JOURNAL OF BACTERIOLOGY, May 1983, p. 976–979
0021-9193/83/050976-04$02.00/0

Vol. 154, No. 2

# NOTES

## Assembly of *Rickettsia tsutsugamushi* Progeny in Irradiated L Cells

TATSUO HASE

*Department of Ultrastructural Studies, Division of Pathology, Walter Reed Army Institute of Research, Washington, D.C. 20012*

Received 7 October 1982/Accepted 1 February 1983

In the assembly of *Rickettsia tsutsugamushi* progeny in irradiated L cells, nascent forms first appear as undemarcated foci in the host cell granular cytoplasm, in which electron-lucent filamentous (f) and electron-dense granular (g) areas differentiate. Morphological observations indicated that the assembly involves formation of a filamentous network in the f area, manufacture of rickettsial ribosomes in the g area, and formation of mildly electron-dense fuzzy zones, along which a double membrane assembles.

Rickettsiae, like viruses, are obligatory intracellular parasites. In contrast to viruses, which release their genetic substance into host cells to utilize cellular biosynthetic machinery, rickettsiae are generally believed to maintain their morphological integrity throughout their life cycles and to depend on host cells for nutritional needs (3). We have recently reported that in the infection of irradiated L cells with *Rickettsia tsutsugamushi*, progeny rickettsiae are assembled within the amorphous granular cytoplasm in a manner analogous to that of viruses (1). Although certain aspects of this unique life cycle need to be clarified, I have elucidated by electron microscopy the assembly process within the host cell cytoplasm.

Mycoplasm-free L929 cells were incubated at 37°C, subjected to 3,000-rad irradiation in a $^{60}Co$ gamma cell, and 24 h later, inoculated with the Gilliam strain of *R. tsutsugamushi* as described elsewhere (1). The infected cells were then harvested daily for up to 7 days and processed for electron microscopy. (The chronological study of the rickettsial growth pattern in host cells is reported elsewhere [1]).

The earliest forms of rickettsia appear in the granular cytoplasm of the infected cell 3 days after infection and are recognizable as poorly demarcated foci where electron-lucent filamentous (f) and electron-dense granular (g) areas differentiate (Fig. 1A). These nascent forms lack limiting membranes; their peripheries either merge with the surrounding granular cytoplasm or are separated from the latter by narrow, electron-lucent spaces. A mildly electron-dense, fuzzy material also accumulates on the peripheries. As the nascent rickettsiae "mature," they assume a round or ovoid shape (Fig. 1B). Their surfaces are covered by zones of fuzzy material or by double membrane segments and membraneless areas (R3 and R4 in Fig. 1B). Faint membranous components, possibly representing incipient membranes, are seen in the fuzzy zones (R3 in Fig. 1B). The rickettsial double membranes are assembled along the fuzzy zones (R2 in Fig. 1A; R3 and R4 in Fig. 1B).

In an assembling rickettsia, the f area consists of a filamentous network in an electron-lucent background; the network seems to become more distinct as the rickettsia matures (R1 to R4 in Fig. 1). The g area contains ribosomes (R1 and R2 in Fig. 1A). Although ribosome size cannot be accurately measured in electron micrographs, ribosomes in the g area appear to be somewhat smaller than those in the surrounding granular cytoplasm and, compared with the loose and often linear arrangement of cytoplasmic ribosomes, are arranged in compact aggregates with other proteinaceous granules. Between the rickettsial g area and the host granular cytoplasm, the fuzzy material accumulates and forms a demarcating zone. In more mature rickettsiae, ribosomes in the g areas are typical of those of procaryotes (R3 and R4 in Fig. 1B).

A crucial finding that supports the occurrence of the rickettsial assembly within the amorphous granular cytoplasm of host cells is the total or partial absence of limiting membranes in a majority of progeny rickettsiae. This has been confirmed through geometric analyses of the

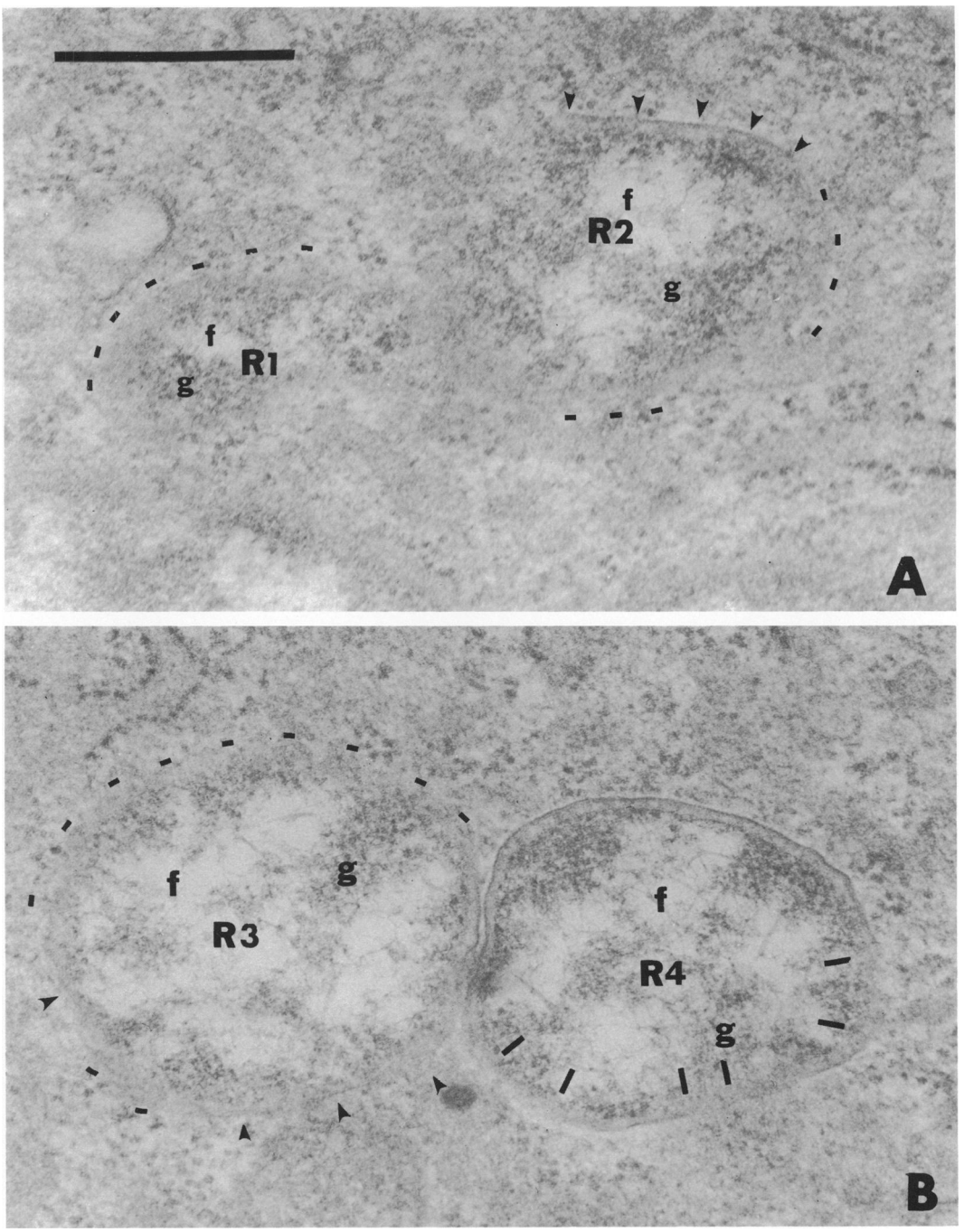


FIG. 1. Rickettsial assembly in the granular cytoplasm of infected L cells. Bar represents 500 nm. (A) R1 and R2, nascent rickettsiae seen as foci where filamentous (f) and granular (g) areas separate. Their borders with the surrounding cytoplasm are vaguely recognizable (dotted lines). R2 shows a segment of outer membrane (arrows). (B) R3, a rickettsia showing a large body covered with a fuzzy zone (dotted line). Faint membranous components are seen in places along the zone (arrows). A short segment of double membrane, consisting of cell wall and cytoplasmic membrane, appears on the surface facing R4. R4, a rickettsia with a well-developed body covered with an almost complete double membrane. The markers include the segments of the rickettsial periphery where the double membrane is still lacking.

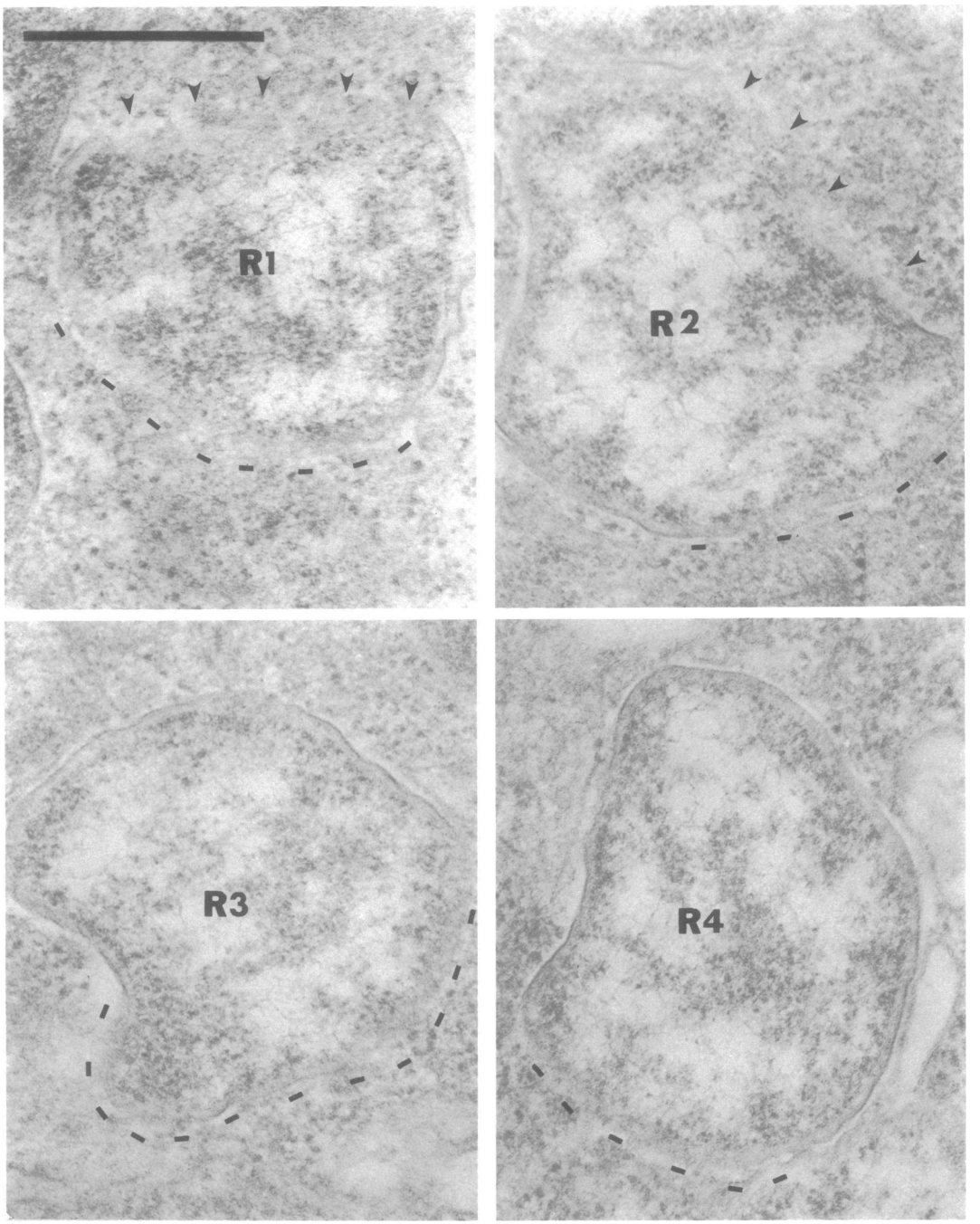


FIG. 2. Membrane coverage of progeny rickettsiae (R1 through R4). Bar represents 500 nm. Arrows in R1 and R2 point to the accumulations of fuzzy material at the membraneless periphery. Dotted lines in R1 through R4 cover the fuzzy zones along which incipient membranes are formed.

circumferences of sectioned rickettsiae and by examination of electron micrographs at various degrees of tilt (1). For example, for the presumed ovoid shapes of R3 and R4 in Fig. 1B, it is inconceivable that the distribution of surface membranes is fully accounted for by an out-of-phase phenomenon. Examination of the progeny periphery reveals that the membraneless parts

are usually covered by zones of fuzzy material or by faint membranous components; all gradations of membrane assembly occur along the fuzzy zones (Fig. 2). The biochemical nature of the fuzzy material is not yet known. The present study reveals an intimate topographical relationship between the fuzzy material and the rickettsial membrane. It is possible that the fuzzy material represents rickettsial membrane lipoprotein which is synthesized in the differentiating interior and secreted to the periphery for membrane assembly.

R1, R2, R3, and R4 in Fig. 1A and B appear by light microscopy to be coccobacillary forms. However the paired rickettsiae have markedly different ultrastructure, suggesting that they are not binary fission pairs; they are, in fact, progeny rickettsiae in different stages of assembly. Undoubtedly, rickettsial assembly occurs in the presence of the rickettsial genetic substance within the host granular cytoplasm. The initial differentiation of small portions of the granular cytoplasm into nascent rickettsial f and g areas may, in fact, reflect arrangement of rickettsial DNA in a filamentous network in the f area and manufacture of rickettsial ribosomes and proteins in the g area. Apparently, discrete rickettsial bodies are then formed, and limiting membranes are assembled on their surfaces. The above suggests that the rickettsial biosynthetic machinery develops concommitantly with differentiation of the cells themselves. It has been demonstrated that mature rickettsiae do have specific limiting membranes (2); accordingly, rickettsial membrane lipoprotein is probably synthesized specifically by the rickettsial biosynthetic machine which develops within the assembling rickettsia. This finding may account for membrane formation lagging behind body formation during progeny rickettsia assembly.

Previously it was thought that viruses are the only infectious particles that assemble in host cells. The results presented here provide evidence for the intracellular assembly of a procaryote.

## LITERATURE CITED

1. **Hase, T.** 1983. Growth pattern of *Rickettsia tsutsugamushi* in irradiated L cells. J. Bacteriol. **154**:879–892.
2. **Silverman, D. J., and C. L. Wisseman, Jr.** 1978. Comparative ultrastructural study on the cell envelopes of *Rickettsia prowazekii*, *Rickettsia rickettsii*, and *Rickettsia tsutsugamushi*. Infect. Immun. **21**:1020–1023.
3. **Weiss, E.** 1973. Growth and physiology of rickettsiae. Bacteriol. Rev. **37**:259–283.