Real-time footprinting of DNA in the first kinetically significant intermediate in open complex formation by Escherichia coli RNA polymerase

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The architecture of cellular RNA polymerases (RNAPs) dictates that transcription can begin only after promoter DNA bends into a deep channel and the start site nucleotide (+1) binds in the active site located on the channel floor. Formation of this transcriptionally competent “open” complex (RPo) by Escherichia coli RNAP at the λPr promoter is greatly accelerated by DNA upstream of base pair −47 (with respect to +1). Here we report real-time hydroxyl radical (OH) and potassium permanganate (KMnO4) footprints obtained under conditions selected for optimal characterization of the first kinetically significant intermediate (I1) in RPo formation. OH footprints reveal that the DNA backbone from −71 to −81 is engulfed by RNAP in I1, but not in RPo; downstream protection extends to approximately +20 in both complexes. KMnO4 footprinting detects solvent-accessible thymine bases in RPo, but not in I1. We conclude that upstream DNA wraps more extensively on RNAP in I1 than in RPo and that downstream DNA (−11 to +20) occupies the active-site channel in I1, but is not yet melted. Mapping of the footprinting data onto available x-ray structures provides a detailed model of a kinetic intermediate in bacterial transcription initiation and suggests how transient contacts with upstream DNA in I1 might rearrange the channel to favor entry of downstream duplex DNA.

Quantitative kinetic–mechanistic studies find that at least two kinetically significant intermediates, generically designated I1 and I2, precede formation of RPo by E. coli RNAP:

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\begin{array}{cccc}
& \text{rapid equil.} & \text{slow} & \text{I1} \\
\text{RNAP} + P & k_1 & k_2 & k_{2} \\
& k_{3} & k_{2} & k_{3} \\
k_2 & k_2 & k_1 & k_{1} \\
k_3 & k_3 & k_{3} & \text{RPo}
\end{array}
\]

where the slowly interconverting states I1 and I2 are rate-limiting in both the forward and back directions (2, 3). In the mechanism shown in Eq. 1, I2 and RPo are characterized by their resistance to a short challenge with a polyanionic competitor such as heparin, which acts to sequester any free RNAP present during the challenge. In contrast, after a 10 to 20 sec challenge with heparin, I1 complexes, which are in rapid equilibrium with free RNAP and promoter sequences, are eliminated from the population. Given the high degree of conservation of bacterial RNAP and promoter DNA sequences, this mechanism is likely to describe the key steps in initiation in most prokaryotes. Moreover, conservation of many elements of sequence, structure, and/or function between bacterial and eukaryotic polymerase (pol II) subunits and transcription factors supports the inference that these bacterial intermediates may be homologs of initiation intermediates formed by pol II (4, 5).

Recently we (6) and Ross and Gourse (7) found that the presence of DNA upstream of the −35 promoter recognition hexamer greatly accelerates (up to ∼60-fold) the rate-determining isomerization step (conversion of I1 to I2). Strikingly, DNase I footprinting of I1 at the strong bacteriophage promoter λPr reveals that when nonspecific DNA upstream of base pair −47 is present, downstream DNA is protected to around +20, and thus bound in the active-site channel of RNAP. However, when DNA upstream of −47 is deleted, I1 is now less “advanced,” with downstream protection ending at +2 (template strand)/+7 (non-template strand) (6). How does DNA upstream of −47 alter downstream interactions in early transcription intermediates? Surprisingly, protection of DNA in I1 from DNase I cleavage extends upstream only to −52 (template) on “full-length” λPr, similar to what is observed in RPo (6). Because DNase I might displace weak upstream interactions in I1 (8, 9), other techniques are required to probe RNP–DNA contacts in this transient intermediate.

Selvi et al. (10) recently reported an elegant “real-time” footprinting study of RPo formation at the T7A1 promoter using x-ray-generated OH and rapid quench mixing. Unlike DNase I, OH is small and nonperturbing of weak interactions (11). It reacts rapidly and exhibits little if any sequence specificity, making it very useful for probing transient protein–DNA interactions (11). Additionally, the rapid rate of OH reaction with the DNA backbone means that fractional protection is approximately proportional to the 

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pecific transcription initiation by Escherichia coli RNA polymerase (RNAP; core subunit composition αββ′ω + σ30 = holoenzyme) at promoter sequences is determined by recognition of DNA (−10 and −35 hexamers) upstream of the start site (+1) by the specificity subunit σ70. Subsequent to binding, a series of large-scale conformational changes in both RNAP and promoter DNA create the initiation-competent open complex (RPo) (1). During these steps, the multisubunit bacterial RNAP acts as an intricate molecular machine and opens ∼14 bp of the DNA double helix. Defining the cascade of conformational changes that occur during initiation is essential to understand sequence- and factor-dependent regulation of the rate of transcription initiation and has important applications in chemical biology and in antibiotic design. However, the intermediates on this pathway are relatively unstable and short-lived and hence are difficult to trap unambiguously. To date, all structural information about complexes known to be on-pathway intermediates in RPo formation has come from chemical and enzymatic DNA footprinting methods.

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fractional occupancy for a short-lived, rapidly equilibrating intermediate like I₁ (10). By following the appearance of protection of the DNA backbone as a function of time (milliseconds to seconds) after mixing at 37°C, this study (10) circumvented potential issues raised by trapping promoter complexes at low temperature (11) after mixing at 37°C, this study (10) circumvented potential issues raised by trapping promoter complexes at low temperature (11)

In the absence of detailed kinetic–mechanistic information for this promoter, Sclavi et al. (10) used the footprinting data from base pair −60 to +20 to infer both the mechanism (e.g., sequence of intermediates and rate constants of their interconversions) and structure of intermediates preceding RPo. Evidence was obtained for three classes of intermediates (and two to three subclasses of the two early intermediates). The mechanistic analysis revealed that, in general, multiple complexes are populated at T7A1 at each time point in the kinetics. Intriguingly, the series of snapshots of open complex formation at T7A1 suggests that the backbone is protected to −70 (nontemplate)/−82 (template) at early times but to only approximately −60 (template/nontemplate) in RPo (see figure 6 in ref. 10). However, Sclavi et al. (10) did not interpret the far-upstream signal on either strand in their structural–mechanistic analysis.

What is the molecular basis of activation of transcription initiation by upstream DNA? Does the extent of upstream interactions change in RPo formation? Does placement of the downstream DNA (−11 to +20) in the active site channel of RNAP in I₁ require that regions of this DNA be single-stranded (i.e., melted)? Here we use kinetic data from nickotcellulose filter binding experiments (1) to select reaction conditions and times where a relatively homogeneous population of either I₁ or RPo exists at the AP₂ promoter. By avoiding complications that might arise from a mixed population or from off-pathway complexes, these -OH footprints obtained in real time provide unambiguous structural information regarding DNA backbone positions protected from cleavage in I₁ and in RPo. Similarly, these conditions allow us to probe the extent of unstacking of thymine bases in I₁ with KMnO₄. The lack of KMnO₄ reactivity of I₁ demonstrates that downstream DNA enters the channel as a duplex and thus must be opened by RNAP in subsequent steps.

**Results**

**Comparative Analysis of DNA Backbone Interactions in I₁ and in RPo by -OH Footprinting.** How does the presence of upstream DNA extend the protection of downstream DNA from +2 (nontemplate)/+7 (template) to approximately +20 in I₁ (6)? Are direct contacts between RNAP and DNA upstream of base pair −47 responsible? Fig. 1 summarizes the -OH footprints of I₁ and of RPo at AP₂ obtained at 25 sec (>70% I₁) and >2,500 sec (>95% RPo) after mixing, respectively (see Methods). Striking differences exist between these footprints in the upstream boundary, in the degree of protection of the far-upstream region of DNA (approximately −65 to −81) and in the degree of protection of the downstream DNA (approximately −17 to +16). Regions of promoter DNA from −82 (nontemplate)/−81 (template) to approximately +20 are protected in I₁, whereas the upstream boundary of moderate DNA backbone protection in RPo ends at −64 (nontemplate) and −59 (template) [Fig. 1c; see supporting information (SI) Fig. 5 and SI Tables 1 and 2]. Very weak protection from −60 to −68 and from −77 to −80 on the template strand is observed in RPo; this protection pattern is consistent with previously reported footprints of RPo at AP₂ (8). Although the downstream boundaries of I₁ and RPo are similar, the downstream region from −17 to +16 is much more strongly protected in RPo than in I₁ (see Fig. 1a and b). Periodic protection centered at −42 (template) and −54 (template) occurs in both I₁ and RPo, and presumably reflects binding of the C-terminal domains of the α subunit (cCTD) (12–14). As shown in Fig. 1, the amount of protection at these positions does not change when I₁ isomerizes to RPo, indicating that the extent of occupancy of each of these sites by an cCTD is the same in I₁ and RPo at AP₂ (see below).

To ensure that the small fraction of RPo (<30%) present under conditions used to footprint I₁ does not significantly contribute to the observed footprint, heparin, an inert competitor for free RNAP, was added to the reaction 10 sec before the generation of -OH. This challenge eliminates the population of the short-lived I₁ complexes but not the long-lived RPo complexes (3). The -OH footprints obtained after a 10 sec heparin challenge show only weak protection of DNA backbone positions protected from cleavage in each of these sites by an cCTD is the same in I₁ and RPo at AP₂ (see below).
Because these regions are within the most strongly protected parts of the OH footprint of RPo, we conclude that this residual protection results from the small subpopulation of RPo, that forms before the heparin challenge (see Methods and SI Methods). Importantly, however, no protection exists outside of these regions, indicating that this small amount of RPo does not significantly contribute to the upstream protection pattern reported for I1 in Fig. 1c.

Comparison of the Extent of DNA Opening in RPo with I1 Using KMnO4 Footprinting. We and others have previously invoked binding in the active site channel of RNAP to explain protection of downstream DNA (to approximately +20) in I1 (3, 9) and in RPo (10, 12, 15, 16). What is the state of the DNA when initially bound in this cleft? The narrow width of the channel (+15 Å) in the crystal structures of free RNAP holoenzyme has prompted proposals that DNA must open before entering (16, 17). However, the acidic N-terminal domain (NTD) of α′ (α1.1), which binds in the channel in the free RNAP in solution (18, 19), is not observed in the available crystal structures. Placing α1.1 in the channel may open the β/β′ jaws (20), which can “flex” as much as 50 Å apart (21). Such an “open” jaw conformation would permit dsDNA to enter before strand separation occurs.

To address whether entry of downstream DNA into the channel in I1 involves extensive DNA opening, KMnO4 was used to probe for unstacked, solvent-accessible thymine bases. Reactions were performed 20 sec after mixing at 17°C (>70% I1 and <30% RPo) and at >2500 sec (>95% RPo; see Methods). At >2500 sec, thymines at +11, +9, and +8 (template; Fig. 2) and at +2, −3, and −4 (nontemplate; SI Fig. 7) are strongly oxidized by the dose of KMnO4 used here. At 20 sec these thymines also exhibit a small amount of KMnO4 reactivity (Fig. 2 and SI Fig. 7). Because this KMnO4 reactivity remains in a sample pretreated with heparin (SI Table 3), we conclude that this signal originates primarily from the small subpopulation of RPo complexes that form in this short time (see Methods and SI Methods), and not to any significant extent from I1. [These signals from reactive thymines in the subpopulation of RPo are observable because base-paired and stacked thymines in dsDNA (e.g., free DNA) are not KMnO4-reactive.] We conclude from these results that promoter DNA in I1 is largely double-helical. Alternatively, open bases in I1 could be buried in interactions with polymerase and therefore not accessible to KMnO4. We do not favor this interpretation because it would require these presumably favorable interactions to exist only in this transient intermediate and to be disrupted to create the much more stable RPo complex.

Discussion

DNA Opening Occurs in the RNAP Active Site Channel in the Conversion of I1 to RPo. Real-time OH and KMnO4 footprinting obviates the need for temperature downshifts or other shifts in solution conditions to stabilize or trap the first intermediate at the AP1 promoter. At the short times after mixing examined here, I1 complexes are >70% and RPo complexes are <30% of promoter DNA. At this point time, downstream protection extends to +20, which can be accounted for only by placing DNA downstream from −11 into the active site channel of RNAP (Fig. 3) (3, 6, 15, 16). Together, the OH and KMnO4 data (Figs. 1 and 2) and DNease I and KMnO4 footprinting of I1 at 0°C (9) provide compelling evidence that opening of the DNA from −11 to +2 occurs only after it binds in the active site channel in I1. Cross-linking experiments on wild-type RNAP

Fig. 2. Potassium permanganate (KMnO4) footprints of RNAP–promoter complexes. Shown are representative results from KMnO4 probing of complexes obtained early (20 sec, >70% I1, <30% RPo) and late (>2500 sec, <5% I1, >95% RPo) in the time course of open complex formation at 17°C. A sequencing (40) and an uncut DNA control were performed. Positions of KMnO4-reactive thymines in RPo on the template strand are labeled. KMnO4 reactivity observed in the 20 sec lanes in the presence and absence of heparin is consistent with the small population of RPo complexes formed at this time after mixing. SI Table 3 quantifies the intensity of bands in this and two other independent experiments. See SI Fig. 7 and SI Table 3 for KMnO4 probing of the nontemplate strand.

Fig. 3. Model of the intermediate complex I1 preceding formation of the transcriptionally competent RPo. The conformation of RNAP (beige) is based on the crystal structure of the homologous T. thermophilus enzyme (Protein Data Bank ID code 1IW7 (16); T. thermophilus β′ residues 138–452, absent in E. coli (28), are not shown). The modeled position of promoter DNA in I1 is based on DNease I (6), OH and KMnO4 footprinting and relevant crystal structures. The two views of I1 demonstrate the agreement between the model and the experimentally determined strong and moderate protection (red and purple, respectively) of the DNA backbone (gray) from OH cleavage (see Fig. 1). Strong protection seen at −12 to −19 likely involves the nonconserved region of E. coli α1* not present in α′ of T. thermophilus. Domains in E. coli subunits β and β′ (represented as blue tear-drops, missing in T. thermophilus) are positioned at the sites of their insertion in the T. thermophilus sequence; these likely protect DNA downstream of +10 from OH cleavage. The upstream surface groove formed by β′ and the NTD of α, and the mobile downstream jaw (E. coli β′ residues 913–1262) and upstream DNA clamp (E. coli β′ residues 808–912), are highlighted in cartoon representations in dark blue, magenta, and gold, respectively. This figure was created using PyMol (41).
and on a mutant RNP lacking the downstream lobe of β (E. coli βΔ186–433) have led to a similar “active” melting proposal (22).

Alternative models of DNA opening during initiation invoke the passive capture of transiently open regions in the –10 hexamer by RNP (16, 17, 23). These proposals are largely based on the conformational state of free RNP captured in the crystal. Although atomic resolution structural data are necessary to establish molecular structure–function relationships, not all aspects of the current set of crystal structures need be relevant for hypotheses regarding RPo formation in solution. In particular, high-salt crystallization conditions (15) and/or crystal packing interactions may disfavor binding of r1.1 in the channel, and this or other aspects of crystallization may induce the “narrow” conformation of flexible/mobile elements of the enzyme. The results reported here and the publication of a crystal structure of a dsRNA ligand bound in the structurally homologous cleft of the yeast RNP (24) demonstrate that double-stranded nucleic acids can indeed access the channel, ruling out a general requirement for DNA opening to precede entry.

Recently, a subassembly of RNP containing only regions 2 and 3 of α(2) and the NTD of β′ was shown to form a KMnO4-reactive complex with an A/T-rich promoter sequence (23). Does formation of this complex mirror the steps in RPo formation by RNP? Unlike opening by the wild-type RNP holoenzyme at APK, formation of this open or distorted complex requires a highly negatively supercoiled DNA template. At this superhelix density, both experiments and DNA supercoiling thermodynamics indicate that the equilibrium fraction of open bases is significant at 37°C (1–5%, primarily A/T-rich regions). Because region 2 of α70 (α2) bound to the NTD of β′ selectively recognizes and binds the nontemplate strand of the –10 recognition hexamer (TATAAT) (25), possibly α2R′ recognizes and stabilizes this A/T-rich region as it transiently opens on negatively supercoiled DNA (but not on linear fragments). Kinetic data for this minimal system are required to define the mechanism of DNA opening at this supercoiled promoter and how it relates to opening by the wild-type RNP machinery.

Structural Model of I1. To explain why moderate to strong far-upstream protection is observed only in I1 and to explore how upstream DNA facilitates placement of downstream dsDNA in the active site channel, we modeled I1 using footprinting data (Figs. 1 and 2), patterns of DNase I hypersensitive cleavage sites (6), and relevant crystal structures (see Methods and SI Methods). In this model (Fig. 3), protection to position –81 (template)/–82 (non-template) in I1 is achieved by wrapping upstream DNA around the outside of RNP. Protection to +20 arises from a sharp DNA bend (~90°) at position –11/–12 that places downstream duplex DNA high in the channel (~50 Å above the active site) (3). Whereas the channel protects downstream DNA from OH cleavage in I1, far greater protection is observed in RPo (Fig. 1 a and b). This difference presumably reflects formation of extensive contacts between RNP and the DNA backbone that stabilize the open bubble (~11 to +2) in RPo.

A critical feature of the model (Fig. 3) is that the trajectory of upstream DNA in I1 is set by the interaction of the ~35 hexamer with α region 4 (α4), the interaction of the proximal αCTD with the region centered at base pair ~62, and the interaction of the distal αCTD with the region centered at base pair ~54 (26). Together these interactions are predicted to bend promoter DNA from positions ~30 to ~57 by ~90–100°, directing the flanking upstream DNA along a path along the outside of β′ that roughly parallels the active-site channel (Fig. 3).

The model predicts that DNA from positions ~60 to ~65 lies next to the C terminus of β′ and the ω subunit; the following helical turn of upstream DNA (from ~71 to ~81) falls in a surface groove formed by the interface between β′ and the NTD of the β′-associated α subunit. This surface groove contains pairs of conserved cationic (β′ R576, R610, K615; α H1128) and anionic (β′ D571 and E616) residues (see SI Methods) positioned to be able to form intramolecular salt bridges in the absence of DNA, and hence modulate the affinity of RNP for the upstream region of promoter DNA (27). Although the OH backbone protection data do not precisely define the phasing of the far-upstream DNA with respect to RNP (see SI Methods), they unambiguously demonstrate that the “backside” of RNP interacts extensively with this DNA in I1, but not in RPo.

Proposal: Upstream DNA Wrapping Triggers Conformational Changes in the RNP Active Site Channel. Results presented here and by Scavvi et al. (10) indicate that the extent of upstream DNA protection from OH cleavage is time-dependent. At early times after mixing, E. coli RNP protects the DNA backbone at T7A1 (10) and APK to approximately ~80 but to only approximately ~60 in RPo. At APK, upstream protection from ~71 to ~81 spans the minor and the major groove for approximately one turn. This pattern is difficult to explain by transient interactions with the αCTDs [as deduced from cross-linking experiments on RPo formed at the lacUV5 promoter (12)] but is completely consistent with wrapping of upstream DNA on RNP, as modeled here (Fig. 3). Moreover, the protection from ~71 to ~81 indicates that the DNA backbone is practically engulfed on all sides, which cannot be accounted for by binding in the surface groove alone.

What domains of RNP are responsible for the protection of far-upstream DNA in I1? One possible candidate is the conserved domain of β′ (E. coli residues 808–912; Thermus thermophilus residues 1106–1218) (Fig. 3), which lies directly above the surface groove in the T. thermophilus RNP crystal structure (16). To explain the upstream protection data, we propose that this domain moves to contact this region of DNA when it wraps on RNP in I1 (Fig. 4). This region, which we propose calling the “upstream DNA clamp,” is likely flexible in solution, as suggested by the prediction that it is partially disordered in free RNP (1). Impor-
tantly, the upstream DNA clamp (magenta in Fig. 4) is N-terminal to and contacts another conserved domain called the “downstream jaw” (E. coli residues 913–1262; T. thermophilus residues 1219–1377) (gold in Fig. 4) or “downstream mobile clamp,” also predicted to be partially disordered in solution (1). In E. coli, the downstream jaw includes the so-called “trigger loop” and a flexibly tethered, independently folded ~200-residue sequence insertion [E. coli residues 940–1139 (28), absent in T. thermophilus], shown as a blue shape in Fig. 4. The “plasticity” of the downstream jaw and the upstream DNA clamp is also suggested by the significant and shape in Fig. 4. The “plasticity” of the downstream jaw and the residues 940–1139 (28), absent in upstream DNA contacts the CTD of explanation for these observations. In this model, of activators (32) significantly increases (activates) or decreases sically bent DNA sequences (e.g., ref. 31) or by moving binding sites degree and/or phasing of upstream bending by introducing intrin-

dent DNA sequences (e.g., ref. 31) or by moving binding sites disfavor the upstream wrap, either directly or indirectly. Unwrap-
ing of upstream DNA is predicted to release the downstream jaw, making it available to fold on downstream DNA in RP, (1). These proposals for a series of coordinated motions in the polymerase machinery involved in DNA opening are currently being tested by kinetic studies on RNAP and promoter variants.

Methods

Buffers. RNAP storage buffer (SB) is 50% glycerol, 10 mM Tris (pH 7.5 at 4°C), 100 mM NaCl2, 0.1 mM DTT, and 0.1 mM EDTA. DNase I storage buffer is 50% (vol/vol) glycerol, 10 mM Tris-HCl, and 5 mM MgCl2. The binding buffer (BB) for hydroxyl radical (·OH) and KMnO4 footprinting experiments is 12.5 mM Tris (adjusted to pH 8 at the temperature of the experiment), 3 mM MgCl2, 60 mM KCl, 0.6 M glycine betaine, and 100 μg/ml BSA. ·OH were generated by using the Fenton reaction [1.73 mM Fe(II), 2.3 mM EDTA, 0.98 mM Na ascorbate, and 0.08% H2O2]. ·OH stop buffer is 9.5 mM thiourea and 1.7 mM EDTA. Solvent-exposed thymines were probed with 4 mM KMnO4; strands at oxidized thymines were cleaved by adding 10% piperidine. KMnO4 stop buffer is 1 M 2-mercaptoethanol and 2.7 M ammonium acetate. TBE buffer and urea leading buffer have been described (8).

RNAP, E. coli K12 RNAP σ70 holoenzyme containing the ω subunit was purified as previously described (35, 36). The promoter-binding activity of RNAP was assayed at the time of the footprinting experiments as described in (37) and was always 60–95%. All RNAP concentrations reported are for the active holoenzyme.

RP, Promoter DNA. Full-length RP promoter DNA fragments were isolated and labeled as described (6). DNA fragments end at −110 on nontemplate-labeled DNA and at −102 on template-labeled DNA; RP DNA sequences extend from −60 to +20. This design eliminates RP from the region upstream of −60 with nonspecific plasmid DNA sequences.

Determination of Fractions of Promoter DNA in I1 and RP Complexes as a Function of Time After Mixing. At 17°C, where the equilibrium constant K1 is a maximum and conversion of I1 to I2 is relatively slow, we previously found that the fraction of promoter DNA in I1 complexes (0.0%) at 15 sec after mixing RP, promoter with 70 nM (excess) RNAP was 0.55 (6). To increase the fraction of I1 in the
time interval (20–35 sec) of the footprinting experiments, we reduced the KCl and MgCl₂ concentrations to those listed in Buffers and added 0.6 M glycine betaine. Based on observed dependences of $K_1$ and $K_2$ on these solute concentration variables (1) we calculate $\theta^c > 0.7$ in the absence of heparin for the time interval and under the conditions of experiments used to footprint I1. See SI Methods.

**-OH Footprinting.** A preliminary attempt to characterize I1 by -OH footprinting was performed at 0°C at equilibrium (38) where the only complex predicted to be populated is I1 (9). Because the signal:noise ratio in these 0°C experiments was marginal, and to avoid the possibility of populating off-pathway complexes at 0°C (39), we identified solution conditions where I1 is >70% of promoter DNA (see above) at early times in the time course of RPo (for specific details of analysis and alignment criteria). RPo footprints reported here are consistent with footprints reported in ref. 7838/H20841/H18528/H9258.

For the conditions of experiments used to footprint I1. See SI Methods for details of analysis and alignment criteria. RPo footprinting was performed at 0°C at equilibrium (38) where the time interval (20–35 sec) of the footprinting experiments, we identified solution conditions where I1 is >70% of promoter DNA (see above) at early times in the time course of RPo. Footprints reported here are consistent with footprints reported in ref. 7838/H20841/H18528/H9258.

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** = protection compressed
++ = strong protection
+ = enhancement
** = protection enhanced
++ = strong protection enhanced

Table 1: OH Footprints of \( I \) and \( Rp \). Template Strand
<p>| Table 2. OH Footprints of I ND = end of gel ** = protection compressed Empty = no protection ++ = strong protection |</p>
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* = strong protection + = weak protection Empty = no protection ^ = enhancement ** = protection compressed

1 = RP and RP 2 = i3 and RP 3 = i5 4 = enhanced
Heparin

RNAP

>2500 s

20 s

Free DNA

Uncut DNA

A+G sequence

Non-template Strand

Davis 17.AI

- +

- +

+ +

+ +

Heparin RNAP

-4

+3

+3

+2

-3