# Response of human DNA polymerase ι promoter to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine

**Huifang Zhu, Yanfeng Fan, Jimin Shao***

*Department of Pathology and Pathophysiology, Zhejiang University School of Medicine, Hangzhou 310058, China.*

*Environmental Toxicology and Pharmacology (IF: 1.051)*

## ABSTRACT

Human Pol ι is a highly distributed, low-fidelity DNA polymerase lacking intrinsic exonuclease proofreading activity, thus its effects are strictly regulated. We predicted and cloned the promoter region of the human POLI gene. Successively, by transfection of deletion constructs of the POLI promoter, we demonstrated that the regions - 848/- 408 and - 30/+215 contained positive regulatory elements, and the region +215/+335 had proximal promoter activity. Overexpression of Sp1 significantly increased the transcriptional activity of the promoter, and mutation of the Sp1 site reversed Sp1-induced promoter transactivation. Quantitative RT-PCR showed that POLI mRNA expression was up-regulated in human amnion FL cells treated by the carcinogen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). Reporter gene assays demonstrated that MNNG also significantly increased the transcriptional activity of the predicted promoter (- 848/+335) and the proximal promoter (+215/+335). However, the promoter with the Sp1 site mutation had no response to MNNG treatment, suggesting that Sp1 plays an important role in the transcriptional regulation of the POLI gene stimulated by MNNG. Our data suggest that abnormal regulation of Pol ι may be involved in the mutagenesis and carcinogenesis induced by environmental chemicals.

## METHODS

- Cell line: Human amnion epithelial cells (FL cells)
- Dose: MNNG 10 μM, DMSO as control.
- Software: FirstEF, Promoter Scan, Neural Network Promoter Prediction, Promoter 2.0, CpG Islands Prediction; P-Match-Public 1.0 public, Match-1.0 Public, SiteGA Site recognition by Genetic Algorithm, TFSEARCH ver.1.3
- Transient transfection and reporter gene assays
- Quantitative real-time RT-PCR

## RESULTS

```
−848  GAGGCTCACA GCAGTTATGT AACCTGCCCA AAGTCACACA GCATGATGGA GGGTCTGGAT
                  VBP or HLF           SREBP     GATA-1      Hand1/E47
−788  TGGAGATTTT GTCTGACTAC AACACTCATG TATCAAATCC TATTCAGTTT TGATGTTAAA
                                       Evi-1
−728  AATTCTGTGG TTGAATTCGT CACAGTGTCT TTAAATAAAA CAACAACAAA CAAAACACAG
      AML-1a/CdxA                      SRY       SRY       SRY
−668  CAAACCTTAA CATAGTTGCT AATCATATGA GTTTACAAGG CCACCTGTGT CTTTACTATC
                                       SF1
−608  AGTTACACTC CTTCCCACCC CATCACATTA ATCACTCAGG TCTAGGGATT TGCAAGTTTG
                  SREBP   GATA-1  CdxA
−548  CTAACAGCCG AATGGAACAA AGACAAAACG CTAAAATTTG TGATGTGGGC CTAACTCCAA
                                       CdxA  SREBP
−488  AGCCTTCAAG AAAAAAAAAA AAAGGCCAGG CGCAGTGGCT CATGCCTGTG ATCTCAACAC
−428  TTTGGGAGGC CTAGGAGGGT GGATCACCTG AGGACAGGAG TTCGAAACCA GCCTGACCAA
      Lyf-1                 deltaE/SREBP
−368  CATGGTGAAA CCTCGTCTCT ACTAAATACA AAAAAATTAG CTGGGCGTGA TGGCGCATGC
      IRF1                                                  GATA-1/GATA-2
−308  CTGTTAATCC CAGCTACTTG GGAGGCTGAG GCAGGAGAAT CGCTTGAATC CGGGAGGCAG
        Bcd             Lyf-1
−248  AGGTTGCAGT GAGCCGGTAT TGCGTCACTG TTGCCCACTC CAGCCTGAGC AACAAGAACG
−188  AAACTCCGTC TCAAAAAAAA AAAAAATAGC CATTTGTGCT TTCAATCTCT CCGCTTCCGG
                                       CdxA                 Elk-1/c-ETS
−128  CCTCCACCCG GCGGGAAAAA ACTCCCGCAC GTGGGCCTCC ACGATTCCTT GGCATGCGTG
                  E2F             USF/N-Myc
−68   TCACTCTGGG CAACAGGCTG GTCGGCCACG ACTGCGCAGG CGAGAGGCAC AGAGCGACTG
                +1
−8    GAGACTGT | AG TCCCCCGGTT TCCCTGGAGA CCAGGCGGAA GCGGCCGGAA GTAGCGCTGC
          c-Rel                                    Elk-1 or c-Est-1(p54)
+53   GGTTGGCAGC GGCGGGATGG AGAAGCTGGG GGTGGAGCCG GAGGAGGAAG GCGCGGCGA
      AML-1a      GATA-1
+113  CGACGACGAG GAAGACGCCG AGGCCTGGGC CATGGAACTG GCGGACGTGG GGCGGCAGC
+173  CAGCTCGCAG GGTGCGCCGC AGCCAGAGGA GCCAGCGGCC TCCTTGGGTG TAAATGAGAA
+233  GGGTGGGGCA GGCGGACGCT CGGGGCGGCG GCCACTGGGG CGCGACCGTC CCCGCCCCAC
                                                              Sp1/MZF1
+293  TCGGCTCCTC TAAGAGAGGG CTGCCTCCCT CTGCCTTGTG TTA
```

**Fig. 1**. Bioinformatics prediction of transcription factor binding sites for the human POLI promoter. The numbers on the left indicate the nucleotide order relative to the transcription start position (tsp, position +1) of the human POLI gene. Putative binding sites of transcription factors are in bold and underlined.

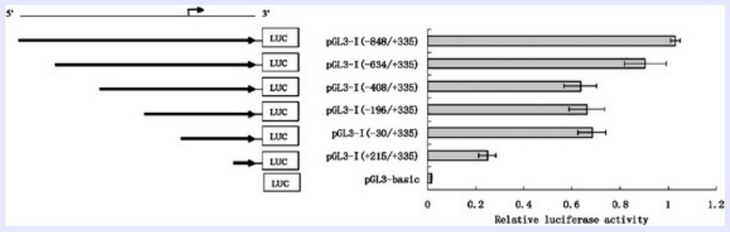


**Fig. 2.** Transcriptional activity analysis of 5-deletion constructs of the human POLI promoter by reporter gene assays. Left: schematic representation of the promoter–reporter constructs containing the promoter fragments of different lengths cloned into pGL3-basic vector. Arrows indicates the transcription start position. The deletion constructs were separately transfected into FL cells with pRL-SV40. Right: relative luciferase activity normalized to pRL-SV40 values for each construct. The results are the average of three independent experiments performed in duplicate.

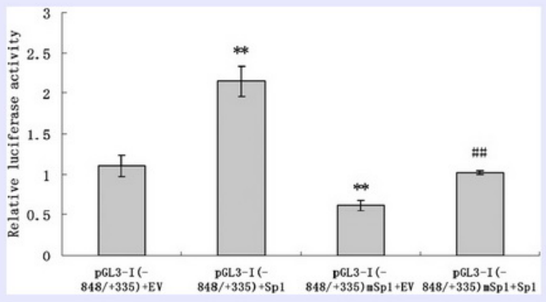


**Fig. 3.** Sp1 mediates the activity of the human POLI promoter. pGL3-I(- 848/+335) or pGL3-I(- 848/+335)mSp1 (containing an Sp1 site mutation) was co-transfected with pcDNA3.1-cMyc (EV) or pcDNA3.1-cMyc-Sp1 (Sp1) into FL cells. The relative luciferase activity was normalized to pRL-SV40 values. The data are the average of three independent experiments performed in duplicate. $^{**}P < 0.01$, compared to cells transfected with pGL3-I(- 848/+335) and pcDNA3.1-cMyc. $^{\#\#}P<0.01$, compared to cells transfected with pGL3-I(- 848/+335)mSp1 and pcDNA3.1-cMyc (Student's *t*-test).

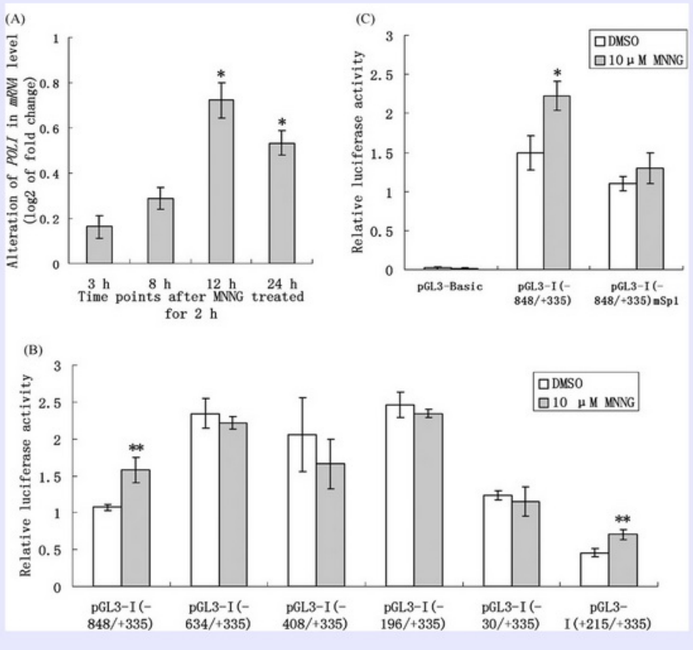


**Fig. 4.** (A) qRT-PCR measurements of transcription of the human POLI gene in response to 10 μM MNNG treatment. (B) Reporter gene assays for transcriptional responses of 5-deletion constructs of the human POLI promoter to 10 μM MNNG. The deletion constructs were separately transfected into FL cells. The day following transfections, cells were treated with 10 μM MNNG in serum-free medium for 2 h, and 12 h later luciferase activity was determined. (C) The reporter plasmid pGL3-Basic, pGL3-I(- 848/+335) or pGL3-I(- 848/+335)mSp1 was transfected into FL cells, the cells were treated with 10 μM MNNG or the control DMSO, and 12 h after treatment, the luciferase activity was measured. The data were analyzed by Student's *t*-test compared to controls (DMSO). $^{*}P < 0.05$, $^{**}P < 0.01$.

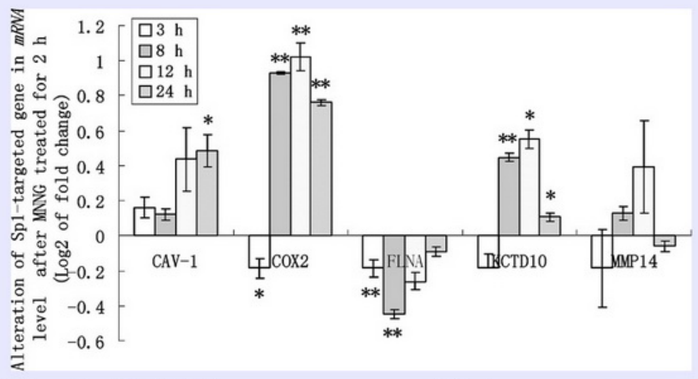


**Fig. 5.** qRT-PCR measurements of Sp1-targeted genes in response to 10 μMNNG treatment. The data were analyzed by Student's *t*-test compared to controls (DMSO). $^{*}P < 0.05$, $^{**}P < 0.01$.

## Conclusion

By analyzing the human POLI promoter sequence with bioinformatics methods, we found that the human POLI promoter region contained a high GC content but no canonical TATA box. The proximal promoter region of human POLI from +215 to +335, relative to the gene transcription start position, contained an Sp1 site. In addition, we showed that Sp1 significantly increased the transcriptional activity of the human POLI promoter, and mutation of the Sp1 site reversed Sp1-induced promoter transactivation, indicating that the Sp1 site plays an important role in regulation of human POLI gene expression.

**Acknowledgment:** This research was supported by the National Natural Science Foundation of China (30770831, J0730856) and Zhejiang Provincial Natural Science Foundation for Excellent Research Groups (R207153).