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# The conserved 3′-flanking sequence, AATGGAAATG, of the wheat histone H3 gene is necessary for the accurate 3′-end formation of mRNA

Norihiro Ohtsubo and Masaki Iwabuchi*
Department of Botany, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan

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## ABSTRACT

**We examined the 3′-flanking regions required for accurate 3′-end formation of wheat histone H3 mRNA using gene expression in transformed sunflower cells. The introduction of mutations into the conserved sequence AATGGAAATG in the 3′-flanking region of plant histone genes, located 22 bp upstream from the polyadenylation site of the wheat H3 gene (TH012), completely abolished the 3′-end formation of mRNA at the authentic 3′ end without affecting the transcription efficiency. However, a 0.8 kbp sequence containing this motif could not produce a normal 3′ end when joined to the 3′ end of the nopaline synthase (NOS) gene instead of its 3′ sequence. The results indicated that this conserved sequence is necessary but not sufficient for the 3′-end formation of H3 or NOS mRNA. Deletion of a 59 bp sequence, located 19 bp upstream from the AATGGAAATG sequence, also reduced the 3′-end formation efficiency by a factor of 10, compared with the efficiency in wild-type gene. We concluded that 3′-end formation of wheat histone H3 mRNA is regulated by multiple sequences including the AATGG-AAATG motif.**

## INTRODUCTION

The cellular levels of histone mRNA are controlled throughout the cell cycle by factors such as the transcription rate, RNA processing, including 3′-end formation, mRNA stability, and mRNA transport from the nucleus to cytoplasm (1–4). With respect to the 3′-end formation of histone mRNA, characteristic features of animal histone genes have been reported. A major class of histone genes is the replication-dependent type, the expression of which is coupled with the cell cycle (1, 5). Animal histone genes of this type lack intervening sequences and the canonical polyadenylation signal sequence AATAAA, and have no poly(A) tails in their mRNAs. Instead, some animal histone genes have unique structures at their 3′ regions that can produce mRNA with a stem-loop structure consisting of a 6-base stem and a 4-base loop, followed by a purine-rich sequence downstream (6, 7). The 3′-end formation of animal histone mRNA also requires the U7 small ribonucleoprotein (snRNP), hairpin-binding factor (HBF) and heat-labile factor (HLF) (8–12). The stem-loop structure in the 3′ region participates in not only 3′-end formation but also in mRNA metabolism and transport to the cytoplasm. When the cell cycle progresses from S to G2, or when DNA synthesis is inhibited, histone mRNA is rapidly degraded (13–15), which requires the hairpin structure (16, 17). Sun *et al.* (18) reported that removing the stem-loop structure from histone genes resulted in increased amount of transcripts in the nucleus. Thus, it is generally thought that the stem-loop structure participates in the cell cycle-specific regulation of histone mRNA in animal cells.

Although little is known about 3′-processing of histone pre-mRNA in higher plants, several lines of evidence have shown that such 3′-processing is distinct from that of animals. A sequence comparison has suggested that the 3′-untranslated region of plant histone genes is much longer than that of animals and it has no typical T-hyphenated palindromic structure (for review, see Ref. (19) ). In addition, plant histone mRNAs have a poly(A) tail at their 3′ ends (20–24) without the typical poly(A) signal sequence AATAAA (25). Chaubet *et al.*, who first found that maize histone H3 and H4 mRNAs contain poly(A) sequences at their 3′-terminus, have shown that a consensus sequence, A/G-ATG(G)AAATG, is located 25 nt upstream from the poly(A) addition sites of the both genes (20, 26). Analogous sequences also have been found in *Arabidopsis* (21), alfalfa (22, 23), rice (27) and wheat (24). Thus, it is speculated that this conserved sequence is a polyadenylation signal for plant histone mRNAs.

In the present study, we examined the role of the conserved sequence AATGGAAATG of the 3′-untranslated region of the wheat histone H3 gene (TH012) in the 3′-processing of pre-mRNA using transformed sunflower cells. Mutational analyses revealed that the conserved sequence participates in 3′-end formation of H3 mRNA, and that both 5′ and 3′ sequences flanking the polyadenylation site are required.

*To whom correspondence should be addressed

## MATERIALS AND METHODS

### Construction of H3 mutant genes and the NOS/H3 chimeric gene

A *Hin*dIII fragment (from −185 to +1403 relative to the cap site), prepared from the wheat histone H3 gene (TH012, Ref. (28)), was cloned into the *Hin*dIII site of the M13mp19 replicative form to obtain M13mp19H3, from which the H3 mutants were constructed.

Two H3 mutants, H3dpm and H3dpmR, in both of which the conserved sequence AATGGAAATG of the wild-type H3 gene was changed, were prepared by oligonucleotide-directed mutagenesis according to Kunkel (29). The H3dpm mutant, having two point mutations at positions +636 and +639, was constructed using the oligonucleotide 5′-TCAGCAATTG-ACATGAAAAA-3′ as a mutagenic primer. To introduce the conserved sequence into the region from +512 to +521, H3dpmR that was point-mutated at positions +512, +513, +520 and +521 of H3dpm was constructed using the procedure described above except that M13mp19H3dpm DNA was used as a template and the oligonucleotide 5′-CTGAGTTCAT-CAATGGAAATGAGTGGTGTTTCAGA-3′ was the primer.

A chimeric gene, NOS/H3, was prepared by fusing the *Sst*II−*Bam*HI fragment from the NOS gene (30) to the *Kpn*I−*Hin*dIII fragment (from +615 to +1403) from the H3 gene after adding a *Bam*HI linker to the *Kpn*I end, although the *Sst*II site of the NOS fragment was replaced by a *Hin*dIII linker.

To construct the internal deletion mutant, IntΔ1, of the H3 gene, a *Hin*dIII−*Bam*HI fragment (from −185 to +555), prepared from a 3′-deletion mutant (3′Δ+555), was fused to a *Bam*HI−*Hin*dIII fragment (from +615 to +1403) from the NOS/H3 gene.

The internal deletion mutant IntΔ2 of the H3 gene, was constructed by oligonucleotide-directed mutagenesis using M13mp19H3 as a template and the oligonucleotide 5′-GAGCC-TTTCCTCTTGAATACCTCTTTAAGCCGCG-3′ which hybridizes simultaneously to the regions from +703 to +720 and from +761 to +777 as the primer.

These modified H3 or NOS/H3 constructs were subcloned into the *Hin*dIII site of pBN19 (31) which is commonly used as an intermediate vector in the *Agrobacterium*-mediated gene transfer system. This vector also contained the NOS gene as an internal control.

### Transformation of sunflower cells

Wild type, mutant and chimeric genes were all introduced into sunflower (*Helianthus annuus*) cells using the pBN19-mediated gene transfer system *via Agrobacterium tumefaciens* strain C58ClCm$^r$, as previously described (32).

### Probes for S1 protection assay

Single-stranded DNA probes were prepared for S1 protection assays using M13 recombinant phages. The probes mpH35 and mpH33, which were used to detect the 5′ or 3′ ends of the wheat H3 transcript, were prepared as described elsewhere (32). The probe mpNOS, for detecting of the 3′ end of the NOS transcript, was also obtained as described previously (31). To identify the 3′ ends of transcripts from the H3 mutants, the *Pst*I−*Pst*I region of mpH33 was replaced by its corresponding region in H3dpm, dpmR, IntΔ1 and IntΔ2, to yield mpDPM, mpDPMR, mpINTΔ1 and mpINTΔ2, respectively. Similarly, to prepare a probe with which to identify the 3′ end of NOS/H3 transcript, a *Hin*cII−*Pst*I fragment (315 bp in length) in the 3′-coding and non-coding sequences of the NOS gene and a 517 bp sequence downstream from +615 of the H3 gene was cloned into M13mp18 to obtain mpNH.

### RNA preparation and S1 protection assay

Total cellular RNA was prepared from transformed sunflower calli as described elsewhere (31). Poly(A)$^+$ RNA was prepared by means of oligo d(T)-cellulose chromatography (33). To quantify transcripts with the normal 3′ end, we performed S1 protection analysis under the conditions described previously (31) except for the conditions under which S1 nuclease digestion proceeded (270 units, 4 min.). Transcripts from the H3 genes, NOS/H3 gene and NOS gene were quantified by measuring the radioactivity of each S1-protected band on dried polyacrylamide gels using an AMBIS Radioanalytic Imaging System (AMBIS Systems Inc.). Processing efficiency was determined by comparing the amounts of processed transcripts from the wild-type H3 gene and the mutants after correcting them for variations in the amount of the NOS transcript.

## RESULTS

### The wheat H3 mRNA synthesized in transformed sunflower calli is polyadenylated

We reported that mRNA from a wheat histone H3 gene, TH012, is accurately transcribed in transformed sunflower cells (32). To examine whether the machinery for 3′-end formation including polyadenylation of mRNA functions correctly in the transformants, we performed S1-protection analysis of RNAs from transformed cells. The total RNA of the transformants harboring the wild-type wheat H3 gene, was fractionated into poly(A)$^+$ and poly(A)$^-$ RNAs by oligo(dT) cellulose chromatography, then defined amounts of the RNA were hybridized with the 3′ probe that detected the normal 3′ end of the H3 mRNA. As shown in Figure 1B, most of the H3 mRNA was present in the poly(A)$^+$ fraction. However, a small amount of readthrough transcripts was also present in the poly(A)$^+$ fraction. The results may be due to the polyadenylation of readthrough transcripts initiated from the vector (discussed below), which we reported in our previous study (31). Thus, the results suggest that the machinery for 3′-processing of pre-mRNA and for poly(A) addition in dicotyledonous plants forms normal 3′-ends in monocotyledonous histone mRNA.

### A 3′-downstream conserved sequence is required for 3′-end formation in wheat histone H3 mRNA

In the wheat H3 gene (TH012), the AATGGAAATG sequence lies between +633 and +642, relative to the transcription initiation site, 22 bp upstream of a poly(A) addition site (Figures 1A and 2A). To examine whether this motif is involved in 3′-end formation, we performed mutational analyses of the conserved sequence.

We first tested the H3 mutant H3dpm, which has two base-substitutions of G→T and A→C at the well-conserved positions +636 and +639, respectively (Figure 2A; also see Ref. 24 ). Transcripts from this mutant were analyzed by means of the S1-protection assay. Figure 2B shows that the amount of the H3dpm transcript with the normal 5′ end is nearly equal to that of the wild-type gene transcript, as determined with the 5′ probe (lanes 1 and 2), indicating that disruption of the conserved sequence did not influence the transcription initiation of this

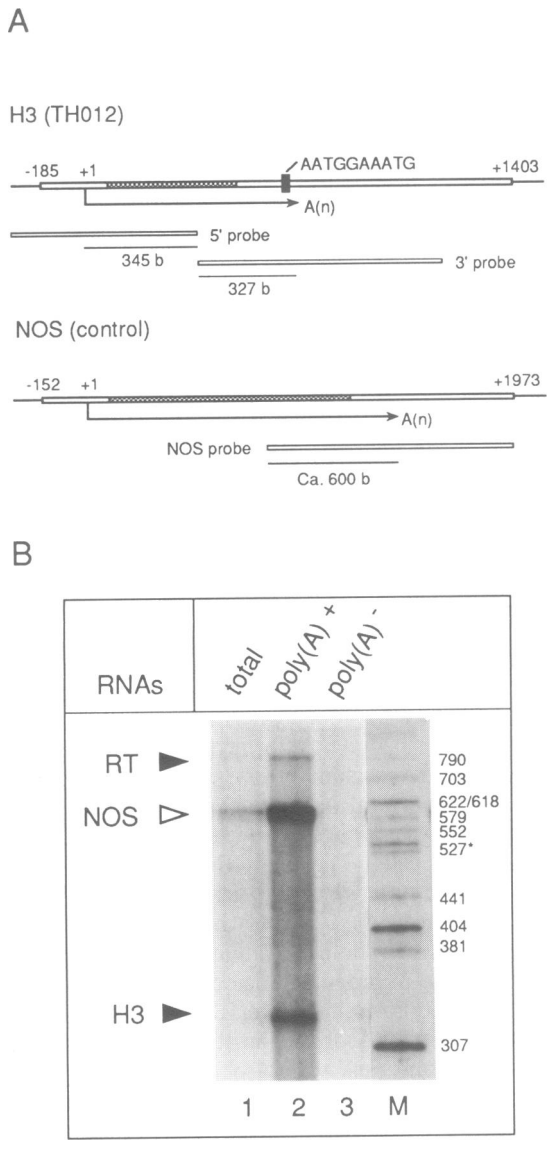


**Figure 1.** S1 analyses of polyadenylated wheat H3 and NOS mRNAs from transformed sunflower cells. **A.** Schematic representation of the structures of the H3 and the NOS gene constructs used for the transformation and the probe DNA in S1 assay. The numbers above each construct represent positions of nucleotides relative to the cap site (+1). The hatched part in each construct indicates the protein-coding region. The filled box represents the putative polyadenylation signal AATGGAAATG of plant histone genes. The bent arrows below the constructs represent the predicted transcripts. Probes for S1-assay are shown below the gene constructs. The 5′ and 3′ probes are used to detect the 5′ and 3′ ends of the H3 transcript, respectively. The NOS probe is to detect the 3′ end of the NOS transcript. Thin lines below the probes indicate the predicted length of S1-protected DNA fragments. **B.** S1-protection pattern of the transcripts from the wheat H3 and the NOS genes. Fifty micrograms of total RNA (lane 1), poly(A)$^-$ RNA (lane 3) or 5 μg of poly(A)$^+$ RNA (lane 2) of transformed sunflower cells carrying the H3 gene was hybridized with a mixture of the 3′ and NOS probes, then treated with S1 nuclease. The S1-protected DNA fragments were separated on a 5% polyacrylamide-9M urea gel. The filled arrowheads indicate the S1-protected 3′ probe fragments corresponding to the H3 transcript with a normal 3′-end (H3) or readthrough transcript (RT). The open arrowhead indicates the S1-protected NOS probe fragment. A mixture of *Hpa*II-digested pBR322 and *Taq*I-digested M13mp19 replicative form DNAs was used as a size marker (lane M). The lengths of each marker fragments are shown on the side. Each strand of a 527 bp fragment derived from the *Hpa*II-digested pBR322 DNA appeared as two separate bands (527 with a small asterisk).

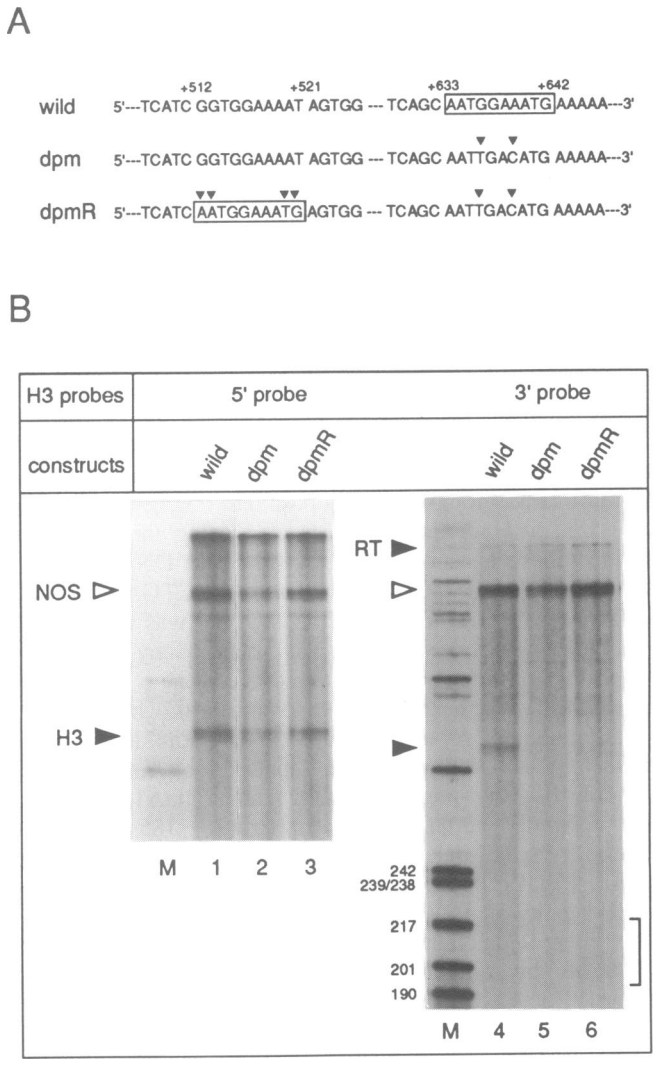


**Figure 2.** S1-analyses of total RNAs from sunflower cells transformed with each of the H3 mutants, dpm and dpmR. **A.** Nucleotide sequences around the substituted bases in the 3′-untranslated region. Gene constructs of dpm and dpmR are identical to that of the wild type (Figure 1A) except for the base substitutions. The sequences that fit the consensus sequence of the polyadenylation signal of plant histone genes are boxed. Substituted bases of the mutant genes are marked by filled triangles. **B.** S1-protection patterns of the H3 transcripts from the wild-type gene and the mutants. Fifty micrograms of total RNA from transformed sunflower cells carrying the dpm or dpmR gene was assayed as described in Materials and Methods. The probes used to detect the H3 mRNA with normal 5′ or 3′ ends and the NOS mRNA with a normal 3′ end were the same as shown in Figure 1A, except that the 3′ probe was base-substituted to completely hybridize with the dpm or dpmR transcript. The filled arrowheads indicate the S1-protected 5′ and 3′ probe fragments corresponding to the H3 transcripts with normal 5′ or 3′ ends (H3), or the readthrough transcript (RT). The open arrowheads indicate the S1-protected NOS probe. A mixture of *Hpa*II-digested pBR322 and *Taq*I-digested M13mp19 replicative form DNAs was used as a size marker (lane M). The lengths of marker fragments less than 300 bp are shown on the side. The bracket beside lane 6 indicates the region where the signal corresponding to the predicted processed product (206 nt or so) from dpmR should appear.

mutant. When we used the 3′ probe in the S1-assays however, no bands corresponding to the normally processed H3 mRNA appeared (Figure 2B, lanes 4 and 5). This indicates that the processing activity was completely abolished by introducing the base-substitutions within the AATGGAAATG sequence. Because there was no change in the steady-state level of mRNA when

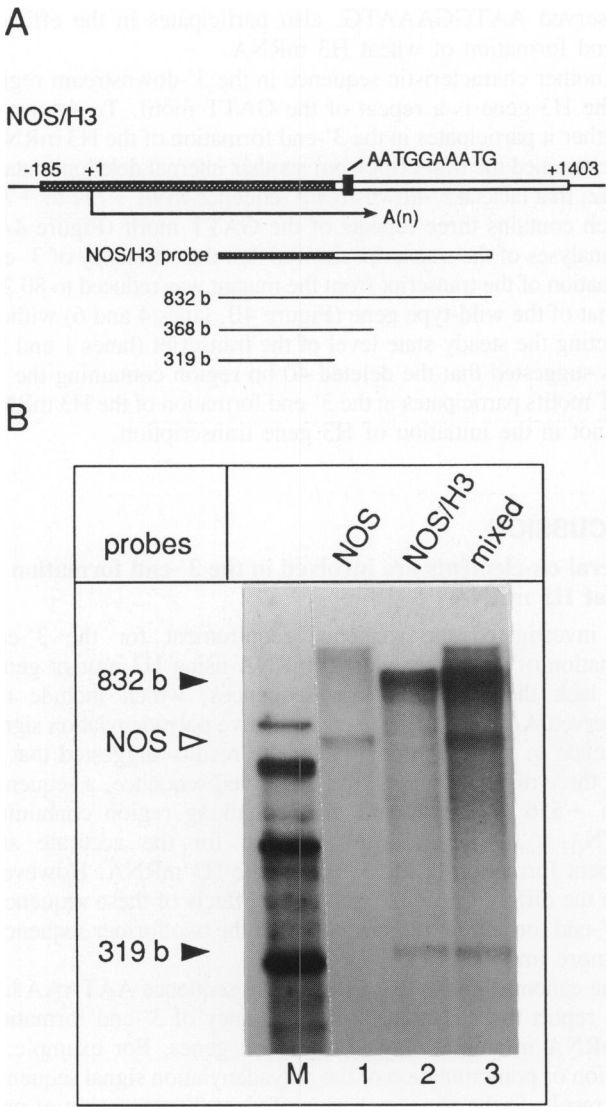


**Figure 3.** S1-analyses of total RNA from sunflower cells transformed with the NOS/H3 chimeric gene. **A.** Schematic structure of the chimeric gene construct and the probe used in the S1 assay. Details of the chimeric gene are described in the text. The numbers above the gene construct represent positions of nucleotides relative to the cap site (+1). The hatched and open parts represent the H3 and the NOS sequences, respectively. The filled box represents the putative polyadenylation signal AATGGAAATG. The bent arrow below the construct represents the predicted transcript. The NOS/H3 probe below the gene construct is used to detect the 3′ end of the NOS/H3 transcript. Thin lines below the probe indicate the predicted lengths of the S1-protected NOS/H3 probe corresponding to the readthrough NOS/H3 (832 b), processed NOS/H3 (368 b), or NOS transcripts (319 b). **B.** The S1-protection pattern of the transcript from the NOS/H3 chimeric gene. The S1-assay was performed as described elsewhere (31), using the NOS (lane 1), NOS/H3 (lane 2) or mixed probes (lane 3). The filled and open arrowheads indicate the S1-protected bands obtained using the NOS/H3 and the NOS probes, respectively. The NOS probe did not hybridize to the NOS/H3 transcripts, and the 319 b band represents the hybridization of NOS/H3 probe to the NOS transcript. A mixture of *Hpa*II-digested pBR322 and *Taq*I-digested M13mp19 replicative form DNAs was used as a size marker (lane M).

assayed with the 5′ probe, we speculated that primary transcripts were readthrough to a far-downstream region, probably to the vector, and not processed to mature mRNA with a normal 3′ end. Thus, the above results suggest that the conserved sequence participates in the normal 3′-end formation of H3 mRNA.

The next question was whether the conserved sequence alone is sufficient for 3′-end formation. Thus, we examined the transcript from the mutant, dpmR (see Figure 2A), which has four point mutations within 121 bp upstream region of the conserved sequence to reconstruct the AATGGAAATG sequence. If this reconstructed sequence works as a processing signal, transcripts of 206 nt (or so) in length should be detected. In Figure 2B, however, no such dpmR mRNA was observed (lane 6), suggesting that the conserved sequence alone is insufficient to produce the 3′-end formation and that *cis*-acting sequence(s) other than this sequence are required.

### A far-upstream sequence of the polyadenylation site is important for the efficient 3′-processing of H3 pre-mRNA

We previously reported (31) that a relatively long 3′ sequence of the wheat H3 gene, which is located downstream of a region corresponding to the 3′ end of the mRNA and which contains multiple GATT motifs ($TTTN_{(13-16)}GATT$), is necessary for accurate 3′-end formation, and we assumed that the multiple G-ATT motifs participate in 3′-processing of the primary transcript. To obtain further evidence for the involvement of the long downstream sequence in 3′-end formation, we examined the transcript of a NOS/H3 chimera, which was constructed by joining the NOS promoter region (ca. 1 kbp) with the 5′-portion (65%) of the NOS coding region to the 3′ sequence (from +615 to +1403) of the H3 gene (Figure 3A). This portion of the H3 gene contains the conserved sequence AATGGAAATG, which corresponds to the 3′ end of the mRNA, and the GATT motifs. Therefore, if transcripts from the NOS/H3 gene are correctly processed to position +664 of the H3 sequence, an S1-protected band of 368 nt should appear. However, S1 analyses showed that the chimeric gene did not produce any transcripts with the authentic 3′ end of the H mRNA and that the transcript was hybridized to the entire region of the NOS/H3 probe (832 nt long; Figure 3B). This indicated that the chimeric gene was transcribed to a far-downstream region as observed in H3dpm. The S1-protected 319 nt band was derived from NOS transcripts hybridized with the NOS/H3 probe and did not represent correctly processed mRNA. Therefore, these results imply that the sequence from +615 to +1403, containing the AATGGAAATG motif, is in itself insufficient to form the authentic 3′ end of the H3 mRNA.

The above results suggest that a far-upstream sequence of the polyadenylation site also participates in the efficient formation of the 3′ end of the H3 mRNA. Therefore, we examined the 3′-end formation of the transcript using an internal deletion mutant IntΔ1, lacking the 59 nt sequence from +556 to +614 (Figure 4A) which corresponded to that (from 50 to 108 nt) upstream of the poly(A) addition site. This deleted sequence contains a TG-rich sequence, 5′-TGGTGGGTGGATGTGTTATG-3′, which is considered to participate in correct 3′-end formation of the mRNA in some higher plants. If transcription of IntΔ1 occurs normally, the length of the transcript from the mutant should be 59 nt shorter than that from wild-type H3 gene. The S1 analyses showed that the proportion of the transcript with an authentic 3′ end among all transcripts from the mutant gene was reduced to 8.9%, compared with that of the wild-type gene transcript (Figure 4B, lanes 4 and 5). In contrast, the steady-state levels of total transcripts were almost equal between the mutant and wild-type genes (Figure 4B, lanes 1 and 2). Therefore, the above results suggests that a sequence upstream of the

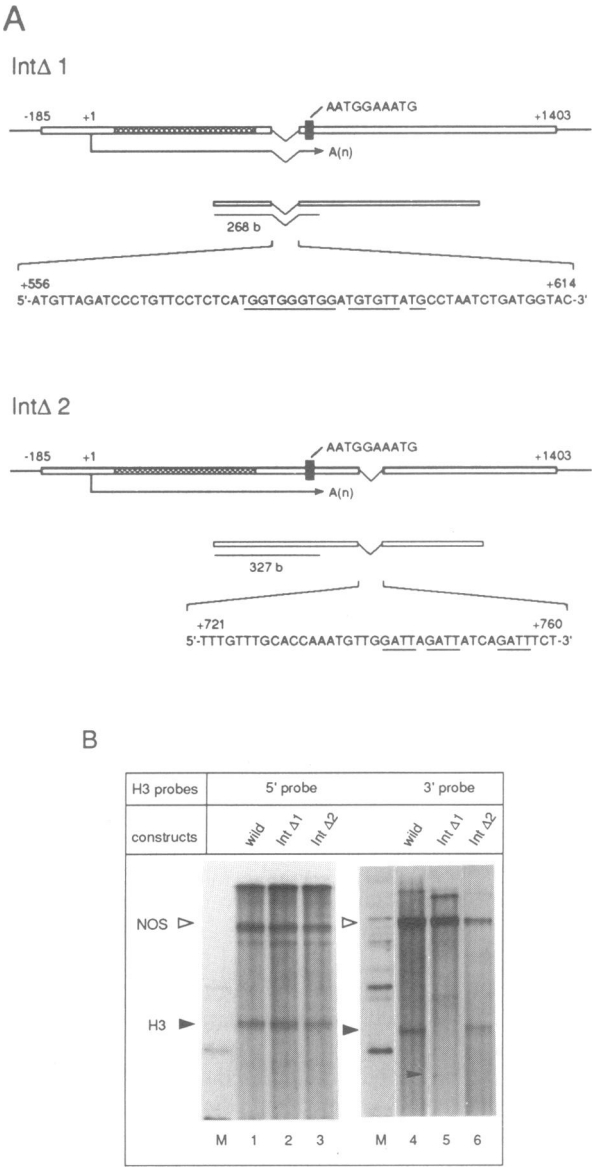


**Figure 4.** S1-analyses of total RNAs from sunflower cells transformed with the internal deletion H3 mutants, IntΔ1 and IntΔ2. **A.** Schematic representation of the structures of the mutant genes and the 3′ probes used for the S1-assay. Numbers above the constructs represent positions of nucleotides relative to the cap site (+1). The hatched part and the filled box represent the protein-coding region and the putative polyadenylation signal AATGGAAATG of plant histone genes, respectively. The bent arrows below the constructs represent the predicted transcripts. The 3′ probes used to detect the mutant H3 mRNAs with a normal 3′ end are shown below the constructs. The thin lines below the probes indicate the predicted length of S1-protected DNA fragment. The deleted sequences are shown below the probes. The TG-rich sequences in the region from +555 to +614 and the 3 repeats of the GATT motif in the region from +721 to +760 are underlined. **B.** S1-protection patterns of the H3 transcripts from the internal deletion mutants. Fifty micrograms of total RNAs of transformed sunflower cells carrying the IntΔ1 or IntΔ2 gene were assayed as described in Materials and Methods. The probes used to detect the H3 mRNA with a normal 5′ and 3 end and the NOS mRNA with a normal 3′ end are shown in Figure 1A, except that the 3′ probe was modified to completely hybridize with the IntΔ1 or IntΔ2 mutant transcript. The filled and open arrowheads indicate S1-protected bands of the H3 mRNA with normal 5′ or 3′ ends and the NOS mRNA with a normal 3′ end, respectively. The small filled arrowhead (lane 5) indicates a band of the IntΔ1 mRNA, which has a normal 3′ end but is 59 nt shorter due to the internal deletion of the transcribed region of the H3 gene. A mixture of *Hpa*II-digested pBR322 and *Taq*I-digested M13mp19 replicative form DNAs was used as a size marker (lane M).

conserved AATGGAAATG, also participates in the efficient 3′-end formation of wheat H3 mRNA.

Another characteristic sequence in the 3′-downstream region of the H3 gene is a repeat of the GATT motif. To determine whether it participates in the 3′-end formation of the H3 mRNA, we examined the transcript from another internal deletion mutant, IntΔ2, that lacks a 3′-downstream sequence from +721 to +760 which contains three repeats of the GATT motif (Figure 4A). S1 analyses of the transcript showed that the efficiency of 3′-end formation of the transcript from the mutant was reduced to 80.2% of that of the wild-type gene (Figure 4B, lanes 4 and 6) without affecting the steady-state level of the transcript (lanes 1 and 3). This suggested that the deleted 40 bp region containing the G-ATT motifs participates in the 3′-end formation of the H3 mRNA but not in the initiation of H3 gene transcription.

## DISCUSSION

### Several *cis*-elements are involved in the 3′-end formation of wheat H3 mRNA

We investigated the sequence requirement for the 3′-end formation of wheat histone H3 mRNA using H3 mutant genes that lack the 3′-downstream sequences, which include the conserved AATGGAAATG, as a putative polyadenylation signal sequence in plant histone genes. The results suggested that at least three distinct regions, the conserved sequence, a sequence from +556 to +614, and the 3′-flanking region containing $TTTN_{(13-16)}GATT$, are all required for the accurate and efficient formation of the 3′ end of the H3 mRNA. However, from the difference in the mutational effects of these sequences on 3′-end formation, we speculate that the two former sequences are more important.

The canonical polyadenylation signal sequence AATAAA has been report to be related to the efficiency of 3′-end formation of mRNA in animal class II and viral genes. For example, a deletion or point mutation of the polyadenylation signal sequence (34) results in the abnormal or inefficient 3′-processing of pre-mRNAs (35, 36). The A/GATG(G)AAATG sequence in the 3′ region of plant histone genes is similar to the canonical polyadenylation signal AATAAA. In contrast to the strict requirement of the AATAAA sequence for polyadenylation of mRNAs in animal cells, the polyadenylation signal for plant gene transcripts may not always be restricted to this sequence (25). In fact, it has been suggested that AATGAA and AATGGAAA function as polyadenylation signals for the *Petunia* Cab gene (37), the 27 kDa maize zein gene (38), and the pea *rbcS* gene (39). Thus, the conserved sequence in the 3′-untranslated region of plant histone genes might be regarded as a variation in the canonical polyadenylation signal.

Mogen *et al.* (40) studied the upstream sequences of the poly(A) addition site of some plant genes in relation to the 3′-processing of pre-mRNA. They showed that sequences other than the AATAAA motif are required for the efficient formation of the 3′ end of the transcript of the CaMV 19S/35S and pea *rbcS* genes. They speculated that multiple *cis*-elements which differ from the AATAAA motif are required for the 3′-end formation of plant gene transcripts (40).

A TG-rich sequence, involved in 3′-end formation of the H3 mRNA, has been identified upstream of the polyadenylation site in some plant genes (40–42). Disruption of the TG-stretch (10 nt) in the 3′-downstream region of the *rbcS*-E9 gene results in

**Table 1.** TG-rich sequences located in the 3′ untranslated region of plant histone H3 and H4 genes.

| Clone | bp* | sequence | reference |
|---|---|---|---|
| Wheat | | | |
| H3 TH012 | -54/-35 | TGGTGGGTGGaTGTGTTaTG | Tabata et al. 1984 |
| H4 TH011 | -66/-36 | TTGTTTaGTTcGcTaTTTccTcTGTaGTTTG | Tabata et al. 1983 |
| H3.TH081 | -50/-27 | TGTTaTGTTGTaGTGTcTGaTGTT | |
| H4.TH091 | -45/-26 | TGGGGTTcaGTGGGcTGTGT | |
| Corn | | | |
| H3.C2 | -60/-39 | TTTTcTacTcTGcTTGTTcTTG | Chaubet et al. 1986 |
| H3 C3 | -94/-44 | TTcTTGGaaGTTTGTacTaGTGGTcGGGTcGTGTGGaaGGaGTGGTGTaGT | Gigot et al. 1987 |
| H3 C4 | -71/-35 | GTTGTTcaGTTGcTGGGTaaccTGaTTGTTTaTcTTG | Chaubet et al. 1986 |
| H4 C7 | -36/-14 | TTGTacTGcaTTTcTTTGTTcTG | Philips et al. 1986 |
| H4 C13 | -36/-14 | TTGTacTGcaTTTcTTTGTTcTG | Gigot et al. 1987 |
| H4 C14 | -36/-23 | GTaGTTTcTGTTGT | Philips et al. 1986 |
| Arabidopsis | | | |
| H3 A713 | -44/-15 | TTTcGTGTTTTaGGGTTGGaTTaGGTTTTG | Chaboute et al. 1987 |
| H3 A725 | -63/-40 | TTGTTTGGTTaaTTTGTGcTaGTT | Chaboute et al. 1987 |
| H4 A748 | -86/-35 | TTTTGTaTTGGGTTTGGcGTTTaGTaGTTTGcGGTaGGTTcTTGTTaTGTGT | Chaboute et al. 1987 |
| H4 A777** | -53/-28 | GTTTGTTTGTTTTTTcTGGaTTTcTT | Chaboute et al. 1987 |
| H4 A777** | -64/-35 | GTaGTTTGcGGTaGcGTTcTTcTTaTGTGT | Chaboute et al. 1988 |
| Alfalfa | | | |
| H3 ALH3-1.1 | -47/-21 | GTTTcTcTaTGGaTTTTGTTaTaTTGT | Wu et al. 1988 |

* bp upstream from the plant putative polyadenylation signal A/GATG(G)AAATG.
** A777 has two putative polyadenylation signals.

the significant reduction of 3′-end formation in its mRNA (43). Our observations of the mutant IntΔ1 (Figure 4) being defective in a similar TG-stretch (10 nt) agrees with this finding. TG-rich sequences in the 3′-downstream regions of many other plant histone H3 and H4 genes have been isolated to date (Table 1).

The importance of the 3′-downstream sequence in gene expression has often been reported in relation to the transcription termination (reviewed in Ref. 44 ) and mRNA stability (reviewed in Ref. 45 ). For example, the AT-rich sequence in the 3′ region of many yeast genes has been suggested as a signal to slow down or stop the chain-elongation of nascent RNA mediated by RNA polymerase II (46). On the other hand, the AU-rich sequence containing an AUUUA repeat in the 3′-untranslated region of small auxin-up mRNAs (*SAURs*) function as a destabilizer of mRNA (47). The present study suggests that the TTTN(13−16)GATT sequence of the H3 gene participates in the transcription termination and the 3′-end formation of mRNA. This would explain the negative effect of the successive 3′-deletion mutants on 3′-processing of the H3 pre-mRNA, observed in our previous study (31, 48). We speculate that nine repeats of the GATT motif in the 3′ region of the H3 gene function as a regulatory element in 3′-end formation of mRNA. More detailed analyses are required to confirm this conclusion.

### The significance of the spatial relationship between the signal sequences in the 3′-downstream region

S1-protection analysis of the transcript of the H3 mutant dpmR. did not detect any H3 mRNA with a normal 3′ end. In this mutant, the position of the conserved sequence AATGGAAATG differed from that in the wild-type gene. There are two possible explanations for the results using the dpmR mutant. The first is that a relatively wide sequence surrounding the AATGGAAATG sequence is needed for 3′-processing of the H3 pre-mRNA. The second is that the distance or order between the conserved sequence and other related *cis*-elements is crucial for 3′-processing. In other experiments, we showed that the chimeric NOS/H3 gene, containing the AATGGAAATG motif and its surrounding sequences, did not produce any mRNA with a normal 3′ end (see Figure 3B). This was also true of IntΔ1 (see Figure 4B). These observations are probably due to the absence of the sequence from +556 to +614, involved in 3′-end formation as mentioned above, in these mutants. The spatial relationship between the sequence from +556 to +614 and the AATGGAAATG motif in the wild H3 gene is reversed in the dpmR mutant. This may be why no 3′-processing of the pre-mRNA occurred. Thus, the second explanation is more plausible.

### Presumed functions of *cis*-sequences and the 3′-downstream region in the histone gene regulation

The results suggested that both the initiation and the elongation of transcription in all the H3 mutants (except for the chimeric NOS/H3 gene) occur normally as in the wild-type gene, but that their transcription products are not always processed to mRNA with an authentic 3′ end. A similar phenomenon was reported by Ingelbrecht *et al.* (49), who showed that the transcript from the neomycin phosphotransferase II gene, which does not carry the normal 3′ region, produced a polyadenylated transcript. Since, in this study, we did not observe any transcripts with an abnormal 3′-end or any degradation products of transcripts from all the H3 mutants, the readthrough transcripts of the mutants are quite stable. Although the reasons for the stabilization of the readthrough products are unknown, it is likely that the three sequences identified 3′-downstream of the H3 gene participate in the termination of transcription and/or 3′-processing of the H3 pre-mRNA, but are not involved in the transcription initiation or the stabilization of mRNA.

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