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The toposome, essential for sea urchin cell adhesion and development, is a modified iron-less calcium-binding transferrin

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Abstract

We describe the structure and function of the toposome, a modified calcium-binding, iron-less transferrin, the first member of a new class of cell adhesion proteins. In addition to the amino acid sequence of the precursor, we determined by Edman degradation the N-terminal amino acid sequences of the mature hexameric glycoprotein present in the egg as well as that of its derived proteolytically modified fragments necessary for development beyond the blastula stage. The approximate C-termini of the fragments were determined by a combination of mass spectrometry and migration in reducing gels before and after deglycosylation. This new member of the transferrin family shows special features which explain its evolutionary adaptation to development and adhesive function in sea urchin embryos: (i) a protease-inhibiting WAP domain, (ii) a 280 amino acid cysteine-less insertion in the C-terminal lobe, and (iii) a 240 residue C-terminal extension with a modified cystine knot motif found in multisubunit external cell surface glycoproteins. Proteolytic removal of the N-terminal WAP domain generates the mature toposome present in the oocyte. The modified cystine knot motif stabilizes cell-bound trimers upon Ca-dependent dissociation of hexamer-linked cells. We determined the positions of the developmentally regulated cuts in the cysteine-less insertion, which produce the fragments observed previously. These fragments remain bound to the hexameric 22S particle in vivo and are released only after treatment of the purified toposome with reducing agents. In addition, some soluble smaller fragments with possible signal function are produced. Sequence comparison of five sea urchin species reveals the location of the cell–cell contact site targeted by the species-specific embryo dissociating antibodies. The evolutionary tree of 2-, 1-, and 0-ferric transferrins implies their evolution from a basic cation-activated allosteric design modified to serve multiple functions.
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Introduction

A potential model system for the study of embryonic cell adhesion and positional information (Wolpert and Gustafson, 1961; Noll et al., 1979) was introduced upon Herbst's discovery (1900), more than a century ago, that in calcium-free seawater sea urchin blastulae dissociate into single cells, which, when returned to normal seawater, reaggregate into normally developing embryos. The molecule responsible for this Ca^{2+}-dependent adhesive activity was isolated by the use of an assay (Noll et al., 1979, 1981; Matranga et al., 1986) in which reaggregation was inhibited by Fabs specific for the cell–cell adhesion site and the reversal of this inhibition by the addition of the active membrane component (Beug et al., 1970). In addition, the activity could be removed from the cell surface with non-cytolytic concentrations of butanol in seawater, a treatment that renders the dissociated cells incapable of reaggregation and development in seawater, unless the cells were resupplied with the dialyzed extract (Noll et al., 1979) or with the purified adhesion glycoprotein, called toposome (Noll et al., 1985).

Surprisingly, the active component was similar to a previously known 22S glycoprotein complex (Malkin et al., 1965; Infante and Nemer, 1968; Ii et al., 1978; Ozaki, 1980; Harrington and Easton, 1982) whose function had been controversial. Past work, lacking a functional assay and assuming its exclusive occurrence in cytoplasmic "yolk granules", sought to assign the 22S complex some nutritional role (Williams, 1967; Shyu et al., 1986). However, no depletion of the 22S particles is observed in cell homogenates of embryos raised under conditions of starvation, a treatment that was expected to force utilization of stored nutrients, but, contrary to expectations, stunted further development (Scott et al., 1990). Hence, this observation is inconsistent with a proposed role in nutrition, but confirms the proposed function as a stored membrane component (Gratwohl et al., 1991). Furthermore, the 22S particles do not decrease during embryonic development, contrary to what is expected for nutrients required for development (Kari and Rottmann, 1985).

Although previous efforts in cloning the gene that generates the 22S particle from *Strongylocentrotus purpuratus* (Shyu et al., 1986, 1987) suggested that it is related to the vitellogenin family, the fragments that were sequenced were too short for an unambiguous identification of the protein structure. Here we describe the sequence of a full-length cDNA of the 22S particle from *Tripneustes gratilla*. Its coding region of 1344 amino acids has no similarity to any known vitellogenin. The cognate protein belongs to the transferrin family, whose members are characterized by two internal repeats that form two lobes connected by a short alpha-helical peptide (Anderson et al., 1987). The 22S particle has the additional novel features of a 280-amino acid intervening sequence within its second lobe and a further C-terminal extension of 240 amino acids. Furthermore, the protein lacks most of the five iron-binding amino acids D, Y, R, Y, and H present at specific positions in iron-transporting transferrins (Baker et al., 1987; Legrand et al., 1988; Baker and Lindley, 1992), which is consistent with the Ca^{2+}-binding function of the 22S protein in cell adhesion rather than transport. We also provide here the entire highly conserved sequence of its homolog in *Paracentrotus lividus*. The iron-less nature of the toposome protein is not unusual for a member of the transferrin family, since other iron-less members of this family continue to be discovered. Two of these are also membrane-associated (Morabito and Moczydlowski, 1994; McNagny et al., 1996), but their functions remain unknown.

The most remarkable feature of the 280-amino acid intervening sequence within the transferrin structure is its central function in early sea urchin development. Developmentally regulated proteolytic modifications in this region, which fail to disrupt the hexameric particle in vivo, have been elucidated and are described below. To understand their significance, it is necessary to relate these post-translational modifications to earlier observations.

The cDNA-derived amino acid sequences reported here are not those of the 22S particle but of its precursor, which is synthesized exclusively in the gonads and gut of the adult animal as a 180–190 kDa glycoprotein (Shyu et al., 1986). From the unfertilized eggs, the toposomes are isolated as 22S particles (Noll et al., 1985), which on reducing SDS gels appear as a single 170 kDa band (Kari and Rottmann, 1985; Noll et al., 1985). We now show that the N-terminus corresponding to this band results from a cut between amino acids 86 and 87 of the precursor, thus generating the mature toposome. By contrast, after fertilization, the particles, while still sedimenting at 22S, are further modified proteolytically. When isolated from the cytoplasm or from purified membranes of blastulae and analyzed by SDS-PAGE, these particles give rise, in addition to the 170 kDa protein, to four fragments ranging in molecular mass from about 70 kDa to 110 kDa with some species-dependent variations. All of the smaller fragments derive from the 170 kDa subunit (Kari and Rottmann, 1985; Noll et al., 1985; Matranga et al., 1986; Armant et al., 1986; Yokota and Kato, 1988; Lee et al., 1989; Scott and Lennarz, 1989; Gratwohl et al., 1991; Mallya et al., 1992), since new synthesis cannot be detected either in the unfertilized eggs or in the embryo up to the pluteus stage (Heifetz and Lennarz, 1979; Shyu et al., 1986). This developmentally controlled modification by proteases in the "yolk" granules (Yokota and Kato, 1988; Lee et al., 1989; Scott et al., 1990) is detectable in *T. gratilla* 6 h after fertilization and reaches its peak at the early gastrula stage (Noll et al., 1985). However, these proteolytic cuts fail to disrupt the hexameric particle, in which the peptide chains are held together by the extensive disulfide bonds characteristic of the transferrin family. Blocking these cuts by inhibitors of thiol-proteases arrests development at the blastula stage (Mallya et al., 1992; H.N. and A.J., unpublished results). Here we present the N-terminal sequences derived by Edman degradation of the five proteolytic fragments from *T. gratilla* in addition to three internal peptides of the major C-terminal fragment of *P. lividus* determined by amino acid sequencing. The approximate C-terminal ends of the fragments from *T. gratilla* were estimated by their band positions in reducing gels and mass spectrometry. An extensive analysis of the structural details of the mature toposome confirms and extends our previous conclusions concerning its function.

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