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COMMENTARY

The Shank family of scaffold proteins

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SUMMARY

Shank proteins make up a new family of scaffold proteins recently identified through their interaction with a variety of membrane and cytoplasmic proteins. Shank polypeptides contain multiple sites for protein-protein interaction, including ankyrin repeats, an SH3 domain, a PDZ domain, a long proline-rich region, and a SAM domain. Binding partners for most of these domains have been identified: for instance, the PDZ domain of Shank proteins interacts with GKAP (a postsynaptic-density protein) as well as several G-protein-coupled receptors. The specific localization of Shank proteins at postsynaptic sites of brain excitatory synapses suggests a role for this family of proteins in the organization of cytoskeletal/signaling complexes at specialized cell junctions.

Key words: Postsynaptic density, Cell junction, PDZ domain, SH3 domain, Ankyrin repeat, SAM domain, Homer, Cortactin, Glutamate receptor, GKAP, PSD-95

INTRODUCTION

Protein complexes of specific composition assembled in particular regions of the cell function as molecular machines that govern subcellular structure, protein targeting and signal transduction. These modular assemblies are often built around one or more central 'scaffold' proteins, which contain multiple domains for protein-protein interaction, such as PDZ domains and SH3 domains (Pawson and Scott, 1997). Recently, a family of scaffold proteins (the Shank family) has been independently identified by several different investigators through the ability of these proteins to bind to a variety of membrane and cytoplasmic proteins. A synthesis of these recent studies is useful because Shank proteins probably play roles in multiple cell biological contexts. Here, we review the current state of knowledge about the expression patterns and the structure and function of members of the Shank family, focusing on the nervous system, where most is known.

STRUCTURE AND DIVERSITY OF THE SHANK FAMILY OF PROTEINS

Multiple genes, primary structure and domain organization

Currently the Shank family has three known members: Shank1, Shank2 and Shank3. The genes that encode these proteins have been independently cloned under different guises and are hence known under other names, such as Synamon, ProSAP, CortBP, Spank, and SSTRIP (see Table 1 for clarification of nomenclature and appropriate references). Here, we use Shank1, Shank2 and Shank3 to designate specific members of the family, and the generic term Shank to refer to the family as a whole.

The Shank proteins are relatively large: the long splice variants (see below) are ~2000 residues in length and >200 kDa in molecular mass. Full-length Shank is characterized by (in N-terminal to C-terminal order) multiple ankyrin repeats, an SH3 domain, a PDZ domain, a long proline-rich region that occupies more than half the protein, and a sterile alpha motif (SAM) domain (see Fig. 1). All these domains can act as sites for specific protein-protein interactions, and the presence of these multiple domains within a single polypeptide is consistent with a function as a scaffolding protein. All known members of the mammalian Shank family appear to share this domain structure, the equivalent domains in different Shank proteins showing amino acid sequence identities of 63-87% (Fig. 1). However, it is unclear at present whether Shank2 contains ankyrin repeats, because the splice variants of this gene characterized so far lack the region N-terminal to the SH3 and PDZ domains (Lim et al., 1999). Indeed, some of the reported sequences of Shank2 (CortBP1 and ProSAP1) lack the SH3 domain as well as the ankyrin repeats (Boeckers et al., 1999a; Du et al., 1998).

Alternative splicing

Shank contains multiple sites for alternative splicing – at least four sites have been identified in Shank1 and Shank2 (Fig. 1A;

Table 1. Alternative names for members of the Shank family*

Gene/protein	Other names	Derivation of name	References
Shank1		SH3 domain and ankyrin repeat containing protein	Lim et al., 1999; Naisbitt et al., 1999; Tu et al., 1999
	Synamon		Yao et al., 1999
	SSTRIP	Somatostatin-receptor-interacting protein	Zitzer et al., 1999a,b
	Spank-1	Name and sequence entered in GenBank #AF159046	
Shank2			Lim et al., 1999; Naisbitt et al., 1999
	CortBP1	Cortactin-binding protein; lacks the ankyrin repeats and SH3 domain	Du et al., 1998
	ProSAP1	Proline-rich synapse-associated protein	Boeckers et al., 1999a
	Spank-3	Name and sequence entered in GenBank #AF159048	
Shank3			Lim et al., 1999; Naisbitt et al., 1999; Tu et al., 1999
	ProSAP2		Boeckers et al., 1999b
	Spank-2	Name and sequence entered in GenBank #AF159047	

*The various proteins grouped as Shank1, Shank2 or Shank3 derive from the same orthologous genes, but their sequences may differ as a result of species differences, alternative splicing or different assignments of translational start sites.

Boeckers et al., 1999a; Lim et al., 1999; Yao et al., 1999; Zitzer et al., 1999a). Most of the inserts found at these splice sites contain small stretches of amino acids, but a few contain alternative translational start or stop codons. Since most of the splice sites are located between the recognizable domains of Shank, the presence of alternative start or stop codons suggests that the domain structure of Shank (and hence its protein-protein interactions) is regulated by alternative splicing (Fig. 1B).

Alternative splicing of Shank appears to be regulated during brain development. For example, RT-PCR analysis has shown that a small insert (SLIDGIDSG) is increasingly incorporated between the SH3 domain and the PDZ domain of Shank1 during postnatal development of rat brain (Lim et al., 1999). In situ hybridization has revealed that in rat cerebellum use of a small insert (KASVRKK) between the PDZ domain and the proline-rich region of Shank2 is maximal in the third week of postnatal development, whereas use of the same insert in cortex decreases (Boeckers et al., 1999a). In contrast, the use of inserts that have alternative translational start or stop codons did not show any prominent changes during postnatal brain development (Lim et al., 1999). The functional significance of alternative splicing of Shank proteins is currently unknown.

EXPRESSION PATTERNS OF SHANK

Tissue distribution

The known Shank genes show distinct patterns of expression. In rat, Shank1 mRNA and protein are expressed almost exclusively in brain (Lim et al., 1999; Yao et al., 1999; Zitzer et al., 1999a). Shank2 mRNA is strongly expressed in brain and at lower levels in kidney and liver (Du et al., 1998; Lim et al., 1999), whereas Shank3 is expressed abundantly in heart and moderately in brain and spleen (Lim et al., 1999).

Shank expression in the brain

In situ hybridization and northern analysis reveal that Shank1 mRNA is expressed abundantly in the cortex, hippocampus and amygdala, moderately in thalamus and substantia nigra, but at

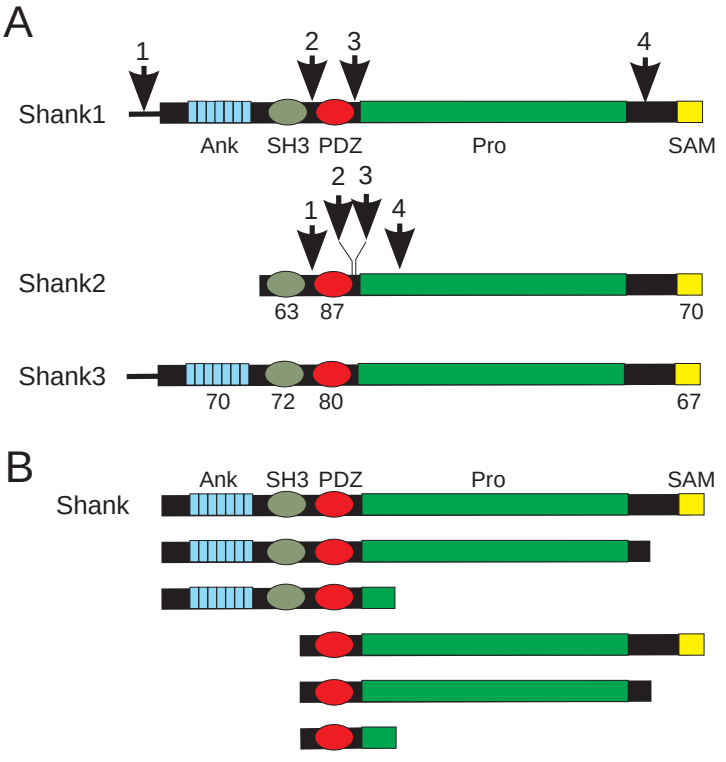

Fig. 1. Multiple genes, domain structure and alternative splicing of Shank. A, Domain organization of Shank family proteins. Sites of alternative splicing in Shank1 and Shank2 are indicated by numbered arrows. Alternative splicing in Shank3 has not been extensively studied. No cDNA clones of Shank2 have yet been reported that contain the ankyrin repeats; this region was therefore omitted from the schematic. The amino acid sequence identity (%) between respective domains of different Shank family members is indicated beneath each domain. For instance, the SH3 domain of Shank2 and Shank3 are 63% and 72% identical to the SH3 domain of Shank1, respectively. Ank, ankyrin repeats 1-7; SH3, Src homology domain 3; PDZ, PSD-95/Dlg/ZO-1 domain; Pro, proline-rich region; SAM, sterile alpha motif. *B*, Splice variants of a generic Shank protein deduced by conceptual translation of cloned cDNAs and splice variants containing alternative translation-start sites and stop codons. These splice forms have not been verified at the protein level and do not necessarily apply to all Shank genes.

low or undetectable levels in the cerebellum, caudate nucleus, corpus callosum and subthalamic nucleus (Zitzer et al., 1999a). Shank2 mRNA is widely expressed in many brain regions, including the cortex, hippocampus, cerebellum**,** olfactory bulb and central gray (Boeckers et al., 1999a). The distributions of Shank3 and Shank2 mRNAs overlap in many brain regions, including the cortex and hippocampus, but they show a complementary distribution in the cerebellum: Shank2 mRNA is expressed in Purkinje cells, whereas Shank3 mRNA is found only in the granule cell layer (Boeckers et al., 1999b). In situ hybridization has shown that the overall distribution of Shank2 mRNA does not change prominently during postnatal development of the brain, although differential splicing can affect the levels of individual splice variants (see above; Boeckers et al., 1999a).

Immunoblot analysis using Shank1- or Shank2-specific antibodies has revealed a remarkable complexity of Shank polypeptides in rat brain: these range in size from 120 kDa to 240 kDa (Boeckers et al., 1999a; Du et al., 1998; Lim et al., 1999; Naisbitt et al., 1999; Tu et al., 1999). It remains to be determined whether these multiple Shank bands are the products of alternative splicing and/or degradation. The pattern of Shank immunoblot bands varies between different brain regions, which is consistent with differential expression of Shank genes and/or splice variants (Lim et al., 1999).

SUBCELLULAR LOCALIZATION OF SHANK

Synaptic localization of Shank in neurons

The postsynaptic density (PSD) is an electron-dense structure associated with the cytoplasmic face of the postsynaptic membrane. Shank polypeptides are highly enriched in PSD fractions purified from rat brain, which suggests that they localize at postsynaptic sites (Boeckers et al., 1999a; Lim et al., 1999; Naisbitt et al., 1999; Zitzer et al., 1999a). This has been confirmed more directly by immunostaining.

In hippocampal neurons in culture, immunostaining with an antibody that recognizes all known Shank isoforms revealed a punctate pattern of Shank immunoreactivity that colocalized with markers of excitatory synapses but not inhibitory synapses (Naisbitt et al., 1999; Yao et al., 1999). A punctate synaptic localization was similarly shown in rat brain labeled with a Shank1-specific antibody – relatively little staining was present in the cell bodies and dendritic cytoplasm of neurons (Lim et al., 1999).

Immunoelectron microscopy (immunoEM) has confirmed the specific concentration of Shank proteins in the PSDs of excitatory synapses (Naisbitt et al., 1999; Boeckers et al., 1999a; Tu et al., 1999). Detailed quantitation of immunogold particles indicates that Shank is uniformly spread across the breadth of the PSD but concentrated in the deeper part of the PSD. The peak density of immunogold labeling occurs ~30 nm inside the postsynaptic membrane (the PSD being 40-50 nm thick; Naisbitt et al., 1999). Thus Shank lies in a deeper part of the PSD than does PSD-95 (a scaffold protein that binds directly to NMDA receptors), the density of which peaks within 5-10 nm of the postsynaptic membrane.

In developing neurons in culture, Shank immunoreactivity is concentrated in growth cones of axons and dendrites, and then accumulates at synaptic junctions (Du et al., 1998; Naisbitt et al., 1999). ImmunoEM has also revealed a change in the subcellular localization of Shank during postnatal rat brain development (Boeckers et al., 1999a): it shifts from small processes and lamellipodia of neurons at postnatal day (P) 5 to the cytoplasm of cell bodies and outgrowing neurites (at P8) and finally concentrates at the PSD (at P10). Light-microscopy studies show that the punctate synaptic pattern of Shank1 immunostaining emerges around P7 (Lim et al., 1999).

The expression and synaptic localization of Shank seem to lag slightly behind that of PSD-95 (E. Kim, C. Sala and M. Sheng, unpublished observations), which is consistent with the idea that recruitment of Shank to the PSD is secondary to its binding to the PSD-95-GKAP complex (see below).

Dendritic localization of Shank mRNA in neurons

The vast majority of mRNAs are localized in the cell bodies of neurons. An intriguing aspect of the subcellular distribution of Shank is the dendritic localization of Shank1 mRNA (Zitzer et al., 1999a; M. Passafaro and M. Sheng, unpublished observation). Since the machinery for protein synthesis is present in dendrites, this suggests that the translation of Shank1 can occur in the postsynaptic compartment; translation of Shank might be regulated locally by synaptic activity (Kiebler and DesGroseillers, 2000; Schuman, 1999; Worley, 1998).

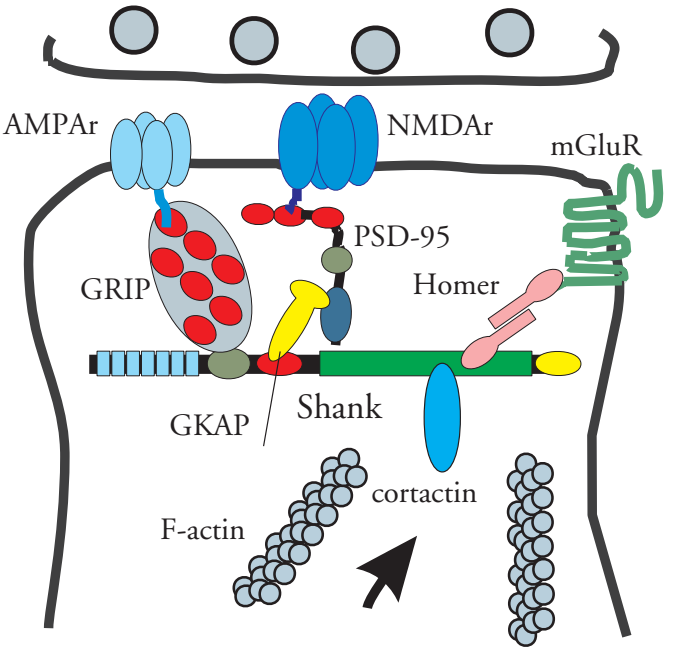

Fig. 2. A postsynaptic protein assembly organized by Shank. Schematic diagram of Shank and its protein-protein interactions in the postsynaptic density of a dendritic spine. The various domains of Shank are shown (see Fig.1 for details). The interaction between cortactin and Shank might be regulated by subcellular redistribution of cortactin (signified by arrow), which in turn affects local F-actin arrangement. PDZ domains are shown as red ovals; SH3 domains are shown as green ovals. AMPAr, AMPA receptor; NMDAr, NMDA receptor; mGluR, metabotropic glutamate receptor.

PROTEIN-PROTEIN INTERACTIONS INVOLVING SHANK

Considerable knowledge has accrued about the protein interactions mediated by individual domains of Shank, but an understanding of their functional significance lags behind. Most known interactions involve the PDZ domain of Shank – indeed, it was through these PDZ-mediated interactions that Shank was isolated in several independent yeast two-hybrid screens.

The PDZ domain

The best-characterized interaction mediated by the PDZ domain of Shank is with GKAP (Boeckers et al., 1999b; Naisbitt et al., 1999; Tu et al., 1999; Yao et al., 1999). GKAP comprises a family of abundant PSD proteins (also known as SAPAPs or DAPs) of undetermined function that bind directly to postsynaptic scaffold proteins PSD-95**,** S-SCAM and nArgBP2 (Hirao et al., 1998; Kawabe et al., 1999; Kim et al., 1997; Naisbitt et al., 1997; Satoh et al., 1997; Takeuchi et al., 1997). Binding to the Shank PDZ domain is mediated by the C terminus of GKAP, which ends in the sequence QTRL. Since this C-terminal sequence is shared by the four known members of the GKAP family (GKAP/SAPAP1-4), all GKAPs probably interact with Shank. Interestingly, however, splice variants of GKAP1 exist that alter the C terminus and thus prevent binding to Shank – for example, GKAP1b, which terminates in a GQSK sequence (Naisbitt et al., 1999). Indeed, overexpression of GKAP1b in neurons inhibits the synaptic localization of endogenous Shank, which suggests that the interaction between the GKAP C terminus and the PDZ domain of Shank is important for the recruitment of Shank to postsynaptic sites.

The PDZ domain of Shank recognizes the consensus C-terminal sequence X-T/S-X-L, in which leucine is the last amino acid residue (Naisbitt et al., 1999). A variety of integral membrane proteins have cytoplasmic C-termini that fit this consensus, and some have been shown to interact directly with Shank. For instance, the group I metabotropic glutamate receptors (mGluRs) terminate with the sequence SSSL (mGluR1α) or SSTL (mGluR5). Both these cytoplasmic tails interact directly with the Shank PDZ domain in yeast two-hybrid and biochemical assays (Tu et al., 1999), but the in vivo relevance of this interaction remains unclear.

Another G-protein-coupled receptor, the somatostatin receptor type 2 (SSTR2), has a cytoplasmic tail that terminates in the related sequence QTSI. SSTR2 also interacts with the PDZ domain of Shank1 (which these investigators named SSTRIP for somatostatin receptor interacting protein) and Shank2 (Zitzer et al., 1999a,b). However, this association was not confirmed in vivo. Curiously, two other somatostatin receptors, SSTR1 (ending in -ITSL) and SSTR3 (-LSHL), showed no binding to Shank in the yeast two-hybrid system (Zitzer et al., 1999a). Thus conformation to the consensus for Shank PDZ binding (X-T/S-X-L) seems insufficient to predict actual binding.

The proline-rich region

Between the PDZ domain and the SAM domain of Shank proteins lies a region of >1000 residues that is rich in proline and serine residues. Proline-rich motifs commonly act as binding sites for SH3, EVH1 and WW domains; thus the proline-rich region of Shank is likely to mediate multiple sets of protein interactions. Two proteins that have been shown to bind to the proline-rich region are Homer and cortactin; in both cases, the Shank interaction was first identified by yeast two-hybrid screens using portions of Homer or cortactin as baits (Du et al., 1998; Tu et al., 1999).

Members of the Homer family of proteins contain a single EVH1 domain that recognizes PPXXF motifs in partner proteins (Tu et al., 1998; Xiao et al., 1998). The Homer EVH1 domain binds directly to Shank1 and Shank3; the –LVPPPEEFAN- sequence in Shank3 (residues 1307-1316) is critical for this interaction (Tu et al., 1999). A similar sequence (PLPPPLEFSN, residues 1563-1572) in Shank1 presumably mediates binding to Homer. Shank2 (which contains two putative sites: PLPPPLEFAN and FLPPPESFDA) also presumably interacts with Homer, but this remains to be shown directly. Homer proteins are required for efficient signaling between mGluRs and IP3 receptors, both of which have PPXXF Homer-binding motifs (Tu et al., 1998; Xiao et al., 1998). Homer physically and functionally links together the phospholipase-C-coupled mGluR with its downstream effector (the inositol 1,4,5-triphosphate receptor), which leads to more efficient release of intracellular calcium in response to receptor stimulation. The Homer-binding motif is found in other proteins, including the ryanodine receptor. Thus, Homer might play a general role in assembling signaling complexes involved in excitation-calcium coupling. Shank might cooperate with Homer in this function, not only by binding directly to Homer but perhaps also by interacting with G-protein-coupled receptors (such as mGluR1/5) that are linked to intracellular calcium release (Tu et al., 1999).

The Shank family of proteins also interact with cortactin – indeed, the previously described cortactin-binding protein (CortBP1; Du et al., 1998) appears to be a splice variant of Shank2 that lacks the ankyrin repeats and SH3 domain (Lim et al., 1999). Originally identified as a substrate of Src tyrosine kinase, cortactin is an F-actin-binding protein that is enriched in cell-matrix contact sites and lamellipodia of cultured cells, as well as in growth cones of neurons (Du et al., 1998; Wu and Parsons, 1993). The subcellular distribution of cortactin is rapidly regulated: cortactin translocates to the cell periphery in a growth-factor and Rac1-dependent manner (Weed et al., 1998), and it redistributes to synapses in response to glutamate stimulation (Naisbitt et al., 1999). Thus cortactin is likely to play a regulatory role in organization of the actin cytoskeleton in the cell cortex and in dendritic spines.

The Shank-cortactin interaction is mediated by the SH3 domain of cortactin, which binds to the proline-based motif (KPPVPPKP) within the proline-rich region of Shank2 and Shank3 (Naisbitt et al., 1999). Sequences in Shank1 that resemble the cortactin SH3-binding consensus are also present (Sparks et al., 1996), but whether Shank1 can bind cortactin remains to be shown directly.

The SAM domain

The C terminus of Shank is occupied by a SAM domain, which is found in a variety of proteins, including Eph receptors. The SAM domains of Shank can bind to each other in a homomeric and heteromeric fashion, which suggests that Shank proteins can multimerize in a tail-to-tail manner. Oligomerization of Shank could allow cross-linking of multiple sets of protein

complexes such as the PSD-95 complex and the Homer-based complex at postsynaptic sites.

The ankyrin repeats and SH3 domain

GRIP is a scaffold protein containing seven PDZ domains that interacts with AMPA receptors and Eph receptors/ligands (Bruckner et al., 1999; Dong et al., 1997; Srivastava et al., 1998; Torres et al., 1998; Wyszynski et al., 1999). We have recently found in GST-pulldown experiments that the SH3 domain of Shank binds to GRIP (M. Passafaro, S. Naisbitt, C. Sala and M. Sheng, unpublished observations). This implies that Shank has the potential to interact indirectly with three major classes of postsynaptic glutamate receptor via their associated proteins: NMDA receptors via the PSD-95/GKAP complex, mGluRs via Homer, and AMPA receptors via GRIP. Proteins that bind to the ankyrin repeats of Shank remain to be identified.

POTENTIAL FUNCTIONS OF SHANK

Despite considerable knowledge about the primary structure, expression patterns and subcellular localization of Shank proteins, the physiological functions of this family of molecules remain unclear. The potential roles of Shank have to be hypothesized largely on the basis of its known interactions with partner proteins and its subcellular distribution. The fact that Shank interacts with GKAP, Homer and GRIP (all of which can be regarded as scaffolding proteins, or components of scaffolds, for specific glutamate receptors) suggests that Shank is a ‘master scaffold’ holding together the NMDA-, mGluR- and AMPA receptor complexes in the postsynaptic specialization. Shank might also link NMDA receptors and mGluRs in a functional sense, facilitating crosstalk between ionotropic and metabotropic glutamate receptor signaling pathways. We envisage that, by contrast with PSD-95, which lies very close to the membrane, Shank plays an organizing role at the intracellular aspect of the PSD, where the PSD interfaces with the cytoplasm. Here, Shank would be poised to interact with actin-regulatory molecules such as cortactin, which translocate to the postsynaptic site from the cytoplasm. Because of its localization and its protein-protein interactions, Shank would be well positioned to link postsynaptic glutamate receptor activity and local cytoskeletal remodeling, particularly within actin-rich dendritic spines.

In developing neurons, Shank colocalizes with cortactin in growth cones (Du et al., 1998; Naisbitt et al., 1999); this raises the possibility that Shank is involved in neurite outgrowth. In non-neuronal cells, Shank might play analogous roles in cell migration and in cortical cytoskeleton regulation. Shank probably also interacts with surface receptors in non-neuronal cells, facilitating signaling to intracellular pathways and contributing to the macromolecular organization of specialized plasmalemma microdomains.

Perspectives

The Shank proteins are emerging as important molecular scaffolds in excitatory synapses of the brain, but they might play additional roles during neuronal development and in non-neural cells. Many more protein partners of Shank will probably be discovered to add to the existing list. The challenge now is to unravel the functions of Shank at the biochemical, cellular and organismal levels by molecular and genetic approaches. In this regard, we note that Shank is highly conserved in evolution. Thus, reverse genetics in mice and in model organisms such as *Caenorhabditis elegans* should contribute greatly to our understanding of the physiological role of this interesting family of proteins.

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