNA itself was an instructive transcriptional force raised further eyebrows, particularly at a time when proteins were considered the engines of transcription, with DNA being a mere landing site and transcriptional template. Testament to the validity of these concepts, not only has the regional DNA melting and ssDNA binding activity originally identified and modeled in *MYC* stood the test of time,[17-24] but genome wide technologies have revealed non-B-DNA as an essential regulatory feature of all promoters.[25-29]

FUBP-interacting Repressor (FIR), the FUBP1 antagonist

The FUBP1 Interacting Repressor (FIR) blocks FUBP1-dependent *MYC* transcription by dampening XPB helicase activity of the general transcription factor (GTF) complex TFIIH,[30] which is last to load promoters and essential for Pol II promoter escape. Thus, while FUBP1 maximizes release of Pol II, repression of TFIIH by FIR late in the transcription cycle would provide a mechanism to safeguard against inappropriate or excessive signal-induced *MYC* activation. Evidence for the FIR–XPB system of *MYC* transcriptional repression *in vivo* has been reported using *Drosophila* models. The homolog of FIR, Hfp, interacts with the XPB homolog (Hay) to decrease the rate of Pol II pause release from the *MYC* promoter, and thus inhibit *MYC* transcription, cell and tissue growth.[31-33]

The *MYC* promoter integrates multiple signaling inputs; serum-stimulation of mammalian tissue culture cells rapidly activates the endogenous *MYC* promoter.[34] Elegant *ex vivo* Chromatin Immunoprecipitation (ChIP) time-course experiments revealed the dynamic events on the *MYC* promoter following mitogen-stimulated *MYC* transcription.[22] *MYC* enhancers and activators load first, FUBP1 is subsequently detected before decreased Pol II loading (i.e. Pol II release) and the peak in *MYC* mRNA.[22] Following the *MYC* mRNA maximum, FUBP1 and FIR initially co-localize, and as *MYC* returns to basal levels, FUBP1 exits and only FIR is detected on FUSE.[22] Pol II depletion and maximal enrichment for FUBP1 occurring before the peak in *MYC* concurs with FUBP1 promoting the Pol II release required to hyper-activate *MYC* transcription.

Developmental function of FUBP1

The function of FUBP1 during development is still relatively obscure. Early studies demonstrated that reduced FUBP1 is required for *MYC* expression and proliferation in *ex vivo* cultured cells.[35,36] Recent *FUBP1* gene trap[37] and knockout studies[38] revealed essential functions in haematopoietic stem cell (HSC) self-renewal, which is associated with anaemia and embryonic lethality. Interestingly, *MYC* mRNA levels vary drastically between individual *ex vivo* cultures of *FUBP1* knockout mouse embryo fibroblasts (MEFs) harvested from different *FUBP1* embryos.[38] The reason for this variability is currently unknown, but

reduced FUBP1 binding on *MYC* might impair recruitment of FIR and prevent *MYC* shut down. Another possibility is redundancy and/or compensatory activity by FUBP2 or FUBP3, which possess transcriptional activation domains and bind FUSE,[39] but as FIR only binds the former,[21] FUBP2 is most likely to compensate for loss of FUBP1. Moreover, as FUBP1 and 2 regulate common target genes, including *MYC*,[21] determining the phenotypic outcome of FUBP1/2 double knockout, and whether FUBP1 loss-of-function leads to heightened FUBP2 activity, will be critical for clarifying the function of FUBP proteins in mammalian development. Moreover, the RNA-processing functions of FUBP family members (mRNA translation, splicing and stabilization, reviewed[4]) will also contribute to these complex phenotypes. Although the sequence and/or structure of FUBP1s RNA targets are yet to be defined *in vivo*, we predict differences in RNA and single-stranded DNA binding affinity would provide FUBP1 further capacity to integrate complex developmental signals.

Drosophila FUBP1/Psi interacts with mediator to control tissue growth during development

The capacity of the *MYC* promoter to integrate developmental signals is fundamental for growth patterning in multicellular animals, with connections between signaling and *MYC* transcription best delineated in *Drosophila*.[40] Until recently, whether Psi (P-element somatic inhibitor), the sole FUBP protein in flies, integrated signaling to drive *MYC* transcription was unknown. Psi has been ascribed functions modulating splicing of transposable P-elements,[41] a function that cannot reflect evolutionary pressures as P-elements only entered the *Drosophila melanogaster* genome ~50 years ago.[42] Indeed, subsequent studies revealed broader roles for Psi in pre-mRNA splicing, including for many genes required for male courtship behavior.[43] Further to these RNA processing functions, and in accordance with Psi behaving in a functionally analogous manner to FUBP1, recent studies revealed a key role for Psi in sustaining developmentally regulated *MYC* transcription and, thus, cell and tissue growth.[5] Moreover, like FUBP1,[44] Psi has potent transcriptional activator capacity *in vitro*, mediated by conserved tyrosine-rich domains (YM1 and YM2 repeats).[5] The Psi interactome is predominantly comprised of Pol II transcriptional machinery (63% of the top 65 Psi-interactors). This included chromatin-remodeling factors (32%) and gene specific transcriptional regulators (12%), however, most Pol II interactors (56%) comprised subunits of the Mediator (MED) complex. MED is well known to interact with Pol II machinery to integrate environmental and developmental cues into transcriptional outcomes.[45] Importantly, the impaired growth phenotype associated with Psi depletion is dependent on MED, being suppressed by increased MED abundance or activity.

Psi is also required for maintaining *MYC* mRNA at endogenous levels, with the latter being significantly decreased following Psi knockdown, and the impaired growth phenotype suppressed by *MYC* overexpression and enhanced by co-knockdown. The potent modification of the Psi knockdown phenotype by MYC overexpression likely reflects MYC's capacity to act as a global transcriptional amplifier.[46,47] In the context of rapidly proliferating wing disk cells the major program of MYC-modulated transcription will include genes required for cell and tissue growth. In accordance with *MYC* downregulation being due to reduced transcriptional activity, Psi depletion decreased enrichment of initiating Pol II (Ser 5 phosphorylated) and elongating Pol II (Ser 2 phosphorylated) on *MYC*. The observation that Psi activates from a single DNA binding site within the minimal promoter (as observed for FUBP1[44]), compared with most G4-activators that require tandem sites, suggests Psi drives *MYC* transcription downstream of pre-initiation complex (PIC) assembly.

Current model for FUBP1/Psi function in transcriptional control

Figure 1 combines observations from mammalian and *Drosophila* systems to model the sequence of events driving maximal *MYC* promoter activation. For basal transcription, Pol II and the GTFs required to form the PIC will load. In a signaling environment conducive to *MYC* transcription, MED interacts with *MYC* enhancers. MED stabilizes the PIC by recruiting TFIIH, which phosphorylates the Pol II-CTD to drive promoter clearance.[48] Increased Pol II activity will drive conformational changes in *MYC*, including supercoiling to cause the torsional stress required for FUSE melting and FUBP1/Psi binding. The latter will also interact with XPB/TFIIH to enable promoter loop formation between TSS and FUSE.[22] As Psi complexes

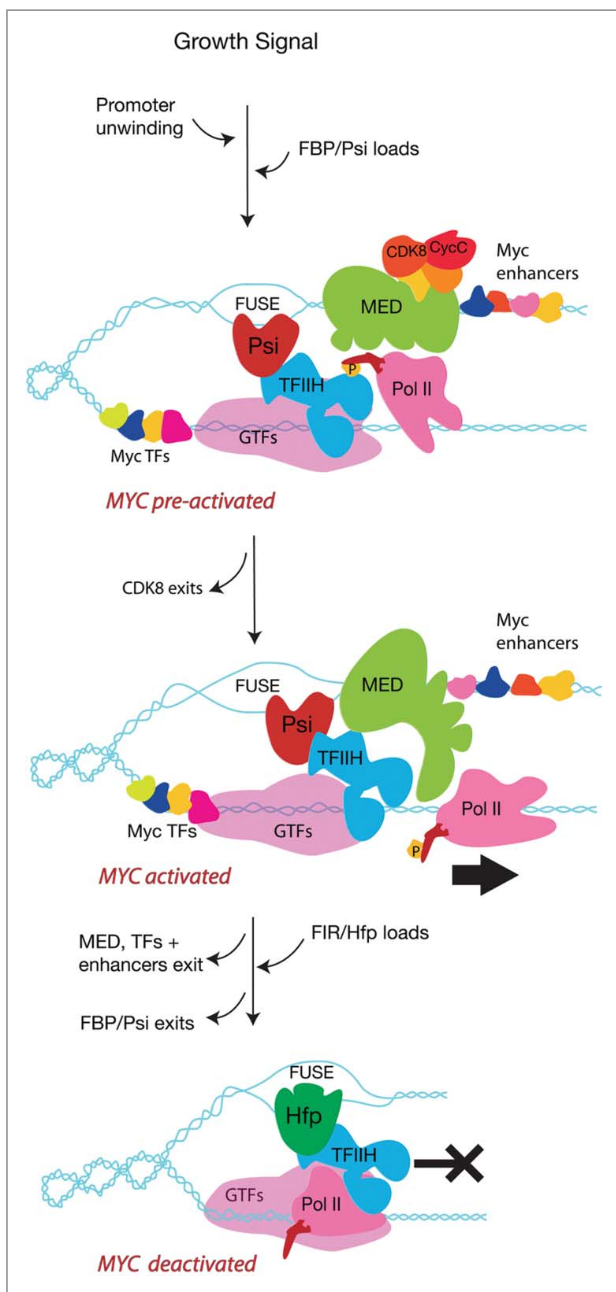

Figure 1. During the initial stages of the transcription cycle the MED complex and hypophosphorylated RNA Pol II holoenzyme are recruited to form the pre-initiation complex (PIC). Following receipt of the appropriate growth stimulating signals, *MYC* enhancers are activated and GTFs (particularly TFIIH) load to drive Ser-5 phosphorylation, activation of RNA Pol II and promoter escape. Increased Pol II activity results in conformational changes in the *MYC* promoter, including supercoiling and generation of the single-stranded FUSE. Structural changes following Psi/FUBP1 binding modulate promoter architecture to facilitate exit of the CDK8 module and maximize Pol II escape and *MYC* transcription. Recruitment of FIR/Hfp inactivates RNA Pol II and returns *MYC* to basal levels.

with MED's inhibitory kinase module, we predict Psi/FUBP1 will first interact with the preactivated *MYC* promoter i.e. with the large MED complex still in residence. Structural changes in ssDNA following Psi/FUBP1 loading will modulate promoter architecture further to facilitate exit of the CDK8 module, thus maximizing MED-driven Pol II activity and *MYC* transcription. Subsequently, FIR/Hfp will be recruited to the *MYC* promoter via binding to ssDNA, FUBP1 and TFIIH to facilitate FUBP1 exit, inactivation of Pol II and return of *MYC* to basal levels.

Concluding remarks and future vision

The concept of KH domain proteins interacting with single-stranded DNA to instruct transcription has been somewhat controversial—both in the transcription field and in the RNA biology field. On the one hand, the transcription community was sceptical of RNA binding proteins being found on DNA, while RNA biologists did not think these proteins should regulate DNA behavior. Here, it is important to step outside of these disciplines that have arisen from our reductionist approach to biology, and draw perspective from the RNA world hypothesis, the most likely origin of nucleic acid-based life.[49] The most logical scenario as organisms made the switch from the RNA to the DNA world is the repurposing of available proteins, an adaption that makes evolutionary sense i.e. rather than make an entirely new set of proteins, the cell adapts existing factors with the capacity to bind and restructure RNA for emerging ssDNA functions. The mounting evidence suggests one fundamental adaption of the FUBP family of KH domain proteins was the capacity to modulate ssDNA structure at key promoters to control transcription.

The extensive biochemical data, generated over the past 20 years, has enabled elucidation of the mechanism by which FUBP1 interacts with GTF machinery to control transcription. The *Drosophila* study of the FUBP1 ortholog, Psi, suggests conservation of FUBP1 function between mammals and flies, and further demonstrates interaction with the Mediator complex. Given the physiologic importance of the FUBP1/Psi-MED interaction to cell and tissue growth during *Drosophila* development, future efforts will be focused on determining the mechanistic basis of this axis of transcriptional control.

The studies highlighted here reveal *MYC* as a major target of FUBP1 and Psi, however, such structural changes enabling maximal transcription are not

unique to *MYC* regulation and will almost certainly be essential for controlling expression of numerous genes. We predict targets will depend upon the transcriptional demands of a given developmental/signaling context, and will most likely consist of genes regulated post-Pol II initiation. Identifying the full spectrum of genes directly regulated by FUBP1 and Psi, and how binding correlates with Pol II loading and non-B DNA formation, is a great imperative.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] Avigan MI, Strober B, Levens D. A far upstream element stimulates c-myc expression in undifferentiated leukemia cells. J Biol Chem 1990; 265:18538-18545; PMID:2211718
[2] Duncan R, Bazar L, Michelotti G, Avigan M, Levens D. A sequence-specific, single-strand binding protein activates the far upstream element of c-myc and defines a new DNA-binding motif. Genes Dev 1994; 8:465-480; PMID:8125259; https://doi.org/10.1101/gad.8.4.465
[3] Bazar L, Meighen D, Harris V, Duncan R, Levens D, Avigan M. Targeted melting and binding of a DNA regulatory element by a transactivator of c-myc. J Biol Chem 1995; 270:8241-8248; PMID:7713931; https://doi.org/10.1074/jbc.270.14.8241
[4] Zhang J, Chen QM. Far upstream element binding protein 1: a commander of transcription, translation and beyond. Oncogene 2013; 32:2907-2916; PMID:22926519; https://doi.org/10.1038/onc.2012.350
[5] Guo L, Zaysteva O, Nie Z, Mitchell NC, Amanda Lee JE, Ware T, Parsons L, Luwor R, Poortinga G, Hannan D, et al. Defining the essential function of FBP/KSRP proteins: Drosophila Psi interacts with the mediator complex to modulate MYC transcription and tissue growth. Nucleic Acids Res 2016; 44:7646-7658; PMID:27207882; https://doi.org/10.1093/nar/gkw461
[6] Shichiri M, Hanson KD, Sedivy JM. Effects of c-myc expression on proliferation, quiescence, and the G0 to G1 transition in nontransformed cells. Cell Growth Differ 1993; 4:93-104; PMID:8494788
[7] Pelicci PG, Knowles DM, Magrath I, Dalla-Favera R. Chromosomal breakpoints and structural alterations of the c-myc locus differ in endemic and sporadic forms of Burkitt lymphoma. Proc Natl Acad Sci USA 1986; 83:2984-2988; PMID:3458257; https://doi.org/10.1073/pnas.83.9.2984
[8] Spencer CA, Groudine M. Molecular analysis of the c-myc transcription elongation block. Implications for the generation of Burkitt's lymphoma. Annal New York Acad Sci 1990; 599:12-28; PMID:2221669; https://doi.org/10.1111/j.1749-6632.1990.tb42360.x
[9] Siebenlist U, Hennighausen L, Battey J, Leder P. Chromatin structure and protein binding in the putative regulatory region of the c-myc gene in Burkitt lymphoma. Cell 1984; 37:381-91; PMID:6327064; https://doi.org/10.1016/0092-8674(84)90368-4
[10] Bentley DL, Groudine M. A block to elongation is largely responsible for decreased transcription of c-myc in differentiated HL60 cells. Nature 1986; 321:702-706; PMID:3520340; https://doi.org/10.1038/321702a0
[11] Spencer CA, LeStrange RC, Novak U, Hayward WS, Groudine M. The block to transcription elongation is promoter dependent in normal and Burkitt's lymphoma c-myc alleles. Gen Dev 1990; 4:75-88; PMID:2307371; https://doi.org/10.1101/gad.4.1.75
[12] Spencer CA, Groudine M. Control of c-myc regulation in normal and neoplastic cells. Adv Cancer Res 1991; 56:1-48; PMID:2028839
[13] Grosso LE, Pitot HC. Transcriptional regulation of c-myc during chemically induced differentiation of HL-60 cultures. Cancer Res 1985; 45:847-850; PMID:2578312
[14] Matunis MJ, Michael WM, Dreyfuss G. Characterization and primary structure of the poly(C)-binding heterogeneous nuclear ribonucleoprotein complex K protein. Mol Cell Biol 1992; 12:164-171; PMID:1729596; https://doi.org/10.1128/MCB.12.1.164
[15] Takimoto M, Tomonaga T, Matunis M, Avigan M, Krutzsch H, Dreyfuss G, Levens D. Specific binding of heterogeneous ribonucleoprotein particle protein K to the human c-myc promoter, in vitro. J Biol Chem 1993; 268:18249-18258; PMID:8349701
[16] Michelotti GA, Michelotti EF, Pullner A, Duncan RC, Eick D, Levens D. Multiple single-stranded cis elements are associated with activated chromatin of the human c-myc gene in vivo. Mol Cell Biol 1996; 16:2656-2669; PMID:8649373; https://doi.org/10.1128/MCB.16.6.2656
[17] Braddock DT, Baber JL, Levens D, Clore GM. Molecular basis of sequence-specific single-stranded DNA recognition by KH domains: solution structure of a complex between hnRNP K KH3 and single-stranded DNA. EMBO J 2002; 21:3476-3485; PMID:12093748; https://doi.org/10.1093/emboj/cdf352

[18] Braddock DT, Louis JM, Baber JL, Levens D, Clore GM. Structure and dynamics of KH domains from FBP bound to single-stranded DNA. Nature 2002; 415:1051-1056; PMID:11875576; https://doi.org/10.1038/4151051a

[19] Kouzine F, Liu J, Sanford S, Chung HJ, Levens D. The dynamic response of upstream DNA to transcription-generated torsional stress. Nat Struct Mol Biol 2004; 11:1092-1100; PMID:15502847; https://doi.org/10.1038/nsmb848

[20] Weber A, Liu J, Collins I, Levens D. TFIIH operates through an expanded proximal promoter to fine-tune c-myc expression. Mol Cell Biol 2005; 25:147-161; PMID:15601838; https://doi.org/10.1128/MCB.25.1.147-161.2005

[21] Chung H-J, Liu J, Dundr M, Nie Z, Sanford S, Levens D. FBPs are calibrated molecular tools to adjust gene expression. Mol Cell Biol 2006; 26:6584-6597; PMID:16914741; https://doi.org/10.1128/MCB.00754-06

[22] Liu J, Kouzine F, Chung HJ, Elisha-Feil Z, Weber A, Levens D. The FUSE/FBP/FIR/TFIIH system is a molecular machine programming a pulse of c-myc expression. EMBO J 2006; 25:2119-2130; PMID:16628215; https://doi.org/10.1038/sj.emboj.7601101

[23] Kouzine F, Sanford S, Elisha-Feil Z, Levens D. The functional response of upstream DNA to dynamic supercoiling in vivo. Nat Struct Mol Biol 2008; 15:146-154; PMID:18193062; https://doi.org/10.1038/nsmb.1372

[24] Benjamin LR, Chung HJ, Sanford S, Kouzine F, Liu J, Levens D. Hierarchical mechanisms build the DNA-binding specificity of FUSE binding protein. Proc Natl Acad Sci USA 2008; 105:18296-18301; PMID:19015535; https://doi.org/10.1073/pnas.0803279105

[25] Kouzine F, Wojtowicz D, Yamane A, Resch W, Kieffer-Kwon K-R, Bandle R, Nelson S, Nakahashi H, Awasthi P, Feigenbaum L et al. Global regulation of promoter melting in naive lymphocytes. Cell 2013; 153:988-999; PMID:23706737; https://doi.org/10.1016/j.cell.2013.04.033

[26] Kouzine F, Gupta A, Baranello L, Wojtowicz D, Ben-Aissa K, Liu J, Przytycka TM, Levens D. Transcription-dependent dynamic supercoiling is a short-range genomic force. Nat Struct Mol Biol 2013; 20:396-403; PMID:23416947; https://doi.org/10.1038/nsmb.2517

[27] Levens D, Corces V. Editorial overview: genome architecture and expression: the nucleus, top and bottom. Curr Opin Genetics Dev 2014; 25:5-7; PMID:24755367; https://doi.org/10.1016/j.gde.2014.04.001

[28] Du X, Gertz EM, Wojtowicz D, Zhabinskaya D, Levens D, Benham CJ, Schäffer AA, Przytycka TM. Potential non-B DNA regions in the human genome are associated with higher rates of nucleotide mutation and expression variation. Nucleic Acids Res 2014; 42:12367-12379; PMID:25336616; https://doi.org/10.1093/nar/gku921

[29] Baranello L, Wojtowicz D, Cui K, Devaiah BN, Chung H-J, Chan-Salis KY, Guha R, Wilson K, Zhang X, Zhang H et al. RNA polymerase II regulates topoisomerase 1 activity to favor efficient transcription. Cell 2016; 165:357-371; PMID:27058666; https://doi.org/10.1016/j.cell.2016.02.036

[30] Liu J, He L, Collins I, Ge H, Libutti D, Li J, Egly JM, Levens D. The FBP interacting repressor targets TFIIH to inhibit activated transcription. Mol Cell 2000; 5:331-341; PMID:10882074; https://doi.org/10.1016/S1097-2765(00)80428-1

[31] Quinn LM, Dickins RA, Coombe M, Hime GR, Bowtell DDL, Richardson H. Drosophila Hfp negatively regulates dmyc and stg to inhibit cell proliferation. Development 2004; 131:1411-1423; PMID:14993190; https://doi.org/10.1242/dev.01019

[32] Mitchell NC, Johanson TM, Cranna NJ, Er ALJ, Richardson HE, Hannan RD, Quinn LM. Hfp inhibits Drosophila myc transcription and cell growth in a TFIIH/Hay-dependent manner. Development 2010; 137:2875-2884; PMID:20667914; https://doi.org/10.1242/dev.049585

[33] Lee JEA, Mitchell NC, Zaytseva O, Chahal A, Mendis P, Cartier-Michaud A, Parsons L, Poortinga G, Levens D, Hannan D, et al. Defective Hfp-dependent transcriptional repression of dMYC is fundamental to tissue overgrowthin Drosophila XPB models. Nat Commun 2015; 6:1-12.

[34] Kelly K, Cochran BH, Stiles CD, Leder P. Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell 1983; 35:603-610; PMID:6606489; https://doi.org/10.1016/0092-8674(83)90092-2

[35] He L, Liu J, Collins I, Sanford S, Levens D. Loss of FBP function arrests cellular proliferation and extinguishes c-myc expression. EMBO J 2000; 19:1034-1044; PMID:10698944; https://doi.org/10.1093/emboj/19.5.1034

[36] Rabenhorst U, Beinoraviciute-Kellner R, Brezniceanu M-L, Joos S, Devens F, Lichter P, Rieker RJ, Trojan J, Chung HJ, Levens DL et al. Overexpression of the far upstream element binding protein 1 in hepatocellular carcinoma is required for tumor growth. Hepatology 2009; 50:1121-1129; PMID:19637194; https://doi.org/10.1002/hep.23098

[37] Rabenhorst U, Thalheimer FB, Gerlach K, Kijonka M, Böhm S, Krause DS, Vauti F, Arnold HH, Schroeder T, Schnütgen F et al. Single-stranded DNA-binding transcriptional regulator FUBP1 is essential for fetal and adult hematopoietic stem cell self-renewal. Cell Rep 2015; 11:1847-1855; PMID:26095368; https://doi.org/10.1016/j.celrep.2015.05.038

[38] Zhou W, Chung YJ, Parrilla Castellar ER, Zheng Y, Chung H-J, Bandle R, Liu J, Tessarollo L, Batchelor E, Aplan PD et al. Far upstream element binding protein plays a crucial role in embryonic development, hematopoiesis, and stabilizing Myc expression levels. Am J Pathol 2016; 186:701-715; PMID:26774856; https://doi.org/10.1016/j.ajpath.2015.10.028

[39] Davis-Smyth T, Duncan RC, Zheng T, Michelotti G, Levens D. The far upstream element-binding proteins comprise an ancient family of single-strand DNA-binding transactivators. JBC 1996; 271:31679-31687; PMID:8940189; https://doi.org/10.1074/jbc.271.49.31679

[40] Er ALJ, May Parsons L, Quinn LM. MYC function and regulation in flies: how Drosophila has enlightened MYC cancer biology. AIMS Genetics 2014; 1:81-98; https://doi.org/10.3934/genet.2014.1.81

[41] Siebel CW, Kanaar R, Rio DC. Regulation of tissue-specific P-element pre-mRNA splicing requires the RNA-binding protein PSI. Genes Dev 1994; 8:1713-1725; PMID:7958851; https://doi.org/10.1101/gad.8.14.1713

[42] Brookfield JF, Montgomery E, Langley CH. Apparent absence of transposable elements related to the P elements of D. melanogaster in other species of Drosophila. Nature 1984; 310:330-332; PMID:6087152; https://doi.org/10.1038/310330a0

[43] Wang Q, Taliaferro JM, Klibaite U, Hilgers V, Shaevitz JW, Rio DC. The PSI–U1 snRNP interaction regulates male mating behavior in Drosophila. Proc Natl Acad Sci USA 2016; 113:5269-5274; PMID:27114556; https://doi.org/10.1073/pnas.1600936113

[44] Duncan R, Collins I, Tomonaga T, Zhang T, Levens D. A unique transactivation sequence motif is found in the carboxyl-terminal domain of the single-strand-binding protein FBP. Mol Cell Biol 1996; 16:2274-2282; PMID:8628294;https://doi.org/10.1128/MCB.16.5.2274

[45] Allen BL, Taatjes DJ. The Mediator complex: a central integrator of transcription. Nat Rev Mol Cell Biol 2015; 16:155-166; PMID:25693131; https://doi.org/10.1038/nrm3951

[46] Nie Z, Hu G, Wei G, Cui K, Yamane A, Resch W, Wang R, Green DR, Tessarollo L, Casellas R et al. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell 2012; 151:68-79; PMID:23021216;https://doi.org/10.1016/j.cell.2012.08.033

[47] Lin CY, Lovén J, Rahl PB, Paranal RM, Burge CB, Bradner JE, Lee TI, Young RA. Transcriptional amplification in tumor cells with elevated c-Myc. Cell 2012; 151:56-67; PMID:23021215;https://doi.org/10.1016/j.cell.2012.08.026

[48] Plaschka C, Larivière L, Wenzeck L, Seizl M, Hemann M, Tegunov D, Petrotchenko EV, Borchers CH, Baumeister W, Herzog F et al. Architecture of the RNA polymerase II-Mediator core initiation complex. Nature 2015; 518:376-380; PMID:25652824; https://doi.org/10.1038/nature14229

[49] Joyce GF. The antiquity of RNA-based evolution. Nature 2002; 418:214-221; PMID:12110897; https://doi.org/10.1038/418214a