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研究種目：基盤研究(B) (特設分野研究)

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課題番号：15KT0087

研究課題名(和文)細胞膜情報処理装置の再構成

研究課題名(英文)Reconstruction of signal transduction machinery in the cell membrane

研究代表者

佐甲 靖志 (Sako, Yasushi)

国立研究開発法人理化学研究所・開拓研究本部・主任研究員

研究者番号：20215700

交付決定額(研究期間全体)：(直接経費) 13,800,000円

研究成果の概要(和文)：上皮成長因子受容体(EGFR)による細胞膜情報処理システムの再構成系を開発し、情報伝達の分子機構を解明することを目的とした。再構成系は直径10~15 nmの微小平面膜であるナノディスクを基盤として作成した。ナノディスク作成法を確立し、特にEGFRの膜貫通・膜近傍領域ペプチドの再構成系による1分子構造ダイナミクス計測から、2量体化が酸性脂質PS、PIP2と膜近傍領域の相互作用によって誘起されること。膜近傍領域T654のリン酸化は酸性脂質との相互作用を介して2量体形成を阻害すること。膜のコレステロールは2量体化を促進するが、異なる構造を持つ可能性、などが示唆された。

研究成果の学術的意義や社会的意義

細胞膜受容体は細胞内外の情報変換を行う重要な蛋白質分子であるが、膜を介した情報伝達は信号分子と受容体の相互作用、受容体の分子構造・会合状態変化、受容体と細胞質分子の相互作用などが、さらに複雑かつ不均一な膜構造・膜脂質組成と絡み合って起こる複雑な反応である。ナノディスクは反応要素を最も小数・単純化できる再構成系であり、リボソームなど再構成膜系を利用した構成的システム研究へ基盤情報を与えることができる。本研究では蛋白質と脂質の協調が反応医御に必要であることが明らかになった。

研究成果の概要(英文)：We have developed reconstitution membrane of EGF receptor (EGFR) for analyzing molecular mechanism of signal transduction across the plasma membrane. EGFR molecules were incorporated into nanodiscs with the diameter of 10-15 nm. Single-molecule FRET imaging of the transmembrane (TM)-juxtamenbrane (JM) domains of EGFR revealed that dimerization of EGFR, which is responsible for signal transduction, is induced by interactions of the JM domain with acidic membrane lipids, and inhibitory effect of the phosphorylation at the JM domain for dimerization requires acidic lipids in the membrane. Cholesterol in the membrane also induced dimerization of EGFR, however, dimer structures suggested to be different from that in membranes without cholesterol.

研究分野：生物物理学

キーワード：シグナル伝達 ナノマシン

2%

W/

(epidermal growth factor receptor: EGFR) ch

w1/Gw23bw/SA6•

EGFR c

EGF

KS4M

EGFR c1fS4

b05/ (4)05/ ) gBK

EGFR b(1A465B

40K05/\$BM(62A)

b0b1B

g

CG•

EGFR tMw/G23w

/b0t05/KZsGG(0)ycA

8b64x12Ab005/t\*(MGAS

rSw30YqvSM(3)G#B(

fc(28w.SAb00SB(0)Bb0%

/50A000ew2A+5AD

000•

EGFR bSBwE2c

(b00Z

1c

>0>,%2b% □

%2c□

61□

(EGFR) (□

bS(□

6K(0)(b(SOM □

G% KS□

EGFR b(□

DKb8□

>□

SK(b□

4)bs(□

bp6MS□

%2□

S□

E07)SSb□

S(KSS02% □

□

□

KS9(6bG% □

KS □

>1>,%2b2

S(c%□

10~15 nm b8□

60K8M□

e2A>05b8g0y

9(ELsb□

6□ %2c>1bM

KS

EGFR rS

cKS

EGFR□

KS

MSP .2Ab

2A=SKS>0b

MSP □

KS

EGFR □

M□ (□

SMA %KIP□

KS

SMA 8KS6b

EGFR SKS

MSP □

□

ERGR #((KSG(AS)>(□

□

GFP%□ EGFR SKSc>rSc>0(bE□

B184E9AG(KE□

Gb:0□

4) EGFR b

MSP S(8M□

EGFR b 238□

(transmembrane: TM) □(2A b 38□

(juxtamembrane: JM)□

49 gbKS>□(

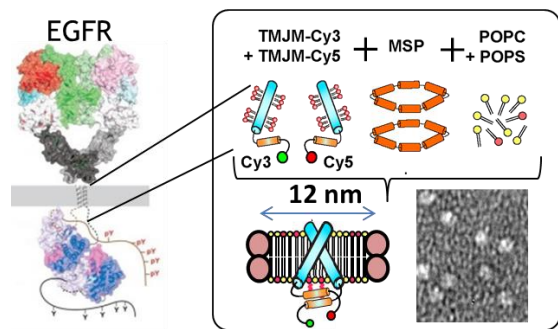


Fig.1. EGFR TM-JM b MSP □  
bS□  
GbS□ (c 04)x00□  
2A)DMGA□  
8KSb786~□

EGFR b 238□ (transmembrane: TM) □(2A b 38□ □  
(juxtamembrane: JM)□ 49 gbKS>□(

N (E68E) C (M) Cy3 (rSc) Cy5 ( )  
 KSeS ( ) JM 8b ( ) 654 6S4 ( ) KKS  
 pT654 KKS ( )  
 G ( ) DPPC 58E ( ) 70% DPPC ( ) 30% DPPS b2A ( ) 50% DPPC ( )  
 50% b2ASISSKS ( )  
 Kbgc ( ) KKS ( )  
 E ( ) MSP .2Ac ( ) 9 ( )  
 (M)A ( ) 86~8M ( )  
 b) Cy3, Cy5 > 0.6b) FRET 0.6 ( )

22%

b) 24K

Fig.1 ( )

gM ( ) ( ) bg ( )

KS ( ) ( )

S ( ) FRET 0.66~

2k ( )

(%) K ( ) 0 K ( )

G ( )

(Fig.2) ( )

. ( ) 8 > 0 b ( )

1:1 ( ) E K ( )

FRET ( )

(x) 0 M ( )

N 6b ( )

FRET ( )

(E) c 0 C ( )

(E > 0.8) ( )

6e9808G ( )

6SKK ( )

C 6E ( )

PC S ( )

p > 0.5 ( )

(E = 0.9) 5 ( )

(E=0.1) b ( )

bb6b8 (x) ( )

( ) JM b) C e 0.7 K ( ) P ( )

pi88S4 ( )

( )

42A PS tS ( )

x) PC bsb ( )

PIP2 M ( )

> 0.5 ( )

( )

KS (Fig. 3) ( ) JM e C b 7 ( ) e ( ) G b) e 4 ( )

2A ( ) JM b) % 80 K ( )

JM 6b > 0.5 gM ( )

EGFR b ( )

4MGgK8 ( )

(Fig.3) ( )

JM f ( )

T654 b 4E ( )

EGFR b 2b 0 ( )

8Sc8 ( )

T654 ( ) K S b S ( ) e ( )

PS fp > 0.5 A Ca K 5 K S p 6 g b (x) ( )

0bS ( )

KK ( )

PC bs S K S ( )

e ( )

PIP2 S 4 (x) b ( )

e 0.9 IS ( )

pT654 e) JM > 0.5 b 0.7 M b e ( )

JM 6% 8 ( )

% K > C b e C 4 2 A b % 8 K 8 G g IS ( )

(Fig. 3) ( )

b) e 4 2 A ( )

JM b) % 8 ( )

JM 6b > 0.5 g ( ) ( ) 2 s I ( )

4Eg 7 M G M ( ) 2 A 2 A ( ) K ( ) 0 ( )

8G ( )

( ) K 8 ( )

Cy3(donor)/Cy5(acceptor)

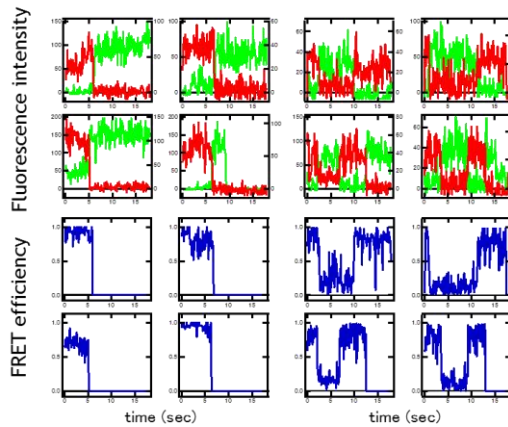
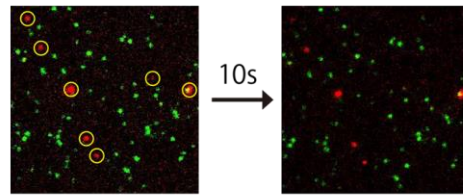


Fig.2 TM-JM 6b > ( ) FRET 0 ( )  
 > 2 ( ) Cy3 (0s) b 2 s ( ) M 4 b ( )  
 e) Cy5 (2e) b) FRET 0.9 ( ) e > ( )  
 ( ) FRET b 6 ( ) JM 6b 27 ( )  
 M ( )

kinetics 08 □ PS  
 □ PIP2 b6S)2 □  
 MG(S □ M □ PS □  
 5505b6b%K4DC  
 2sGhPK □ PIP2 □cp6g  
 )KSS4598 □  
 EGFR □ PIP2 □  
 □K8G%8C □  
 )e(h) □  
 (S4M □ JM □  
 PIP2 b%8E%05g  
 KMGgK8M □  
 KSE □ PLC<sub>γ</sub>, PI3K □ PIP2  
 aI(g) □  
 >05g7I □ >AgpMh □

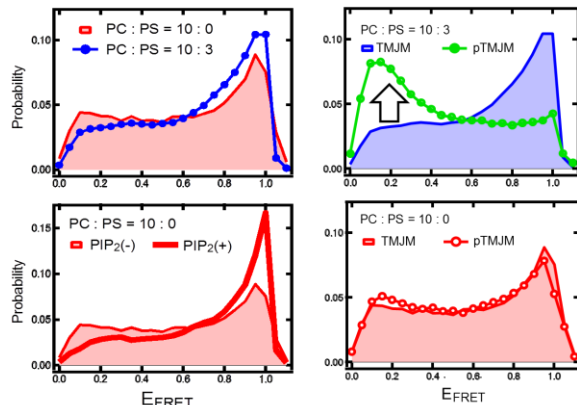


Fig. 3. TM-JM05/bS4(x FRET 088) JM 6b273E PS(e V ) H 8 c  
 C 05/g BK Z •  
 PIP2(e W ) w [05/bm 01  
 • PS μ twē pT654 v6605  
 /gBcöaM@ (V ) PC bsbwē  
 pT654 b0ē (W )

3 \$1e ...  
 711=00 □ 4 □

1. \*Okamoto, K. and \*Sako, Y. (2019) Single-molecule Förster resonance energy transfer measurement reveals dynamic partially ordered structure of the epidermal growth factor receptor C-tail domain. *J. Chem. Phys. B.* 123, 571-581. doi: 10.1021/acs.jpcc.8b10066. 1w
2. Yasui, M., Hiroshima, M., Kozuka, J., \*Sako, Y., and \*Ueda, M. (2018) Automated single-molecule imaging in living cells. *Nat. Comm.* 9, 3061, doi:10.1038/s41467-018-05524-7 (1-11) 1w
3. Hiroshima, M., Pack, C.-g., Kaizu, K., Takahashi, K., Ueda, M., and \*Sako, Y. (2018) Transient acceleration of epidermal growth factor receptor dynamics produces higher-order signaling clusters. *J. Mol. Biol.* 430, 1386-1401, doi: 10.1016/j.jmb.2018.02.018. 1w
4. Maeda, R., Sato, T., Okamoto, K., Yanagawa, M., and \*Sako, Y. (2018) Lipid-protein interplay in dimerization of the juxtamembrane domains of epidermal growth factor receptor. *Biophys. J.* 114, 893-903. doi: 10.1016/j.bpj.2017.12.029. 1w

9 □

1. Hiroshima, M., Yasui, M., Kozuka, J., Sako, Y., and Ueda, M. In cell automated single-molecule analysis and its extensive applications. (2018.9.15) 56 GD  
 “New trends in bioanalysis based on single molecule biophysics”
2. (yw/b4 6 (18.4.21) “ 17 GD  
 4c93/4
3. (yw/b/ (18.3.10) 56 GD  
 □ 42 G64 Sn 3
4. (yw/b/ (18.2.10) 18 GD  
 G64 b3a 11/4

5. Maeda, R., Sato, T., Okamoto, K., and Sako, Y. (2017.9.20) Lipid-protein cooperativity in the regulation of juxtamembrane domain dimer formation in epidermal growth factor receptor. " 55 G<sub>TP</sub> 3D "New detergents, liposomes, and nanodiscs as membrane-mimetic environments. 4

6. Okamoto, K., Maeda, R., and Sako, Y. Single-molecule FRET measurement for EGFR-RAS-MAPK signal transduction pathway. (2017.9.3-8) "Deciphering complex energy landscape and kinetic network from single molecules to cells: a new challenge to make theories meet experiments" Dijon, France

7. Yoshida, S. (www/b4/b4) (2017.6.13) " 69 G<sub>TP</sub> 3D 3D 3D 3D

8. Yoshida, S. (www/b4/b4) (2016.12.10) 3D 3D N4S)r/T 3D 3D 3D

9. Yoshida, S. (www/b4/b4) (2016.9.5) " 25 G<sub>TP</sub> 3D 3D 3D

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3D 2)

(1)2(,\*

2(13

3D

3D 2 6 3D

4 3D

3D

8 E>8

(2) % \*

% B

B

B ↓ % c % b 0 \ 2i B M \_ 6 M 0 x 2i c % b x % b b it. \_  
B \ b 03. \_ ö B 2B \_ 6 M 0 x 2i c % b \_ rM