定量的電子顕微鏡法による脂肪滴成長過程の検討

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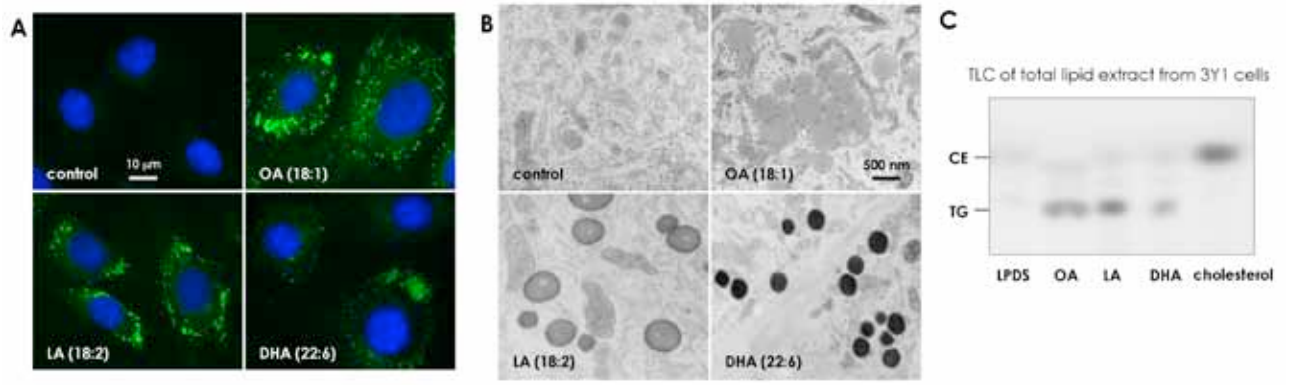
要旨：
脂肪滴はほとんど細胞に見られ、細胞の生存環境に応じて形成、成長、退縮する動的なオルガネラである。脂肪滴のコアとなるトリグリセリド、コレステロールエステルなどは小胞体膜で合成されるが、脂肪滴の形成、成長のメカニズムは明らかでない。我々は脂肪滴の成長過程を可視化し、定量的に検索するための新たな方法を開発し、おもに線維芽細胞での脂肪滴成長メカニズムについて検討した。

この方法は脂肪酸の不飽和度に応じて、トリグリセリドへの四酸化オスミウムの結合性が変化し、その結果、電顕で観察される脂肪滴の電子密度が変わることを利用する。あらかじめ細胞にオレイン酸（ＯＡ；炭素数１８、不飽和結合１個）あるいはドコサヘキサエン酸（ＤＨＡ；炭素数２２、不飽和結合６個）を取りこませると、それぞれ電子密度の低い脂肪滴と高い脂肪滴ができる。その後、最初とは別の脂肪酸を細胞に与えると、脂肪滴の電子密度は経時的に変化する。グリッドと細胞外空間の２点を標準点とすることにより、個々の脂肪滴の電子密度を数値化し，定量的に比較することができる。

この方法でラット 3Y1 線維芽細胞を検索すると、ＯＡ→ＤＨＡという順番に投与した場合の脂肪滴はほぼ一様に低電子密度から高電子密度へという変化を示し、ＤＨＡだけでできる高電子密度の脂肪滴が独立に観察されることはなかった。ＤＨＡ→ＯＡという順番に投与した場合も同様であり、またノコダゾール処理で微小管を脱重合させ、脂肪滴の運動を極少にした細胞でも電子密度変化の一様性は保たれた。これらの結果は、線維芽細胞の脂肪滴の成長が、個々の脂肪滴局所におけるトリグリセリド産生・供給によって起こると考えると説明できる。一方，3T3-L1 分化脂肪細胞においては、脂肪滴の電子密度の変化は一様に起こらず、直径によって異なっていた。このことは線維芽細胞と脂肪細胞では脂肪滴の成長過程に差があることを示唆する。

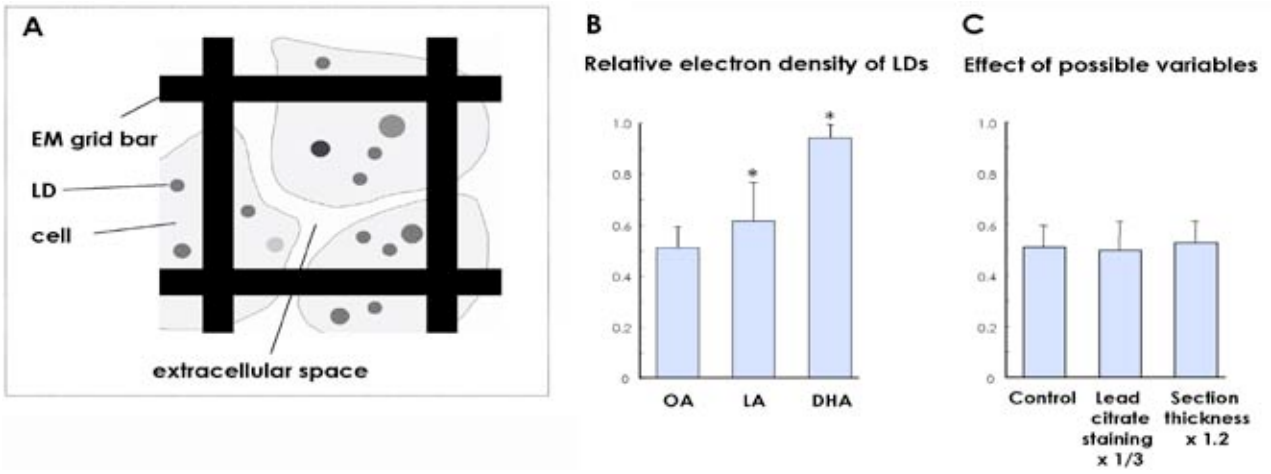
Fig. 1.　De novo formation of LDs in 3Y1 cells



3Y1 fibroblasts were cultured in 2% LPDS for 2 d to deplete LDs, and then treated with 100 μM OA, LA, or DHA for 12 h.

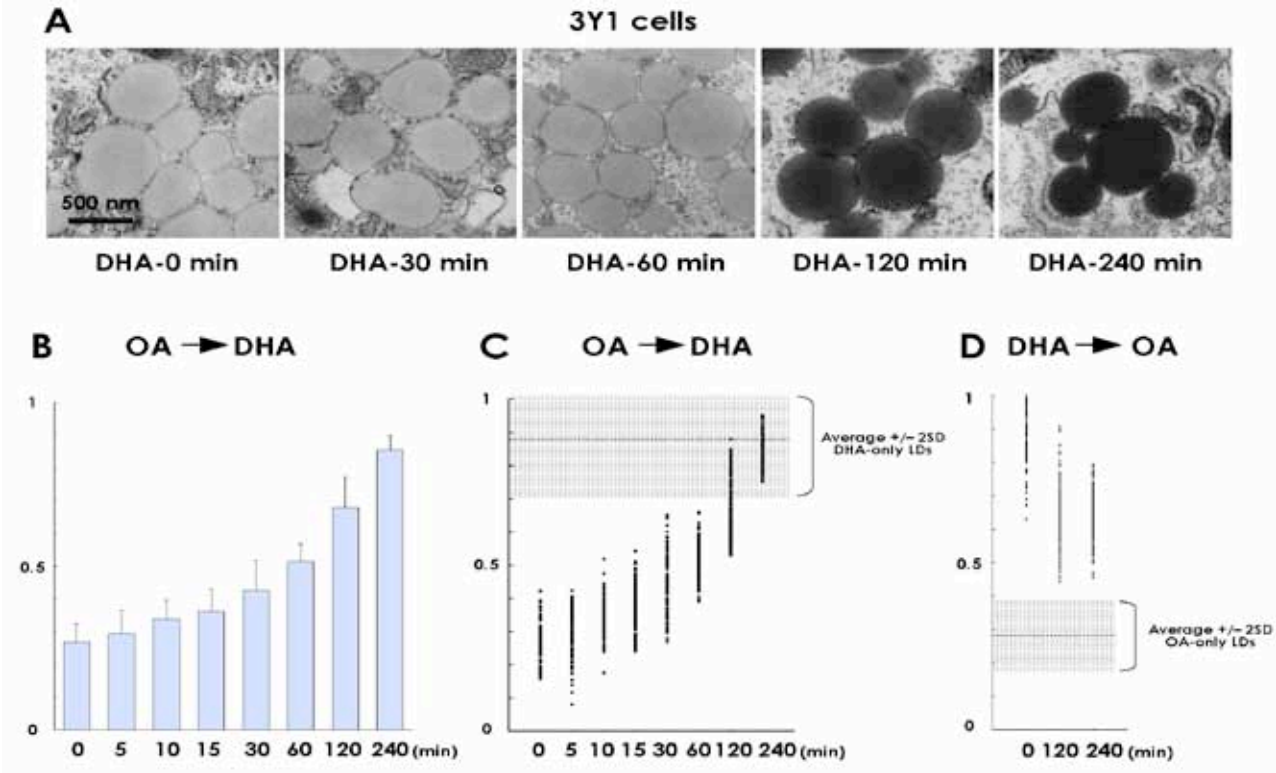
A. Fluorescence microscopy. LDs and nuclei were stained with BODIPY493/503 (green) and DAPI (blue), respectively, after formaldehyde fixation. After 2 d of culturing in 2% LPDS, LDs were rarely observed (control). However, new LDs formed rapidly after treatment with fatty acids. OA induced new LDs most effectively, followed by LA and DHA.
B. Conventional electron microscopy of ultrathin sections. The electron density of LDs differs prominently in accordance with fatty acid treatment. Electron density was the highest in cells treated with DHA, followed by LA and OA.
C. Thin layer chromatography of total lipids extracted from 3Y1 cells. Two days of culturing in 2% LPDS reduced TGs and CEs to minimum levels. After incubation with 100 μM OA, LA, or DHA for 12 h, a TG band became apparent but CE did not change. When cells were treated with 0.2 mM methyl-β-cyclodextrin-cholesterol complex for 12 h, CE, but not TG, became prominent.

Fig. 2. Quantitative measurement of LD electron density in electron micrographs of 3Y1 cells.



A. The method of quantification. The electron density of LDs was measured with a relative scale, with the extracellular space and a grid bar in the same image field as representing density points of 0 and 1, respectively.
B. The electron density of LDs in cells treated with OA, LA, or DHA for 12 h was quantified as described in A. The average electron density of LDs in LA- or DHA-treated cells differed significantly from that in OA-treated cells (Student's *t* test; $*p < 0.01$). The numbers in the y-axis indicate the relative electron density in this and the following graphs.
C. The effect of possible variables on LD electron density. OA-treated cell specimens were either electro-stained with lead citrate for one-third time shorter (left) or sectioned 1.2 times thicker (right) than a control sample from the same specimen. Neither the length of lead staining, nor a difference in section thickness, affected LD electron density.

Fig. 3. The time-dependent change in LD electron density in 3Y1 cells.



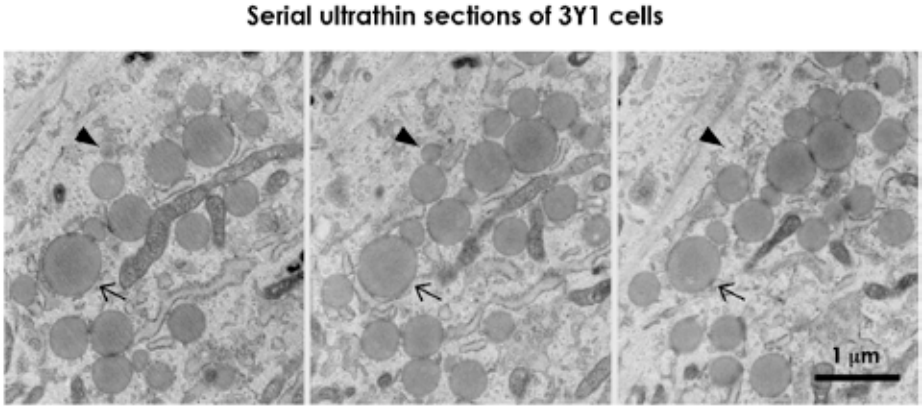
A. Cells were cultured with OA for 12 hr, rinsed, and treated with DHA. Representative electron micrographs before, and 30, 60, 120, and 240 min after the DHA addition are shown. The LD electron density was low at 0 min, but increased gradually after DHA was added to the medium.
B. The average electron density of LDs treated as in A. Ten images were taken in random areas, and the electron

density of all the LDs in each field quantified by the method described for Fig. 2A. The average electron density increased in a time-dependent manner. Note the small standard deviation at each time point. The electron density at any time point is significantly different from that of the adjacent time point, e.g., 0 min vs. 5 min ($p < 0.01$ by Student's *t*-test).

C. The point plot of electron density for all LDs found in ten randomly chosen cells treated as in A. The shaded zone indicates the average (〒 2 standard deviations) of the LD electron density in cells treated with DHA alone for 12 hr. Note that LDs of high electron densities did not occur as an independent population.

D. Cells were cultured with DHA for 12 hr, rinsed, and treated with OA for either 120 or 240 min. The point plot of electron density for all LDs before and after OA treatment is shown. The shaded zone shows the average (〒 2 standard deviations) LD electron density in cells treated with OA alone for 12 hr. The result indicates that LDs made of the OA ester alone do not form by the OA treatment of 120 or 240 min.

Fig. 4. Serial ultrathin sections of 3Y1 cells cultured with OA for 12 h and then with DHA for 60 min.



The LDs in the field show a homogenous change in density. The LD marked by an arrow is more than three times larger than the LD marked by an arrowhead. These results show that LDs differing in diameter by 3–4 times, or 27–64 times different in volume, contain existing OA and newly synthesized DHA esters in comparable ratios.

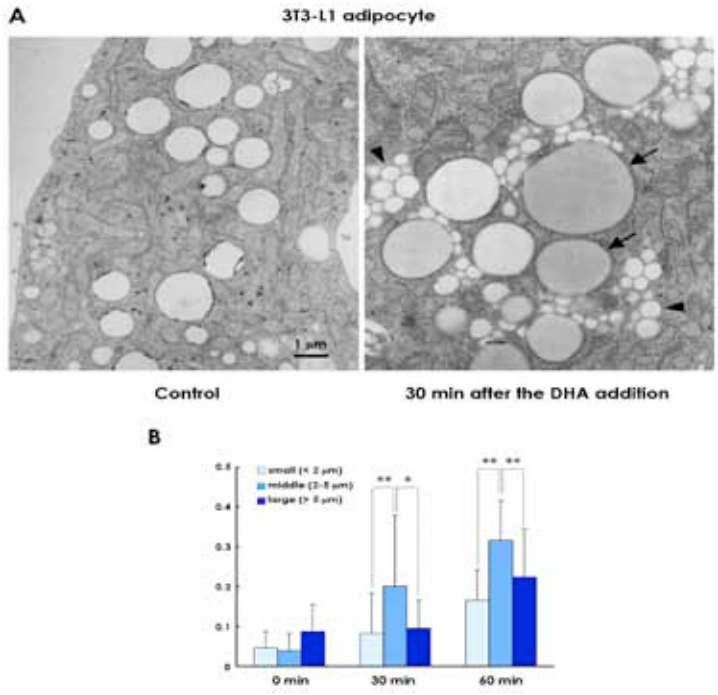
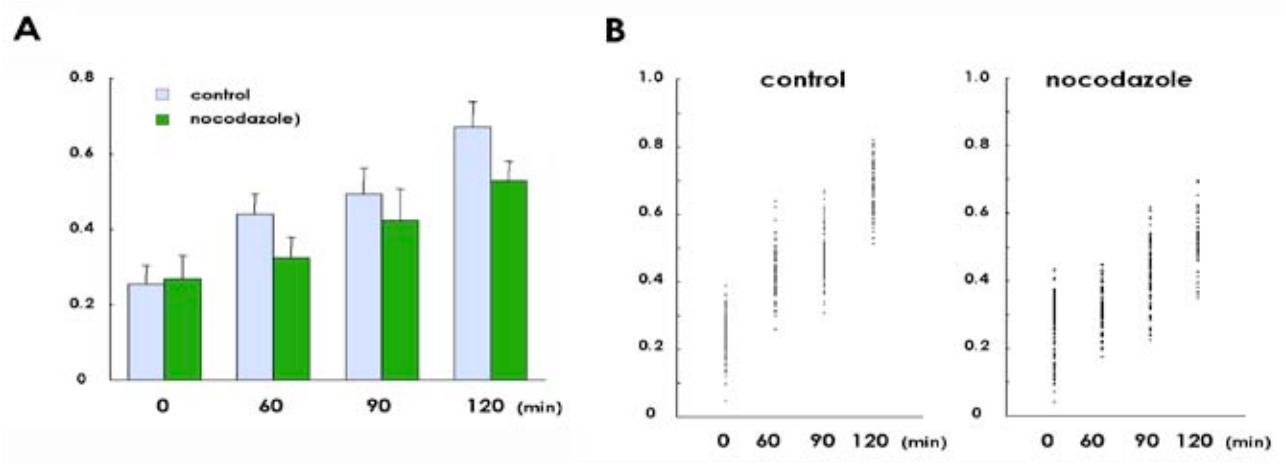


Fig. 5. The electron density of LDs in 3T3-L1 adipocytes treated with DHA.

A. Representative electron micrographs before (left) and 30 min after (right) treatment with DHA . LDs before DHA treatment were invariably electron-lucent, whereas those in DHA-treated cells were heterogeneous in electron density. Small LDs (arrowheads) generally had a lower electron density than medium-sized LDs (arrows).

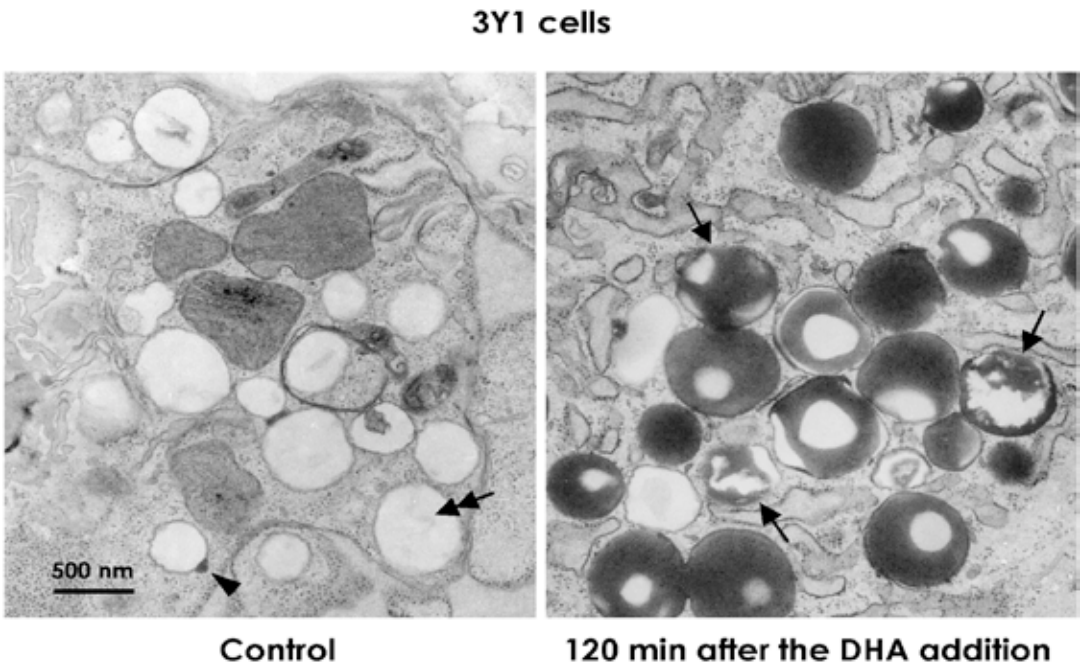
B. LDs were categorized into three groups according to their diameter, and their electron density 30 and 60 min after DHA treatment was quantified as for 3Y1 cells. After DHA, medium-sized LDs (2-5 mm in diameter), had an electron density higher than that of both smaller (less than 2 mm) and larger (more than 5 mm) LDs (*$p < 0.05$, and **$p < 0.01$ by Student's *t*-test, respectively.).

Fig. 6. The effect of nocodazole on the time-dependent change in electron density for 3Y1 LDs after DHA treatment.



A. Cells were cultured with OA for 12 hr, rinsed, and treated with DHA. One group of cells was administered 10 mM nocodazole during the last 1 hr of OA culturing, and DHA medium containing nocodazole was applied. The average electron density of LDs increased at a slightly slower rate in nocodazole-treated cells than in control cells, but the standard deviation of LD electron density was similarly small in both samples.
B. The point plot of LD electron densities after DHA treatment, in the absence (left) or presence (right) of 10 mM nocodazole. Even in the nocodazole-treated sample, LDs with high electron densities was not observed as an independent population.

Fig. 7. Incorporation of DHA esters into CE-rich LDs.



3Y1 cells were cultured with 0.2 mM methyl-b-cyclodextrin-cholesterol complex for 12 hr, and then treated with or without DHA for 120 min. Representative electron micrographs are shown. CE-rich LDs were electron-lucent in the center, but occasionally appeared hazy (double arrow) and/or had compact electron-dense materials in the rim (arrowhead). After DHA treatment, some LDs became homogenously electron-dense, whereas others had zones of variously shaped high and low electron density (arrows).

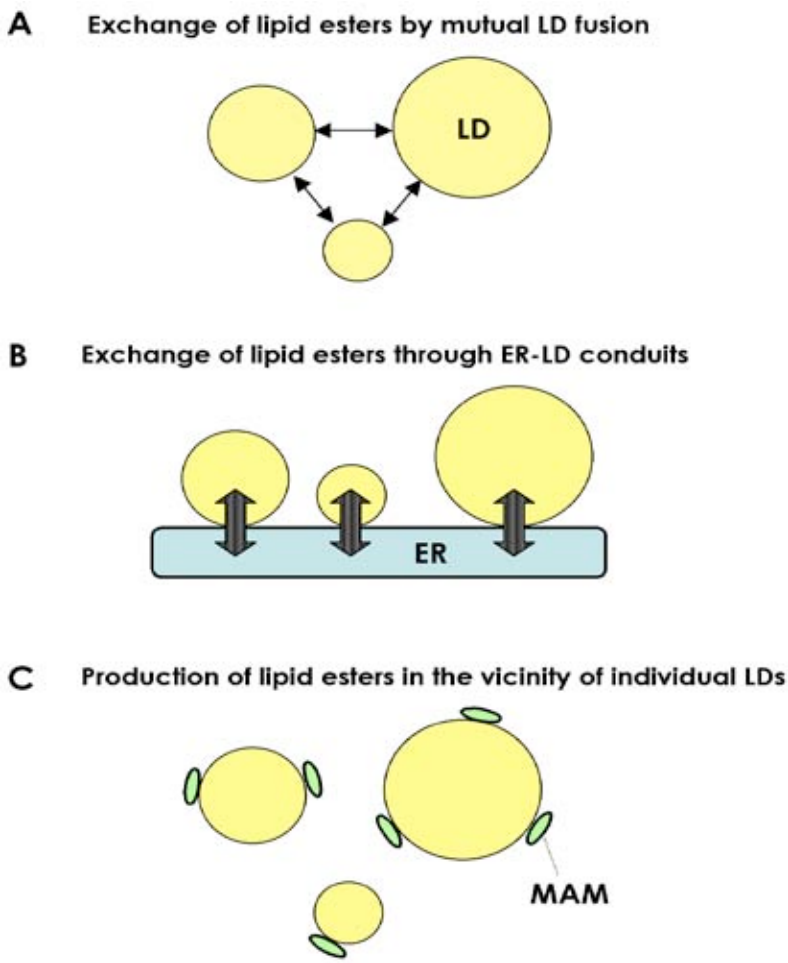


Fig. 8. Schemes for three possible mechanisms of LD growth with a uniform incorporation of TGs.

A. Exchange of TGs by mutual fusion of LDs.
B. Exchange of newly synthesized and existing TGs through persistent ER-LD conduits.
C. Synthesis of TGs in the mitochondria-associated membrane (MAM) within the local vicinity of individual LDs.

Conclusion:

線維芽細胞の脂肪滴は局所で作られる

トリグリセリドが付加されて大きくなる