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Origins and development
of the septin field

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INTRODUCTION

In this chapter, I have told the story of the septin field from its conception in Lee Hartwell’s and Breck Byers’s laboratories (1967–1976), through its long gestation, awkward birth, and short childhood, mostly in my own laboratory (1977–1993), and finally through its adolescence in a growing number of laboratories until its coming of age was marked (in 2001) by a session’s being devoted to it at a major meeting (see Appendix C). Although there are many parts of the story that I find embarrassing and guilt-provoking (even many years after the events), I have told the unvarnished truth as best I can reconstruct it. I think that there is value in having an accurate historical record of a scientific field; if nothing else, this account should clarify aspects of the septins’ early history that would otherwise be quite mysterious to a newcomer. I think that this story also illustrates a great and reassuring truth about science: it moves forward even though the people involved, and their behaviour, are often imperfect.

CONCEPTION, GESTATION, AND BIRTH

In 1966, Lee Hartwell, then a beginning Assistant Professor at the University of California, Irvine, began isolating a large collection of temperature-sensitive-lethal (ts) mutants of the budding yeast Saccharomyces cerevisiae. His inspiration was the work done on ts mutants of bacteriophage T4 in Bob Edgar’s lab at Caltech, where Lee had done undergraduate research. A publication summarizing
the properties of 400 such mutants appeared soon (Hartwell, 1967); it included
brief descriptions of two of the mutants that would later define the septins, namely
\textit{ts310} and \textit{ts471} (later \textit{cdc10} and \textit{cdc12}), described at this time as defective
‘in cell-wall formation’. In 1967, another new faculty member at Irvine, Cal
McLaughlin, teamed up with Lee, and the two spent the next several years char-
acterizing mutants with defects in RNA and protein synthesis (e.g. Hartwell \textit{et al.},
1970b); this collaboration continued after Genetics Department Chairman Herschel
Roman lured Lee to the University of Washington in 1968.

Meanwhile, I had been diverted from my intended graduate study of population
ecology by fabulous courses in genetics (Matt Meselson) and cell biology (Keith
Porter) that I was required to take as a first-year student. I had also fallen in love
with yeast as an experimental organism on the basis of a lab course taught by
Nick Gillham, a \textit{Chlamydomonas} geneticist who had learned about yeast through
some exposure as a postdoc. Given my somewhat contrary personality, the fact
that no one else in the Boston area was working on yeast at that time only added
to its appeal. I solved my immediate problem by working with Guido Guidotti, a
protein chemist who was willing to sponsor my study of some yeast proteins (‘so
long as they’re interesting’), but I soon also began planning to do a postdoc in
Seattle, which at the time had the only concentration of yeast geneticists (three
labs) in the country. In November 1968, I approached Herschel at a meeting and
soon had arranged to join his Department as a postdoc when I finished my PhD;
he kindly agreed to sponsor me although my proposed project (a genetic analysis
of the enzymes that I was studying as a graduate student) was unrelated to his
own research.

In subsequent correspondence, Herschel soon began pushing me toward the
laboratory of his new recruit, but I resisted because I wasn’t excited by the papers
on RNA and protein synthesis. However, when I visited Seattle in September
1969, Lee told me how he, undergraduate Brian Reid, and first-year graduate
student Joe Culotti had recently realized that because of yeast’s budding mode
of reproduction, microscopic inspection of the \textit{ts} mutants after temperature shift
would allow recognition of mutants with a variety of specific cell-division-cycle
(\textit{cdc}) defects. Luckily, I had the wit required (it didn’t take much) to see that this
project was more exciting than my original plan, and Lee and I soon agreed that I
would join his lab. Thus, in July 1970, I happily became Lee’s first postdoc, just
a month after the first report on the \textit{cdc} mutants was published (Hartwell, Culotti
and Reid, 1970a). This paper included a description of a third mutant that would
later help to define the septins; it was named \textit{cdc3} and described as defective in
‘cell separation’. During the next three exhilarating years, I worked mostly on the
nutritional and growth control of cell-cycle initiation, but I used the \textit{cdc} mutants in
many of my experiments, and we talked endlessly about them in the lab (often to
the short-term detriment of our experiments!). I was particularly captivated by the
mutants with grossly abnormal cell morphologies that indicated defects in cyto-
plasmic rather than nuclear processes (Figure 1.1). These mutants included one that
could continue growth and the nuclear cycle but not make buds (\textit{cdc24}: Hartwell
CONCEPTION, GESTATION, AND BIRTH

Figure 1.1 The first attempt to organize the events of the yeast cell cycle into dependent and independent pathways (Figure 3 of Hartwell et al., 1974, a now-famous paper that was originally rejected without review by *Nature*). Events defined by particular *cdc* mutants are indicated by the *CDC* gene number. iDS and DS, initiation and continuation of DNA synthesis; mND and lND, medial and late nuclear division; BE and NM, bud emergence and nuclear migration; CK and CS, cytokinesis and cell separation; HU and TR, the DNA-synthesis inhibitors hydroxyurea and trenimon; MF, the mating pheromone α factor. Note that *cdc12* was not included among the cytokinesis mutants because the alleles available at this time were too leaky to allow full characterization. Reproduced with permission from Hartwell et al., (1974) *Science*, 183, 46–51, Copyright © 1974 Elsevier et al., 1974) and four that made abnormally elongated buds with multiple nuclei but could not complete cytokinesis (*cdc3, cdc10, cdc11* and *cdc12*: Hartwell, 1971; Figure 1.2). When I began my own lab at The University of Michigan in 1975 (after a second postdoc in Zürich that focused further on nutritional control), I soon decided to concentrate on these mutants, a decision that was the more

Figure 1.2 Images from the first systematic description of the *ts* mutants that would later define the septins (Hartwell, 1971); the cells had been incubated for several hours at restrictive temperature and then stained with Giemsa to reveal their nuclei. The defect in cytokinesis was clearly recognized at this time, using a newly developed assay to discriminate cytokinesis from the somewhat later process of separation of the daughter cell walls. (b) *cdc3*; (c) *cdc11*. Similar images were also presented for *cdc10* and *cdc12* mutants. Reproduced with permission from Hartwell, L.H. (1971) *Exp. Cell Res.*, 69, 265–76, Copyright © 1971 Elsevier
appealing to me because it seemed that everyone else (including Lee) was more interested in the mutants with defects in DNA replication and/or nuclear division. My early years at Michigan were dominated by the struggle to get a lab up and running on a miniscule budget, cope with a heavy teaching load, and finish the projects related to nutritional and growth control of the cell cycle. The time and resources available for the morphogenesis mutants were put into further characterizing *cdc24* (Sloat and Pringle, 1978; Sloat, Adams and Pringle, 1981) and screening for more mutants with defects in bud emergence. These efforts were quite rewarding (as reviewed elsewhere: Pringle *et al*., 1995; Pringle, 2006), but they delayed work on *cdc3, 10, 11, and 12*, and thus the birth of the septins. However, the mutant screen did yield, as a by-product, 28 new mutants with the same phenotype as *cdc3, 10, 11, and 12*. We were disappointed when all 28 proved to have mutations in the four known genes, but this did help to convince us that the phenotype was highly specific, so that our intended studies would be of manageable scope. Also, we recovered *cdc11* and *cdc12* alleles (including the now widely used *cdc12-6*: Adams and Pringle, 1984) that were tighter, and thus more useful in temperature-shift experiments, than those available previously. 

During this period, there were also other developments important for what would become the septin field. First, Breck Byers and his outstanding technician Loretta Goetsch, working a few doors down from the Hartwell lab, undertook systematic electron-microscopic (EM) analyses of both wild-type yeast and the various *cdc* mutants. In wild-type cells, they observed seemingly filamentous structures in close apposition to the plasma membrane in the mother-bud neck (Byers and Goetsch, 1976a; Byers, 1981; Figure 1.3a and b; see also Figure 3.4 of Chapter 3 by McMurray and Thorner). In addition, in the *cdc3, 10, 11, and 12* mutants, these images have better contrast than those published earlier (Byers and Goetsch, 1976a). (c–e) ImmunoEM images obtained using antibodies against (c) Cdc3p and (d and e) Cdc10p (J. Mulholland, B. Haarer, S. Ketcham, D. Preuss, J. Pringle and D. Botstein, unpublished results). (a) and (b) reproduced from Byers, B. (1981) with permission from Cold Spring Harbor Laboratory Press (c–e) ImmunoEM images obtained using antibodies against (c) Cdc3p and (d and e) Cdc10p (Reproduced with permission from Mulholland J., Haarer B., Ketcham S., Preuss D., Pringle J. and Botstein D., unpublished results).
In Saccharomyces cerevisiae, a highly ordered ring of 1005 filaments underlies the inner surface of the plasma membranes within the neck connecting the bud with the cell. The role of this ring in the cell cycle was investigated by determining whether the ring was present or absent in several temperature-sensitive mutant strains (characterized by L. R. Hartwell et al., 1973 Genetics 74, 267) defective in specific phases of the cell division cycle. These cdc mutants were brought to the terminal phenotype by shifting them from the permissive to the restrictive temperature and were prepared for thin-section electron microscopy. Staining mutant in 24 different cdc genes caused arrest of the cell division cycle with one or more buds present. The filamentous ring was found to persist in 20 of these strains. The remaining 4 strains, in which no ring could be detected, represent the same mutants as those which are specifically defective in cytokinesis. Cells of these latter strains form buds and undergo nuclear division but fail to separate their cytoplasmic masses at the restrictive temperature. The suggested requirement for the filamentous ring in cytokinesis was further examined by determining the rate at which the ring was lost following the shift to the restrictive temperature. The loss of the ring occurred with the same kinetics as the loss of the potential for continued cytokinesis. These results further substantiate the essential role of the filamentous ring in cytokinesis. (Supported by NIH grants GM 18134 and GM 20790).

Figure 1.4 The abstract that reported that certain cdc mutants lacked the ‘neck filaments’ (Byers and Goetsch, 1976b); note that the names of these mutants are not actually mentioned in the abstract, although this information was provided when the poster was presented at the International Congress on Cell Biology (B. Byers, personal communication). Reproduced from Byers, B. and Goetsch, L. (1976a) J. Cell Biol., 69, 717–21, with permission from Rockefeller University Press.
realize that the several plasmids isolated by rescue of a cdc11 mutant all actually contained \textit{CDC12} (Pringle \textit{et al.}, 1986)! However, we had the clones sorted out by 1986, and Brian had been able to fuse portions of \textit{CDC12} in frame to \textit{E. coli lacZ} and \textit{trpE} (because we didn’t yet have sequence information, he cloned random fragments of \textit{CDC12} into the fusion vectors and screened for clones that produced a fusion protein), use the fusion proteins to make Cdc12p-specific antibodies, and use these antibodies to demonstrate that Cdc12p localized to the mother-bud neck (Haarer and Pringle, 1987). This demonstration required Brian to overcome another confusing artefact that resulted from the presence in most rabbit sera of antibodies that recognized a yeast cell-wall component (probably chitin) that is also localized to the neck and often remains there during the preparation of cells for immunofluorescence. Thus, we had two moments of high excitement in this project: first, when the unpurified antiserum (but not a control serum) gave us staining at the neck, the exact place where we expected to find Cdc12p, and second, when affinity-purified antibodies also stained the neck. Of course, these highs were separated by the profound low of realizing that the first results were an artefact!

Before long, we had also obtained good antibodies to Cdc3p, 10p, and 11p, and shown that these proteins also localized to the neck (Figure 1.5a and b; Kim, Haarer and Pringle, 1991; Ford and Pringle, 1991). Importantly, we demonstrated that all four proteins were lost simultaneously from the neck when any one of the four \textit{ts} mutants was shifted to restrictive temperature; taken together with the results of Byers and Goetsch (see above), this suggested that Cdc3p, 10p, 11p, and 12p formed a complex that contributed to the filament-like structures seen by EM. Further support for this conclusion was obtained in 1991, when Jon Mulholland and Daphne Preuss in David Botstein’s lab obtained some beautiful immuno-gold images using the antibodies that we provided (Figure 1.3c–e). Although we unfortunately did not find a vehicle for publication of these images, they were shown widely and mentioned in a review (Longtine \textit{et al.}, 1996), and they helped establish two points that were not clear from fluorescence images, namely that the proteins of interest, like the filaments described by Byers and Goetsch, are closely associated with the plasma membrane and are present throughout the neck region (so that the structure in which they are found during most of the cell cycle is an hourglass-shaped band and not a pair of discrete rings, despite the common appearance of fluorescence images resulting from the geometry of the neck).

Meanwhile, in the summer of 1986, Brian and others in the lab had begun sequencing the four genes, another process that was rather laborious in those days. In Brian’s report to his thesis committee in December 1986 (which I still have), all four genes were described as partially sequenced, but there was no hint that any sequence similarities had been found. But in early February 1987, I wrote letters of recommendation for Brian that described the ‘very interesting result’ that Cdc3p, 10p, 11p, and 12p formed a family of related proteins. So although I have no record of the precise date, the ‘Eureka moment’ must have been in January 1987. It took quite a bit longer actually to finish the sequencing of both strands and to feel confident that we had all the bugs out of both this and the original
Figure 1.5 Excerpts from abstracts for talks at the biannual Cold Spring Harbor Yeast Cell Biology Meetings of 1987 (a), 1989 (b) and 1991 (c). In (c), cdc103+ was the gene later named spn1 (Longtine et al., 1996); the Drosophila gene was sep1 (Fares, Peifer and Pringle, 1995); and the mammalian (mouse) genes were Sept1 (Nottenburg, Gallatin and St. John, 1990) and Sept4 (Kato, 1990), originally called DIFF6 and H5. For a Cold Spring Harbor cytoskeleton meeting in April 1991, we submitted an abstract nearly identical to (c), except that we did not yet know about the mouse genes. Although we were very slow to publish our results, we were not secretive about them! Reproduced with permission from the authors.
characterization of the clones, but we were comfortable with presenting the central results at a major meeting in August 1987 (Figure 1.5a). (After this presentation, Jeremy Thorner referred, prophetically, to the project as a ‘gold mine’, but most other people did not seem very interested in this out-of-the-mainstream work.)

It also took many months of hard work to write it all up: the work had been done by seven people (most of whom had already left the lab) over a five-year period; the story had some intrinsic complications; and methods had evolved rapidly during the years that the work was being done (so that the ways we had actually done things were not always how we would have done them at the time of writing). However, by July 1990, we thought that we had satisfactorily addressed every complication and produced a pair of tightly written manuscripts, which we submitted with great pride to Genetics (Figure 1.6). In a telephone conversation on about August 25, the editor indicated that the reviews were generally positive (as indeed they proved to be), and on this basis we described the papers as ‘in press’ when we submitted the Cdc3p-localization manuscript (Kim, Haarer and Pringle, 1991) on August 27. Thus, it was with shock bordering on disbelief that I received an editorial decision letter on August 29 that said, among other unpleasant things, that the manuscripts were ‘intensely irritating to read’ and that I should have known that the two manuscripts would need to be combined into a single manuscript. (Actually, this thought had never even crossed my mind.) The letter was the more shocking in that it came from an editor with whom I had worked on other projects and thought of as someone who shared my deeply held beliefs about the importance of addressing, rather than glossing over, the technical details and complications of a study. I couldn’t really see how to re-write the material as

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This will acknowledge receipt on 3/27/90 of your manuscript submitted for publication in GENETICS and entitled:

Isolation and Characterization of CDC3, CDC11, and CDC12, Three Genes Controlling Morphogenesis in the S.c. Cell Cycle

This will acknowledge receipt on 7/30/90 of your manuscript submitted for publication in GENETICS and entitled:

The S.c. CDC3, CDC10, CDC11, and CDC12 Genes Encode a Family of Similar Proteins

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Figure 1.6 The two manuscripts in which we attempted to report the cloning and sequencing of the original four septin genes were submitted to Genetics in July 1990. The postcards acknowledging receipt are shown. Authors of the first paper were Sue Lillie, Brian Haarer, Laird Bloom, Kevin Coleman, and myself; authors of the second paper were Brian Haarer, Stuart Ketcham, Susan Ford, David Ashcroft, and myself.
a single paper of reasonable length, and although it was years before I gave up on publishing these papers, both life (I moved from Michigan to North Carolina in 1991, and none of the people involved in this phase of the project moved with me) and science (see below) moved on without its ever getting done. We did talk widely about the results, refer to them in the 1991 protein-localization papers, and provide details and reagents to the few who were interested. We also entered the sequences into the public databases, although, embarrassingly, this didn’t happen until May 1993 (as a result of a miscommunication and a lack of follow-up by me, rather than any attempt to be secretive), with some consequences as described below.

At some point in 1989 or 1990, we began in the lab to refer to the protein family as ‘septins’ (for their role in septation), but we were too diffident to apply this term in our 1991 publications, a 1992 paper in which we proposed to introduce the term was aborted (see below), and we published nothing more on septins until 1995. Thus, when publication of the Neufeld and Rubin (1994) Pnut paper (see below) elicited the minireview by Sanders and Field (1994), Sylvia and Chris had to call to ask if we had yet given the proteins a name. I told them, and they kindly used it (with attribution) in their review, thus introducing this convenient term into the literature.

CHILDHOOD

As the *S. cerevisiae* septin picture was coming into focus in the late 1980s, we naturally began to wonder whether such proteins were also found in other organisms. EM observations by Soll and Mitchell (1983) had shown that *Candida albicans* also had ‘neck filaments’, but even if these proved to contain septins (as indeed found later: see Chapter 5 by Gladfelter and Sudbery), this didn’t seem to expand our horizons very much. Thus, we turned to the fission yeast *Schizosaccharomyces pombe*, which is more distant phylogenetically from *S. cerevisiae* and also distinct morphologically in that it divides by medial fission rather than by budding. I thought that we would be competent to handle another yeast in the lab, and this proved more-or-less true, although we also got a huge assist when *S. pombe* expert Peter Fantes came from Edinburgh on sabbatical early in the project. I also thought that the comparison of septin function in these two yeasts might be highly informative, just as the comparative analysis of cell-cycle control had been (Hartwell, 2002; Nurse, 2002); this has arguably been the case, albeit in the rather perverse way of revealing to us mostly the complexity of septin biology and the major gaps in our understanding of cytokinesis (see below and Chapter 5)!

Our initial approach, in 1988, was to screen our antibodies against the *S. cerevisiae* proteins for cross-reaction with *S. pombe* proteins; we found a plausible potential homologue only with an anti-Cdc3p antibody (Figure 1.7a). A long struggle by Annette Healy and Hyong Kim with the λgt11 expression-vector system then led eventually to the cloning of the corresponding *S. pombe* gene (*spn1*),
and we had enough sequence information to report the result at 1989 summer meetings (Figure 1.5b). Tom Pugh joined the project at this point, and he, Hyong, and Peter (and later Omayma Al-Awar) proceeded both to investigate Spn1p function and to isolate four additional genes (spn2-spn5) by a combination of PCR (new to the lab at this time) with degenerate primers and rescue of the S. cerevisiae septin mutants using a library of S. pombe cDNAs in an appropriate expression vector. They found that Spn1p localized to the division site (Figure 1.7b and c) but that knockout of spn1 did not block cytokinesis or septum formation, although it did produce a delay in the separation of the daughter cells. We reported our progress at 1991 summer meetings (Figure 1.5c) and began drafting a manuscript, but our zeal to finish it was reduced by the supposition that the mild phenotype of the spn1 mutant probably resulted from redundancy in function with one or more of the other septins.

At this critical juncture came the move to North Carolina and the dispersal of the S. pombe group, of whom only Omayma moved with me. She was joined in a few months by Maria Valencik, and the two of them began to look for additional genes (finding spn6) and to construct the required single, double, and multiple mutants (which was challenging in those days because of a paucity of selectable markers for S. pombe gene knockouts and the lack, at the time, of a PCR method for generating the knockout constructs). Discouragingly, even the multiple mutants still displayed only the mild phenotype of cell-separation delay, leaving the puzzle, which endures to this day (see below and Chapter 5), as to why the seemingly conserved septin array at the division site is essential for cytokinesis in some cell types (such as S. cerevisiae) and non-essential in others.

Figure 1.7 Initial identification and localization of an S. pombe septin (H. Kim, A. Healy, T. Pugh, P. Fantes, and J. Pringle, unpublished results). (a and b) Recognition of Spn1p in immunoblots. (a) Antibodies raised against Cdc3p (Kim, Haarer and Pringle, 1991) recognized Cdc3p in a blot of S. cerevisiae proteins (lane 1) and a protein of similar molecular weight in a blot of S. pombe proteins (lane 2). As Cdc3p and Spn1p are only ~45% identical in amino-acid sequence, this cross-reaction sounds a cautionary note for investigators working in organisms with multiple septins. (b) After cloning spn1, Spn1p-LacZ and Spn1p-TrpE fusion proteins were used to raise and affinity purify antibodies specific for Spn1p, as shown by staining blots of proteins from wild-type cells (lane 1), an spn1 deletion strain (lane 2), and a strain carrying a high-copy spn1 plasmid (lane 3). (c) The antibodies used in (b) were also used for immunofluorescence staining of wild-type S. pombe cells; the cells shown were fixed at the time of cytokinesis. Reproduced with permission from Kim, H.B., Haarer, B.K. and Pringle, J.R. (1991) J. Cell Biol., 112, 535–44, Copyright © 1991 Elsevier
Maria understandably became discouraged and eventually abandoned the project for a look at the mouse septins (see below); Omayma bravely soldiered on and completed what became half of her thesis, but it was not until 2001 that work by Jürg Bähler and Jian-Qiu Wu had filled in all the gaps (including the identification of spn7 based on a partial sequence released by the S. pombe genome project). In the meantime, we had described many of the results at the 1993 Cold Spring Harbor Yeast Meeting, deposited the sequences in the public databases (although not until August 1995), and described some of the results in our 1996 review (Longtine et al., 1996). Although other groups have now begun to work on the S. pombe septins (see Chapter 5), and we have provided them with information and reagents, we still have considerable material that really ought to be published, and I still have hopes, but with a trail so long and complicated it is not a simple matter.

Meanwhile, armed with degenerate PCR and the knowledge that the septins were evolutionarily ancient at least in the fungi, we had been emboldened to look farther afield, and by mid-1990 Johnny Fares had identified a Drosophila gene (sep1), which we reported (eliciting no great interest) at meetings in April and August 1991 (Figure 1.5c). Functional analysis of the Drosophila septin was stymied for some time because we had no experience with flies and no appropriate collaborator at Michigan, Johnny’s first attempts to generate antibodies failed, and Johnny himself also had several yeast projects that took much of his time.

Then, in May 1991, there was an exciting development: after four years of checking the sequence databases intermittently without success, we noticed a mouse sequence that was clearly homologous to the yeast and fly septins. Nottenburg, Gallatin and St. John (1990) had been trying to clone the gene for a glycoprotein thought to be involved in the binding of lymphocytes to endothelial layers. They recovered a cDNA (‘DIFF6’) whose expression levels in different cell lines matched those of the glycoprotein of interest, but, to their disappointment, the clone did not encode that protein, and they found no DIFF6 homologues when they searched the databases (for the reasons described above). Thus, DIFF6 (now SEPT1) has the honour of being the first septin whose sequence was published and entered in the public databases. [Kato (1990) described H5 (now Sept4) as one of a set of mouse cDNAs isolated on the basis of their interesting expression patterns in the brain, but the sequence was not entered into the databases until August 1991; Steensma and van der Aart (1991) sequenced a chromosome region that included CDC10 as an early step in the S. cerevisiae genome project, but their sequence (checked against ours, which they knew about from our meeting presentations) wasn’t deposited until May 1992.]

Immediately after seeing the DIFF6 sequence, I called Tom St. John (whom I knew slightly from his earlier work on yeast) and told him the septin story, which he was pleased to hear. Remarkably, he had also recently been contacted by George Miklos, a Drosophila geneticist in Australia who had also sequenced a gene encoding a Drosophila homologue. George’s group was interested in several genes defined by mutations that produced behavioural and/or neurological phenotypes
and mapped near the centromere of the X chromosome. To identify the genes, they had cloned this chromosome region and were characterizing all the transcription units. Between the genes that proved to correspond to the mutations small optic lobes (Delaney et al., 1991) and sluggish (Hayward et al., 1993) were two other transcribed regions that did not appear to correspond to any of the mutations of interest; one of these had shown the homology to DIFF6 that prompted the contact with the St. John group. On May 14, I sent large packets of information on the septins (including various sequence alignments) to both the St. John and Miklos groups; arriving at the latter without prior warning, I suppose that it produced a stir, especially given that our sep1 proved to be identical to their gene (whose sequence we had not yet seen when I sent our packet). In any case, a cordial and informative letter arrived by fax on May 22 and was followed by a phone call, and we soon agreed to publish a joint paper reporting that proteins homologous to the ‘neck-filament-associated proteins’ in yeast were present in both flies and mammals. I was excited, because I thought that this little paper would markedly raise the level of interest in this family of proteins. I proposed that because St. John and co-workers had not been able to say anything interesting about the DIFF6 sequence when they published it, but had put out the sequence that allowed us all to make contact, they should now be included as co-authors, and this proposal appeared to be accepted (albeit with some reluctance).

Work began on the paper but proceeded rather slowly (too slowly for George’s satisfaction) because of the disruption caused by my move in August to North Carolina and the other pressures (notably the editing of the Cold Spring Harbor yeast monograph) that I was facing at that time. George also objected to several aspects of my proposed organization of the paper, as well as to my rather hesitant proposal to call the proteins ‘septins’ (arguing that this was premature without knowledge of their function in animal cells). Nonetheless, by April 1992, after lots of hard work and patient negotiation, I thought that we had a manuscript that all could agree on, and I shipped it off to Australia. Several weeks later, I received a fax announcing George’s intention to withdraw his group from the paper; the main sticking point was the inclusion of St. John and co-workers as co-authors, which was now described (to my astonishment) as ‘outrageous’. This was the last straw for me, and I made no further attempt to rescue the joint publication. The Miklos group entered their sequence into the public databases in July 1992 but never published any of their other information on sep1, which is a shame because they apparently had genetic data (based on deletion analysis of the region) that would have shed valuable light on Sep1 function. George apparently also considered himself and his group to be the injured parties (which I could never understand), and even after we had finally obtained a good antibody and an appropriate collaborator and published our paper on Sep1 localization and possible functions (Fares, Peifer and Pringle 1995), they declined to acknowledge the septin name or the progress in the field and used their own terminology (‘innocent bystander’ for sep1 and the ‘innocent bystander family’ for the septins) on through their last publication in the area (Maleszka, De Couet and Miklos, 1998).
During the early 1990s, several other groups encountered mammalian septin genes during studies of other topics (Kumar, Tomooka and Noda, 1992; Nakatsuru, Sudo and Nakamura, 1994; H. Zoghbi, personal communication), but except for an unsuccessful attempt by Makoto Noda’s group (in collaboration with yeast expert Kuni Matsumoto) to rescue the yeast septin mutants using their mouse \textit{Nedd5} (now \textit{Sept2}) clone (we sent strains in October 1993, along with lots of information about the yeast and fly septins, and learned of the negative results in December), there was little indication of interest in actually studying septin function. (Although the December letter mentioned that ‘we are raising antibody’, we didn’t learn until much later that Makoto Kinoshita in the Noda lab was leading a serious effort to investigate NEDD5 function, as described below.) Thus, when Maria Valencik became frustrated with the \textit{S. pombe} project (see above), I encouraged her proposal to begin looking at mammalian septin localization even though our lab was not well equipped for studies of either mice or cultured mammalian cells. We obtained the \textit{Diff6} cDNA from Tom St. John and an \textit{H5} cDNA from Huda Zoghbi in November 1993 and began trying to make antibodies using fusion proteins, eventually succeeding with both the amino- and carboxyl-terminal halves of \textit{DIFF6}. (The eventual success was with rabbit antibodies, but this did not happen until after we had first followed the enthusiastic advice of a colleague and tried to raise antibodies in chickens, yielding about half a coldroom full of eggs but no useful antibodies!) Maria used the cDNAs and antibodies to get a variety of interesting results (Figure 1.8), but we still had only fragments of a story when she left the lab in August 1995 (for family reasons and an opportunity to learn mouse methods in a more appropriate environment). Thus, although we briefly described the principal results in our 1996 review (Longtine \textit{et al.}, 1996), no full account of this work was ever published.

\section*{EARLY ADOLESCENCE}

Although a few other groups had happened upon septin genes, as described above, attempts to explore septin function were essentially confined to my laboratory for about 10 years, which of course meant that progress was rather slow and there was little interest by the outside world. Although a paper on \textit{cdc10} mutations and yeast budding patterns (Flescher, Madden and Snyder, 1993) was more important than was apparent at the time (see below), I think that the maturation of the septin field really began with the analysis of \textit{Drosophila} septin function by Neufeld and Rubin (1994). In a complex screen for mutations affecting eye development, Tom Neufeld had recovered a mutation that he named \textit{pnut}, following the traditional (and maddening to outsiders) practice in \textit{Drosophila} genetics of giving whimsical names to genes; in this case, the name was based on the superficial resemblance of some cells that had failed cytokinesis, and thus had several nuclei, to peanuts in the shell (T. Neufeld, personal communication). When the gene was sequenced in early 1992, database searches revealed only the \textit{DIFF6} and \textit{H5} sequences
Figure 1.8 Some early observations on mammalian septins (M. Valencik and J. Pringle, unpublished; Longtine et al., 1996). (a) SEPT1 concentrates in the cleavage furrow (arrow) of at least some dividing mammalian cells (an EL4 lymphoid cell is shown; similar observations were made on NB41A3 neuroblastoma cells). Note that in the coloured originals, the propidium iodide-stained telophase chromosomes (*) were clearly distinguished from the Bodipy-labelled secondary antibody used in the SEPT1 staining. (b) SEPT1 is particularly concentrated in cells of the central nervous system. SDS-PAGE and immunoblotting were performed on protein extracts from various organs of a dissected mouse (1, thymus; 2, spleen; 3, cerebrum; 4, cerebellum; 5, spinal cord; 6, lung; 7, stomach; 8, liver; 9, pancreas; 10, kidney; 11, heart). (c) SEPT1 is concentrated in the growth cones of differentiating PC-12 neural cells in culture. As in (a), the red-fluorescent nucleus (*) and green-fluorescent antibody staining (around the tips of the multiple growth cones) were distinct in the original. (d) Evidence for differential expression and differential splicing of Sept1 and Sept4 in different tissues. A commercially obtained blot of poly A+ RNAs from various mouse organs (shown are 1, heart; 2, brain; 3, spleen; 4, lung; 5, testis) was hybridized to radiolabelled Sept1 (left) and Sept4 (right) cDNAs. Reproduced with permission from Longtine, M.L. et al. (1996) Curr. Opin. Cell Biol., 8, 106–19

and no connection to the yeast proteins or any biological function (see above). Nonetheless, Tom and Gerry were soon in contact with me. (We have been unable to reconstruct exactly how this happened; the path presumably led through Nottenburg and St. John, although George Miklos – who visited the Rubin lab at about this time – may also have been involved.) In March and April 1992 (by phone and mail, and when I visited Berkeley for a seminar that had been arranged months earlier for other reasons), I passed on everything that we knew at that point about the septins, including our progress with Drosophila Sep1. Additional exchanges of information and reagents followed, so that by the time the Pnut paper was submitted in early 1994, Tom and Gerry were able to provide extensive context that added to the impact of their own highly interesting results.

The Pnut paper was important for several reasons. First, it was the first conspicuous public announcement that this family of proteins existed in animals and not
just in budding yeast. [As described above, we had presented this fact at meetings but not in any publication. And although Flescher, Madden and Snyder (1993) had been able to point out that Cdc10p-like proteins existed in flies and mammals, this paragraph was buried within their paper and unlikely to have been noticed by many.] Second, it presented both protein-localization and mutant-phenotype data that were highly informative about septin function in flies. In particular, it showed that the septins were involved in cytokinesis in animal cells as well as in yeast, while also suggesting strongly that they had other roles, notably in the nervous system. Third, it helped to get Chris Field and Tim Mitchison interested in the septins (Sanders and Field, 1994), paving the way for their later seminal contributions to the field (see below).

The Rubin lab did not continue studies of the fly septins after Tom Neufeld’s graduation, but my lab carried on with the indispensable collaboration of Mark Peifer, a card-carrying fly geneticist who had joined UNC as an Assistant Professor within a few months of my own move there from Michigan. A combination of genetic, protein-localization, and biochemical studies (Fares, Peifer and Pringle, 1995; Adam, Pringle and Peifer, 2000; Shih et al., 2002; see Chapter 6 by Field et al.) produced additional important information, including (i) evidence for differentiation of function among the fly septins; (ii) evidence that the septins may be non-essential for cytokinesis in some cell types even though they are essential in others; (iii) evidence that Sep2, unlike Pnut, is dispensable for development to adulthood [although this issue is complicated by the possibility of functional redundancy with the very similar (73 % sequence identity) Sep5]; and (iv) evidence that Pnut, Sep1, and Sep2 are all particularly highly concentrated in non-dividing cells of the embryonic central nervous system, suggesting strongly (as did the preliminary data for mouse; see Figure 1.8) that the septins must have important roles in at least some cell types that are unrelated to cytokinesis.

Unfortunately, despite this progress and the promise of much more, the fly septin project waned at UNC: Mark lost interest as the other projects in his lab gained momentum; Johnny Fares, Jenny Adam, and Hsin Shih all graduated and were not replaced by other students; and postdoc Karen Hales received an irresistible offer of a faculty position while she still had a year left on her fellowship (and when she was just on the verge of getting the sep5 mutant that would have allowed the critical sep2 sep5 double mutant to be constructed). [We never attempted to generate a sep1 mutant because of the (unrealized) expectation that the Miklos group would eventually publish something on this topic (see above).] I thought that the fly septin project might revive when I moved from UNC to Stanford in 2005, but with more and more of my own attention devoted to our new study of the dinoflagellate-cnidarian symbiosis (we have cloned two anemone septin genes, but it is unlikely that they will become a major focus of the project), I now think that this is unlikely. Thus, it seems to me that study of the septins in the genetically tractable Drosophila system, where septin mutations even give some strong phenotypes, represents an extraordinary opportunity for an enterprising investigator who wants to do something different and important.
Meanwhile, our labours in *Drosophila* had also allowed us to contribute in a small but important way (the anti-Sep1 and anti-Sep2 antibodies) to the first serious and successful effort at a biochemical analysis of the long-presumed septin complexes, the breakthrough study by Chris Field, Tim Mitchison, and co-workers (Field *et al.*, 1996). As a research associate in Bruce Alberts’s lab, Chris had been using biochemical approaches to the study of *Drosophila* cytoskeletal proteins (discovering, among other things, anillin: Field and Alberts, 1995; and see Chapter 6), so that she was well positioned to take a similar approach to the fly septins once her (and husband Tim’s) attention had been drawn to these proteins. This study established or solidified the evidence for multiple important points, including (i) the several septins in a given cell type (in this case, at least Pnut, Sep1, and Sep2) do form a physical complex with each other; (ii) as suggested by their sequence motifs, the septins can bind and hydrolyse GTP; and (iii) the septin complexes can form at least short filaments *in vitro* (a point of particular interest given the paucity of evidence for septin filaments *in vivo* other than in *S. cerevisiae*). With Tim’s student Jen Frazier leading the way, the approach was soon extended to the *S. cerevisiae* septins (Frazier *et al.*, 1998), leading to similar conclusions (except that much longer filaments could be formed *in vitro*) and a solid beginning for yeast septin biochemistry (for further discussion, see Chapters 3 and 6).

Field *et al.* (1996) also extrapolated from their structural observations to propose a new model for septin-filament organization in yeast, in which the filaments would run longitudinally along the mother-bud axis rather than in a helix around the neck as Byers and Goetsch (1976a) had proposed and we all had thought ever since. At the time, I thought (and politely mentioned to Chris and Tim) that this model was not justified by the data and indeed was almost certainly totally wrong. However, I soon changed my tune when Mark Longtine showed that cells defective in the protein kinase Gin4p displayed a reorganization of the septins (into thick bars running through the neck) that I found (and, indeed, still find) easy to explain if the septin filaments are longitudinal and hard to explain if they are helical (Longtine, Fares and Pringle 1998). Although the jury is still out on the ultimate validity of this model (see the detailed discussion in Chapter 3), there can be no doubt that it, and the experiments and discussions it has spawned, have contributed greatly to the development of our understanding of septin organization.

There were several other important developments in the septin field during the mid-1990s. First, studies by Beth DiDomenico and Yigal Koltin in *Candida albicans* (first communicated to me in late 1991; later published by DiDomenico *et al.*, 1994), by Michelle Momany and co-workers in *Aspergillus nidulans* (Momany *et al.*, 1995; Momany and Hamer, 1997), and by Michael Glotzer and Tony Hyman in *Xenopus laevis* [first communicated to me in October, 1994; abstract and poster presented in 1996 (Glotzer and Hyman, 1996); eventually published in part in 2002 (Mendoza, Hyman and Glotzer, 2002)], taken together with the *S. pombe* and *Drosophila* data, made it seem certain that the septins would prove to be ubiquitously present in both fungi and animals.
Second, Fatima Curcková in Kim Nasmyth’s lab performed a genetic screen to identify yeast proteins that were critical in cells deficient in G1 cyclins; she identified mutations both in \textit{CDC12} and in the novel gene \textit{CLA4} and showed that \textit{cla4} mutations affected septin organization (Curcková et al., 1995). Because Cla4p proved to be a protein kinase in the PAK family, activated by Cdc42p and/or other Rho-type GTPases, this was the first step in trying to understand how Cdc42p controls the spatially polarized localization of the septins (among other things), an effort that continues into the present (see Iwase et al., 2006, and references cited therein).

Third, in mid-1995 I received an invitation to write a short review on ‘recent progress’ with the septins for \textit{Current Opinion in Cell Biology}. Recognizing that enough other people were becoming interested that a true septin field was beginning to emerge, and acknowledging (even if tacitly and grudgingly) that some of our early work would probably never be published in detail, I decided that we should try to put the new field on a sound footing by providing a comprehensive review of what was known about the septins to that point (including our own unpublished results and personal communications from others as needed to make the state of the art clear). The resulting paper (Longtine et al., 1996) was rather different from (and about twice as long as!) what the commissioning editors had envisaged, but after some discussion they agreed to publish it anyway. In re-reading this review a dozen years after it was written, it still seems to me that it did quite well in summarizing both what was known at the time and the challenges that lay ahead.

Fourth, some important general points about septin function were revealed by studies of the rather specialized process of yeast ascospore formation. To ask if there were additional septins in \textit{S. cerevisiae} beyond Cdc3p, 10p, 11p, and 12p, Johnny Fares had used degenerate PCR. He recovered and began to study a fifth septin gene. The knockout strain had no detectable vegetative phenotype but had a defect in spore formation, which caused me to start calling friends in the sporulation field. From Mary Clancy [from whose lab Tom Pugh (see above) had come to mine], we learned that our gene was identical to the previously named \textit{SPR3} (Ozsarac et al., 1995; Fares, Goetsch and Pringle, 1996), which had been identified much earlier by Mary and Pete Magee (Clancy et al., 1983) as one of a set of genes with \textit{SP}orulation \textit{Regulated} patterns of gene expression. Because Mary’s (and collaborator Ian Dawes’s) focus was on the mechanisms of transcriptional regulation (Kao et al., 1989; Ozsarac et al., 1997), there was little information about the function of Spr3p. However, an important role for the septins during sporulation was suggested strongly by the very strong transcriptional induction of \textit{SPR3}, \textit{CDC10} (Kaback and Feldberg, 1985; see above – previously these data had appeared to make no sense), and a sixth septin gene that was revealed by the yeast genome project and named \textit{SPR28} (to connect it to \textit{SPR3} and minimize the number of gene-name acronyms) when it also proved to be induced strongly during sporulation (De Virgilio, DeMarini and Pringle, 1996). Although the fascinating process of ascospore formation, and the role of the septins in this
process, remain poorly understood (Fares, Goetsch and Pringle, 1996; De Virgilio, DeMarini and Pringle, 1996; Moreno-Borchart et al., 2001; Neiman, 2005), these early studies provided the first solid evidence for two important general points, namely that an organism can express different subsets of its septin genes, and form complexes containing different sets of septin proteins, in different cell types. Because Maria Valencik’s early studies of the mouse septins had also pointed to at least the first of these conclusions (Figure 1.8), we felt comfortable in making this point quite strongly in Longtine et al. (1996).

Fifth, several different lines of investigation led to development of the scaffold model for septin function. In retrospect, the first intimations of this mode of septin action had come much earlier. In the course of their long-running studies of septum formation, Enrico Cabib and his co-workers had reported, based on EM observations that I myself found rather difficult to evaluate, that ‘septum-like structures’ formed at ectopic locations in the cdc3, 10, 11, and 12 mutants (Slater, Bowers and Cabib, 1985). At about the same time, Alison Adams observed by fluorescence microscopy (using a specific dye) that these mutants displayed a diffuse deposition of chitin, in contrast to the normal tight localization of this cell-wall component to a ring around the neck and the primary layer of the septum (Adams, 1984). This observation suggested that Cdc3p, 10p, 11p, and 12p were involved in the localization of the chitin synthase(s), an hypothesis that was reinforced when we later observed that in cells changing shape in response to mating pheromone, the diffuse deposition of chitin (Schekman and Brawley, 1979) was correlated with the presence of the septins in a zone that was much more diffuse than the tight band seen in vegetative cells (Ford and Pringle, 1991; Kim, Haarer and Pringle, 1991). Alison had also observed that the normal concentration of actin at the neck during cytokinesis failed to occur in the cdc3, 10, 11, and 12 mutants (Adams and Pringle, 1984). However, this observation was difficult to interpret at the time (and indeed remained so until 1997, when we finally began to understand the various actin structures that form at the neck during cytokinesis). Thus, in the early 1990s, we had three dots, but two of them were a little fuzzy, and so far as I can reconstruct, we had not connected them into any general model for septin function.

This situation might have changed when Mike Snyder and his co-workers analysed a cdc10 mutant that they had isolated in a screen for genes whose products interacted with Spa2p, which they were studying intensively at the time (Flescher, Madden and Snyder, 1993). On the basis of the mutant’s abnormal budding pattern, they proposed (correctly as it turned out) that the septins functioned to localize to the division site some protein(s) that marked future budding sites. However, at the time this paper had little impact (at least on me), partly because the putative marker protein(s) were purely hypothetical at this point and partly because the cell-polarization side of my lab (and of my own brain) felt that the methods used in Mike’s lab to score budding patterns made their conclusions difficult to evaluate. In any event, it was not long before work in my own and Ira Herskowitz’s laboratories identified some of the proteins involved in marking
potential budding sites and showed that they indeed localized to the division site in a septin-dependent manner (Chant et al., 1995; Sanders and Herskowitz, 1996). In addition, at about this time, Jamie Konopka found that Afr1p, a protein induced by mating pheromone and involved in producing the change of shape in responding cells, interacts with Cdc12p and co-localizes with the septins both in mating cells and (when ectopically expressed) in vegetative cells (Konopka, DeMattei and Davis, 1995), providing another example of a protein whose localization appeared to depend upon the septins.

Thus, by 1996, there were lots of dots. However, from re-reading our 1996 review, it does not appear that we had connected them into a coherent general model until a combination of genetic and protein-localization experiments by Doug DeMarini made a persuasive case that the chitin synthase Chs3p was anchored at the neck by a hierarchical scaffold in which Chs3p bound its activator Chs4p, which bound the newly identified Bni4p (necessary for Chs3p localization but not for its activity), which bound the septin complex through Cdc10p (DeMarini et al., 1997). Additional support for the scaffold model soon came from other studies, such as the demonstration by Erfei Bi (Bi et al., 1998) and by Lippincott and Li (1998) that the actin structures at the division site include an actomyosin contractile ring and that the myosin component of this ring is recruited to the budding site early in the cell cycle in a septin-dependent manner. However, I think that the result that really crystallized the scaffold model was the demonstration by Mark Longtine that when the septins reorganize in the absence of Gin4p (see above), the various proteins that display septin-dependent localization undergo a parallel reorganization (Longtine, Fares and Pringle, 1998).

From this time forward, additional examples of septin-dependent localization accumulated rapidly, so that by the time Amy Gladfelter bravely led an effort to summarize the known information, there were at least 22 proteins that were known to localize to the neck, in a variety of temporal and spatial patterns, and with a very wide variety of functions, in a septin-dependent manner (Gladfelter, Pringle and Lew, 2001). A similar effort today would certainly at least triple this number, so I think it is well established that at least one role of the septins is to provide a scaffold for the recruitment of other proteins and probably also for their organization at the site to which they have been recruited. However, I also think that it remains a little embarrassing that we still know so little about the molecular details of this recruitment and about which proteins actually interact directly with the septins! (See additional discussion in Chapters 8 and 9).

LATE ADOLESCENCE

Although new areas of cell biology are often pioneered by studies in model organisms, they are not usually considered mature (unless they are plant or microbe-specific) until they have captured the attention of mammalian cell biologists. For the septins, the first big step in this direction was the publication by
Makoto Kinoshita and his co-workers (Kinoshita et al., 1997); this first detailed exploration of mammalian septin function had required more than three years of work since the Noda laboratory began raising antibodies to ‘NEDD5’ (now SEPT2) in 1993 (see above). Among other things, the data presented indicated that septins are important for cytokinesis in mammalian cells as well as in yeast and flies, that GTP binding and perhaps hydrolysis are important for septin function, and that the septins have distinct functions in interphase cells that involve interactions with the actin cytoskeleton. (See further discussion in Chapters 8 and 10.) Other important developments soon followed and included the following.

1. The first links of the septins to human disease (another step toward the full maturity – and funding! – of a field) appeared. The first publication appears to have been the report that a translocation implicated in the genesis of acute myeloid leukaemia fuses the \textit{MLL} gene to \textit{SEPT5} (Megonigal et al., 1998). However, other studies suggesting oncogenic roles also for \textit{SEPT9} and \textit{SEPT6} soon appeared (Osaka, Rowley and Zeleznik-Le, 1999; Kalikin, Sims and Petty, 2000; Russell et al., 2000; Sorensen et al., 2000; Borkhardt et al., 2001), as did a report that the septins are concentrated in tangled fibres in the senile plaques in the brains of Alzheimer’s disease victims (Kinoshita et al., 1998), suggesting a septin role in this disease. Meanwhile, Barbara Zieger, Jerry Ware, and their co-workers observed that \textit{SEPT5} was expressed at high levels in platelets, suggesting a possible role in haemostasis and hence in bleeding disorders (Zieger, Hashimoto and Ware, 1997; Yagi et al., 1998), a hypothesis that was later supported when alterations of secretory function were observed in platelets from a \textit{Sept5} knockout mouse (Dent et al., 2002). Relationships between the septins and human diseases are discussed further in Chapters 9, 11, 12, 14, and 15.

2. The very high concentrations of septins in the central nervous system cells of \textit{Drosophila} (Neufeld and Rubin, 1994; Fares, Peifer and Pringle, 1995) had suggested strongly that the septins had roles unrelated to cytokinesis (which these cells do not do) and hinted that they might have roles in vesicle trafficking (of which these cells do a lot). These ideas gained strong support as papers began to appear documenting both the high concentrations of various septins in non-dividing cells of the mammalian brain and the association of these septins with structures and proteins involved in vesicle trafficking (Caltagarone et al., 1998; Hsu et al., 1998; Kinoshita et al., 1998; Yagi et al., 1998; Beites et al., 1999; Kinoshita, Noda and Kinoshita, 2000; Xue et al., 2000). Understanding exactly what the septins are doing in neurons and in relation to vesicle trafficking there and in other types of cells remain major challenges for the field, as discussed further in Chapters 8, 11, and 15.

3. Bill Trimble, a highly accomplished investigator of vesicle trafficking, became the first major recruit to the septin field from mainstream
mammalian cell biology when he leaped in with both feet (and four papers) in 1999 (Beites et al., 1999; Trimble, 1999; Xie et al., 1999; Zhang et al., 1999). This initial burst of activity from Bill’s lab provided, among other things, evidence for differentiation of mammalian septin function in different tissues, high levels of expression of some septins in brain, involvement of the septins in vesicle trafficking and secretion, and direct binding of the septins to phospholipids as at least part of the mechanism for septin-membrane association.

4. Ian Macara, a highly accomplished investigator of the many functions of small GTPases, was led to study the septins when they were found as binding partners of the Borg proteins, which his lab had identified previously as apparent effectors of Cdc42 function during cell polarization (Joberty et al., 2001). Ian’s entry into the field had significance well beyond the direct (and substantial) impact of the research contributions from his lab. First, it further legitimized the septins as an object of study by mainstream cell biologists. Second, it was Ian, after he participated in the 2001 ASCB Meeting workshop (see below and Appendix C), who said ‘enough is enough’ to the nightmare of mammalian septin names and led the successful effort to rationalize and simplify the taxonomy and nomenclature of the genes and proteins (Macara et al., 2002). It would be difficult to overstate the value that this contribution has already had, and will continue to have, to the field, and it should be stressed that Ian’s leadership could not have been successful without the cooperation of essentially everyone in the field, all of whom should feel proud of their participation in this community-spirited action. An update on this nomenclature can be found in Appendix B.

As the mammalian septin field was taking off, there were also important developments on several other fronts. First, Tri Nguyen, John White, and their co-workers discovered that the Caenorhabditis elegans unc-59 and unc-61 genes, which John had identified many years earlier on the basis of mutants with uncoordinated movements (White, Horvitz and Sulston, 1982), encoded the worm’s two and only two septins and that even a mutant carrying null mutations of both genes produced viable adult worms (Nguyen and White, 1996; Nguyen et al., 2000). This work raised important questions about septin evolution (why do yeasts, flies, and mammals have multiple septins and the worm just two?), structure (do these two septins form assemblies similar to those of the multi-protein assemblies in other organisms, and, if so, how?), and function (why are the worm septins non-essential for cytokinesis in most cells even though they localize to the cleavage furrows as in other cells?) that still engage the field.

Second, studies by Yves Barral, Mike Snyder, and co-workers (Barral et al., 2000) and by Peter Takizawa, Ron Vale, and co-workers (Takizawa et al., 2000) established the important point that, at least in yeast, the septins function to restrict the mobility of integral membrane proteins and other cortical components and thus
allow the cell to maintain distinct mother-cell and bud polarized cortical domains. Whether the septins form the diffusion barrier themselves or by recruiting other proteins that form the actual barrier is still unclear, although some arguments favour the former model (see further discussion in Chapter 4).

Third, as in so many other areas of cell biology, the ability to view the behaviour of the septins in real time in living cells by using GFP and other fluorescence tags opened up the study of septin dynamics (Cid et al., 2001; Lippincott et al., 2001). Continuation of these studies in many laboratories, and in other organisms as well as yeast, has provided many insights into septin assembly, organization, and function, as discussed further in various other chapters in this book.

**CONCLUDING REMARKS**

Most of the program for the American Society for Cell Biology’s Annual Meeting is set by the society president and program committee, but a modest number of ‘member-initiated’ workshops can be organized for the Saturday afternoon at the beginning of the meeting. In the summer of 2001, Chris Field called me to suggest that there was enough activity and interest in the emerging septin field to justify organizing such a workshop for the December 2001 meeting. I agreed with enthusiasm, and the workshop was duly proposed to the ASCB, approved, and organized (with Chris doing most of the work). As the program shows (Appendix C), it brought together many of the people who had done interesting work on the septins. Not many things could have caused me to miss this event, but the invitation to Stockholm for the celebration of Lee Hartwell’s Nobel Prize was one of them. My absence did not appear to detract much from what proved to be a very valuable session with multiple beneficial consequences, including the attraction of other investigators into the field, and I think that this session really marked the emergence of the septins as a mature (if still growing) field. From this point forward, to summarize progress with the septins requires a book (i.e. this one) and not a single chapter, so I end my history at this point.

Since the 2001 session, there have been two International Septin Workshops (see Appendix C), each of which has been delightful both for its science and for its camaraderie. The first of these workshops was conceived and catalysed by my two co-editors, Hilary Russell and Peter Hall. These workshops have illustrated the growth of the field, our progress, and also how much there is still to do before we really understand the structure, dynamic assembly, and multiple functions (and dysfunctions) of this class of proteins. The next Septin Workshop should be at least as exciting, and I am looking forward to it keenly. In the meantime, for my own part, I will continue, with my group, to try to understand better the question with which we began, and to which I think we still do not really have a very satisfying answer: what do the septins do in cytokinesis, and why is this role essential in some cell types and not in others?
ACKNOWLEDGEMENTS

It is a pleasure to thank the many members of my own group, our collaborators, and the other interested scientists who have helped to build the septin field over the years. To those who have suffered because various parts of the developing story were not published in a timely way (or at all), I can only apologize and express my relief that the field has developed so well despite these lapses. I would also like to thank the National Institute of General Medical Sciences, which has generously supported our work from a time long before it was clear whether the septins would ever turn out to be relevant to human health.

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