TLS, EWS and TAF15: a model for transcriptional integration of gene expression

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Abstract
Multifunctional proteins are demonstrating that gene expression is not a series of compartmentalized events beginning with transcription and culminating in delivery of mature mRNA into the cytoplasm, but an integrated pathway of transcription, splicing, RNA metabolism and subcellular targeting of translation. One such multifunctional family is made up of the RNA-binding proteins TLS, EWS and TAF15. These three proteins each contribute a potent transcriptional activation domain to oncogenic fusion proteins, and the formation of these fusion genes are thought to be the primary causes of their associated cancers. Wild-type TLS, EWS and TAF15 can function as classical transcription factors in addition to their better-known functions in splicing and mRNA transport. The interaction between TLS and the stress-response protein YB-1 is an example of how these proteins can induce a multi-faceted change in gene expression, as they can interact to induce changes in both transcription and splicing of target genes. Investigating the multiple functions of TLS, EWS and TAF15 will enhance our understanding of gene expression as a whole, and also allow us to better understand how these proteins may be contributing to the oncogenic pathways the associated fusion proteins initiate.

Keywords: TLS; EWS; TAF15; YB-1; cancer; transcription

TET RELATED GENES AND CANCER
Gene expression can be broadly considered to begin with the initiation of transcription and culminate in the delivery of mature RNA to the cytoplasm for translation into protein. Analysis of proteins involved in the regulation of gene expression is typically categorized by specific functions, such as transcription, splicing or mRNA processing factors. More recently, proteins are being identified that are involved in the regulation of gene expression at multiple levels, and this is redefining the way in which we understand the integration of these processes. Instead of being a series of segregated events, we are now beginning to understand that transcription, mRNA processing and subcellular targeting of translation may be directly modulated and that this added layer of complexity may serve to integrate multiple transcription targets and gene expression in response to external cellular signals. One such family of these multifunctional proteins is comprised of TLS (translocated in liposarcoma)/FUS, EWS (Ewing’s sarcoma) and TAF15/TAFII68, and is known as the TET family [1]. This unique family of RNA-binding proteins are structurally and functionally related, and are defined by the presence of an N-terminal SYGQ-rich region, an RNA-recognition motif, a C2/C2 zinc finger motif and at least one RGG-repeat region [2].

TLS and EWSR1 were initially identified as components of the fusion genes HLS–CHOP and EWSR1–FLI-1 found in myxoid liposarcoma [3, 4]
and the Ewing’s sarcoma family of tumours, respectively [5]. Subsequently, all three family members have been found in a variety of cancer-associated fusion genes (Table 1). In each case, the N-terminus of TLS, EWS or TAF15, which have all been found to be potent transcriptional activation domains [6–8], is fused to the DNA-binding domain of a transcription factor, and form aberrant transcription factors. The formation of these fusion genes are thought to be the primary causes of these cancers, because of the high frequency and specificity with which some of these fusions are found within the cancers. Over 90% of myxoid liposarcomas contain the TLS–CHOP fusion [9], and around 85% of Ewing tumours carry the EWSR1–FLI-1 fusion [10]. In most of the remaining Ewing tumours, EWSR1 is translocated with other members of the ETS transcription factor family (Table 1). TLS, EWSR1 and TAF15 can replace each other in some of the fusions, indicating a functional relationship between the homologues (Table 1).

Transgenic mouse models TLS–CHOP have also shown that the presence of this fusion is enough to initiate its associated cancer. Mice, ubiquitously expressing TLS–CHOP or CHOP–TLS, specifically develop tumours that almost completely recapitulate the phenotype of human myxoid liposarcoma [11, 12]. The presence of the N-terminus of TLS is required for oncogenesis, as mice overexpressing only CHOP do not develop liposarcoma [12]. The N-terminus of TLS can still promote liposarcoma development in mice when the N-terminus and CHOP are overexpressed as physically separate domains [13], suggesting that the N-terminus of TLS has a function separate from that of the fusion gene, and may function in a dominant-negative manner against the wild-type function of TLS. This observation emphasizes the need for a better understanding of the normal function of TLS, and by corollary, EWS and TAF15. It is the focus of this review to explore the role of the TLS, EWS and TAF15 proteins in the regulation of transcription and briefly discuss some of our preliminary results involving the gene-specific transcriptional modulation of gene expression by the TLS protein.

**TET ‘RIBONUCLEAR PROTEINS’ AND GENE TRANSCRIPTION**

The initial association of TLS and EWS to gene expression was through the ability of their SYGQ-rich regions to function as potent transcriptional activation domains [6–8], is fused to the DNA-binding domain of a transcription factor, and form aberrant transcription factors. The formation of these fusion genes are thought to be the primary causes of these cancers, because of the high frequency and specificity with which some of these fusions are found within the cancers.
RNA, and both single-stranded and double-stranded DNA [1, 3, 6, 14, 15]. Few proteins have been characterized to date that are capable of binding both RNA and DNA [16]. These unique qualities of the TET family members are most likely pivotal to their functions in mammalian gene expression. Given their nucleic acid-binding properties, it is not surprising that these proteins have been shown to affect splicing [17] and RNA shuttling [18, 19]. Furthermore, these proteins have also been shown to interact with the ribonucleoproteins hnRNPA1 and hnRNPC1/C2, proteins involved in RNA maturation [20]. However, these proteins have also been implicated in the process of gene transcription. The TLS, EWS and TAF15 proteins have been shown to associate with integral components of the transcriptional pre-initiation complex, which is essential for the induction of transcription. Specifically, they interact with RNA polymerase II enzyme, a key regulator of eukaryotic transcription, and the TFIID complex, a DNA-binding component of the general transcriptional machinery, and thus, they have been implicated in early transcriptional initiation and elongation [1, 21, 22]. TLS and EWS can also associate with several gene-specific transcription factors, which suggest a more specialized, gene-specific regulatory role for the proteins in the regulation of gene expression. The TLS protein is an interaction partner to several nuclear hormone receptors, including the glucocorticoid, oestrogen and thyroid hormone receptors, as well as the retinoid X receptor-α [23]. It has been shown to interact with PU.1 in vivo, resulting in functional consequences for both transcription and splicing [24]. It has also been identified as an interaction partner to the p65 subunit of NF-κB. When exogenously expressed together, TLS and NF-κB were able to co-activate in vitro expression from the intercellular adhesion molecule-1 (ICAM-1) gene promoter [25]. The Spi-1 (PU.1) and NF-κB proteins are major transcription factors that regulate signal transduction pathways often disrupted in cancer. The PU.1 oncogene is an important transcription factor in the differentiation of the white blood cells [26–28], while NF-κB is a key regulator of apoptosis [29, 30]. A binding map of various TLS interaction partners is shown in Figure 1.

The EWS protein interacts with the POU homoeodomain transcription factor Bm-3a [31, 32], an important factor in neuronal differentiation. Interestingly, EWS activates in vitro transcription of a specific isoform of Bm-3a [33]. Additionally, EWS and the histone acetyl-transferase proteins CREB-binding (CBP) and p300 transcriptional activator proteins have been shown to interact and co-transactivate several in vitro promoters in a cell-type specific manner [34]. Finally, the ZFM1 protein, a known transcriptional repressor, was identified as an interaction partner of all the three TET proteins in a yeast two-hybrid screen [35]. Taken together, the literature indicates that TLS, EWS and TAF15 may play an important role in the recruitment of both general and more specialized factors for the initiation of transcription.

**Figure 1:** An interaction map for TLS. TLS can be divided into its SYGQ-rich transcriptional activation domain and its C-terminal RNA-binding domain. This diagram shows, to which of these two regions some of its interaction partners preferentially bind. In the oncogenic fusions, the C-terminal domain of TLS and potentially, the associated interaction partners, are lost.
TLS IS A TRANSCRIPTIONAL MODULATOR OF GENE EXPRESSION

To better understand the normal cellular function of the TLS protein, we have previously generated TLS-deficient mice and showed that TLS, among its other functions, is required for genomic stability [36]. We hypothesize that TLS may work to integrate a transcriptional response to several stresses to the cell to maintain the integrity of the genome. As such, we are currently investigating TLS’ role as a transcriptional modulator to the stress-induced Y-box-binding-1 (YB-1) transcription factor and are seeking to identify potential target genes to better understand the mechanism in which TLS regulates gene expression.

The YB-1 (Y-box binding) protein is a multifunctional protein that is known to be involved in transcriptional regulation and mRNA processing.
It has been identified as an interaction partner of TLS, EWS and the RNA polymerase II enzyme [38]. It was demonstrated that a potential role for the TLS and YB-1 proteins in splicing exists and even suggested that the two serve as a link between transcription and splicing [38]. Upon activation by cytotoxic [39] and genotoxic [40] stresses, YB-1 is able to bind its target gene promoters through two distinct elements: the single stranded response element (RE-1) [41], and the highly conserved Y-box, or inverted CCAAT box [39, 41–43]. The YB-1 has been shown to regulate transcription of several target genes, including MMP-2 [44] and MDR-1 [45, 46].

Given the functional role for TLS in maintaining genomic stability and YB-1’s transcriptional regulation of target genes in response to DNA damage, we were particularly interested in examining whether or not the TLS–YB-1 interaction can modulate YB-1-directed transcriptional activation. To address this directly, we decided to test a model using a putative YB-1 responsive target gene, MMP-1. The MMP-1 promoter contains both the conserved RE-1 element found in the YB-1 responsive MMP-2 gene and also a consensus Y-box. To our knowledge, this is the first reported example of a promoter that contains both types of YB-1 binding elements. For this reason, we rationalized that the MMP-1 was an ideal target gene to use, to test our model.

Our results show that TLS modulates YB-1-directed activation of the MMP-1 gene by further enhancing expression levels from a luciferase reporter construct. Specifically, we have shown a further 2-fold activation of YB-1-directed expression of the MMP-1 gene when TLS is coexpressed. Whether this indicates a synergistic effect or an additive effect remains to be elucidated. These results support a mechanism by which TLS may modulate the expression of a large set of target genes by interacting with gene-specific transcription factors. Identification of TLS-modulated target genes will provide important insights into mechanisms by which TLS regulates the biological response to DNA damage or drives the transformation process in the protein-associated fusion cancers.

Multifunctional proteins are integral in bridging cellular processes by providing avenues for cross-talk between core components of individual processes. The TET sub-family of RNA-binding proteins, TLS, EWS and TAF15, are excellent examples of multifunctional proteins. They appear to function from the initiation of transcription through to the delivery of the mature mRNA to the cytoplasm. The exact mechanisms that permit a single protein to participate in the multiple levels of gene expression like this are poorly defined. The study of the TET family members will undoubtedly reveal novel concordances between cellular processes that were once thought to occur independently. Furthermore, these investigations will also provide information as to the roles of TLS, EWS and TAF15 in their associated fusion proteins and the pathogenesis of the resulting cancers.

Key Points

- TLS, EWS and TAF15 are highly related and distinct sub-set of ribonucleoproteins, collectively known as the TET genes.
- The chromosomal translocations that result in the fusion of the amino transactivation domain of TET proteins with the DNA binding domain of ETS-related transcription factor proteins are the common determinants of cancer.
- In addition to mRNA processing, TET proteins also directly interact with bona fide DNA transcription factors and can function as transcriptional co-factors.
- TLS modulates YB-1-directed gene transcription
- Multifunctional TET proteins may serve to modulate gene expression by integrating the processes of transcription, splicing, RNA metabolism and subcellular targeting of translation of specific target genes.

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References


32. Thomas GR, Latchman DS. The pro-oncoprotein EWS (Ewing’s Sarcoma protein) interacts with the Bmi-3a POU transcription factor and inhibits its ability to activate transcription. *Cancer Biol Ther* 2002;1:428–32.


