eage exhibits an overall structural-functional phenotype, but has no completely specific marker. Consequently, there is no in vitro or in vivo model of M cell development available for study. Kernéis *et al.* (*1*) now lead us forward on two fronts: They identify a system in which enterocytes can be induced to switch to an M cell phenotype; and they demonstrate that the information available for this phenotype switch is provided by lymphocytes derived from the Peyer's patches that underlie M cells. The next step will be the identification of the triggering molecule or molecules, which could be used to transiently augment mucosal antigen uptake, an ability that could have a major impact on methods and efficiency of oral vaccination.

Although M cells are committed samplers of lumenal antigen at mucosal surfaces, other pathways for transepithelial delivery of ingested antigen exist as well. Enterocytes normally constitute most of the surface area of the intestine, and it is possible that antigen is shuttled directly across (transcellular) or between (paracellular) this major cell population (see the figure). For example, transient, reversible increases in tight junction permeability to lumenal peptides occur naturally as a consequence of activation of certain apical membrane transport systems (*3*). For example, enhanced peptide permeability of the paracellular pathway by activation of an apical glucose transporter can successfully enhance immune responsiveness to specific lumenal antigens in a model of mast cell–mediated mucosal anaphylaxis (*4*). Other forms of short-term perturbation of the tight junction barrier, for example, by a cholera-derived toxin (ZOT), are likewise capable of enhancing delivery of peptides by way of the paracellular pathway (*5*).

Antigen movement across the enterocyte may also be a regulated event. Using cholera toxin as a model by which movement of an apically bound protein can be traced biochemically, Lencer *et al.* have demonstrated that model enterocytes are capable of direct transcytosis of apically bound cholera toxin B subunit (*6*). In addition, this B subunit, which directs its own transcytosis, is a potent adjuvant for orally delivered antigens (*7*). Indeed, under certain conditions, enterocytes themselves can directly present antigen (*8*). Together, these observations suggest that delivery of oral vaccines might also be enhanced by harnessing the transcellular pathway of the major enterocyte population for antigen delivery and perhaps even initial antigen processing.

A key consideration concerning antigen delivery either across enterocytes converted to the M cell phenotype, or by the paracellular or transcellular routes of unmodified enterocytes, is the immunologic microenvironment of the immediate subepithelial space (see the figure). It is doubtful that induction of new M cells alone, without parallel induction of underlying lymphoid follicles, would have the same functional consequences for antigen delivery as would a normal M cell–lymphoid follicle organization. Additionally, because intestinal immune responses may be cellular or secretory and can result in both inflammation and tolerance, consideration of the underlying immunological microenvironment to which an antigen is delivered will be critical. For example, transgenic animals in which the junctions of enterocytes have been disrupted (by expression of a targeted, dominant-negative mutation of the critical junctional organizing protein E-cadherin) develop a morphologically detectable cellular immune response when junctions in both the superficial (villus) and deep (crypt) mucosa are affected (likely permitting paracellular leak of antigen throughout the mucosa) (*9*). In contrast, similar perturbations restricted to the superficial mucosa display no comparable induction of an immune response. These studies imply that exposure to lumenal antigen may have markedly different consequences depending on the mucosal subcompartment in which exposure takes place and emphasize the importance of the subepithelial microenvironment in determining immunological responses.

Cytokines and other soluble or cell surface signals can drastically modify the function of enterocytes, as well as the expression of enterocyte surface molecules thought to be integral to epithelial–immune cell interactions (*10*). If the trigger for the enterocyte to M cell conversion shown by Kernéis *et al.* is a cytokine, it may turn out that lymphocyte-derived mediators alone can redirect vesicular trafficking pathways in epithelial cells, potentially providing another way to improve the efficiency of oral vaccination. Strategies that expand this efficiency enough to allow bulk movement of antigen may follow, a feature that would also permit improved oral drug delivery.

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MOLECULAR BIOLOGY

Telomerase and Retrotransposons: Which Came First?

Thomas H. Eickbush

Evolution is opportunistic. New cellular mechanisms can evolve from any genetic material available within a cell. This adaptability means that self-replicating genetic elements, such as transposable elements or viruses (cellular parasites), could be recruited for important cellular functions. But this opportunism could work both ways. A gene that supplies a cellular function could become a parasite, if given the ability to self-replicate. An important key to our understanding of which scenario applies to telomeres—specialized structures at the ends of chromosomes—is provided on page 955 of this issue (*1*) and in a previous issue of *Science* (*2*). Because conventional DNA polymerases cannot complete the synthesis of both strands of a blunt-ended DNA template, early eukaryotes adopted the telomere as a mechanism to stably maintain the ends of linear chromosomes. The new reports provide a clear connection between telomerases, the enzymes that synthesize telomeres, and retrotransposons, small elements of DNA that can autonomously move from one part of the genome to another.

Eukaryotic telomeres are composed of tandem arrays of short nucleotide sequences (*3*). The probable mechanism of telomere sequence addition was first revealed by identification of the RNA subunit of telomerase and the demonstration that this RNA provides the template for nucleotide addition (*4*). A short region of the RNA subunit is repeatedly copied with the 3′ hydroxyl at the DNA terminus as a primer. Because the puta-

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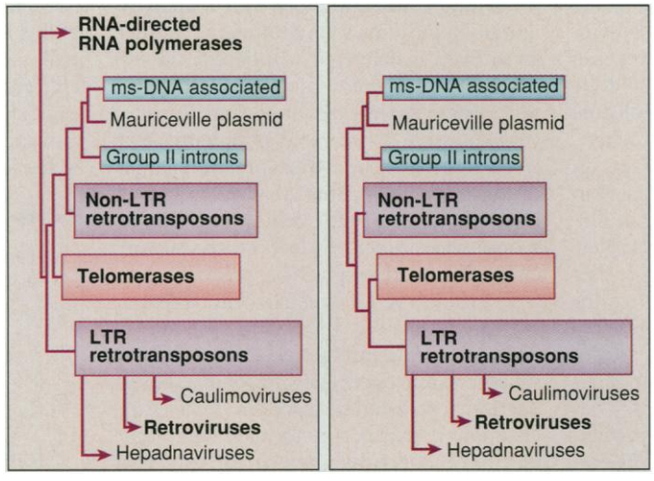
tive polymerase for telomere sequence addition uses an RNA template, it was postulated that this catalytic component could be similar to the reverse transcriptases encoded by retroviruses and retrotransposable elements. In a beautiful series of experiments that used a direct biochemical approach in *Euplotes aediculatus* and a genetic approach in *Saccharomyces cerevisiae*, the first telomerase catalytic subunits were identified (*2*). The *S. cerevisiae* protein was also implicated as a catalytic subunit in an independent study (*5*). By sequence homology this subunit has now also been identified in *Schizosaccharomyces pombe* and in humans, suggesting the universality of this subunit and the mechanism of telomere addition (*1*).

Sequence comparison of these telomerase catalytic subunits revealed that they do indeed contain the conserved domains common to all known reverse transcriptases (*1*, *6*). By molecular phylogenetic analysis, these telomerase sequences fit snugly within a phylogenetic tree of all known reverse transcriptases (see the figure). The major branch on which the telomerases reside contains the eukaryotic retrotransposable elements without long-terminal repeats (known as the non-LTR retrotransposons), group II introns, Mauriceville plasmid of mitochondria, and the reverse transcriptase associated with multicopy single-stranded DNA (ms-DNA) of bacteria. All members of this non-LTR or prokaryotic branch of the tree have retrotransposition mechanisms that differ radically from those used by the LTR-retrotransposons and retroviruses, which are located on the other major branch of the tree. Consistent with their phylogenetic location, the critical step of telomere addition is strikingly similar to the retrotransposition mechanism used by the non-LTR retrotransposons and the group II introns. Although non-LTR retrotransposons do not in general insert at the ends of chromosomes, they use an encoded endonuclease that cleaves within chromosomal DNA. This newly generated DNA end is then used as the primer for reverse transcription so that the cDNA is polymerized directly onto the target site (*7*). This process has been termed target-primed reverse transcription. Group II introns use a variation of this mechanism, in which the RNA subunit is also used as a catalyst in the endonuclease cleavage, but the target-primed reverse transcription step is the same (*8*).

Thus telomerases and non-LTR retrotransposons are related by both the similarity of their catalytic mechanisms, in which the 3′ hydroxyl group of a DNA end is used to prime reverse transcription, and the phylogenetic relation of their sequences. This relation is further strengthened by the remarkable instance in which non-LTR retrotransposons have apparently replaced telomerase for telomere addition. *Drosophila melanogaster* does not contain typical telomerase repeats, but maintains its telomeres as a result of the non-LTR retrotransposons TART and HeT-A, which target the ends of chromosomes (*9*).



A phylogenetic tree of retroelements. The tree in the left panel has been rooted by using RNA-directed RNA polymerases (*1*). The tree in the right panel has the same topology, but the RNA-directed RNA polymerase sequences are removed and the prokaryotic-mitochondrial retroelements root the eukaryotic retroelements. The length of each box corresponds to the divergence within that group. The amino acid sequences of the seven domains common to all reverse transcriptases were used to generate the tree (*6*). Arrows at the bottom indicate the three independent origins of viruses from LTR retrotransposons.

Non-LTR retrotransposons and telomerases appear evolutionarily related, but which came first in early eukaryotes? There are two approaches to rooting the evolutionary tree of reverse transcriptase sequences (*6*). The first would be to use another polymerase sequence as the ancestral outgroup. Assuming that our current DNA world evolved from an RNA world, RNA-directed DNA polymerases would most likely have evolved from an RNA-directed RNA polymerase. Consistent with this assumption, RNA-directed RNA polymerases have greatest sequence similarity to reverse transcriptases (*10*). If these RNA polymerases root the tree (left panel of the figure), the structure of the first retroelement is unclear. However in this rooting, telomerases preceded the non-LTR retrotransposons, supporting a scenario in which a cellular gene in early eukaryotes gave rise to a parasite. Two arguments shake this rooting of the tree. First, the sequence similarity between RNA and DNA polymerases is sufficiently low that there is considerable uncertainty in the location of this branch (*6*). Second, this rooting implies that the mitochondrial and bacterial reverse transcriptases evolved from eukaryotic elements. Convincing arguments can be made to suggest that the prokaryotic-mitochondrial elements are more ancient than eukaryotic elements (*11*).

An alternative rooting of the reverse transcriptase tree, which does not require a transfer of sequences from eukaryotes to prokaryotes, simply uses the prokaryotic retroelements to root the tree of eukaryotic reverse transcriptases (right panel of the figure). This rooting implies that non-LTR retrotransposons gave rise to the telomerases. Thus in early eukaryotes a parasite was recruited by the cell to supply an important function. The *D. melanogaster* case can be viewed as a recent example of a similar event. In order to support this origin of telomerase, it would be necessary to show that non-LTR retrotransposons date back to the origin of eukaryotes. Resolving the ultimate origin of reverse transcriptases will be difficult because of the low level of sequence identity among polymerases. In the meantime, the discovery that the catalytic subunit of telomerase is a reverse transcriptase fuels the argument that retrotransposons have had major influences in shaping eukaryotic genomes.

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