Unlike my two distinguished friends, I did not set out on my scientific career with the intention of studying the cell cycle, and had no idea that the winding road of discovery would lead in that direction. On the contrary, I was interested in the control of protein synthesis, thanks to my Ph.D. advisor, Asher Korner, who studied the effects of insulin on protein synthesis in the rat. When I joined his laboratory in 1964, Asher used to perform one experiment every week, comparing the activity of ribosomes from the livers of control and hypophysectomised rats. On my first day, Asher suggested that I spend some time in the library to look for an interesting project, which I did, finding papers about nephrotic rats whose livers greatly increased their synthesis of serum albumin to compensate for its loss through leaky glomerular membranes. How, I wondered, did the ribosomes “know” that the albumin levels were low, and how was that “knowledge” translated into enhanced albumin synthesis? As far as I know, this would still make a good research project, so it is probably lucky that my first attempts to make some rats nephrotic by injecting them with a kidney homogenate were completely unsuccessful.

IN THE BEGINNING: RABBIT RETICULOCYTES

I was also very fortunate that Louis Reichardt, recently graduated from Harvard, had decided to spend the year in Cambridge, and we shared a bench together. Louis had learned how to work with rabbit reticulocytes, and was trying to make a cell-free system for globin synthesis that would respond to added haem by increasing the rate of globin synthesis. Ironically, while Louis was not successful in his project, either, Hildegard Lamfrom and Paul Knopf working up the road at the MRC Laboratory for Molecular Biology reported that they were able to make a cell-free extract of reticulocytes that briefly maintained a high rate of protein synthesis; I don’t believe we were aware of their efforts at the time. And in the spring of 1965, several of us attended our first scientific meeting, a few hundred yards away in the Chemistry Laboratory. The Symposium was devoted to haemoglobin synthesis and was introduced, very appropriately, by Henry Borsook from Caltech. Borsook had shown in the middle 1950s that reticulocytes needed to be sup-
plied with iron salts in the medium to maintain a high rate of protein synthesis, but his talk on the 8th April 1965 was entitled "Early development of the echinoid egg compared with erythropoiesis" (1). He contrasted the restriction of developmental options found in red blood precursors with the unfolding pattern of development seen in fertilized sea urchin eggs. What caught my attention was his excellent account of the changes in protein synthesis that follow soon after sea urchin eggs are fertilized. But there was no hope of studying sea urchins in Cambridge, and it was a talk by Vernon Ingram that got me started in research. Ingram spoke about the relationship between haem and globin synthesis, and presented some preliminary data that he interpreted to indicate that ribosomes formed a queue behind the sequence in globin that would form the haem pocket. He used the methodology that had been introduced by Howard Dintzis in his brilliant experiments which showed that proteins were made from N- to C-terminus (2). When we got back to the laboratory, we realised that Ingram had interpreted his data exactly backwards, and that, if his data were correct, they meant that ribosomes raced down the mRNA until the haem was added and then slowed right down. So Tony Hunter and I decided to repeat the experiments, making some improvements to the experimental approach, building our own electrophoresis tank and teaching ourselves the rudiments of protein chemistry, encouraged by Alan Munro. The results were slow in coming, but eventually proved beyond doubt that the ribosomes paid no attention whatsoever to when the haem was added and were normally distributed completely at random along the globin messenger RNA (3). Certain tricks allowed us to show that our methods would have enabled us to see queues, had they existed.

One day, I let a centrifuge run too long and we accidentally discovered that α-globin chains were made by smaller polysomes than β-chains (4), and we set out to discover the cause by comparing the rates of elongation of the two chains. By omitting an extremely elementary control, we wrongly concluded that β-chains took longer to translate than α-chains, a misinterpretation that was later corrected by Harvey Lodish (5). This was a sharp lesson about control experiments. We missed another trick, too, which would have been to discover that both globin chains were initiated with a methionine residue. In this case, our mistake was not to believe our own results and to ascribe them wrongly to contamination. Fortunately, Richard Jackson and Tony Hunter later corrected this oversight before anyone else (6).

In 1968, I left Cambridge and went to work in New York with Irving M. London, who was then the chairman of the Department of Medicine at the Albert Einstein College of Medicine. Irving had worked with David Shemin, the man who had elucidated the biosynthetic pathway of haem, and was passionately interested in the question of how haem regulated globin synthesis. About this time, Howard Schulman discovered that addition of haemin to Lamfrom and Knopf's lysate sustained prolonged high rates of protein synthesis (7) and it was a simple matter to confirm this result and start a programme designed to find out how the haem was working in molecular terms. Many people contributed to this endeavour, in particular Marco Rabinowitz
at the NIH who discovered a dominant inhibitor of protein synthesis that formed when the reticulocyte lysates were incubated for long periods at 37° in the absence of added haemin (8). The molecular nature of this inhibitor and the basis of its mode of action were to remain obscure for another five or six years, and as so often, in my experience of research, the clues and answers came from indirect attacks in unexpected quarters, rather than a full frontal assault. For Marco’s inhibitor proved very difficult to purify.

The first clue came from a collaboration with a neighbour in the Department of Medicine, Nechama Kosower. Nechama’s husband Ed was a chemist who had suggested the use of a mild oxidizing agent called “diamide” to cure sickle cell anaemia (I cannot reconstruct the train of thinking). The diamide rapidly converted glutathione to its oxidized form and (reversibly) inhibited protein synthesis in intact reticulocytes. We assumed that the effect on protein synthesis was somehow connected with the loss of GSH, and started to use the cell-free lysate system to investigate. One day it occurred to me that the inhibition could be due to the formation of GSSG rather than loss of GSH, and I tested the effects of adding GSSG to the lysate in the presence of optimal levels of haem. To my surprise and joy (because it had not occurred to the others), minute amounts of GSSG caused an inhibition of protein synthesis that was very reminiscent of that seen when haem was omitted (9). Protein synthesis at first occurred at a high rate, and then initiation shut off after a few minutes. Just as with omitting haem, protein synthesis could be restored after its shut-off, in this case by addition of a reducing agent like dithiothreitol. At some point, it was found that addition of N-ethyl maleimide was a very good way of turning on the Rabinowitz inhibitor, so a finger of suspicion pointed at critical –SH groups; but this apparent clue never really led anywhere. Richard Jackson and I (Figure 1) returned to study the GSSG effect some years later, and discovered that the inhibition of protein synthesis was due to loss of reduced NADP and glucose-6-phosphate, which took us off into studies of thioredoxin and thioredoxin reductase. Affinity chromatography using 2',5' ADP Sepharose (which binds many NADP-requiring enzymes) proved extremely useful in this analysis and the whole episode was a very good and enjoyable series of lessons in practical biochemistry of a rather old-fashioned kind (10, 11).

At the time of the collaboration with the Kosowers, research on the mechanism of action of haem on protein synthesis was not going well, and as a diversion I decided that it would be fun to see if the reticulocyte lysate could translated added poliovirus RNA, and if so, to work out the gene order using essentially the same methods that Tony Hunter and I had employed in our studies of globin synthesis. I discovered that there was a group in Harry Eagle’s Department of Cell Biology who worked on poliovirus, and went and begged some RNA from Don Summers. Analysis of the reaction on the newly-invented SDS-polyacrylamide gel showed no sign of any newly-synthesized high molecular weight product(s), however, and I suspected (correctly, as it turned out many years later) that factors from the nucleated cells that poliovirus normally infected were required for the initiation of polio protein syn-
thesis. To test this idea, I collaborated with Ellie Ehrenfeld, and made the exciting discovery that poliovirus-infected HeLa cell cytoplasm contained a potent inhibitor of reticulocyte lysate protein synthesis (12). The inhibition looked almost exactly like that seen when haem was omitted or GSSG added. What was different, however, was the identity of the inhibitor, which had the remarkable properties of heat stability, sedimentation at 20S in a fairly sharp peak, and resistance to digestion with DNase, RNase and proteases. We were baffled for several weeks by this mysterious substance, whose assay was very tedious (and non-linear), because of course RNase and proteases were deadly inhibitors of protein synthesis and every assay involved re-isolating the inhibitor on sucrose gradients to separate it from the various agents used to probe its identity. In the end, after a depressing period when we deduced that we must be dealing with a giant complex carbohydrate, whose analysis we knew ourselves unable to conclude, we realised that double-stranded RNA had all the properties of our unknown. (13). We consulted with Jerry Hurwitz on the 9th floor, who suggested we used micrococcal nuclease, and not to forget to add the calcium, which sowed the seed of the nuclease-treated reticulocyte lysate assay system for eukaryotic mRNA that Hugh Pelham eventually developed (14).

The micrococcal nuclease made the inhibitor disappear completely, and the next step was to see if the effect was specific for polioviral dsRNA. I bought some poly I:C from Sigma and tested it from 1 mg/ml down in 3-fold steps and was puzzled to find that only the very lowest concentration seemed
to cause significant inhibition. It was reasonable to suppose that I had simply placed the samples back-to-front in the scintillation counter, so I repeated the experiment with a much wider range of concentrations of the synthetic dsRNA and was extra-careful with labelling of the samples. The results were startling. Not only did high levels of dsRNA not inhibit protein synthesis, but the levels required for inhibition were staggeringly low: we later calculated that it would only take one molecule of polivirus replicative form dsRNA to completely inhibit protein synthesis by the millions of ribosomes present in a HeLa cell. The dsRNA therefore had to be acting catalytically and not as we at first imagined by mimicking the 5' end of mRNA and titrating out some initiation factor. We could explain the lack of inhibitory effect of high levels of dsRNA by supposing that two molecules of the putative pro-inhibitor had to bind side-by-side on the RNA, and later back in Cambridge, Hugh Robertson and Tony Hunter found that a stretch of about 50 nucleotides of perfectly double-stranded RNA was required to activate the inhibitor (15), a pre-echo of much more modern times that explain why it is important for 'RNAi' to be short: otherwise, protein synthesis is generally inhibited.

The similarity of the kind of inhibition caused by lack of haem or addition of GSSG or dsRNA strongly suggested that they must have an underlying mechanism in common, and the similarities only increased when I left the Bronx and returned to Cambridge at the end of 1970 to be reunited with my old friends Tony Hunter and Richard Jackson. By then, they had discovered that protein synthesis was initiated by a special methionine tRNA, Met-tRNAf, which provided an important new tool for investigating the pathway of initiation of protein synthesis and its control by the strange collection of disparate inhibitors. Our next great insight came when Steve Legon and Chris Darnbrough found that a previously ignored complex between Met-tRNAf and 40S ribosomes disappeared before protein synthesis shut off (16). It was then fairly simple to show that these complexes formed without the need for mRNA, and we realised that ribosomes must find the start of mRNA by scanning the message with the anticodon of the initiator tRNA. Since it was established dogma that tRNA only bound to ribosomes at the instruction of mRNA, our paper met with a hostile and sceptical reception on first submission, and we needed help from Mike Mathews, another ex-Kornerite, who was by then working at the MRC Laboratory of Molecular Biology and had developed a method for assaying mRNA.

INTRODUCTION TO PROTEIN KINASES

But here again we got stuck for a while, largely because we were using the reticulocyte lysate as an assay system, and either total protein synthesis or the formation of the 40S-Met-tRNAf complexes as the read-out. We did not seriously attempt to dissect partial reactions, largely because none of us had proper biochemical training, and we had got a long way simply analysing this wonderfully active cell-free system. At this stage, I don't think we seriously tried even to purify any of the inhibitors, and did not seriously speculate on
what the underlying molecular explanations might be. It would have been too far. Instead, Steve Legon followed up a clue that cyclic AMP might have some connection with the various inhibitory conditions, and began to test all manner of purines, many of which alleviated the effects of leaving out the haem or adding dsRNA (17). These phenomenological effects were further pursued by Ken Balkow, who had joined the laboratory shortly before a tremendous fire consumed the entire laboratory, and forced our departure to a teaching laboratory in Herman Lehmann's clinical biochemistry Department at the Addenbrooke's Hospital. The destruction of all previous results and the new close contact with molecular biologists, in particular with John Gurdon's group, were highly beneficial, and once the laboratory was set up and running again, progress was quite rapid. Richard Jackson began to get evidence that ATP might be necessary for the inhibitor of initiation to form, and realised that it should be possible to test whether ATP was also necessary for the inhibitor to act by using a partial reaction, the binding of Met-tRNA\textsubscript{f} to 40S ribosomal subunits, which required GTP but not ATP. This proved successful, and inhibition clearly required ATP, something that would never have emerged from studies of bulk protein synthesis. From there it was a very short step to adding labelled ATP to the inhibition reactions and seeing a new phosphorylated band form. This was the work of a talented new graduate student, Paul Farrell. We also discovered that both the haem-regulated inhibitor and the dsRNA-activated inhibitor tended to undergo self-phosphorylation. Moreover, the identity of the target for phosphorylation emerged almost at once, because the molecular weight of the major target band corresponded suspiciously closely to one of the subunits of eIF-2 which had recently been isolated by Theo Staehelin and his colleagues Hans Trachsel and Bernhard Erni at the Basel Institute for Immunology. This protein formed a complex with Met-tRNA\textsubscript{f} and GTP, and delivered the tRNA to the 40 S ribosome. It was a very satisfying explanation for the previous 5 years of accumulated phenomenology, and it simply had to be right (18). I went to Basel for a week or two to learn how to make and handle the factor, in the process for the first time seeing at first hand how proteins were purified. It was not long before we had convinced ourselves that phosphorylation of eIF-2 was responsible for the inhibition of protein synthesis, but there was one remaining puzzle. Phosphorylation of eIF-2 did not seem to impair its ability to form complexes with Met-tRNA\textsubscript{f} and GTP, nor its ability to deliver initiator tRNA to the 40S ribosome. Gradually it emerged that what went wrong was the ability of the eIF-2 to recycle, specifically to exchange its GDP for new GTP. Once again, our lack of proper biochemical skills prevented us from making any significant contribution to the identification of the GDP/GTP exchange factor known as eIF-2B. Similarly, despite considerable efforts, I failed miserably to purify the haem-regulated protein kinase or to understand its mode of activation, and we fared no better with the dsRNA-activated protein kinase now known as PKR.

Getting to the bottom of this kind of regulation of protein synthesis was very satisfying. We had been faced with a long-standing mystery and worked it out. The answers made sense and looked as though they might have rather
wider applicability. During my stay in New York, I had spent a couple of weeks one summer in Gordon Tompkins's laboratory at UCSF looking at "the pleiotypic program" (19) close up, and had seen how extremely easy it was to inhibit pol lysome formation by all kinds of mild abuse of nucleated cells. Could it be that the global control of protein synthesis seen in cultured cells starved of amino acids or glucose, or subjected to sudden changes in temperature, were also simply due to phosphorylation of eIF-2? I grabbed an opportunity presented by Tom Humphreys to help teach the Embryology Course at the Marine Biological Laboratory, Woods Hole in the summer of 1977 in order to get my hands on some sea urchin eggs, and back home in Cambridge we set new graduate students working on making cell-free systems from nucleated cells to see if what was true of reticulocytes was universal. Progress was very disappointing, in every case rather seeming to suggest that the answer was no. About the only interesting thing to emerge from these studies was another protein kinase, which Tony Walker and Carl Anderson discovered by adding DNA to cell-free extracts of HeLa cells (20). We failed to appreciate that this needed free ends of DNA, as Steve Jackson later found (21), such was our inexperience with DNA. It was in any case quite clear that this had nothing to do with the control of protein synthesis.

The sea urchin eggs were more difficult to work with than I had imagined, too, and this was not helped by the equipment and layout of the MBL's embryological classroom. The idea was to study fertilization in as many different phyla and organisms as possible, using the simplest possible equipment and a microscope. Biochemical approaches were not much in vogue, and running gels impossible at first. There was no liquid nitrogen or -80˚ freezers, and Eppendorf tubes and Gilson pipettors were nowhere to be seen. Moreover, the season for sea urchin eggs was short, and I had no experience. For example, I had always thought that blood and sea water had similar ionic compositions, but this turned out to be quite false, sea water being much more concentrated. So what should one use as a homogenisation medium to have any hope of obtaining an active cell-free system in which to study protein synthesis? By then, Richard Jackson and I had almost completely defined the salient ions and low molecular weight compounds that were present in the reticulocytes (22), and we had a keen appreciation of how important they were for the maintenance of high-rate protein synthesis, to say nothing of its physiological control.

SEA URCHIN AND CLAM EGGS

So the next summer was spent in Cambridge, and I missed both the sea and the exhilaration of learning new things, for though Woods Hole had been a disappointing research environment for what I wanted to do, it was the perfect place to learn cell and molecular and developmental biology because of the stream of expert lectures on a wide variety of topics, together with the opportunities for discussion in labs, bars and beaches. So I had no problem in agreeing to return there in 1979 (Figure 2), except that I now insisted on a
–80’ freezer being provided and went with a suitcase full of all the little things I might need – tubes and tips and gel-plates and even a peristaltic pump – so that I could set up a lab away from home. During that summer, Dennis Ballinger and I followed up a hint from two years before from Tom Aune that changes in phosphorylation could be seen in sea urchin eggs after fertilization (23). This proved to be very useful experience, in fact, because running SDS polyacrylamide gels of fertilized sea urchin eggs was not completely trivial, owing to the powerful proteases present in the head (acrosome) of the sperm. But even more rewarding was helping Eric Rosenthal and Joan Ruderman with their analysis of the changing patterns of protein synthesis found in clam oocytes. Bruce Brandhorst had recently made detailed studies of sea urchin eggs, and had concluded that there was little if any evidence of a change on the kinds of proteins made before and after fertilization (24). But clams were quite another matter, probably because whereas sea urchin eggs are shed in a kind of G0-like state, having completed meiosis in the ovary, clam eggs are in fact G2-arrested oocytes, and fertilization causes them to complete meiosis, rather as progesterone acts on frog oocytes except with a different time-scale. Joan and Eric had beautiful gels showing a spectacular change in the synthesis of at least three prominent bands on a 1-dimensional gel after fertilization, and it was easy to show that the mRNA encoding these proteins was present before fertilization, by cell-free assay in the reticulocyte lysate. It was also a simple matter to demonstrate that the mRNAs were in the untranslated “mRNP” compartment in the oocytes, and shifted onto poly-

Figure 2. The Embryology Course, MBL, 1979. Included in the picture are the author, Tom Humphreys, Joan Ruderman, Eric Rosenthal, Andrew Murray, Ed Southern and Gerry Rubin.
somes by the time the oocytes had completed meiosis, about 40 minutes after fertilization (25). This was quite an important advance, being one of the first really striking demonstrations of authentic translational control, a field that had been somewhat lagging behind the general control of protein synthesis in reticulocytes for want of clear examples to study. But we also began to wonder why these cells needed to upregulate protein synthesis or to change the kinds of proteins they were making after fertilization. Considering what a long time the eggs had had to fill up with ribosomes, polymerases and so on, and considering that they were not going to increase in mass until they began to eat, many hours if not days later, it was puzzling to know what these new proteins might be doing.

My introduction to cell cycle control was provided by a clear, scholarly and beautiful seminar given by John Gerhart one afternoon in the summer of 1979. He told the embryology class about the properties of MPF and of his struggles to purify it (26). I was captivated by the idea that a biochemical approach might provide insights into a cell cycle transition, that there might actually be an enzyme which literally catalysed mitosis. However, outrageous such an idea seemed in many ways, it seemed from all that was known that this was rather likely to be true. But although attractive, there did not seem any obvious way to get from what I was studying to MPF, and the appearance of the new bands in the clams occurred too late to correspond to what was known about MPF in frogs, the focus being on meiosis rather than the cleavage divisions. Like Borsook’s lecture twelve years before, this talk made a deep and lasting impression without having the least influence on the work I was doing at the time.

I spent the next two summers teaching and researching in Woods Hole as a member of the faculty of the Physiology Course, first with Ken van Holde and then Joel Rosenbaum as director. Eric Rosenthal and Andrew Murray helped with the teaching, and Dennis Ballinger spent time in the lab working on the ribosomal phosphorylation, although we gradually came to appreciate that this was not the secret of the increase in protein synthesis: for one thing, this was not a universal concomitant of fertilization in sea urchin eggs, and for another, the phosphorylated ribosomes did not enjoy privileged access to the mRNA. So, although the teaching was fun – showing the students how to handle reticulocyte lysates, make and assay mRNA from strange sea creatures, and run beautiful SDS-polyacrylamide gels – no frontiers were being pushed back. I was envious of Andrew Murray, who together with his advisor Jack Szostak was defining the minimal elements of chromosomes in yeast (27).

THE DISCOVERY OF CYCLIN

Thus it was in late July, 1982, with the teaching over and the sea urchin season drawing to a close (Figure 3), that I planned and executed the experiment that changed my life and got me straight into studying the cell cycle. It was, as far as I can recall (and even three weeks later its original purpose was already unclear, as judged by a rather detailed letter home to Richard Jackson) de-
signed to see if there were any differences in the pattern of protein synthesis in properly fertilized sea urchin eggs compared to eggs activated by the calcium ionophore, A23187. By this time, I knew exactly how to do the experiment and to prepare the samples for the gel analysis. I added $[^{35}S]$ methionine to suspensions of the eggs in Millipore-filtered sea water in a 50-ml beaker. Samples were removed at intervals into trichloracetic acid, and the protein precipitates washed with acetone before dissolving in sample buffer. I did not want to miss any transients, and also wanted to build some redundancy into the experiment. In retrospect, it is amazing that this simple experiment hadn’t been done before – after all, we had done exactly the same thing using $^{32}$PO$_4$ as the label – why we had never used $[^{35}S]$ methionine, if only as a kind of class demonstration, is a mystery. I will return to the question of why nobody else did it, either, at the end of this account, in a footnote about gels.

The autoradiogram showed something very odd and unexpected, for although most of the bands got stronger and stronger as time went by, one band did not show this, the expected behaviour. It started as one of the strongest, but at a certain point it faded away. It was difficult to think of any other explanation than that it underwent specific (in the sense that no other bands were affected) proteolysis at some point in the early development of the fertilized egg. The parthenogenetically activated samples did not show such clear disappearance.

That very evening, I chanced to meet John Gerhart again at the wine and cheese party that follows after the Friday evening Lecture. He told me about the experiments on MPF that he, Mike Wu and Marc Kirschner had been
doing (28), and the priceless information that, although the first appearance
of MPF in frog eggs provoked by the injection of MPF did not require protein
synthesis, the second wave of MPF corresponding to the second meiotic divi-
sion did require new proteins to be made. This was electrifying, because it
suggested that a protein was consumed at the end of meiosis I so that new
protein synthesis was required to replenish it. This kind of behaviour was, of
course, exactly what I had seen that morning in the behaviour of cyclin. It is
worth noting that between the two meiotic divisions there is no replication of
DNA, so that the explanation for a protein synthesis requirement during the
cell cycle that was much in the air at the time, that of making new histones to
package up the DNA, did not apply. It seemed far more likely that if MPF was
turned on by some kind of post-translational modification, it was turned off
by the most drastic of all such modifications, proteolysis. Yet we stopped short
of concluding that MPF must contain cyclin as one of its subunits: that would
be only one of many possible explanations, and it seemed extremely impos-
able that we should have hit so lucky by chance. Nevertheless, in the course of
this, easily the most exciting conversation of my scientific life, the outline of
the now conventional view of the cell cycle as depending in part on program-
med proteolysis was born, more or less fully fledged. It was on July 22nd,
1982. I must say that such “eureka” moments are very, very rare in my expe-
rience. It normally takes several weeks of experiments to tease out the truth
even when you have a really pretty good idea of what is going on.

It was of course clear that the experiment must be repeated, and that we
should check cell cycle progression as best we could at the same time, using a
nice healthy batch of eggs that underwent synchronous division. We needed
to check the comings and goings of cyclin in relation to the cell cycle. Tom
Evans, a Cambridge project student, had come with me that summer to act as
an assistant, and he and I set to work to do as much as we possibly could be-
fore the Arbacia egg season ended. We soon confirmed the behaviour of cy-
clin, and showed that it was continuously synthesized and periodically almost
completely degraded, about 10 minutes before the fertilized eggs divided. We
tried but failed to assess the metaphase to anaphase transition, and we added
every known inhibitor of cell cycle progression (as we naively understood
them at the time) to see if they affected the oscillations of cyclin. Dan Distel,
another student, was doing a project that wasn’t going well, so I suggested
that he take a look in clam eggs to see if any proteins came and went. Much to
our surprise and delight, his lovely gel of August 17th showed that the two
larger translationally regulated proteins A and B were both cyclins, whereas C
behaved conventionally, and accumulated steadily. By labelling later embryos
and then “chasing” with the protein synthesis inhibitor, emetine, it was pos-
sible to see that cyclin was still disappearing well into cleavage. We announced
our findings at the Annual General Meeting of the MBL (29), but later kept
quiet about this short note, lest it prevent publication in a “proper” journal.

The apparent size of sea urchin cyclin judged by its gel mobility was very
close to that of tubulin, and fortunately for us there were a number of real ex-
erts in the course that summer. John Kilmartin, who was a friend of Joel
Rosenbaum's, had recently obtained an excellent anti-tubulin monoclonal antibody which he had attached to Sepharose, so it was easy to see if cyclin did or did not bind, and my graduate student Sarah Bray found first, that cyclin was by no means entirely soluble, second, that it did not bind to the column, and third, that another of the labelled proteins, corresponding to band C of the clam as it later turned out, was quantitatively retained by the column, along with the tubulin. We assumed at first that it was a newly-synthesized microtubule-associated protein, but in fact, its C-terminus fortuitously matched the epitope that the antibody recognised, an early lesson in the use of antibodies in research. Later, Nancy Standart made good use of this antibody and we found it could be used for affinity chromatography of what turned out to be the small subunit of ribonucleotide reductase (30). Ironically, both this enzyme subunit and the cyclins shared the property that they were just one subunit of a two-subunit protein of which the other subunit was laid down in eggs as part of the maternal endowment, but we were not to know this until much later.

Although there seemed to be a suspiciously close relationship between cyclin and MPF, and the behaviour of cyclin easily explained how MPF was turned off at the end of mitosis, we were miles from proving any such thing. Indeed, the universality of MPF was by no means established at that time. The only way forward was to clone, sequence and express cyclin to find out what it was, as well as to investigate the consequences of its non-appearance, which we imagined might be possible using antibodies, for example. But there were no people or grants or indeed sea urchins in Cambridge and any progress had to wait until the next summer. I wrote up the initial observations and sent them to Cell, who replied that they would publish the paper, but in "nothing like its present form". One of the reviewers said that it represented "wild speculation based on dubious logic", and I still blush when I look at even the revised version that appeared in the late spring of 1983 (31), although the Discussion ended on a deliberately guarded note: "The parallels between the behavior of MPF and cyclin are striking, but whether there is a direct correspondence between the physiological entity and the chemical one remains to be determined". We did not over-interpret the data.

By the next Spring, the whole episode felt like a dream that seemed increasingly too good to be true. I tried to explain how exciting this was to everyone I met, but the general reaction was sceptical. It had never (as far as I know, or have been able to find in fairly extensive reading of the old literature) been suggested before that cell cycle control might involve proteolysis and it was inconceivable that such specific intracellular proteolysis was even possible, for the recently-described ubiquitin system had been defined in terms of a mechanism for degrading unfolded or denatured proteins. Not until Michael Glotzer left an autoradiograph to expose over a long ski weekend in 1990 did the first signs appear that programmed proteolysis could occur by the ubiquitin pathway (32).

Jonathan Pines did his "Part II Project" with me in the spring of 1983, and agreed to join me as a graduate student to work on cyclin in the autumn.
Meanwhile, another project student, Richard Cornell, came with me to Woods Hole as my assistant that summer, Jonathan having decided to bicycle across the U.S.A., ending up briefly in Woods Hole at the summer’s end. When Richard and I got to the MBL, the first thing we tried was simply to repeat the famous experiment. Much to my relief, cyclin was still there and still showed exactly the same behaviour, and Richard set about trying to find out exactly when it started to disappear: before or after the metaphase to anaphase transition? We were helped by Yoshio Masui, the master of MPF, who was on the faculty of the Embryology Course downstairs and very kindly showed us how to do orcein staining. Elayne Bornslaeger looked at the effects of inhibiting DNA synthesis on the behaviour of cyclin, and confirmed that this prevented the cells entering mitosis, completely stabilizing cyclin. It became clear that cyclin disappeared very close to the time of the metaphase-anaphase transition, but frustratingly we had no way to specifically block its disappearance and examine the consequences.

By this time, of course, I was trying to find out everything that was known about cell cycles and their control. I read reviews and books and talked to all sorts of people, but none of them shed much light on what I was trying to understand. Nobody had ever suggested that something might have to go away in order to keep the cycle turning, as far as I could see. In discussions with people, everyone agreed that this wasn’t such a ridiculous idea. Moreover, it fitted in very well with what was known about the role of protein synthesis in early development. As Wagenaar and Mazia and Wagenaar reported (33), following on Hultin’s original observations (34), protein synthesis was needed for entry into mitosis during early cleavage, and was required in the first half of every cell cycle, quite consistent with the idea that protein or proteins needed to be replaced by new synthesis after they had been degraded, although of course the proteins in question could have been used up in other ways – assembly into chromatin, for example.

During that summer, Andrew Murray looked hard for cyclins in budding yeast, but was unable to detect any by the kind of pulse-chase methods that worked so well in sea urchins. By this time, too, Eric Rosenthal had succeeded in identifying clones for clam cyclin A in cDNA libraries (35), whereas we hadn’t even started to make libraries from sea urchins, and material was in short supply. Back in Cambridge, nobody in the Biochemistry Department had any experience of recombinant DNA work, and indeed, the rules and regulations were still rather tight: special rooms and dedicated equipment were required for this kind of work, and in any case, surprisingly few people yet appreciated the revolution that was taking place. We were regarded as hopelessly trendy, and when I ventured to ask Marion Purvis, the Departmental Administrator if there was some way to keep frogs, she replied helpfully “Over my dead body”. It did not look very promising, and by the end of Jon’s first year, we were no nearer to cloning cyclin. Then Jeremy Minshull (Figure 4) started as a graduate student in the laboratory, and we began to investigate the “hybrid arrest of translation” that we were going to need to identify the cyclin clones, and also perhaps to knock out cyclin synthesis in sea urchin
eggs. We got M13 shotgun clones for TMV that Philip Goelet had used in his determination of the sequence of this classic virus (36), and began to test if antisense clones could block synthesis of TMV protein programmed by viral RNA. We very quickly discovered two things. First, the circular M13 DNA needed to be cut into pieces in order to work (we used HaeIII, which can cut single-stranded DNA). Second, we discovered that although a clone that corresponded to the extreme 5' end of the viral RNA was pretty efficient at preventing protein synthesis, ones that started further along the mRNA hardly had any effect at all – unless one added RNase H to cut the RNA strand of the DNA-RNA hybrids that had formed. It turned out that the levels of RNase H in reticulocyte lysates were extremely low, and that the hybrid arrest that had previously been reported used wheat germ extracts, which were rich in this enzyme (37). We got side-tracked into doing rather detailed studies on the inhibition of globin synthesis by short oligonucleotides, defining times, concentrations, temperatures and specificities (for example, what would a single mismatch do?); these were indeed to prove useful later.

By this time, Jonathan had made a small cDNA library in M13 virus from *Arabidopsis* mRNA and using Jeremy’s protocols found a clone that would specifically ablate cyclin synthesis among the first 50 or so that he tried. This was not really surprising, considering that cyclin looked as though it comprised about 5% of the total synthesis after fertilization. From there, of course, it was a fair-
ly simple matter to obtain full-length *Arbacia* cyclin clones by more conventional means, and the complete sequence of cyclin B emerged at Christmas 1986 (38), by which time it was possible to compare sequences with Spisula (clam) cyclin A, for clone 1T55 had been sequenced by Kevin Farrell and Joan Ruderman in 1983-4. The two cyclins showed strong homology over their last 200 or so residues, but looked like nothing in any of the sequence databases at the time. They were clearly not protein kinases, although immunoprecipitates of clam cyclin A seemed to possess protein kinase activity. This was difficult to interpret without much fuller characterization of the early antisera, and I do not think the idea that cyclin might be associated with another protein that was a protein kinase seriously – or even playfully – entered our minds at this stage. I knew all about these developments, because I had stopped teaching in Woods Hole in 1983 and worked with Joan during the summers of 1984 and 1985, characterising the comings and goings of (labelled) cyclins during meiosis in clam oocytes. We learned how to fix cells and observe chromosomes and to define the points of no return for protein synthesis in relation to the various meiotic and mitotic landmarks. I was entranced by the chromosomes, and understood meiosis properly for the first time in my life. We often used parthenogenetic activation to look at meiosis, partly because it was more reliable than adding sperm and partly because it was easier to watch how the female pronuclei behaved without the confusion of the sperm, which tended to stick to the surface of the eggs and make for less aesthetically pleasing views. We discovered, however, that it was important to wash out the calcium ionophore quite early, or strange things happened that we never fully understood. And the A23187-activated oocytes got stuck at the first mitotic division. Without a second spindle pole, a monaster formed, and the cells remained in M-phase for very long periods, interestingly with high levels of cyclin B yet low levels of cyclin A. This was very reminiscent of what happened when colchicine was added. What was very gratifying was that inhibiting protein synthesis eventually led to a reduction in the level of cyclin B, and exit from M-phase, the only time that inhibiting protein synthesis ever speeded up cell cycle progression. I thought this was pretty spectacular, but I don’t think many people understood. Moreover, I was pleased by the thought that colchicine’s stabilisation of mitotic chromosomes was really due to stabilisation of cyclin, and only indirectly due to inhibition of tubulin polymerisation. Not many people knew that! Less pleasing, however, was the discovery that there was not a perfect correlation between the time of disappearance of the cyclin in these experiments and the decondensation of the chromosomes or the re-formation of the nucleus. There were clearly other things going on that we were not seeing, and interpreting all the curious phenomenology surrounding the meiotic divisions in relation to the requirements for protein synthesis was quite impossible. In many ways the nicest experiments we did in those two summers was measuring how long the proteolysis window stayed open in mitosis in these lovely objects (39). As we had previously found in the sea urchin egg, inhibiting DNA synthesis completely stabilised cyclins A and B alike. Once cells entered mitosis, however, cyclin A went away a minute or two
before cyclin B, which in turn went down about 30 seconds or so before the chromosomes visibly parted at anaphase. Proteolysis then remained on for about 5 minutes, after which the cyclins could accumulate again. We couldn’t see any other labelled proteins showing this kind of behaviour, although we began to suspect that if Nature had invented such an exquisitely specific proteolysis machine, it was unlikely that it was used exclusively for cyclins, and we wondered, quite rightly as it has much more recently turned out, if the “glue” that held sister chromosomes together might not suffer the same fate as the cyclins. But this really was wild speculation. I should add, too, that we never thought to check if this 5-minute proteolysis window applied to anything else than clam and sea urchin eggs, for the simple reason that we had no idea if yeast or human cells contained cyclins. There was a real possibility that these proteins had evolved to control the rapid early cleavage cell cycles of marine invertebrate eggs, and were found nowhere else.

THE LINK WITH MPF

So the next great advance came from a simple experiment by Katherine Swenson and Joan Ruderman in the Spring of 1986 (40). They asked what would happen if synthetic mRNA made from clone 1T55, encoding clam cyclin A, was injected into a frog oocyte. The answer was extremely gratifying, not to say electrifying, for the oocytes behaved exactly as if they had been injected with MPF. This made people sit up and take note of cyclin. At the same time, the result was puzzling in terms of MPF for two reasons. First, it was well-known at the time that stage VI frog oocytes contained "pre-MPF” whose activation by a starter-dose of active MPF did not require protein synthesis. Indeed, the pathway of activation was quite mysterious, although it was suspected to involve changes in the phosphorylation of something to do with MPF. Thus, although Katherine’s spectacular result implied that cyclin was indeed intimately involved in the control of entry into M-phase, it hinted that cyclin was rather peripheral to MPF itself. This tentative conclusion was only reinforced by data in the paper showing that clam oocytes, which also contained pre-MPF, did not contain any detectable cyclin A until well after the first appearance of MPF. Probably it was thinking along these lines that made us favour the idea that cyclin was “anti-anti-MPF”, and a jotting from a notebook of 1985 shows an attempt to make sense of things in these terms (Figure 5).

The summer of 1986 found me in Berkeley, California with the authors of Molecular Biology of the Cell, beginning to work seriously on the companion Problems Book with John Wilson. In those days, one had to go to libraries to read the literature, and I was lucky to get John Gerhart’s blessing to use the excellent little library up the hill in Stanley Hall. Even better was Mike Wu’s offer one day to show me a real live MPF assay, which I eagerly accepted, and the thought that I could get some maternal mRNA from Eric Rosenthal, who was by then a postdoc with Fred Wilt in the main Zoology Department. We quickly confirmed Katherine and Joan’s result using poly A+ RNA from Urechis
caudo and Mike was surprised, because in all his time doing mRNA assays for the Bay Area Community, he had never once observed oocyte maturation. We followed this up with an experiment to see if mRNA from Xenopus eggs made Xenopus oocytes mature, which it did. Creeping back to the incubator at 10 in the evening to see if it worked was extremely naughty and thrilling. Here was suspicious evidence that vertebrates probably had cyclins, too; a great encouragement to Jeremy Minshull after I returned to Cambridge in September. It was a little bit surprising that nobody had thought of looking for the mRNA for MPF earlier, and rather frustrating that there was no easy way to take matters further without very much more work along the cloning and sequencing path. During that summer, both Andrew Murray and Marc Kirschner came to the rented house where we were staying, and we discussed how to unravel the relationship between cyclins and MPF. Andrew’s idea was make a cell-free system based on the one first described by Lohka and Masui (41) that would test the ability of cyclin synthesis to “drive” the cell cycle. It was impossible to imagine doing this in Cambridge, and we agreed that he could have a cyclin clone to test as soon as we had one. Jeremy and I would concentrate on the antisense approach, at which we felt we were getting pretty competent.

Careful comparison between the sea urchin cyclin B and clam cyclin A sequences showed a short stretch of very high homology even at the DNA level, and we designed a minimally redundant antisense oligonucleotide based on this sequence. A test of this oligonucleotide on a variety of starfish, sea urchin and frog mRNA preparations showed very promising results. Bands of the
right size went away. After Christmas, Jeremy came back and within weeks we had our first (short) frog cyclin B clones. At this point, I sent him and Jon back to Berkeley to try the effects of cyclin B and of the antisense oligonucleotides on frog oocyte maturation, under the expert tuition of John Gerhart and Mike Wu. Jon’s mRNA worked beautifully to induce oocyte maturation, but Jeremy’s antisense experiments were not as convincing and we began to suspect that frogs might possess a larger repertoire of cyclins than we had imagined. Indeed, back in England, Jeremy started collaborating with Alan Colman in Birmingham, who was very interested in the MPF story (and in whose laboratory John Shuttleworth cloned the Cdc2 homologue “MO15”, which we later found to be the missing CDK activating kinase (42). They soon found that although the antisense oligonucleotides were working perfectly, the oocytes’ ability to mature in response to progesterone was unimpaired. This was a setback, but not a terminal setback. After all, if cyclin were MPF, we knew that the oocytes already contained the protein, so abating the mRNA would not make any difference. And we had only obtained cyclin B clones: doubtless there would be cyclin A as well, whose synthesis might be vital for the activation of MPF. It was to take almost 10 years to sort out this point (43). In fact, Jeremy kept on isolating and sequencing cyclin clones from *Xenopus* and then testing their role in oocyte maturation by antisense ablation. We stopped after B1, B2 and A1, thereby missing B3, B4, B5 and A2 to say nothing of cyclins D, E and F. We knew then that there were more B-type cyclins to be found. Jon Pines finished his thesis in March 1987 and left Cambridge for Tony Hunter’s laboratory at the Salk Institute with the mission of identifying the human cyclins. He was soon writing home with news of success (44). Meanwhile, Nancy Standart went to Roscoff to see if starfish, the other great source of MPF, had cyclins. Sure enough, there were two rounds of synthesis and destruction corresponding to the two meiotic divisions, although protein synthesis was not necessary for starfish oocyte maturation. It is obvious from the discussions of the papers during that period that we were completely obsessed by the question of the relationship between cyclins and MPF (45). Equally obvious is our bafflement.

**THE CONNECTION WITH YEASTS**

As soon as the signature sequences of cyclin were known, I began scanning protein sequences in papers about the cell cycle, and also asking Paul Nurse if any of their genes looked like ours. The answers were always negative, and DNA sequencing was not usually very high up the priority lists of geneticists at the time. In fact, it was Mark Solomon in Marc Kirschner’s laboratory who spotted that Bob Booher’s sequence of fission yeast Cdc13 corresponded to a B-type cyclin. This caused great excitement and a certain amount of skulduggery, as it was forbidden to tell Paul Nurse of the news, although as luck would have it I visited Oxford shortly thereafter. As usual, I enquired if any of their genes corresponded to cyclins, and was told that they did not. I went off on holiday and came back to find Booher’s paper in the August number of the
EMBO Journal (46), where the sequence was plain for all to see, although there was no mention of cyclins. Shortly after, a couple of notes drawing attention to the homology appeared in Cell (47, 48), and the whole story began to make sense.

In 1987, Alan Colman was teaching in Woods Hole and organised a small symposium about MPF, where Jim Maller showed the famous picture of his and Fred Lohka’s purification of Xenopus MPF (49). Their purest fractions contained two bands and displayed histone kinase activity. The lower band had a molecular weight around 32,000 and the larger band was a shade small for cyclin B, but I promised Jim that as soon as we had antibodies, we would see if it was cyclin, a promise we later kept with the help of Jean Gautier (50). It turned out that Jim’s lab and mine used different recipes for SDS-polyacrylamide gels that largely accounted for the difference in mobility.

The key developments as far as I was concerned took place in parallel in Cambridge and in San Francisco. In Cambridge, Jeremy and I enlisted the help of Julian Blow to help with frog extracts, and succeeded in blocking cyclin synthesis with carefully chosen oligonucleotides that had minimal effects on the synthesis of other proteins in the lysates. We found that these reagents did not affect DNA replication, but did block entry into mitosis (51). Andrew Murray’s approach was much bolder and the results more impressive. Jon Pines’s sea urchin cyclin clone could “drive” several trains of cell cycles in an RNase-treated Xenopus egg extract, and moreover, if the N-terminus was removed by genetic engineering, the cyclin (famously known as ∆90) could drive the extract into mitosis, but such a cyclin was stable and the extracts were stuck in M-phase (although there was confusion – which remains to this day, in fact – about whether the extracts were in metaphase or anaphase) (52, 53).

Marcel Dorée and I have recently discussed elsewhere how it emerged that cyclins and Cdc2 were partners (54), and indeed, Marcel’s purification of starfish MPF, which he projected as a dried-down gel at a meeting in St. Andrew’s University in Scotland in April 1989 (55), set the seal on the matter after a period of confusion during which there had been claims that Cdc2 alone was required for MPF activity. Full resolution of the matter came later from biochemical studies of the activation of CDK2 involving in parallel my laboratory, in particular Randy Poon, Jörg Adamczewski and John Shuttleworth, and that of Marcel Dorée. We found that GST-CDK2, provided by Li-Huei Tsai, could be slightly activated by cyclin A, and that the activation was tremendously enhanced by immunoprecipitates of MO15 (56, 42). It was not too long before the structures of the components and of the whole complex were determined, which explained very clearly why CDKs required cyclins for activation (Figure 6).

I have said very little about what we knew from the yeast genetic approaches, even though we were well aware of them. I had met Lee Hartwell when I was still at Einstein, and knew Paul Nurse quite well from the time he was in Sussex. But as I have made clear, it was the aquatic creatures, the clams, sea urchins, frogs and starfish that really inspired me, and the allure of MPF

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which attracted me to studying the cell cycle. It is interesting to note that none of the classical $\text{cdc}$ mutations in budding yeast corresponded to cyclins (although Steve Reed, Fred Cross and Bruce Futcher quickly identified them by various ingenious approaches). Nor did the crucial CDK activating kinase reveal itself through genetics. In fact, the cell cycle field in general provided an extremely useful meeting place for the two cultures.

One final comment. The decade starting in about 1986 was a fantastic experience for anyone working on the cell cycle. Discoveries emerged from all sides and unexpected quarters at a headily bewildering rate. The culture was generous and open, and the field attracted extremely talented scientists who were very much fun to work with and talk to. I would like to thank them. This Nobel prize honours them all.

A FOOTNOTE ABOUT GELS

Why did nobody run a timed series of $^{35}$S-methionine labelled fertilized sea urchin or clam eggs on a 1-dimensional SDS-polyacrylamide gel between 1970 and 1982? One answer, of course, is that very few people worked on these cells; but more important, perhaps, are simple technical reasons. Thus, in the late 1960s, Paul Gross and his colleagues were interested in patterns of protein synthesis in cleaving sea urchin eggs and in mammalian cell cycles (57-59), but because the SDS gel had not yet been developed, and the slab gel not quite invented, the resolution of individual proteins and timed comparisons were not possible. The next serious investigator of the question of

Figure 6. The structure of CDK2 bound to a C-terminal fragment of cyclin A. Coordinates from IJST by A. A. Russo, P. D. Jeffrey & N. P. Pavletich.
whether fertilized sea urchin eggs synthesized different proteins from unfer-
tilized eggs was Bruce Brandhorst in the mid-1970s (60), by which time the
high-resolution 2-dimensional gel system had been invented by Pat O’Farrell
(61). Two serious obstacles stood in the way of detecting cyclins on 2-dimen-
sional gels. First, as we later discovered, cyclins are difficult, if not impossible
to focus in the isoelectric dimension of the system, and they appear as ugly
smears if they appear at all. Second, it is not easy to compare multiple 2-di-
-dimensional autoradiograms, and the idea of taking many closely-spaced time
points would be both difficult to achieve and rather pointless in order to add-
ress the questions being asked at the time. I was very lucky.

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