パラグラフライティング

研究とは

ポスターの作り方

発表のポイント

なぜ研究するのか？

知的好奇心

社会的課題

学術的課題

「研究」とは

【研究】

◇問題になる事柄についてよく調べて事実を明らかにしたり理論を打ち立てたりすること

（新明解国語辞典より）

◇ある特定の物事について、人間の知識を集めて考察し、実験、観察、調査などを通して調べて、その物事についての事実を深く追求する一連の過程のことである。

（Wikipediaより）

研究とは何か

普遍性・論理性・客観性・再現性

・何らかの学術的問題を提起する
・その問題の解決に貢献する
・その成果を他者に伝える

論文（報告）作成と**発表**が研究者の「業績」である

読んでもらわなくてはならない！
　＆
わかってもらわないといけない‼

論文の読み方（読まれ方）

① キーワードで探す
② タイトルを読む
③ 要旨Abstractを読む

An abstract is a short summary of your (published or unpublished) research paper, usually about a paragraph (c. 6-7 sentences, 150-250 words) long. A well-written abstract serves multiple purposes:

- an abstract lets readers get the gist or essence of your paper or article quickly, in order to decide whether to read the full paper;
- an abstract prepares readers to follow the detailed information, analyses, and arguments in your full paper;
- and, later, an abstract helps readers remember key points from your paper.

The Writing Center University of Wisconsin – Madisonより

論文の読み方（読まれ方）

1. the **context** or background information for your research; the **general topic** under study; the **specific topic** of your research
2. the **central questions** or statement of the **problem** your research addresses
3. **what's already known** about this question, what **previous research** has done or shown
4. the main **reason(s)**, the exigency, the **rationale**, the **goals** for your research—Why is it important to address these questions? Are you, for example, examining a new topic? Why is that topic worth examining? Are you filling a gap in previous research? Applying new methods to take a fresh look at existing ideas or data? Resolving a dispute within the literature in your field? . . .
5. your research and/or analytical **methods**
6. your main **findings**, **results**, or **arguments**
7. the **significance** or **implications** of your findings or arguments.

The Writing Center University of Wisconsin – Madisonより

論文の読み方（読まれ方）

の研究において、 が問題になっている。そこで を調べた。 対象
手法は で、
結果は であった。 実験・結果
すなわち、 ということが明らかになった。 結論

「科学英語論文の読み方入門」
2020, 1 大阪大学図書館

要旨の分析

背: 研究の背景
仮: 仮説・命題
方: 方法
果: 結果
考: 考察
結: 結論

論文の読み方（読まれ方）

の研究において、 が問題になっている。そこで を調べた。 対象
手法は で、
結果は であった。 実験・結果
すなわち、 ということが明らかになった。 結論

わかってもらわないといけない‼

→ 論文の書き方

雑誌の紙面(例)

表紙

Volume 53, Issue 2

February 2012

題名

著者名

所属機関名

略語説明

研究概要

キーワード

序章

PCP
PLANT & CELL PHYSIOLOGY

CRYPTIC PRECOCIOUS/MED12 is a Novel Flowering Regulator with Multiple Target Steps in Arabidopsis

Yuri Imura[1], Yasushi Kobayashi[2,3], Sumiko Yamamoto[2,4], Masahiko Furutani[5], Masao Tasaka[5], Mitsutomo Abe[1,6] and Takashi Araki[1,*]

[1]Graduate School of Biostudies, Kyoto University, Kyoto, 606-8501 Japan
[2]Graduate School of Science, Kyoto University, Kyoto, 606-8502 Japan
[3]Department of Molecular Biology, Max Planck Institute for Developmental Biolog
[4]Genome Informatics Laboratory, CIB-DDBJ, National Institute of Genetics, ROIS, S
[5]Graduate School of Biological Sciences, Nara Institute of Science and Technology,
[6]Department of Biological Sciences, Graduate School of Science, The University of
*Corresponding author: E-mail, taraqui@lif.kyoto-u.ac.jp; Fax, +81-75-753-6470.
(Received December 19, 2011; Accepted January 5, 2012)

Rapid Paper

The proper timing of flowering is of crucial importance for reproductive success of plants. Regulation of flowering is orchestrated by inputs from both environmental and endogenous signals such as daylength, light quality, temperature and hormones, and key flowering regulators construct several parallel and interactive genetic pathways. This integrative regulatory network has been proposed to create robustness as well as plasticity of the regulation. Although knowledge of key genes and their regulation has been accumulated, there still remains much to learn about how they are organized into an integrative regulatory network. Here, we have analyzed the *CRYPTIC PRECOCIOUS* (*CRP*) gene for the Arabidopsis counterpart of the MED12 subunit of the Mediator. A novel dominant mutant, *crp-1D*, which causes up-regulation of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), *FRUITFULL* (*FUL*) and *APETALA1* (*AP1*) expression in a *FLOWERING LOCUS T* (*FT*)-dependent manner, was identified in an enhancer screen of the early-flowering phenotype of 35S::*FT*. Genetic and molecular analysis of both *crp-1D* and *crp* loss-of-function alleles showed that MED12/CRP is required not only for proper regulation of *SOC1*, *FUL* and *AP1*, but also for up-regulation of *FT*, *TWIN SISTER OF FT* (*TSF*) and *FD*, and down-regulation of *FLOWERING LOCUS C* (*FLC*). These observations suggest that MED12/CRP is a novel flowering regulator with multiple regulatory target steps both upstream and downstream of the key flowering regulators including FT florigen. Our work, taken together with recent studies of other Mediator subunit genes, supports an emerging view that the Mediator plays multiple roles in the regulation of flowering.

Keywords: Arabidopsis • Flowering • *FLOWERING LOCUS C* • *FLOWERING LOCUS T* • Mediator • MED12.

Abbreviations: *AP1*, *APETALA1*; bZIP, ba region/leucine zipper; *CCT*, *CENTER CITY*; CDK, cyclin-dependent kinase; CycC, cyclin C; *CO*, *CONSTANS*; *CRP*, *CRYPTIC PRECOCIOUS*; EMS, ethylmethane sulfonate; *FLC*, *FLOWERING LOCUS C*; *FT*, *FLOWERING LOCUS T*; *FUL*, *FRUITFULL*; *GCT*, *GRAND CENTRAL*; GFP, green fluorescent protein; GUS, β-glucuronidase; *HEN*, *HUA ENHANCER*; *LFY*, *LEAFY*; *MAB*, *MACCHIBOU*; MED, Mediator complex subunit; MS, Murashige and Skoog; *NRT1.7*, *NITRATE TRANSPORTER 1.7*; *PFT*, *PHYTOCHROME AND FLOWERING TIME*; *PID*, *PINOID*; qRT–PCR, quantitative reverse transcription–PCR; RT–PCR, reverse transcription–PCR; 35S, Cauliflower mosaic virus 35S RNA promoter; SAM, shoot apical meristem; *SOC1*, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*; *STM*, *SHOOTMERISTEMLESS*; *SULTR2;1*, *SULFATE TRANSPORTER2; 1* promoter; *TSF*, *TWIN SISTER OF*

The nucleotide sequences reporte submitted to DDBJ under acce CCT/CRP locus (AB690343) and (AB690341 and AB690342).

Introduction

The plant life cycle is divided into distinct developmental phases by the morphological and functional features of the organs formed at the flank of the shoot apical meristem (SAM) (Araki 2001, Poethig 2003, Bäurle and Dean 2006). The proper timing of the transition from leaf-forming vegetative phase to flower-forming reproductive phase, called flowering, is especially important for reproductive success. Studies of Arabidopsis (*Arabidopsis thaliana*) have shown that regulation of flowering is orchestrated by inputs of multiple

Editor-in-Chief's choice

Plant Cell Physiol. 53(2): 287–303 (2012) doi:10.1093/pcp/pcs002, available FREE online at www.pcp.oxfordjournals.org

雑誌の紙面(Figure)

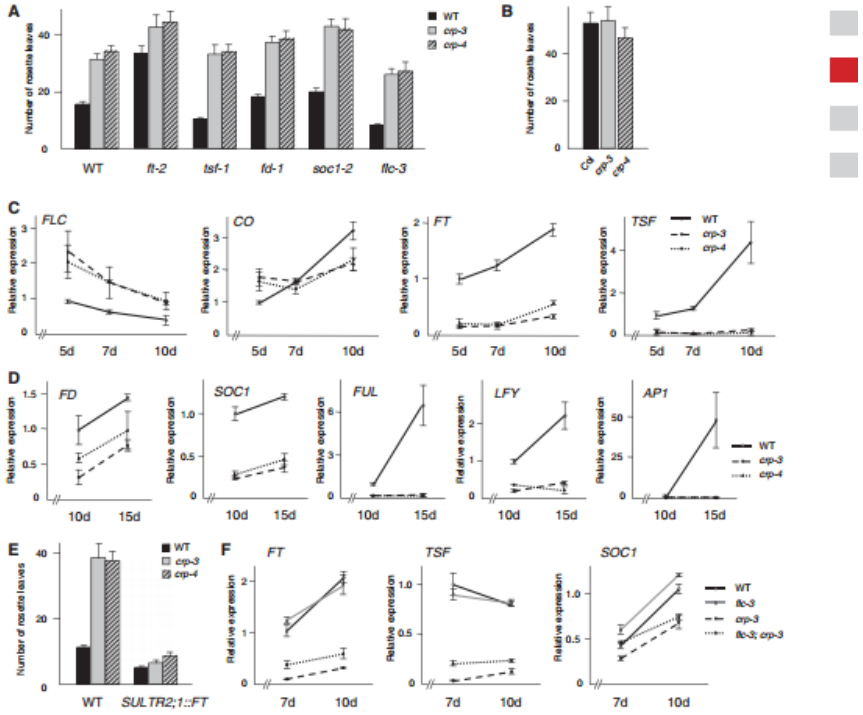


Fig. 2 Effect of *crp* loss-of-function mutations on the flowering phenotype. (A) Flowering time of *crp* loss-of-function mutants and flowering-time mutants with wild-type *CRP*$^+$ or *crp* loss-of-function alleles under long-day conditions. (B) Flowering time of *crp* loss-of-function mutants under short-day conditions. (C) *CO*, *FLC*, *FT* and *TSF* expression in wild-type Col, *crp*-3 and *crp*-4 plants. Aerial parts of seedlings grown under long-day conditions were harvested on days 5, 7 and 10 for qRT–PCR analysis. (D) *FD*, *SOC1*, *FUL*, *LFY* and *AP1* expression in wild-type Col, *crp*-3 and *crp*-4 plants. Shoot apical regions of seedlings grown under long-day conditions were harvested on days 10 and 15 for qRT–PCR analysis. (E) Flowering time of *crp* loss-of-function mutants with or without the *SULTR2;1::FT* transgene. There is a statistically significant difference (Student's *t*-test, $P < 0.005$) between *CRP*$^+$ and *crp* in the *SULTR2;1::FT* background. (F) *FT*, *TSF* and *SOC1* expression in wild-type Col, *flc*-3, *crp*-3 and *flc*-3; *crp*-3 plants. Plants were grown under long-day conditions and aerial parts were harvested on days 7 and 10 for qRT–PCR analysis. In A, B and E, the numbers of rosette leaves are the average of at least seven plants. Error bars indicate the SD. Additional data and statistics of the data are summarized in **Supplementary Tables S2–S4**. Error bars in C, D and F indicate the SEM ($n = 9$).

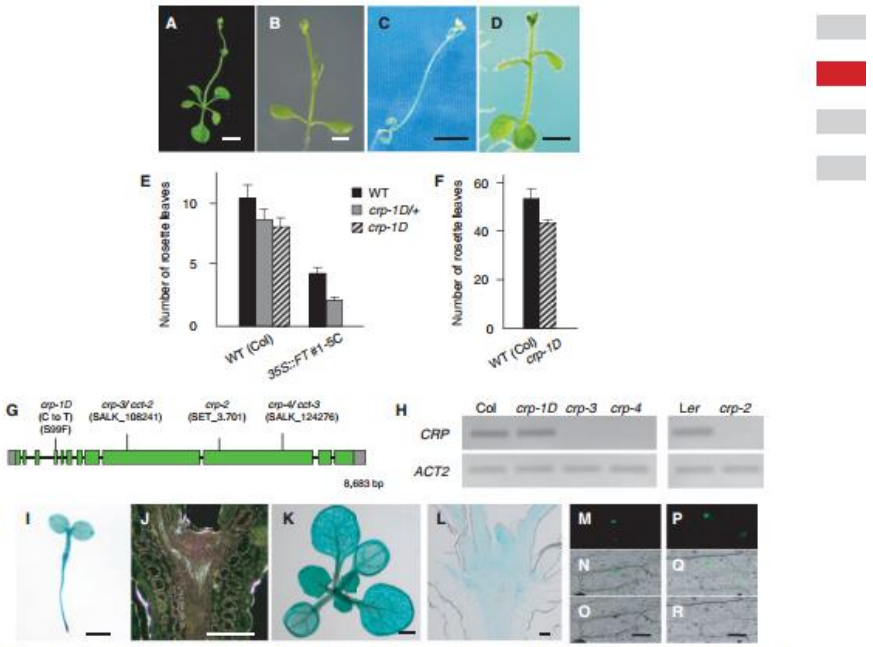


Fig. 1 Flowering phenotype of the *crp-1D* mutant and gene structure and expression patterns of *CRP*. (A–D) Effect of *crp-1D* and *soc1-101D* on the early-flowering phenotype of *35S::FT*. *35S::FT* #1-5C (A), *35S::FT* #1-5C/—; *crp-1D*/+ (B), *35S::FT* #1-5C; *crp-1D* (C) and *35S::FT* #1-5C; *soc1-101D* plants (D). 'Transgene symbol/—' and 'mutation symbol/+' indicate the hemizygote and heterozygote, respectively. (E) Flowering time of *35S::FT* #1-5C/—; *crp-1D*/+ plants under long-day conditions. There is a statistically significant difference (Student's *t*-test, $P < 0.005$) with *CRP*$^+$ (solid bars). (F) Flowering time of the *crp-1D* mutant under short-day conditions. There is a statistically significant difference (Student's *t*-test, $P < 0.005$) between the two genotypes. (G) *CRP* gene structure and positions of mutations and T-DNA or Ds insertions in *crp* alleles. Boxes and lines indicate exons and introns of *CRP*, respectively; gray and green boxes represent untranslated regions and coding regions, respectively. (H) Semi-quantitative RT–PCR analysis of *CRP* expression in the wild type and *crp* mutants. Plants were harvested on day 10 (Col background; left panel) or 7 (*Ler* background; right panel). (I–L) Spatial expression patterns of *CRP*. Whole-mount preparation and longitudinal section through the SAM of 3-day-old (I, J) and 10-day-old (K, L) *CRP::GUS* seedlings. Plants were grown under long-day conditions and subjected to GUS staining for 12–48 h. (M–R) Subcellular localizaton of *CRP* and crp-1D proteins. Onion epidermal cells were bombarded with GFP fusion constructs, *35S::CRP-GFP* (M–O) and *35S::crp-1D-GFP* (P–R). Dark field images (M, P), bright field images (N, Q) and merged images (O, R). Scale bars: 5 mm in A and C, 2 mm in B and D, 1 mm in I and K, and 0.1 mm in J, L, O and R. In E and F, the numbers of rosette leaves are the average of at least 11 plants. Error bars indicate the SD. Additional data and statistics of the data are summarized in **Supplementary Tables S1** and **S2**.

雑誌の紙面

MED12 and MED13 belong to the CDK8 module of Mediator which contains CDK8 and CycC subunits. There has been no report of a mutant phenotype and functional analysis of *CycC* orthologs in plants (Wang et al. 2004). In Arabidopsis, *HUA ENHANCER3* (*HEN3*) encodes the CDK8 subunit, and is expressed in proliferating tissues, such as the SAM, young leaves and floral buds (Wang and Chen 2004, Wang et al. 2004). However, *hen3* mutants do not show pleiotropic developmental defects such as those observed in *cct/crp* and *gct/mab2* mutants. HEN3 protein interacts with LEUNIG (LUG) and SEUSS (SEU), which repress *AG* and are involved in auxin signaling during floral organ development (Navarro et al. 2004, Pfluger and Zambryski 2004, Gonzalez et al. 2007). Interestingly, *crp* and *gct/mab2* mutants showed floral defects reminiscent of *lug* and *seu* mutants (Liu and Meyerowitz 1995, Franks et al. 2002, Gillmor et al. 2010) (Fig. 6G–J). It was reported that *gct; kan* double and *hen3; hua1; hua2* triple mutants showed enhanced loss of floral organ identity, although *gct/mab2* or *hen3* single mutants did not show major defects in floral organ specification (Wang and Chen 2004, Gillmor et al. 2010). These observations suggest that floral organ development may represent a shared developmental program regulated by the CDK8 module proteins of the Mediator possibly through modulation of the auxin response.

Does Mediator contribute to feed-forward loops in flowering regulation?

Flowering of monocarpic plants such as *A. thaliana* is an irreversible phase transition. It has been suggested that feed-forward loops constructed from key regulators are important to minimize external noises to achieve robustness of developmental systems (Mangan and Alon 2003, Dekel et al. 2005, Pastore et al. 2011). As summarized in a simplified model (Supplementary Fig. S7), MED12/CRP, and possibly also MED13/MAB2, are involved in multiple steps in the regulation of production of florigen (FT and TSF proteins) and in multiple downstream steps of florigen action. Similarly, it has been shown that a core subunit of the Mediator, MED25/PFT1, regulates multiple steps both upstream and downstream of FT florigen. These observations and the possible involvement of

ion of the *FLC*
ll et al. 2010)
Mediator may
a key factor
motif. Further
bunits of the
Mediator in flowering and id of transcription factors
involved will be important ste est this hypothesis.

Materials and Methods

Plant materials and growth conditions

Col-0 and *Ler* were used as wild types. T-DNA insertion alleles of *CRP* in the Col background (SALK_108241, *crp-3*; and SALK_124276; *crp-4*) were obtained from the Arabidopsis Biological Resource Center (ABRC). *crp-3* and *crp-4* were back-crossed with wild-type Col-0 three times prior to analysis. *crp-3* and *crp-4* correspond to *cct-2* and *cct-3*, respectively (Gillmor et al. 2010). A Ds-transposon insertion allele in the *Ler* background (SET_3.701; *crp-2*) was kindly provided by Dr. V. Sundaresan (Parinov et al. 1999), and was backcrossed with wild-type *Ler* three times prior to analysis. *crp-1D* was isolated as an enhancer mutation of the 35S::*FT* #1-5C in the Col background (Kobayashi et al. 1999). Mutagenesis was carried out by soaking seeds in 0.1% EMS for 16 h. The resulting M_1 population (5,000 seeds) was sown and self-fertilized, and the M_2 population was screened for enhancers of the early-flowering phenotype in constant light conditions (24°C). *crp-1D* L was obtained by five backcrosses with wild-type *Ler*. *CRP::GUS* and 35S::*CRP* (see below for the transgene construction) were generated in this work. The *ft-2* allele from the *Ler* accession was introgressed into Col by five backcrosses to generate *ft-2* (Col). Previously published plant materials used in this work are as follows: 35S::*FT* #11-1 (a strong line), 35S::*FT* #1-5C (a weak line), *SULTR2;1::FT* #1-a, 35S::*TSF* #4-1 (a weak line), 35S::*LFY* (DW151.2.5C), 35S::*AP1*, *soc1-101D*, *flc-3*, *tsf-1*, *fd-1* (Col). *soc1-2*, *ful-2*, *lfy-1*, *pid-3* are in the Col background; *mab2-1* and *pid-2* are in the *Ler* background. Further information is given in Supplementary Table S11.

For analysis of the flowering time phenotype, plants were grown on soil or half-strength Murashige and Skoog (MS; Wako) medium supplemented with 0.5% sucrose and 0.4% Gellan Gum (Wako) at 22°C under long-day (16 h light/8 h dark) conditions with white fluorescent light (~80 μmol m^{-2} s^{-1}) or short-day (8 h light/16 h dark) conditions with white fluorescent light (~100 μmol m^{-2} s^{-1}). Flowering time was measured by counting rosette and cauline leaves. Bars in the figures show the number of rosette leaves. For expression analysis, plants were grown on half-strength MS medium supplemented with 0.5% sucrose and 0.4% Gellan Gum. Seeds were stratified by keeping them at 4°C for 2–4 d and then transferred to 22°C long-day conditions, which was defined as day 0. Plants were harvested at Zeitgeber time (ZT) 15 on each day.

RT–PCR analysis

RNA was extracted using TRIzol reagent (Invitrogen) and was treated with RNase-free DNase I (Invitrogen) according to the manufacturer's instructions. Total RNA (0.5 μg) was reverse-transcribed in a 20 μl reaction mixture using SuperScript III (Invitrogen). After the reaction, 10 μl of the mixture was diluted with 240 μl of water, and 5 μl aliquots were analyzed. Quantitative RT–PCRs (qRT–PCRs) were performed using SYBR Premix Ex Taq II (TAKARA). Primers used in this study are listed in Supplementary Table S12. qRT–PCR results normalized to *ACT2*, *NRT1.7* or *STM* show the average of nine different reactions (biological × technical triplicate), except for the data shown in Fig. 3A (only technical triplicate). Relative expression was obtained as the ratio to the level in wild-type Col harvested on the first day of the experiment. Semi-quantitative RT–PCR was performed using the primers listed in Supplementary Table S13. PCR products were electrophoresed on an agarose gel and visualized by ethidium bromide staining.

Plasmid construction and transgenic plants

To construct *CRP::GUS*, the *GUS* coding sequence from pBI101 was inserted downstream of the 2.0 kb *CRP* promoter fragment (2.0 kb sequence upstream of the presumptive initiation ATG codon) amplified by PCR from Col using the primers listed in Supplementary Table S14. To clone *CRP* and *crp-1D* cDNA, the coding region of *CRP* from Col and *crp-1D*, respectively, was amplified by PCR using the primers listed in Supplementary Table S14 and recombined into the *Sal*I and *Xho*I sites of the pENTR1A vector (Invitrogen). To construct *CRP-GFP* and *crp-1D-GFP*, the coding region of sGFP was fused, in-frame, to the C-terminus of CRP or crp-1D in the pENTR vector as described above and, after sequencing, this translational fusion construct was cloned into the binary vector pGWB2 (Nakagawa et al. 2007) using LR Recombination Reactions (Invitrogen). To construct 35S::*CRP*, the *CRP* coding sequence in the pENTR1A vector was transferred to the binary vector pGWB2 (Nakagawa e ion Reactions (Invitrogen) the constructs we *tumefaciens* strain pM ed by the floral dip proce

GUS staining, histological analysis and microscopy

CRP::GUS line #13.4 was chosen for analysis. Samples were collected at ZT15 from plants grown in long-day conditions. For GUS staining, tissues were incubated at 4°C for 15 min in 90% acetone, rinsed with phosphate-buffered saline (PBS), infiltrated with staining solution (0.5 mg ml^{-1} X-Gluc, 100 mM sodium an-ide, 5 m vacuu s h in the c m-ples we For section eries, embedded in Technovit 7100 (Heraeus Kulzer) and sectioned at a thickness of 4 μm with a microtome.

For visualization of seedling vasculature, plants were fixed and rehydrated as described previously (Aida et al. 1997). Gynoecium morphology was observed using a scanning electron microscope (TM3000 Miniscope, Hitachi).

Particle bombardment and GFP fluorescence detection

35S::*CRP-GFP* and 35S::*crp-1D-GFP* were used as bioluminescent reporter constructs. These plasmid vectors were introduced into onion cells using the particle bombardment PDS-1000/He Biolistic Particle Delivery System (Bio-Rad). A 1 μg aliquot of plasmids was mixed with 8 μl of a pre-washed 1 μm diameter gold particle suspension (60 mg ml^{-1}), 3.3 μl of $CaCl_2$ (2.5 M) and 3.3 μl of spermidine (0.1 M). After incubation, the particles were washed with 80% ethanol and resuspended in 10 μl of 100% ethanol. The DNA-coated particles were fired into the onion cells using a 1,100 p.s.i. rupture disk. Onion cells were incubated in the dark at 23°C for 24–40 h after particle delivery and the epidermal cells were observed with a confocal laser scanning microscope (FV1000, Olympus), excitation wavelength 48 500–600 nm.

Suppleme

Supplementa

Funding

This work was supported by the Ministry of Education, Culture, Sport, Science and Technology of Japan [18370018 and 19060012 to T.A.]; the Japan Science and Technology Agency [the CREST program (to T.A.); the Mitsubishi Foundation [to T.A.].

Acknowledgments

We thank Dr. V. Sundaresan and the Arabidopsis Biological Resource Center for providing seeds of the Ds-insertion allele and T-DNA insertion alleles, respectively, Drs. Y. Daimon and Y. Tomita for excellent technical assistance and helpful discussions, and Dr. J. Ito and members of the Araki lab for discussion and comments. Thanks are also due to Dr. T. Oyama for the use of the particle bombardment facility.

References

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y. et al. (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* 309: 1052–1056.

Adrian, J., Farrona, S., Reimer, J.J., Albani, M.C., Coupland, G. and Turck, F. (2010) cis-Regulatory elements and chromatin state coordinately control temporal and spatial expression of *FLOWERING LOCUS T* in Arabidopsis. *Plant Cell* 22: 1425–1440.

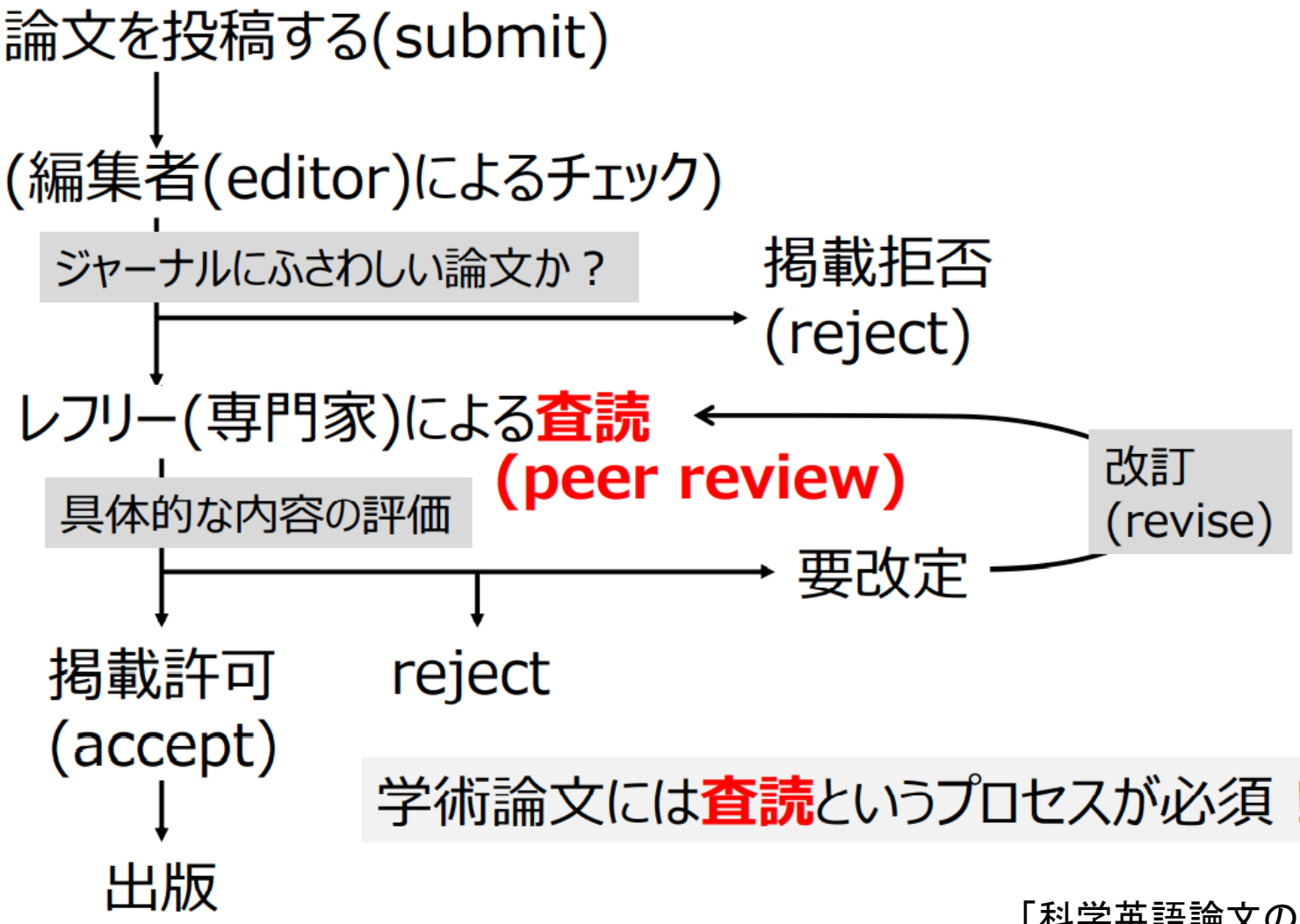
Aida, M., Ishida, T., Fukaki, H., Fujisawa, H. and Tasaka, M. (1997) Genes involved in organ separation in Arabidopsis: an analysis of the *cup-shaped cotyledon* mutant. *Plant Cell* 9: 841–857.

Araki, T. (2001) Transition from vegetative to reproductive phase. *Curr. Opin. Plant Biol.* 4: 63–68.

Atwell, S., Huang, Y.S., Vilhjálmsson, B.J., Willems, G., Horton, M., Li, Y. et al. (2010) Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature* 465: 627–631.

Autran, D., Jonak, C., Belcram, K., Beemster, G.T., Kronenberger, J., Grandjean, O. et al. (2002) Cell numbers and leaf development in

論文が出版されるまで



「科学英語論文の読み方入門」
2020, 1 大阪大学図書館

論文の読み方（読まれ方）

[　　]の研究において、[　　]が問題になっている。そこで[　　]を調べた。 対象
手法は[　　]で、
結果は[　　]であった。 実験・結果
すなわち、[　　]ということが明らかになった。 結論

わかってもらわないといけない‼

→ 伝わるように整理する。

次回までの課題

ポスター(の下書き)

① A3の紙に、項目の位置を決める。
② 実験のタイトル(仮説・命題)、結果(、考察)を１枠に書く。
(考察は仮説に対応しているか？
ad hocな仮説や正体不明の要素は含めない)

スライド(の下書き)

① A5の紙１枚に1スライド。
② 実験のタイトル(仮説)、結果、考察を書く。
③ 文字は20ポイント以上が望ましい。

ポスター・スライド作成の注意点

グラフ、表を見やすく！

＊グラフの縦軸・横軸を太くする
＊グラフの軸の目盛りを明確にする。
＊軸の項目・単位の文字を大きくする。

スライドは情報を最小限に！

＊スライド1枚あたり30秒～1分で説明する。
＊文章は最小限にする。

参考文献

岡本尚也 2017「課題研究メソッド」啓林館

酒井聡樹 2013「これから研究を始める高校生と指導教員のために」共立出版

戸田山 2011「『科学的思考』のレッスン」NHK出版

中谷宇吉郎 1958「科学の方法」岩波新書