The development of eosinophilia is regulated by interleukin (IL)-5. The biological specificity of eosinophilia suggests a tight and independent regulation of IL-5 expression. A number of regulatory regions in the 5′-end of the IL-5 gene have been described; many of them are involved in the regulation of other genes, and it is not clear how the specific expression of IL-5 is regulated. In this study, we report the finding of a novel 3′-regulatory element. Data base analysis of a 2-kilobase fragment of the 3′-end of the mouse IL-5 gene revealed the presence of a 40-base pair-long repetitive sequence that consists of four direct repeats of ATGAATGA distributed in a symmetrical manner. This sequence, named mouse downstream regulatory element-1 (mDRE1), was shown to be protected in DNase I footprinting assays in vitro. Electrophoretic mobility shift assays using specific antibodies identified the transcription factors Oct-1 and Oct-2 as responsible for the formation of the specific complexes with mDRE1 and nuclear extracts from both EL4 and primary T-cells. Competition electrophoretic mobility shift assays with oligonucleotides containing different numbers of ATGAATGA repeats showed that Oct-1 and Oct-2 bind to different motifs in the mDRE1 sequence. Deletion of mDRE1 from a 9.5-kilobase IL-5 gene construct significantly decreased the expression of the luciferase reporter gene, suggesting that it plays a positive role in the expression of the IL-5 gene.

Eosinophilia is a unique specific phenomenon regulated by interleukin (IL)-5. The biological specificity of eosinophilia involves increases in eosinophil numbers and activation state, without any changes in other cell types. Besides their role in the immune response against helminths (1–3), eosinophils are the principal cause of tissue damage that leads to the symptoms of asthma and other allergic diseases (4). IL-5 controls the production of eosinophils from the bone marrow and is also involved in their differentiation and activation. It is a cytokine produced mainly by Th2 cells, but mast cells and eosinophils are also known to produce it (5–7). The expression of IL-5 is tightly regulated and tissue-specific, and it is present in all cases of eosinophilia (8).

Like the majority of cytokines, the expression of IL-5 is regulated at the transcriptional level (9, 10). As most of the research on the regulation of IL-5 expression targets the promoter region of the gene, all known elements that regulate its expression are located in that region. However, transcription regulatory elements can be present in the 3′-end of genes. The 3′-untranslated region of the CD2 gene contains an enhancer that provides T-cell-specific expression to this gene or any other to which it is attached (12). In cytokine genes, the tumor necrosis factor-α-responsive element renders tissue-specific tumor necrosis factor-α-induced expression to the tumor necrosis factor-α gene in cells of the central nervous system, but not in mononuclear cells (13). In the case of the IL-4 gene, a silencer region present in the 3′-flanking region of the gene renders Th2 specificity to IL-4 expression through the binding of STAT6. As Th1 cells are not capable of nuclear expression of STAT6, these cells cannot overcome the 3′-silencer activity and therefore are not able to express IL-4 (14).

Previous studies in this laboratory demonstrated that the 3′-untranslated region has little or no effect on the post-transcriptional control of IL-5 expression (10). In this study, we investigated the role of elements present in the 3′-flanking region of the IL-5 gene and show that they are involved in the transcriptional control of the gene. We identified a new regulatory element located in the 3′-flanking region of the mIL-5 gene. This murine downstream regulatory element-1 (mDRE1) is 40 bp long, consists of four direct repeats of ATGAATGA distributed in a symmetrical manner, and was shown to be protected in DNase I footprinting assays. Deletion of this sequence from a 9.5-kb construct containing the whole mIL-5 gene decreased the promoter activity upon PMA/cAMP stimulation by 3.5-fold, suggesting that this part of the 3′-untranslated region is involved in the positive regulation of the gene. Using nuclear extracts from the thymoma-derived EL4 cell line and primary T-cells, we have shown that transcription factors Oct-1 and Oct-2 bind to the mDRE1 sequence. Together, these data suggest that mDRE1 is a new regulatory element involved in the regulation of IL-5 expression and that its activity may be regulated by octamer factors.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Stimulation—**EL4 and primary murine cells were grown in RPMI 1640 medium supplemented with 100 μg/ml Eagle’s medium nonessential amino acid solution (Life Technologies, Inc.), 1 mM sodium pyruvate, 2 mM l-glutamine, 75 μM monothioglycerol (Sigma), and 10 mM Hepes. 5% fetal calf serum was added to EL4 cell medium. Primary murine cell cultures were supplemented with 10% fetal calf serum and 100 units/ml recombinant human IL-2. EL4 cells were stimulated with 10 ng/ml PMA and 1 μM cAMP. Primary T-cells were stimulated with 5 μg/ml concanavalin A (ConA).

**DNase I Footprinting Assay—**The 220-bp fragment of the 5′-flanking region of the mIL-5 gene (+4958 to +5178) was subcloned into the XbaI/SacI sites of the pSPT7 vector (Promega). The resulting construct was subsequently digested
with restriction enzymes XhoI and HpaI, for labeling of the coding strand, or with BglII and PvuII, for labeling of the noncoding strand. The fragments were treated with shrimp alkaline phosphatase for dephosphorylation of the 5'-terminus and end-labeled with $[^{32}P]$ATP.

The DNase I footprinting assays were carried out using the DNase I footprinting system supplied by Life Technologies, Inc. The protection protocol followed the instructions supplied with the kit. Nuclear extracts from unstimulated EL4 cells were used as the source of proteins, and the incubation was carried out at room temperature for 20 min in 20 mM Hepes, pH 7.9, 0.1 M KCl, 0.2 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 8 mM MgCl2, and 0.5 mg of poly(dI-dC).

**Plasmids and Site-directed Mutagenesis**—A 9.5-kb fragment of the murine IL-5 gene (from positions −3861 to +5612) was cloned into the p-Poly-III expression vector (15), and the luciferase reporter gene was inserted at position +1. Site-directed mutagenesis was carried out using the Transformer™ site-directed mutagenesis kit supplied by CLONTECH (Palo Alto, CA). The mutagenesis protocols followed the instructions supplied with the kit. The oligonucleotides used in the mutagenesis reaction were as follows: mutagenic primer, 5'-GGC TCA GGG TGA ACA GGA GAG GGC CTC CAT TAA TAA AGT GCT TGC-3'; and selection primer, 5'-CGA TAA GGA TCC GCC GAC GGA-3'.

**Transient Transfection and Luciferase Assay**—EL4 cells were transfected with 10 μg of the IL-5/luciferase constructs by electrophoresis as described previously (16). Each experiment was performed in duplicate. After transfection, the cells were either left unstimulated or stimulated with 10 ng/ml PMA and 1 mM cAMP for 12 h. The luciferase assay was performed as described (17). The background obtained from the lysis buffer was subtracted from each sample. Errors represent S.D. The magnitude of the luciferase activity varied greatly between experiments. The mean of the activity of the mutant construct is expressed as the ratio of the mutant to the wild type with an S.D. calculated over five experiments.

**Preparation of Nuclear Extracts and EMSA**—Nuclear extracts used in EMSA were prepared following the method described by Schreiber et al. (18) with the following modifications. Protease inhibitor mixture, 1 mM Na3VO4, and 0.5 mM dithiothreitol were added to the reaction buffers just prior to lysis. Protein concentration was determined using the Bio-Rad protein assay. Standard binding reactions contained 5 μg of nuclear extracts, 60 mM KCl, 8 mM MgCl2, 12 mM Hepes, pH 7.9, 0.1 mM EDTA, 1 mM dithiothreitol, 0.5 μg of poly(dI-dC), 12% glycerol, and 25 fmol of $[^{32}P]$-end-labeled oligonucleotide probe. Probe preparation, DNA-
protein binding reactions, and polyacrylamide gel electrophoresis were performed as described by Karlen and Beard (19).

The following oligonucleotides were used as probes or competitors (one strand shown). The wild-type oligonucleotide for mDRE1, spanning nucleotides +4539 to +4579 of the mIL-5 gene, was 5'-ATG AAT GAG AGG ATG AAT GAA TGA TGG AA TGG GAG-3'. The mDRE1 deletion, substitution, and insertion mutants are given in Tables II and III. All oligonucleotides were synthesized using standard phosphoramidite chemistry by Macromolecular Resources (Colorado State University). Other competitor oligonucleotides included the AP1 consensus oligonucleotide (5'-GGG CTG CTT GAG GAA GTA TAA ACT GAC CGC CCG CGG CCC GT-3'), the Oct-1 consensus oligonucleotide (5'-TCT CGA GAA TCT ACT AGT ACA GAG-3'), the AP2 consensus oligonucleotide (5'-GAT CGA ACT GAC CGG CGG CCC GT-3') (all obtained from Promega), the c-Ets consensus oligonucleotide (5'-GGG CTG CTT GAG GAA GTA TAA GAA T-3'), and the GATA consensus oligonucleotide (5'-CAC TTG ATA ACA GAA AGT GAT AAC TCT-3') (both obtained from Santa Cruz Biotechnology). In supershift experiments, polyclonal antibodies to Jun/AP1 (reactive with all Jun members), Oct-1, Oct-2, Ets1/2, and c-Myb (Santa Cruz Biotechnology) were added to the reaction mixture and incubated for 1 h on ice prior to the addition of the probe.

**RESULTS**

Palindromic and repetitive sequences are well known to display regulatory activities in the expression of a number of genes. To identify potential regulatory elements in the mIL-5 gene, a pattern recognition search was performed in the first 2 kb of the IL-5 3'-untranslated region, commencing at the stop codon (+3772). An interesting pattern of direct repeats was found downstream of the polyadenylation site. This sequence is 40 bp long (from positions +4539 to +4578) and consists of four direct repeats of ATGAATGA (Fig. 1A). The first and last pairs of repeats are separated from the core by four nucleotides, so the whole structure is symmetrical. Using the program TFSEARCH (20), this sequence showed homology to the binding motifs of AP1, Oct-1, GATA1/2, and c-Ets (Fig. 1A). Interestingly, all these transcription factors have been described to be involved in the regulation of IL-5 (16, 21–23, 32).

**DNase I Footprinting Assay—**To determine if nuclear proteins were able to bind to the repetitive sequence, a 220-bp fragment (from positions +4585 to +5178) was cloned into the pSP72 vector and used as template in DNase I protection assays with nuclear extracts from the murine T-cell line EL4. This cell line was used in the original characterization of IL-5 and has been widely used to study gene regulation (31). Four protected regions (sites I–IV) were observed in the IL-5 sequence in experiments with unstimulated EL4 cells (Fig. 1, B and C). Alignment of protected regions from coding and non-coding strands indicated that sites I and III are complementary and cover 47 bp, including the repetitive ATGAATGA sequence in its entirety (Fig. 1A). In contrast, sites II and IV are not complementary to each other. In addition, site II overlaps part of the vector sequence and was shown to be nonspecific. This indicates that the repetitive motif (sites III/III) is involved in protein binding.

**Repetitive Motif Regulates the IL-5 Gene—**A 9.5-kb fragment of the mIL-5 gene (from positions −3861 to +5612) (Fig. 2) was cloned into the p-Poly-III plasmid with the luciferase reporter gene inserted immediately downstream of the IL-5 cap site. The protected 47 base pairs were deleted by site-directed mutagenesis from this construct, and both wild-type and deletion constructs were transiently transfected into EL4 cells. Luciferase activity levels produced in unstimulated cells transfected with the wild-type or mutant construct were not significantly different from background levels. However, following cell stimulation with PMA/cAMP, a combination known to induce optimal levels of IL-5 in EL4 cells (24), the mean of the expressed activity of the mutant construct relative to the wild-type construct in five independent experiments (Table I) was 29.85 ±
1.4%. This suggests that the deleted sequence (mDRE1) is important for PMA/cAMP-induced expression of the IL-5 gene and functions as a positive regulatory element.

Formation of Specific Complexes with mDRE1—EMSA utilizing a double-stranded radiolabeled oligonucleotide corresponding to the mDRE1 sequence was performed with nuclear extracts from unstimulated EL4 cells. Four complexes were identified, three of which (C1, C2, and C3) were competed with excess unlabeled mDRE1 (Fig. 3A, lanes 2–4), but not with an unrelated AP2 consensus sequence (Fig. 3A, lanes 5–7), suggesting specific binding. The formation of complexes C1 and C3 was reproducible in all experiments; however, the major complex, C2, seemed to be accompanied by a second, faster migrating band that was competed with unlabeled mDRE1, but not complexes C1, C2, and C3 with mDRE1 and nuclear extracts from both unstimulated (UNSTM; lane 2) and PMA/cAMP-stimulated (lane 6) EL4 cells. The AP1 consensus sequence competed for the formation of only complex C2 (lanes 3 and 7), and the c-Ets consensus sequence did not interfere with complex formation (lanes 4 and 8). B, GATA and AP2 consensus sequences did not interfere with complex formation with mDRE1 and nuclear extracts from PMA/cAMP-stimulated EL4 cells. All competitors were present at a 100-fold molar excess. C, the consensus sequence for Oct-1 competed for the formation of complexes C1, C2, and C3 with nuclear extracts from ConA/IL-2-stimulated primary T-cells. The Oct-1 consensus oligonucleotide was used at a 100-fold molar excess.

Fig. 4. Competition EMSA with the mDRE1 oligonucleotide. A, the consensus sequence for Oct-1 competed for the formation of complexes C1, C2, and C3 with mDRE1 and nuclear extracts from both unstimulated (UNSTM; lane 2) and PMA/cAMP-stimulated (lane 6) EL4 cells. The AP1 consensus sequence competed for the formation of only complex C2 (lanes 3 and 7), and the c-Ets consensus sequence did not interfere with complex formation (lanes 4 and 8). B, GATA and AP2 consensus sequences did not interfere with complex formation with mDRE1 and nuclear extracts from PMA/cAMP-stimulated EL4 cells. All competitors were present at a 100-fold molar excess. C, the consensus sequence for Oct-1 competed for the formation of complexes C1, C2, and C3 with nuclear extracts from ConA/IL-2-stimulated primary T-cells. The Oct-1 consensus oligonucleotide was used at a 100-fold molar excess.

Fig. 5. A, supershift EMSA performed with the mDRE1 oligonucleotide and nuclear extracts from unstimulated (UNSTM; lanes 1–6) and PMA/cAMP-stimulated (lanes 7–12) EL4 cells; B, supershift EMSA performed with nuclear extracts from ConA/IL-2-stimulated primary T-cells. Extracts were incubated for 1 h with antibodies specific for the transcription factors indicated, prior to addition of the labeled mDRE1 probe.
with AP2. This complex was not present in all EMSAs, and its intensity was weak and variable; so it is not further discussed. The intensity of complex C2 varied in the different assays, but it was always present. Although C1, C2, and C3 were constitutively expressed in EL4 cells, the bands increased in intensity following stimulation (Fig. 4A), but no additional complexes were formed.

Nuclear extracts from unstimulated primary T-cells did not form any complexes with mDRE1 (Fig. 3B, lanes 1–3). However, extracts from ConA/IL-2-stimulated primary cells formed three specific complexes with similar gel migration patterns obtained with extracts from EL4 cells (Fig. 3B, lanes 4–6). The intensity of complex C1 varied, but it was consistently present in all EMSAs. These results show that the nuclear factors binding to mDRE1 are constitutively expressed in EL4 cells and increase after stimulation, whereas in primary T-cells, there is no detectable constitutive expression, but they are strongly induced after cell activation.

Oct-1 and Oct-2 Bind to mDRE1—As described above, a series of possible binding sites for known transcription factors were present in the mDRE1 sequence. To determine, if any of these were forming the specific complexes with mDRE1, consensus sequences were tested for the ability to inhibit complex formation in EMSA.

As shown in Fig. 4A (lanes 2 and 6), only the Oct-1 consensus sequence was able to compete for the binding of the specific complexes C1, C2, and C3 from both unstimulated and PMA/cAMP-stimulated EL4 cells. The AP1 consensus oligonucleotide was able to compete for the binding of complex C2 (Fig. 4A, lanes 2 and 7), whereas c-Ets (lanes 4 and 8) and GATA (Fig. 4B, lane 2) did not compete for any of the complexes. When extracts from ConA/IL-2-stimulated primary T-cells were tested, the Oct-1 consensus sequence competed for the binding of C1, C2, and C3 (Fig. 4C).

To further characterize the proteins that bind to mDRE1, we performed supershift assays. Addition of anti-Oct-1 antibody clearly abolished the formation of only complex C1 with nuclear extracts from either stimulatory condition (Fig. 5A, lanes 2 and 8). Anti-Oct-2 antibody eliminated only the C3 band (Fig. 5A, lanes 3 and 9), whereas anti-Jun antibody interfered with the formation of complex C2, but did not totally abolish it. Antibodies against Ets1/2 and c-Myb had no effect (Fig. 5A, lanes 4–6 and 10–12). These results indicate that Oct-1 forms C1, Jun forms C2, and Oct-2 forms C3.

Similar results were obtained when nuclear extracts from ConA/IL-2-stimulated primary T-cells were used. As shown in Fig. 5B, anti-Oct-1 and anti-Oct-2 antibodies interfered with the binding of only complexes C1 and C3, respectively, confirming that the same proteins form complexes with mDRE1 in both the cell line and primary T-cells.

Oct-2 Binds to the Central Repeats of the mDRE1 Sequence—To determine if the Oct-1 and Oct-2 transcription factors were binding to the same motifs in the mDRE1 sequence, oligonucleotides containing different numbers of repeats (Table II), with or without a flanking sequence, were used as competitors in EMSA. The results (Fig. 6A) show that the minimal sequence necessary for competition with complex C3-Oct-2 contains two ATGAATGA repeats and that a flanking sequence is also necessary (Oligo5). This sequence was called the “minimal binding sequence” (MBS). The nature of the flanking sequence is probably unimportant, as an oligonucleotide containing the same flanking sequence, but one copy of ATGAATGA (Oligo5), could not compete for the formation of any of the complexes (data not shown). None of the oligonucleotides were able to compete for the formation of complex C1-Oct-1 with labeled mDRE1, showing that Oct-1 and Oct-2 bind to different motifs in the mDRE1 sequence.

To determine which nucleotides were important for formation of the major complex C3-Oct-2, substitution mutations were generated within the MBS. These mutants (Table III)
Octamer Factors Bind to a 3′-Element in the mIL-5 Gene

were used as competitors in EMSA using radiolabeled mDRE1 as probe. The substitution of TG for CT in mut1 totally abolished the capacity of the oligonucleotide to compete for complex C3-Oct-2 (Fig. 6B, lanes 2–4). Similarly, the substitution of TG for CT in mut4 also abolished the capacity of the oligonucleotide to compete for complex C3-Oct-2 (data not shown). The same substitution on mut9 (Fig. 6B, lanes 6–8) and mut3 (data not shown), however, did not interfere with the ability of the sequence to compete for C3-Oct-2. As shown in Fig. 6B (lanes 6–8), mut9 was able to compete for the formation of complex C1-Oct-1 with mDRE1. A computer analysis of this sequence showed that an 80% homologous Oct-1-binding motif had been created in this mutant, which probably explains its capacity to compete for complex C1-Oct-1. The substitution of a pair of AA for CT in mut7, mut8, and mut10 also abolished the capacity of these mutants to compete for binding of complex C3-Oct-2, whereas the same substitutions in mut11 had no effect (data not shown).

Taken together, these results suggest that the important nucleotides for protein recognition are symmetrically distributed within the sequence (Fig. 6C) and that the substitution of only two of them is enough to interfere with protein binding. The nature of the flanking sequence is unimportant, as mutations in this region did not alter the capacity to compete for formation of complex C3-Oct-2 (data not shown).

To determine if the spacing between the repeats was important for complex formation, oligonucleotides with two, four, or six thymines inserted between the central repeats (Table III) were produced. Insertion of two nucleotides between the repeats (mut12) was enough to interfere with protein binding to the sequence, as the mutant was not able to compete for formation of complex C3-Oct-2 with labeled mDRE1 (Fig. 6B, lanes 9–11). The same was true with insertion mutants mut13 and mut14. These results show that spacing between the repeats is of importance for protein recognition of the element, as any disruption of its continuity abolishes the capacity of the mutants to form complex C3-Oct-2.

**DISCUSSION**

The expression of cytokine genes is regulated at the transcriptional level. Although the best known transcription regulatory elements are present in the 5′-flanking region, a series of regulatory elements have been described in the 3′-end of genes. The work presented here describes the finding of a novel 3′-flanking element (mDRE1) that plays a positive role in the regulation of the murine IL-5 gene.

Data base analysis of a 2-kb fragment of the 3′-flanking region of the mIL-5 gene revealed the presence of a 40-bp-long repetitive sequence that consists of four direct repeats of ATGAAATGA distributed in a symmetrical manner. This sequence was shown to be protected in *in vitro* DNase I footprinting assays with nuclear extracts from EL4 cells. When the sequence corresponding to mDRE1 was deleted from the complete 9.5-kb mIL-5 gene, the expression of the luciferase reporter gene was significantly decreased in comparison with the wild-type construct when expressed in EL4 cells. This indicates that mDRE1 plays a positive role in the expression of mIL-5.

EMSAs using oligonucleotides corresponding to the mDRE1 sequence showed the formation of at least four complexes, three of which (C1, C2, and C3) were specific and consistently formed. Competition analysis indicated that complexes C1 and C3 belong to the octamer family of transcription factors, and supershift assays showed that Oct-1 and Oct-2 were forming complexes C1 and C3, respectively. The formation of complex C2 could be competed with both Oct and AP1 consensus sequences, and the addition of anti-Jun antibody interfered with, but did not abolish, the formation of this complex. Thus, some of the proteins forming complex C2 may belong to the Jun family.

Although Oct-1 and Oct-2 are not present in nuclear extracts from unstimulated primary T-cells, they are constitutively expressed at a low level in EL4 cells as described previously (25, 26). In both cases, they are induced after stimulation. Nuclear extracts from stimulated primary T-cells displayed the same pattern of complex formation as those from EL4 cells, and competition and supershift assays indicated that Oct-1 and Oct-2 formed complexes C1 and C3, respectively.

Competition with oligonucleotides containing different numbers of ATGAAATGA repeats indicated that Oct-1 and Oct-2 bind to different motifs in mDRE1. Oct-2 binds to the central repeats, whereas Oct-1 appears to bind to the flanking region. Mutations in the MBS showed that the nucleotides important for protein recognition are distributed symmetrically in the sequence and that spacing between the repeats is critical for protein binding, as any disruption of its continuity interfered with the capacity of the mutants to compete for formation of the Oct-2 complex.

Recent data from our laboratory revealed that the Oct-1 and Oct-2 transcription factors also bind to the conserved lymphokine element-0 sequence located in the 5′-flanking region of the IL-5 gene. The fact that octamer factors, and in particular Oct-2, can be found to bind to sequences in the 5′- and 3′-flanking regions of the IL-5 gene suggests the possibility that they might work together, in the context of the whole IL-5 gene, to regulate the expression of the gene. One possibility is that the octamer factors play a role in the remodeling of the chromatin in the IL-5 locus in a similar way as STAT6 binds to both the promoter (27) and the 3′-flanking region of the IL-4 gene (14). In this case, it has been proposed that the binding of STAT6 is necessary for long-range remodeling of the IL-4 locus during Th2 differentiation (28).

In conclusion, this work describes the finding of a novel downstream regulatory element (mDRE1) that plays a positive role in the regulation of the IL-5 gene. The activity of mDRE1 seems to be regulated by octamer factors. Thus, further investigation is necessary to determine the mechanisms of action of these factors in the regulation of the IL-5 gene.

**TABLE III**

Mutant oligonucleotides used in EMSA

| Mutant        | Sequence                                |
|---------------|-----------------------------------------|
| Substitution mutants | GAGGATGATGGAATGGAATGGAATAA |
| mut1          | GAGGATGATGGAATGGAATGGAATAA |
| mut2          | GAGGATGATGGAATGGAATGGAATAA |
| mut3          | GAGGATGATGGAATGGAATGGAATAA |
| mut4          | GAGGATGATGGAATGGAATGGAATAA |
| mut5          | GAGGATGATGGAATGGAATGGAATAA |
| mut6          | GAGGATGATGGAATGGAATGGAATAA |
| mut7          | GAGGATGATGGAATGGAATGGAATAA |
| mut8          | GAGGATGATGGAATGGAATGGAATAA |
| mut9          | GAGGATGATGGAATGGAATGGAATAA |
| mut10         | GAGGATGATGGAATGGAATGGAATAA |
| mut11         | GAGGATGATGGAATGGAATGGAATAA |
| Insertion mutants | GAGGATGATGGAATGGAATGGAATAA |
| mut12         | GAGGATGATGGAATGGAATGGAATAA |
| mut13         | GAGGATGATGGAATGGAATGGAATAA |
| mut14         | GAGGATGATGGAATGGAATGGAATAA |

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