Cell multiplication: editorial overview

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A remarkable year for cell cycle studies

Although the mechanisms and control of cellular repro-
duction have been studied for many years, it seems clear
that the year under review has been one of the most re-
markable yet in terms of the breadth and depth of the
insights it has brought us. It also seems clear that this
is no accident, and that we can expect more remark-
able years of discovery in the near future. The reason
is, of course, that the development of recombinant-DNA
technology (in conjunction with other technical advances
on a broad front) has triggered a revolution in cell biol-
ogy that is comparable in its profundity to that brought
on by the rapid development of cell fractionation and
electron microscopy procedures in the late 1940s and
early 1950s. The flames of the present revolution are be-
ing fanned by the growing appreciation of the degree to
which fundamental mechanisms are conserved through-
out the eukaryotic world.

The power of genetic and recombinant DNA approaches

Although recombinant DNA technology has facilitated
cell biological studies in many ways, its most profound
impact derives from its having rendered practical each
of two complementary genetic strategies, both of which
are abundantly illustrated in the reviews that follow. One
of these strategies,'forward genetics', has of course been
pursued vigorously throughout this century. It begins
with the collection of interesting mutants, and has the
great advantage that the mutations may affect proteins un-
dreamt of by biochemists, too rare to be studied easily
during waking hours, or both. Thus, totally unknown ter-
ritories can be (and often have been) penetrated. How-
ever, until the advent of recombinant DNA technology,
there was little real hope of charting these territories
at the molecular level. Today, however, the mutational
identification of an interesting gene can generally be fol-
lowed by the physical isolation of that gene as a recom-
binant clone. This in turn leads to the sequence of the
gene product, to its overproduction for facilitation of bio-
chemical studies, and to the generation of antibodies that
allow (among other things) the immunolocalization of
the gene product. The information so obtained may not
lead immediately to a molecular understanding of gene
product function, but it certainly eases the path.

The alternative strategy, 'reverse genetics', has really only
been thinkable since the advent of recombinant DNA
technology. In this strategy, one begins with an inter-
esting protein (or a messenger RNA with an interest-
ing pattern of expression) and then uses an appro-
priate trick to isolate the corresponding gene. *In vitro*
manipulations of the gene, followed by its transfection
back into the appropriate cells, allow investigation of
the functions *in vivo* of whole proteins and of their
individual domains. The results are usually informative
and sometimes startling, as in the discoveries that some
eukaryotic cells can survive without clathrin (Payne and
Schekman, *Science* 1985, 230:1009–1014; Lemmon and
Jones, *Science* 1987, 238:504–509; Payne *et al.*, *J Cell
Biol* 1988, 106:1453–1461) and that *Dictyostelium* amoe-
bae can crawl without myosin heavy chain (De Lozanne
and Spudich, *Science* 1987, 236:1086–1091; Knecht and
Loomis, *Science* 1987, 236:1081–1086).

The conservation of fundamental mechanisms and its implications

Although the past year has seen great progress on many
fronts in the cell cycle field, surely the most remarkable
discoveries relate to the control of mitosis and the re-
alization of how closely the mechanisms of this control
are conserved throughout the eukaryotic world. The *Sac-
charomyces cerevisiae CDC28* gene and the *Schizosac-
charomyces pombe cdc2$^+$* gene were originally identified
by temperature-sensitive mutations that arrest cells at the
critical G_1 control point known as Start (Hartwell *et al.*,
Science 1974, 183:46–51) and at mitosis (Nurse *et al.*,
Mol Gen Genet 1976, 146:167–178), respectively. Later
work showed that *CDC28* was also involved in the con-
trol of mitosis (Piggott *et al.*, *Nature* 1982, 298:391–393)
and that *cdc2$^+$* was also involved in the control of Start
(Nurse and Bissett, *Nature* 1981, 292:558–560), and that
the two genes were closely homologous and cross-func-
tional (Beach *et al.*, *Nature* 1982, 300:706–709; Booher
and Beach, *Mol Cell Biol* 1986, 6:3523–3530), despite the
vast phylogenetic gap between these species (Huysmans
et al., *Nucl Acids Res* 1983, 11:2871–2880). This suggested
that the fundamental mechanisms might be common to
all eukaryotes, a view that was dramatically supported
when Lee and Nurse (*Nature* 1987, 327:31–35) identified
a human homologue by virtue of its ability to comple-

Abbreviations
cAMP — cyclic adenosine monophosphate; cAPK — cAMP dependent protein kinase; MPF — maturation-promoting factor.

ment an *S. pombe cdc2* mutation. This startling result was followed quickly by the equally startling discoveries that the *Xenopus* homologue of *CDC28/cdc2⁺* was a central component of the long-elusive maturation-promoting factor (MPF) and that the *CDC28/cdc2⁺*/MPF homologues of various cell types all appear to interact with the local versions of 'cyclin', a protein originally detected by virtue of its striking pattern of continuous synthesis followed by abrupt post-mitotic breakdown in the cleaving eggs of marine invertebrates (see reviews by Fantes, pp 250–255 and Hunt, pp 268–274 in this issue).

These and other observations suggest that there is a deep conservation of fundamental mechanisms among eukaryotes; in some areas, this may extend to prokaryotes as well (see the reviews by Masters, pp 241–249 and Blow, pp 263–267). This in turn suggests that it is entirely appropriate to continue using a wide variety of cell types and experimental techniques to study fundamental cell biological problems. It's not so bad that *Xenopus* is a geneticist's nightmare if the proteins identified biochemically in frogs can be studied genetically in yeasts; it's not so bad that yeast chromosome cytology is difficult if the proteins identified genetically in yeast can be studied cytologically in mammalian cells. To pick just one concrete and topical example, the fascinating new proteins identified in mammalian cells by immunofluorescence screening of scleroderma and monoclonal antibodies (e.g. Cooke *et al., J Cell Biol* 1987, 105:2053–2057) will be difficult to understand if they can only be studied in mammalian cells. However, if homologues can be found in yeasts, then the exploration of protein function should be greatly facilitated.

In stressing the conservation of fundamental mechanisms, we should also not overlook the wide range of apparent variations in cell cycle organization and control as seen especially among the lower eukaryotes (see review by Berger, pp 256–262, in this issue). To the extent that these variations reflect only superficial differences in the expression of highly conserved mechanisms, they should nonetheless be instructive in revealing the degree of flexibility of those mechanisms. If deep differences in mechanism are involved, this will be all the more interesting, as there is clearly more to life than its least common denominator.

The diversity of cell cycle problems and progress

Studies of cellular reproduction must address two distinct categories of questions: (1) questions about the mechanics of the process (*how* does a cell reproduce, once it has undertaken to do so?) and (2) questions about the overall control of the process (what determines *whether and when* a cell will reproduce?). In organising this series of reviews, we attempted to subdivide the cell cycle field in such a way that all major questions in both categories would be addressed. However, inevitably, this has not happened. There has been too much progress on too many different problems, and the constraints of time and space have forced the authors to be selective rather than comprehensive. Although their choices of topics to

consider are all well justified, in many cases other choices could have been made with equal justification. In the remainder of this overview, I have attempted to fill in some of the gaps (with the explicit caveat that I have certainly not filled them all) and offered some of my own perceptions as to the status of certain problems in this field.

The mechanics of cellular reproduction

Identification of cell cycle genes and proteins

We cannot thoroughly understand any machine without a fairly complete inventory of its parts. The cell cycle is no exception, and a continuing effort to identify genes and gene products that function at specific stages of the cycle is clearly vital. In this regard, at least three developments are worthy of special note. First, recent successes suggest that it may soon be routine (even in cell types other than yeast!) to clone by complementation genes that have originally been identified by conditional-lethal mutations that arrest the cell cycle (Ittmann *et al., Mol Cell Biol* 1987, 7:3386–3393; Sekiguchi *et al., EMBO J* 1988, 7:1683–1687; Osmani *et al., Cell* 1988, 53:237–244). Second, recent successes emphasize the potentially rich harvest obtainable by starting with conditional-lethal cell cycle mutations and seeking interacting genes using a battery of tactics including conventional suppression, suppression by overexpression of heterologous sequences, unlinked non-complementation, and synthetic lethality (Regan and Fuller, *Gen Dev* 1988, 2:82–92; Stearns and Botstein, *Genetics* 1988, 119:249–260; Toda *et al., Gen Dev* 1988, 2:517–527; Adams *et al., Science* 1989, 243:231–233; Hays *et al., Mol Cell Biol* 1989, 9:875–884; Bender and Pringle, *Proc Natl Acad Sci USA* 1989, in press). Third, immunological screening using banks of scleroderma and monoclonal antibodies is beginning to identify many interesting proteins that can then be studied using reverse genetics (e.g. Cooke *et al.,* 1987; Snyder and Davis, *Cell* 1988, 54:743–754).

Macromolecule synthesis through the cell cycle

Heintz (pp 275–279) has reviewed the considerable progress made toward understanding the temporal regulation of gene expression during the mammalian cell cycle. It is worth noting that this issue is also being studied successfully in yeast and other lower eukaryotes (Breeden, *Trends Genet* 1988, 4:249–253; Berger, pp 256–262). However, it is also worth noting that the significance of such regulation in cell cycle progression and control remains problematic. In particular, both of two common assumptions (that genes important in cell cycle control must exhibit temporal regulation of transcription; that genes exhibiting temporal regulation of transcription must be important in cell cycle control) are almost certainly wrong. Firstly, it seems abundantly clear that the levels of gene products can vary during the cell cycle for many reasons other than periodic transcription (Heintz, pp 275–278; Evans *et al., Cell* 1983, 33:389–396). Secondly, it seems equally clear that many proteins of indisputable importance in the regulation of cell cycle pro-

gression vary little or not at all in their levels through the cycle (Aves *et al., EMBO J* 1985, 4:457–463; Simanis and Nurse, *Cell* 1986, 45:261–268; Brizuela *et al., EMBO J* 1987, 6:3507–3514; Lee *et al., Nature* 1988, 333:676–679), are normally present (or can easily be tolerated) in vast excess over the amount needed to complete the cell cycle in progress (Byers and Sowder, *J Cell Biol* 1980, 87:6a; Sclafani *et al. Mol Cell Biol* 1988, 8:293–300), or both. Third, cells seem remarkably ambivalent about whether particular proteins are produced continuously or in a cell cycle-specific fashion (Creanor *et al., J Cell Sci* 1983, 61:339–349; White *et al., EMBO J* 1986, 5:1705–1709; Berger, pp 256–262) and remarkably tolerant of the continuous production of proteins that are normally produced in a cell cycle-specific fashion (Han *et al., Cell* 1987, 48:589–597; Han *et al., EMBO J* 1988, 7:2211–2219; McIntosh *et al., Mol Cell Biol* 1988, 8:4616–4624). In summary, it appears that cells exploit mechanisms at all possible levels to regulate cell cycle progression, but that the most generally important mechanisms involve the regulation of gene product activity or assembly. Although such regulation no doubt involves numerous specific mechanisms, covalent modifications such as phosphorylation (Fantes, pp 250–255; Hunt, pp 268–274; Russell and Nurse, *Cell* 1987, 49:569–576) and ubiquitination (Murti *et al., Proc Natl Acad Sci USA* 1988, 85:3019–3023; Goebl *et al., Science* 1988, 241:1331–1335) seem certain to be important.

Spatial organization in the cell cycle

Interest in the temporal organization of the cell cycle should not blind us to the fact that the maintenance and generation of appropriate spatial organization during the cycle is equally important. Important progress in understanding morphogenesis in the cell cycle has been made both in bacteria (Sommer and Newton, *J Bacteriol* 1988, 170:409–415; de Boer *et al., J Bacteriol* 1988, 170:2106–2112; Hahnenberger and Shapiro, *J Bacteriol* 1988, 170:4119–4124) and in yeasts. In *S. cerevisiae*, we have learned much about the functions of the cytoplasmic microtubules, which are not involved in polar growth of the cell surface but do function in nuclear migration and spindle orientation (Huffaker *et al., J Cell Biol* 1988, 106:1997–2010; Jacobs *et al., J Cell Biol* 1988, 107:1409–1426), as well as much about the proteins that associate with actin to carry out polarized cell surface growth (and perhaps other processes; Drubin *et al., J Cell Biol* 1988, 107:2551–2561; Magdolen *et al., Mol Cell Biol* 1988, 8:5108–5115; Adams *et al.,* 1989; Liu and Bretscher, *Proc Natl Acad Sci USA* 1989, 86:90–93). It is particularly pleasing that yeast appears to contain a full complement of the actin-associated proteins that have been described in other cell types, which should open this whole complicated system to facile genetic investigation. We have also begun to learn more about an apparently novel type of cytoskeletal element that functions in morphogenesis at the bud neck in *S. cerevisiae* but is probably present in other eukaryotic cells as well (Haarer and Pringle, *Mol Cell Biol* 1987, 7:3678–3687; Kim *et al., J Cell Biol* 1989, in press;Haarer *et al.,* personal communication).

Cell division

Spindle function during mitosis was reviewed recently by Mitchison in this series (*Curr Opin Cell Biol* 1989, 1:67–74) and eukaryotic cytokinesis will by reviewed by Salmon in the next issue (*Curr Opin Cell Biol* 1989, in press). In addition, it should be noted that continuing progress is being made in understanding both prokaryotic cell division (de Boer *et al.,* 1988; Nathan and Newton, *J Bacteriol* 1988, 170:2319–2327; Phoenix and Drapeau, *J Bacteriol* 1988, 170:4338–4342; Beall *et al., J Bacteriol* 1988, 170:4855–4864) and the mechanisms of disassembly and reassembly of the nuclear envelope during mitosis (Newport and Spann, *Cell* 1987, 48:219–230; Sheehan *et al., J Cell Biol* 1988, 106:1–12; Wilson and Newport, *J Cell Biol* 1988, 107:57–68).

The control of cellular reproduction

Growth control versus cell cycle control

In considering the overall control of cellular reproduction, it is essential to remember the differences in lifestyle between unicellular organisms and the individual cells of multicellular organisms. The former are typically programmed to grow (i.e. increase in mass) as rapidly as the supply of energy and nutrients from the environment allows and to divide as often as the cell mass and essential components can be duplicated. In contrast, the latter must reproduce at rates consistent with their roles in the organism as a whole. Moreover, it is not only cell division but also cell growth that must be controlled — an uncontrolled increase in cell mass would be just as inappropriate in such cells as would uncontrolled division. This refusal to grow must be maintained even though the cells are (usually) bathed in a nutrient-replete environment in the organism. Thus, the cell's decision as to whether or not to reproduce is intimately tied to its decision as to whether or not to grow, and much interest attaches to the study of the 'growth factors' that seem to control both decisions (Westermark and Heldin, pp 279–285), as evidenced in part by the uncontrolled growth and division produced by oncogenes that resemble growth factors or their receptors (Sigal and Gibbs, pp 286–290).

The foregoing arguments suggest that unicellular organisms do not require *growth* control but only *cell cycle* control. How then do we explain the function of the *S. cerevisiae RAS*/cyclic adenosine monophosphate (cAMP)/cAMP-dependent protein kinase (cAPK) system, mutational perturbations of which can clearly produce a G_1-specific block of the cell cycle, apparently by first blocking growth? The details of this complicated system of interacting components continue to be worked out with impressive speed (Toda *et al.,* 1988; Marshall *et al., Mol Cell Biol* 1988, 8:52–61; Wilson and Tatchell, *Mol Cell Biol* 1988, 8:505–510; Powers *et al., Mol Cell Biol* 1989, 9:390–395). Two recent developments are particularly intriguing. First, cells appear to be reasonably normal if they totally lack cAMP, so long as they contain an unregulated but appropriately attenuated cAPK catalytic subunit (Cameron *et al., Cell* 1988, 53:555–566); this suggests

the existence of cAMP-independent mechanisms whose regulatory roles parallel those of the cAMP-dependent ones. Second, there is increasing evidence that *RAS* plays a second role in yeast in addition to its role in regulating adenylate cyclase (Toda *et al*, *Cell* 1987, 50:277–287; Wigler *et al*, *Cold Spring Harb Symp Quant Biol* 1988, in press); this resurrects the possibility that direct information about the functions of *ras* in mammalian cells may be gained by studying yeast.

Despite this progress, the ultimate role of the *RAS*/cAMP /cAPK system in yeast physiology remains somewhat obscure. I think that the most reasonable view at present is that this system exists not for the overall control of growth but for coordination of its different aspects. That is, by allowing a coordinate shut-down (or activation) of different aspects of macromolecule synthesis, it allows the cell to avoid the unbalanced growth that might otherwise result as environmental conditions change.

G₁ control, mitotic control, and cell cycle oscillators

The observation that certain subsets of cell cycle events can continue periodically even when other subsets of events are blocked has made the concept of a 'fundamental cell cycle oscillator' seductive (Creanor and Mitchison, *J Cell Sci* 1986, 86:207–215; Kimelman *et al*, *Cell* 1987, 48:399–407). However, I think that this is a somewhat misleading perception based on: (1) insufficient attention to the degree to which the events of the cell cycle are organized as multiple interconnected chains of dependent events (Pringle and Hartwell, *In* The Molecular Biology of the Yeast *Saccharomyces*, edited by Strathern, Jones and Broach. Cold Spring Harbor Laboratory, 1981, pp 97–142; Hirano *et al*, *EMBO J* 1986, 5:2973–2979; Ohkura *et al*, *EMBO J* 1988, 7:1465–1473), and (2) excessive attention to certain cell types that are highly programmed for rapid divisions. It is important to remember that the cleaving egg represents but a moment in the life cycle and that the nutrient-replete environments that we provide for microorganisms in the laboratory are presumably the exception rather than the rule in nature. For most cells, most of the time, each trip around the cell cycle is an adventure to be undertaken only when the environment has been carefully assessed for suitability. A substantial weight of evidence suggests that this assessment normally occurs at a control point in G₁.

This line of reasoning thus also leads me to a viewpoint that may seem rather heterodox in the current flush of (entirely justifiable) excitement about mitotic control (see above). That is, I think it is reasonable to regard this control more as a checkpoint (or coordination point) in a system that is already committed to reproduction than as a point at which fundamental decisions about whether to reproduce or not are taken. After all, even in *S. pombe*, where mitotic control has been so elegantly studied (Fantes, pp 250–255), nutritional limitation (Nasmyth, *J Cell Sci* 1979, 36:155–168) or the need to choose between asexual and sexual reproduction (Nurse and Bissett, 1981) quickly makes manifest the existence of a decision point in G₁. It is only fair to note that this argument

begs the fascinating questions of how and why the same proteins (the *S. cerevisiae* CDC28 and *S. pombe* cdc2+ products) function *both* in mitotic control and at Start in G₁.

G₀ versus G₁

It has long seemed reasonable to suppose that cells that opt not to reproduce exit from the cell cycle (normally from G₁) into a discrete G₀ state. Although non-proliferating cells do indeed generally differ from actively proliferating cells in many ways, it has proven surprisingly difficult to identify unequivocally gene products that function specifically in the transition to or from the hypothetical G₀ state. For example, after a long search, Johnston, Singer and colleagues finally identified a yeast mutant that seemed to be defective specifically in the G₀ to G₁ transition (Drebot *et al*, *Proc Natl Acad Sci USA* 1987, 84;7948–7952). However, genetic analysis of this mutant revealed that it contained two unlinked mutations, one of which blocked the cell cycle of actively proliferating cells as well as the first cycle upon emergence from stationary phase, and the second of which suppressed the first in actively proliferating cells. Thus, it seems unlikely that either of the genes identified is specifically involved in the G₀ to G₁ transition.

An alternative approach is to screen for proteins or messenger RNAs that are expressed differentially in non-proliferating or proliferating cells (or during transitions between the two states), and indeed many potentially interesting species have been found in this way (Lau and Nathans, *Proc Natl Acad Sci USA* 1987, 84:1182–1186; Philipova *et al*, *Biol Cell* 1987, 60:1–8; Tominaga, *FEBS Lett* 1987, 226:53–57; Wang, *J Cell Physiol* 1987, 133:151–157; Ching and Wang, *Proc Natl Acad Sci USA* 1988, 85:151–155; Ryder *et al*, *Proc Natl Acad Sci USA* 1988, 85:1487–1491; Rollins *et al*, *Proc Natl Acad Sci USA* 1988, 85:3738–3742; Schneider *et al*, *Cell* 1988, 54:787–793; Linzer and Mordacq, *EMBO J* 1988, 6:2281–2288; Ryseck *et al*, *Exp Cell Res* 1989, 180:266–275; Ryseck *et al*, *Exp Cell Res* 1989, 180:537–545; and references cited therein). However, it seems fair to say that it remains unclear (and may be rather hard to determine) how many of these species, if any, actually function in *regulating* the transitions between the proliferating and non-proliferating states. The application of reverse genetic procedures to sporulating yeast has shown that inferring differential function from differential expression is a risky business (Kaback and Feldberg, *Mol Cell Biol* 1985, 5:751–761; Yamashita and Fukui, *Mol Cell Biol* 1985, 5:3069–3073; Law and Segall, *Mol Cell Biol* 1988, 8:912–922).

Conclusions

The pace of discovery in the cell cycle field during the past year has been staggering. However, as should be apparent from this and the reviews that follow, there is also no dearth of interesting problems left to study. This field will clearly keep us busy for many years to come.