Nature Reviews Molecular Cell Biology | Published online 31 May 2017; doi:10.1038/nrm.2017.54

Journal club

CYTOPLASMIC MOVEMENTS OUTSIDE THE LIVING CELL

Inspired by the study of Allen and colleagues, who showed that cytoplasm from ruptured amoebae can continue to stream outside the living cell, Thompson and Wolpert devised a method to isolate cytoplasm from *Amoeba proteus*, which had the capacity to gel and undergo remarkably life-like movements outside the living cell! They used centrifugation to prepare concentrated cytoplasm from cells that had been chilled for 24 h. Movements began spontaneously when the extract was warmed to room temperature in the presence of ATP. Electron micrographs by their colleague E.H. Mercer showed that gelled extracts contained "spongy material and fibrils," which they thought might be interchangeable (Wolpert, Thompson & O'Neill). Thompson and Wolpert noted "this system seems to offer a very favourable starting material for the study of the molecular basis of amoeboid movement."

> "some were skeptical that anything could be learned about cellular movements after homogenizing cells"

At that time, no molecules that produce cellular movements had been purified from non-muscle cells. Furthermore, some were skeptical that anything could be learned about cellular movements after homogenizing cells to extract such molecules. Thompson and Wolpert's observations should have put these vitalistic prejudices to rest, but their paper received little notice — just two citations during the 1960's. I was told years later that others were unable to reproduce their experiments.

Thompson and Wolpert inspired my own entry into the field. Unaware that their work was in doubt, I reproduced their observations of ATP-dependent movements in extracts of *A. proteus*. Together with my mentor Susumo Ito, we used better fixation methods for electron microscopy to show that soluble subunits in the extracts assemble a gel of actin filaments when warmed to room temperature and supplemented with ATP. These filaments interact with thick filaments (presumed to be myosin II) to produce movements in the extract. The use of the extract was key, because intact amoebae react poorly to fixatives, which destroy the structures relevant to their movements. This laid the groundwork for studies of the actin cytoskeleton and its role in cell motility.

Thomas D. Pollard
Department of Molecular Cellular and Developmental Biology
Yale University
thomas.pollard@yale.edu

The author declares no competing interests.

ORIGINAL ARTICLES Allen, R. D., Cooledge, J. W. & Hall, P. J. Streaming in cytoplasm dissociated from the giant amoeba *Chaos chaos*. *Nature* **187**, 896–899 (1960) | Thompson, C. M. & Wolpert, L. The isolation of motile cytoplasm from *Amoeba proteus*. *Exp. Cell Res.* **32**, 156–160 (1963) | Wolpert, L., Thompson, C. M. & O'Neill, C. H. in *Primitive Motile Systems in Cell Biology* (eds Allen, R.D. & Kamiya, N.) 143–171 (Academic, 1964) | Pollard, T. D. & Ito, S. Cytoplasmic filaments of *Amoeba proteus*. I. The role of filaments in consistency changes and movement. *J. Cell Biol.* **46**, 267–289 (1970)