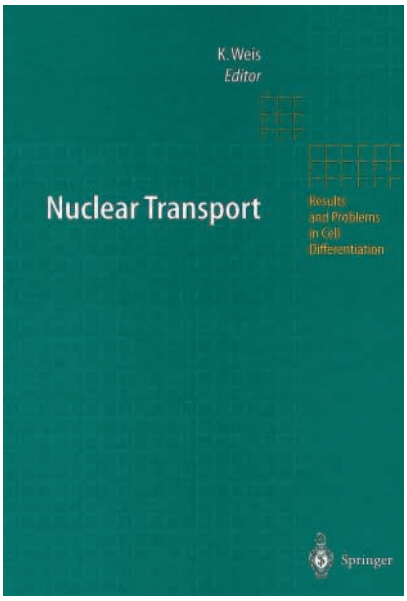
Molecular machinery of nuclear trafficking



Nuclear Transport (Results and Problems in Cell Differentiation Vol. 35)

edited by Karsten Weis

Springer-Verlag (2002) 197 pages. ISBN 3-540-42368-0
£72.50/$110

Nuclear trafficking was a major growth area in cell biology during the past decade. Karsten Weis has assembled a fascinating series of review chapters by many of the leading authorities in the field that serves as an elegant testimony to just how much has been achieved and how firmly established many of the key features of the process have become. In many ways the fact that such a book could now be written marks the end of the beginning of this field and signals its evolution into one of the more established trafficking areas, in which the emphasis has inevitably shifted from identifying the basic components of the transport machinery to establishing how they interact to generate function. For cell biologists wishing to pursue such functional investigations, this will be a valuable background reference collection.

The basic structure of the nuclear pore complexes (NPCs) that mediate nuclear trafficking, and the proteins from which they are constructed, is reviewed by Strambio-de-Castillia and Rout (from a yeast perspective) and also by Fahrenkrog and Aebi (for vertebrates). Medium-resolution EM structures of both yeast and vertebrate NPCs have been obtained and considerable information has become available about the location of individual nucleoporins. Many nucleoporins contain distinctive 'FG' sequence repeats that are thought to interact with a range of soluble components of the transport machinery, possibly concentrating material for transport and facilitating translocation through NPCs as well as imposing a level of selectivity on the process.

One of the unexpected developments during the past decade was the realization that the NPC itself probably does not function primarily as a gating mechanism or by directly transporting cargo macromolecules between the nuclear and cytoplasmic compartments. Instead, nuclear trafficking relies on a range of soluble components. Transport factors bind their cargo in one compartment and then move through the NPCs to the other compartment, where the cargo is released. The transport factor then recycles back through the NPC to participate in another round of transport. In many instances, this process is orchestrated by the Ras-superfamily GTPase Ran. Bischoff et al. describe in detail how the nucleotide state of Ran is controlled by its nuclear guanine-nucleotide-exchange factor (RCC1) and cytoplasmic GTPase-activating protein (RanGAP). The nucleotide state of Ran is crucial to defining the interactions between transport factors, their cargoes and nucleoporins, and is fundamental to the sorting mechanism that defines the directionality of transport.

Conti provides a comprehensive overview of the progress made on the structures of many of the components of the importin-based pathways that are crucial for nuclear protein import. Many nuclear proteins have a classical nuclear localization sequence that is recognized by importin-α, which acts as an adapter to the importin-β transport factor. This interaction is favoured in the cytoplasm, where Ran is primarily in the GDP-bound form, but is disrupted by RanGTP in the nucleus. The structural basis for these interactions, and also those between importin-β and FG-nucleoporins, has now been established and Ran is thought to modulate the various interactions by introducing a conformational change on binding to importin-β.

Nuclear protein export is mediated by exportins, such as CRM1, which are homologues of importin-β and are reviewed by Fornerod and Ohno. Cargo molecules carry defined nuclear export signals analogous to those used for import, and again the formation of cargo-carrier complexes in the nucleus and their dissociation in the cytoplasm is orchestrated by Ran. The export of U snRNAs is also mediated by these transport factors. The nuclear export of tRNA employs a different transport factor (Los1p in yeast) and is reviewed comprehensively by Simos et al. The nuclear export of mRNA is still an area of considerable controversy and, in addition to CRM1 having a role (discussed by Fornerod and Ohno), Izaurralde describes how transport factors of the Tap/Mex67 family may function in this process. These transport factors are multidomain proteins in which different modules can bind mRNA containing the CTE element, mRNA through adapters, or FG-nucleoporins.

Cullen contributes a fascinating chapter outlining how retroviruses have been used to study the nuclear export of both mRNA and proteins. It is striking how complementary virology and nuclear trafficking have been in this instance, with each giving fundamental insights into the other. This chapter is complemented by Schüller and Ruis, who review a broad range of systems in which nuclear trafficking is regulated.

Overall, this book serves as a landmark collection of reviews summarizing the identification of the components of the nuclear trafficking machinery. It will certainly be a fundamental reference collection for researchers engaged in defining precisely how the machinery works as well as for those working in a broad range of related areas, such as signalling and the cell cycle, in which nuclear trafficking forms a crucial component of the overall function. It is

a little unfortunate, perhaps, that there are not also contributions from some of the central architects of the remarkable progress made in the past decade, such as Görlich, Gerace and Blobel, but this does not prevent this collection being an extremely valuable resource. As always, one cannot help being disappointed by the delay in actually getting works like this published: most references are to studies from the 1990s and there are only a small number of papers from 2000. It is a pity that publishers do not seem to appreciate the value of immediacy in such a topical area as this, and unfortunately in some areas, such as mRNA export, the field has already moved on. But, better late than never, and this fine collection will make a valuable addition to the libraries of workers in the nuclear transport field.

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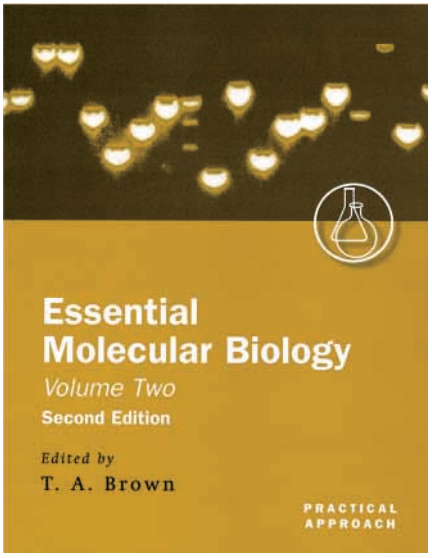
How to clone – part 2



Essential Molecular Biology, Vol. 2, 2nd edn

edited by T. A. Brown

Oxford University Press (2001) 289 pages. ISBN 0-19-963644-3
£32.50

About 6 months ago I reviewed the first volume of this updated two-part series from T. A. Brown (Plant, K., 2001. Can't clone, won't clone. *J. Cell Sci.* 114, 1797). I used cookery as my theme, hence the title given to this review of 'part 2', which is a reference to Delia Smith's 'How to Cook' series of books for the complete culinary novice. So, to continue my analogy, what you, the potential purchaser, really want to know is whether this second and final volume will complete your transformation from molecular Egg-Boiler to Cordon Bleu.

Volume 1 covered the real basics (DNA and RNA isolation, electrophoresis, cloning, and such microbiology as we molecular biologists can manage); so you will need to buy both volumes if you are starting from scratch. Volume 2 continues where Volume 1 finished, explaining how to make and screen libraries, various types of blotting and detection, sequencing, the polymerase chain reaction, transcript mapping and the expression of recombinant proteins in bacteria. This range is broadly the same as that of the first edition, although the chapter on protein expression has replaced one on DNA-protein interactions. Comparing the other chapters with the original, I found that often there were relatively few changes, which in some cases was more surprising than in others.

The section of the book where a shortage of recent innovations was particularly noticeable is the one describing the polymerase chain reaction (PCR). In my experience, many non-molecular biologists find that their first introduction to molecular techniques involves PCR, whether they are clinicians interested in mapping or diagnosing genetic disorders, biochemists seeking to generate mutant proteins or microbiologists speeding up the identification of clinical pathogens. In this substantially re-written chapter, Brown gives a good description of the principles of PCR and how to optimise it. However, there have been many improvements in basic PCR technology over the years, including new proof-reading enzymes and buffering systems, which are not discussed here. For those interested in the RNA products of genes there is very little on RT-PCR and nothing other than a reference for the twin techniques of 5′ and 3′ RACE. Because of this short-fall, the book pushes those wishing to isolate cDNA products towards the library-screening approach, which in this day and age is certainly not the easiest and most cost-effective route.

Apart from the somewhat deficient PCR chapter, I have to say I liked this book. As I said of the first volume, one of the nice things about Brown's books is their no-nonsense style. You'll find that this book goes beyond being just a series of recipes and protocols. It is full of sound advice to help you plan, execute and control your experiments. It gives you plenty of options (for example, there are many possibilities for labelling your DNA probe) and the information required to help you chose which will best suit your experiment. Brown's approach of building from the basics to prevent an overdependence on kits is one of which I whole-heartedly approve and in the long run it is well worth the effort. It will make you a far better molecular biologist!

So, to come back to our original questions, should you purchase and will it turn you into Egon Ronay? Well, to the first question I'd have to say pretty much the same as I said about the first volume. If you are a novice (even an expert novice!) looking for a good manual then this one is better than most, and it will certainly keep you moving in the right direction if you started with Volume 1. However, if you are simply wondering whether to upgrade your 10-year-old first edition, I am surprised to say there aren't really enough changes in here to merit the cost. As to making us all Masterchefs, well, I don't think that was ever Brown's (or indeed Smith's) intention. I suspect that if he succeeds in encouraging a few more non-molecular biologists to forgo the pre-prepared meals-for-one and actually weigh out a few ingredients he will be satisfied. For your final transformation, it is probably necessary to move on to the more specialised books or to start to create your own recipes! Happy cooking!

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