 Nucleic Acids Research, 1999, Vol. 27, No. 15 **3173–3182**

Transcriptional pause, arrest and termination sites for RNA polymerase II in mammalian N- and c-*myc* genes

Richard G. Keene[1,3], Anja Mueller[2], Robert Landick[1,4] and Lucille London[1,*]

[1]Department of Biology and [2]Department of Chemistry, Box 1134, Washington University, Lindell and Skinker, St Louis, MO 63130, USA, [3]Department of Molecular Biology, NC20, Cleveland Clinic Foundation, Cleveland, OH 44195, USA and [4]Department of Bacteriology, University of Wisconsin-Madison, 1550 Linden Drive, Madison, WI 53706, USA

Received March 15, 1999; Revised and Accepted June 16, 1999

ABSTRACT

Using either highly purified RNA polymerase II (pol II) elongation complexes assembled on oligo(dC)-tailed templates or promoter-initiated (extract-generated) pol II elongation complexes, the precise 3' ends of transcripts produced during transcription *in vitro* at several human c- and N-*myc* pause, arrest and termination sites were determined. Despite a low overall similarity between the entire c- and N-*myc* first exon sequences, many positions of pol II pausing, arrest or termination occurred within short regions of related sequence shared between the c- and N-*myc* templates. The c- and N-*myc* genes showed three general classes of sequence conservation near intrinsic pause, arrest or termination sites: (i) sites where arrest or termination occurred after the synthesis of runs of uridines (Us) preceding the transcript 3' end, (ii) sites downstream of potential RNA hairpins and (iii) sites after nucleotide addition following either a U or a C or following a combination of several pyrimidines near the transcript 3' end. The finding that regions of similarity occur near the sites of pol II pausing, arrest or termination suggests that the mechanism of c- and N-*myc* regulation at the level of transcript elongation may be similar and not divergent as previously proposed.

INTRODUCTION

A growing list of genes transcribed by RNA polymerase II (pol II) are regulated at the level of transcript elongation including important genes involved in cellular proliferation such as the *myc* gene family (1–3). Expression of the c-*myc* gene is regulated, at least in part, by changes in the ability of pol II to transcribe through template sequences that block elongation. Quiescent or differentiated cells reveal significant blocks to elongation during transcription through c-*myc* first exon or first intron sequences whereas pol II in proliferating cells transcribes through the c-*myc* gene more efficiently (4–6). Similarly, at least some cells over-express N-*myc* through loss of transcriptional attenuation within the first exon of the mammalian N-*myc* gene (7).

Control of transcript elongation is complex and probably occurs at two steps. One step is promoted by intrinsic signals within genes that dictate blocks to elongation in three ways: (i) pausing can occur prior to incorporation of the next NTP, (ii) arrest can occur where pol II halts in a form not capable of spontaneously resuming elongation in the absence of transcript cleavage or (iii) termination can occur where the nascent transcript and polymerase are released from the DNA template. Pausing and arrest of pol II at intrinsic sites requires no accessory factors and results exclusively from protein:nucleic acid interactions between pure pol II and pure nucleic acid components in the DNA or in the nascent transcript (8). Another step at which transcript elongation is modulated is through association of any of several distinct proteins with pol II that increase or decrease the ability of pol II to elongate through sequences that cause blocks to elongation (9,10). For instance, TF-IIF (11–14) and S-III (15,16) reduce pausing by stimulating the rate of pol II elongation whereas S-II allows arrested pol II elongation complexes to read through intrinsic sites of blockage (17).

Understanding pol II elongation control mechanisms will require knowledge of the protein:nucleic acid interactions that block elongation and the mechanisms by which elongation factors promote read through of polymerases blocked at these sites. There is a ubiquitous role of runs of Us in promoting pol II blockage in that the most efficient arrest and termination events occur during transcription through phased runs of several Ts in the non-transcribed DNA strand such as those found at the c-*myc* TII site (8,18,19). However, additional signals in the RNA or DNA can cause transcriptional pausing, arrest or termination of pol II. For instance, pol II pausing occurs at +62 of the HIV transcription unit in response to an RNA hairpin in the nascent transcript distinct from the TAR element (20). Furthermore, pol II pauses downstream of other potential RNA hairpins and addition of runs of Us at the transcript 3' end promoted more efficient elongation blockage and termination (21).

To investigate which intrinsic signals in the nucleic acid block pol II elongation, we determined the precise 3' ends of several pause, arrest or terminated transcripts formed during transcription of c- and N-*myc* templates. We found that several sites of pol II pausing, arrest and termination in the c- and N-*myc* genes have conserved DNA or RNA sequences surrounding the

*To whom correspondence should be addressed at present address: Department of Microbiology and Immunology, Medical University of South Carolina, 173 Ashley Avenue, PO Box 250504, Charleston, SC 29425, USA. Tel: +1 843 792 5014; Fax: +1 843 792 2464; Email: londonl@musc.edu

RNA 3' end. This result was striking since c- and N-*myc* were reported to be unrelated in first exon sequences (22).

MATERIALS AND METHODS

In vitro transcription in extracts

HeLa cells were obtained from the Cell Culture Center (Minneapolis, MN). Extracts were prepared with the inclusion of 0.5 mM phenylmethylsulfonylfluoride (PMSF, Sigma, St Louis, MO) in all buffers (23). All preinitiation reactions between extract and DNA template were assembled, pulse labeled, and sarkosyl washed in bulk (typically 10–20 reactions). HeLa nuclear extract (3 µl/reaction) was added to reactions prewarmed to 30°C containing 20 mM HEPES (pH 7.9), 8 mM $MgCl_2$, 12% glycerol, 60 mM KCl, 10 U inhibitase/ml (5'→3'), 0.5 mM DTT, 20 µg/reaction creatine phosphokinase, 5 mM creatine PO_4 and 20 µg/ml immobilized template on Dynabeads (Dynal, Great Neck, NY) in a final volume of 20 µl per reaction. On the AdMLP-c-*myc* template, transcription was initiated by addition of 5 µl nucleoside triphosphates (NTPs) per reaction which brought ATP, GTP and UTP to 200 µM each and contained 10 µCi of [α-^{32}P]CTP (Amersham, Arlington Heights, IL; 3000 Ci/mmol) for 1 min prior to sarkosyl washing. On the AdMLP-N-*myc* template, transcription was initiated by addition of 5 µl NTPs per reaction which brought ATP, GTP and CTP to 200 µM each and contained 10 µCi of [α-^{32}P]UTP (Amersham; 3000 Ci/mmol) for 1 min prior to sarkosyl washing. Sarkosyl washing was performed by dilution of extract-generated elongation complexes into 20 vol of BC100 [20 mM HEPES (pH 7.9) 20% glycerol, 100 mM KCl, 0.2 mM EDTA, 1 mM DTT) at room temperature containing 1% sarkosyl. After mixing, complexes were centrifuged at 8500 *g* for 2 min and the buffer removed by pipette using a magnet to immobilize the magnetic bead. Sarkosyl was removed by washing three times with BC100 at room temperature in a total volume equal to the original sarkosyl rinsing volume. Sarkosyl washing removes the bulk of stimulators and inhibitors of elongation present in crude extracts (14,24), any trace contaminants and NH_4^+ ions present in the storage buffer of the pure pol II preparation. Following sarkosyl washing, elongation complexes were equilibrated in transcription buffer supplemented with an additional 24 mM KCl and 70 mM potassium glutamate, unless otherwise indicated, and chased at 30°C at NTP concentrations shown for the indicated times. Transcription reactions were terminated by proteinase K treatment, phenol extraction and ethanol precipitation (25). Transcripts were electrophoresed on discontinuous 4%/15% 0.5× TBE 7 M urea polyacrylamide gels (composed of equal portions of a 4% gel poured above a 15% gel in 29 or 48 cm plates).

RNA sequencing ladders formed on any of the immobilized templates were generated using 0.8 mM of a given 3'-dNTP (Boehringer Mannheim Biochemicals, Indianapolis, IN) and 1 mM each NTP as chase NTPs to elongation complexes for 20 min. Polyacrylamide gels (4–8%) with RNA sequencing ladders were run in 0.5× TBE buffer and 8.3 M urea on 48 cm plates at 55 W and dried before autoradiography and exposure to PhosphorImager screens.

RNA pol II purification and *in vitro* transcription using pure pol II

Calf thymus was obtained from ANTEC (Tyler, TX). Pol IIA was purified using immobilized 8WG16 antibody (26).

For *in vitro* transcription using pure pol II, transcription templates were oligo(dC)-tailed at the indicated restriction site (27) and cut at a downstream restriction site (*Xba*I for c-*myc* templates and *Eco*RI for N-*myc* templates) and filled in using dATP, dGTP and dCTP at 40 µM and biotin-14-dUTP (BMB) at 20 µM and 0.2 U of Klenow fragment (NEB) per µg of DNA for 30 min at 30°C. After filtration through Sephadex G50 (Pharmacia) to remove unincorporated biotin-dUTP, transcription templates were immobilized to Dynabeads (Dynal) following the manufacturer's instructions. Transcription reactions contained pure pol II (40 µg/ml) incubated with 1 µg of immobilized oligo(dC)-tailed template in 20 µl for 5 min at 30°C in buffers with 50 mM NH_4Cl and 37.5 mM NH_4SO_4 during initiation (18). Transcription was initiated by adding 5 µl NTPs per reaction which brought ATP and GTP to 0.8 mM, UTP to 0.4 mM and CTP to 1.2 µM including 15 µCi of 3000 Ci/mmol [α-^{32}P]CTP (Amersham), from 1 to several min prior to sarkosyl washing (28).

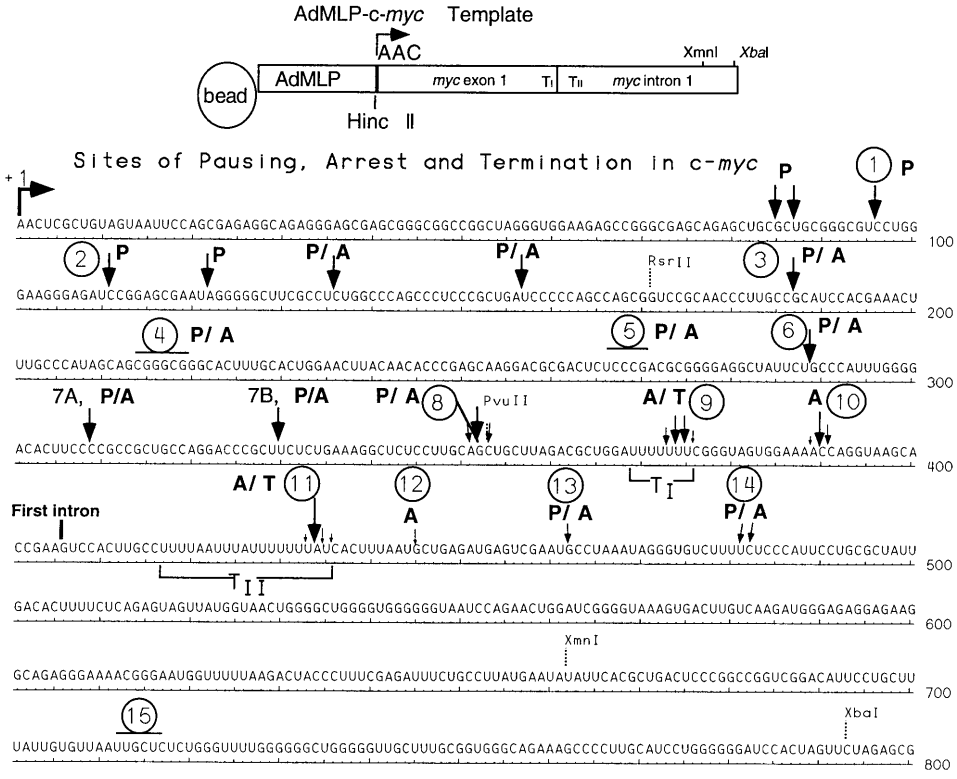
On the *his* template, transcription was initiated on templates oligo(dC)-tailed at the *Taq*I site and transcripts pulse-labeled in the presence of ATP and GTP at 0.8 mM and CTP at 0.7 µM unlabeled CTP plus 30 µCi of 3000 Ci/mmol [α-^{32}P]CTP (Amersham) for 24 min followed by the addition of CTP to 50 µM for 1 min prior to washing in 20 vol of BC100. For experiments on the *his* template and samples in Figure 3, the oligo(dC)-tails were removed by treatment with up to 50 U/ml exonuclease VII (United States Biochemical, Cleveland, OH) in 50 µl BC100 for 1.5 h at 37°C. Prior to chase, treated samples were washed three times with 50 µl BC100.

Quantitation of arrest efficiencies and computer sequence analysis

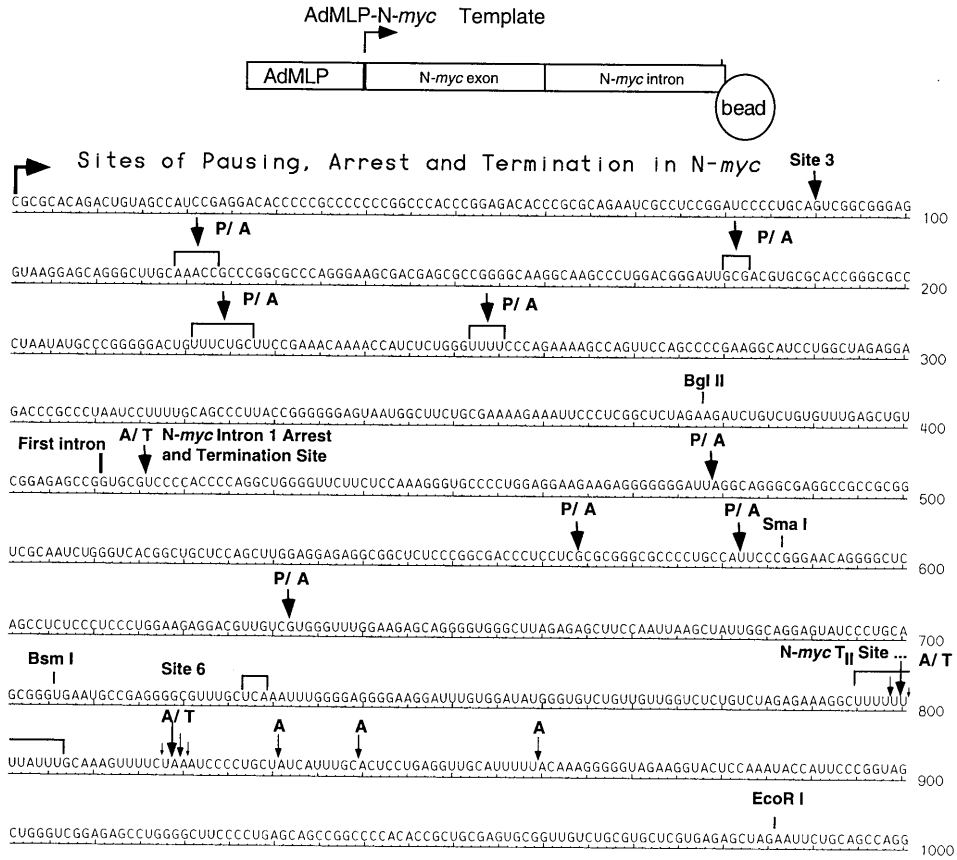
Gels were scanned by a PhosphorImager (Molecular Dynamics) and arrest efficiencies were calculated as described (25). GCG programs BESTFIT and FOLD were used to determine the percent similarity and stability of potential RNA structures, respectively (29). For BESTFIT, the default settings for gap lengths and gap weight were used.

Figure 1. (Opposite) c- and N-*myc* transcription templates. (**A**) An AdMLP-c-*myc* transcription template (pRL558) (25) was immobilized to streptavidin–Dynabeads by biotinylation at the upstream *Xho*I. (**B**) An AdMLP-N-*myc* transcription template was immobilized to streptavidin–Dynabeads by biotinylation at the downstream *Eco*RI. Arrows above template sequences denote sites of pausing and arrest in c- or N-*myc* mapped precisely using pure pol II. The 3' ends of transcripts at some sites were not assigned precisely but lie within the region of template indicated by brackets drawn above the *myc* sequence. P, a site of pure pol II pausing; P/A, a site of pure pol II pausing and low-efficiency (<6%) arrest; A, a site of pure pol II arrest; A/T: a site of > 6% arrest of pure pol II and arrest and termination for extract-generated elongation complexes. Circled numbers correspond to sites mapped in Figure 2.

A


AdMLP-c-myc Template
AAC
XmnI
XbaI
bead
AdMLP
myc exon 1
TI
TII
myc intron 1
Hinc II
Sites of Pausing, Arrest and Termination in c-myc
+1
P
1 P
2 P
P
P/ A
P/ A
RsrII
3 P/ A
4 P/ A
5 P/ A
6 P/ A
7A, P/A
7B, P/A
P/ A
8
PvuII
A/ T 9
A 10
A/ T 11
12 A
13 P/ A
14 P/ A
TI
First intron
TII
15
XmnI
XbaI
AACUCGCUGUAGUAAUUCCAGCGAGAGGCAGAGGGAGCGAGCGGGCGGCCGGCUAGGGUGGAAGAGCCGGGCGAGCAGAGCUGCGCUGCGGGCGUCCUGG
GAAGGGAGAUCCGGAGCGAAUAGGGGGCUUCGCCUCUGGCCCAGCCCUCCCGCUGAUCCCCCAGCCAGCGGUCCGCAACCCUUGCCGCAUCCACGAAACU
UUGCCCAUAGCAGCGGGCGGGCACUUUGCACUGGAACUUACAACACCCGAGCAAGGACGCGACUCUCCCGACGCGGGGAGGCUAUUCUGCCCAUUUGGGG
ACACUUCCCCGCCGCUGCCAGGACCCGCUUCUCUGAAAGGCUCUCCUUGCAGCUGCUUAGACGCUGGAUUUUUUUCGGGUAGUGGAAAACCAGGUAAGCA
CCGAAGUCCACUUGCCUUUUAAUUUAUUUUUUUAUCACUUUAAUGCUGAGAUGAGUCGAAUGCCUAAAUAGGGUGUCUUUUCUCCCAUUCCUGCGCUAUU
GACACUUUUCUCAGAGUAGUUAUGGUAACUGGGGCUGGGGUGGGGGGUAAUCCAGAACUGGAUCGGGGUAAAGUGACUUGUCAAGAUGGGAGAGGAGAAG
GCAGAGGGAAAACGGGAAUGGUUUUUAAGACUACCCUUUCGAGAUUUCUGCCUUAUGAAUAUAUUCACGCUGACUCCCGGCCGGUCGGACAUUCCUGCUU
UAUUGUGUUAAUUGCUCUCUGGGUUUUGGGGGGCUGGGGGUUGCUUUGCGGUGGGCAGAAAGCCCCUUGCAUCCUGGGGGGAUCCACUAGUUCUAGAGCG
100
200
300
400
500
600
700
800

B


AdMLP-N-myc Template
AdMLP
N-myc exon
N-myc intron
bead
Sites of Pausing, Arrest and Termination in N-myc
Site 3
P/ A
P/ A
P/ A
P/ A
Bgl II
First intron
A/ T
N-myc Intron 1 Arrest and Termination Site
P/ A
P/ A
P/ A
Sma I
P/ A
Bsm I
Site 6
N-myc TII Site ...
A/ T
A/ T
A
A
A
EcoR I
CGCGCACAGACUGUAGCCAUCCGAGGACACCCCGCCCCCCCGGCCCACCCGGAGACACCCGCGCAGAAUCGCCUCCGGAUCCCCUGCAGUCGGCGGGAG
GUAAGGAGCAGGGCUUGCAAACCGCCCGGCGCCCAGGGAAGCGACGAGCGCCGGGGCAAGGCAAGCCCUGGACGGGAUUGCGACGUGCGCACCGGGCGCC
CUAAUAUGCCCGGGGGACUGUUUCUGCUUCCGAAACAAAACCAUCUCUGGGUUUUCCCAGAAAAGCCAGUUCCAGCCCCGAAGGCAUCCUGGCUAGAGGA
GACCCGCCCUAAUCCUUUUGCAGCCCUUACCGGGGGGAGUAAUGGCUUCUGCGAAAAGAAAUUCCCUCGGCUCUAGAAGAUCUGUCUGUGUUUGAGCUGU
CGGAGAGCCGGUGCGUCCCCACCCCAGGCUGGGGUUCUUCUCCAAAGGGUGCCCCUGGAGGAAGAAGAGGGGGGGAUUAGGCAGGGCGAGGCCGCCGCGG
UCGCAAUCUGGGUCACGGCUGCUCCAGCUUGGAGGAGAGGCGGCUCUCCCGGCGACCCUCCUCGCGCGGGCGCCCCUGCCAUUCCCGGGAACAGGGGCUC
AGCCUCUCCCUCCCUGGAAGAGGACGUUGUCGUGGGUUUGGAAGAGCAGGGGUGGGCUUAGAGAGCUUCCAAUUAAGCUAUUGGCAGGAGUAUCCCUGCA
GCGGGUGAAUGCCGAGGGGCGUUUGCUCAAAUUUGGGGAGGGGAAGGAUUUGUGGAUAUGGGUGUCUGUUGUUGGUCUCUGUCUAGAGAAAGGCUUUUUU
UUAUUUGCAAAGUUUUCUAAAUCCCCUGCUAUCAUUUGCACUCCUGAGGUUGCAUUUUUACAAAGGGGGUAGAAGGUACUCCAAAUACCAUUCCCGGUAG
CUGGGUCGGAGAGCCUGGGGCUUCCCCUGAGCAGCCGGCCCCACACCGCUGCGAGUGCGGUUGUCUGCGUGCUCGUGAGAGCUAGAAUUCUGCAGCCAGG
100
200
300
400
500
600
700
800
900
1000

RESULTS

Extract-generated elongation complexes paused, arrested or terminated during transcription of the c-*myc* gene

Our experimental approach was to map the positions of pausing, arrest and termination in the *myc* family genes using either extract- or pure pol II-generated transcription complexes. Transcription elongation can be blocked in three ways. During pausing RNA pol II stops until it resumes elongation either spontaneously or by the action of another molecule, but it does not irreversibly terminate transcription. Arrest occurs when pol II halts in a form not capable of spontaneously resuming elongation without releasing the nascent RNA transcript. Paused transcripts can be distinguished from arrested transcripts since only paused transcripts will elongate in the presence of chase NTP concentrations. Termination occurs when the transcription complex dissociates and the nascent RNA transcript is released. Termination can be distinguished from arrest since released RNA transcripts will be found in the supernatant fraction of washed, immobilized transcription complexes whereas arrested transcripts which are still associated with the transcription complex will be found in the template fraction.

For transcription in extracts, the Adenovirus major late promoter (AdMLP) was fused to c-*myc* sequences (Fig. 1A, AdMLP-c-*myc*, pRL558) (25) to allow initiation in crude HeLa nuclear extracts. All transcription was mediated by pol II since only limited elongation occurred after 30 min in the presence of 1 mM NTPs and 2 μg α-amanitin/ml (Fig. 2, lane 1). Extract-generated pol II elongation complexes assembled on an immobilized c-*myc* template revealed a distribution of transcript lengths (up to 67 nt long) that survived the 1% sarkosyl wash (Fig. 2, lane 2).

Transcriptional pausing of 1% sarkosyl washed extract-generated complexes occurred at several c-*myc* template positions. Prominent paused transcripts can be observed with lengths of 154, 158, 191, 212, 230, 270, 286 and 294 nt, during the time course at 100 μM each NTP (Fig. 2, lanes 3–7 and 9, respectively). All of these transcripts were chased with the addition of 1 mM each NTPs for the last 10 min of elongation (Fig. 2, lane 8). Either arrest or termination of extract-generated elongation complexes and not simply extended pausing occurred to produce the 31, 44, 56, 91, 112, 166, 181, 335, 370, 426 and 476 nt transcripts since addition of a 10 min chase at 1 mM each NTP after 20 min did not allow most of the transcription complexes blocked at these sites to resume elongation (Fig. 2, lane 8). To generate either arrested or terminated transcripts in the absence of paused transcripts, a 30 min elongation in the presence of 1 mM NTPs was performed (Fig. 2, lane 10). The majority of arrested or terminated transcripts produced from this reaction were of sizes 91, 112, 370 and 426 (TI and TII) nt, and minor sites of arrest or termination of sizes 31, 44, 56, 166 and 335 nt. After centrifugation of the immobilized transcription template, terminated transcripts could be distinguished from arrested transcripts. Terminated transcripts of sizes 91, 112, 166, 335 and 426 (c-*myc* TII site) nt were revealed in the supernatant fraction (Fig. 2, lane 11) whereas arrested transcription complexes which yielded small amounts of the 31, 44 and 56 nt transcripts remained template bound (Fig. 2, lane 12). Transcripts at the c-*myc* TI site (370 nt) resulted from both arrest and termination since some transcripts were released from the template (Fig. 2, lane 11) and others were still template bound (Fig. 2, lane 12). S-II had little effect on the amount of termination at TI or TII (370 and 426 nt) and did not prevent termination at sites where exclusively termination occurred (91, 112, 166 and 335 nt transcripts) (Fig. 2, lanes 10, 11 and 13). S-II allowed arrested transcription complexes, such as those that produced the 31, 44 and 56 nt transcripts, to chase (Fig. 2, lane 13).

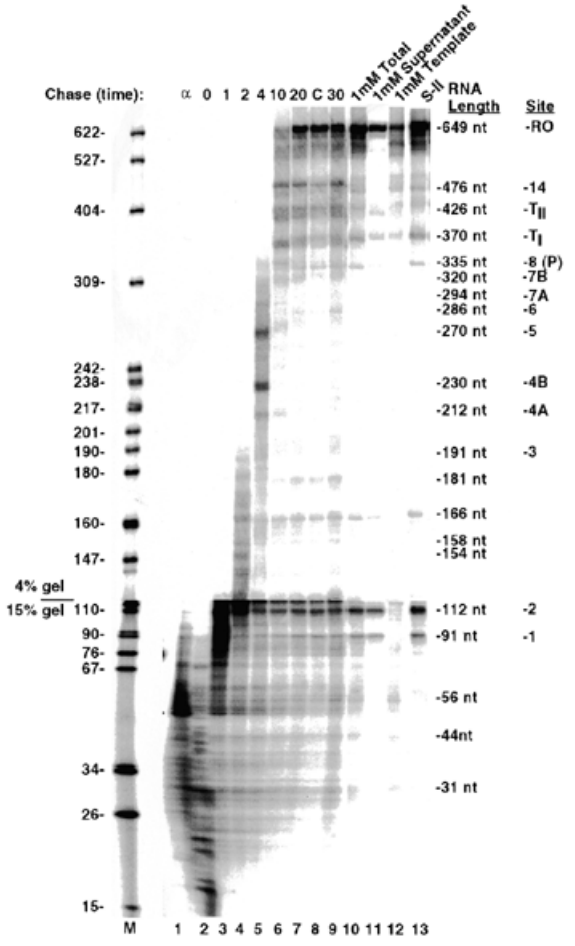


Figure 2. Transcripts produced by sarkosyl washed extract-generated pol II elongation complexes on an AdMLP-c-*myc* template. Transcripts were produced in transcription buffer supplemented with 70 mM potassium glutamate and 24 mM additional KCl. Lane 1, transcripts produced in the presence of 2 μg α-amanitin/ml and 1 mM NTPs for 30 min. Lane 2, transcripts produced by washed extract-generated elongation complexes prior to chase. Lanes 3–7, transcripts produced at the indicated times during elongation at 100 μM each NTP. Lane 8, duplicates lane 7 followed by an additional chase for 10 min at 1 mM each NTP. Lane 9, transcripts produced after 30 min at 100 μM each NTP. Lane 10, transcripts produced at 1 mM each NTP for 30 min. Lanes 11 and 12, duplicate lane 10 followed by centrifugation of the transcription template to separate supernatant fractions containing terminated transcripts (lane 11) or template fractions containing arrested transcription complexes (lane 12). Lane 13, duplicates lane 10 but with 20 ng S-II added immediately after chase NTPs. M, PBR322 *Msp*I marker DNA fragments of the sizes indicated at the left. The c-*myc* transcript lengths and transcriptional pause, arrest and termination sites are indicated at the right and correspond to sites indicated in Figure 1A.

Pure pol II-generated elongation complexes paused or arrested during transcription of the c-*myc* gene

A number of transcription templates were used to evaluate pure pol II transcription of the c-*myc* gene. For the c-*myc* sites of pausing and arrest shown in Figure 1A, the precise 3' ends of transcripts at and prior to site 3 were determined using pure pol II initiated on c-*myc* templates oligo(dC)-tailed at the *Hin*cII site. The 3' ends of transcripts at sites 6–10 were determined using pure pol II initiated on c-*myc* templates oligo(dC)-tailed at the *Rsr*II site. The 3' ends of transcripts at sites 11–14 were determined using pure pol II initiated on c-*myc* templates oligo(dC)-tailed at the *Pvu*II site. Pure pol II transcription complexes either showed pausing or arrest but not termination on the c-*myc* template and arrows above the template sequences denote these sites (Fig. 1A). Sites 1–8 and more proximal sites are primarily pause and low-efficiency (<6%) arrest sites for pure pol II elongation complexes (Fig. 1A). More efficient arrest of pure pol II elongation complexes occurred at sites TI and TII. While extract-initiated pol II arrested as well as terminated at the c-*myc* TI site and terminated at the TII site, pure pol II exclusively arrested at both these sites (Figs 1A and 3).

We determined the precise 3' ends of transcripts at the TII site using pure pol II initiated on c-*myc* templates oligo(dC)-tailed at the *Pvu*II site (Fig. 1A) by direct alignment of arrested transcripts with an RNA sequencing ladder (Fig. 3, lanes 7–10). Transcripts arrested primarily at the c-*myc* TII site at the downstream edge of the run of 7 Us (U433, the seventh U, prior to A434) (Fig. 3, lane 3). Blockage was not due to transcriptional pausing since addition of chase NTPs to 1 mM (Fig. 3, lane 4) or transcription in the presence of 1 mM NTPs for 30 min did not promote elongation (Fig. 3, lane 5). However, either addition of S-II to already stalled transcription complexes (data not shown) or addition of 50 ng S-II added with chase NTPs (Fig. 3, lane 6) enhanced synthesis of runoff transcripts and relieved almost all the arrested transcripts at TII. Thus, TII transcripts produced by pure pol II resulted from transcriptional arrest and not termination. Transcriptional arrest of pure pol II occurred with 38 ± 12% efficiency at TII (n = 3) in the presence of 1 mM NTPs for 30 min (Fig. 3, lane 5).

Purified pol II produces two types of transcription complexes during transcription on oligo(dC)-tailed templates: one with displaced transcripts and another with transcripts present as RNA–DNA hybrids (30). More than 50% of the transcription complexes produced on the oligo(dC)-tailed *Pvu*II c-*myc* template produced displaced transcripts resistant to RNase H (Fig. 3, lane 11). Some transcripts were present as RNA–DNA hybrids resistant to RNase T1 (Fig. 3, lane 12). The 3' ends of transcripts present as either displaced transcripts (Fig. 3, lane 11) or RNA–DNA hybrids (Fig. 3, lane 12) arrested at the identical positions within TII. However, transcription complexes that synthesized transcripts as RNA–DNA hybrids arrested more efficiently at TII, as well as near the end of the transcription template, than those that produced displaced transcripts (Fig. 3, lanes 11 and 12).

One other site of c-*myc* pausing occurred downstream of a potential RNA hairpin in the c-*myc* transcript [Figs 1A (site 6) and 6]. To map this site precisely, we formed pure pol II elongation complexes on a c-*myc* template oligo(dC)-tailed at the *Rsr*II site. During a time course at 100 µM NTPs for 1, 2 or 6 min (Fig. 4A, lanes 2–4), paused transcripts aligned with an RNA sequencing ladder (Fig. 4A, lanes 7–10) after U288 with a small amount of pausing after G289. A small amount of transcriptional arrest was evident after a 20 min elongation followed by a 10 min chase (Fig. 4A, lane 5). Addition of S-II allowed the small fraction of arrested transcription complexes to synthesize runoff (Fig. 4A, lane 6).

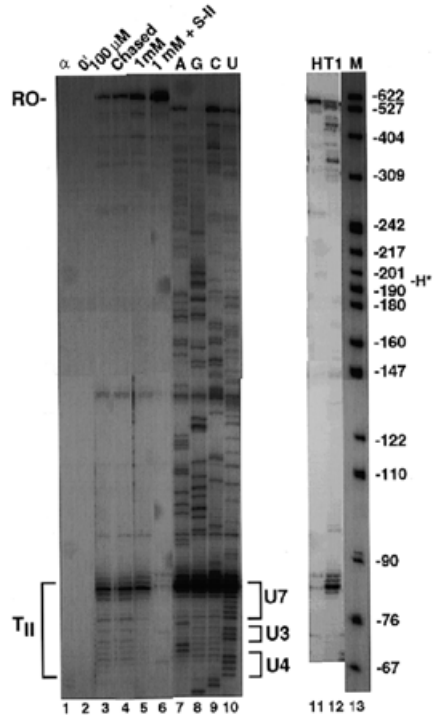


Figure 3. Determination of the precise 3' ends of transcripts produced by pure pol IIA arrested at the c-*myc* TII site. Lane 1, transcripts produced in the presence of 2 µg α-amanitin/ml and 100 µM NTPs for 30 min. Lane 2, transcripts produced by initially pulsed-labeled pure pol II elongation complexes prior to chase. Lane 3, transcripts produced after elongation of pure pol II elongation complexes at 100 mM NTPs for 30 min. Lane 4, duplicates lane 3 but reactions were chased at 1 mM each NTP for the final 10 min. Lanes 5 and 6, transcripts produced after elongation of pure pol II elongation complexes at 1 mM NTPs (lane 5) or 1 mM NTPs and 50 ng S-II for 30 min (lane 6). Lanes 7–10, transcripts produced in the presence of the indicted 3' dNTPs and rNTPs to generate an RNA sequencing ladder. Lane 11, transcripts produced as in lane 5 followed by a 10 min digestion with 6.25 U RNase H/ml (H). Lane 12, Transcripts were produced as in lane 5 followed by digestion with 5 U RNase T_1/ml (lane 12, T_1). In lanes 11 and 12, the oligo(dC)-tails were removed from transcription templates by exonuclease VII digestion prior to the NTP chase. Lane 13, PBR322 *Msp*I marker DNA fragments with sizes indicated at the right.

Pure pol II paused near the *Salmonella his* pause RNA hairpin

Since pol II pausing downstream of potential RNA secondary structures has been previously observed (12,30), we investigated the pol II response to a known RNA hairpin-dependent pause site for *Escherichia coli* RNA polymerase. The *Salmonella his* operon leader pause site was chosen since *E.coli* RNA polymerase recognizes the pause RNA hairpin as a component of the pause site (31). Pure pol II elongation complexes were assembled on a wild type pCL185 *his* template oligo(dC)-tailed at the *Taq*I site (32,33). Transcripts resulting from pausing were evident during the time course (Fig. 4B, lanes 7–11 and Fig. 6).

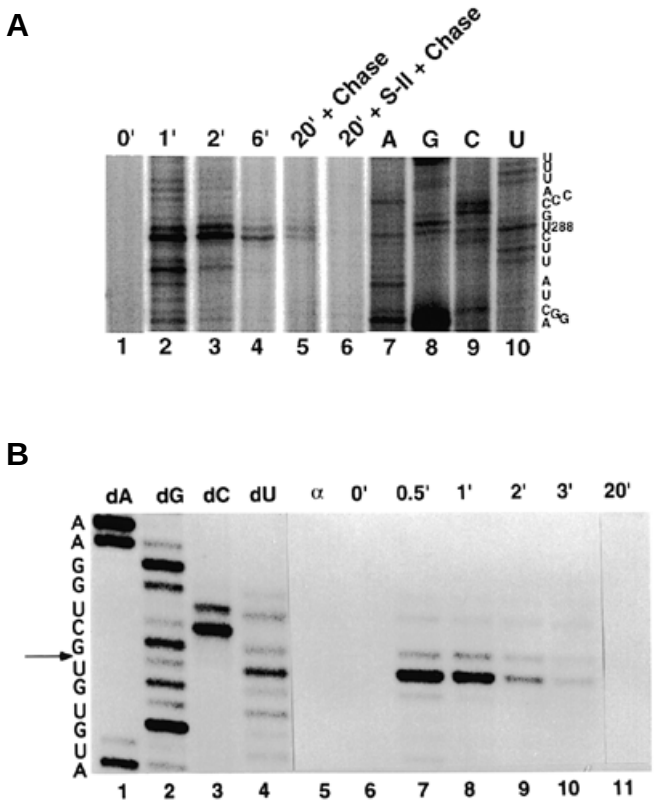


Figure 4. (**A**) Transcriptional pausing at c-*myc* site 6. Lane 1, transcripts produced by initially pulse-labeled pure pol II elongation complexes prior to chase. Lanes 2–4, transcripts produced at 100 μM each NTP for 1, 2 or 6 min, respectively. Lane 5, transcripts produced for 20 min at 100 μM each NTP followed by a 10 min chase at 1 mM each NTP. Lane 6, duplicates lane 5 but S-II (50 ng) was added during the final 10 min chase. Lanes 7–10, transcripts produced in the presence of the indicated 3' dNTPs and rNTPs to generate an RNA sequencing ladder. (**B**) Transcripts pausing at the *Salmonella his* leader operon pause site. Lanes 1–4, transcripts produced in the presence of the indicted 3' dNTPs and rNTPs to generate an RNA sequencing ladder. Lane 5, transcripts produced after 20 min in the presence of 2 μg α-amanitin/ml. Lane 6, transcripts produced by initially pulsed-labeled pure pol II elongation complexes prior to chase. Lanes 7–11, transcripts produced at 500 μM ATP, CTP, UTP and 50 μM GTP on the wild type *his* template (pCL185) after the indicated elongation time. The site of pol II pausing following U99 is indicated by an arrow.

Alignment of the paused transcripts with the RNA sequencing ladder (Fig. 4B, lanes 1–4) revealed that pausing occurred primarily after U99. Duplicate time courses conducted at a limiting concentration of 50 μM GTP (Fig. 4B) as well as time courses conducted at a less limiting GTP concentration of 100 μM (data not shown) revealed that transcriptional pausing occurred with an average apparent half life of 64 and 69 s, respectively, following U99.

Extract-generated elongation complexes paused, arrested and terminated at distinct N-*myc* sites during elongation

We examined sites of pausing, arrest and termination in the N-*myc* gene since N-*myc* is subject to regulation at the level of transcript elongation, even though the first exon was reported to be unrelated to c-*myc* (22). The AdMLP promoter was fused to N-*myc* templates at the *Bss*HII site (+185 relative to the most upstream of several transcriptional start sites (34) in the N-*myc* first exon) to generate the AdMLP-N-*myc* template (Fig. 1B). Most initially labeled transcripts were shorter than 48 nt (Fig. 5A, lane 2) and transcription was due to pol II since only limited elongation occurred in the presence of 2 μg α-amanitin/ml (Fig. 5A, lane 1). A number of discreet transcripts including transcripts of 219, 324, 357, 388, 484, 522 and 575 nt, resulting from either brief or extended pausing were apparent during the time course in the presence of 100 μM NTPs (Fig. 5A, lanes 2–7).

After either a 10 min chase with 1 mM NTPs or after elongation for 30 min at 1 mM NTPs, transcripts of lengths 30, 48, 109, 123 and 677 nt, of low-efficiency blockage accumulated (Fig. 5A, lanes 8 and 9). However, a 438 nt transcript (intron 1 A/T site) resulted from termination (17% efficiency) since these transcripts were released from immobilized templates exclusively into the supernatant fraction (Fig. 5A, lane 10). An 816 nt transcript (N-*myc* TII) fractionated partially with the template fraction as arrested transcription complexes (Fig. 5A, lane 11) and partially as terminated transcripts released into the supernatant (Fig. 5A, lane 10; 45% combined arrest and termination efficiency). Addition of 20 ng S-II with chase NTPs (Fig. 5A, lane 12) could not prevent termination of the 438 nt transcripts (Fig. 5A, lane 12). Additionally, the 438 nt transcript (intron 1 site) and the 816 nt (N-*myc* TII site) transcripts accumulated in the presence of high salt (Fig. 5A, lane 13). Under these conditions, both the 438 and 816 nt transcripts were primarily released from the immobilized template during transcription at 400 mM KCl (Fig. 5A, lane 14).

Pure pol II elongation complexes paused or arrested at distinct N-*myc* sites during elongation

For N-*myc* sites of pausing and arrest shown in Figure 1B, the 3' ends of transcripts at sites prior to the *Bgl*II site were determined using pure pol II initiated on N-*myc* templates oligo(dC)-tailed at a *Bsa*A1 site (in the AdMLP) 60 nt upstream of +1 on the template shown. The 3' ends of transcripts at sites residing within template sequences numbered 400–600 were determined using pure pol II initiated on N-*myc* templates oligo(dC)-tailed at the *Bgl*II site. The potential 3' ends of transcripts at site 6 were determined using N-*myc* templates oligo(dC)-tailed at the *Sma*I site. However, we were unable to precisely determine the sites of pure pol II pausing at this site due to multiple start sites (R.G.Keene, unpublished observation). The 3' ends of transcripts arrested at the N-*myc* TII site were determined using pure pol II initiated on N-*myc* templates oligo(dC)-tailed at the *Bsm*I site.

Using pure pol II initiated on N-*myc* templates oligo(dC)-tailed at the *Bgl*II site, a site of pure pol II blockage was mapped precisely to just after G415 which is 5 nt downstream from the intron 1 splice donor site (Fig. 1B). Pure pol II underwent exclusively arrest and not termination after G415 since S-II could promote read through (data not shown). This site is within 23 nt of the 438 nt terminated transcript mapped with extract-initiated pol II. This discrepancy between the estimated size of the N-*myc* transcript deduced using DNA size standards in Figure 5A (438 nt) versus the size determined precisely using pure pol II (415 nt) most likely resulted from sizing the abnormally GC-rich N-*myc* transcripts against DNA size standards.

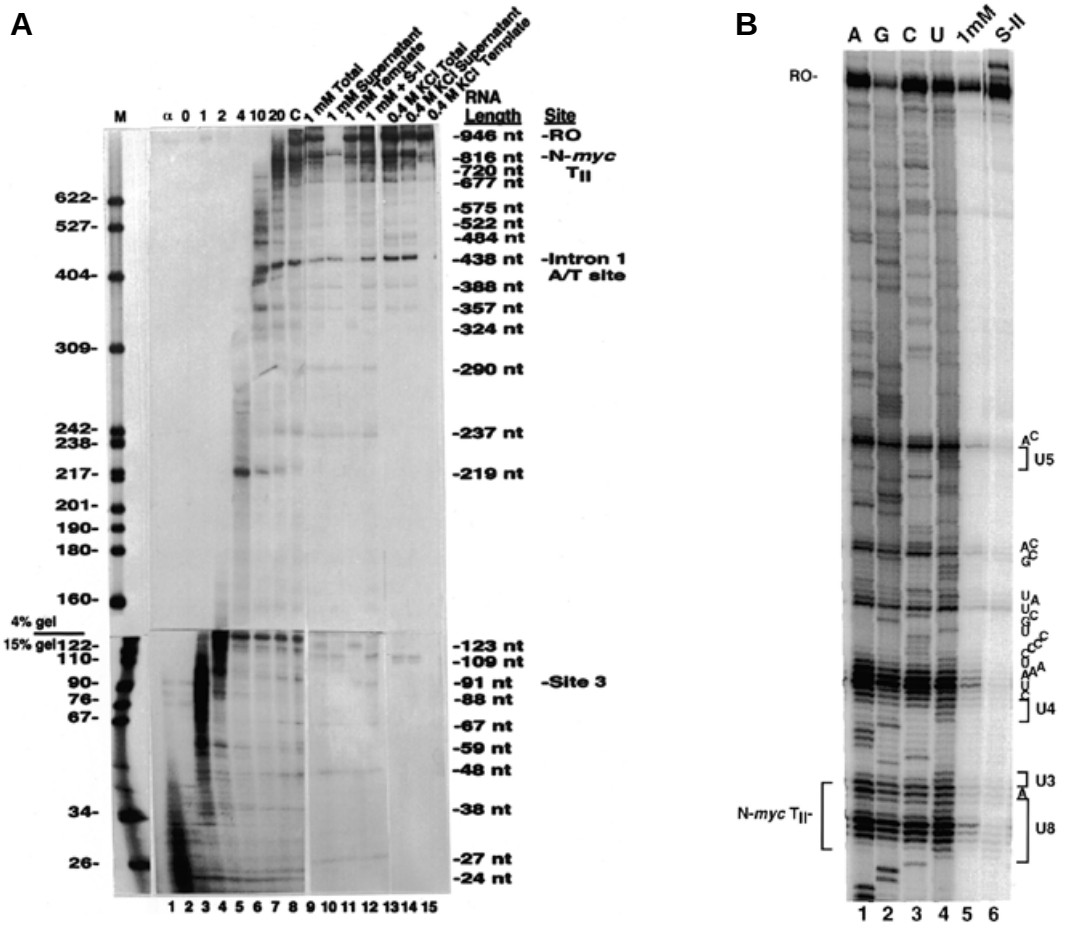


Figure 5. (**A**) Transcripts produced by sarkosyl washed extract-generated pol II elongation complexes on the AdMLP-N-*myc* template. Lane 1, transcripts produced in the presence of 2 μg α-amanitin/ml and 1 mM NTPs for 30 min. Lane 2, transcripts produced by washed extract-generated elongation complexes prior to chase. Lanes 3–7, transcripts produced after 1, 2, 4, 10 or 20 min, respectively, in the presence of 100 μM NTP. Lane 8, duplicates lane 7 followed by an additional chase for 10 min at 1 mM each NTP. Lane 9, transcripts produced in the presence of 1 mM each NTP for 30 min. Lanes 10 and 11, duplicates lane 9 followed by centrifugation of the transcription template to separate supernatant (lane 10) or template fractions (lane 11). Lane 12, duplicates lane 10 but with 20 ng S-II added immediately after chase NTPs. M, PBR322 *Msp*I marker DNA fragments of the sizes indicated at the left. The N-*myc* transcript lengths and transcriptional pause, arrest and termination sites are indicated at the right and correspond to sites indicated in Figure 1B. (**B**) Determination of the precise 3' ends of transcripts produced by pure pol IIA arrested at the N-*myc* T_{II} site. Lanes 1–4, transcripts produced in the presence of the indicated 3'-dNTP and each rNTP to generate an RNA sequencing ladder. Lane 5, transcripts produced after 30 min elongation in the presence of 1 mM each NTP. Lane 6, duplicates lane 5 but S-II (50 ng) was added just after chase NTPs.

Transcripts produced at the N-*myc* TII site were mapped precisely using pure pol II on templates oligo(dC)-tailed at the *Bsm*I site. This site corresponds to the 816 nt transcript observed after extract-generated transcription (Fig. 5A). The template sequence shows a resemblance to the c-*myc* TII site and other pol II arrest and termination sites in that phased runs of eight and three Ts are present in the non-transcribed DNA strand (Fig. 1B) (19). Pol II blockage occurred after the fifth U within the run of eight Us at the N-*myc* TII site and in the region of template downstream of the run of eight Us following synthesis of the sequence UUUUCU, just prior to incorporation of an A (Fig. 5B, lane 5). Pure pol II blockage at the N-*myc* TII site was due almost exclusively to arrest and not termination since elongation factor S-II relieved arrest to levels below 6% (Fig. 5B, lane 6). Pol II arrested with 28% total efficiency at the upstream and downstream region of the N-*myc* TII site shown in Figure 1B.

DISCUSSION

The *myc* family of cellular oncogenes, c-, N- and L-*myc*, encode three related nuclear phosphoproteins whose changes in expression coincide with critical developmental transitions in many cell types (22). Since the disregulation of *myc* family genes have been shown to play a key role in tumorigenesis, the mechanism by which transcription of these genes are regulated is important (22). This study revealed several significant findings regarding pol II behavior during transcription of c- and N-*myc* sequences. First, any type of pol II elongation complex that we tested generally recognized the same c- or N-*myc* sites of blockage in some way by exhibiting either pausing, arrest or

termination. This is notable since the pol II transcription complexes had purities ranging from those partially purified from crude nuclear extracts to those assembled with highly purified pol II. Furthermore, we found that pure pol II transcription complexes recognized the same c- or N-*myc* sites in spite of seemingly different structures since transcription complexes that produced either displaced transcripts or transcripts as RNA–DNA hybrids, which can form on oligo(dC)-tailed templates (29,35), both paused or arrested at precisely the same sites in agreement with earlier findings (30). Second, the distinct pol II transcription complexes we studied showed qualitatively different behaviors at these sites. Pure pol II underwent either pausing or arrest (but almost no termination at any site including the TII site), whereas high salt washed or sarkosyl washed extract-generated complexes paused and arrested but were also capable of termination at several sites, even in the absence of elevated ionic strengths. This observation is consistent with the stable association of a transcript release factor with a fraction of the extract-generated pol II complexes that enhances transcriptional termination by paused or arrested polymerases. A pol II transcript release factor, N-TEF that causes termination of *Drosophila* pol II elongation complexes at several template positions where promoter-proximal pausing ordinarily occurs has been described (36).

There is no single conserved feature in the vicinity of all pause, arrest, or termination sites mapped by us or by others (8,17,37–40). These sites fall into three broad classes of similarity near the positions of blockage: (i) sites that are U-rich, (ii) sites that are both U- and C-rich and (iii) sites following potential RNA hairpins. The c- or N-*myc* TII sites are examples of U-rich sites where pure pol II arrest occurs (or termination for extract-generated pol II complexes) after synthesis of a run of exclusively Us at the transcript 3' end. The different efficiencies and positions of arrest among different U-rich sites that have been mapped do not correlate simply with the length of the U-run (30). But revealed that both the precise position and efficiency of transcriptional arrest is altered by imposing different flanking sequences placed upstream (19) or downstream of the run of Us (14,19,30,37,38,41). In our studies, even though the c-*myc* TI and TII sites both contain a run of seven Us, arrest of pure pol II occurred primarily after the fifth and sixth U within TI and almost exclusively following the seventh U within TII. At the N-*myc* TII site arrest occurred most prominently after the fifth U within a potential run of eight Us whereas for the *Salmonella* ρ-independent *his* terminator, arrest occurred most prominently just after the second and the fifth U within the run of nine Us. Together, these observations reveal important, but poorly understood, sequence context effects for U-rich pol II arrest sites.

In addition, each U-rich arrest site also promoted some transcriptional arrest 11–16 bp downstream of the last U within the run of Us that constituted the major site of arrest. For the c-*myc* TII site the downstream arrest was only 4% as efficient as the arrest after U433, the seventh U. However, at some sites, such as the c-*myc* TI, N-*myc* TII and downstream of the *his* terminator, the downstream arrest was as efficient or nearly as efficient as arrest events within the long run of Us. There is no common feature in the region of the downstream arrest events at the different U-rich sites. Rather, the feature in common at all these sites is the presence of an upstream oligo (rU)-run in the transcript. One hypothesis that explains the arrest downstream of the long run of Us is that the long oligo (rU)-run may remain stably bound within a tight binding site of pol II. Models where polymerase is composed of an active site accompanied by tight and loose transcript binding sites bounded by clamps that slide along the DNA template have been proposed (31,42,43). Tight binding of an RNA binding domain of pol II to an oligo (rU)-run in the transcript could subsequently cause difficulty in further translocation of the transcription complex and could cause the arrest that we always observed downstream of the longest downstream run of Us. Yeast pol II bound a poly-U40 RNA in binary complexes 2-fold more tightly than RNA not comprised of exclusively U-residues (44), an observation consistent with tight binding of oligo (rU) runs to a pol II RNA binding domain.

The second class of pol II pausing and low-efficiency (<6%) arrest in c- and N-*myc* sequences generally occurred at sites of nucleotide addition to a pyrimidine or to pyrimidine-rich (U- or C-rich) regions of the transcript. Alignment of 20 nt upstream and downstream of the c- and N-*myc* pure pol II pause sites mapped precisely revealed that for 71% of the sites, pausing and low-efficiency arrest occurred following either a U or a C. This observation is consistent with addition of nucleotides to a pyrimidine and in particular to a U (at ~50% of the sites) at the transcript 3' end as a slow step in pol II elongation. Elongation through pyrimidines appears to be a slow step in pol II catalysis where pyrimidine-rich regions of template reveal the greatest effects of the pol II inhibitor α-amanitin on transcriptional elongation (45). In addition, while any of the NTPs was equally likely to be the incoming NTP, the nucleotide at position –7 was usually a C (at 67% of the sites) and position –4 was rarely an A (at only 4% of sites) and at position –1 was typically a U or a C (71% of the sites). These preferences could reflect effects mediated at least in part through decreased stability of an ~8 nt RNA–DNA hybrid in the transcription complex which may lead to pol II pausing.

The third class of sites of blockage in *myc* genes occurred downstream of potential RNA secondary structures (Fig. 6). Pol II pausing occurred on the human c-*myc* template following U288, just prior to a G, 8 nt downstream of a potential RNA hairpin followed by the downstream DNA sequence AUUUGGGGA. The mouse c-*myc* gene promotes blocks to elongation between this conserved potential RNA hairpin and the downstream AUUUGGGGA sequence in *Xenopus* oocytes (46). This region of the mouse c-*myc* gene is 82% conserved with the human c-*myc* gene within the region of template encoding the potential RNA hairpin through the AUUUGGGGA sequence. The human N-*myc* gene also contains a potential RNA hairpin upstream of the same conserved AUUUGGGGA sequence (Fig. 6). Mutational analysis reveals that the RNA hairpin and the downstream run of Ts of the mouse N-*myc* homolog of this site contributes to attenuation *in vivo* (7). However, we were unable to precisely determine the sites of pure pol II pausing in the human N-*myc* gene at this site due to multiple start sites on templates oligo(dC)-tailed near this site (R.G.Keene, unpublished observation). However, transcriptional arrest of extract-generated complexes on the N-*myc* templates to produce the 720 nt N-*myc* transcript is consistent with pol II pausing and arrest at this RNA hairpin site.

We also showed that pure pol II pausing occurred downstream of the Salmonella *his* pause site, an RNA hairpin-dependent pause site for *E.coli* RNA polymerase (31). Since

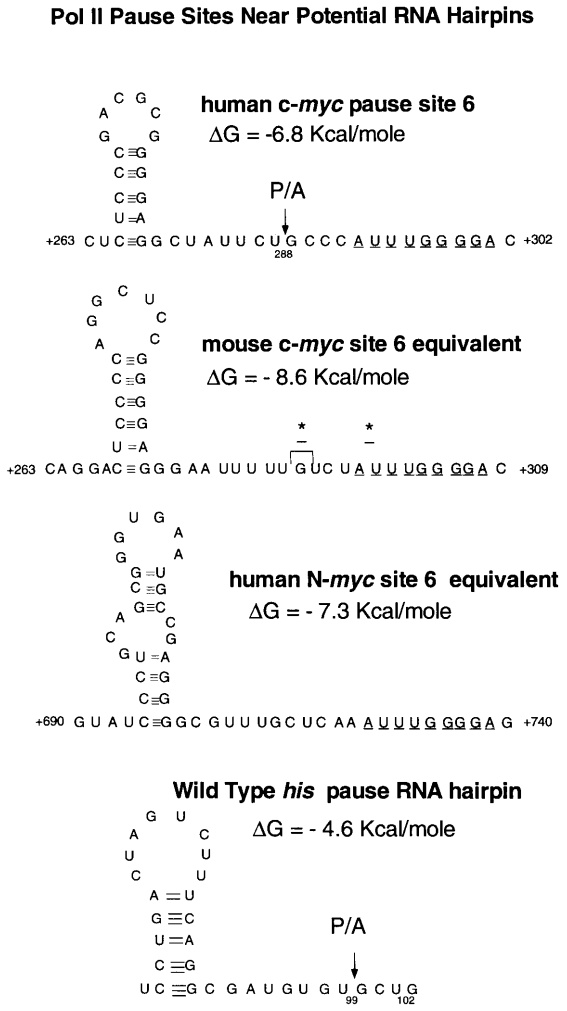


Figure 6. Positions of transcriptional pausing near potential RNA hairpins. The site of pausing and low-efficiency arrest (P/A) downstream of the human c-*myc* site 6 potential RNA hairpin is indicated by an arrow and the sites of blockage downstream of the mouse potential RNA hairpin (46) designated by asterisks, are shown. The conserved AUUUGGGGA sequence is underlined. Pol II pauses at the *Salmonella his* pause site after U99 whereas *E.coli* RNA polymerase pauses after U102. The wild type *his* potential RNA hairpin is shown.

pol II pausing and arrest downstream of potential RNA hairpins can clearly occur (30,47), an intriguing possibility is that at least some sites of pausing for pol II may have a similar multipartite architecture to prokaryotic RNA hairpin-dependent pause sites such as the *Salmonella his* pause site. Here, an RNA hairpin causes *E.coli* RNA polymerase to pause. The upstream RNA hairpin, the 3'-proximal RNA or DNA sequence prior to the pause site, the identity of the 3'-proximal NTP and the NTP to be added, as well as DNA sequences up to 14 nt downstream of the pause site influence the duration or efficiency of pausing (31). One trend evident from our work is that when pol II pausing occurred downstream of either the site 6 potential RNA hairpin in c-*myc* or at the *his* pause site it occurred following a pyrimidine 8 nt downstream of the end of the potential RNA hairpin.

Previous reports that c- and N-*myc* first exon sequences had no significant similarity produced speculation that the mechanism of control of transcript elongation by pol II may be divergent within the c-*myc* family members (22). Our observation that at least some sites of pol II pausing and arrest in the c- and N-*myc* first exon and first intron occur within similar sequences suggests that the mechanisms involved in c- and N-*myc* elongation control may be conserved at least at the level of the *cis*-acting signals present in the nucleic acids that promote blocks to pol II elongation. It is known that blocks to elongation occur within intragenic regions of the mammalian c-*myc* first exon and first intron sequences when assayed in nuclei (4,48,49), in *Xenopus* oocytes (50,51) or *in vitro* (18,25). The production of a strong block to pol II elongation in *myc* family genes *in vivo* may require the presence of an array of several pause, arrest and termination sites rather than simply one site, such as the TII site, working alone. Template titration experiments are consistent with this view of *myc* attenuation occurring at several sites since increased efficiency of blockage at several c-*myc* template positions, which appear to correspond to the c-*myc* pause sites 3 through 11 that we mapped precisely in this study, occurred when greater amounts of c-*myc* templates were injected into *Xenopus* oocytes (50,51). A requirement for an array of sites of blockage, rather than one single site of blockage, may explain the lack of ability of studies to attribute efficient c-*myc* attenuation onto any single site when minimal sequences were assayed *in vivo* (48). The array of pause and arrest sites we characterized within the first exon and first intron of the mammalian c- and N-*myc* genes could serve one, or possibly both, of two purposes in gene regulation. First, an array of transcriptional pause, arrest and termination sites would serve to prevent pol II elongation complexes that are not exceptionally elongation competent from completing transcript synthesis through a c- or N-*myc* coding region. Second, particular intragenic sites of pausing, arrest and termination could act as sites where pol II transcription complexes can undergo conditional modifications that allow either continued transcript elongation or abortion of transcript synthesis.

ACKNOWLEDGEMENTS

We thank Caroline Kane, David Price, Nancy Thompson and Craig Pikaard for suggestions on this manuscript. Special thanks to Donal Luse for suggestions and assistance with figures. This work was supported by grants from the NSF and ACS to R.L. L.L. was supported by an IRSA from the NCI. R.G.K. was supported by a NIH pre-doctoral training grant to Washington University.

REFERENCES

1. Spencer,C.A. and Groudine,M. (1990) *Oncogene*, **5**, 777–785.
2. Kerppola,T.K. and Kane C.M. (1991) *FASEB J.*, **5**, 2833–2842.
3. Krumm,A. and Groudine,M. (1995) *Science*, **269**, 1400–1401.
4. Bentley,D.L. and Groudine,M. (1986) *Nature*, **321**, 702–706.
5. Eick,D. and Bornkamm,G.W. (1986) *Nucleic Acids Res.*, **14**, 8331–8346.
6. Krumm,A., Meulia,T. and Groudine,M. (1993) *Bioessays*, **15**, 659–665.
7. Xu,L.Y., Meng,Y., Wallen,R. and DePinho,R.A. (1995) *Oncogene*, **11**, 1865–1872.

8. Kane,C.M., (1994) In Conaway,R.C. and Conaway,J.W. (eds), *Transcription: Mechanisms and Regulation.* Raven Press, New York, pp. 279–296.
9. Uptain,S.M., Kane,C.M. and Chamberlin,M.J. (1997) *Annu. Rev. Biochem.*, **66**, 117–172.
10. Shilatifard,A., Conaway,J.W. and Conaway,R.C. (1997) *Curr. Opin. Genet. Dev.*, **7**, 199–204.
11. Sluder,A.E., Greenleaf,A.L. and Price,D.H. (1989) *J. Biol. Chem.*, **264**, 8963–8969.
12. Bengal,E., Flores,O., Krauskopf,A., Reinberg,D. and Aloni,Y. (1991) *Mol. Cell. Biol.*, **11**, 1195–1206.
13. Izban,M.G. and Luse,D.S. (1992) *J. Biol. Chem.*, **267**, 13647–13655.
14. Wiest,D.K., Wang,D. and Hawley,D.K. (1992) *J. Biol. Chem.*, **267**, 7733–7744.
15. Bradsher,J.N., Jackson,K.W., Conaway,R.C. and Conaway,J.W. (1993) *J. Biol. Chem.*, **268**, 25587–25593.
16. Garrett,K.P., Tan,S., Bradsher,J.N., Lane,W.S., Conaway,J.W. and Conaway,R.C. (1994) *Proc. Natl Acad. Sci. USA*, **91**, 5237–5241.
17. Reines,D. (1994) In Conaway,R.C. and Conaway,J.W. (eds), *Transcription: Mechanisms and Regulation.* Raven Press, New York, pp. 263–278.
18. Kerppola,T.K. and Kane,C.M. (1988) *Mol. Cell. Biol.*, **8**, 4389–4394.
19. Kerppola,T.K. and Kane,C.M. (1990) *Biochemistry*, **29**, 269–278.
20. Palangat,M., Meier,T.I., Keene,R.G. and Landick,R. (1998) *Mol. Cell.*, **1**, 1033–1042.
21. Bengal,E. and Aloni,Y. (1989) *J. Biol. Chem.*, **264**, 9791–9798.
22. DePinho,R.A., Schreiber-Agus and,N. and Alt,F.W. (1991) *Adv. Cancer Res.*, **57**, 1–45.
23. Shapiro,D.J., Sharp,P.A., Wahli,W.W. and Keller,M.J. (1988) *DNA*, **7**, 547–558
24. Izban,M.G. and Luse,D.S. (1992) *Genes Dev.*, **6**, 1342–1356.
25. London,L., Keene,R.G. and Landick,R., (1991) *Mol. Cell. Biol.*, **11**, 4599–4615.
26. Thompson,N.E., Aronson,D.B. and Burgess,R.R. (1990) *J. Biol. Chem.*, **265**, 7069–7077.
27. Ausubel,F.M. (ed.) (1995) *Current Protocols in Molecular Biology*. John Wiley & Sons. New York, NY.
28. Keene,R.G. (1996) Ph.D. Thesis, Washington University in St Louis.
29. Devereux,J., Haeberli,P. and Smithies,O. (1984) *Nucleic Acids Res.*, **12**, 387–395.
30. Dedrick,R.L., Kane,C.M. and Chamberlin,M.J. (1987) *J. Biol. Chem.*, **262**, 9098–9108.
31. Chan,C.L. and Landick,R. (1994) In Conaway,R.C. and Conaway,J.W. (eds), *Transcription: Mechanisms and Regulation.* Raven Press, New York, pp. 297–321.
32. Chan,C.L. and Landick,R. (1989) *J. Biol. Chem.*, **264**, 20796–20804.
33. Chan,C.L. and Landick,R. (1993) *J. Mol. Biol.*, **233**, 25–42.
34. Kohl,N.E., Legouy,E., DePinho,R.A., Nisen,P.D., Smith,R.K., Gee,C.E. and Alt,F.W. (1986) *Nature*, **319**, 73–77.
35. Gu,W., Powell,W., Mote,J. and Reines,D. (1993) *J. Biol. Chem.*, **268**, 25604–25616.
36. Xie,Z. and Price,D.H. (1996) *J. Biol. Chem.*, **271**, 11043–11046.
37. Hawley,D.K., Wiest,D.K., Holtz,M.S. and Wang,D. (1993) *Cell. Mol. Biol. Res.*, **39**, 339–348.
38. Izban,M.G. and Luse,D.S. (1993) *J. Biol. Chem.*, **268**, 12874–12885.
39. Giardina,C., Perez-Riba,M. and Lis,J.T. (1992) *Genes Dev.*, **6**, 2190–2200.
40. Kash,S.F. and Kellems,R.E. (1994) *Mol. Cell. Biol.*, **14**, 6198–6207.
41. Samkurashvili,I. and Luse,D.S. (1996) *J. Biol. Chem.*, **271**, 23495–23505.
42. Chamberlin,M.J. (1994) *New Models For The Mechanism Of Transcription Elongation and its Regulation.* The Harvey Lectures, **88**, 1–21.
43. Landick,R. and Roberts,J.W. (1996) *Science*, **273**, 202–203.
44. Johnson,T.L. and Chamberlin,M.J. (1994) *Cell*, **77**, 217–224.
45. Geiduschek,E.P. and Tochini-Valenti,G.P. (1988) *Annu. Rev. Biochem.*, **57**, 873–914.
46. Bentley,D.L. and Groudine,M. (1988) *Cell*, **53**, 245–256.
47. Resenkov,O., Kessler,M. and Aloni,Y. (1989) *J. Biol. Chem.*, **264**, 9953–9959.
48. Wright,S. and Bishop,J.M. (1989) *Proc. Natl Acad. Sci. USA*, **86**, 505–509.
49. Krumm,A., Meulia,T., Brunvand,M. and Groudine,M. (1992) *Genes Dev.*, **6**, 2201–2213.
50. Spencer,C.A. and Kilvert,M.A., (1993) *Mol. Cell. Biol.*, **13**, 1296–1305.
51. Meulia,T., Krumm,A. and Groudine,M. (1993) *Mol. Cell. Biol.*, **13**, 5647–5658.