Research Article

World Journal on Immunology

Transcription Begins With The Traffic Controller

Anne Gegonne, Ballachanda N. Devaiah and Dinah S. Singer*

Experimental Immunology Branch; NCI; NIH; Bethesda, MD USA

Corresponding author:
Dinah S. Singer,
Experimental Immunology Branch; NCI; NIH; Bethesda, MD USA

Received Date: 01 April 2024
Accepted Date: 16 April 2024
Published Date: 22 April 2024

Citation:
Dinah S. Singer. Transcription Begins With The Traffic Controller. World Journal on Immunology 2024.

1. Introduction:

RNA polymerase II (RNAP II)-dependent genes require an orderly sequence of events leading to initiation, elongation, and termination for productive transcription to occur. The RNAP II machinery is assembled into a preinitiation complex (PIC) prior of transcription initiation.The general transcription factors (GTFs) TFIIB, TFIIE, TFIIF, and TFIIH are sequentially recruited once the general transcription factor TFIID binds to the core promoter to initiate 1PIC assembly. The connection of Mediator, the elongation factor P-TEFb, the bro-modomain protein BRD4, and RNAP II completes PIC assembly.1-4 When the PIC is completed, The first NTPs are included to begin transcription. The C-terminal domain (CTD) of RNAP II is phosphorylated by a sequence of kinases that phosphorylate it during the transition from initiation to productive elongation. These kinases are CDK7/TFIIH, which phosphorylates Ser5 of the CTD heptad repeat, BRD4, which phosphorylates Ser2, and CDK9/PTEFb, which phosphorylates Ser5/2 during elongation.5. These several instances of phosphorylation provide a platform on the CTD.for the enlistment of several complexes required for maturation and processing of developing RNA.5-7 It is well recognized that a series of events is required for the commencement and extension of productive transcription, but little is known about the regulatory mechanisms that guarantee that each stage is successfully finished before the subsequent one starts. According to recent research, TAF7, a transcription factor, is essential for this control.Either the TATA binding protein (TBP) or a TBP-related protein (TRF1, TRF2, TRF3) along with more than a dozen TBP Associated Factors (TAFs) make up the TFIID complexes that bind to the core promoter.1,8–10 TAF7 is a 55 kDa protein that is one of the TAFs present in the TFIID complex. TAF7 was once believed to be only a structural element of TFIID, but research from our laboratory has shown that it also plays a far wider role as an enzyme activity regulator, controlling the activities of several other enzymes necessary for transcription initiation. Our current understanding of TAF7, its relationships with other transcription factors, and its function in controlling transcription initiation and cellular proliferation is outlined in the sections that follow.

2. TAF7 Controls the Start of Transcription

TAF7 and TAF1 are bonded within TFIID.Only the 250 kD TAF1 exhibits enzymatic activity among the TAFs; it possesses an acetyl-transferase activity, two different kinase activities, and mapping to the amino- and C-terminal domains.11,12 TAF1 itself serves as a substrate for TAF1 kinase activity.as well as TAF7.11,13 Though its biological substrate is still unknown, TAF1's intrinsic acetyl trans-ferase (AT) activity is crucial for transcription in both vitro and in vivo. Even though it has been discovered that TAF1,14, and 15 have acetylated the histone H3 linked to the cyclin D1 promoter on K9 and K14, histones may not be the functionally significant target of TAF1AT activity. In fact, transcription of bare DNA in vitro requires TAF1 AT activity.16 TAF7 and TAF1 interact through the AT domain of TAF7 as well as the RAPiD domain, which is also where the TFIIF RAP70 subunit interacts. Our initial research showed that TAF7 binding to TAF1 impairs TAF1's crucial AT therefore preventing the commencement of transcription. In vitro and in vivo, exogenous TAF7 inhibited the expression of an MHC class I gene that was TAF1-dependent.17 A recent work that demonstrated that TAF7 overexpression inhibits the production of the cyclin D and cyclin A genes and represses TAF1 AT activity supported and expanded on these findings.18The canonical TFIID holocomplex is transcriptionally suppressed because TAF7 binding to TAF1 reduces the AT activity necessary for transcription. This begs the contradictory question: if TFIID is dormant, how does transcription begin? Two possibilities exist: Before PIC assembly, either i) TAF7 separates from TFIID or ii) TAF7 staysconnected to TFIID during PIC assembly but is later freed. We have demonstrated that the latter is true: TAF7 is released from the PIC concurrently with initiation and upon completion of assembly.

Thirteen As a result, TAF7 functions as a negative regulator of the initial stage of transcription initiation and may delay premature or abortive start until all prerequisite elements are present.The discovery that TAF7 is released from TFIID implies that TFIID is a highly dynamic complex as opposed to the static, architectural one that was previously thought to exist.Not all transcription is TAF1 reliant, even if TAF7 controls TAF1-dependent promoters. For instance, only 54% of the RNAs in the HEK293 kidney fibroblast line require TAF1.Notably, within the same cells, 65% of Transcripts require TAF7.19 A subgroup of genes is therefore TAF7-dependent but TAF1-independent. Similar differences have been observed in yeast, where TAF7 controls 27% of the transcripts whereas TAF1 is only necessary for 14% of them.20 What TAF7 targets in TAF1-independent transcription is thus raised. A well-characterized instance of TAF1-independent transcription is the transcription of MHC class I

genes triggered by γ-interferon.Translation of the basal MHC class I gene is reliant on TAF1. However, class I transcription becomes independent of TAF1 once cells are treated with γ-interferon (IFN). The IFN-induced transcriptional co-activator, CIITA, takes over its functions. CIITA contains AT activity, just like TAF1. Additionally, it interacts with TAF6, TAF9, and TAF19, indicating that CIITA might form a structure like TFIID. These features enable CIITA to get around the need for TAF1.21 Crucially, TAF7 binds to CIITA, suppressing transcription and preventing its AT action. Therefore, TAF7's control over transcription initiation goes beyond how it affects TFIID.Remarkably, TAF7 independent genes make for 31% of the TAF1-dependent genes. It is yet unknown what controls these genes' transcriptional initiation.

3. The Major RNAP IICTD Kinases Are Regulated by TAF7

Apart from controlling TAF1, TAF7 also engages in interactions, modulates, and is modulated by the kinases that phosphorylate the RNAP II CTD in the first stages of transcription. Repeats of the heptamer Y1S2P3T4S5P6S7 make up the CTD; the number of repeats varies depending on the organism (26 in yeast, 52 in mammals).CDK7/TFIIH, BRD4, and CDK9/PTEFb, respectively, phosphorylate the CTD Ser5/7, Ser2, and Ser5/2 residues. This is required for the recruitment of several complexes involved in nascent RNA processing. At the earliest stage of transcription initiation, when RNAPII is still at the promoter and concurrent with the production of the initial oligo-nucleotides, CDK7/TFIIH phosphorylates Ser5. BRD4 phosphorylates Ser2 before the switch to efficient extension. CDK9/PTEFb mediates further Ser5/2 phosphorylation during elongation.5, 6Tropically, TAF7 interacts to all three CTD kinases and inhibits them.5,22 The inhibition of TAF7 is exclusive to these kinases as well as the CTD substrate. It increases the PTEFb phosphorylation of SPT5, a part of the DSIF complex that transitions early in transcription from a negative to a positive regulator, but it has little effect on CDK2, a minor CTD kinase.22, 23Although TAF7 binding controls the activity of the CTD kinases, TAF7 is also phosphorylated by the three CTD kinases: P-TEFb phosphorylates TAF7 in its central domain, while CDK7 and BRD4 phosphorylate TAF7 in its C-terminal domain (unpublished data).

13, 24Additionally, TAF7 is phosphorylated.by TAF1, which causes TAF7 to be released from TFIID when transcription starts.13, 18 While TAF1 phosphorylates TAF7 at several locations, new data indicates that TAF7 Ser264 is the primary site.18 Every one of these kinases phosphorylates TAF7, which in turn affects the kinase's subsequent capacity to inhibit the other CTD kinases. This results in a regulatory cascade that maintains an ordered progression through the transcription initiation steps.24 Drawing from these findings, we have postulated that TAF7 is a crucial transcriptional regulator, acting as a checkpoint regulator that modifies the enzymatic activities necessary for transcriptional initiation and elongation, guaranteeing that each stage of the process is finished before moving on to the subsequent one.TAF7 controls the transcription of both TAF1-dependent and -independent genes, as was already mentioned. It is yet unknown which general mechanism(s) TAF7 uses to control TAF1-independent genes. Nonetheless, one potential regulation mechanism is provided by TAF7's interaction with the main RNApolymerase II CTD kinases.

4. Transcripts Required for TAF7-dependent Cell Proliferation

Not all transcription is TAF7 dependent, as was already mentioned. T cell maturation in the peripheral and thymus provided us with a perfect model system to study.whether proliferating cells or differentiating cells were correlated with TAF7-dependent transcription. Thymocyte differentiation phases are identified by the expression of two co-receptor molecules on the cell surface, CD4 and CD8. Double negative thymocytes, or DN, are the most immature thymic precursors; they do not express either CD4 or CD8. After the T cell receptor β gene (TCRβ) is rearranged, DN thymocytes proliferate rapidly and develop into "double positive" (DP) thymocytes, which express both CD4 and CD8. Without additional proliferation, DP thymocytes undergo subsequent differentiation into either mature single positive CD4 (CD4SP) or CD8 (CD8SP) thymocytes.25There are significant differences in the impact of TAF7 deletion at different phases of thymocyte development. elimination of TAF7 at the DNDN thymocytes with TAF7 deletions exhibit lower levels of incorporation of BrdU in comparison to their wild-type counterparts. Additionally, the introduction of a trans-gene encoding the survival protein Bcl2 does not prevent cell loss in TAF7-deleted DN thymocytes (unpublished data).26 On the other hand, deletion of TAF7 in the DP positive stage has very little effect on thymocyte development because it does not require proliferation to transition to the SP stage. Undoubtedly, DP thymocytes lacking TAF7 undergo differentiation into CD4 and CD8 SP thymocytes, which thereafter leave the thymus and settle in peripheral lymphoid organs. T cells lacking TAF7 in peripheral lymphoid organs can de novo transcribe cell surface markers like CD69 that are indicative of the TCR activation pathway, but they cannot multiply in response to activation stimuli.26 ConsequentlyWhile TAF7 is not necessary for all transcription, it is necessary for proliferation throughout the whole T cell developmental cycle.

TAF7 is necessary for the growth of numerous different cell types, either whole or partially.Significantly slower proliferation occurs when TAF7 is depleted in HEK293 kidney cells22 or in hamster ovary CHO cells (unpublished findings). After TAF7 deletion, mouse embryonic fibroblasts (MEFs) cease to proliferate.26 TAF7 germline deletion in miceis fatal to the embryo between 4.5 and 5.5 days after coitus, when the embryo implants and experiences a significant cellular growth.26 When combined, these results imply that TAF7 is necessary for all cells to proliferate.TAF7 is required for proliferation, although not during a particular stage of the cell cycle. MEFs with TAF7–/– are inhibited during the cell cycle.26Moreover, TAF7 overexpression in MEFs does not result in a particular cell cycle arrest (unpublished data). Consequently, even though TAF7 interacts with TAF1 and BRD4, the mutation or deletion of either leads to a cell cycle arrest at the G1/S junction; yet, the deletion of TAF7 has no such effect.TAF7-dependent transcription is seen in young, proliferating cells, which is consistent with its crucial function in

proliferation. But during differentiation, this need becomes less important. Hence, almost all genes are impacted by the worldwide suppression of transcription that occurs when TAF7 is deleted from proliferating MEFs.26 This isn't because of a flaw in the TFIID complex; in these cells, the holo-TFIID complex is still being built even in the absence of TAF7. The majority of the 65% of genes that are TAF7-dependent in the highly differentiated but still proliferating HEK293 kidney cell line are those needed for cell growth and proliferation.19 When peripherally at restTAF7 deletion affects just a tiny percentage (~2.5%) of all transcripts, as T cells do not require TAF7 to survive. Numerous TAF7-dependent genes in mature T cells are responsible for encoding TCR signaling pathway components.26Consequently, TAF7 deletion largely impacts a group of genes linked to T cell function in terminally differentiated, non-proliferating T cells, but it has a broad influence on transcription in immature, proliferating cells (i.e., MEFs and DN thymocytes). TAF7's transcriptional control of the genes necessary for cell development and proliferation is reflected in its biological effects.

5. TAF7 is Transcription Initiation's Traffic Controller.

According to our research, TAF7 is essential for both transcription start and proliferation. Its numerous interactions with TFIID/TAF1, CIITA, BRD4, CDK7/TFIIH, and CDK9/PTEFb—components of the RNAP II transcription machinery—all lead to the suppression of their enzymatic activity. Our research leads us to develop a model in which TAF7 acts as a "traffic controller" at the beginning of transcription, making sure that all general transcription factors arrive and operate in the right order (Fig. 1). As we have shown in the instance of TAF1, the recovery of the critical TAF1 AT activity and the release of TAF7 occur simultaneously with the commencement of transcription. TAF7's suppression of TAF1 AT duringPreinitiation complex assembly may help avoid premature transcription initiation, which could lead to an unsuccessful termination if all required components are not recruited. We further hypothesize that TAF7 binding and associated suppression of the remaining factors' activities—CDK7, BRD4, and CDK9—have a comparable purpose, namely to delay the premature termination of their functions.Based on this model, TAF7 would be released in the following order: first, from TFIIH to permit CTD Ser5/7 phosphorylation and the recruitment of capping enzymes; next, from BRD4 to permit CTD Ser2 phosphorylation and the recruitment of splicing factors; and last, from PTEFb to permit Ser5/2 phosphorylation and the ongoing recruitment ofduring elongation, the ment of transcript maturation factors. Thus, TAF7-mediated regulation of the general transcription factors' enzymatic activities guarantees a systematic advancement through both transcription initiation and the cell cycle.It is yet unknown how TAF7 specifically carries out its regulatory function. The possibility that a single TAF7 molecule recruits itself into a preinitiation complex via TFIID and controls every downstream enzyme activity is unknown. As an alternative, distinct TAF7 molecules may target and release each of the CTD kinases in turn during the preinitiation complex's construction. The latter option would necessitate a molar excess of TAF7 in comparison to the other TFIID constituents. According to preliminary findings, this is the case: There exists a molar excess in several cells.TAF7 in comparison to TBP (unpublished observations). TAF7 has also been found in TFIID free cellular fractions.22The varied regulatory roles of TAF7 on the same gene at different transcriptional stages are implied in the current model. Nevertheless, research has not yet been conducted to ascertain if TAF7 operates sequentially on a single gene or whether its inhibition of TAF1 AT activity can be distinguished from its inhibition of CD kinase activities and use of other genes.Subsequent research endeavors will center on comprehending the exact mechanisms underlying TAF7's transcription control.

6. Conclusion:

TAF7 is becoming known as a crucial regulator of transcription start and cell proliferation, despite its initial classification as a structural element of TFIID. It is yet unknown how TAF7 inhibits proliferation and its direct gene targets in both differentiated and undifferentiated cells at the molecular level.be decided. It's noteworthy to note that the HIV transactivator Tat and the multitude of tasks currently identified to TAF7 exhibit striking similarities. It is therefore tempting to hypothesize that TAF7 is Tat's normal cellular homolog and that it may share some of Tat's undefined extra activities.

7. Acknowledgments:

We express our gratitude to our lab members for their serious talks and our regret to the researchers whose work could not be referenced because of space constraints. The NIH's Intramural Research Program, the National Cancer Institute, and the Center for Cancer Research provided funding for this study.

References:

1. Thomas MC, Chiang CM. The general transcriptionmachinery and general cofactors. Crit Rev BiochemMol Biol 2006; 41:105-78; PMID:16858867; http://dx.doi.org/10.1080/10409230600648736.
2. Conaway RC, Conaway JW. Function and regula-tion of the Mediator complex. Curr Opin Genet Dev2011; 21:225-30; PMID:21330129; http://dx.doi.org/10.1016/j.gde.2011.01.013.
3. Taatjes DJ. The human Mediator complex: a versa-tile, genome-wide regulator of transcription. TrendsBiochem Sci 2010; 35:315-22; PMID:20299225;http://dx.doi.org/10.1016/j.tibs.2010.02.004
4. Dey A, Nishiyama A, Karpova T, McNally J, OzatoK. Brd4 marks select genes on mitotic chroma-tin and directs postmitotic transcription. Mol BiolCell 2009; 20:4899-909; PMID:19812244; http://dx.doi.org/10.1091/mbc.E09-05-0380.
5. Devaiah BN, Lewis BA, Cherman N, Hewitt MC,Albrecht BK, Robey PG, et al. BRD4 is an atypicalkinase that phosphorylates serine2 of the RNA poly-merase II carboxy-terminal domain. Proc Natl AcadSci U S A 2012; 109:6927-32; PMID:22509028;http://dx.doi.org/10.1073/pnas.1120422109.
6. Egloff S, Dienstbier M, Murphy S. Updating the RNApolymerase CTD code: adding gene-specific layers.Trends Genet 2012; 28:333-

41; PMID:22622228;http://dx.doi.org/10.1016/j.tig.2012.03.007.

7. Hsin JP, Manley JL. The RNA polymerase II CTDcoordinates transcription and RNA processing.Genes Dev 2012; 26:2119-37; PMID:23028141;http://dx.doi.org/10.1101/gad.200303.112.
8. Cler E, Papai G, Schultz P, Davidson I. Recentadvances in understanding the structure and func-tion of general transcription factor TFIID. Cell MolLife Sci 2009; 66:2123-34; PMID:19308322; http://dx.doi.org/10.1007/s00018-009-0009-3.
9. D'Alessio JA, Wright KJ, Tjian R. Shifting playersand paradigms in cell-specific transcription. Mol Cell2009; 36:924-31; PMID:20064459; http://dx.doi.org/10.1016/j.molcel.2009.12.011.
10. Müller F, Zaucker A, Tora L. Developmental regula-tion of transcription initiation: more than just chang-ing the actors. Curr Opin Genet Dev 2010; 20:533-40; PMID:20598874; http://dx.doi.org/10.1016/j.gde.2010.06.004.
11. Dikstein R, Ruppert S, Tjian R. TAFII250 is abipartite protein kinase that phosphorylates the basetranscription factor RAP74. Cell 1996; 84:781-90;PMID:8625415; http://dx.doi.org/10.1016/S0092-8674(00)81055-7.
12. Mizzen CA, Yang XJ, Kokubo T, Brownell JE,Bannister AJ, Owen-Hughes T, et al. The TAF(II)250subunit of TFIID has histone acetyltransferase activ-ity. Cell 1996; 87:1261-70; PMID:8980232; http://dx.doi.org/10.1016/S0092-8674(00)81821-8.
13. Gegonne A, Weissman JD, Zhou M, Brady JN,Singer DS. TAF7: a possible transcription initiationcheck-point regulator. Proc Natl Acad Sci U S A2006; 103:602-7; PMID:16407123; http://dx.doi.org/10.1073/pnas.0510031103.
14. Dunphy EL, Johnson T, Auerbach SS, Wang EH.Requirement for TAF(II)250 acetyltransferaseactivity in cell cycle progression. Mol Cell Biol2000; 20:1134-9; PMID:10648598; http://dx.doi.org/10.1128/MCB.20.4.1134-1139.2000.
15. Hilton TL, Li Y, Dunphy EL, Wang EH. TAF1 his-tone acetyltransferase activity in Sp1 activation of thecyclin D1 promoter. Mol Cell Biol 2005; 25:4321-32; PMID:15870300; http://dx.doi.org/10.1128/MCB.25.10.4321-4332.2005.
16. Weissman JD, Howcroft TK, Singer DS.TAF(II)250-independent transcription can be con-ferred on a TAF(II)250-dependent basal promoter byupstream activators. J Biol Chem 2000; 275:10160-7; PMID:10744699; http://dx.doi.org/10.1074/jbc.275.14.10160.
17. Gegonne A, Weissman JD, Singer DS. TAFII55binding to TAFII250 inhibits its acetyltransferaseactivity. Proc Natl Acad Sci U S A 2001; 98:12432-7; PMID:11592977; http://dx.doi.org/10.1073/pnas.211444798.
18. Kloet SL, Whiting JL, Gafken P, Ranish J, WangEH. Phosphorylation-dependent regulation of cyclinD1 and cyclin A gene transcription by TFIID sub-units TAF1 and TAF7. Mol Cell Biol 2012; 32:3358-69; PMID:22711989; http://dx.doi.org/10.1128/MCB.00416-12.
19. Devaiah BN, Lu H, Gegonne A, Sercan Z, ZhangH, Clifford RJ, et al. Novel functions for TAF7,a regulator of TAF1-independent transcription. JBiol Chem 2010; 285:38772-80; PMID:20937824;http://dx.doi.org/10.1074/jbc.M110.173864.
20. Shen WC, Bhaumik SR, Causton HC, Simon I, ZhuX, Jennings EG, et al. Systematic analysis of essentialyeast TAFs in genome-wide transcription and preini-tiation complex assembly. EMBO J 2003; 22:3395-402; PMID:12840001; http://dx.doi.org/10.1093/emboj/cdg336.
21. Raval A, Howcroft TK, Weissman JD, Kirshner S,Zhu XS, Yokoyama K, et al. Transcriptional coacti-vator, CIITA, is an acetyltransferase that bypassesa promoter requirement for TAF(II)250. Mol Cell2001; 7:105-15; PMID:11172716; http://dx.doi.org/10.1016/S1097-2765(01)00159-9.
22. Gegonne A, Weissman JD, Lu H, Zhou M, DasguptaA, Ribble R, et al. TFIID component TAF7 func-tionally interacts with both TFIIH and P-TEFb.Proc Natl Acad Sci U S A 2008; 105:5367-72;PMID:18391197; http://dx.doi.org/10.1073/pnas.0801637105.
23. Yamada T, Yamaguchi Y, Inukai N, Okamoto S,Mura T, Handa H. P-TEFb-mediated phosphoryla-tion of hSpt5 C-terminal repeats is critical for proces-sive transcription elongation. Mol Cell 2006; 21:227-37; PMID:16427012; http://dx.doi.org/10.1016/j.molcel.2005.11.024.
24. Devaiah BN, Singer DS. Cross-talk among RNAPolymerase II kinases modulates C-terminal domainphosphorylation. J Biol Chem 2012; 287:38755-66; PMID:23027873; http://dx.doi.org/10.1074/jbc.M112.412015.
25. Bosselut R. CD4/CD8-lineage differentiation in thethymus: from nuclear effectors to membrane signals.Nat Rev Immunol 2004; 4:529-40; PMID:15229472;http://dx.doi.org/10.1038/nri1392.
26. Gegonne A, Tai X, Zhang J, Wu G, Zhu J, YoshimotoA, et al. The general transcription factor TAF7 isessential for embryonic development but not essentialfor the survival or differentiation of mature T cells. Mol Cell Biol 2012; 32:1984-97; PMID:22411629;http://dx.doi.org/10.1128/MCB.06305-11.
27. Hayashida T, Sekiguchi T, Noguchi E, SunamotoH, Ohba T, Nishimoto T. The CCG1/TAFII250gene is mutated in thermosensitive G1 mutants of theBHK21 cell line derived from golden hamster. Gene1994; 141:267-70; PMID:8163200; http://dx.doi.org/10.1016/0378-1119(94)90583-5.
28. Mochizuki K, Nishiyama A, Jang MK, Dey A, GhoshA, Tamura T, et al. The bromodomain protein Brd4stimulates G1 gene transcription and promotes pro-gression to S phase. J Biol Chem 2008; 283:9040-8; PMID:18223296; http://dx.doi.org/10.1074/jbc.M707603200.