deleterious effects upon suckling animals.

There are other mutations in the inbred mouse which involve metallo-ion transport. The *pallid* (*pa*) mutant involves manganese transport to the inner ear (*11*), *quaking* (*qk*) is associated with an inability to concentrate copper in the brain (*12*), and *mottled* (*mo*) produces a condition similar to the human disease, *Menke's kinky hair*, in which absorption of intestinal copper is reduced (*13*). Also, mice with *sex-linked anemia* (*sla*) exhibit a reduced ability to absorb intestinal iron (*14*). A mutation, designated *toxic milk* (*tx*), has been reported to lead to an increased accumulation of tin in the milk (*15*).

A defect in the metabolism of zinc in the milk has also been shown to underlie the inherited human disease, *acrodermatitis enteropathica* (AE) (*16*). This recessive autosomal trait, characterized by all symptoms of dietary zinc deficiency, is expressed at the onset of weaning from human milk to bovine milk or to other foodstuffs and is completely overcome by dietary zinc supplementation (*16*). Since zinc levels of bovine milk are generally higher than those of human milk, the manifestation of AE suggested to Eckhert and co-workers (*17*) that zinc of bovine milk is present in a form different from that in human milk. This suggestion was supported by their observations that most of the zinc in bovine milk was associated with high-molecular weight fractions, whereas zinc in human milk was associated with low-molecular weight fractions. Although the *lethal milk* syndrome is not identical to AE, it may be useful for the eventual understanding of AE.

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Assembly of Type C Oncornaviruses: A Model

Abstract. *The salient features of this model for oncornavirus assembly are that uncleaved precursor molecules to the internal virus polypeptides possess specific recognition sites both for viral envelope constituents already inserted in the cell membrane and for the viral RNA. After orderly alignment of these components at the budding site, virus maturation proceeds through specific proteolytic cleavage of the precursor components and association of the resultant molecules into the characteristic type C virion substructures revealed by electron microscopy.*

Analysis of the composition and organization of virion structural proteins has often been useful for constructing a preliminary model for virus assembly (*1*). On the basis of the fine structure of avian and murine type C oncornaviruses, the molecular arrangement of the virion structural components in the particle, as well as the available data concerning the biosynthesis of these components, a model for the assembly of the virus can be proposed. We suggest a rela-
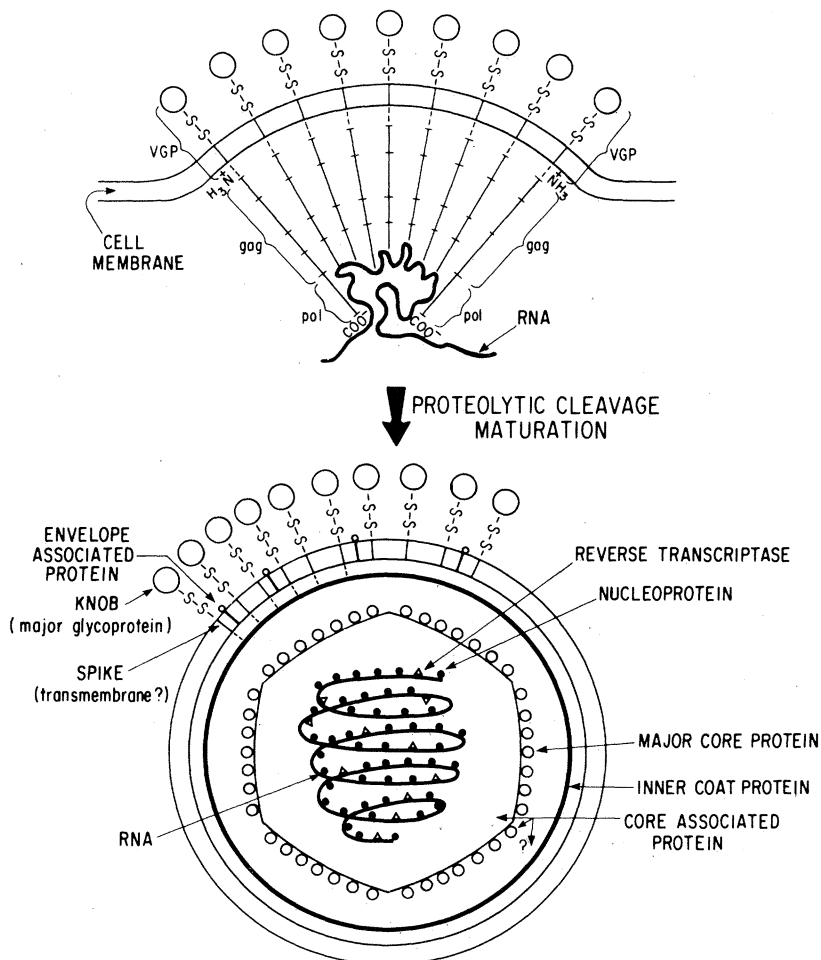

Fig. 1. A model for the assembly of type C oncornaviruses. Table 1 should be consulted for a more detailed designation of the individual murine and avian polypeptides which are indicated schematically here.

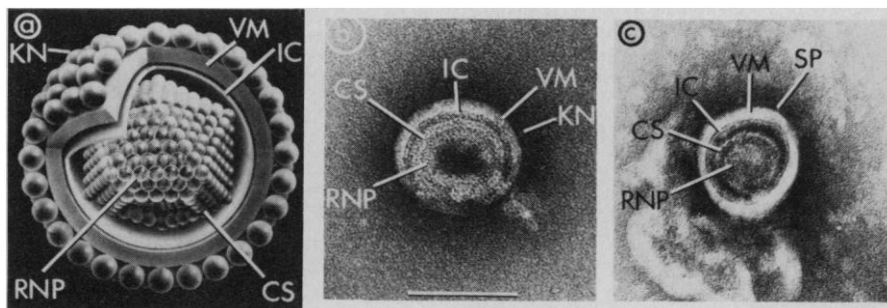0036-8075/78/0113-0183$00.75/0 Copyright © 1978 AAAS

Fig. 2. Morphological features of type C oncornaviruses. (a) Three-dimensional reconstruction of Friend murine leukemia virus (FLV). The cut-away of the icosahedron permits visualization of the ribonucleoprotein. (b) Electron micrograph of FLV after staining with uranyl acetate. The bar represents 100 nm. (c) Electron micrograph of avian myeloblastosis virus performed as in (b). *KN*, knobs; *VM*, viral membrane; *IC*, inner coat; *CS*, core shell; *RNP*, ribonucleoprotein; *SP*, spikes.

tively simple scheme for this process, which includes the following events (see Fig. 1).

1) Complexes consisting of the virus envelope components migrate to the cell surface and are inserted in the membrane at the site of virus budding.

2) Uncleaved precursor molecules to the internal virus polypeptides are then transported to the budding site where one end of the molecule is joined to the virus envelope complex, while the other end associates with the virus RNA.

3) As viral maturation progresses, proteolytic enzymes cleave the precursor molecules at the appropriate sites, generating the individual structural elements of the virus.

4) The respective virion components then associate with themselves or with other molecules (or both) to form the substructures found in the mature virus such as the envelope, inner coat, core-shell, and ribonucleoprotein complex (see Fig. 2).

The morphological features of type C oncornaviruses are exemplified by the model for Friend murine leukemia virus (FLV) depicted in Fig. 2, which is based on fine structure analysis of the particles and their subunits by means of various electron microscopic techniques (2, 3).

The surface of the particle consists of a lipid bilayer (*VM*) derived from the host cell from which project the loosely attached surface knobs (*KN*) (Fig. 2b). Avian type C viruses are arranged similarly, except that the outer knobs are connected to the particle through a readily visible thin spike (*SP*) and thus project outward further from the virion surface (Fig. 2c). Electron microscopic investigations with murine as well as avian type C viruses (2, 3) have revealed the presence of a thin layer, the inner coat (*IC*), just beneath the viral envelope. The envelope and inner coat surround the virus core, which is composed of an outer shell (*CS*) of hexagonally arranged subunits within which lies an internal electron-opaque structure [nucleoid, or more precisely, ribonucleoprotein (*RNP*) complex] consisting of a filamental strand in the form of a spiral.

The localization of the major structural polypeptides and glycoproteins of type C viruses relative to the virus structural subunits discussed above is summarized in Table 1, beginning with the exterior molecules and proceeding inwardly to the center of the particle. The data supporting this scheme come from a number of studies from several laboratories (2, 3).

Considerable information is now available with regard to the genesis of the individual structural elements from their respective precursor molecules. In each virus system, two distinct precursor forms for the major structural components have been described: one for the envelope constituents of the virus (env) and a second for the internal virion polypeptides. The latter precursor polyprotein has been termed "gag" by several investigators to signify its content of group-specific (gs) antigens. However, the antigenicities of the molecules on this precursor are certainly not exclusively group-specific.

Concerning the env precursor, England *et al.* (4) have demonstrated that in avian oncornaviruses a poorly glycosylated precursor (gp90) can be identified, which presumably through disulfide bond formation and proteolytic cleavage produce the viral glycoprotein (VGP) complex (gp85-S-S-gp35) (Fig. 3). In the murine system, an analogous situation seems to occur in which a glycosylated precursor (gp90) is evidently processed in a similar manner to form a gp71-S-S-p15E complex (5–7) (Fig. 3). Murine p15E appears to be further cleaved to give rise to the serologically and biochemically related envelope protein p12E (5–8), which, in contrast to p15E, is not linked by disulfide bonds to gp71 (9). The specific cleavage of the murine env precursor appears to take place prior to or coordinate with its insertion in the membrane (10). This processing may be analogous to the scheme in influenza virus where an HA precursor is cleaved to HA_1-S-S-HA_2; the heavily glycosylated HA_1 component seems to be more exposed at the virion surface, while the hydrophobic HA_2 component is embedded in the lipid bilayer (11). Of considerable interest is that these cleavages impart some of the important biological functions of the respective molecules (12).

Precursor molecules to the internal structural polypeptides of the virus have also been characterized in both systems. In avian viruses this material (pr76) gives rise to the proteins p27, p19, p15, and p12 (13). The analogous components are contained in the corresponding murine precursor (pr70), and include p30, p15, p12, and p10 (14) (see Table 1). It was recently reported that uncleaved gag pre-

Table 1. Structural components of avian and murine oncornaviruses. Localization of structural polypeptides is based on the studies by Bolognesi (3, 34) and Montelaro *et al.* (9).

Virion polypeptide		Structural component	Virion substructure
Avian	Murine		
gp85—S	gp71—S	Knob	
gp35—S	p15E—S	Spike	Envelope
p10	p12E	Envelope associated	
p19	p12	Inner coat*	Inner coat*
p27	p30	Core shell	Core exterior
p15	p15C	Core associated	
p12	p10	Nucleoprotein	Ribonucleoprotein complex
p91(β)	p70(α)	Reverse transcriptase	
p64(α)			

*The assignment of viral phosphoproteins (p19 and p12) to the inner coat does not exclude the possibility that a small number of molecules (< 1 percent) may bind to viral RNA during maturation, as suggested by the studies of Sen and Todaro (36). Moreover, the evidence that murine p12 represents the inner coat is based largely on analogy to the avian system. Murine p15C has properties which make it a likely candidate as well.

cursor molecules were first identified on free polyribosomes and subsequently on membrane bound polyribosomes, presumably near the cell surface (15). Cleavage of the gag precursor appears to take place predominantly in cells actively producing virus (16), with two possible exceptions (17).

A number of studies have attempted to determine if the env and gag precursor molecules arise from a larger precursor form containing both sets of information. For the most part, this has not been found to be the case although molecules larger than either precursor have been identified (5-7). For example, a 200,000-dalton precursor that contains protein sequences related both to the gag polypeptides and virion polymerase (gag-pol) and is present in 20-fold less quantities than pr70 has been detected in Rauscher murine leukemia virus (18). Other studies suggest that env and gag may be coded for by separate messenger RNA molecules which are derived from the virion 35S genome (19) by nucleolytic processing. This would be consistent with the finding of noncoordinate synthesis of these molecules in uninfected cells (20) as well as in transformed nonproducer cells (21).

Pertinent to the mechanism of virus assembly are the bonding interactions between structural components during the budding process. With other enveloped viruses, there appears to be a specific recognition and noncovalent association between certain envelope and internal virion molecules. This interaction may be only temporary and serve to align components at the budding site, but the respective molecules could retain their affinity for one another even in the mature virion. Provocative evidence for such a relationship in avian oncornaviruses is the finding that p19 remains associated with the VGP complex after isolation from the virus by immune precipitation with antiserum to the major glycoprotein (gp85) (22). This associa-

tion is not by disulfide bonds and appears to be noncovalent (9).

For avian viruses, p19 represents the NH2-terminal polypeptide of the gag precursor (14) while the gp35 is the innermost component of VGP after its insertion into the cell membrane (Table 1 and Fig. 3). Furthermore, studies by Rohrschneider et al. (23) emphasize the critical role of avian p19 in virus synthesis since mutants with defective p19 molecules fail to assemble properly. On this basis we suggest that recognition between the interior portion of the transmembrane protein and the NH2-terminal polypeptide of the gag precursor is required for proper virus assembly. Such an arrangement would ensure uniform alignment of the gag (and gag-pol?) precursor molecules and provide optimum positioning for interaction with the incoming viral RNA. Subsequent proteolytic cleavage of the precursor molecules and specific association between the resulting structural components would then produce the substructures that can be identified in mature virus. In accord with this assembly scheme is that the sequence of the structural components from the exterior to the interior of the mature virus corresponds relatively well with their order on the respective precursor molecules (14, 16, 18, 24).

Support for this model for oncornaviruses comes from studies by Cheung et al. (25) where examination of newly formed virions (rapid harvest virus) revealed a series of high-molecular-weight polypeptides that disappeared with time while the major structural proteins increased somewhat in quantity. This would be consistent with the processing of the gag precursor during virus maturation as suggested above. Investigators working with mammalian viruses have reported the presence of nonglycosylated gag precursor molecules, which apparently escaped proteolytic cleavage, in purified virions (5-7, 26). More recently, evidence has been reported that gag

precursor molecules as well as precursor-specific proteases are present in Rauscher leukemia virus, and that appropriate incubation of these particles results in cleavages to yield the characteristic structural components (27). In fact, it appears that virus structural components (avian p15) may be involved in this proteolytic activity (28). Taken together, these observations support the notion that cleavage of the gag precursor occurs at a relatively late stage of virus morphogenesis and that the cleavage process is intimately associated with budding at the plasma membrane. Such a mechanism may explain why gag processing is usually associated with only virus-producing cells (16).

The proposed assembly model for oncornaviruses also predicts an equimolar ratio for the structural components derived from the gag precursor. Although accurate stoichiometric calculations are difficult for the complex oncornaviruses, which always contain a substantial number of partially degraded particles, estimated molar ratios range from about 1 : 1 to 1 : 2 for the various internal components (29). The ratio of gag polypeptides to polymerase molecules in purified virions is about 50 : 1, the same ratio as reported for gag to gag-pol precursors. Moreover it would appear that the virus RNA is not required for virion assembly since murine leukemia virus produced in the presence of actinomycin D lacks 60S to 70S RNA, but contains normal amounts of virion structural polypeptides, including polymerase (30). Taken together, these results suggest that the gag-pol molecule is not a nonspecific readthrough product, but serves to mediate the incorporation of a small number of polymerase molecules into the virion.

Our model further suggests that, in the absence of virus glycoproteins, it is unlikely that virus particle synthesis could even occur. This would appear to be incompatible with studies by Hanafusa and

Fig. 3. A scheme for the biosynthesis of oncornavirus envelope components based on the studies of England et al. (4), Moelling and Hayami (37), Naso et al. (5), and Karshin et al. (7). The cleavage of gp35 to generate p10 is a speculation based on the pathway established for murine viruses where more than 90 percent of p15E is cleaved to p12E. FLV gp71 remains noncovalently bound to p12E; the avian glycoprotein complex evidently does not remain associated with p10 and is presumably released from the virus (9).
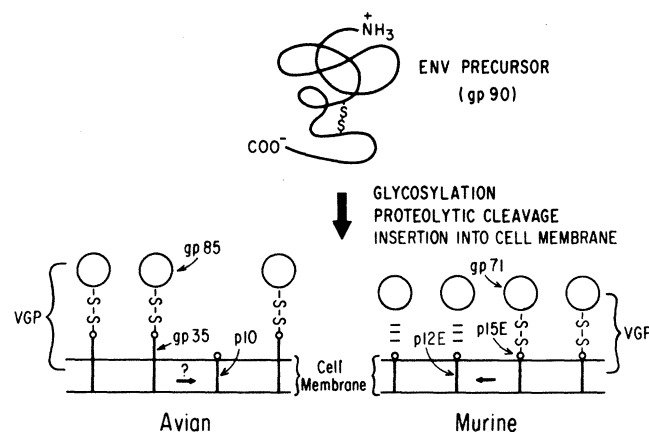
co-workers (*31*) which demonstrated that defective Rous sarcoma virus particles synthesized in chicken factor–negative cells had no detectable gp85 or gp35 by analysis on polyacrylamide gels. However, it could not be excluded that very small amounts of the glycoprotein or a portion of the molecules with markedly different electrophoretic properties, possibly coded by fraction of undeleted envelope genetic material, may have been present. In this context, studies (*32–34*) strongly imply a stringent requirement for recognition by the viral core proteins of homologous envelope components during formation of pseudotypes of vesicular stomatitis and murine leukemia viruses. Interestingly, the studies by Witte and Baltimore (*32*) indicate that it is sufficient for the glycoproteins to be present in very small amounts for the assembly process to occur.

Although we have restricted our discussion to type C oncornaviruses, the recently established (*35*) precursor-product relationship between mouse mammary tumor virus type A particles and mature type B particles suggests that this class of oncornaviruses may assemble by mechanisms similar to those proposed for type C particles.

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Costae of *Tritrichomonas foetus*: Purification and Chemical Composition

Abstract. *The costa is an intracellular organelle common to all trichomonads. Costae from* Tritrichomonas foetus *have been purified by a method which involves lysis of* T. foetus *with the heat-stable hemolysin produced by* Pseudomonas aeruginosa, *followed by differential centrifugation. Analysis of the purified costae demonstrated that the organelle is composed of 95 percent carbohydrate and 5 percent protein. The carbohydrate moiety, probably a polysaccharide, consisted of glucose (95 percent), mannose (0.4 percent), glucosamine (1.4 percent), ribose (0.6 percent), and an unidentified sugar (2.6 percent). The kinetosomal complex was attached to the costa after initial lysis of cells but was separated from the costa during purification.*

Protozoa of the family Trichomonadidae are of particular interest because some members, *Tritrichomonas foetus* and *Trichomonas vaginalis*, are transmitted by a venereal mode to cattle and humans, respectively. The ultrastructure of the organism is complex and has been studied in detail by both light and electron microscopy (*1*). However, none of the observed organelles have been purified so that their chemical composition can be determined, a prerequisite to understanding structural and functional relationships.

We have developed a procedure for purification of the costa, a supportive organelle common to all trichomonads, and have determined its chemical composition. In addition, we have obtained evidence of a structural association among costae, kinetosomes, and flagella.

In our research we employed the parasite of bovines, *Tritrichomonas foetus*.

The culture of *T. foetus* was isolated from an infected bull in a herd located near Baton Rouge, Louisiana. The culture was maintained in NIH thioglycollate broth (Difco Laboratories, Detroit) amended with 5 percent filter-sterilized, heat-inactivated bovine serum. To grow large quantities of cells, liter quantities of the same medium in screw-cap erlenmeyer flasks were inoculated with a 10 percent volume of a mature culture. The cultures were incubated for 18 to 20 hours at 37°C. The cells were harvested by centrifugation and washed twice in 0.85 percent NaCl. For purification of costae, 1.0 ml of packed washed cells was diluted with 3.0 ml of saline, and the cells were lysed by the addition of 800 units of purified heat-stable hemolysin produced by *Pseudomonas aeruginosa* (*2, 3*) in a volume of 1.0 ml. After lysis of the trichomonads, 1.0 mg each of phospholipase C, deoxyribonuclease, and ri-