PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Introduction to Programmed proteolysis and the control of cell division. A Discussion Meeting held at the Royal Society on 4 and 5 November 1998

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THE ROYAL SOCIETY

Introduction

Since the earliest days of biochemistry, proteases have been prime objects of study. Their purification, crystallization, mechanism of action and control have all provided many a good living and many a fine training for many a long year. The focus has shifted gradually from the extracellular digestive enzymes found in animal stomachs and duodenums, to the acid hydrolases of the lysosomes (De Duve & Wattiaux 1966) and the cathepsins and peptidases found within cells (Barrett 1980), to the turnover of cytoplasmic enzymes by the ubiquitin system of marking proteins for cytoplasmic proteolysis (Hershko & Ciechanover 1998). The proteasome, the large protein complex that is responsible for the disposal of ubiquitinated proteins, has been purified, crystallized and its structure determined at atomic resolution (Groll *et al.* 1997). These are great scientific achievements, but as so often happens, pushing back the frontiers only reveals fresh puzzles, of which the present volume records our struggles to understand, at a Discussion Meeting held at the Royal Society in November 1998 in London. The main focus of the meeting was the role of proteolysis in the control of the cell cycle, a topic that, strangely, did not exist until quite recently. In his classic book, *The biology of the cell cycle* (Mitchison 1971), Murdoch Mitchison devoted considerable space to the then all-but-fruitless searches for periodic enzyme activities, and a decade earlier, the late Daniel Mazia had written compellingly (Mazia 1959) about the changes that must occur at the onset of mitosis in metazoan cells, with references to some of the earlier theories that tried to explain the mitotic cycle in terms of changing energy fluxes or the oxidation state of glutathione. In retrospect, it is surprising that nobody pointed out something that now seems obvious: that for cells to be different at different stages of the cell cycle, they not only need to make new proteins appropriate to the new state, but must degrade those proteins that uniquely characterized the previous condition. Otherwise, one could expect in a normal growing cell at best a twofold oscillation in protein level if a stable protein stopped being made. It is hard to generate irreversible step functions out of that. It began to emerge that proteolysis was a crucial mechanism for cell-cycle control in the early 1980s, when the abrupt disappearance of the mitotic cyclins was first observed (Evans *et al.* 1983), and the cell-cycle arrest imposed by ubiquitin ligase mutants was discovered (Finley *et al.* 1984). Prior to that, the importance of proteolysis in the control of cell-cycle transitions was quite unsuspected, and despite the landmark discovery of the ubiquitin system for intracellular proteolysis in the late 1970s (Hershko & Ciechanover 1982), it would have seemed highly improbable that the half-life of particular proteins could be regulated over a range of several orders of magnitude in response to signals generated within cells. Studies of the stability of ornithine decarboxylase, a well-known enzyme with a very short but regulated half-life, indicated a special degradative pathway which did not require ubiquitin (Elias *et al.* 1995; Glass & Gerner 1987; Murakami *et al.* 1992; Rosenberg-Hasson *et al.* 1989). It emerged slowly that ubiquitinylation could be highly regulated, and that cyclin proteolysis used this pathway (Glotzer *et al.* 1991; Hershko *et al.* 1991). We now know that there are at least two quite distinct systems for specific protein ubiquitinylation. The destruction box pathway identified by Glotzer *et al.* (1991) requires the giant, multisubunit anaphase-promoting complex (APC/cyclosome) (Irniger *et al.* 1995; King *et al.* 1995, 1996; Sudakin *et al.* 1995). The slightly simpler F-box pathway was identified (Bai *et al.* 1996) as a ubiquitin-dependent system for highly specific culling of particular phosphorylated proteins at particular times in the cell cycle. We also begin to see hints that such a useful mechanism for turning things on and off inside cells has a much wider application than ever suspected before, as in the control of p53 levels in response to DNA damage (Haupt *et al.* 1997; Kubbutat *et al.* 1997), in Professor Ohsumi's studies on autophagy in yeast (Mizushima *et al.*

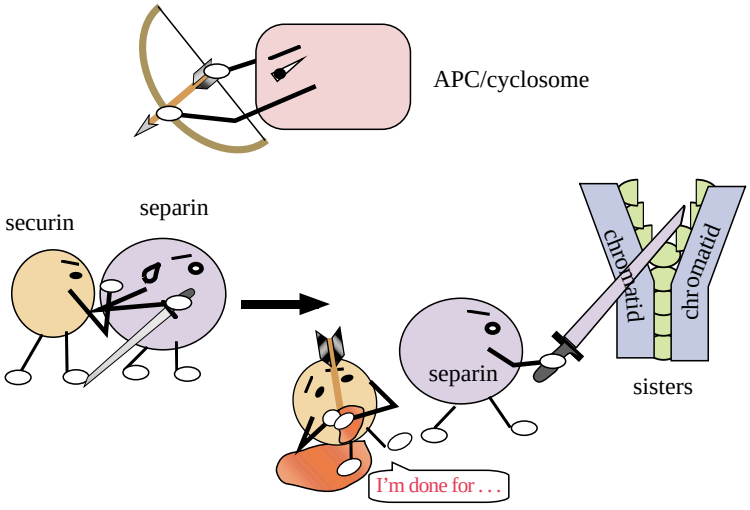

Figure 1. Cartoon by K.Kumada.

1998), and, since the meeting took place, in the control of cellular responses to hypoxia (Maxwell *et al*. 1999). Underlying all these systems are the tagging systems exemplified by ubiquitin and its relatives (Hershko & Ciechanover 1998; Saitoh *et al*. 1997). The wealth of detail recorded in the present issue should not obscure some underlying difficult questions about how proteins selected for programmed proteolysis are recognized with such precision, or precisely how the proteasome attacks its targets, or how protein tagging systems other than ubiquitin are working. We do not yet know the answers, and we hope that students browsing through the discussions after each paper will get a sense of where the fog still lies.

Finally, a note about the cover cartoon. It was drawn by Kazuki Kumada when he was a graduate student with Professor Mitsuhiro Yanagida. The cartoon depicts two separate acts of highly regulated and specific proteolysis in the control of chromosome segregation at the metaphase–anaphase transition. The names have been changed to reflect current usage: securin is Cut2 in *S. pombe*, or Pdsl in *S. cerevisiae*, and it contains target signals for the APC. The role of separin (Cutl in *S. pombe*, Espl in *S. cerevisiae*) in the separation of sisters seems to be accurately foretold in the light of recent results from Kim Nasmyth's laboratory suggesting that Sccl, one of the proteins required for sister chromatid cohesion, undergoes specific proteolytic cleavage in a reaction that depends on separin (Uhlmann *et al*. 1999). It remains to be seen if separin is actually a protease or not. Labelling on the original cartoon was partially in Japanese; we hope nothing was lost in the translation.

The organizers would like to thank all those who took part in the meeting, and especially the staff of the Royal Society of whom Kaye Pudney deserves singling out for bearing the brunt of the administration.

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