New functions for the NHERF family of proteins

Commentary

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The sodium-hydrogen exchanger regulatory factor, NHERF (also called EBP50), was isolated initially as a necessary cofactor in cAMP-associated inhibition of the renal brush border sodium-hydrogen exchanger (NHE3) (1). Shortly thereafter, a second member of this family was cloned and characterized (NHERF2, also called E3KARP and TKA1) (2). NHERF and NHERF2 contain two tandem protein-protein interactive PDZ domains. Following the demonstration that these proteins bind ezrin, a new model was developed for regulation of NHE3 signaling by cAMP (3, 4), in which NHERF (or NHERF2), ezrin, and protein kinase A form a multiprotein signal complex linking NHE3 to the actin cytoskeleton. The formation of this complex is proposed to facilitate the phosphorylation and downregulation of NHE3.

Recent studies have confirmed the basic elements of the model (4). This form of signal transduction, not previously considered in control of epithelial transporters, has generated considerable interest (5), and recent experiments have documented an association between NHERF and NHERF2 with a number of other transporters (4). While the precise relation between NHERF and these transport proteins remains to be determined, there are already preliminary data to suggest that NHERF interacts with these proteins in ways not directly linked to its role as an adaptor protein.

A remarkable offshoot of the isolation of NHERF has been the discovery of its involvement with a broad array of biologic systems. At least three of the effects of NHERF are distinct from its regulation of renal transporters and deserve special comment. First, Katzenellenbogen and coworkers reported that estrogen stimulates the expression of NHERF in estrogen receptor-positive but not receptor-negative breast cancer cell lines and that antiestrogens can block this effect (6). Concurrent with these studies, we had isolated a large fragment of the mouse *NHERF* promoter and, given the above results, were surprised to find that it contains no recognizable estrogen response element and that estrogen fails to stimulate *NHERF* reporter constructs transiently expressed in estrogen receptor-positive cell lines (7). The mechanism of estrogen stimulation of NHERF remains to be determined; nonetheless, it is suggested that NHERF is involved in the signal-transduction pathway of estrogen. Recently, Stemmer-Rachamimov et al. reported a correlation between expression of NHERF and of estrogen receptors in breast carcinoma specimens (8). Others have made similar observations. The array of proteins stimulated by estrogen is finite, and identification of a new estrogen-responsive protein with a possible link to breast cancer is likely to stimulate research into the possible role of NHERF in breast, endometrium, and other estrogen-stimulated tissues.

The relation between NHERF and the cystic fibrosis transmembrane regulator (CFTR) has been another area of increasing interest. CFTR functions as a cAMP-regulated chloride channel, and mutations in CFTR are causal in cystic fibrosis. CFTR contains a C-terminal NHERF consensus sequence and the two proteins bind with high affinity, an interaction that may help explain CFTR's ability to regulate other transport proteins, including the epithelial sodium channel, the renal outer medullary potassium channel, and NHE3. Recent experiments have postulated two, not necessarily exclusive, roles for NHERF in CFTR function. Guggino, Stanton, and coworkers have proposed that NHERF functions as a membrane retention signal for CFTR (9). Foskett and coworkers suggest that NHERF facilitates the dimerization of CFTR and, as a consequence, full expression of chloride channel activity (10). As is the case for the relation between NHERF and estrogen signaling, the associations between NHERF and CFTR and their relevance to the complex physiologic derangements seen in cystic fibrosis are still unfolding.

NHERF2 and the glomerular barrier

The third area of research related to the NHERF proteins involves their interactions with a variety of receptors, including the β_2-adrenergic receptor and, possibly, the P2Y purinergic receptor (11). NHERF regulates the fate of the retrieved receptors by sorting them to either recycling endosomes or lysosomal degradatory pathways. NHERF2 is heavily expressed in vascular structures, and it is tempting to speculate that the NHERF family of proteins plays an important role in the function of this tissue (2). In this issue of the *JCI*, Takeda et al. in the laboratory of Marilyn Gist Farquhar report an association between podocalyxin, ezrin, and NHERF2 (12). Podocalyxin is expressed on the surface of glomerular podocytes and, by virtue of its negative charge, is important in maintaining the integrity of the glomerular barrier to proteins. Significantly, Takeda et al. also show that the NHERF/podocalyxin/ezrin/actin assembly is disrupted in a disease model characterized by proteinuria.

These results are of considerable interest. The original identification of NHERF2 was as a protein interacting

with the PDGF receptor. Recent experiments have established that NHERF and NHERF2 function to permit dimerization of subunits of the PDGF receptor with the consequent activation of tyrosine kinase activity and downstream signaling events (13). Certain forms of glomerulonephritis are associated with marked upregulation of the PDGF receptor, and it has been suggested that this upregulation is of pathophysiologic importance. Although it has not been established that NHERF2 abundance is increased in clinical or experimental renal diseases, the abundance of the protein in normal tissue would suggest that it would not be rate-limiting. Thus, it might be hypothesized that the linkage of NHERF2 to the PDGF receptor would facilitate formation of a cytoskeleton-associated signaling complex that activates downstream signals including the mitogen-activated protein kinase pathway, resulting in proliferation of glomerular cells, the production of matrix material, and cellular remodeling.

The biology of the NHERF family of proteins and related PDZ proteins is just beginning to be understood. The relation between these proteins and defined disease processes is also incompletely defined. The development of NHERF and NHERF2 knockout mice may prove especially useful in defining phenotypic changes and pointing investigators to other organs and biochemical systems that might require participation of members of the NHERF family of proteins.

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