Inorganic phosphate, arsenate, and vanadate enhance exonuclease transcript cleavage by RNA polymerase by 2000-fold

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Inorganic Pn, the simplest soluble form of phosphorus, plays central roles in cellular energetics and metabolism, as well as in biological structure and regulation (for review see ref. 1). It is believed that Pn participated in the principal processes that led to the appearance of life on Earth (1–4). Disruption of phosphate homeostasis is associated with human disorders (5). The high intracellular concentration of Pn, 50–500 mM (5, 6), underscores its biological significance. In the cell, phosphate forms anhydrides (e.g., pyrophosphate, acylphosphates, and nucleoside-5′-di- and triphosphates) and esters with the hydroxyl groups of a variety of cellular metabolites (e.g., sugars, lipids, and proteins) as well as DNA and RNA diesters.

RNA, synthesized by RNA polymerase (RNAP) from a DNA template, encodes the genetic information that directs cell functioning. Although RNA synthesis is the principal activity of RNAP, the enzyme can also degrade RNA in the reverse reaction of pyrophosphorolysis or by hydrolytic cleavage. In the present study we report that Pn strongly catalyzes RNA hydrolysis by RNAP, suggesting an additional important role for this anion in cell biology.

RNA synthesis is carried out through ordered polymerization of NTPs by RNAP using one DNA strand as template. During NTP polymerization by transcription elongation complex (TEC; shown in Fig. L4) the RNA product transiently binds to template DNA strand, forming an RNA:DNA hybrid 9–10 bp long (7). Advancement of TEC along the template is frequently delayed by pausing. Some pauses are due to backtracking, that is, reversible back-translocation of transcribing RNAP (7, 8). When RNAP is backtracked the RNA 3′ segment is disengaged from the RNA:DNA hybrid and extruded into the secondary channel (Fig. L4), thus occluding the active center and preventing transcription elongation. Backtracking is promoted by occasional incorporation of noncognate RNA residues, DNA damage, and roadblocks conferred by DNA binding proteins. Bacterial factors GreA and GreB (9–11) and eukaryotic factor TFIIIS (12) rescue backtracked complexes by stimulating an RNAP exonuclease activity that removes the disengaged RNA segment by hydrolytic cleavage. RNAP is also able to eliminate the 3′ terminal RNA residue in pretranslocated TEC by exonucleolytic cleavage (13). Cleavage is stimulated by noncomplementary nucleotides (13). Unlike endonucleolytic cleavage, the physiological role of exonucleolytic RNA degradation is not readily apparent and remains to be explored.

Both RNA synthesis and degradation by RNAP occur in the same active center (14), situated at the cross-section of the RNAP main channel that secludes the RNA–DNA hybrid and the secondary channel (Fig. L4). The latter supplies the NTP substrates for transcript synthesis and also accommodates the disengaged RNA segment in RNAP backtracked complexes. The channels are divided by the bridge helix, a long β′ subunit α-helix.

All RNAP reactions proceed through an S2,2 mechanism (13–15), which involves two Mg2+ ions (Fig. 1) chelated by an invariant aspartate triad of β′ subunit residues (D460, D462, and D464). One Mg2+ ion (Mg-I) saturates the active center, whereas the other (Mg-II) binds transiently and must be recruited through additional coordination for each round of catalysis (13). In the nucleotidyl transfer reaction Mg-II is stabilized by chelation with phosphate groups of the NTP substrate. In transcript cleavage it is coordinated with carboxylate residues of Gre or TFIIIS transcription cofactors, which approach the active center through the secondary channel, or by the triphosphate residue of noncognate NTP substrate bound at the E-site of the active center (13). In RNA synthesis Mg-I activates the 3′-hydroxyl of the terminal RNA phosphate | vanadate | arsenate | transcript cleavage | RNA polymerase

Significance

To understand how RNA polymerase (RNAP) can synthesize or degrade RNA, and how these processes are regulated, it is necessary to elucidate the catalytic mechanisms of these reactions. Catalysis is sensitive to external factors that modulate the rate of polymerization or hydrolytic attack on the RNA. Here we report that a ubiquitous cellular metabolite, inorganic phosphate, stimulates RNAP exonuclease transcript cleavage activity nearly 2000-fold. This finding represents a stunning example of how simple cellular molecules can reprogram an enzyme’s active center by providing functional groups and suggests an additional important role for inorganic phosphate in cell biology.

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residue and the α-phosphate of an NTP substrate for nucleophilic attack, whereas Mg-II promotes release of pyrophosphate (Fig. 1B). The two Mg$^{2+}$ ions switch roles for RNA hydrolytic cleavage. Thus, Mg-II activates the attacking water and Mg-I aids in release of the leaving group (Fig. 1C). In addition to the above-mentioned aspartate triad, two neighboring acidic residues, βE813 and βD814, as well as a basic cluster (βK1073, βR678, βR1106, and β′R731), are engaged in both substrate binding and catalysis (11, 13, 16).

RNAP catalytic activity is regulated by small compounds, as exemplified by the inhibitor tagetitoxin (17) and noncognate NTPs (13), which bind to the active center and change Mg-II coordination. The analogous action mechanism of P$_i$ and its structural mimics on

Fig. 1. Location and models of action for the RNAP active center. (A) The active center performing P$_i$-stimulated RNA hydrolysis. Catalytic Mg$^{2+}$ ions are indicated. (B) Nucleotidyl transfer reaction. The dotted lines represent coordination bonds. Arrows indicate the migration of electron density. (C and D) Models for P$_i$ action in hydrolytic RNA cleavage and RNA phosphorolysis, respectively. (E and F) Structural models for C and D, respectively.
transcription reported in this study represents a stunning example of how simple cellular molecules can reprogram the RNAP active center by providing additional functional groups.

Results

P<sub>i</sub> Stimulates Exonuclease Transcript Cleavage in TEC. In experiments exposing TEC to different factors we noticed nascent transcript cleavage that was dependent upon the presence of phosphate in the reaction mixture. Such cleavage had not been previously reported, and we therefore decided to investigate this reaction in detail. TEC10C (18) was constructed bearing a 3′-labeled C (Fig. 2a) and incubated at ambient temperature. As seen from ion-exchange (Fig. 2b) and silicagel TLC analyses (Fig. 2c), the major phosphate-induced RNA degradation product comigrated with [α-<sup>32</sup>P]CMP. We thus identify the product as [α-<sup>32</sup>P]CMP originating from 3′exonuclease transcript cleavage. Exonucleolytic cleavage was also observed in TEC12C assembled on a natural T7 A1 promoter template using an incomplete set of NTP substrates (Fig. S1). A radioactive RNA degradation product comigrated with CDP, suggesting that this product originates from RNA phosphorylisis by nucleophilic attack of phosphate on the phosphodiestar bond. P<sub>i</sub> failed to induce exonuclease cleavage of a terminal mismatched RNA residue (Fig. S2).

We next tested compounds structurally related to phosphate: arsenate and vanadate (Fig. 2d). As seen from Fig. 2d, vanadate, and to a lesser extent arsenate, stimulated hydrolytic cleavage, but the close structural analog, sulfate, did not. The latter can be explained by a reduced number of ionized groups and their more acidic character in sulfate anion. The fold enhancement under these conditions (10 mM reactants [HXO<sub>2</sub>]<sup>−</sup> and 10 mM Mg<sup>2+</sup>) was about 800, 2,000, and 170 for phosphate, vanadate, and arsenate, respectively. To further characterize their RNAP exonuclease-enhancing activities TEC10C was incubated with various concentrations of the above anions. The dependence of the reaction rate on anion concentration (Fig. 3a) revealed saturation curves with apparent K<sub>A</sub> values equal to 5 mM, 0.4 mM, and 20 mM for phosphate, vanadate, and arsenate, respectively. Transcript cleavage experiments were performed at 0°C due to high rate of the reaction at 37°C (60- to 80-fold higher than at 0°C).

P<sub>i</sub> and Its Mimics Increase Retention of the Catalytic Mg-II Required for Transcript Cleavage. The rate of the exonuclease reaction is enhanced by factors that increase retention of the catalytic Mg-II ion that is required for hydrolytic cleavage, and which is weakly bound in the active center (13). We thus determined the effect of Mg<sup>2+</sup>, arsenate, and vanadate on RNA cleavage rates in the presence and absence of P<sub>i</sub> and its structural mimics. As shown in Fig. 3b, the anions tested dramatically reduced the Mg<sup>2+</sup> dependence of the reaction, suggesting that they enhance Mg<sup>2+</sup> binding. Indeed, the calculated K<sub>1/2</sub> values for Mg<sup>2+</sup> were ~10 mM, 5 mM, and 20 mM in the presence phosphate, vanadate, and arsenate, respectively, and >100 mM for the nonstimulated reaction.

From these results we calculate the maximal reaction enhancement factor for all anions tested (at saturating concentration of both the reactant anions and Mg<sup>2+</sup>) to be about 2,000. Remarkably, the magnitude of this enhancement approaches that for prokaryotic Gre (11) and eukaryotic TFIIS (19) factors in the transcript endonucleolytic cleavage reaction (3,000- to 4,000-fold).

P<sub>i</sub> Does Not Stimulate Endonucleolytic Transcript Cleavage or Endonucleolytic Phosphorolysis. Only pretranslocated TEC can support exonuclease activity. Endonuclease activity is observed in backtracked TEC. We asked whether P<sub>i</sub> stimulates endonucleolytic cleavage by comparing P<sub>i</sub> to GreA, a factor known to promote this reaction. For these experiments we have generated the backtracking-prone TEC11A. Upon incubation in transcription buffer the RNAP intrinsic endonuclease reaction released 3′terminal dinucleotide pCPA from RNA11A. However, P<sub>i</sub> had little, if any, effect on this reaction (Table 1 and Fig. S3, lane 5). Conversely, in contrast to P<sub>i</sub>, GreA only marginally stimulated exonuclease cleavage of TEC10 (Table 1 and Fig. S3, lane 6).

Effect of pH on P<sub>i</sub>-Stimulated Exonuclease. Deprotonation of the attacking water at high pH stimulates nucleic acid activity; this stimulation decelerates as the pH reaches and exceeds the pK<sub>a</sub> value of bound water. However, the effect of pH on hydrolytic RNA cleavage is complex, since Mg-II binding is affected by pH (13). Indeed, retention of Mg-II can be enhanced through additional coordination by the neighboring carboxylate residue of the active center, D814 and, possibly, E813 (Fig. 1c). At physiological pH, however, βD814 is salt-bridged with the closely positioned βR1106 and is not available for coordination (Fig. 1c). Deprotonation of βR1106 can explain the abrupt upturn in the exonuclease pH-dependence curve at the pH range 9–10 by releasing the carboxylate side chain(s) for additional Mg-II coordination (13). The above two effects of pH on hydrolytic cleavage can be dissected by elimination of the salt bridge through the R1106A substitution (discussed below), which makes Mg-II binding pH-independent. This allows calculation
of the attacking water (9.0) by measuring the reaction rate at various pH with mutant TEC (13).

Fig. 4 shows the effect of pH on P nonsuppressed RNA cleavage. Raising the pH from 6 to 7 only marginally stimulated cleavage, and there was no further increase above pH 7. An apparent decrease in the reaction rate within the pH interval 7–9 was due to competing phosphorolytic cleavage. The cleavage rate was about half of the plateau value at about pH 6, suggesting that this is the pKₐ for the attacking water. This is in striking contrast to nonstimulated exonuclease cleavage, where the apparent pKₐ of the attacking water is about 9.0 (13). Therefore, in addition to enhancing Mg-II binding, P introduces a working model to explain P nonsuppressed transcript degradation (Fig. 1 E and F). The atomic coordinates for the modeling were those of yeast backtracked TEC (Protein Data Bank ID code 3G70) (20). We chose this structure over an apparently more relevant X-ray structure (116V) of pretranslocated TEC (the exonuclease substrate) because the latter depicts a catalytically inactive state in which the contacts of the 3′ terminal RNA residue in the active center are not established (21). To generate the structure of a functional pretranslocated TEC we deleted the terminal scissile phosphoester group as the oxygen atom of the Mg-II coordinated phosphate in phosphorolysis (Fig. 1 C and D, respectively). Therefore, the hydrolytic coordination mode for the phosphate is achieved by “phosphorolytic” state isomerization, which frees an Mg-II valence for binding the attacking water (Fig. 1 C–F). Both these activities of P are mechanistically and functionally related to those observed for pyrophosphate: pyrophosphorolysis and pyrophosphate-induced exonuclease RNA hydrolysis characterized in our previous study (13).

Modeling of the Phosphate–Induced Reactions. We have constructed a working model to explain P nonsuppressed transcript degradation (Fig. 1 E and F). The atomic coordinates for the modeling were those of yeast backtracked TEC (Protein Data Bank ID code 3G70) (20). We chose this structure over an apparently more relevant X-ray structure (116V) of pretranslocated TEC (the exonuclease substrate) because the latter depicts a catalytically inactive state in which the contacts of the 3′ terminal RNA residue in the active center are not established (21). To generate the structure of a functional pretranslocated TEC we deleted the terminal scissile phosphoester group as the oxygen atom of the Mg-II coordinated phosphate in phosphorolysis (Fig. 1 C and D, respectively). Therefore, the hydrolytic coordination mode for the phosphate is achieved by “phosphorolytic” state isomerization, which frees an Mg-II valence for binding the attacking water (Fig. 1 C–F). Both these activities of P are mechanistically and functionally related to those observed for pyrophosphate: pyrophosphorolysis and pyrophosphate-induced exonuclease RNA hydrolysis characterized in our previous study (13).

Table 1. Rate constants for transcript cleavage reactions with WT and mutant RNA at 0°C in TEC10 and TEC11 at 10 mM MgCl₂, in the absence or presence of 2.5 mM P, or 0.1 μM GreA

| Reaction | k, h⁻¹/enhancement factor |
|----------|---------------------------|
| Exo, TEC10, WT | 0.0047 |
| Exo, TEC10, WT, P₁ | 1.2 |
| Exo, TEC10, WT, P₁, enhancement | 255 |
| Exo, TEC10, βR1106A | 0.332 |
| Exo, TEC10, βR1106A, P₁ | 1.0 |
| Exo, TEC10, βR1106A, P₁, enhancement | 3.3 |
| Exo, TEC10, βE813, D814/AA | 0.0028 |
| Exo, TEC10, βE813, D814/AA, P₁ | 0.014 |
| Exo, TEC10, βE813, D814/AA, P₁, enhancement | 5.0 |
| Exo, TEC10, GreA | 0.007 |
| Exo, TEC10, GreA, enhancement | 1.4 |
| Endo, TEC11 | 0.14 |
| Endo, TEC11, GreA | 4.1 |
| Endo, TEC11, GreA, enhancement | 2.9 |
| Endo, TEC11, P₁ | 0.18 |
| Endo, TEC11, P₁, enhancement | 1.3 |

Table 1. Rate constants for transcript cleavage reactions with WT and mutant RNA at 0°C in TEC10 and TEC11 at 10 mM MgCl₂, in the absence or presence of 2.5 mM P, or 0.1 μM GreA

Nuclease reaction in WT or mutant TEC k, h⁻¹/enhancement factor

The data represent the average of three independent measurements.
used the following structural constraints for modeling: (i) Bound P$_i$ must be within coordination bond distance to Mg-II of the active center, (ii) one coordination valence of the chelated Mg-II must remain available for the attacking water, and (iii) the hydrolytic attack must proceed codirectionally with the displacement of the leaving group (Fig. 1C). The arrangement of the catalytic Mg-II ion relative to the reactant groups, precipitated by the $S_2$ mechanism, was as in our previous modeling (13). These constraints in the context of the X-ray structure placed P$_i$ unequivocally in the active center.

In the resulting model (Fig. 1 C and E) P$_i$ fits into a small cleft between the βR1106 and βR678 side chains and Mg-II. This forms a sandwich-like structure, in which the Arg residues flank the P$_i$ ligand. P$_i$ stacks to the βR1106 side chain in this cavity so that three hydroxyl groups of the P$_i$ are within salt-bridging distance to three guanidinium-group nitrogens, among which the positive charge of the group is distributed. Two of these P$_i$ hydroxyl groups simultaneously salt-bridge with βR678. This orientation poses the fourth P$_i$ oxygen within hydrogen-bonding distance to the attacking water, thus explaining the observed enhanced dissociation constant for the reactive water (discussed above). The interaction network between P$_i$ and the Arg residues poses two P$_i$ hydroxyl groups to coordinate Mg-II, which explains observed enhanced metal ion retention in the presence of P$_i$. Moreover, the remaining coordination valence of Mg-II can be used to chelate D814 (Fig. 1E), which is consistent with the involvement of D814 in Mg-II coordination under conditions where the salt bridge between this residue and R1106 is eliminated (discussed above). Such P$_i$ docking also accounts for the effect of the active center mutations on RNA cleavage. Thus, the βR1106A substitution reduces the P$_i$ binding by eliminating the bulk of P$_i$ salt-bridging interactions and by widening the cavity in which the ligand binds. The βD813, D814/AA mutation can affect P$_i$ binding by changing the orientation of the βR1106 side chain involved in interactions with P$_i$. In fact, the βD814 residue is in immediate contact with βR1106 (Fig. 1E), which is reinforced by salt-bridging between these two residues. Therefore, substitution of Asp by a smaller Ala side chain simultaneously creates a gap in the active center and eliminates the salt bridge.

For modeling RNA phosphorolytic cleavage (Fig. 1 D and F) we postulate that the P$_i$ orientation in this reaction is the same as that for the attacking phosphate part of a pyrophosphate molecule in the related pyrophosphorolytic reaction. We suggest that these alternate phosphate coordination modes at Mg-II exist in equilibrium. Transition from a hydrolytic to a phosphorolytic complex is achieved by rotation of P$_i$ around its two Mg-II coordination bonds. This is accompanied by displacement of the coordinated attacking water by a P$_i$ oxygen, which was originally hydrogen-bound to the water (Fig. 1E). As suggested by molecular modeling, this transition would distance P$_i$ from βR1106 and βR678 (Fig. 1F), thus destabilizing the complex. The equilibrium between the two P$_i$ coordination modes is therefore expected to favor the more stable hydrolytic orientation. This is consistent with significantly higher efficiency of P$_i$-induced RNA hydrolytic cleavage (ca.10-fold) compared with P$_i$ phosphorylation (Figs. 2 and 4).

**Discussion**

The most striking finding reported here is the dramatic stimulation by orthophosphate and its structural mimics, arsenate and vanadate, of hydrolytic exonuclease RNA cleavage by RNAP in a TEC. We demonstrate that these compounds act by recruiting the Mg-II ion of the active center, which, unlike Mg-I, is delivered by substrates or cofactors for each act of catalysis. Stimulation of exonuclease by E site-bound noncognate NTP substrates likewise results from recruitment of Mg$^{2+}$ (13). Thus, our work suggests that RNAP catalysis is regulated in general by modulation of the coordination of active center Mg$^{2+}$ ion. The magnitude of stimulation by P$_i$ and its structural mimics is remarkable—comparable to that observed for specialized transcription cleavage protein factors Gre and TFIIIS. Despite the mechanistic analogy, P$_i$ and Gre enhance nucleic acid cleavage in opposite modes. The former stimulates exonuclease and the latter endonuclease cleavage reactions, as explained in our previous studies (16). Recall that the substrate for exonuclease is pretranslocated TEC, whereas endonuclease acts on backtracked TEC. A follows from molecular modeling, the disengaged 3′ RNA segment in a backtracked complex blocks the P$_i$ binding site. In contrast, Gre-mediated catalysis in backtracked TEC relies on interactions of the first 3′ disengaged RNA residue in the active center. These interactions set the active center for catalysis by attracting Mg-II in intrinsic and Gre-assisted reactions (16). We also observe some small enhancing action of GreA factor on exonuclease RNA cleavage. This is consistent with the effect of Gre and TFIIIS factor on exonuclease hydrolysis observed for yeast and *Escherichia coli* enzymes (22, 23).

The biological role of the two P$_i$-mediated processes characterized in this study is not known. Nevertheless, the high intracellular P$_i$ concentration (50–500 mM) and reasonable Km (5 mM) for P$_i$ in RNA phosphorylation and hydrolytic cleavage suggest that this metabolite can efficiently execute both reactions in vivo. We demonstrated that P$_i$ does not support exonuclease cleavage of 3′ misincorporated RNA residue, which excludes the role of P$_i$ in proofreading. Also, P$_i$ does not stimulate endophosphorylase RNA cleavage in backtracked TEC. However, a related pyrophosphate (PP$^i$)-induced cleavage was observed in yeast Pol-II backtracked TEC (24). This implies that P$_i$ can act as a PP$^i$ analog in eukaryotes, thus assisting proofreading. Since the intracellular P$_i$ concentration is about 1,000-fold greater than that of PP$^i$, PP$^i$-promoted nucleolytic cleavage might have a physiological role in eukaryotes by complementing TFIIIS factor in hydrolytic RNA cleavage. In addition, P$_i$ might act in conjunction with transcription termination factors that lock TEC in the pretranslocated state (18). By stimulating exonuclease RNA cleavage in such complexes P$_i$ would shorten the RNA-DNA hybrid, thereby facilitating TEC decay and transcript release. Finally, active center-bound orthophosphate can suppress RNAP backtracking and transcriptional arrest by stabilizing the pretranslocated TEC state through phosphodiester coordination (Fig. 1C).

Taking into account the average intracellular concentrations of P$_i$ and NTP, as well as their Km, in the reactions of RNA degradation and extension by RNAP, it is expected that P$_i$ can affect the rate of transcription by competing with NTP substrate binding at the active center. However, NTP and other chelating molecules in the cell can scavenge Mg$^{2+}$, whose concentration in unbound form is very low (25), thus reducing the concentration of P$_i$–Mg$^{2+}$ complex, which is likely the active center-binding form of P$_i$. This should favor RNA extension, since NTPs bind Mg$^{2+}$ stronger than P$_i$. Also, nucleotidyl transfer reaction proceeds with significantly higher maximal rate than RNA hydrolysis. Nevertheless, a regulatory role of P$_i$ on transcription via competition with NTP substrates cannot
be excluded. This mechanism could operate in starved cells, when P\textsubscript{i} content in the cell is high and the NTP concentration is low (26).

Vanadate and arsenate have attracted considerable attention. Thus, vanadate was used as a mimic for the transition phosphate state in various enzymatic reactions (27), while arsenate has been validated as a phosphate analog in some enzymatic systems (28, 29). In this study we report a previously unknown activity of these compounds, that is, their strong stimulation of RNA degradation in transcription complexes. The ability of arsenate and vanadate to greatly accelerate hydrolytic RNA cleavage by RNAP is a striking example of their potential biological activity.

**Materials and Methods**

All chemicals were from Sigma-Aldrich. His\textsubscript{a}-tagged RNAP was purified from the RL721 E. coli strain. Mutant RNAPs were obtained as previously described (13). Ribonucleoside-5′-triphosphates were from Pharmacia. [\textsuperscript{\textalpha-32}P]CMP was obtained from [\textalpha-\textsuperscript{32}P]CTP, 3,000 Ci/mmol (MP Biomedicals) by treatment with snake venom phosphodiesterase (New England BioLabs). Oligonucleotides were from Oligos. Radioactive products of RNA degradation reaction by RNAP were resolved by electrophoresis, or by TLC on PEI cellulose or silica gel plates (Sigma-Aldrich) and quantified by phosphomagerography using a Molecular Dynamics device (GE Healthcare). Molecular modeling was performed using WebLab ViewerLight 4.0 (Molecular Simulations Inc.). TEC9 was assembled essentially as previously described (18). Detailed protocol is provided in SI Materials and Methods.

**Effect of Phosphate, Vanadate, Arsentate, and Sulfate on Exonuclease Transcript Cleavage.** Reaction mixtures (8 μL) containing labeled TEC10C in 20 mM Tris-HCl pH 8.0, and 0.1 M NaCl were supplemented with 1 μL of 0.1 M sodium phosphate, vanadate, arsenate, or sulfite with pH adjusted to 8.0. The reaction was started by addition of 1 μL of 0.1 M MgCl\textsubscript{2} and carried out at 0 °C. After 20 min incubation the reaction was quenched and analyzed by PEI cellulose TLC as described above.

**Determination of the K\textsubscript{s} for Phosphate, Vanadate, and Arsentate in Exonuclease RNA Cleavage.** TEC10C in transcription buffer (TB) lacking Mg\textsuperscript{2+} was supplemented with orthophosphate or its mimics to final concentrations indicated in Fig. 3A. The reaction was started by addition of MgCl\textsubscript{2} to a final concentration of 10 mM and, after incubation at 0 °C, quenched and analyzed as described above. Incubation time was 10 min, 20 min, and 40 min for vanadate, orthophosphate, and arsenate, respectively. The reactions were quenched and analyzed as described above.

**Determination of the K\textsubscript{s} for Mg\textsuperscript{2+} in the Reaction of Exonuclease RNA Cleavage in the Presence of Phosphate, Vanadate, and Arsentate.** The reaction was carried out at 0 °C as described above in the presence of 5 mM P\textsubscript{i} or its mimics. The concentration of Mg\textsuperscript{2+} in the reaction mixtures is indicated in Fig. 3B. The incubation time was 5, 10, and 40 min for vanadate, phosphate, and arsenate, respectively. The control reaction was performed under the same conditions but in the absence of the reaction anions.

**Comparative Effect of P\textsubscript{i} and GreA on Exonuclease and Endonuclease Transcript Cleavage.** Reactions were performed at 0 °C with labeled TEC10C or TEC11A in TB in the presence of 2.5 mM P\textsubscript{i} or 0.1 μM GreA. The incubation time was 10 min and the reactions were quenched as previously described.

**Effect of Active Center Mutations in RNAP on P\textsubscript{i}-Induced Transcript Cleavage.** TEC9A with mutant enzymes was assembled as for WT RNAP. Elongation of TEC9A with [\textalpha-\textsuperscript{32}P]CTP was performed at 20 °C for 1 h, followed by washing with TB lacking MgCl\textsubscript{2}. TEC\textsubscript{m} were supplemented with 10 mM MgCl\textsubscript{2} and 2.5 mM P\textsubscript{i} and kept at 0 °C. Incubation times were 15 h for jE614A/8D11A, 1 h for jR1106A, and 15 min for WT RNAP TEC\textsubscript{m}. The reaction products were resolved by TLC on PEI cellulose in 0.1 M potassium phosphate (pH 4.1) and detected by phosphomagerography.

**Effect of pH on P\textsubscript{i}-Induced Transcript Degradation.** Reactions were performed with TEC10C obtained as described above. The desired pH was created by using the following buffers: MES pH 6.0, Hepes pH 7.0, Tris pH 8.0, and taurine pH 9.0 at 20 mM concentration. The mixtures were supplemented with 10 mM MgCl\textsubscript{2} and 2.5 mM P\textsubscript{i} and kept for 20 min at 0 °C. The reaction products were analyzed by TLC on PEI cellulose in 0.1 M potassium phosphate (pH 4.1).

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