Current Trends in Immunology
Vol. 18, 2017

Review

Transcription elongation factor ELL2 in antibody secreting cells, myeloma, and HIV infection: a full measure of activity

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ABSTRACT

Transcription elongation is an important regulatory step in development, differentiation, and cancer. The ELL (eleven-nineteen lysine-rich leukemia) proteins are encoded by three closely related genes (ELL1, 2 and 3) essential in controlling transcription elongation as part of the super elongation complexes (SEC) for mRNAs or the little elongation complexes (LEC) for snRNAs. A conserved portion of the ELL2 protein interacts with the central portion of the AFF4 scaffold protein (at its elbow region) to stabilize the SEC in HIV infections. In antibody secreting cells, ELL2 has an important role in production of the secretory-specific Ig heavy chain, the unfolded protein response, and glycosylation. At least 25 tumor tissue types have mutations in ELL2. Having too much or too little ELL can cause cancer; this dichotomy may potentially be explained by cells expressing varying levels of the transcriptional targets of the SEC or the levels of a number of ELL-interacting protein factors in those tumors such as EAF2, EAP30/ SNF8, HIF-1 alpha, RB, SIAH1, or c-MYC which can be ubiquitinylated by ELL1. Recent studies have linked expression mutations in ELL2 with a non-secreting form of familial multiple myeloma. We conclude that ELL2 can serve not just as a factor to enhance Ig heavy chain mRNA processing but also as an important target for some forms of cancer and for growth regulation of lymphocytes.

KEYWORDS: transcription, elongation, ELL-family, B cells, cancer, myeloma, MYC

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ABBREVIATIONS

ASC, antibody secreting cell; ELL, eleven-nineteen lysine-rich leukemia; LEC, little elongation complex; MLL, multiple lineage leukemia; MM, multiple myeloma; RNAP-II, RNA polymerase II; SEC, super elongation complex.

1. Introduction: ELL genes and their associates, elbow to elbow

The ELL proteins (eleven-nineteen lysine-rich leukemia proteins 1, 2, or 3) are part of a large complex of transcription elongation factors acting in concert, called the SEC or super elongation complex. Many of the factors in the SEC were originally discovered as fusion partners with the MLL gene in mixed lineage leukemia wherein the NH2 portion of MLL is linked to the COOH portion of the SEC elongation factor [1-3]. MLL, aka KMT2A, encodes a histone H3 lysine 4 (H3K4) methyl-transferase which mediates chromatin modifications that result in transcriptional activation. The common enzymatic component of the SEC (pTEFb = CyclinT + cdk9) phosphorylates the long carboxyl terminal domain (CTD) of RNA polymerase II (RNAP-II) and the negative elongation factors. Then PAF factors are added to the complex [4] and RNAP-II leaves its paused state just downstream of the transcription start site and engages in productive mRNA synthesis [1]. Some proteins in the SEC hold the complex together (AFF4 or AFF1) or are themselves lysine methyl transferases for histone H3K79 (DOT1L), see review [5]. The ELL proteins enhance the rate of RNAP-II transcription and reduce pausing *in vitro* [3, 6, 7]. So when the MLL histone methyl-transferase

activity commandeers one of the SEC proteins by gene fusion in cancer, it inadvertently brings along all the SEC partners, by protein: protein interactions, to activate abnormal Hox transcription, spur elongation, and thereby promote tumorogenesis [8]. The ELL1 portion of the fusion protein in MLL was shown to contribute to the pathogenesis of acute leukemia in part because it bound to p53 [9]; p53 binding is a common feature among the many MLL-fusion partners like ENL, AF9, and AF10 [10]. ELL1 expression is high in human bone marrow, placenta and testis but remains relatively constant through B and T cell development. ELL1 was also identified as a component of a little elongation complex (LEC) with other proteins like Ice 1 and Ice 2 and RNAP-II; the LEC is responsible for the synthesis of snRNAs [11].

The related genes ELL2 (1) and ELL3 [12] were isolated by virtue of their homology to ELL1; all ELL family members have a characteristic transcription elongation activity encoded in the conserved NH2 terminal portion of the molecule. The COOH terminal region in all three ELL proteins contains a domain with a conserved fold like that of the occludin ZO-1-binding domain [3, 6]. Occludin protein is required for correct assembly of tight junction barriers but thus far has no known role in transcription. ELL2 is 640 amino acids long while ELL3 is 400 amino acids long. ELL3 protein lacks the majority of the central disordered protein domain compared with ELL1 and ELL2 but is 50% sequence identical to them in at the start and end of the molecule. ELL1 and ELL2 differ slightly throughout their sequence, resulting in differential interactions with different partners in a yeast two-hybrid study [13]. ELL3 was originally described as testis specific but was later found in embryonic germ (ES) cells, B cells, and various cancers [12, 14-16]. ELL3 is highly expressed in germinal center-derived lymphoma cells in which depletion of ELL3 results in disruption of DNA synthesis and ultimately cell death [17].

The AFF1 and AFF4 subunits of the SEC have been shown to reside in separate super elongation complexes; the AFF1-SEC is more potent in supporting HIV-1 transcription while the AFF4-SEC is more important for HSP70 induction [18]. Both AFF1 and 4 have been implicated in acute lymphoblastic leukemia as well as in fragile X syndrome [19]. The genes regulated by AFF1-SEC and AFF4-SEC are largely non-overlapping and perform distinct functions [18]. The AFF1 and ELL2-containing version of the SEC, while low in abundance, plays a predominant role in reversing HIV latency [20]. HIV-1 Tat and the host AFF1 complex cooperate for coordinate activation of HIV-1 transcription at the viral Tar elements [21]. As a scaffold for the SEC, AFF interactions with pTEFb have been mapped to its NH2-terminal 73 amino acids [22, 23] while the interaction site with ENL or AF9 maps to residues 761-774, near the COOH end of AFF [24]. Recently ELL2 was shown to interact with amino acids 301-350 of AFF4 *via* ELL2's COOH terminal occludin-like domain, creating an arch-shaped three-helix fold (alpha helix 1 aa 553-578, alpha helix 2 aa 584-602, and alpha helix 3 at aa 607-638 of ELL2) structures seen previously in the COOH of occludin [25]. See figure 1, discussed in [26], for portions of ELL2 described in the text.

The authors called the region of AFF4 that interacts with ELL2 the $AFF4^{ELLbow}$. It is perhaps ironic to note that "ell" is actually defined alternatively as an L-shaped extension, an obscure word for elbow, or a unit of measure (often for cloth, originally called a cubit) that approximates the length of a man's arm from the elbow to the tip of the middle finger. By joining "at the elbows" ELL2 and AFF4, or the highly similar AFF1, stimulate transcription. The AFF4-ELL2 interface is imperfectly packed, leaving a cavity that may allow for potential binding by small molecules to alter its activity. It is important to note that ELL2 levels are limiting in the tissue culture cells HEK 293, HeLa, and Jurkat 2D10 (T cell line), where these interaction studies were conducted. In primary T cells the ELL2 levels might differ significantly based on the level of T cell differentiation. It is also interesting to note that infected regulatory T cells persist following retroviral therapy [27], perhaps because of their high levels of ELL2 expression. AFF1 is upregulated in plasma cells while AFF4 is not [28]; whether the AFF1 and AFF4 complexes serve different roles in those cells has not been explored.

Among all the SEC subunit proteins, ELL2 is rate limiting and uniquely controlled at the level of protein stability. The RING domain protein SIAH1, but not the homologous SIAH2, was identified as

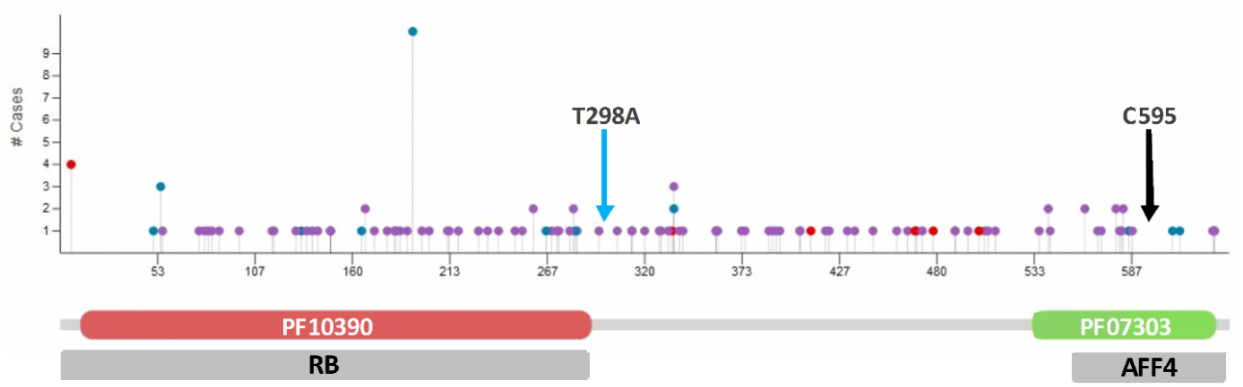

Figure 1. Features of the ELL2 gene described here. Colored pins denote the locations of the 156 identified cases of ELL2 mutant cancers where red represents frameshift mutants, blue represents gain of a stop codon, and purple represents a missense mutation. Amplitude of each pin signifies the number of cases with each mutation, as indicated by the y-axis to the left. The blue arrow shows the location of the Thr298Ala missense variant identified as a multiple myeloma risk allele. The red region indicates the transcription elongation domain at the NH2 terminus of the gene, which corresponds to Pfam family 10390 (PF10390 = RNA polymerase II elongation factor ELL). The occludin-like domain at the C terminus of the gene shown in green corresponds to the Pfam family 07303 (PF07303 = Occludin/RNA polymerase II elongation factor, ELL domain). The portions of the gene that interact with RB and AFF4 are highlighted by labeled gray boxes. In the largely homologous ELL1, an active site of its ubiquitin ligase domain is in the C terminus of the gene at C595, indicated here with a black arrow.
NCI web sites where the original can be found: (https://portal.gdc.cancer.gov/) Data Analysis, Visualization, and Exploration (DAVE) tools (https://gdc.cancer.gov/analyze-data/gdc-dave-tools).

the E3 ubiquitin ligase for ELL2 poly-ubiquitination and proteasomal degradation. SIAH1 cannot access and ubiquitinate ELL2 bound to AFF4, although, at high concentrations, it also degrades AFF4 and AFF1 to destroy the whole SEC. Prostratin and HMBA, two well-studied activators of HIV transcription and latency, enhance ELL2 accumulation and SEC formation largely through decreasing SIAH1 expression and ELL2 poly-ubiquitination [29]. Due to its short half-life the effects of ELL2 may be transitory. ELL2 turnover may be important when activated B cells (plasmablasts) travel to the germinal center and undergo affinity maturation.

ELL 1 and 2 have been shown to bind to EAF1 and EAF2 and thereby link with MED26 [30], a component of the mediator complex that serves as a docking site for the transcription elongation factors and the SEC. MED26 interacts first with the basal transcription factor TFIID in the initiation phase of RNA polymerase complex formation. Then MED26 exchanges TFIID for the SEC [30] or LEC [31] to drive the polymerase transition to the elongation stage of transcription. EAP30 (snf8, Dot3; VPS22) is a component of the endosomal sorting complex required for transport II (ESCRT-II), involved in the movement of ubiquitinylated transmembrane proteins to the lysosome for degradation; it was also found to interact with ELL1 in a region that can depress the activity of RNAP-II, which is not present in the portion of ELL in the MLL fusion protein [32].

2. Elongation and RNA processing are linked

Transcription elongation and the ELL proteins have emerged as important regulatory partners in a number of genetic expression systems including development [33], inflammation [34] and cancer [35]. Several human diseases have also been linked to transcription elongation [1, 36]. RNAP-II can load onto a gene near the promoter with basal transcription factors but then pause or stall there, awaiting the addition of transcription elongation factors. These factors can modify or unwind the nucleosomes, modify the carboxyl-terminus of the polymerase itself, phosphorylate negative elongation factors, and/ or add extra proteins to the complex that will enable the resulting mRNA product to be spliced or polyadenylated [37]. Even in plants, RNAP-II complexes exist that coordinate mRNA elongation and processing [38]. But transcription

elongation and mRNA splicing have complex and thus far enigmatic interactions [39].

Early experiments indicated that rapid elongation of RNAP-II always led to the skipping of weak splice sites [40]. Those experiments were performed using DRB, an adenosine analog, which blocks elongation both as a chain terminator as well as interfering with the phosphorylation of DSIF, one of the factors in the stalled polymerase complex; thus elongation was slowed by enhancing stalling. The authors also used drugs to deacetylate histones, slowing down the polymerases by keeping chromatin more compact [41, 42]. When global alternative splicing in the knockdown of ELL2 by shRNA was investigated [43], the authors concluded that despite the high frequency of transcripts whose processing was modulated by ELL2, they did not observe a consistent pattern of exon skipping or first poly(A) site use on pre-mRNA processing events, with the exception of the already known Ig heavy chain. However, they did not identify which of the alternatively spliced genes were direct versus indirect ELL2 targets. More recent experiments employed global gene analyses and mutations in the RNA polymerase itself which changed the elongation rate by changing the catalysis rate for ribonucleotide addition [39]. In those experiments the authors concluded that fast elongation could either increase or decrease inclusion of a particular exon depending on the sequence of the gene; likewise slower elongation could result in inclusion or exclusion of exons. Thus splicing may depend on the sequence of each gene as well as the pre-RNA folding and protein associations with splicing factors. How the elongation factors may help the splicing factors load onto the transcribed RNA for some but not all genes remains to be determined.

Many genes have multiple poly(A) sites and regulation can occur on them in a developmental or differentiation-specific fashion [44]. For example, the Ig heavy chain gene undergoes polyadenylation at the promoter distal membrane-specific site in B cells and shifts to the predominate use of the promoter proximal secretory poly(A) site in antibody secreting cells [45]. In HeLa nuclear extracts last-intron splicing and cleavage polyadenylation are functionally coupled and the RNA sites interact with each other as they exit the polymerase, which may induce a polymerase pause [46]. Paused polymerase was found just 3' of the secretory-specific poly(A) site in plasma cell lines expressing the IgM heavy chain on a plasmid [47, 48] but not on the endogenous IgG2a gene [49]. The differences in these results may be attributed to the fact that IgM requires more stringent regulation because the IgD C-region is immediately downstream or, alternatively, on the location of the Ig substrate, where the endogenous gene may be elongated at a higher rate because all the surrounding Ig enhancers minimize pausing [50]. We noted that histone H3K79 methylation is enhanced beyond the IgG2a heavy chain internal enhancer in plasma cells but not in B cells [28]. Methylation of histone H3K79 aids in DNA unwinding from the nucleosome. Opening of chromatin structure by DOT1, the methylase, carried along in the SEC with ELL2, may enhance the use of the promoter-proximal, secretory-specific poly(A) site by allowing polyadenylation factors to access the nascent RNA more readily.

3. ELL2 in lymphocytes and cancer, measuring RNA and survival

3.1. Role of ELL2 in B cells

How individual, activated B cells in either the Marginal Zones (MZ) or the Follicles (FO) choose between division, death, antibody-secreting cell (ASC) development, and class switching is unknown, and the molecular basis of this heterogeneity is still a mystery [51]. The relationship between the short-lived cycling plasmablasts and the long-lived antibody secreting cells also remains unclear. Among the ELL-family members, only ELL2 is highly up-regulated in dendritic cells, suppressor T cells, in response to androgens [52], and in the transition to antibody-secreting cells [53] After its induction in antibody-secreting cells, ELL2 directs the selection of the secretory-specific poly(A) site resulting in the production of the secretory-specific form of the Ig heavy chain mRNA and higher overall Igh expression. Mice lacking ELL2 only in the B cell compartment are deficient in Ig heavy chain transcript levels and processing to the Ig secretory-specific form. The B-cell conditional knockouts in ELL2 are also deficient in the production of bone-marrow-derived plasma cells [53]. The Igh mRNA gets measurably shorter and significantly more abundant when ELL2 is

expressed while the plasmablasts do not progress to a long-lived state without ELL2; are these processes linked directly or indirectly through ELL2? The finding that expression of BCMA (TNFRSF17), a growth factor receptor, is controlled by ELL2 indicates one pathway for ELL2 to increase longevity [43]. At the same time, the observation that ELL1 can act as an E3 ubiquitin ligase for c-MYC [54], an essential growth regulator in lymphocytes [55], indicates a pathway towards cell death. Balancing those signals by ELL2 and ELL1 levels of expression may lead to cycles of growth vs quiescence or death.

ELL2 is required *in vivo* for both the T-independent (marginal zone) and the T-dependent/recall responses (involving the follicles) [53]. If B cells reside and are stimulated in the marginal zone by T-independent antigen engagement of the B-cell receptors, or through stimulation with the toll-like receptors or Ig plus C3d, they will differentiate into antibody secreting cells with a high probability of resulting in short-lived plasma-blasts. Activated marginal zone ASCs persist for only a few days after activation. They die rapidly either through an inability to deal with internal reactive oxygen species formed because of the large amount of secretory-specific antibody molecules they produce and/or because they fail to upregulate receptors for survival signals.

B cells that initially travel to the spleen or lymph node follicles require a more complex set of reactions with T cells in order to be stimulated by antigen. B cells take up antigen and can serve as antigen-presenting cells to interact with T cells *via* MHC II, antigen and the T-cell receptor. Engagement of the B cell surface receptor CD40 molecule can also occur *via* contact with T cell surface CD40 ligand (CD154). Secretion of cytokines including IL-2, -4, and -5 made by interacting T cells further activates the B cells. CD40 engagement results in B cell activation, isotype switching, and somatic hyper-mutation upon passing through a germinal center in the absence of antibody secretion. Those B cells then later differentiate into long-lived antibody-secreting cells or memory cells. The antibody-secreting cells from B cells stimulated in follicles can acquire CXCR4+ and can home to specific CXCL12+ niches in the bone marrow to become long-lived ASCs. Long life for ASCs depends on soluble factors like BAFF, BCMA, and APRIL made by the bone marrow stroma and a “touch” of autophagy (Atg5 expression) to repair damage in the endoplasmic reticulum [56]. Knocking out ELL2 in mice reduced the number of $IgG1^+$-secreting bone marrow cells [53], which is one measure of a memory response. Thus ELL2 is important for both short-lived ASCs and long term/memory responses.

What other genes are involved in the ELL2 regulatory pathway? Might they influence cell death vs survival? Genome-wide association studies (GWAS) of 23 altered immunoglobulin G (IgG) *N*-glycosylation phenotypes uncovered ten genome-wide significant loci, of which five were novel including ELL2 [57]. The implication is that ELL2 is also controlling genes for IgG glycosylation and perhaps other important molecules. When ELL2 is not expressed there is also decreased expression of ATF6, BiP, light chain, ELL1, the UPR pathway, and POU2AF1 (OCAB, BOB1, OBF1) [53]. Genes bound by OCAB have the octamer sequence; those genes may be targets of ELL2-enhanced elongation, either directly or indirectly. When ELL2 was decreased by shRNA in a plasma cell line, approximately 12% of the transcripts were differentially spliced, including BCMA (Tnfrsf17) and many small nuclear RNAs [43], presumably important for viability. POU2AF1-deficient mice showed strain-specific, partial blocks at multiple stages of B-cell maturation and a complete disruption of germinal center formation in all strains [58]; in another study of POU2AF1-deficient mice there was no block in the formation of antibody-secreting cells *in vitro* but the T-dependent response was impaired [59]. Understanding the action of ELL2 on POU2AF1 and their potential collaboration at inducing genes with the octamer box may be important for resetting the time-to-live versus the time-to-die clock in B cells.

3.2. ELL2 loss in some forms of multiple myeloma

Multiple myeloma (MM) is the second most common hematological malignancy; it displays a clonal expansion of plasma cells (long-lived ASC) in bone marrow, a hypoxic niche [60]. Many myelomas arise as a result of the aberrant rearrangements in subclass switching and somatic hyper-mutations that occur on the Ig heavy chain genes joining with oncogenes such as c-MYC,

RAS, p53, cyclin D1, or FGFR3 [61]. In these MM cells the overexpressed oncogene contributes substantially to the malignancy. This type of MM can be distinguished from another form of MM, the hyper-diploid form, in which trisomies of some regions occur. In addition, there have been 17 independent MM risk loci identified by GWAS [62] with the locus at 5q15 (ELL2) showing the most robust associations with the disease. Loss of ELL2 expression and MM are strongly linked in tumors that have lost the ability to secrete Ig [62, 63]. In one study, in a Nordic population the MM risk allele was found to have a Thr298Ala missense variant; this site resides in a domain of ELL2 required for its transcription elongation activity (see Figure 1). The patients harboring this allele have reduced levels of immunoglobulins IgA and IgG and, potentially, an increased risk of bacterial meningitis [63]. In another study mutations were found in 5q15 (the human ELL2 locus) within a predicted enhancer element; the authors showed that the enhancer physically interacts with the transcription start site of ELL2 by promoter capture Hi-C. The 5q15 risk allele is associated with reduced enhancer activity and lowered ELL2 expression [62]. In addition in 505 MM patients there was a strong relationship between the lowered level of ELL2 and reduced levels of the mRNAs for BiP, ATF6, ELL1 and POU2AF1 (Bob1, Oca-B, OFB1), with $P = 3 \times 10^{-6}$ to 5.7×10^{-10} [64]. We have previously shown that these are some of the genes downregulated in ELL2 conditional knockout B cells [53]. Thus it seems clear that ELL2 has an important role in Ig secretion, the unfolded protein response (BiP and ATF6), and plasma cell development (POU2AF1). Whether these mutations in ELL2 are the sole cause of the MM is not clear. Understanding what protein:protein interactions of the ELL-family members and oncogenes occur in these cells may shed further light on the generation of the myelomas.

3.3. ELL2, myc, low oxygen, and cancer

At least 156 tumors have mutations in ELL2 (Figure 1), encompassing 25 types of cancer, as reported in the Genomic Data Commons (GDC) Data Portal by the National Cancer Institute [26]. For example, ELL2 is expressed in gliobastoma-multiforme tumors, with high levels of ELL2 correlating with shorter median patient survival [64]. ELL2 and its regulated genes are expressed at a high level in neuroendocrine prostate tumors, while paradoxically, ELL2 is down-regulated in prostate adenocarcinomas [52]. Conditional deletion of ELL2 in prostate epithelia cells results in murine prostatic intraepithelial neoplasia or mPIN, that is, ELL2 loss can produce increased epithelial proliferation. Loss of ELL2 activity is also associated with a unique form of human myeloma, a tumor of a plasma cell as discussed above. Meta-analysis of Hispanic and non-Hispanic white cohorts identified a significant single nucleotide polymorphism linked to ELL2 in salivary gland carcinomas [65]; what effect this polymorphism has on the level of ELL2 expression is unknown. The existence of several different partners for ELL2 or significant targets in different cells might explain the apparent discrepancy in the level of ELL2 alone either driving or suppressing cancer. There is ample evidence that ELL1 or ELL2 can interact with HIF-1 alpha, c-MYC, and the tumor suppressor protein retinoblastoma (RB) in different tumors. ELL2 was shown to interact with the oncogenic RB protein by co-immunoprecipitation mediated by the NH2-terminus of ELL2 and C-terminus of RB (Figure 1). They act together to suppress prostate cancer progression [66].

ELL1, along with HIF-1 alpha, regulates and responds to hypoxia in PC3 prostate cancer cells [67]. HIF-1 alpha is a transcription factor induced in conditions of low oxygen aka hypoxia. During hypoxia, HIF-1 alpha, CCNC (cyclin C), and CDK8 in the mediator complex act along with the SEC to accelerate transcription of a subset of hypoxia-inducible genes in many cell types [68]. The genes in cancer cells influenced by HIF-1 alpha include those involved in mitochondrial function, glucose transporters, hexokinase and those involved in glycolytic flux [69]. It has been suggested that it is the binding of ELL and HIF-1 alpha proteins directly to each other that modulates the functions of each in hypoxia [69]. Which portions of each protein are involved in this interaction have not been mapped. ELL overexpression inhibits expression of HIF-1 alpha protein and its downstream gene expression pattern under normoxia; but the inhibition of cell growth by ELL was alleviated under hypoxia. In

another study, HIF-1 alpha gene expression was significantly increased in prostate tissue in ELL2 conditional knockout mice vs wild type controls within intra-epithelial cells [52]. One might imagine that this interaction with HIF-1 alpha could be relevant for splenic B cells undergoing rapid growth following stimulation until maximal ELL2 levels are achieved, in which case cell growth could be inhibited in normoxia but alleviated by hypoxia.

The oncogene c-MYC is a transcription factor for cellular growth regulation and for metabolism genes; c-MYC has been linked to many neoplasia because of the duality of its effects [70]. MYC is frequently translocated in multiple myeloma and Burkitt's lymphoma leading to its aberrant expression [70]. The half-life of the MYC mRNA and protein are both short (less than 30 minutes). C-MYC has been shown to undergo ubiquitination and degradation by the proteasome but can be targeted for destruction differently in different cell types [71]. Mutations in the coding region of MYC, particularly at the Thr58 phosphorylation site in some Burkitt's lymphomas (to the phosphorylation dead Thr58A mutation), reduce ubiquitin addition to MYC and turnover leading to increased transformation [55]. ELL1 was identified as an E3 ubiquitin ligase for c-MYC in HEK293 cells, promoting degradation of MYC in the presence or absence of phosphorylation [54]. The region of ELL1 involved in the MYC binding was finely mapped to amino acids 583-614 encompassing the homologous region of ELL2 that interact with the AFF4 elbow. Mutation of the C595 to an A reduces the degradation of MYC (see Figure 1). Overexpression of ELL1 inhibited both the transcriptional activity of c-MYC and cell proliferation. The region of ELL mapped to contain the active C595 is highly conserved between human and zebrafish ELL1 and with ELL2 but not ELL3 [12]. Whether ELL2 can also serve as an E3 ubiquitin ligase for MYC has not been demonstrated but it seems significant that when ELL2 is diminished, ELL1 levels are reduced as well in the myeloma cells. Thus there may be a double loss of the E3 ubiquitin ligase activity, which may temporarily enhance the life-time of B cells in the ELL2 knockout. This could occur even though ELL3 has been decreased by blimp-1 induction as it would be in normal stimulated B cells [72]. Loss of ELL3 in germinal center cells in the presence of ELL1 and ELL2 can cause death.

Hypoxia and MYC expression are inter-related. Cells encounter oxygen deprivation (hypoxia) in various physiological and pathological contexts. Adaptation to hypoxic stress occurs in part by suppressing MYC. Hypoxia has been reported to inhibit MYC through multiple means, including disruption of MYC transcriptional complexes and decreased MYC protein abundance. Enhanced proteasomal degradation and cathepsin-mediated proteolysis are important mechanisms for hypoxic MYC inhibition in human colon carcinoma cells. MYC overexpression in hypoxic cells promotes cell cycle progression but also enhances cell death *via* increased expression of the pro-apoptotic genes NOXA and PUMA. Collectively, these results indicate that hypoxic cells promote MYC degradation as an adaptive strategy to reduce proliferation, suppress biosynthetic processes, and promote cell survival under low O2 tension [73].

4. Summary

Taken together then there appears to be strong evidence for an interaction of hypoxia, MYC expression, and ELL in directing lymphocyte proliferation and its cessation. T and B cells undergo a controlled burst of proliferation following antigen stimulation before returning to quiescence. Investigation of the role of MYC expression following the activation of T cells revealed a division-independent timed process for decreased c-MYC production and independently, an induced, programmed time to die mechanism. Forced expression of MYC therefore did not lead to unlimited cell growth in these T cells because it was over-ridden by the time to die cycle [74]. Candidates for the time to die mechanism include Bim and Bcl-2. The short life span of a plasmablast following B cell stimulation may be a result of the induction of large amounts of ELL2 for Ig secretion and glycosylation and, as a side product, the ubiquitination of MYC. Hypoxic conditions would add to the MYC degradation. The ability of normal, stimulated B cells to survive the large amount of induced ELL2, and subsequent MYC degradation, may require resetting the time to die signals. Finding BCMA as an ELL2 target suggests a reprogramming event so that the cells can receive growth signals from the environment

independent of MYC. Induction by ELL2 of POU2AF1 and cyclin B2 is another way the survival signals may be activated [53]. Cyclin B2 is primarily associated with the Golgi region which would be induced in cells secreting large amounts of protein. Cyclin B2 also binds to transforming growth factor beta RII [75]. Obviously there is much that needs to be learned about ELL2 targets and their interactions.

5. Future directions

Understanding exactly how elongation factors work to regulate alternative RNA processing is a major unanswered question. The biochemical data showing that those factors change the rate of elongation comes solely from *in vitro* studies, so analysis of *in vivo* rates of transcription plus and minus the elongation factors using newly developed tools is in order. The role of elongation factors in modulating the addition of splicing factors and polyadenylation factors to the nascent RNA should also be explored more deeply. ELL2 and HIV interactions in regulatory, helper, and cytotoxic primary T cells should be studied further to assist our understanding of HIV infection and latency in those populations. The partners and targets of ELL2 and their expression in the various cancers also need to be investigated for a fuller understanding of how the level of ELL2 can be either too high or too low in cancer. The interactions of MYC, BCMA, and POU2AF1 with ELL1 and 2 should be examined further in the context of lymphocyte programmed cell growth versus the programmed time to die mechanisms. The remarkable findings of ELL and MYC interactions along with altered ELL2 expression in multiple myeloma are important indicators that ELL2 plays a vital role not just in Ig secretion but also in controlling cell fate.

ACKNOWLEDGEMENTS

The work was supported by 1R21AI124241 from the National Institutes of Health NIAID to C. M., Department of Immunology funds, and a shared resources grant P30CA047904 to the University of Pittsburgh Cancer Institute.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

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