mosomes were much less compact. In addition, it was observed that CP60, a protein that shuttles between centrosomes and an END-like compartment[9], co-localizes with EAST and is inappropriately localized in mutant *east* larvae. Together, these data indicate that EAST may be a component of an expandable extrachromsomal matrix, the assembly of which may be dependent in part upon the EAST protein.

Although the studies using heat-shocked larvae and *east* mutants suggest that EAST is necessary for END regulation, they do not provide any information as to whether increased EAST expression is sufficient for expansion of this compartment. To explore this possibility, Wasser and Chia induced specific overexpression of EAST in salivary glands using the GAL4 system. Interestingly, induction of massive EAST overexpression in salivary glands resulted in a significant decrease in salivary-gland length and a high level of variability in the DNA content of polytene chromosomes, implying an effect on DNA replication. In addition, overexpression of EAST alone, in the absence of heat shock, induced morphological changes in the nuclei that were very similar to those observed in heat-shocked embryos, and long-term overexpression of EAST resulted in an END that extended well beyond chromosomal boundaries. Although it is possible that EAST overexpression could have induced the synthesis or assembly of other proteins, the simplest interpretation of these experiments is that the EAST protein alone is sufficient to induce separation of polytene chromosomes and an expansion of the END.

Actin is a minor constituent of nuclei, although its nuclear function is unknown[3]. Wasser and Chia made the surprising finding that nuclei containing high levels of EAST accumulate increased levels of actin. A striking demonstration of this fact is seen in Figure 6 of their paper, which shows the differential accumulation of nuclear actin in wild-type larvae and in larvae overexpressing EAST. Perhaps the most puzzling observation is that this nuclear actin does not react with phalloidin and is, therefore, probably unpolymerized G-actin, although it may also be in a novel, polymerized state that is unable to bind to phalloidin. These results indicate that expansion of the END by overexpression of EAST may either cause G actin to migrate to the nucleus from the cytoplasm, or may effect an overall increase in actin synthesis, leading to the accumulation of actin in the nucleus.

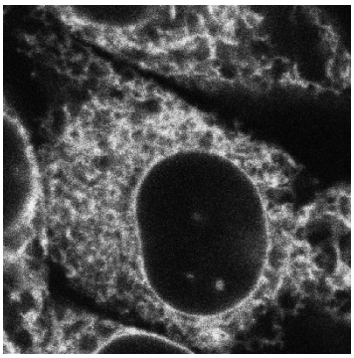
Of course, it was important to determine whether regulation of the END by EAST is specific to salivary-gland nuclei. Showing a similar function for EAST in non-polytene diploid nuclei proved substantially more troublesome, however, because of the compact nature of the chromosomes. To address this question, Wasser and Chia overexpressed EAST in germ cells, using a

Origins of antigenic peptides

Peptides derived from cellular proteins are continuously exposed at the cell surface, in association with MHC class I molecules. Recognition of these MHC–peptide complexes by circulating lymphocytes is critical to the immune system's tolerance of self-derived antigenic peptides, and to the onset of immune reactions against exogenous peptide antigens, such as viral antigens. Antigenic peptides were thought to be derived from the proteolytic degradation of a fraction of total cellular proteins. However, the results of two studies recently published in *Nature* (Reits *et al. Nature* **404,** 774–778, 2000; Schubert *et al. Nature* **404,** 770–774, 2000) indicate that the generation of antigenic peptides may instead depend upon continuous protein synthesis, the antigens themselves being created through the degradation of up to 30% of these newly synthesized proteins. Although apparently wasteful, this system would ensure rapid, efficient presentation of antigens to the immune system, regardless of the half lives of individual proteins.

The transporters associated with antigen processing (TAPs) allow antigenic peptides that have been generated in the cytosol to be transported into the lumen of the endoplasmic reticulum (ER), where they can form complexes with MHC class I molecules. These complexes are, in turn, transported to the plasma membrane. Reits *et al.* investigated the relationship between the motility of a TAP tagged with green fluorescent protein (TAP–GFP) and its activity, and found that its motility decreases in situations in which peptide translocation is blocked (such as in the presence of peptides with very large side chains). The picture shows the subcellular location of TAP–GFP in living Mel JuSo cells, melanoma cells used as antigen presenters.

Thus, measurements of diffusion can be used to visualize conformational changes, a finding that has potentially broad applications. Reits *et al.* used the motility of TAP–GFP as a readout for intracellular peptides *in vivo*, and found that one-third of all cellular TAP proteins are normally busy translocating peptides; this proportion increases to 100% during an acute influenza infection. Continuous protein translation seems to be the source of substrates for TAP, as TAP–GFP motility was blocked by inhibitors



of protein synthesis, such as cycloheximide. This finding supports the idea that antigenic peptides are derived from 'freshly made' proteins. TAP–GFP motility was also correlated with the transport of MHC class I molecules from the ER to the plasma membrane, a result that was anticipated, as MHC class I peptides can only translocate to the plasma membrane once they are complexed with an antigenic peptide.

Defective ribosomal products (DRiPs) are polypeptides that fail to fold properly as a result of errors in translation or of defective post-translational modifications. They have previously been suggested to be a source of antigenic peptides. Schubert *et al.* monitored the recovery of protein contents after transiently labelling newly synthesized proteins, in the presence or absence of protease inhibitors and of inhibitors of protein synthesis. They were thus able to show that up to 30% of newly synthesized proteins are DRiPs, that some of them have indeed been targeted for proteolytic degradation (by conjugation with ubiquitin), and that some of them are viral antigens (as shown by experiments using virus-transfected cells).

Overall, the results from these two studies form a body of evidence indicating that protein synthesis may be the principal means by which antigenic peptides are generated.

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