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Direct observation of apolipoprotein B refolding at single molecule level by ultra sensitive fluorescence microscopy and solution transmission electron microscopy CHIA-CHING CHANG, HSUEH-LIANG CHU, HSING-YUAN LEE, National Chiao Tung University, TSAI-MU CHENG, Taipei Medical University, GONG-SHEN CHEN, Mackay Memorial Hospital, FU-RONG CHEN, National Tsing Hua University — Apolipoprotein (apo) B is the only protein of low-density lipoprotein (LDL). The huge size and extreme hydrophobicity of apoB make examination of its lipidation process an experimental challenge. In this study, we showed that apoB lipidation and its intermediates could be observed at single molecule level by an on-path folding process. When carboxyl-terminal-truncated mutants apoB-29 and apoB-48, representing the amino-terminal 29% and 48%, respectively, of the full-length apoB (apoB-100), were used for comparison, we observed that the refolded apoB-100 resembled both native LDL and VLDL precursors. Thus the process of lipidation recapitulates that of pre-VLDL assembly, *in vitro*. These results suggest that the assembly of mature VLDL requires involvement of factors in addition to apoB-100 and lipids. Using solution transmission electron microscopy (TEM), we were able to detect incorporation of hydrophobic super-paramagnetic iron oxide nanoparticles into apoB-100 particles at the initial, but not final, stage of refolding. The current study thus demonstrates that VLDL assembly can be monitored at single molecule level, too.

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