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# Positive Factor 1 (PF1) from oat is an HMGY- and H1 histone-like protein that binds a functionally defined AT-rich DNA element in the oat phytochrome A gene (*PHYA3*) promoter

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Phytochrome A is a member of a plant regulatory photoreceptor family (1). The down-regulation by phytochrome of the transcription of its own *PHYA3* gene, is one of the most rapid light-mediated effects on transcription reported to date in plants (2). Two positive elements designated PE1 (−367 bp to −346 bp) and PE3 (−111 bp to −81 bp) necessary for high level expression of the oat *PHYA3* gene, were defined using linker-scan mutagenesis in a transient expression assay (3).

We report the sequence of $pO_2$, a previously cloned cDNA encoding a protein that binds to the AT-rich PE1 region in the oat *PHYA3* gene promoter (4). $pO_2$ was originally obtained by screening a λgt11 cDNA expression library prepared from polyA+ RNA isolated from the shoots of etiolated oat seedlings. Screening was performed with double stranded synthetic oligonucleotides containing the sequence of the PE1 region of the oat *PHYA3* gene promoter (4). $pO_2$ encodes a protein with the DNA binding characteristics expected for oat PF1, the nuclear factor that interacts *in vivo* with the PE1 region (4) and is therefore designated hereafter as PF1 (Figure 1).

Oat PF1 is 79% similar and 69% identical to rice PF1, another PE1-binding protein encoded by a recently described cDNA (5). Oat PF1 contains three repeats of the nonapeptide: K/GRG/PRGRPP/AK. This sequence is very similar to the 'A-T hook' motif, a DNA binding domain characteristic of all HMG I and HMG Y proteins (6, 7, 8) that are known to bind dAdT-rich DNA, as oat and rice PF1 do (4, 5). Oat PF1 is also related to the protein encoded by the soybean SB16 cDNA which contains three 'AT-hooks' (65% similarity, 51% identity) (9), as well as to the pea histone H1 protein (50% similarity, 29% identity) (10), mostly in the $NH_2$ terminal region. As in reported HMG Y proteins, oat PF1 lacks the distinctive hydrophobic region present in mammalian HMG I proteins (a.a. 110–116 of the rice PF1 sequence) (Figure 1). The absence of acidic regions that may function as activation domains (11) is notable in the oat PF1 sequence.

The role of PF1 in the transcriptional activation of the oat *PHYA3* gene has yet to be determined. The analysis of the *cis* elements of the oat *PHYA3* gene indicating a synergistic interaction between PE1 and PE3 for full expression of the gene (3) predicts that neither PF1 nor PF3 are factors efficient in transcriptional activity by themselves. Thus the combined activities of both PF1 and PF3 and perhaps coactivators or adaptors may be necessary for maximal expression of the oat *PHYA3* gene.

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                1                                                          60
Human HMG-I     ..........  ..........  ..........  ..........  ..........  ..........
Human HMG-Y     ..........  ..........  ..........  ..........  ..........  ..........
SoybeanHMGY-a   MaTEE.....  .....vnKpq  slPpYPEMIv  ktlEaLnEpn  GSNKSAISKy  IEttYgeL..
SoybeanHMGY-b   ..........  ..........  ..........  ....aLnEad  GSNKSAISKy  IEttYgeL..
Rice PF1        MaTEEdAstm  aaaeadpKpa  atPsYPEMIl  aaiEaLdDrn  GSNKtAISqh  IEgkYegLlP
Oat PF1         MsTEEvA...  .......Ksl  llPpYPEMIl  eaiEsLnDkv  GSNKSAISKy  IEgkYgd.mP
    Consensus   M-TEE-A---  -------K--  --P-YPEMI-  ---E-L-E--  GSNKSAISK-  IE--Y--L-P

                61                                                         119
Human HMG-I     ..........  ...MsESssk  s..sqplask  qekdgteKRG  RGRPrKqPpv  spgtalvgsq
Human HMG-Y     ..........  ...MsESssk  s..sqplask  qekdgteKRG  RGRPrKqPp.  ..........
SoybeanHMGY-a   .pdatvLgsH  LnkMkDSgel  sFkqNnymka  d.pnappKRG  RGRPpK.Pk.  ........tp
SoybeanHMGY-b   .pdetvLgsH  LnkMkESgel  aFkqNnymka  d.pnappKRG  RGRPpK.Pk.  ........vp
Rice PF1        PahpslLtaH  LarMkqtgel  aFskNnyfrg  ddpslppKRG  RGRPpK.Pkd  aaaaaaapap
Oat PF1         PthgslLtaH  LarMkEtgel  lFlkNnyfrt  dapdappKRG  RGRPpK.vkd  .......pna
    Consensus   P-----L--H  L--M-ES---  -F--N-----  -------KRG RGRP-K-P--  ----------

                120                                                        178
Human HMG-I     kePseVptpk  RPRGRPkgsk  Nkgaaktrkt  tTtpgrkPRG  RPKKleKeEe  Egisqessee
Human HMG-Y     kePseVptpk  RPRGRPkgsk  Nkgaaktrkt  tTtpgrkPRG  RPKKleKeEe  Egisqessee
SoybeanHMGY-a   lpPgtVvSpp  RPRGRPpkdp  NaPpkspkak  aTpgsgRPRG  RPKKvpr...  ..........
SoybeanHMGY-b   lpPgtVvSpp  RPRGRPpkdp  NaPpkspkak  aTpatgRPRG  RPKKvar...  ..........
Rice PF1        apaapaaSsp  RPRGRPpk.p  kdPlaeavak  aTsgmpRaRG  RPpKkaKvEq  Edpigapaaa
Oat PF1         ppPppkpSsg  RPRGRPak.a  kdPeaea...  .........a  nPpKkpK...  ......aapa
    Consensus   --P--V-S-  RPRGRP  --  N-P-------  -T---  RPRG RPKK--K-E-  E---------

                179                             213
Human HMG-I     eq........  ..........  ..........  .....
Human HMG-Y     e.........  ..........  ..........  .....
SoybeanHMGY-a   .sPAvaapta  vssgRpRGRP  PKVKPqltev  sves.
SoybeanHMGY-b   .sPAvpspta  vstgRpRGRP  PKVKPqltev  sves.
Rice PF1        saPAaaaeaa  ppvkRgRGRP  PKVrPaapvg  epaaa
Oat PF1         ptPApaadgs  tpakRgRGRP  PKVrPaepta  a....
    Consensus   --PA------  ---  R-RGRP  PKVKP-----  -----
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**Figure 1.** Alignment of amino acid sequences of human HMG I-Y (6, 7) and soybean (9), oat and rice (5) HMG I-Y-like proteins. The positions of the nonapeptide repeats are shown as black boxes on the consensus sequence. Computer analyses were done using programs of the University of Wisconsin Genetics Computer Group (12). The oat PF1 sequence has been deposited with GenBank under accession number L24391.

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