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Midwifery and assisted reproduction in *Dictyostelium* and *Entamoeba*

Cytokinesis is the terminal stage in the life of a replicating cell whereby two daughter cells are formed by the physical division of the mother cell. This conceptually simple event is mediated by a complex interplay between the microtubular mitotic spindle, the acto-myosin cytoskeleton and membrane fusion. Cytokinesis in animal cells is believed to be coupled to the cell cycle and is initiated after the inactivation of mitotic cyclin-dependent kinases. Typically, a mitotic spindle forms and the duplicated chromosomes are aligned at the equator of the cell. At anaphase, the chromosomes move away towards the poles and contractile ring components (non-muscle myosin II and actin) assemble at the cleavage furrow around the equator. During telophase the furrow constricts and the midbody forms at the intercellular bridge that connects the nascent daughter cells. Complete separation of the two newborns occurs with the severing of the midbody. Interesting deviations from this 'classical' model are seen in special situations such as syncytial divisions and cellularization, asymmetric cell division and incomplete cytokinesis. Variations in the event are noted during development as studied in flies (*Drosophila*) and roundworms (*Caenorhabditis elegans*). Variations have also been seen in cases of polyploidy such as megakaryocytes and giant trophoblasts in the mouse (for reviews see Balasubramanian *et al* 2000; Glotzer 2001).

Recent work has spotlighted cytokinesis in two different amoeboid cells, *Dictyostelium* and *Entamoeba*. *Dicytostelium* is able to grow both in suspension and as adherent cells on a surface. It exhibits three different modes of cytokinesis (A, B and C) during different conditions (Nagasaki *et al* 2002). Cytokinesis A refers to a myosin II-dependent and adhesion-independent division method which wild type cells use to proliferate. Cytokinesis B is myosin II-independent and adhesion-dependent. Both methods are coupled to the cell cycle and are possibly used by wild type cells during different environmental conditions. Under standard laboratory conditions cell division occurs within 3–4 min following nuclear division; mononucleate cells are formed in both cytokinesis A and B. Cytokinesis C is adhesion-dependent and myosin II-independent but different from cytokinesis B since it occurs 30 min or more after nuclear division is complete.

In an elegant study using genetic dissection combined with time-lapse microscopy, Nagasaki *et al* (2002), have shown the differences in the molecular mechanisms of these three modes of cell division. Single and double mutants of *mhcA* (myosin II), *amiA* (a gene involved in chemotaxis, communication between cAMP receptor and adenylyl kinase) and *corA* (coronin) were used to demonstrate the subtleties of the three processes. When grown in suspension, myosin II null mutants were unable to carry out cytokinesis. Others had shown earlier that cells in suspension that laced functional myosin II became multi-nucleated and lysed eventually. Cytokinesis B was disrupted in double mutants of *mhcA* and *corA* or *mhcA* and *amiA* but the phenotype of cells lacking both *amiA* and *corA* function was similar to cells lacking either amiA or coronin but not both. This pointed to the two genes functioning in a synergestic, rather than additive, manner. Furthermore, amiA and coronin appear to play largely unessential roles in cytokinesis A. However, cells defective in both cytokinesis A and cytokinesis B showed very low rates of cell cycle coupled cytokinesis, suggesting that these are the two major pathways for cell cycle coupled division in *Dictyostelium* growing in suspension or on substrate.

An unusual phenomenon was noted in mutants which were defective in cytokinesis A and B. When these cultures growing on substrates, became dense and entered mitosis, a neighbour cell often crawled towards the equatorial region of the mitotic cell and apparently helped the mitotic cell to divide (Nagasaki *et al* 2002). This parallels a similar observation on another amoeba, *Entamoeba*

invadens by Biron *et al* (2001). *E. invadens*, a reptilian parasite, is used as a model for the human pathogen *Entamoeba histolytica*. It grows at 25°C and can be induced to differentiate to cysts in axenic culture. Biron and co-workers demonstrated that in about one-third of the dividing cells contraction of the cleavage furrow was halted before physical separation was complete. The connected daughter cells overcame this problem with the help of 'midwife cells', which are chemotacticaly recruited for mechanical intervention. They also identified a likely chemical factor responsible for attracting 'midwives' and hypothesize that it was secreted by cells unable to complete cytokinesis.

The same study indicated that at low cell densities midwifery was less likely and abortive divisions and tripolar divisions more frequent. Intriguingly, a threshold number of amoebae are needed for *Entamoeba* cultures to grow and proliferate (unpublished observations). Thus dilution to a single cell almost never yields a successful clonal population, pointing to a possible need for 'midwives'. The central question is what do these 'midwives' do? Do they provide an extra surface for attachment? Do they adhere to two sides of the cleavage furrow and supply additional traction force? Is there membrane fusion between the cytokinesing cells and the midwives? Is there exchange of cytoplasmic factors? It seems most likely that the midwives simply supply additional supportive traction.

Why does the dividing cell require – and invoke – assistance? It is not yet established if assistance from other cells is the rule or whether it happens to a specific subset of cells or whether it is a random phenomenon. In asexual reproduction each daughter cell is supposedly an exact replica of the mother cell. What leads to the formation of cytokinesis-defective cells in a population of cells undergoing independent division? Are there epigenetic mechanisms at work here – perhaps a form of genomic imprinting? Are some cells predisposed to be 'midwives'? The organisms classified under the generic name 'amoeba' are traditionally labelled 'primitive'. Recent evolutionary studies have demonstrated this to be incorrect (Samuelson 2002). In evolution, the cooperativity shown by dividing amoebae may have been the earliest form of intercellular communication within members of the same species. The phenomenon may have been a forerunner of complex cellular networks, perhaps even rudimentary neural networks. What drives *Entamoeba* to depend on cooperativity may also tell us something about its differentiation and survival in the human host.

References

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