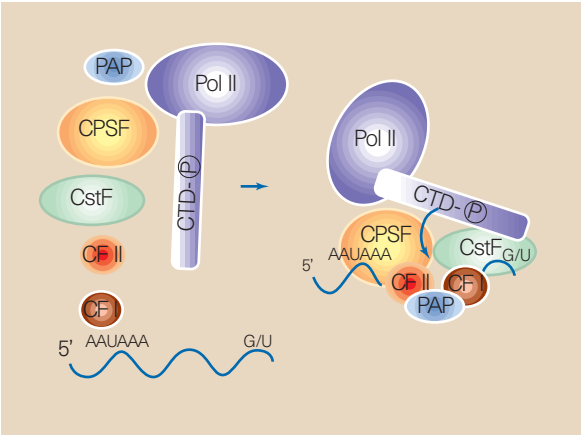

Figure 2 **Model for activation of 3′ processing by the pol II carboxy-terminal domain (CTD). Hirose and Manley[3] have shown that the CTD is part of the protein complex that carries out cleavage and polyadenylation to produce a mature messenger RNA transcript. CPSF, cleavage/polyadenylation specificity factor; CstF, cleavage-stimulation factor; CFI, cleavage factor I; CFII, cleavage factor II; PAP, poly(A)polymerase.**

maturation of the primary transcript[9].

Hirose and Manley provide an insight into how the mRNA factory operates. They followed up the curious observation that, in a purified system, cleavage at the poly(A) site is stimulated by creatine phosphate and by phosphoamino acids. They reasoned that these compounds may mimic a phosphoprotein that allosterically activates the cleavage reaction. An attractive candidate phosphoprotein is pol II — the CTD undergoes a cycle of hyperphosphorylation and dephosphorylation (mainly at residues two and five of the YSPTSPS repeat[10]) as pol II cycles through transcriptional initiation, elongation and termination. Sure enough, the authors found that phosphorylated pol II and recombinant CTD stimulate cleavage *in vitro*. Unexpectedly, however, the unphosphorylated CTD was also effective.

These experiments show that the CTD stimulates 3′ processing in the absence of transcription, although it is not clear whether the CTD is acting in the same way as creatine phosphate. Hirose and Manley also found that the cleavage reaction was inhibited when they immunodepleted pol II from a crude nuclear extract, after dissociating poly(A) factors from the CTD with high salt. Moreover, they could restore cleavage activity by adding back pure pol II. In other words, the CTD stimulates cleavage in a crude extract, as well as in a purified system.

But how does the CTD stimulate cleavage in the absence of transcription? Does it promote the formation of a stable complex (within which cleavage can then occur), or does it contact polyadenylation factors only fleetingly, triggering cleavage? Assembly of a complex — which might comprise CstF, CPSF, CFI, CFII and PAP, together with the RNA (Fig. 2) — may be rate limiting both *in vivo* and *in vitro*. The CTD could accelerate assembly of the complex by acting as a scaffold that positions the proteins optimally, all in the same place at the same time. A scaffold might even impose an order of assembly of the polyadenylation machine.

Hirose and Manley suggest that the CTD is not just a passive scaffold but an active participant in the cleavage reaction. According to this model, which is more consistent with the effect of creatine phosphate, the CTD behaves as a cofactor or allosteric activator. One possible target for allosteric activation is CstF, which binds the CTD *in vitro*[4]. The importance of this interaction for scaffolding or allosteric activation could be tested using CTD mutants that do not bind CstF.

It is now clear that pol II stimulates 3′ processing, both *in vivo* and *in vitro*, through the CTD. Conversely, poly(A) factors may affect pol II by influencing the decision to terminate or elongate the RNA chain. Termination depends on transcription of the poly(A) signal[11], poly(A) factors[12] and the CTD (ref. 4). Signalling from the polyadenylation machine to the polymerase may ensure that the chain is terminated only after pol II has reached the end of a gene. So, a two-way line of communication between pol II and the 3′ processing factors may be built into the mRNA factory.

Working out how the machines in the mRNA factory interact with one another is a big challenge. Hirose and Manley[3] have provided the first *in vitro* system to study one aspect of the complex communication system that integrates different nuclear processes through protein–protein interactions. We can look forward to the development of more *in vitro* systems, which will reveal the details of how the CTD affects splicing and capping as well as polyadenylation. □

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Daedalus

Zero-tolerance policing

Millions of potentially lethal cell mutations occur in each of us every day. We notice nothing; the immune system arrests and executes the offenders instantly. But with advancing age, its vigilance slackens. Mutations start to go unchecked; and we succumb to the melancholy neoplastic or degenerative diseases of old age.

So Daedalus is extending his scheme of last week for priming the immune system against all normal biological proteins. He now wants to prime it against abnormal proteins as well — in particular, the abnormal ones expressed on the surfaces of malfunctioning human cells. DREADCO volunteers are now providing small biopsy tissue samples of blood, lung, gut, and so on, to be cultured in the usual way. The cultures will then be attacked with mutagens, ultraviolet light, X-rays, and such, to induce as many mutations in them as possible. The battered and misfiring cells will express all sorts of crazy and altered proteins on their surfaces. These will be extracted, and injected back into the volunteer from whom the cells came. This 'auto-immunization' will prime his immune system against such cell mutations. Should a cell in his gut or liver start to go crazy in one of those pre-identified ways, the immune system will spot the abnormality at once. It will stamp on the rogue cell instantly.

Sadly, auto-immunization can never be a perfect defence. There are too many different body tissues, each with too many ways of going wrong, for any vaccine to contain all the proteins expressed by all these errors. But not all errors are equally likely, or equally deadly if they do occur. The human genome can suffer about 10 billion possible single-site mutations; yet there are only some 30,000 recognized illnesses of all kinds. The deadly diseases are those in which a mutated cell starts to multiply, or to trigger similar changes in its neighbours. So Daedalus will tune his treatment to his customers. If all your ancestors died of liver cancers, you'll include some liver cells in your tissue sample; if Dr Alzheimer stalks your family tree, you'll make sure that brain cells are well represented in it. And the mutagenic regime itself will be chosen to provoke the most appropriate type of cell damage. The resulting immunization will be an excellent defence against the disease of your fears. Sadly, it won't make you immortal. But you'll last much longer, and finally die of something you weren't expecting.

David Jones