Chapters 14-16. Weaver, 1999. Mol Biol X107A.

Excerpts from:
Ch 14-16. Post-transcriptional Events.
  Ch 14. … I. Splicing
  Ch 15. … II. Capping and polyadenylation
  Ch 16. … III. Other events

Most of this handout is from a previous class. That year we did cover these chapters very briefly, in sequence. I have updated the core sections only minimally. However, I have updated the Further Reading section, to list some more recent references, largely grouped by topic. I have not referred to the articles in the core sections.

This handout, then, is considerably more informal than the regular ones, but I hope it may be of some use to those who want to explore these topics, beyond our course, on their own. Feedback appreciated, as to whether it is worthwhile to maintain a supplementary handout such as this.

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Reading note. We will cover only selected excerpts from these three chapters. The main purpose of our coverage is to establish the existence of some of these events, not to analyze them in detail. I hope to spend about one class period on these three chapters total. The only parts I expect you to read are:

- Ch 14: pp 413-4, introductory remarks only.
- Ch 15: Sections 1 (Capping) and 2 (polyadenylation)… just enough to understand what these modifications are, with some introduction to their function. The function will be discussed more in Ch 17. Skip Sect 3.
- Ch 16: Sections 1-2, enough to establish that these processes occur. Section 5, an introduction to mRNA stability.

Coverage discussed below, as reinforced in class, should help guide you.

⇒ You are not formally responsible for this material on the final exam.

Clark & Russell: pp 140-3 for RNA processing; pp 403-5 for ribozymes.

A. Highlights

- RNA processing
  - Relevant to synthesis and degradation of all classes of RNA.
  - Includes: changes in chain length (both ends; middle); base modifications.
  - Immediate concerns:
    - Cleavage of tRNA and rRNA from precursors
    - Role of ribozymes
    - Polyadenylation and capping of eukaryotic mRNA.
    - Also see next item.

- mRNA degradation: functional inactivation vs physical degradation; regulation; recognition sequences, nucleases, factors.

- Processing and degradation may share features.

B. RNA processing; introduction

Three classes of RNA are needed for protein synthesis (m, r, t). The central dogma describes that RNAs are made by transcription of the DNA template (Ch 6, 10). However, it turns out that "production of functional RNA" does not stop with the simple act of copying RNA from DNA.

There are several events that fall into the general category of RNA processing. The general idea is that the "final" RNA product (the RNA molecule that does the job) is made from an RNA transcript, by processing (post-transcriptional modifications).

RNA processing events include cleavages, base modifications, and additions.

Further, it turns out that RNA degradation is related to RNA processing.

C. tRNA and rRNA processing; ribozymes

(Ch 16 Sect 1-2)

tRNA and rRNA are cut out from large precursors. Leaders, trailers, and intergene spacers are all removed, by enzymes that presumably recognize general structural features in the precursor.

Fig 16.2 gives an example of how processing of an rRNA precursor may occur. (This should not be taken as definitive.)

Of particular interest is that one of the tRNA processing enzymes, RNase P (Fig 16.6), is part RNA as well as part protein. Bacterial RNase P is ~85% RNA, and the catalytic activity is in the RNA part of the enzyme (Fig 16.7). Historically, this result was our first clue that RNA molecules can play the role of enzyme. RNA molecules that serve as enzymes are termed ribozymes. We briefly noted the existence of ribozymes in Ch 3 handout Sect I (Fall 2000), and saw that the ribosome is a ribozyme in Ch 18-19.

Some of the splicing processes are catalyzed by ribozymes (Ch 14).

> The discovery that RNA can serve as an enzyme showed that RNA can carry out all the fundamental processes now done by DNA, RNA and protein. Thus it is plausible that primordial life involved only RNA (as the only biopolymer); later, some activities of the primitive RNA were acquired by DNA and proteins, which do some things better than RNA does them. This general view of a primitive "RNA world" dominates most current thinking about the origin of life. Of course, most parts of that story remain open.

D. mRNA processing

Extensive processing of mRNA is typical of eukaryotes. Major types of processing include:

- Addition of 5′ cap (and its modification by methylations).
- Addition of 3′ poly(A).
- Removal of intragene spacers (intervening sequences = introns).

We discuss these three types of eukaryotic mRNA processing in the following three sections.

There is only occasional processing of bacterial messages. There are examples where bacterial mRNA processing serves a regulatory role. For example, mRNA processing can break a polycistronic mRNA into simpler messages, each of which has its own decay characteristics..

> (One might suggest that the coupling of translation and transcription in bacteria, in a common compartment, limits the opportunity for intermediate processing.)

The increased complexity of eukaryotic mRNA processing provides additional sites for regulation: any processing step is potentially a site for regulation. Regulation of

polyadenylation and splicing is well documented. Cap removal is part of some mRNA degradation pathways; we would suspect that cap removal will turn out to be a regulated event. In any case, a cap identifies an RNA as mRNA.

E. Capping

(Ch 15 Sect 1)

What. The cap is a structure at the 5′ end of eukaryotic mRNAs. The basic cap structure is a guanosine nucleotide, added "backwards" to the 5′ end; that is, the linkage is 5′ to 5′. Fig 15.3 shows the basic structure. Variant caps differ in methyl groups, on the G and on the nearby riboses; this variation is not important for us.

How. The cap structure is added during transcription, probably very early (chain length of new RNA below 30 nucleotides; p 462). Fig 15.5 outlines the steps, which are not particularly interesting.

> (Capping is closely associated with Pol II. A few non-mRNAs are made by Pol II; they are also capped. p 463.)

Why. Weaver lists four functions for the cap in his introductory statement on Function, p 462. It will help if you generally think of the cap as a marker for mRNA. Thus the cap directs the nascent RNA to be treated as a mRNA. The cap directs transport to the cytoplasm, the site of translation. The cap is a key recognition site for the initiation of translation; we will examine this in Ch 17. The cap is involved in mRNA degradation, in that decapping is often a key step in initiating degradation; see Sect J, below. Weaver also notes that capping may direct the RNA to the splicing apparatus. This indicates how interrelated the various processing steps may be.

F. Polyadenylation

(Ch 15 Sect 2)

What. Eukaryotic mRNAs typically have a 3′ tail, consisting of entirely A. In higher eukaryotes, the poly(A) tail is about 200 nucleotides long (p 465).

How. The poly(A) tail is not encoded in the genome. At some point during transcription, the polyadenylation complex [including the poly(A) polymerase itself] recognizes the nascent mRNA, cleaves it, and then attaches the poly(A) tail. Fig 15.15.

> Note, therefore, that the end of the mRNA (prior to the tail) is not determined by transcriptional termination events, but rather by the polyadenylation system. The exact fate of the transcription apparatus is not entirely clear; it is assumed that the Pol continues for some distance, but is eventually released.

The mRNA signals for polyadenylation are complex (Figs 15.20 & 25), and can be regulated. A given gene may include more than one possible polyadenylation signal. Regulatory systems would determine which is actually used, but the point is that more than one type of functional mRNA can be made from the same gene as a result of alternative polyadenylation.

Why. The 3′ poly(A) tail is involved in mRNA stability (Sect J, below). Interestingly, it is also involved in translational initiation (Ch 17).

> It may be useful perspective to note that the presence of both a 5′-cap and a 3′-tail attest that an mRNA is intact.

G. Splicing

(Ch 14) *You are not responsible for this section or for Ch 14 of Weaver*.

Splicing involves the removal of intervening sequences (introns) from the interior of an otherwise useful RNA. In the case of mRNA, the introns may represent non-coding regions embedded within coding regions.

This is not an X107A topic. It has many complex aspects. We may note briefly…

- There are different types of splicing.
- In some types of splicing, the splicing activity is in RNA molecules. That is, some splicing enzymes are ribozymes. Recall Sect C.
- The mechanism of splicing for nuclear mRNA (the type that probably interests you the most) is mechanistically closely related to that for the so-called Group II self splicing introns -- even though the modern apparatus for the former process is much more complex. Weaver notes this in his discussion of Group II introns, pp 449f.
- Splicing must be perfectly precise. This is most obvious when it involves mRNA coding regions.
- Splicing is highly regulated, via the formation of a complex splicing apparatus.
- As a corollary of regulated splicing, there may be alternative splicing: the same pre-mRNA may be spliced more than one way, thus may code for more than one functional protein. See subsection on Alternative Splicing, p 443.

  Alternative splicing may be a major process in humans, helping to explain our surprisingly small number of "genes". There are estimates that 1/3 of human genes may be subject to alternative splicing. Analysis of all the genes on one chromosome (#22) revealed an average of 2.6 distinct transcripts per gene (IHGSC, 2001; p 898).

H. Protein splicing

[Not in Weaver.]

The term splicing usually refers to removal of intervening sequences from RNA. However, a protein splicing phenomenon also occurs occasionally. Intervening sequences in proteins, removed by protein splicing, are called inteins.

For more about inteins and protein splicing, see the Intein Database web site, listed in Sect M.

I. Organizing transcription and processing; the CTD

Weaver makes some comments about how the various processes are interrelated.

An interesting idea that is developing is the key role of the Pol II CTD; recall Ch 10. The broad idea is that the composition of the Pol changes during transcription. More specifically, it seems that various factors are carried along on the CTD. For example… polyadenylation factors are CTD-associated; after a polyadenylation site is reached, these factors leave Pol, and change the Pol to a state that is competent for termination.

J. mRNA degradation

(Ch 16 Sect 5)

Broad perspective on mRNA degradation

- It is a general characteristic of mRNA that it is unstable. This is "always" true, relative to the so-called stable species, tRNA and rRNA. In fact, the stability of mRNA varies widely. In general, eukaryotic messages have longer lifetimes than prokaryotic messages. (This is partly a reflection of the different growth rates commonly found, but that does not explain the entire difference in mRNA stability.)

- More interestingly, mRNAs for different genes have different lifetimes, and for some genes the mRNA lifetime varies with conditions. Thus we are interested in how mRNA is degraded not just for its own sake, but because it is part of the story of gene regulation.

In Ch 16 Sect 5, Weaver focuses on some examples that show regulation of mRNA stability in eukaryotes. To complement this, I will show one example from bacteria, along with some information about how bacterial mRNAs are degraded. Then I will show an overview of mRNA degradation pathways from yeast, where much more mechanistic information is available.

Broad ideas on how mRNA is degraded

- Functional inactivation and physical degradation are distinct. An mRNA may be functionally inactivated by a "critical hit", presumably from an endonuclease. Following such cleavage, other nucleases can further degrade the fragments, to recycle the nucleotides; this can be done by known exonucleases.

- The inactivating cleavages may be at specific sites or structures, and are potentially regulatable. Regulation might involve regulating the activity or specificity of the inactivating nuclease or the accessibility of the inactivation site in the mRNA.

- For eukaryotic mRNAs, the "specific sites or structures" referred to above may include the 5′-cap and the 3′-poly(A) tail, the characteristic special features of this class of RNA.

Bacteria

We have noted that the direction of mRNA synthesis and use is the same, 5′→3′. 5′→3′ degradation of mRNA might be an appealing idea, for consistency. However, no exonuclease with that specificity has been found in bacteria. [There is such an enzyme in eukaryotes.]

Processing reactions (as in cleavage of an RNA from a long precursor; Sect C) and degradation both involve endonucleolytic cleavages, with some specificity. Both recognize RNA sequence or 2° structure. Not surprisingly, it seems that the same enzymes may be involved in both processes. Work in E. coli has identified two endonucleases that are important in mRNA degradation; both are also involved in processing of stable RNA (p 499). These are RNase E and RNase III.

Polynucleotide phosphorylase may be the major exonuclease that degrades RNA to nucleotides. Interestingly, much of it is found in a complex that includes RNase E and a helicase.

Hairpin sequences may destabilize mRNA by serving as a target site for RNAse III, which is specific for DS RNA. On the other hand, some hairpins can stabilize mRNA from exonucleolytic degradation. Genetic engineers sometimes add stabilizing 3′ hairpins to a gene in an attempt to increase protein expression.

Eukaryotes

The cases that Weaver discusses involve specific regulatory mechanisms, and do not necessarily provide any general perspective on mRNA degradation.

It is virtually certain that the poly(A) tail is relevant to stability of eukaryotic mRNA. A correlation between tail length and stability is common. Some general degradation pathways involving the poly(A) tail are recognized. However, many details remain obscure. There is a poly(A)-binding protein (PABP), which protects mRNA from degradation. When the tail is

too short to bind PABP, the mRNA is rapidly degraded. This is a good story, but leaves open how tail length is determined, either initially or later.

Another good story is that of destabilizing sequences, especially in genes for growth factors. Growth factor mRNAs are quite unstable, by eukaryotic standards; that even seems reasonable. A destabilizing sequence can be identified by genetics. And moving this sequence to other mRNAs makes them unstable. The destabilizing sequence seems to promote poly(A) shortening, which then presumably promotes degradation. The destabilizing sequence is in some proto-oncogenes, genes that can mutate to cause cancer. In fact, some cancer mutations in these genes involve loss of the destabilizing sequence.

A variety of mRNA degradation pathways are found in yeast. These pathways involve various combinations of deadenylation and decapping, and various nucleases. It seems likely that the major normal pathway starts with deadenylation (maybe coupled to the extent of translation), followed by decapping, and then 5′→3′ exonucleolytic degradation. However, the bigger story may be the variety of pathways, each of which may be particularly important under certain circumstances.

K. tRNA and rRNA base modifications

tRNA base modification is an important type of post-transcriptional processing. We discuss this in Ch 19.

There is also some base modification in rRNA, which Weaver briefly notes on p 499.

L. Further reading

> These papers are briefly listed, often without annotation if the title is clear. Most are grouped by topic. Most of the topics are listed by their order in the handout above.

ribozymes (Sect C)

C Guerrier-Takada et al, Phenotypic conversion of drug-resistant bacteria to drug sensitivity. PNAS 94:8468, 8/5/97. Interesting application of RNase P. They develop plasmids that code for a small RNA that will bind to an mRNA of interest. When the new RNA binds to the mRNA, it creates an RNase P cleavage site -- thus inactivating the mRNA. In their model system, they use this method to inactivate a gene for drug resistance.

L P Landweber et al, Ribozyme engineering and early evolution. BioScience 48:94, 2/98. Feature article in a journal aimed at a broad audience of biologists. Ribozymes -- enzymes based on RNA molecules -- exploit the ability of RNA molecules to fold up into structures about as complex as proteins. It is a plausible scenario that primitive life depended on ribozymes. Landweber et al review both natural and engineered ribozymes, and discuss the methods used to develop new ribozymes in the lab.

P J Unrau & D P Bartel, RNA-catalyzed nucleotide synthesis. Nature 395:260, 9/17/98. (+ News, Robertson & Ellington, p 223.) Another demonstration of the versatility of ribozymes, supporting the idea that primitive life may have been RNA-based.

N K Tanner, Ribozymes: the characteristics and properties of catalytic RNAs. FEMS Microbiology Reviews 23:257-275, 6/99.

J J Rossi, Ribozymes in the nucleolus. Science 285:1685, 9/10/99. News. Therapeutic applications of ribozymes would undoubtedly depend on proper targeting. Here, they target ribozymes to the nucleolus by attaching them to small RNAs known to be transported to the nucleolus.

S J Freeland et al, Molecular evolution: Do proteins predate DNA? Science 286:690, 10/22/99. News. We noted that the existence of ribozymes has led to the idea that RNA was the primordial biopolymer. This news item discusses the inevitable next part of that story: which came next, DNA or protein? They discuss several arguments, on both sides. The one they think is the most persuasive is that the production of deoxyribonucleotides from ribonucleotides requires a difficult enzymatic step that may well be beyond the capability of a ribozyme.

J Rogers & G F Joyce, A ribozyme that lacks cytidine. Nature 402:323, 11/18/99. So who cares? Proteins use 20 subunits, ordinary ribozymes use four. Here they stretch that to three -- to show that high diversity of subunits is not critical in developing enzymes. They chose to omit C, since it is the least stable of the normal bases (remember the problem of deamination of C to U).

E Finkel, Biochemistry: DNA cuts its teeth - as an enzyme. Science 286:2441, 12/24/99. News. If RNA can serve as an enzyme, how about DNA? No examples of DNA-enzymes are yet known from Nature, but lab work has succeeded in developing them. This news item discusses the therapeutic potential of DNA-enzymes, in a rat model. If they work, DNA-enzymes would have the advantage over RNA-enzymes of lower production cost and greater stability.

W G Scott, RNA structure, metal ions, and catalysis. Current Opinion in Chemical Biology 3:705–709, 12/99.

capping (Sect E)

C D Lima et al, Structure and mechanism of yeast RNA triphosphatase: an essential component of the mRNA capping apparatus. Cell 99:533, 11/24/99. The first step in cap addition is converting the natural triphosphate end of the new RNA to a diphosphate; Fig 15.5a.

polyadenylation (Sect F)

Y N Osheim et al, EM Visualization of transcription by RNA polymerase II: Downstream termination requires a poly(A) signal but not transcript cleavage. Mol Cell 3:379, 3/99. A

reasonable interpretation is that Pol changes composition as it passes a polyadenylation site, and becomes termination-competent.

J Zhao et al, Formation of mRNA 3′ ends in eukaryotes: mechanism, regulation, and interrelationships with other steps in mRNA synthesis. Microbiol & Molec Biol Rev 63:405. 6/99. Major review, primarily on polyadenylation and related processes.

F E Kleiman & J L Manley, Functional interaction of BRCA1-associated BARD1 with polyadenylation factor CstF-50. Science 285:1576, 9/3/99. BRCA1 is one of the genes implicated in breast cancer. Its precise function is not clear, though it has been implicated in DNA repair. Here they show an interaction of the BRCA1 protein with polyadenylation. The role of this interaction is not clear.

S M L Barabino & W Keller, Last but not least: Regulated Poly(A) tail formation. Cell 99:9, 10/1/99. Minireview.

splicing (Sect G)

T Misteli & D L Spector, RNA polymerase II targets pre-mRNA splicing factors to transcription sites in vivo. Mol Cell 3:697, 6/99. One issue in RNA processing is understanding how the processes are related, physically or temporally. Here, they show that some splicing factors are associated with the CTD of Pol II (recall Ch 10). Mutant Pol lacking the CTD shows defective splicing. Thus splicing is coupled to transcription, in that the Pol delivers splicing factors.

P Cramer et al, Coupling of transcription with alternative splicing: RNA Pol II promoters modulate SF2/ASF and 9G8 effects on an exonic splicing enhancer. Mol Cell 4:251, 8/99.

S Wu et al, Functional recognition of the 3′ splice site AG by the splicing factor $U2AF^{35}$. Nature 402:832, 12/16/99. (+ related articles, p 835 & 838.)

D R Edgell et al, Barriers to intron promiscuity in bacteria. J Bacteriol 182:5281-5289, 10/00. Minireview. Some types of introns are mobile, but what determines/limits the mobility? They usefully explore the issues, without clear conclusions.

IHGSC (International Human Genome Sequencing Consortium), Initial sequencing and analysis of the human genome. Nature 409:860, 2/15/01.

Organizing the processes (Sect I)

J D Lewis & D Tollervey, Like attracts like: getting RNA processing together in the nucleus. Science 288:1385, 5/26/00. Review; part of a feature section on Nuclear Dynamics. A general discussion of the organization of RNA processing within the nucleus. The emerging view is that there are no fixed sites for processing events, but foci with specialized activities develop simply as materials accumulate in an area.

mRNA decay (Sect J)

The articles listed here include examples from bacteria and yeast, plants and animals.

R-F Wang et al, Escherichia coli mrsC is an allele of hflB, encoding a membrane-associated ATPase and protease that is required for mRNA decay. J Bact 180:1929, 4/98. (+ accompanying paper, p 1920, in which they isolate the mutant strain studied here.) They identify another gene involved in mRNA degradation. As best they can tell so far, it seems to code for a membrane-bound protease. Why? They don't know. One possibility is that the protease activates some RNase.

G A Mackie, Ribonuclease E is a 5′-end-dependent endonuclease. Nature 395:720, 10/15/98. RNase E is the enzyme that initiates degradation of many mRNAs in E. coli. Part of its specificity is that it requires a free 5′ end. Also see Bricker & Belasco (1999), below.

J E G McCarthy, Posttranscriptional control of gene expression in yeast. Microbiol & Molec Biol Rev 62:1492, 12/98. Posttranscriptional control includes issues other than mRNA decay. In fact, much of this review is on control of translational initiation. The final section reviews mRNA decay.

G Laroia et al, Control of mRNA decay by heat shock-ubiquitin-proteasome pathway. Science 284:499, 4/16/99. They deal with an mRNA that is destabilized by a AU-rich element. A protein binds this element, somehow marking the mRNA for degradation. During heat shock, the protein is complexed by heat shock proteins, thus stabilizing the mRNA.

A L Bricker & J G Belasco, Importance of a 5′ stem-loop for longevity of papA mRNA in Escherichia coli. J Bact 181:3587, 6/99. The pap genes are relevant to the pathogenesis of E. coli; they encode proteins that help the bacterial cells adhere to epithelial cells in the urinary tract. The papA and papB genes are transcribed into a single message, which is then cleaved into two pieces. The piece coding for papA is stabilized by a hairpin. Recall Mackie (1998), above.

Y Chiba et al, Evidence for autoregulation of cystathionine γ-synthase mRNA stability in Arabidopsis. Science 286:1371, 11/12/99. Arabidopsis is a model plant system.

G A Farr et al, Protection against 3′-to-5′ RNA decay in Bacillus subtilis. J Bact 181:7323, 12/99. They study engineered RNA molecules, and find that different features may block different nucleases.

S Tharun et al, Yeast Sm-like proteins function in mRNA decapping and decay. Nature 404:515, 3/30/00.

M Gao et al, Interaction between a Poly(A)-specific ribonuclease and the 5′ cap influences mRNA deadenylation rates in vitro. Mol Cell 5:479, 3/00.

C Spickler & G A Mackie, Action of RNase II and polynucleotide phosphorylase against RNAs containing stem-loops of defined structure. J Bacteriol 182:2422-2427, 5/00.

M J Ruiz-Echevarria & S W Peltz, The RNA binding protein Pub1 modulates the stability of transcripts containing upstream open reading frames. Cell 101(7):741, 6/23/00. A study of factors that promote decay of mRNAs containing premature stop codons. This study is with yeast, and is for a cytoplasmic system.

C Bousquet-Antonelli et al, Identification of a regulated pathway for nuclear pre-mRNA turnover. Cell 102:765, 9/15/00. They find a system for degrading unspliced pre-mRNAs in yeast. Nucleases working from both ends of the RNA are involved, but the major player is the exosome. The exosome is a complex of several ribonucleases, possibly all degrading from the 3′ end. This complex is involved in both processing and degradation, both cytoplasmic and nuclear. The paper includes a good discussion of different degradation pathways.

C Grosset et al, A mechanism for translationally coupled mRNA turnover: interaction between the poly(A) tail and a c-fos RNA coding determinant via a protein complex. Cell 103:29, 9/29/00.

editing (Ch 16 Sect 4)

> RNA editing is a change in the informational content of an RNA after transcription, other than those other types of change already considered (e.g., splicing). Weaver discusses a bizarre phenomenon in trypanosomes, in which multiple U's are added to or deleted from the mRNA. When this was first described, it seemed to be a significant challenge to the Central Dogma. As the mechanism, which Weaver discusses, became clear, we realized that the RNA information really was in the DNA, just in an unusual way. This bizarre phenomenon is restricted, so far as I know, to protozoans. However, other examples of RNA editing, usually involving single base changes to an mRNA, are known in higher organisms. These editing events are mechanistically unrelated to the trypanosome editing, but are of biological significance. A few examples follow.

M Higuchi et al, Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. Nature 406:78, 7/6/00. RNA editing is an essential function in mammals, relating to brain function.

M J Palladino et al, A-to-I pre-mRNA editing in Drosophila is primarily involved in adult nervous system function and integrity. Cell 102(4): 437, 8/18/00.

A Longacre & U Storb, A novel cytidine deaminase affects antibody diversity. Cell 102(5):541, 9/1/00. Minireview.

K Bourara et al, Generation of G-to-A and C-to-U changes in HIV-1 transcripts by RNA editing. Science 289:1564, 9/1/00.

Also recall Schwartz et al (1999; Ch 2 FR) and Gerber & Keller (1999; Ch 19 FR).

transport to/from nucleus

S Nakielny & G Dreyfuss, Transport of proteins and RNAs in and out of the nucleus. Cell 99:677, 12/23/99. Major review. The nuclear pore complex mediates transport in both directions.

M Damelin & P A Silver, Mapping interactions between nuclear transport factors in living cells reveals pathways through the nuclear pore complex. Mol Cell 5:133, 1/00.

G Blobel & R W Wozniak, Structural biology: Proteomics for the pore. Nature 403:835, 2/24/00. News. Details of the yeast nuclear pore complex.

V Zennou et al, HIV-1 genome nuclear import is mediated by a central DNA flap. Cell 101:173, 4/14/00. HIV is a member of a (small) subclass of retroviruses that can replicate in non-dividing cells. To do this, it needs an active system to get the genome to the nucleus (rather than passively entering during M phase). This nuclear import seems to depend on a special intermediate in reverse transcription.

A-B Shyu & M F Wilkinson, The double lives of shuttling mRNA binding proteins. Cell 102(2):135, 7/21/00. Minireview. Discusses mRNA binding proteins in nucleus and cytoplasm.

mRNA localization

R P Brendza et al, A function for kinesin I in the posterior transport of oskar mRNA and staufen protein. Science 289:2120, 9/22/00. mRNA localization within cells is known to be important. Here they show the involvement of a motor protein, which transports cargo along microtubules. Whether the transport system directly binds mRNA, or whether something more complex is happening, is not known. Recall Vale & Milligan (2000; Ch 1 FR) for an introduction to kinesin.

S-B Choi et al, Messenger RNA targeting of rice seed storage proteins to specific ER subdomains. Nature 407:765, 10/12/00. Rice grains contain two major storage proteins, which are stored in different compartments. Here they show that the compartmentalization of the proteins depends on proper location of the mRNAs. One of the mRNA targeting events depends on the presence of an initiation codon, but not on translation. Thus the initiation complex may be involved in targeting.

protein degradation

M Kanemori et al, The ATP-dependent HslVU/ClpQY protease participates in turnover of cell division inhibitor SulA in Escherichia coli. J Bact 181:3674, 6/99. We discussed how GTP binding and hydrolysis can control an activity cycle of a protein (Ch 17 Sect H (Fall 2000)). Another approach is to make and degrade the protein. Of course, this is more practical for proteins with very long cycle times, such as once per generation.

A F Kisselev et al, Proteasome active sites allosterically regulate each other, suggesting a cyclical bite-chew mechanism for protein breakdown. Mol Cell 4:395, 9/99. Interaction of the various proteolytic enzymes within the proteasome.

X H Liang et al, Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402:672, 12/9/99. They suggest that defective protein degradation may be one aspect of cancer development.

M S Brown et al, Regulated intramembrane proteolysis: A control mechanism conserved from bacteria to humans. Cell 100:391, 2/18/00. Review. The paradigm is the role of cholesterol in regulating its own synthesis. The general idea is that the signal causes proteolysis of a membrane-spanning protein, releasing an intracellular fragment which then serves as a TF.

K J Travers et al, Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101(3):249, 4/28/00. How cells respond to misfolded proteins. (This paper was called to my attention by a recent student, whose friend is a co-author.)

D M Lonard et al, The 26S proteasome is required for estrogen receptor-α and coactivator turnover and for efficient estrogen receptor-α transactivation. Molecular Cell 5(6):939, 6/00. An example of regulated proteolysis, as part of a regulation system.

<u>miscellaneous</u> (some not on current topic)

T Hunter, Signaling - 2000 and beyond. Cell 100:113, 1/7/00. Review. Signal transduction pathways - how extracellular signals are converted into gene responses. Another of the Millennium Reviews in this issue.

P Uetz et al, A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature 403:623, 2/10/00. (+ News, Oliver, p 601.) The "two-hybrid assay" is a method for finding interactions between two proteins. The assay is based on a gene regulation system in yeast; the two hybrids being tested effectively become artificial members of the regulation system: gene function occurs only if the two proteins interact, to complete the regulatory signal. Weaver introduces the two hybrid assay in Fig 14.36. Here, Uetz et al systematically examine all yeast proteins for interactions, using a high throughput version of the two hybrid assay. This is an example of "proteome" analysis.

1) T Gura, A silence that speaks volumes. Nature 404:804, 4/20/00. News. 2) B L Bass, Double-stranded RNA as a template for gene silencing. Cell 101(3):235, 4/28/00. Minireview. A story about a confusing phenomenon that has received a lot of attention recently. A simple version of the story… a virus infects a cell, viral RNA is made, for one reason or another some of that RNA is DS, the cell mounts a defense against that RNA sequence. The defense, though it is stimulated by DS RNA, will inactivate mRNA (SS) of the appropriate sequence. Thus the production of DS RNA causes genes to be shut off. The phenomenon is known variously (by workers in different fields) as post transcriptional gene silencing (PTGS), co-suppression, RNA interference, and quelling.

<u>M. Computer resources</u>

InBase, the Intein Database. History and general information on protein splicing, Sect H. The site is maintained by New England BioLabs.

http://www.neb.com/inteins/intein_intro.html

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8/6/01