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Regulated Incorporation of Two Different Metal Ions into Programmed Sites in a Duplex by DNA Polymerase Catalyzed Primer Extension**

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Abstract: *Metal-mediated base pairs formed by the coordination of metal ions to natural or artificial bases impart unique chemical and physical properties to nucleic acids and have attracted considerable interest in the field of nanodevices. Ag^I ions were found to mediate DNA polymerase catalyzed primer extension through the formation of a C–Ag^I–T base pair, as well as the previously reported C–Ag^I–A base pair. The comparative susceptibility of dNTPs to Ag^I-mediated enzymatic incorporation into the site opposite cytosine in the template was shown to be dATP > dTTP ≫ dCTP. Furthermore, two kinds of metal ions, Ag^I and Hg^{II}, selectively mediate the incorporation of thymidine 5′-triphosphate into sites opposite cytosine and thymine in the template, respectively. In other words, the regulated incorporation of different metal ions into programmed sites in the duplex by DNA polymerase was successfully achieved.*

Metal-mediated base pairs formed by the coordination of metal ions to natural or artificial bases impart unique chemical and physical properties to nucleic acids. For example, an increase in duplex and triplex stability[1–3] and the assembly of programmable arrays of one or several kinds of metal ions into a duplex have been reported.[4] Metal-mediated base pairs have thus attracted considerable interest in the field of nanodevices, for example, in the context of electronic wires,[5] magnetic devices,[6] and DNA-based logic gates.[7]

Recently, it was reported that Hg^{II} and Ag^I ions coordinate to natural mismatched base pairs in oligodeoxynucleotide (ODN) duplexes and stabilize the base pairs through the formation of metal-mediated base pairs, such as T–Hg^{II}–T (Figure 1), U–Hg^{II}–U, and C–Ag^I–C.[8–10] Hence, we focused on the biological relevance of metal-mediated base pairs and discovered that in the presence of Hg^{II} ions, DNA polymerases incorporate 2′-deoxythymidine 5′-triphosphate (dTTP) into the sites opposite thymine (T) in the template strand to synthesize the full-length product through the formation of a T–Hg^{II}–T base pair.[11] A metal-mediated base pair consisting of an artificial base was reported to be recognized by DNA polymerase as well.[12] Furthermore, we found that Ag^I ions promoted the enzymatic incorporation of 2′-deoxyadenosine 5′-triphosphate (dATP) into the sites opposite cytosine (C) in the template strand through the formation of a C–Ag^I–A base pair.[13] This result was quite unexpected because an Ag^I ion was reported to selectively increase the thermodynamic stability of duplexes containing a C–C mismatched base pair.[10] We found that Ag^I ions also increase the thermodynamic stability of duplexes containing a C–T mismatched base pair, probably through the formation of a C–Ag^I–T base pair (Figure 1).[14]

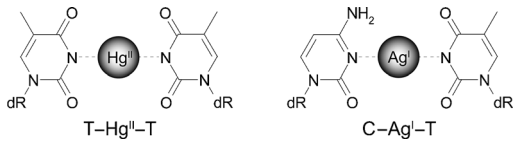

Figure 1. Structures of the T–Hg^{II}–T and C–Ag^I–T base pairs. dR = deoxyribose.

Herein, we report on a primer extension reaction in which DNA polymerase catalyzes the formation of a C–Ag^I–T base pair within the DNA duplex to synthesize full-length product. The reaction involving the incorporation of dTTP into the sites opposite C in the template was highly specific for Ag^I ions. From the comparative study of the Ag^I-mediated incorporation of dNTPs into the site opposite C in the template, the strictly preferential incorporation of dATP and dTTP over dCTP was observed. In combination with the previously reported enzymatic formation of a T–Hg^{II}–T base pair within the DNA duplex, the formation of a C–Ag^I–T base pair enabled the accurately regulated enzymatic incorporation of dTTP into the sites opposite T and C in the template through the addition of Hg^{II} and Ag^I ions, respectively. The two different kinds of metal ions (Ag^I and Hg^{II} ions) were successfully incorporated into the programmed sites in the same duplex as metal-ion-mediated mismatched base pairs by DNA polymerase.

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To elucidate the compatibility of the C–Ag^I–T base pair with DNA polymerases, a primer extension reaction was carried out with 3′→5′-exonuclease-deficient Klenow fragment (KF exo$^-$). In the absence of Ag^I ions, KF exo$^-$ stalled at the 19-mer site, however, the enzyme incorporated dTTP or dCTP into the site opposite C in the template to elongated the stalled product to yield the full-length product at increasing concentrations of Ag^I ions (Figure 2a). The reversed reaction,

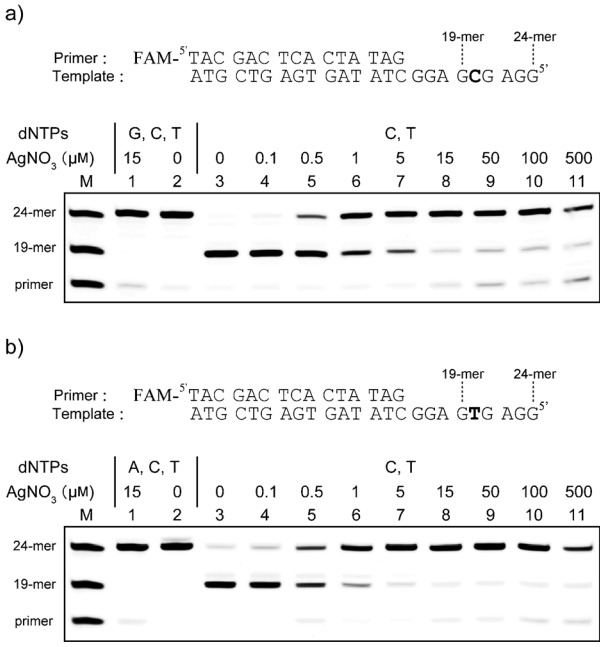


Figure 2. PAGE analysis showing the effect of Ag^I ion concentration on the primer extension reaction catalyzed by KF exo$^-$. The reaction mixtures contained 20 μM dNTPs and 0.4 units of KF exo$^-$ in the presence or absence of various concentrations of $AgNO_3$. M indicates markers for the primer, 19-mer, and 24-mer. FAM = 6-carboxyfluorescein.

Ag^I-mediated dTTP or dCTP incorporation into the site opposite T, also proceeded in the presence of Ag^I ions (Figure 2b). To confirm the kind of dNTP incorporated by KF exo$^-$, single-nucleotide insertions were carried out with the primed templates shown in Figure 3. For the Ag^I-independent incorporation of dNTP into the site opposite C in the template, dGTP was incorporated by the enzyme to yield the 20-mer ($n+1$) product through the formation of a canonical Watson—Crick (G–C) base pair, regardless of the presence or absence of Ag^I ions (Figure 3a, lanes 3 and 4). For Ag^I-dependent reactions, KF exo$^-$ incorporated dATP into the site opposite C in the template through the formation of a C–Ag^I–A base pair in the presence of Ag^I ions (Figure 3a, lane 1) as reported.[12] Furthermore, the enzyme incorporated dTTP into the site opposite C in the template in the presence of Ag^I ions to yield the 20-mer ($n+1$) product (Figure 3a, lane 7). Unexpectedly, no incorporation of dCTP into the site opposite C was observed under these conditions (Figure 3a, lane 5), although Ag^I ions show stronger stabilizing effects for duplexes containing a C–C mismatch than for those containing a C–T mismatch.[10] The reverse reaction, the Ag^I-

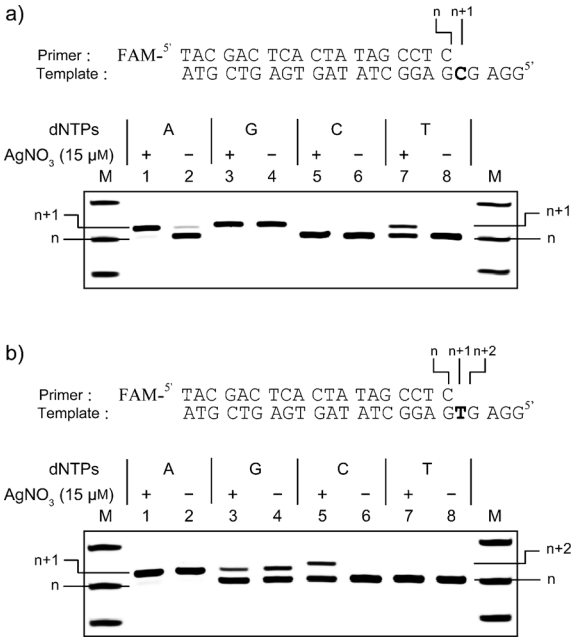
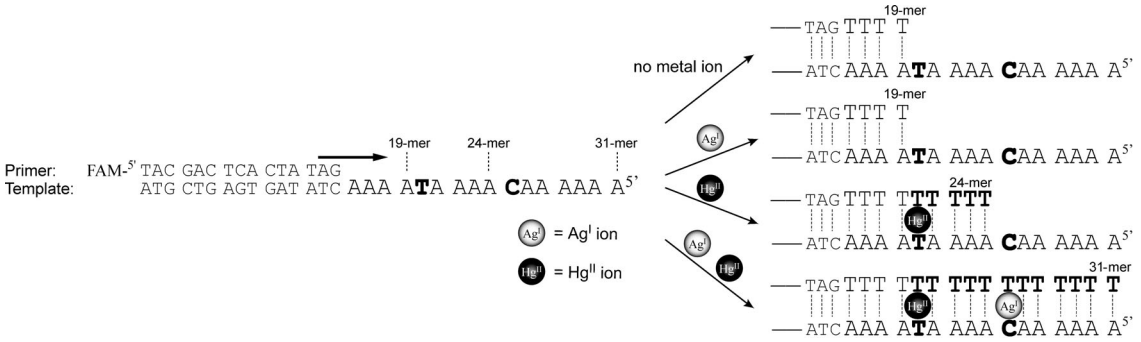


Figure 3. "PAGE analysis of single-nucleotide insertions into the sites opposite C (a) and T (b) in the template strands catalyzed by KF exo$^-$. The reaction mixtures contained 10 μM dNTPs and 0.2 units of KF exo$^-$ in the presence or absence of 15 μM $AgNO_3$. M indicates markers for the 15-mer, primer, and 24-mer.

mediated incorporation of dCTP into the site opposite T in the template also proceeded in the presence of Ag^I ions (Figure 3b, lane 5). The compositions of the full-length products of the Ag^I-promoted reactions in Figure 2 were confirmed by MALDI-TOF mass spectrometry (see Figure S1 in the Supporting Information).

To investigate the effects of other metal ions, we performed the primer extension reactions in the presence of Mn^{II}, Fe^{II}, Fe^{III}, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}, Cd^{II}, Au^{III}, Hg^{II}, Tl^I, or Pb^{II} (Figure 4). The reactions proceeded to yield a full-length product only in the presence of Ag^I and Hg^{II} ions. Ag^I ions promoted both the extension reactions to yield the full-length products (24-mer; Figure 4a and b). By contrast, only the incorporation of dTTP or dCTP into the site opposite T, and not the incorporation of dTTP or dCTP opposite C, proceeded in the presence of Hg^{II} ions (Figure 4b). We then confirmed that dTTP was selectively incorporated into the site opposite T in the template by the Hg^{II}-mediated reaction (see Figure S2 in the Supporting Information) as reported.[11] These results indicate that the incorporation of dTTP into the site opposite C in the template is highly specific to Ag^I ions.

In addition to the previously reported C–Ag^I–A base pair, we demonstrated the Ag^I-mediated formation of a C–Ag^I–T base pair within the DNA duplex by DNA polymerase. The formation of a C–Ag^I–C base pair was not observed in this work (Figure 3a) or in previous work,[12] despite the fact that Ag^I ions have been reported to selectively stabilize the C–C mismatched base pair among 16 kinds of matched and mismatched base pairs.[10] Therefore, we compared the enzymatic incorporation of dATP, dTTP and dCTP into the site opposite C in the template by using various templates



Scheme 1. A schematic illustration of the regulated incorporation of Ag^{I} and Hg^{II} ions into programmed sites in the duplex.

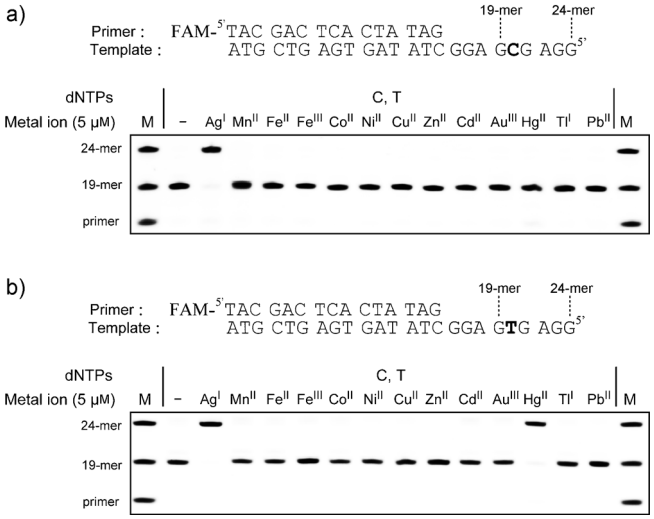


Figure 4. PAGE analysis showing the effect of various metal ions on the primer extension reaction. The reaction mixtures contained 20 μM dTTP and dCTP, 0.4 units of KF exo⁻, and 15 μM various metal ions. M indicates markers for the primer, 19-mer, and 24-mer.

possessing different sequences in the upstream region of the incorporation site (see Figure S3 in the Supporting Information). In the presence of Ag^{I} ions, dATP was incorporated into almost all of the primed templates except for Duplexes 4 and 10, which may form a hairpin structure between the C-rich upstream and G-rich downstream regions of the incorporation site. The incorporation of dTTP was somewhat dependent on the upstream sequence of the incorporation site. However, dCTP was not incorporated into any of the primed templates under these assay conditions. Indeed, a lower efficiency for the incorporation of dCTP into the site opposite C in the template was recently reported.[15] The use of larger amounts (0.8 units) of the enzyme and a higher concentration of dCTP (20 μM) allowed the incorporation of dCTP into some primed templates to form a C–Ag^{I}–C base pair (see Figure S4 in the Supporting Information). These results indicate that dTTP is a much better substrate for this reaction than dCTP. Hg^{II} ions deprotonate the imino protons of two thymine residues to form a neutral T–Hg^{II}–T base pair, whereas Ag^{I} ions should deprotonate one imino proton to form neutral base pairs such as C–Ag^{I}–T. The positively charged C–Ag^{I}–C base pair may thus not easily be recognized as a "correct" substrate by DNA polymerases. This may suggest that the absence of a positive charge on a metal-mediated base pair is important for its polymerase-catalyzed integration into the duplex.

We have demonstrated that the incorporation of dTTP into the site opposite C in the template through the formation of a C–Ag^{I}–T base pair is strictly specific to Ag^{I} ions (Figure 3a and Figure 4a). In combination with the enzymatic formation of a T–Hg^{II}–T base pair within the DNA duplex, this reaction could make the regulated extension of primers and the regulated incorporation of different metal ions into the same duplex possible because an Ag^{I} ion mediates the incorporation of dTTP only opposite C and not opposite T in the template (Figure 3). We thus designed a novel metal-ion-triggered primer extension system, which enables the regulated incorporation of two different metal ions (Ag^{I} and Hg^{II} ions) into programmed sites in a duplex (Scheme 1). The results are shown in Figure 5. In the absence of Hg^{II} ions, KF exo⁻ did not catalyze the extension reaction at the site opposite T in the template to yield the 19-mer product, regardless of the presence or absence of Ag^{I} ions (lanes 2 and 3). However, by adding Hg^{II} ions, a T–Hg^{II}–T base pair was formed and the enzyme went through the site opposite T and subsequently stalled at the site opposite C to yield the 24-mer product (lane 4). The addition of both Ag^{I} and Hg^{II} ions promoted extension at the sites opposite T and C in the template to yield the full-length 31-mer product (Lane 5). The compositions of these stalled and full-length products were

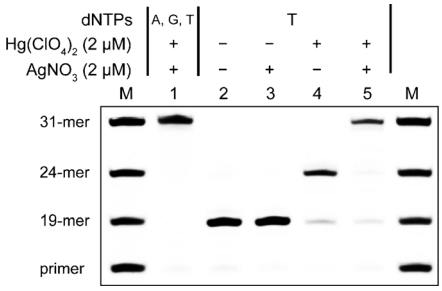


Figure 5. PAGE analysis of the regulated incorporation of Ag^{I} and Hg^{II} ions into the programmed sites by KF exo⁻. The reaction mixtures contained 30 μM dNTPs and 0.4 units of KF exo⁻ in the presence of 2 μM $AgNO_3$ and/or $Hg(ClO_4)_2$. M indicates markers for the primer, 19-mer, 24-mer, and 31-mer.

confirmed by MALDI-TOF mass spectrometry (see Figure S5 in the Supporting Information). Furthermore, the metal-ion-triggered extension reaction with a primed template in which the C and T sites were exchanged with each other proceeded in a similar manner (Figure S6 in the Supporting Information). For both template configurations, the two different kinds of metal-mediated base pairs (T–Hg^{II}–T and C–Ag^{I}–T) were successfully formed at the programmed sites in the newly synthesized double-stranded DNA.

In conclusion, we demonstrated that Ag^{I} ions mediate DNA polymerase catalyzed primer extension through the formation of a C–Ag^{I}–T base pair, as well as the previously reported C–Ag^{I}–A base pair. The comparative susceptibility of dNTPs to Ag^{I}-mediated enzymatic incorporation into the site opposite C in the template was shown to be dATP > dTTP ⩾ dCTP. In combination with T–Hg^{II}–T formation, C–Ag^{I}–T formation was used in a novel metal-ion-triggered primer extension system. The specific formation of two different kinds of metal-mediated base pairs was thus achieved at programmed sites in duplex DNA. To our knowledge, this is the first report of the successful regulated enzymatic incorporation of different metal ions into programmed sites in duplex DNA. Our findings open up the possibility of regulating a multistep replicating system with metal ions. Moreover, this enzymatic approach may overcome the difficulties in the preparation of large double-strand DNA molecules base-paired through selective interstrand coordination with different kinds of metal ions.[16]

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[1] a) G. H. Clever, C. Kaul, T. Carell, *Angew. Chem.* **2007**, *119*, 6340 – 6350; *Angew. Chem. Int. Ed.* **2007**, *46*, 6226 – 6236; b) G. H. Clever, M. Shionoya, *Coord. Chem. Rev.* **2010**, *254*, 2391 – 2402; c) Y. Takezawa, M. Shionoya, *Acc. Chem. Res.* **2012**, *45*, 2066 – 2076.

[2] a) K. Tanaka, M. Shionoya, *J. Org. Chem.* **1999**, *64*, 5002 – 5003; b) E. Meggers, P. L. Holland, W. B. Tolman, F. E. Romesberg, P. G. Schultz, *J. Am. Chem. Soc.* **2000**, *122*, 10714 – 10715; c) S. Atwell, E. Meggers, G. Spraggon, P. G. Schultz, *J. Am. Chem. Soc.* **2001**, *123*, 12364 – 12367; d) K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Shiro, M. Shionoya, *J. Am. Chem. Soc.* **2002**, *124*, 12494 – 12498; e) C. Switzer, S. Shinha, P. H. Kim, B. D. Heuberger, *Angew. Chem.* **2005**, *117*, 1553 – 1556; *Angew. Chem. Int. Ed.* **2005**, *44*, 1529 – 1532.

[3] a) K. Tanaka, Y. Yamada, M. Shionoya, *J. Am. Chem. Soc.* **2002**, *124*, 8802 – 8803; b) Y. Takezawa, W. Maeda, K. Tanaka, M. Shionoya, *Angew. Chem.* **2009**, *121*, 1101 – 1104; *Angew. Chem. Int. Ed.* **2009**, *48*, 1081 – 1084.

[4] a) K. Tanaka, G. H. Clever, Y. Takezawa, Y. Yamada, C. Kaul, M. Shionoya, T. Carell, *Nat. Nanotechnol.* **2006**, *1*, 190 – 194; b) G. H. Clever, T. Carell, *Angew. Chem.* **2007**, *119*, 254 – 257; *Angew. Chem. Int. Ed.* **2007**, *46*, 250 – 253; c) F.-A. Polonius, J. Müller, *Angew. Chem.* **2007**, *119*, 5698 – 5701; *Angew. Chem. Int. Ed.* **2007**, *46*, 5602 – 5604; d) S. Johannsen, N. Megger, D. Böhme, R. K. O. Sigel, J. Müller, *Nat. Chem.* **2010**, *2*, 229 – 234.

[5] S. Liu, G. H. Clever, Y. Takezawa, M. Kaneko, K. Tanaka, X. Guo, M. Shionoya, *Angew. Chem.* **2011**, *123*, 9048 – 9052; *Angew. Chem. Int. Ed.* **2011**, *50*, 8886 – 8890.

[6] a) K. Tanaka, A. Tengeiji, T. Kato, N. Toyama, M. Sionoya, *Science* **2003**, *299*, 1212 – 1213; b) G. H. Clever, S. J. Reitmeier, T. Carell, O. Schiemann, *Angew. Chem.* **2010**, *122*, 5047 – 5049; *Angew. Chem. Int. Ed.* **2010**, *49*, 4927 – 4929.

[7] a) R. Freeman, T. Finder, I. Willner, *Angew. Chem.* **2009**, *121*, 7958 – 7961; *Angew. Chem. Int. Ed.* **2009**, *48*, 7818 – 7821; b) H. Pei, L. Liang, G. Yao, J. Li, Q. Huang, C. Fan, *Angew. Chem.* **2012**, *124*, 9154 – 9158; *Angew. Chem. Int. Ed.* **2012**, *51*, 9020 – 9024.

[8] a) A. Ono, H. Torigoe, Y. Tanaka, I. Okamoto, *Chem. Soc. Rev.* **2011**, *40*, 5855 – 5866; b) Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami, A. Ono, *J. Am. Chem. Soc.* **2006**, *128*, 2172 – 2173; c) Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima, A. Ono, *J. Am. Chem. Soc.* **2007**, *129*, 244 – 245.

[9] S. Johannsen, S. Paulus, N. Düpre, J. Müller, R. K. O. Sigel, *J. Inorg. Biochem.* **2008**, *102*, 1141 – 1151.

[10] a) A. Ono, S. Cao, H. Togashi, M. Tashiro, T. Fujimoto, T. Machinami, S. Oda, Y. Miyake, I. Okamoto, Y. Tanaka, *Chem. Commun.* **2008**, 4825 – 4827; b) H. Torigoe, I. Okamoto, T. Dairaku, Y. Tanaka, A. Ono, T. Kozasa, *Biochemie* **2012**, *94*, 2431 – 2440.

[11] H. Urata, E. Yamaguchi, T. Funai, Y. Matsumura, S. Wada, *Angew. Chem.* **2010**, *122*, 6666 – 6669; *Angew. Chem. Int. Ed.* **2010**, *49*, 6516 – 6519.

[12] C. Kaul, M. Müller, M. Wagner, S. Schneider, T. Carell, *Nat. Chem.* **2011**, *3*, 794 – 800.

[13] T. Funai, Y. Miyazaki, M. Aotani, E. Yamaguchi, O. Nakagawa, S. Wada, H. Torigoe, A. Ono, H. Urata, *Angew. Chem.* **2012**, *124*, 6570 – 6572; *Angew. Chem. Int. Ed.* **2012**, *51*, 6464 – 6466.

[14] H. Urata, E. Yamaguchi, Y. Nakamura, S. Wada, *Chem. Commun.* **2011**, *47*, 941 – 943.

[15] T. Tian, S. Peng, H. Xiao, Y. Long, B. Fu, X. Zhang, S. Guo, S. Wang, X. Zhou, S. Liuc, X. Zhouc, *Chem. Commun.* **2013**, *49*, 10085 – 10087.

[16] J. Müller, *Nature* **2006**, *444*, 698.

