Coordinate regulation of RARgamma2, TBP, and TAFII135 by targeted proteolysis during retinoic acid-induced differentiation of F9 embryonal carcinoma cells

ABSTRACT

These observations indicate that appropriately timed proteolysis of TBP and TAFII135 is required for normal F9 cell differentiation. Hence, in addition to transactivators, targeted proteolysis of basal transcription factors also plays an important role in gene regulation in response to physiological stimuli.

INTRODUCTION

Background RNA polymerase II (pol II) transcription factor TFIID comprises the TATA-binding protein (TBP) and a set of TBP-associated factors (TAFIIs) []. At least 12 TAFIIs have been identified in TFIID and cloning of their cDNAs has shown an evolutionary conservation of TAFIIs from yeast to mammals []. TAFIIs are not only components of the TFIID complex, but a subset of TAFIIs are also found in the SAGA, PCAF, TFTC/STAGA complexes which lack TBP []. TAFII function in living cells has been studied in yeast where the use of temperature sensitive (TS) mutants has shown that many TAFIIs are required for transcription of the majority of yeast genes []. In contrast, TS lesions in TAFII145, TAFII150, and TAFII90 have a less dramatic effect affecting the expression of only a specific subset of genes mainly involved in the cell cycle [] (for reviews see. In mammalian cells, a TS mutation in TAFII250 shows that one of the functions of this protein is cell cycle regulation []. Genetic experiments indicate that TAFII30 is required for the viability of mouse F9 embryonal carcinoma cells as well as for their differentiation into parietal endoderm. In the absence of TAFII30, undifferentiated F9 cells die through apoptosis, but TAFII30 is not required for survival of retinoic acid differentiated F9 cells. Several studies have also focused on TAFII135. TAFII135 comprises 1083 amino acids and contains multiple functional domains. At least four glutamine-rich domains have been described. Sp1 and CREB interact with distinct glutamine-rich domains of TAFII135 and TAFII135 acts as a coactivator in vitro for these activators. In transfected cells, subdomains of TAFII135 can act as dominant negative repressors of CREB activity []. It has further been suggested that some neurodegenerative diseases may result from sequestration of TAFII135 by expanded polyglutamine domains and consequent interference with CREB activity. TAFII135 also contains two conserved regions, CR-I and CR-II, which are shared with the Drosophila homologue dTAFII110 and mammalian TAFII105 The CR-II region is also shared with the yeast homologue yTAFII48 and contains a histone fold domain required for heterodimerisation with hTAFII20/yTAFII68. The CR-II domain plays an essential role in the ability of TAFII135 to potentiate ligand-dependent transactivation by the the receptor for all-trans retinoic acid (RAR) in transfected mammalian cells. Aside from these studies, little is known concerning the role of TAFII135 in more physiological situations. An increasing body of evidence indicates that targeted 26S proteasome-mediated proteolysis of transcription factors is an integral part of the transactivation process. There is a very tight relationship between the potency of activation domains and their stability []. Activation domains and sequences required for degradation overlap and mutations in the VP16 activation domain which impair its function result in enhanced protein stability. Similarly, ligand-dependent targeted proteolysis of several nuclear receptors has been observed []. In the estrogen receptor, the RARα, and the RXRα, deletion of the α-helix H12 of the

ligand binding domain which is essential for ligand-dependent activation stabilises these proteins showing that proteolysis and transactivation are intimately linked []. In the case of nuclear receptors, their targeted proteolysis in the presence of ligand may be a mechanism for attenuating the physiological response to the ligand. It has also been suggested that targeted proteolysis is a means of regulating other physiological responses, such as signaling through STAT factors and heat shock. Although targeted proteolysis of transcriptional activators has been investigated, it is not known whether components of the basal transcription apparatus are subject to this type of regulation in response to physiological stimuli. We show here that TBP and TAFII135 are selectively depleted in extracts from T-RA differentiated F9 cells and from differentiated C2C12 cells. This depletion is due to the selective targeting of TBP and TAFII135 for proteolytic degradation since depletion is blocked when cells are treated with proteasome inhibitors. During F9 cell differentiation, degradation of TBP and TAFII135 is concomitant with that of the RARγ2, a critical activator in primitive endoderm differentiation. These results reveal a novel pathway controlling the intracellular levels of these two TFIID components and show that in F9 cells RA not only induces targeted proteolysis of the RARγ2, but also of the basal transcription factors which mediate transcriptional activation. We further show that stable ectopic expression of TAFII135 in F9 cells delays the targeted degradation of endogenous TAFII135, TBP, and the RARγ2 in response to T-RA. The cells have an enhanced growth rate and their differentiation into primitive endoderm is impaired at an early stage, but they readily differentiate into parietal endoderm. Treatment of these cells with T-RA also induces the appearance of a population of cells with an atypical elongated morphology, distinct from that of the primitive endodermal cells, which have not been previously documented with wild type F9 cells, and which are resistant to differentiation with bt2cAMP.

CONCLUSION

Conclusions We report for the first time that components of the basal transcription machinery are subject to regulation by targeted proteolysis in response to a physiological stimulus. Our results point to a critical role of TAFII135 in F9 cell physiology. It is downregulated during F9 cell differentiation and expression of TAFII135 at elevated levels promotes cell growth, impairs the normal T-RA response, and induces a novel differentiation pathway.