Current Biology, Vol. 13, R717–R718, September 16, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00650-X

Cytokinesis: GAGs Form the Walls that Separate Our Parts Dispatch

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Cytokinesis, the final step of cell division, involves the formation of membranous barriers that partition cytosol and organelles between the resultant daughter cells. Recent studies reveal a crucial role for the extracellular glycosaminoglycan chondroitin in the completion of the cleavage furrow in dividing *Caenorhabditis elegans* embryos.

Animal cell cytokinesis is often viewed as the plain Jane sister of the more seemingly glamorous field of mitosis. Perhaps this is because a cursory examination of the fundamental process of cell cleavage suggests that it is simply the consequence of the constriction of an equatorial belt of actomyosin filaments. Recent studies, however, have revealed that the mechanisms of cytokinesis have unanticipated layers of richness, diversity and complexity. Two new papers [1,2] report evidence for another such layer. These two independent studies have shown that the synthesis and extracellular deposition of the glycosaminoglycan (GAG) chondroitin are necessary for the completion of cytokinesis in early embryos of the nematode worm *Caenorhabditis elegans* [1,2].

GAGs, also known as mucopolysaccharides, are a large structurally diverse family of long, unbranched chains of repeating disaccharide units which are often attached to extracellular core proteins. Chondroitin is usually sulphated, although this does not seem to be the case in *C. elegans* [2]. Protein–GAG complexes, known as proteoglycans, are ubiquitous components of extracellular structures and the cell surface. The negatively charged GAGs take up extended molecular configurations and attract low molecular weight cations, thereby forming a hydrated gel-like matrix that resists compressive forces [3]. The GAG heparin sulphate also plays a key role in regulating signal transduction through several pathways [4–6]. However, no such role has been ascribed to chondroitin or chondroitin sulphate.

The two new *C. elegans* studies [1,2] had quite separate starting points, but converged to a single gene (*T24D1.1*) which was shown to encode a chondroitin synthase (ChSy). In one study [2], genome database analysis was used to identify a single *C. elegans* ortholog of the human *ChSy* gene. In contrast to other organisms, the *C. elegans* genome has only one *ChSy* ortholog, *T24D1.1*, suggesting that the *C. elegans* ChSy may catalyze both initiation and subsequent elongation of the chondroitin polymer [2]. Immunohistochemistry revealed the presence of chondroitin on the surface of embryonic blastomeres and in the gonads of adult worms.

Suppression of *T24D1.1* expression in *C. elegans* by RNA interference (RNAi) markedly reduced the levels of chondroitin — but increased the levels of heparin sulphate — and gave rise to cytokinesis failures in early embryos. A deficiency in the *T24D1.1* gene was isolated and shown to cause defects in vulval morphogenesis in the homozygous progeny of heterozygous mothers. These homozygotes produced eggs that failed to develop, probably because of cytokinesis failures. To confirm that the cytokinesis failures were due to a failure of chondroitin deposition, cultured blastomeres were treated with chondroitinase ABC. This treatment again caused cleavages of the blastomeres to fail, although interestingly the furrows initially appeared to ingress normally but then subsequently regressed, indicating a failure of scission, the final breaking of the intercellular canal and resealing of the daughter cells [7].

The second study [1] began with a forward genetic screen for mutants that affect vulval morphogenesis in *C. elegans*. A collection of genes had been identified previously that, when mutated, give rise to a squashed vulva phenotype. Seven of these *sqv* genes were shown to control the biosynthesis of both chondroitin and heparin sulphate [8,9]. The eighth gene, known as *sqv-5* was identified in the current study as *T24D1.1* and found to be required for the synthesis of chondroitin, but not heparin sulphate [1].

The embryonic consequences of loss-of-function of *sqv-5* were not discussed by Hwang *et al.* [1]. In an earlier study, however, mutations of *sqv-1*, which encodes a UDP-glucuronic acid decarboxylase, and *sqv-7*, which encodes a nucleotide sugar transporter, were found to produce failures of cytokinesis [8]. These failures were manifested as a swelling of the early zygote which eliminates the extracellular space within the eggshell, and a failure to initiate cytokinesis. This phenotype is reminiscent of mutants that exhibit failures in osmoregulation [10] and may be rather different from the late-cytokinesis phenotype produced by suppressing expression of the ChSy gene *T24D1.1* [2].

GAGs are synthesized in the Golgi and delivered to the cell surface by means of the secretory pathway. Disruption of this pathway in *C. elegans* by treatment with Brefeldin A rapidly produces failures of scission [11]. Possibly, this observation could be explained by a failure to deliver chondroitin to the cell surface in Brefeldin A-treated cells. If so, this indicates that it is newly synthesized and transported chondroitin that is required for cytokinesis, rather than the presence of chondroitin in the pre-existing glycocalyx on the cell surface. Interestingly, secretion of the GAG hylan is also highly polarized toward the cleavage plane in dividing sea urchin zygotes [12].

The implication of chondroitin, and perhaps other GAGs as a necessary component of cytokinesis (at

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least in *C. elegans*), comes as something of a surprise. Although chondroitin is an abundant and bulky component of the glycocalyx on the cell surface, it is not obvious why this structure should be required for cytokinesis. Previous studies have emphasized that the process of cytokinesis is dependent on the presence of a number of proteins, many of them associated with the spindle midzone, but all of these proteins are intracellular [13]. The role of the spindle midzone in scission has, however, been suggested to be similar to that of the phragmoplast in plant cytokinesis [11].

Plants have rigid cell walls and cytokinesis proceeds through the phragmoplast-mediated transport of Golgi-derived vesicles to the equatorial plane to form the cytokinetic organelle known as the cell plate. Maturation of the cell plate is accompanied by the removal of the polysaccharide, callose (β-1,3-glucan) and the deposition of cellulose (β-1,4-glucan) and Golgi-derived pectins within the lumen of the cell plate [14]. Interestingly, mutations that affect the function of *Arabidopsis* proteins required for cell wall biosynthesis, including CYT1 and KORRIGAN/RSW2 [15–18], inhibit the completion of plant cytokinesis. Furthermore, synthesis of the extracellular polysaccharide, chitin, and bacterial peptidoglycan are also known to be required for cytokinesis in the budding yeast *Saccharomyces cerevisiae* [19] and the bacterium *Escherichia coli* [20], respectively. These observations suggest that the synthesis of extracellular matrixes may be an evolutionarily highly conserved component of prokaryotic and eukaryotic cytokinesis.

A possible role for the cell-surface polysaccharides in animal cytokinesis is to consolidate and strengthen the newly synthesized septal membrane or cleavage furrow. As these membranes are being formed by targeted vesicle fusion, it seems likely that nascent membranes from newly separated cells could be at risk of re-fusing again, unless prevented from doing so by a wall of GAGs that may act to keep the daughter membranes separate.

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