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Signal Transduction Pathways in *Entamoeba histolytica*: PKA Activity and Translocation During the Interaction of Trophozoites with FN

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Introduction

Signal transduction mechanisms have been demonstrated to operate in *Entamoeba histolytica* trophozoites after diverse external stimuli (1). Interaction with FN transduces information into the amebas that activates the phosphoinositide as well as the adenylyl cyclase signaling pathways (2,3). cAMP has been shown to have a modulatory effect on the organization of the actin cytoskeleton to form specialized adhesive structures, and also in the regulation of actin mRNA levels (4). In both processes, the participation of proteins phosphorylated by a cAMP-dependent kinase (PKA) has been inferred by comparison with similar phenomena observed in other eukaryotic cells (5). Here, we present evidence for the presence and cAMP-dependent activation of PKA in trophozoites and the nuclear translocation of its catalytic subunit after stimulation of the cells with FN.

Materials and Methods

Trophozoites of *Entamoeba histolytica* HM-1:IMSS were grown at 37°C in axenic TYI-33 medium containing 10% bovine serum and harvested at the logarithmic phase of growth. Cell extracts were prepared by lysis of 2.5×10^6 trophozoites in 1 mL of 50 mM Tris, pH 7.5, 5 mM EDTA buffer containing a cocktail of thiol-proteinase inhibitors. Ten-microliter aliquots were assayed for PKA activity measuring the incorporation of [γ^{32}P] ATP to Kemptide, a specific peptide substrate of PKA, using the kit provided by Gibco BRL (Gaithersburg, MD, USA). Radioactivity incorporated into the Kemptide was quantified by liquid scintillation in aliquots precipitated on phosphocellulose filters (6).

Immunoblots were made after separation of the proteins in trophozoite extracts by 10% SDS-PAGE and transference onto nitrocellulose paper. A polyclonal antibody prepared against the catalytic subunit of PKA was obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA. Positive bands were visualized by chemiluminescence. The same antibody was used for immunofluorescence staining of control and fibronectin (FN)- , forskolin (FK)- , or dibutyryl cAMP (dcAMP)-stimulated trophozoites. Trophozoites were treated with FK or dcAMP for 4 h in medium without serum or plated on FN-coated glass coverslips for 30 min. To visualize structured actin distribution, trophozoites were stained with rhodamine phalloidin after incubation with the anti-PKA antibody and an FITC-tagged second antibody. Adhesion assays of trophozoites to FN-coated substrates, in the presence of PKA activators and inhibitors, were performed as previously reported (2).

Results and Discussion

As shown in Table 1, trophozoite extracts have a basal PKA activity that can be inhibited with the specific PKA inhibitor H89 and activated by addition of cAMP. Live cells treated with dibutyryl cAMP showed stimulation of the PKA basal activity. Analysis of the cell extracts by immunoblot (Figure 1A) revealed the presence of a peptide of approximately 20 kDa, apparently corresponding to the catalytic subunit of PKA in *Entamoeba histolytica.* As internal control for all PKA experiments, MDCK cell extracts were utilized. Figure 1A also shows that the antibody recognizes the catalytic subunit of MDCK cells reported to be a peptide of ~40 kDa. Cells plated on FN or treated with the adenylyl cyclase agonist FK, or with dbcAMP, when immunostained with

Table 1. PKA activity in trophozoite extracts

Assay	Kemptide[a]	H89[b]	cAMP[c]	% of Basal activity
1	—	—	—	100
2	+	—	—	151
3	+	+	—	103
4	+	—	+	195

[a]Specific PKA substrate; [b]300 nM; [c]1 μM.

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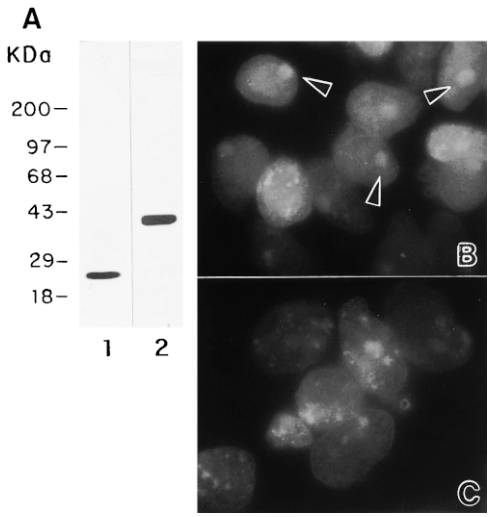

Figure 1. Identification of PKA catalytic subunit in *Entamoeba histolytica* cell extracts and live cells. (A) Immunoblot of amebic (1) and MDCK cell extracts (2). Molecular weight markers are indicated at left; (B, C) immunolocalization of PKA catalytic subunit in cells stimulated by FN interaction and in cells plated on uncovered glass surfaces, respectively. Arrowheads point to the nucleus in the cells. Bar corresponds to10 μm.

the same antibody, showed translocation of the catalytic subunit from the cytoplasm to the nucleus, and in some cells, a particular aggregation of vacuoles. In control cells, the subunit colocalized with cytoskeletal-membrane fractions or appeared free in the cytosol (Figures 1B and C). This translocation coincided with the time at which activation of the actin gene transcription is initiated. It has also been reported (4) that FK and dbcAMP promote adhesion to FN-coated substrates. We report now that PKA inhibitors have a deleterious effect on it. Our results suggest that PKA-phosphorylated proteins are involved in the reorganization of the actin cytoskeleton that follows the activation of the adenylyl cyclase signaling pathway initiated by the FN/trophozoite interaction.

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