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ABP50: An Actin-Binding Elongation Factor 1α From *Dictyostelium discoideum*

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Abstract ABP50 is a polypeptide elongation factor 1α from *Dictyostelium* that is associated with the actin cytoskeleton. Upon chemotactic stimulation, ABP50 undergoes a dramatic cytoplasmic redistribution into newly formed surface projections and in vitro binds to and bundles actin filaments. Many questions are raised by this interaction pertaining to the spatiotemporal regulation of protein synthesis and cytoskeletal organization by extracellular signals.

Key words: cytoskeleton, protein synthesis, morphogenesis, chemotaxis, cell motility

For efficient translation of a mRNA the distribution of the translational machinery bears equal importance to that of the message itself. In this regard, it is of interest to determine if translational components display a distribution pattern correlating to that of the message. In other words, are all the players present in the same place at the same time such that translation becomes feasible? What factors influence this contemporaneous distribution?

A role for the actin cytoskeleton in translation has been suggested [for reviews see Hesketh and Pryme, 1991; Bag, 1991]. For example, actin mRNA and ribosomes bind to actin filaments [Singer et al., 1989; Howe and Hershey, 1984], and translation of messages associated with the cytoskeleton appears to be more efficient [Cervera et al., 1981]. As yet the significance of these observations is unclear. One major question is, does the cytoskeleton exist merely as a passive framework for the translational apparatus or does it serve a more active function? Conversely, do translational components affect the dynamics and organization of the actin cytoskeleton itself?

ABP50 is a 50 kD polypeptide, isolated from the cellular slime mold *Dictyostelium discoideum*, which bundles actin filaments in vitro [Demma et al., 1990]. Cloning and sequencing complementary DNA obtained from a *Dictyostelium* expression library revealed that ABP50 is elongation factor 1α (EF-1α) [Yang et al., 1990]. This communication will discuss some issues pertaining to the interaction between an EF and the actin cytoskeleton and the potential significance to the regulation of protein synthesis and cell physiology. A large number of questions remain unanswered.

ABP50 was first identified by its ability to cosediment with filamentous actin (F-actin) from high speed supernatants of *Dictyostelium* cell extracts [Hock and Condeelis, 1985]. Scanning densitometry of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels prepared from vegetative cells showed that ABP50 comprises 1% of total cell protein and Scatchard analysis of the binding of ABP50 to F-actin in vitro gave a K_d of 2.1 μM with a molar ratio at saturation of 1:1 [Demma et al., 1990]. When mixed together, ABP50 and F-actin form bundles within seconds that are visible with light microscopy and can be sedimented by low speed centrifugation. The high cellular concentration of ABP50 (75 μM) implies that it is the major F-actin bundler in *Dictyostelium*.

Affinity-purified polyclonal antibodies raised against ABP50 were used to screen a *Dictyostelium* λgt11 expression library [Yang et al., 1990]. Two clones were isolated which overlapped by 95% of the coding region. Sequence comparisons show that ABP50 shares 76% identity with hu-

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man EF-1α. While the two clones code for the same amino acid sequence, they differ in the third codon at 34 sites. Consistent with these results is the presence of two genes by Southern blotting (unpublished observations). The composite complementary DNA is 1.8 kb and matches in size a single band revealed by Northern analysis of total RNA. Functionally, ABP50 acts identically to rabbit EF-1α in an in vitro translation assay using rabbit cofactors.

The intracellular distribution and association of ABP50 with the cytoskeleton changes in response to the natural *Dictyostelium* chemoattractant cyclic AMP. Whereas the distribution of ABP50 is diffuse in resting cells as revealed by immunofluorescence, in stimulated cells ABP50 becomes colocalized with F-actin to newly formed surface projections [Dharmawardhane et al., 1991]. In resting cells, at pH 6.8, 10% of total cell ABP50 is associated with the detergent-insoluble cytoskeletal pellet; however, after cyclic AMP stimulation this association increases to 20%. Immunoprecipitation of ABP50 from the detergent-soluble supernatants cosediments monomeric actin (G-actin) in a molar ratio of 1:1. In vitro binding assays of ABP50 with G-actin complexed to Sepharose beads provides a K_d of 90 nM. Thus, based on the relative concentrations of ABP50 and G-actin in *Dictyostelium* cytoplasm, 99% of soluble ABP50 should be bound to G-actin. If all ABP50 is complexed with some form of actin, what is the impact upon cytoskeletal organization and protein synthetic activity? How is this association regulated?

EFFECT OF ABP50 ON THE ACTIN CYTOSKELETON

In vitro ABP50 combines with F-actin to form large bundles. Electron microscopic studies of such bundles reveals two types of patterns: "checkerboard" with 6.9 nm interfilament spacing and "line" with 10 nm spacing [Demma et al., 1990]. Tilting of the specimen interconverts these patterns demonstrating that they result from different views of the same structure [Owen et al., 1992]. Bundle cross sections display three types of filament packing: liquid, hexagonal, and square. The latter regions represent the structure responsible for the "checkerboard" patterning. A crossbridge bonding rule can be deduced for square-packed bundles which predicts ABP50 crosslinks actin filaments rotated 90° relative to each other. This bonding rule is different from that of other known actin-bundling proteins and as a result square-packed bundles would tend to exclude other bundling proteins. Thus, square-packed bundles provide a unique microenvironment within the cytoplasm, which implies partitioning of actin bundlers to distinct intracellular compartments.

What is the significance of compartmentalization of actin bundles? Predominantly, the formation of these bundles occurs at the leading edge of the moving cell, a major site of actin polymerization. As the mRNA of actin is located in the same region of motile fibroblasts [Sundell and Singer, 1991], is it valid to infer that some cytoskeletal proteins are synthesized in the same compartment in which they are utilized?

While actin filament/ABP50 bundles exist in filopodial surface projections of *Dictyostelium,* it remains to be determined if such bundles are square packed. Is it possible that the protein synthetic machinery requires a square-packed bundle for binding or that translational components only bind as a functioning unit to such bundles? The actin filament organization required for ribosomal or mRNA binding is unknown. Intuitively, one would expect that a large organelle like a ribosome could not gain access to ABP50 crosslinking the interior of a bundle of filaments spaced 10 nm apart. Is a special feature of square-packed bundles "breathability" so that such access is possible? Or are bundles coated by ABP50 sufficient for ribosomal association? Implicit in this view is the removal of internal ABP50 from "active duty" in elongation events. In addition, are other members of the apparatus; e.g., EF-1β or EF-1γ, actin-binding proteins? Yet, perhaps ABP50 crosslinked actin filaments are unrelated to protein synthesis and only serve a unique structural role related to the increased cell motility response to chemotactic stimulation.

An increase in G-actin polymerization is associated with chemotactic stimulation in a number of cell types. What is the impact on actin dynamics if a large percentage of G-actin is complexed to ABP50? Free G-actin is rarely present in cytoplasm due to the favorable conditions for polymerization. To control the amount of F-actin in cells, the amount of free G-actin must be tightly regulated. This is partly done by monomer sequestering proteins that inhibit complexed G-actin from participating in polymerization events. Two examples are profilin [Carlsson et al., 1977] and thymosin β4 [Safer et al., 1991], both of which reversibly bind to G-actin and

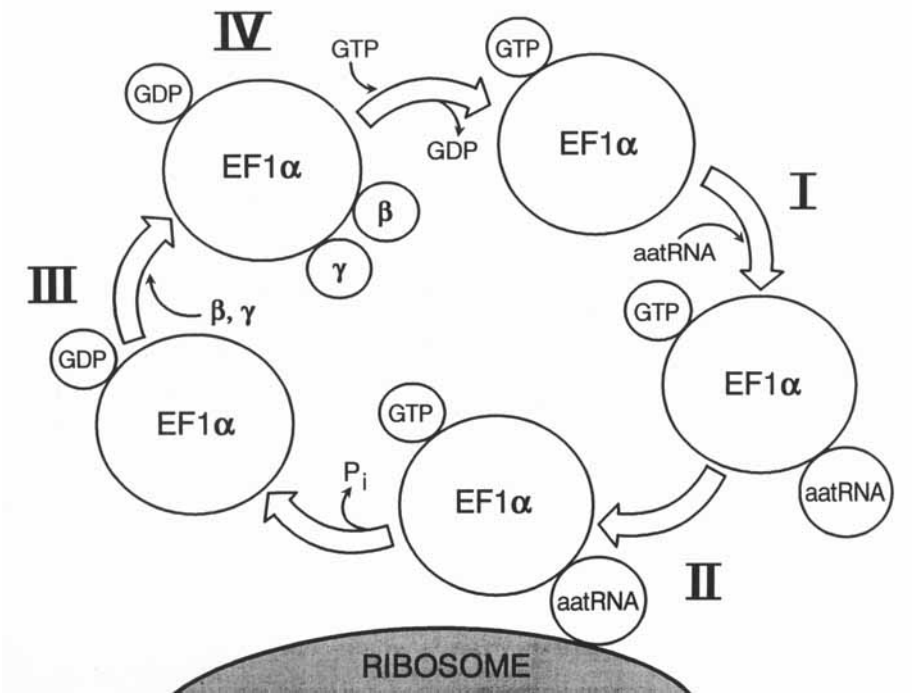

Fig. 1. Schematic representation of the eukaryotic elongation cycle involving EF-1α. The sites of putative actin influence are indicated by Roman numerals (see text for details).

reduce rates of actin polymerization in vitro. Perhaps ABP50 should be added to this list. What is the nature of the interaction between actin, ABP50, profilin, and thymosin β4? If there is antagonism by ABP50, does the actin monomer increase its polymerization activity? At present, the effects of ABP50 on actin polymerization kinetics are unknown.

EFFECT OF ACTIN ON ABP50 ACTIVITY

ABP50 serves a structural role in *Dictyostelium* by forming unique actin filament bundles, but another major function of the molecule is as an EF in nascent polypeptide synthesis. Does the actin cytoskeleton alter the elongation activity of *Dictyostelium* EF-1α? A model for putative sites of actin influence in peptide elongation can be created based on the above observations and on the specific functions ascribed to EF-1α (Fig. 1). As currently understood, GTP-EF-1α catalyzes aminoacyl-tRNA binding to the ribosomal A-site. Subsequent GTP hydrolysis releases GDP-EF-1α. Upon association of free GDP-EF-1α with the β and γ subunits, GDP is exchanged for GTP.

ABP50 binding to G-actin is affected differentially by guanine nucleotides. GDP (1 mM) has no effect on the formation of an ABP50-G-actin complex, whereas millimolar GTP reduces binding by 80% [Dharmawardhane et al., 1991]. Accordingly, little influence by G-actin on aminoacyl-tRNA (site I) or ribosome binding (site II) would be expected. However, G-actin binding to GDP-EF-1α might affect nucleotide exchange by either directly producing a conformational change that alters the nucleotide affinity (site IV) or indirectly by blocking association with the β-γ subunits (site III). Thus, G-actin effects on translation rate may occur upstream of aminoacyl-tRNA binding to the ribosome.

F-actin binding is not guanine nucleotide sensitive [Dharmawardhane et al., 1991] and so in principle could be associated with EF-1α throughout the elongation cycle. Therefore, G-actin may play an inhibitory role while the F-actin microenvironment may be permissive to peptide elongation. Upon chemotactic stimulation and concomitant formation of F-actin (and decrease in total G-actin), is there an increase in protein synthesis associated with the leading pole of the moving cell?

A comparison between the deduced amino acid sequence from *Dictyostelium* and the directly determined sequence from rabbit reticulo-

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0                                                   *                   *
MEFPESEKTH INIVVIGHVD AGKSTTTGHL IYKCGGIDKR VIEKYEKEAS EMGKQSFKYA
^^^MGK.... .......... S......... ...Q...... T...F....A ....G.....

60                        *
WVMDKLKAER ERGITIDIAL WKFETSKYYF TIIDAPGHRD FIKNMITGTS QADCAVLVIA
..L....... ........S. .........V .......... .......... .......IV.

                                                                depactin
120                                                             ├──────────
SPTGEFEAGI AKNGQTREHA LLAYTLGVKQ MIVAINKMDE KSTNYSQARY DEIVKEVSSF
AGV....... S......... .......... L..GV....S TEPP...K.. E..K....TY
                                                      *

180          actin
──┤    ├──────────┤
IKKIGYNPEK VAFVPISGWN GDNMLERSDK MEWYKGP^^^ ^^^^^^^^^T LLEALDAIVE
........DT .......... ......P.AN .P.F..WKVT RKDGNASGT. ......C.LP
                                             *

240
      actA
├──────────┤
PKRPHDKPLR IPLQDVYKIG GIGTVPVGRV ETGIIKPGMV VTFAPAGLST EVKSVEMHHE
.T..T..... L......... .......... ...VL..... .....VNVT. ..........

                    actobindin
300              ├──── * ────┤
QLPEARPGDN VGFNVKNVSV KEIKRGMVAG DSKNDPPQET EKFVAQVIVL NHPGQIHAGY
A.SQ.L.... .......... .DVR..N... ....N..M.A AG.T....I. ......S...
   #

350
SPVLDCHTAH IACKFTEIVD KVDRRTGAVV AKEGTAAVVL KNGDAAMVEL TPSRPMCVES
A......... .....AQLKE .I...S.KKL EDGPKF^^^. .S....I.DM V.GK......
                  #

410
FTEYPPLGRF AVRDMRQTVA VGVIKSTVKK APGKAGDKKG AAAPSKKK
.SD....... .......... .....AVD.. .A.AGKVT.S .QKAQ.A.
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Fig. 2. Alignment of the deduced amino acid sequence of *Dictyostelium* ABP50 (**top line**) and the directly determined sequence of rabbit reticulocyte EF-1α [from Dever et al., 1989] (**bottom line**). Areas of post-translational modification are indicated: * = methylation and # = ethanolamine addition. Gaps in sequence required to maintain alignment are shown by ^. Regions that contain homology to other actin-binding proteins are correspondingly labeled.

cyte EF-1α [Dever et al., 1989] shows interesting similarities (Fig. 2). Not surprisingly, there is a great deal of identity in the amino-terminal third of the protein. This region in bacterial EF-Tu has been shown to be responsible for guanine nucleotide binding [Jurnak et al., 1990]. The remaining sequence is less similar but may contain the actin-binding sites. Based on sequence homology with other actin-binding proteins, there are four putative actin-binding regions in the ABP50/EF-1α sequence: depactin-like #162-183 [Sutoh and Mabuchi, 1989]; actin-like #187-198 [Vandekerckhove and Weber, 1980]; *Listeria* actA-like #240-253 [Kocks et al., 1992]; and actobindin-like #315-326 [Vandekerckhove et al., 1990]. At present there is no

evidence that these regions in ABP50 are responsible for either G- or F-actin binding. Scattered among these putative actin-binding sites are the residues implied to participate in aminoacyl-tRNA binding based on chemical crosslinking studies [Kinzey et al., 1992]. Together, these observations would suggest that cobinding of actin and aminoacyl-tRNA to EF-1α are mutually exclusive events. If true, then one would expect a dramatic influence of actin on protein synthetic activity by reducing ribosomal aminoacyl-tRNA binding. What determines the differential binding of actin vs. aminoacyl-tRNA and how is this regulated? Is it purely a function of the guanine nucleotide state?

REGULATION OF ABP50-ACTIN BINDING

It is clear that ABP50 changes its intracellular distribution upon chemotactic stimulation. What second messenger elements are responsible for this redistribution? The signal transduction cascade linked to the cyclic AMP receptor in *Dictyostelium* is a prototypical G protein-phospholipase C-diacylglycerol pathway [for review see Van Haastert et al., 1991]. Interestingly, EF-1α is a substrate of protein kinase C [Venema et al., 1991] which is activated by diacylglycerol. As yet, no effect on peptide elongation has been attributed to EF-1α phosphorylation. Effects on actin binding have not been investigated.

Rabbit EF-1α contains several post-translational modifications detected by direct sequencing (see Fig. 2) [Dever et al., 1989]. Most numerous are six methylated lysine residues. In addition, there are two sites of ethanolamine addition to glutamic acid (#303, 366). It is noteworthy that a trimethylated lysine lies in the actobindin-like region. Again the significance of these modifications is unknown; however, some evidence exists that methylation increases the interaction of EF-1α with the β and γ subunits [Sherman and Sypherd, 1989]. It is unknown whether *Dictyostelium* ABP50 contains similar modified residues.

The activity of other actin crosslinking proteins has been shown to be affected by polyphosphoinositides. Actin filament crosslinking activity by muscle α-actinin is increased by phosphatidylinositol 4,5-bisphosphate [Fukami et al., 1992], whereas it decreases the same activity of filamin [Furuhashi et al., 1992]. Most recently, carrot EF-1α and ABP50 were shown to activate carrot phosphatidylinositol kinase [Yang et al., 1993], suggesting a possible crosstalk between the cytoskeleton, protein synthetic, and lipid signaling pathways. Can the actin-binding activity of ABP50 also be influenced by phospholipids?

In conclusion, the study of the interaction between the cytoskeleton and members of the protein synthetic complex is still in its infancy. There is a great deal of tantalizing evidence for such interactions that needs to be placed within the greater context of other metabolic systems in the cell and the changing external environment to which the cell is exposed. For example, it is insufficient to say that mRNA binds to actin filaments; it is equally crucial to know what the cell was doing when the binding occurred, e.g., chemotaxis, mitosis, etc. Only with this more global view can the disparate observations made to date gel into a comprehensible scheme.

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