Isolation and sequence of a cDNA encoding the cap binding protein of wheat eukaryotic protein synthesis initiation factor 4F

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Three of the eukaryotic protein synthesis initiation factors (eIF[1]), eIF-4A, eIF-4B and eIF-4F, bind to the 5′ end of mRNA and catalyze ATP-dependent unwinding of the secondary structure of the mRNA prior to the binding of the 40S ribosomal subunit to the mRNA (1–4). One of these factors, eIF-4F, contains a small subunit (cap binding protein, eIF-4E) that binds to the m^7G cap of capped mRNAs in the absence of eIF-4A, eIF-4B and ATP (1, 3). The genes or cDNAs for several cap binding proteins have been sequenced for yeast (5, 6), mouse (7), human (8) and rabbit (GenBank accession no. X61939).

A partial cDNA for the cap binding subunit (p26) of wheat eIF-4F was obtained from a wheat sprout cDNA expression library (λZAP, Stratagene) by screening with affinity purified antibody to p26 (9). Screening of $> 5\times10^6$ plaques yielded only one partial cDNA for p26. Both strands of the cDNA (~800 nt, EMBL accession number Z12616) were sequenced with Sequenase V2 (USB). The cDNA encodes a polypeptide missing ~15 residues from the amino-terminus. Attempts are being made to obtain a full length cDNA.

A comparison of the amino acid sequences of wheat cap binding protein (p26) to the yeast and mouse cap binding proteins is shown in Figure 1. There is ~40% identity to both yeast and mammalian (human, mouse, rabbit) cap binding proteins by computer analysis (Microgenie V7, Beckman). The number and positions of the tryptophan residues are conserved and there is one additional tryptophan residue in the wheat p26. Ueda *et al.* (10, 11) have proposed that a glutamic acid residue in human cap binding protein which is three amino acids to the carboxy-terminal side of tryptophan 5, participates in the binding of the m^7G of mRNA. There is no acidic residue in this region of the wheat p26. There is a serine residue in the same vicinity as Ser53 of the mammalian cap binding proteins. The phosphorylation of Ser53 in mammalian cap binding proteins has been shown to affect activity and play a role in regulation of cell growth (12–16). It is not known at this time whether phosphorylation of plant cap binding proteins occurs or has any effect on activity or cell growth.

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wheat 26  ---------- -----EGEIA DDGDGSSAAA AGRITAHPLE MAWTFWFDNP .QGKSRQVAWG STIHPIHTFS TVEDFWGLYN NIHNPSKLNV GADFHCFKNK IEPKWEDPIC
mouse 4E  MATVEPETTP TTNPPPAEEE KTESNQEVAN PEHYIKHPLQ NRWALWFFKN .D.KSK..TWQ ANLRLISKFD TVLDFWALYN HIQLSSNLMP GCDYSFFKDG IEPMWEDEKN
yeast 4E  MSVEEVSKKP EENVSVDDTT ATPKTVLSDS AHFDVKHPLN TKWTLWYTKP AVDKSE..SWS DLLRPVTSPQ TVEEFWAIIQ NIPEPHELPL KSDYHVFRND VRPEWEDEAN
                                                  *1 *2        ▲    *3                  *4                          *5 ♦

          ANGGKW..T. ISCGR.GKSD TFWLHTLLAM IGE.QF.DFG DEICGAVVSV RQKQERVAIW TKNAAN.EAA QISIGKQWKE FLDYKDS..I GFIVHEDAK. RSDKGPKNRY TV
          KRGGRWLITL NKQQRRSDLD RFWLETLLCL IGE.SFDDYS DDVCGAVVNV RAKGDKIAIW TTECENRDAV .THIGRVYKE RLGLPPKIVI GYQSHADTAT KSGSTTKNRF VV
          AKGGKW..SF QLRGKGADID ELWLRTLLAV IGETIDEDDS .QINGVVLSI RKGGNKFALW TK.SEDKEP. LLRIGGKFKQ VLKLTDDGHL EFFPHSSANG RHPQPSITL. ..
               *6                *7                                  *8                *9
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Figure 1. Comparison of the amino acid sequences for wheat cap binding protein (p26) of eIF-4F (EMBL accession no. Z12616) with cap binding proteins from yeast (Genbank accession no. M15436) and mouse (Genbank accession no. P20415). The peptide sequences obtained from trypsin digests of p26 are underlined. The conserved tryptophan residues are marked (*) and numbered. The position of the mammalian Ser53 is marked (Δ). The glutamic acid residue described by Ueda *et al.* (10, 11) is marked (diamond).