

1-1 論文を作る

論文は研究成果を発表するものである。本文を書く、図を作る、文献を入力する。皆さんは、それぞれの論文作成のステップで、どのようなソフトを使っているのだろうか？

冒頭の論文はわたしが1999年にNatureに発表した食欲を刺激するホルモン“グレリン”の発見の論文である1-1-1～1-1-5。論文発表の舞台裏については、今は廃刊になったMolecular Medicineにエッセイを書いているので、図書館で探して読んで欲しい。

この論文の評価は結構高く、グレリンに関する研究論文はPubMedで検索すると2015年1月の段階で

7,300編を超えている。このグレリン発見の論文には、医学・生物学分野の科学論文に典型的なFigureをいくつか含んでいる。それは棒グラフ、線グラフ、写真などの画像である。

わたしは機会があれば多くの研究者に、どのワープロソフトを使って本文を書いているか、どのソフトで図を作成しているかを質問する。その結果、ワープロソフト以外は研究

1-1-1

Ghrelin is a growth-hormone-releasing acylated peptide from stomach

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Small synthetic molecules called growth-hormone secretagogues (GHSs)¹⁻³ stimulate the release of growth hormone (GH) from the pituitary^{4,5}. They act through GHS-R, a G-protein-coupled receptor for which the ligand is unknown. Recent cloning of GHS-R^{6,7} strongly suggests that an endogenous ligand for the receptor does exist and that there is a mechanism for regulating GH release that is distinct from its regulation by hypothalamic growth-hormone-releasing hormone (GHRH)^{4,5}. We now report the purification and identification in rat stomach of an endogenous ligand specific for GHS-R. The purified ligand is a peptide of 28 amino acids, in which the serine 3 residue is *n*-octanoylated. The acylated peptide specifically releases GH both *in vivo* and *in vitro*, and *O*-*n*-octanoylation at serine 3 is essential for the activity. We designate the GH-releasing peptide 'ghrelin'.

ghrelin apart from two amino acids both rat and human indicates that GH release from the pituitary may be regulated not only by hypothalamic GHRH, but also by ghrelin.

わたし(著者)です。

第3章「タッチタイピングをマスターする」

1-1-2

第2章「2-10 複雑な線の図を描く」
Illustratorの「ペンツール」が威力を発揮する。

analysis did not increase $[Ca^{2+}]_i$ (Fig. 2b). Moreover, RP-HPLC showed that natural ghrelin eluted about 10 min later than the synthetic 28-residue peptide (desacyl ghrelin) (Fig. 2a, top and middle panels). This indicates that Ser 3 in natural ghrelin must be modified by a hydrophobic moiety. To identify the hydrophobic moiety of Ser 3, we subjected purified ghrelin to electrospray ionization mass spectrometry. The observed M_r of purified ghrelin was $3,314.9 \pm 5.9$ (28 mass units higher than the M_r (3,189) calculated for the 28-residue sequence [X = Ser]). This indicated that a hydrogen atom of the hydroxyl group in Ser 3 was replaced by a $C_{15}H_{31}CO$ moiety (127 mass units). In other words, the hydroxyl group of Ser 3 residue is octanoylated. To verify the deduced structure, we synthesized an (*O*-*n*-octanoyl Ser 3) peptide, and found that it is identical to natural ghrelin in its behaviour under chromatography, mass-spectrometric pattern and GH-releasing activity. As clearly seen in Fig. 2a (bottom panel), natural ghrelin co-migrates with the synthetic *n*-octanoylated peptide on RP-HPLC. Furthermore, amino-terminal peptides (Gly¹-Ile⁴-Phe⁴-Ile⁴), prepared by chromotrypsin digestion of both natural ghrelin and synthetic *n*-octanoylated peptide also showed the same retention times on RP-HPLC and identical fragmentation patterns when analysed on a time-of-flight mass spectrometer. In CHO-GHSR2 cells, synthetic ghrelin clearly induced an increase in $[Ca^{2+}]_i$ that was as potent as that induced by natural ghrelin (Fig. 2b), and stimulated GH secretion from rat primary pituitary

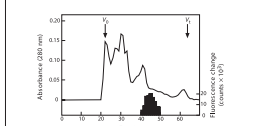


Figure 1 RP-HPLC (flow rate 0.5 ml/min) of RP18 fraction from 40 μg rat stomach. Black bars indicate fluorescence change using $[Ca^{2+}]_i$ sensor in CHO-GHSR2 cells. Active fractions were eluted around M 3,000. K, total volume.

letters to nature

cells (described below). Thus, the complete structure of rat ghrelin is unambiguously identified as an (*O*-*n*-octanoyl-Ser 3)-peptide (Fig. 2c). Octanoylation has not been observed previously in peptide modification. The fact that the *n*-octanoyl moiety is essential for the activity of ghrelin indicates that the post-translational mechanism for acyl-modification should be urgently elucidated. It is also interesting that no structural homology with ghrelin is observed among synthetic GHSs.

Ghrelin potently induced an increase in $[Ca^{2+}]_i$ in CHO-GHSR2 cells (50% excitatory concentration (EC₅₀) 2.5×10^{-10} M), whereas GHRH had no effect on the cells under the same conditions. The threshold for the ghrelin-induced increase in $[Ca^{2+}]_i$ was 10^{-10} M (Fig. 3), but the $[Ca^{2+}]_i$ increase was depressed in the presence of 10⁻⁷ M [D-1²⁵-D²⁷]-GHRH-6, an antagonist specific for HS-R^{8,9}. A high dose of ghrelin, however, regenerated the $[Ca^{2+}]_i$

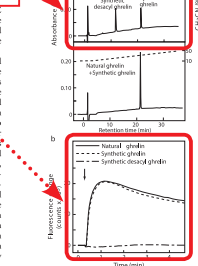


Figure 2 Identification of the *n*-octanoyl modification in ghrelin. a, Chromatographic comparison on RP-HPLC of natural ghrelin (top panel), synthetic ghrelin and desacyl ghrelin (middle panel), and co-purifier of natural and synthetic ghrelin (bottom panel). Black bar indicates $[Ca^{2+}]_i$ -increasing activity (top panel). Desacyl ghrelin is a synthetic 28-residue peptide (X = Ser), which has the sequence deduced from cDNA analysis. Each peptide (2 μg) was applied on a subnanogram stream (flow rate, 1 ml min⁻¹). Solvent system, a linear gradient elution from A to B for 40 min. A, 0.1% TFA in A; B was 10% TFA. b, For 40 (n = 10), 10 (n = 4) and 1 (n = 1) min. Natural ghrelin co-migrates with synthetic ghrelin in the present conditions (bottom panel). c, Time course for changes in $[Ca^{2+}]_i$ in CHO-GHSR2 cells induced by natural ghrelin, synthetic ghrelin and desacyl ghrelin. Each peptide (10⁻⁷ M) was added at the time indicated by the arrow. d, Structure of rat ghrelin.

室（研究者ごと？）によって使われているソフトは千差万別である。なかには図を描くのに「まだこんな古いソフトを！」と驚くこともしばしばある。

またわたしは「ブラインドタッチ（タッチタイピング）で文章を打てるか？」とよく質問する。結果は10人に聞いて、よくて1人が正統なタッチタイピングで打てるという低率である。ほとんどの研究者は昔から自己流で何となくキーを打ってきたから、特に不自由ないと思っている。自慢ではないが（いや、自慢かもしれないが）わたしは、正統なタッチタイピングで文章が打てる。しかもプロにはかなわないが、かなりのスピードで打てること自負している。たいていの研究者よりは速いのではないかな？ これまでに他の研究者

1-1-3

letters to nature

change to the level observed in the absence of the antagonist. These results indicate that ghrelin is competitively inhibiting antagonist.
Although ghrelin had no sequence homology to an biologically active peptide, we found an amino acid identical to that of rat ghrelin in rat expressed sequence (EST; GenBank accession no. AJ549172). On the basis of sequence, we designed primers for the polymerase chain reaction (PCR) and amplified a cDNA fragment from rat stomach cDNA as the template. The fragment was used as a screening probe on a rat stomach cDNA library. A cDNA of 501 base pairs (bp) long was

第2章「2-7 棒グラフを描く」
基本的なグラフを描いて、Illustratorに慣れよう。

第2章「2-11 S字曲線を描く」
Illustratorの「ペンツール」を使って、なめらかなS字曲線を描く。

library under low-stringency conditions. Isolated human cDNA was found to encode human prepro-ghrelin of 117 amino acids (Fig. 4). Amino acid identities between rat and human prepro-ghrelins are 82.9%. Only two amino acids are replaced in the 28-residue ghrelin segment, indicating that ghrelin is highly conserved between species. We purified human ghrelin from human stomach extract, and verified by synthesis that human ghrelin, like rat ghrelin has an *N*-octanoyl modification. Ser 3 (details will be reported

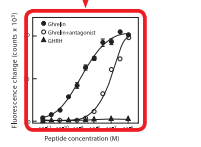


Figure 3 Dose-response relationship of $[Ca^{2+}]_i$ change in CHO-GHFR2 cells. The concentration of Ghrelin was 100 nM. The antagonist (GHR11) concentration was 10⁻⁶ M. GHR11 was also assayed. Data points are means \pm s.e.m. of triplicate for each experiment.

| Human | 1 | MPSVPTVYVLELLEGLNMLGLANLSTSTL | 100 |
|-------|----|-------------------------------|-----|
| Rat | 1 | SPHDTNKKKESKPPKAPLQALADMR | 60 |
| Rat | 1 | MYSSATLCSLLSLWVWVWVWVWVWVWV | 100 |
| Human | 31 | EPHDTNKKKESKPPKAPLQALADMR | 60 |
| Human | 41 | DRGMEAEAEELRDFNAPFVGLKSLSA | 90 |
| Human | 41 | DRGMEAEAEELRDFNAPFVGLKSLSA | 90 |
| Human | 51 | YVQVQVSLGLQDLTLLKAEKAPAK | 117 |

Figure 4 Alignment of amino acid sequences of human and rat prepro-ghrelin. The identical residues are shaded. The asterisk indicates a signal peptide. The first amino acid is a cleavage site of a signal peptide. The open arrowhead indicates N-terminal processing site. Ghrelin sequences are boxed. Asterisks indicate modification sites.

being estimated as 2.1×10^{-10} M for ghrelin and 0.6×10^{-10} M for GHR11. To confirm whether ghrelin specifically stimulates GH release, we analysed which hormones were released from pituitary cells by ghrelin. We found that the ghrelin specifically stimulated GH release in anaesthetized rats, only GH concentration at the maximum levels at 4–10 min after injection (Fig. 5). These results indicate that ghrelin is a GH-specific releasing

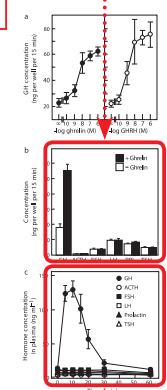


Figure 5 Effects of ghrelin on pituitary hormones. *in vivo* and *in vitro*. Dose-response relationship of GH concentration in pituitary cells induced by ghrelin (top panel) and GHR11 (open bars) (*in vitro*). GH concentration represents means \pm s.e.m. of triplicate assays. *b*, Effects of 400 ng (10⁻⁶ M) ghrelin on hormone secretions from rat primary pituitary cells. Each hormone concentration represents means \pm s.e.m. of quadruplicate assays. *c*, Time course of *in vivo* GH release.

第2章「2-8 線グラフを描く」
オーソドックスなグラフを描こう。

1-1-4

letters to nature

第2章「2-12 画像を含んだ図」

region from the neck to the base of the oxyntic gland (Fig. 6a–d). Ghrelin-immunoreactive cells in the stomach have the same distribution as that found in the hypothalamus (Fig. 6e–g). The distribution pattern and morphological features of the labelled and

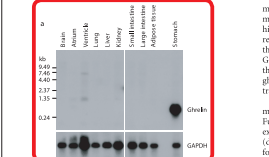


Figure 6 Distribution of ghrelin-immunoreactive cells. *a*, *b*, Ghrelin-immunoreactive cells in the stomach have the same distribution as that found in the hypothalamus. *c*, *d*, Immunohistochemical staining of the stomach. *e*, *f*, Immunohistochemical staining of the hypothalamus. *g*, Immunohistochemical staining of the hypothalamus. Scale bars: *a*, *b*, 100 μ m; *c*, *d*, 40 μ m; *e*, *f*, 200 μ m; *g*, 40 μ m.

unostained cells that ghrelin cells are endocrine cells, we found that ghrelin cells in healthy human blood considerable plasma concentration (11.72 \pm 37.2 pmol ml⁻¹) ($n = 6$). Taken together with the fact that ghrelin, when injected intravenously, induces GH release (Fig. 5c), it is highly likely that this molecule is produced in small secreted from stomach, circulating in the blood stream to act on the pituitary.

Although no detectable northern blot signal was observed in mRNA from whole brain (Fig. 6f), RT-PCR amplification of ghrelin mRNA revealed the presence of this transcript in brain. Immunohistochemical analysis performed after colchicine treatment revealed that ghrelin-immunoreactive neurons were present in the hypothalamic arcuate nucleus (Fig. 6h, i). Considering that RT-PCR is known to exist in hypothalamic regions, including the arcuate nucleus and pituitary gland, these results suggest that ghrelin in the arcuate nucleus may act on the hypothalamus or be transported to the anterior pituitary gland.

Thus, the occurrence of ghrelin in both stomach and hypothalamus will give a new dimension to the regulation of GH release. Further, our RT-PCR analysis indicates that GHR11 is also expressed in heart, lung, pancreas, intestine and adipose tissue (data not shown). Ghrelin may thus have multifaceted roles, for example the cardiovascular system¹ and metabolism.²

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第4章「EndNoteで文献を入力する」
これでやっかいな文献入力におさらば!

antennae primers. Sense primer: 5'-TTGAGCCGACGACACAGCA3'. Antennae primers: 5'-ATCTCGACGACGACGACGACGAC3'. RT-PCR was performed with the above primers. The amplified fragment was used as a screening probe for a rat stomach cDNA library. Approximately 2.5 \times 10⁶ recombinant phages were screened. Both strands of cloned cDNAs of rat ghrelin were sequenced. Human stomach cDNA library was constructed from a lung human stomach cDNA library with a DNA synthesis kit (Pharmacia). Full-length cDNAs of human ghrelin cDNA were isolated from the human stomach cDNA library using the above primers.

In vitro assay of pituitary hormone releasing activity
Anterior pituitary neurons were obtained from a 6-week-old male Sprague-Dawley rats. After dispersion by collagenase, the above medium, and 2.5 \times 10⁶ cells were seeded in poly-D-lysine well tissue culture flasks. The cells were cultured for 3–4 days. The culture medium was then replaced with a DMEM containing medium, and the cells were incubated for 18 h at 37°C. Aliquots of the resulting incubation medium were collected for ELISA. Concentrations of pituitary hormones were measured with assay kits (RiataK, BIA (Geneva), GH, lutealizing hormone (LH), luteinizing hormone (LH), prolactin (PRL) and

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20. Bögler, G. V. et al. Reconstitution of pituitary hormone release and metabolic improvement by infusion of growth hormone-releasing pituitary neurotrophic releasing hormone in rats with pituitary gland dysfunction. *J. Neuroendocrinol.* **11**, 131–137 (1999).
21. Schwob, R. S. & Nagler, R. D. HPLC: a new instrument for accurate, high-throughput optical scanning. *J. Neuroendocrinol.* **11**, 131–137 (1999).
22. Gnanapavan, S. et al. Ghrelin specifically stimulates GH release in anaesthetized rats. *Proc. Natl. Acad. Sci. USA* **96**, 367–371 (1999).
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24. Dan, Y. et al. Ghrelin, an orexigenic hypothalamic peptide, acts on various endocrine, autonomic and reproductive systems. *Endocrinology* **135**, 209–215 (1994).
25. Dan, Y. et al. Ghrelin stimulates GH release in anaesthetized rats. *Endocrinology* **135**, 203–208 (1994).

Central inputs mask multiple adult neural networks within a single embryonic network

Yes! Le Douarin, Valérie S. Fillion, & Pierre Meyrand

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It is assumed that, after construction of basic network architecture in embryos, immature networks undergo progressive maturation to acquire their adult properties. We examined this assumption in the context of the lobster stomatogastric nervous system. In the lobster, the neuronal population that will form this system is at first organized into a single embryonic network. It generates a single rhythmic pattern. The system then splits into different functional adult networks controlled by central descending systems. These adult networks produce multiple motor programmes, distinctively different from the single output of the embryonic network. We show here that the single embryonic network can produce multiple adult-like programmes. This occurs after the embryonic network is silenced by removal of central inputs, then pharmacologically stimulated to restore rhythmicity. Furthermore, restoration of the flow of descending information reversed the adult-like pattern to an embryonic pattern. This indicates that the embryonic network possesses the ability to express adult-like network characteristics, and descending information reverses it from doing so. Functional adult networks may therefore not necessarily be derived from progressive ontogenetic changes in their themselves, but may result from maturation of descending systems that unmask pre-existing adult networks in an embryonic system.

The stomatogastric nervous system, STNS, of the embryonic (as well as the adult) lobster, *Homarus gammarus*, which controls the rhythmic motions of the stomodaeum and foregut, respectively, consists of four interconnected ganglia, one of which is the stomatogastric ganglion (STG). In mid-embryonic development, the STG contains of adult complement of 30 identified neurons, most of them motor neurones. The adult STG population is organized into two different networks, the gastric and the pyloric, that are well

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がキーボードで文章を打っているのを横から眺めていて、「俺より速く打てる！」と感嘆したことはない。

またキーボードが速く打てても、論文の最後には、あの面倒な引用文献が待っている。しかも雑誌ごとに文献のスタイルが違っているため、投稿論文がリジェクトされて別の雑誌に再投稿するときにはまた最初か

ら文献を入力する、あるいは文章を変更したため文献の番号がズレてしまっていて混乱した、などの経験はないだろうか？

気になったので、何人かの研究者に現在どのようなソフトを使って論文を作成しているのかを聞いてみた。以下、そのうちの5名の回答。

研究者 A

(研究者の標準的な回答だと思う)

【ワープロ】 Word

【図の作成】 PowerPoint で作成。

【文献入力】 EndNote を使っている。

【オンラインストレージ】

使っていない。

研究者 B

【ワープロ】 Word

【図の作成】 PowerPoint。用紙サイズを A4 にして、最終的に PDF あるいは tiff ファイルに変換する。グラフは Excel から移す。

【文献入力】 EndNote を使っている。

【オンラインストレージ】

使っていない。

研究者 C

【ワープロ】 Word

【図の作成】 Illustrator で作図している(なんとわたしが以前に Illustrator の使い方を教えたらしい?!)。

【文献入力】 EndNote を使っている。

【オンラインストレージ】

使っていない。

研究者 D

【ワープロ】 Word

【図の作成】 PowerPoint で作成して、PDF や tiff ファイルに変換する。

【文献入力】 手入力。

【オンラインストレージ】

使っていない。

研究者 E

【ワープロ】 Word

【図の作成】 Photoshop か、PowerPoint で作成。

【文献入力】 手入力。

【オンラインストレージ】

使っていない。

このようにワープロはほとんどの方は Word を使用しているが、図の作成に Illustrator を使っていたのは 1 名だった。予想通り PowerPoint を使って作図している人が多かった。またこの 5 名以外で Dropbox を使っている方が何名かいたが、オンラインストレージはほとんどの研究者が使っていない。タッチタイピングができると自信を持って答えてくれたのは、わたしの研究室のメンバー以外では 1 人だった。おそらくもっと多くの方に聞いてみても、同じような傾向になるだろう。

さて、この本の構成は、実際にわたしが発表した論文の作成に沿って、図の作成から、本文、文献の記入、投稿用ファイルの作り方までを、できるだけ実例のままに説明し、実際に読者が論文作成のときにすぐに役立つ

つようにしてある。

この本で使用したソフトはすべて Mac 版 (OSX10.9.3) の

- ① Illustrator (Illustrator CS6)
- ② Photoshop (Photoshop CS6)
- ③ Word (Word X for Mac)
- ④ EndNote (EndNote X7)
- ⑤ Acrobat (Acrobat 6.0)

である。これらのソフトは、入手も簡単だし、論文作成のために最もポピュラーなものだ。しかし、どのソフトも機能が多く、解説書が分厚く、「とてもこんなに覚えきれない」「使いこなせない」と嘆くかもしれない。しかし、科学論文作成に使うだけならば、それほど高機能な使い方は必要なく、使うべき機能はすごく限られているので、実はマスターするのは簡単だ。ぜひ以下の章でそのことを実感して欲しい。