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Regulin, a Cytoskeleton-Associated Protein Affecting Phosphorylation–Dephosphorylation *

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Translational regulation at the molecular level has been studied intensively with components from mammalian reticulocytes. Since the discovery of translational control by phosphorylation–dephosphorylation of initiation factor eIF-2 [1–3] and possibly 40 *S* ribosomal subunits [4–7], relevant protein kinases and recently counteracting phosphatases have become targets of research. The extent of phosphorylation of a given protein depends on a dynamic equilibrium between the activities of the protein kinase and phosphatase. This equilibrium reaction is schematically depicted for eIF-2 in Fig. 1.

Potentially, the equilibrium can be shifted by activation or inhibition of either the kinase or the phosphatase. Regulation of the protein kinases and phosphatases involved in translational control is poorly understood. It appears that both types of enzyme occur in latent forms in vivo. The mechanisms of activation of the heme-regulated eIF-2α kinase in reticulocytes or of the double-stranded RNA-dependent eIF-2α kinase in interferon-sensitive cells are still unclear. Almost nothing is known about the inactive form and occurrence of protein phosphatases in intact cells. In vitro, phosphatases generally can be activated by high, nonphysiologic concentrations of Mn^{2+} [8–12], by protease treatment [13, 14], or by denaturing agents [15].

We have partially purified and characterized a 76 000 daltons phosphatase from reticulocytes that counteracted the heme-controlled eIF-2α kinase [11], and recently isolated to homogeneity a 56 000 daltons, Mn^{2+}-dependent phosphatase that is most active with phosphorylated 40 *S* ribosomal subunits [12]. Using monoclonal antibodies, we have identified a 230 000 daltons, protease-sensitive protein, which we have named regulin, that stimulates the activity of this phosphatase [16]. Figure 2 shows that regulin extracted from the membrane fraction with spectrin is distinct from the 220 000 and 240 000 daltons α- and β-spectrin subunits. Regulin can be separated from spectrin by ion exchange chromatography in urea, followed by antibody affinity chromatography using monoclonal antibodies against the regulatory

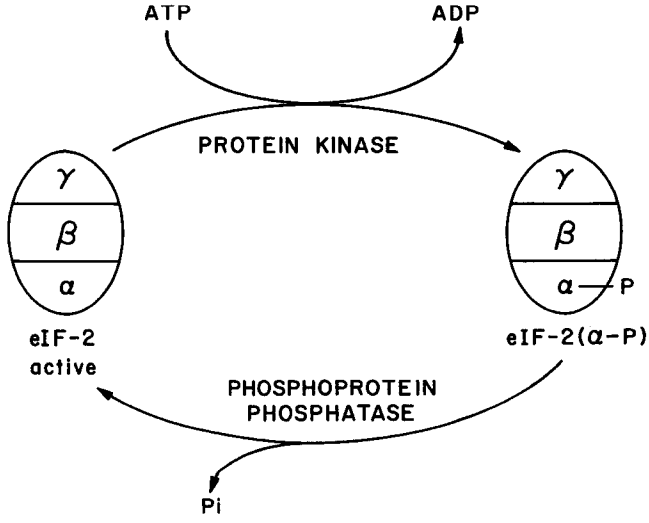

Fig. 1. Phosphorylation–dephosphorylation of eIF-2

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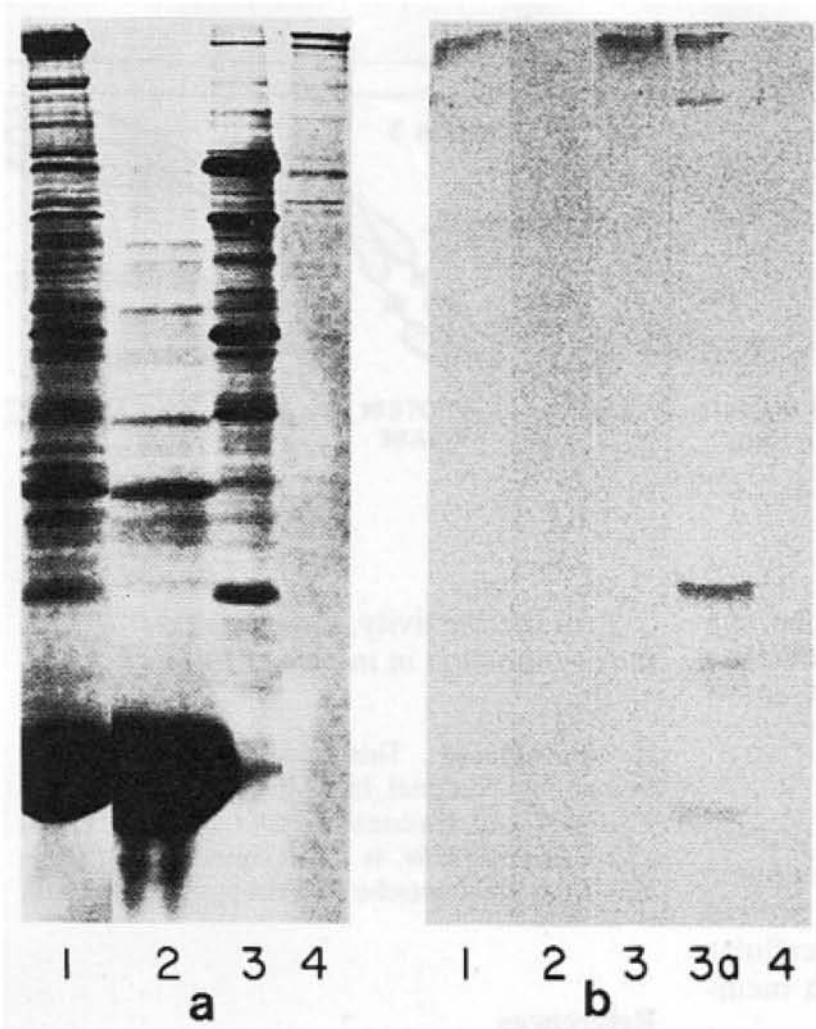

Fig. 2a, b. Separation of regulin from spectrin. Peptides of a crude spectrin preparation extracted from the reticulocyte membrane (*lane* 1) or fractions derived from it (*lanes* 2–4) were separated on 15% polyacrylamide gels in SDS and either stained with Coomassie Blue (**a**) or transferred electrophoretically to nitrocellulose, then incubated with monoclonal anti-regulin antibodies (**b**) as described [16]. Antigen peptides were visualized by dianisidine (cf. [17]). The spectrin preparation was made 6 *M* in urea, then loaded on a DEAE–cellulose column equilibrated in 40 m*M* Tris-HCl (pH 7.5), 25 m*M* KCl, 5 m*M* β-mercaptoethanol, and 6 *M* urea. The wash fraction (*lane* 2) contained mostly hemoglobin. Then proteins were eluted stepwise with the same solution, but containing 100 m*M* (*lane* 3) and 300 m*M* KCl (*lane* 4). *Lane* 3 contains regulin, *lane* 4 mostly spectrin. Lane 3a (100 m*M* KCl fraction) is from an identical preparation from which protease inhibitors were omitted

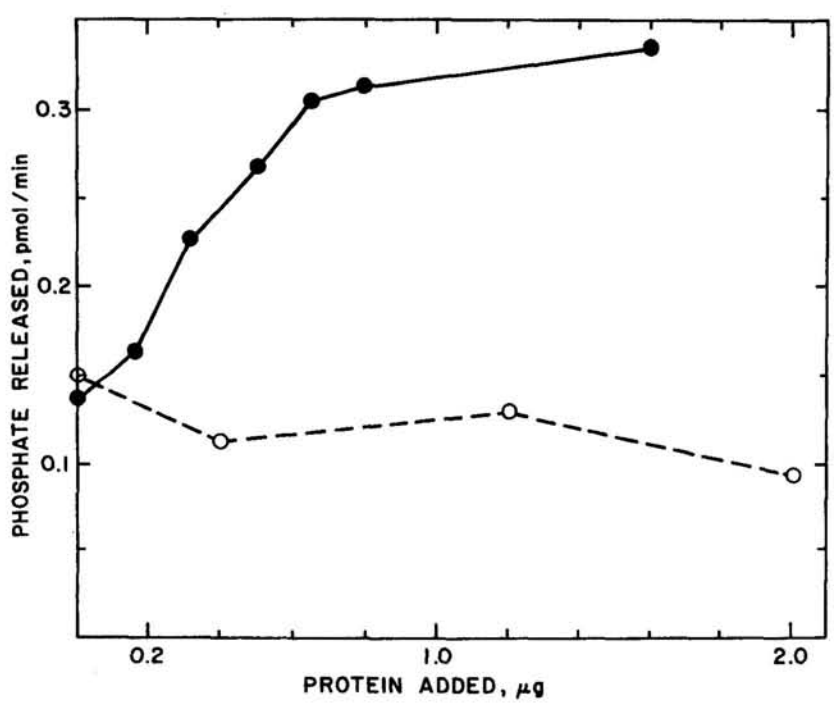

Fig. 3. Stimulation of phosphatase activity by regulin. The 56 000 daltons phosphatase was isolated and its enzymatic activity determined as described [12]. Regulin (*full circles*) or spectrin (*open circles*) were added in amounts indicated on the abscissa

protein. Regulin peptides are identified after separation of peptides by SDS–gel electrophoresis followed by Western blotting and ELISA with monoclonal antibodies (cf. [16]). The antigen–antibody complex is detected by a second antibody to which peroxidase is linked using dianisidine and H_2O_2 as substrate (cf. [17]). Regulin is a 230 000 daltons peptide that is very sensitive to proteolysis. If the cells are lysed in the absence of protease inhibitors, regulin is quickly degraded (Fig. 2B, lane 3a). It is

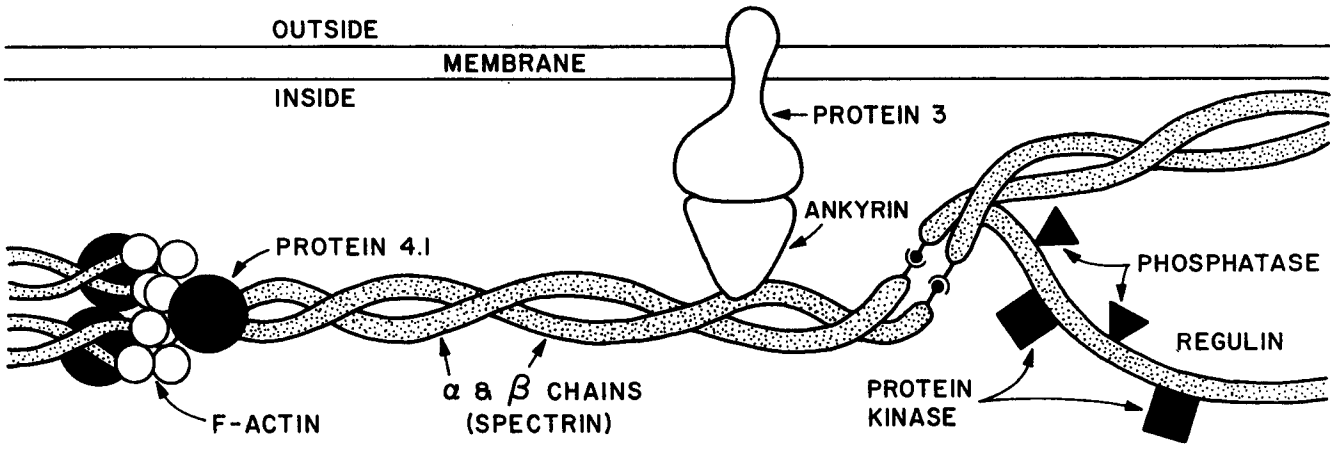

Fig. 4. Model for the association of regulin with spectrin in the red cell cytoskeleton. Illustration of the membrane-associated proteins is modified from [19]

not established whether or not this proteolytic processing of regulin has a physiologic function such as activation of protein kinase or phosphatase by extracellular stimuli that are transmitted through membrane receptors.

After reticulocytes are lysed and fractionated, regulin and its degradation products are found in part in the postribosomal supernatant and appear to copurify through most chromatographic steps with the phosphoprotein phosphatase and the heme-controlled eIF-2α kinase activities. Highly purified regulin – but not spectrin – stimulates the enzymatic activity of the homogeneous 56 000 daltons phosphatase as shown in Fig. 3.

Based on the results here and elswhere [12, 16], we suggest the model depicted in Fig. 4, indicating membrane–cytoskeleton interaction in red cells. It shows regulin associated with spectrin in the cytoskeleton and functioning to organize and modulate the activity of heme-regulated eIF-2α kinase and the protein phosphatase involved in translational control. Evidence has been presented which indicates that mRNA and polysomes active in protein synthesis also are bound to subcellular cytoskeletal structures, as reviewed recently by Trachsel and co-workers [18]. Thus, it appears likely that these elements for protein synthesis, as well as the factors that control their activity, are also organized on the cytoskeleton in intact cells.

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