Adenoviral E1A Function through Myc

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Abstract

The study of DNA tumor viruses has been invaluable in uncovering the cellular nodes and pathways that contribute to oncogenesis. Perhaps one of the best-studied oncoproteins encoded by a DNA tumor virus is adenovirus E1A, which modifies the function of key regulatory proteins such as retinoblastoma (Rb) and the chromatin remodeling protein p400. Although the interaction of E1A with Rb has long been known to target regulation of the E2F transcription factors, the downstream target of the E1A-p400 interaction has remained elusive. We have recently reported that a critical downstream link of the E1A-p400 nexus is the oncprotein transcription factor c-Myc. Through its interaction with p400, E1A stabilizes Myc and promotes formation of Myc-p400 complexes on chromatin, leading to activation of Myc target genes. These findings point to an important role for p400 in Myc function and reveal that E1A drives oncogenesis by tapping into two important transcriptional networks: those of E2F and Myc. [Cancer Res 2009;69(1):6–9]

DNA Tumor Viruses: Keys to Unlocking Molecular Mechanisms

DNA tumor viruses, such as SV40 and adenovirus, have long been used as tools with which to probe critical cellular processes, including oncogenic transformation. Because of their small genomes and striking biological effects, it is generally assumed that DNA tumor viruses have evolved to target the minimal number of cellular nodes and pathways required for transformation. Thus, by studying these viruses over the years, researchers have been able to probe mechanisms of cancer initiation as well as expose the underlying biological events. There is a rich legacy of exploiting DNA tumor viruses to study genome architecture, eukaryotic transcription, DNA replication, and oncogenesis. Work done on DNA tumor viruses in the 1970s and 1980s led to the development of techniques such as the use of ethidium bromide staining for DNA and RFLP analysis, as well as revealing pre-mRNA splicing, transcriptional regulation, and oncogene cooperation (1). Thus, much of what we take for granted in modern-day molecular biology and cancer research has come from studying DNA tumor viruses.

One of the best-studied DNA tumor viruses is adenovirus, which usually causes upper respiratory tract infections, particularly in children. Although adenovirus is not thought to cause cancer in humans, its early gene products are particularly effective at transforming mammalian cells \textit{in vitro}. Much of the research on adenovirus has focused on the E1A protein of adenovirus-5 (Adv-5), a small, tractable protein that efficiently collaborates with the adenovirus E1B protein to drive oncogenic transformation. Adv-5 E1A is expressed as two alternatively spliced isoforms, referred to as 12S and 13S, and shares four highly conserved regions (CR1-CR4) with E1A proteins from other serotypes. These four conserved regions have been studied extensively and are thought to influence distinct cellular processes that, together, contribute to tumorigenesis (2). By focusing on these regions, researchers were able to move beyond E1A to the cellular proteins with which it interacts and exploits to exert its effects. The most notable of these cellular proteins is the retinoblastoma (Rb) protein (3), which was identified as a cellular factor that interacts with E1A via CR1 and CR2, regions essential for the ability of E1A to promote cell cycle progression. It is now clear that, under normal conditions, Rb functions as a tumor suppressor by sequestering the cell cycle regulator E2F and limiting its role in inducing proliferation. On adenoviral infection, however, binding of E1A to Rb strips it away from the E2F complex, which in turn leads to ectopic activation of E2F and its target genes and loss of growth control. These early studies of the E1A-Rb connection thus revealed an important regulatory pathway that has subsequently been found to be deregulated in most human cancers.

In addition to Rb, E1A interacts with an impressive collection of cellular proteins, including TBP, CBP/p300, p400, YY1, and CDK8, to name a few (2). Although it can be difficult to dissect the individual contributions of each of these interactions to E1A function, a growing body of evidence indicates that interaction of E1A with chromatin remodeling proteins such as CBP/p300 (4) and p400 (5) is important for the biological activities of E1A. In the first case, it has been argued that interaction of E1A with CBP/p300 regulates its association with the anaphase-promoting complex APC, which in turn influences cell cycle progression during mitosis or early G\textsubscript{1} phase of the cell cycle (6). For the E1A-p400 interaction, however, the downstream effector network was unknown. Our recent studies (7) have led to the realization that the cellular oncoprotein c-Myc is an important target of the E1A-p400 nexus and that E1A can function through p400 to exploit the transcriptional program of Myc.

Myc as a Downstream Effector of E1A

Myc is an oncoprotein transcription factor that promotes tumorigenesis by activating and repressing a wide set of target genes that control cell growth and proliferation (8). The amount of Myc in a normal cell is very low and is tightly controlled at both the transcriptional and posttranscriptional levels. We have previously found that one mechanism regulating Myc levels is ubiquitin-mediated proteolysis and that tumor-derived mutations within Myc that subvert this process are associated with aggressive oncogenic activity (9, 10). Moreover, we and others have also found that the activity of transcription factors such as Myc can be profoundly regulated by their ubiquitylation status (11–15) and that in some cases proteolysis of these factors is linked to their
ability to activate transcription (16). Because of the importance of ubiquitin-mediated proteolysis in influencing Myc levels and activity, therefore, it is important to identify and understand the molecular processes through which Myc ubiquitylation and stability are regulated.

Recently, Lohr and colleagues (17) reported that E1A stabilizes Myc during the course of adenoviral infection. To explore the underlying molecular mechanism through which this occurs, we performed a structure-function analysis of E1A, using a set of “classic” E1A mutants developed by others, and probed for regions in the protein that are necessary for Myc stabilization. This analysis identified residues 26 to 35 of E1A as being uniquely required for its ability to block Myc turnover. Given the modular nature of E1A, we reasoned that loss of interaction with a specific cellular protein might underlie the inability of the Δ26-35 E1A mutant to stabilize Myc. The NH$_2$ terminus of E1A interacts with a slew of proteins, including Rb, 19S proteasome subunits, CBP/p300, TBP, p400, and TRRAP, but through comparison of different sets of E1A mutants that disrupt the various interactions, we were able to determine that p400 interaction is uniquely required for the effects of E1A on Myc. p400 is part of a nucleosome-remodeling complex that the Livingston group has shown is important for the activity of both E1A and Myc (5). The common connection of Myc and E1A to p400, and the ability of other chromatin-remodeling proteins to regulate Myc stability (18), led us to hypothesize that p400 may underlie stabilization of Myc by E1A. Indeed, our subsequent studies showed that p400 is required for the ability of E1A to stabilize the Myc protein and that forced expression of p400 attenuates Myc ubiquitylation. Based on these results, we have concluded that p400 is an important regulator of Myc stability and that interaction of p400 with E1A promotes its ability to stabilize Myc.

What is the functional significance of the E1A-p400-Myc connection? Our subsequent studies showed that E1A promotes the stable association of Myc and p400 both in solution and, critically, on chromatin at Myc target genes. Consistent with the role of p400 as a cofactor for the transcriptional activity of Myc, this enhanced Myc-p400 interaction leads to activation of a set of Myc target genes. In a sense, therefore, E1A uses p400 to “hijack” Myc and its activities. We believe that the E1A-Myc connection is important for the activity of E1A because E1A cannot stimulate apoptosis in the absence of Myc and because increased expression of Myc can compensate for transformation defects that are associated with loss of the E1A-p400 interaction. Taking our observations together, we conclude that E1A drives oncogenesis, in part, by using p400 to tap into the transcriptional program of Myc and elicit a Myc-like response (Fig. 1).

**Coopting a Cellular Oncprotein**

The functional similarities between Myc and E1A have been appreciated for many years (19). Both proteins have the ability to...
promote ectopic S-phase entry and cell proliferation. Both can block differentiation, and both cooperate with oncogenes such as Ras to drive cellular transformation. Despite the overlapping repertoires of Myc and E1A, the underlying biological basis of this phenomenon has been unclear. Our demonstration that E1A can function through Myc, thus regulating Myc target genes, provides one explanation for this overlap and shows how a viral oncoprotein can recruit a cellular oncoprotein to exert its effects.

The regulation of Myc by adenovirus E1A raises several interesting issues about the underlying processes at work and how these processes affect cell growth control pathways. One intriguing issue is the mechanism through which E1A acts on p400 to stimulate its interaction with Myc. We favor the idea that E1A acts by forming a ternary complex between itself, p400, and Myc and that it is this complex that assembles on Myc-regulated genes to activate their expression. Although the Livingston group (5) has shown that p400 associates with distinct populations of E1A and Myc in solution, it is possible that such a complex forms only transiently or is stabilized within the context of chromatin. The concept that E1A acts within the context of chromatin is not without precedent. Work from the Harter group (20) has shown that E1A associates with E2F-dependent target genes and that this association leads to changes in chromatin structure that permit gene activation. In this instance, E1A is not simply acting to sequester Rb family proteins but is playing an active role in setting the appropriate epigenetic state for E2F-dependent transcription. We hypothesize that E1A similarly acts at Myc target genes to influence their chromatin structure via p400. Indeed, we have detected E1A at several Myc target genes by using chromatin immunoprecipitation, consistent with the idea that E1A acts on Myc that is promoter bound. If this notion is correct, it raises the interesting possibility that E1A could influence Myc function not simply through p400 but also through the unique set of other transcription factors with which it interacts (2). In this way, E1A does not just stimulate Myc activity but, through recruitment of new cofactors, could qualitatively change the manner in which Myc functions and how its target genes are regulated.

An additional unanswered question is the mechanism through which p400 stabilizes the Myc protein. Curiously, Myc stability is known to be regulated by the histone acetyltransferases hGCN5 and TIP60 (18), establishing a precedent for how a chromatin modifier can influence Myc turnover. In the latter instance, however, hGCN5/TIP60 function by acetylating Myc, which could possibly block availability of lysine residues within the protein for ubiquitin conjugation. Given that p400 resides within the TIP60 complex (5), it is possible that Myc acetylation is a relevant mechanism in the E1A-p400-Myc nexus. Alternatively, it is possible that p400 binds directly to the transcriptional activation domain of Myc, the same region that signals Myc ubiquitination (21). In this case, stabilization could occur by steric hindrance, with p400 blocking binding of either the Fbw7 (22) or Skp2 (12, 15) ubiquitin ligases to their cognate degrons within Myc.

Regardless of the mechanism through which p400 stabilizes Myc, however, the observation itself raises a curious paradox. Numerous studies have implied that the ubiquitination/destruction of proteins such as Myc is coupled to their activity and in some cases may be required for activator function (13, 14). At least two ubiquitin ligases that target Myc also serve as coactivators for Myc function (8, 11, 12, 15). If indeed ubiquitination promotes Myc activity, can E1A, which attenuates Myc ubiquitination via p400, stimulate Myc activity? We suggest that stimulation of the p400-Myc interaction by E1A serves to obviate the requirement of Myc ubiquitination in Myc activity. This could occur either if p400 bypasses the need for ubiquitination by initiating a different mode of Myc activity or if ubiquitination itself is a signal that normally acts to promote or stabilize the p400-Myc interaction. In either case, E1A both stimulates Myc activity and disconnects it from a potent mode of cellular regulation that integrates multiple signaling pathways (23, 24) that typically keep Myc in check. Interestingly, E1A may have multiple means to disconnect Myc activity from destruction. Myc is stabilized by phosphorylation of Ser62 (24) within its degron. This phosphorylation is carried out by extracellular signal-regulated kinase (ERK), which in turn is induced by E1A (25). Thus, in addition to promoting the p400-Myc interaction, E1A may also stabilize Myc by activation of ERK. As it is likely that some cancer-associated Myc mutants are stabilized by accumulation of Ser62 phosphorylation (24), further study of E1A and p400 may thus provide important insight into the mechanism through which highly aggressive mutants of Myc function. An important future goal will be to study the parallels between the effects of E1A on Myc versus the tumor-derived Myc cancer mutants. It will also be important to determine how p400 and Myc ubiquitination figure in this context.

As mentioned earlier, the utility of studying DNA tumor viruses stems in large part from their ability to shed light on the most influential and vulnerable cellular processes that modulate growth control. Just as the interaction of E1A with Rb revealed the importance of the Rb-E2F pathway in control of the cell cycle and cancer progression, we believe that interaction of E1A with p400 highlights the importance of the p400-Myc connection to the same processes. Previous work has shown that p400 is a novel regulator of the p53-p21 cellular senescence pathway (26), and led to the notion that up-regulation of p400 could subvert normal senescence mechanisms. Our data imply that, conversely, increases in p400 levels would also lead to a profound deregulation of Myc target gene expression, which could collaborate with a block in senescence to drive transformation. Although there are no data currently pointing to altered p400 expression in human cancers, the duality with which perturbations in p400 could influence tumorigenesis makes an examination of p400 expression and regulation in tumors an attractive idea.

A Left Jab, Right Hook Strategy for Transformation

E1A is a remarkable protein. Despite its small size (~250 amino acids), it has evolved to target a surprising number of host cell factors. As a viral strategy, the ability of E1A to tap into the Myc pathway is both highly effective and economical; as little as nine amino acids in E1A (26-35) could allow it to modulate expression of many of the hundreds to thousands of genes that are under the control of Myc. Together with a plethora of previous studies, our work reveals that E1A simultaneously and synergistically activates at least two critical growth-controlling transcriptional networks: Myc and E2F. This "double whammy" likely lies at the heart of the oncogenic potency of E1A and further highlights the utility of DNA tumor viruses as roadmaps to understanding cancer initiation and progression.

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3 Unpublished data.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 8/5/2008; revised 9/12/2008; accepted 9/14/2008.

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