Driving Innovation in Japan: Unveiling the Journey of 692 Academic Entrepreneurs

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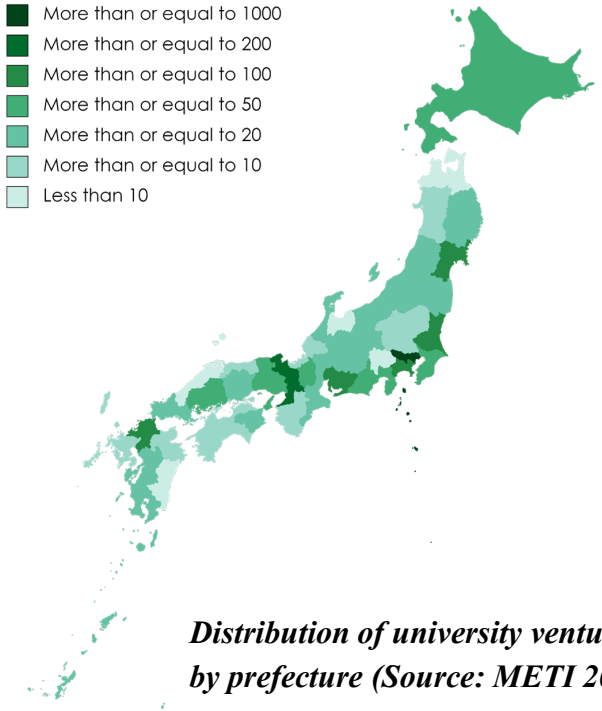
Overview



Distribution of university ventures in Japan by prefecture (Source: METI 2022)

Introduction

Research Project

Data

Methodology

Results

Discussion

Conclusion

Introduction

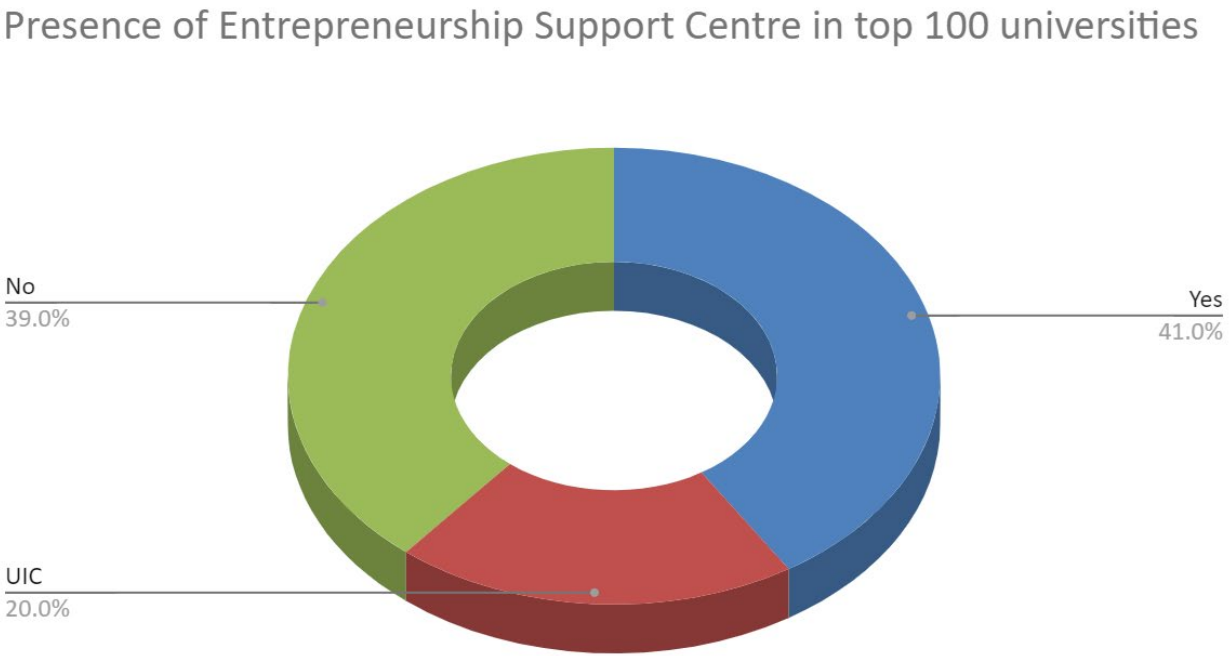
“Beyond Limits, Unlock our Potential” start-up strategies in 2019 (to enhance the start-up ecosystem of Japan)

Importance of academic start-ups in the **6th Science and Technology (STI) Basic Plan** (2021-2025)

Third pillar of Prime Minister Kishida’s “**New Capitalism**” focusing on investment in startups

Japan’s new **¥10 trillion (euro 65 billion)** national endowment fund to boost research in universities will go live at the start of the new financial year (April 2025), first selected candidate **Tohoku University**

Supporting Structures within top 100 Universities



Source: Oo and Carraz (2023)

Main forms of support include:

- Legal support
- Management consultation
- Business consultation
- Technical consultation
- Co-working space
- Entrepreneurship education
- Access to networks & mentors
- Networking events

Wait…

According to the 2021 Global Entrepreneurship Monitor (GEM) study, **16,52%** of working age **Americans**, **7,70%** of **French**, **6,27%** of **Japanese,** were actively engaged in starting a business or were the owner/manager of a business that is less than three years old (Total Entrepreneurial Activity - TEA).

Global Average (13,53%); Income level average(11,32%)

Source: https://www.gemconsortium.org

Attitudes towards Entrepreneurship?

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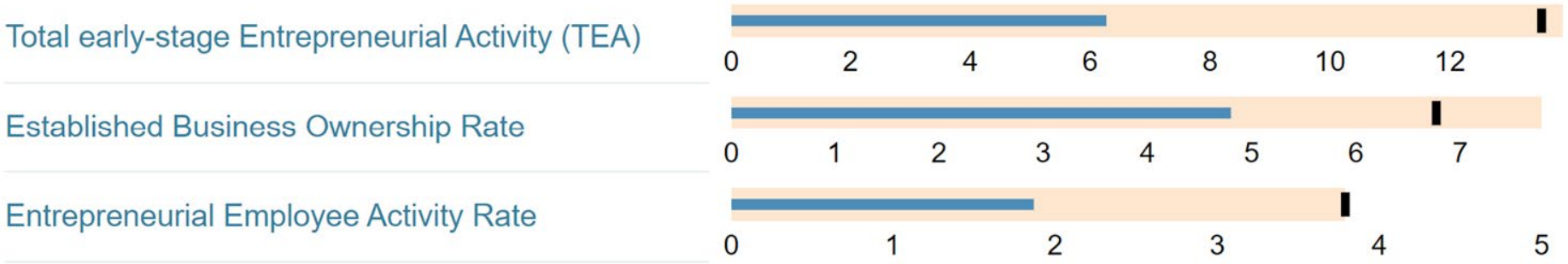
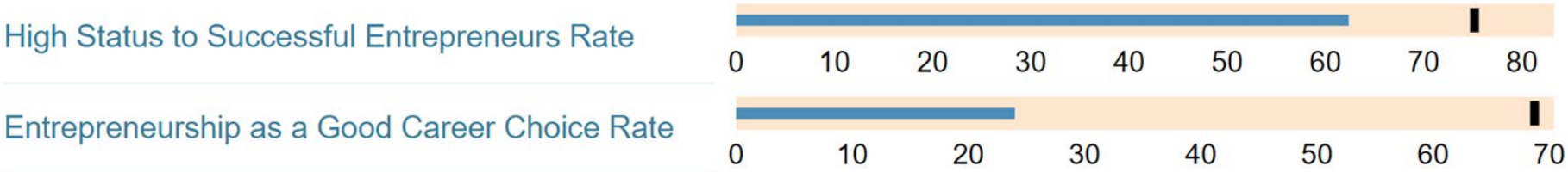
Attitudes towards Entrepreneurship?

Activity



Societal Values



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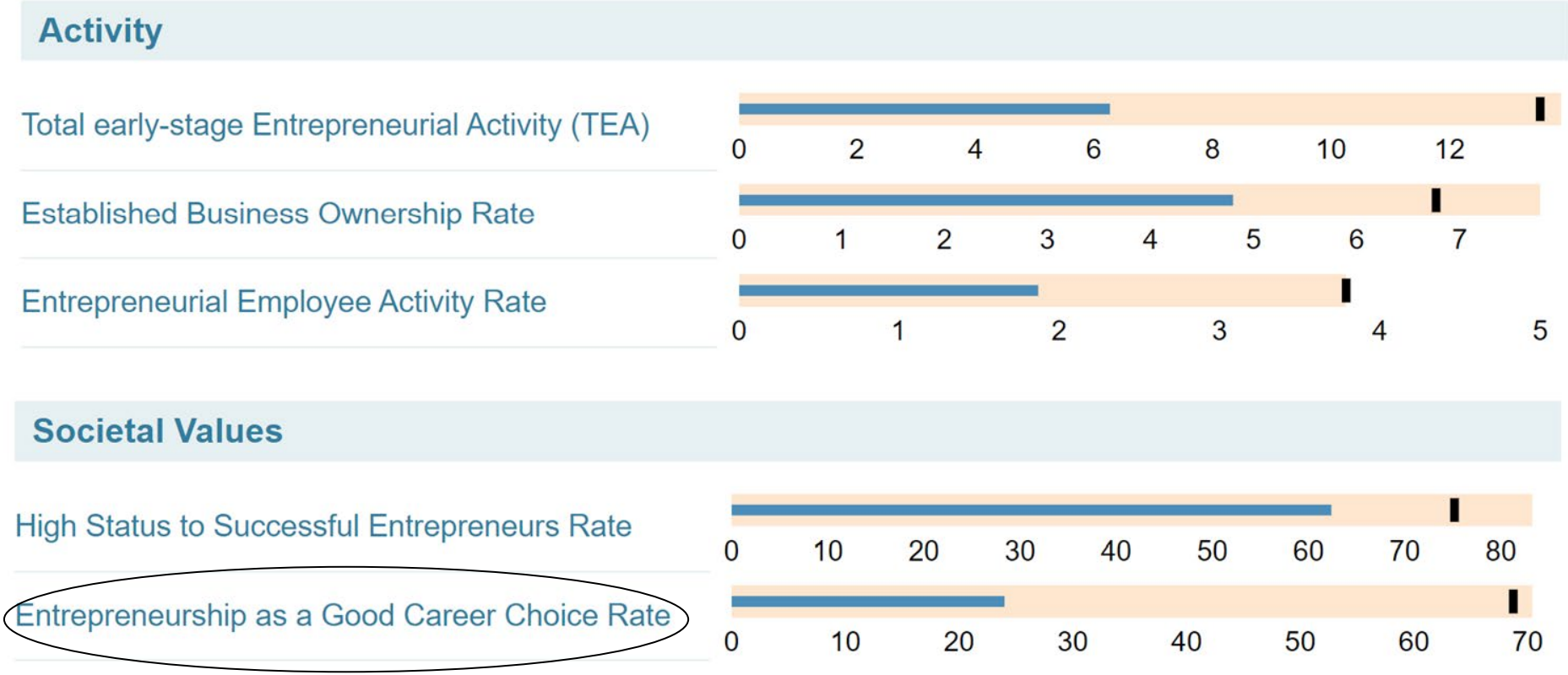
Attitudes towards Entrepreneurship?



Source: https://www.gemconsortium.org

Research Project

An In-depth Analysis of **Academic Entrepreneurship** in Japan: Understanding **Motivation**, **Impact**, and **Progress** in a Context of strong Institutional Support."

Data Collection

Bi-annual survey run by the METI

Creation of DB focused on individual entrepreneurial researchers

Origin of the project … 2 years ago

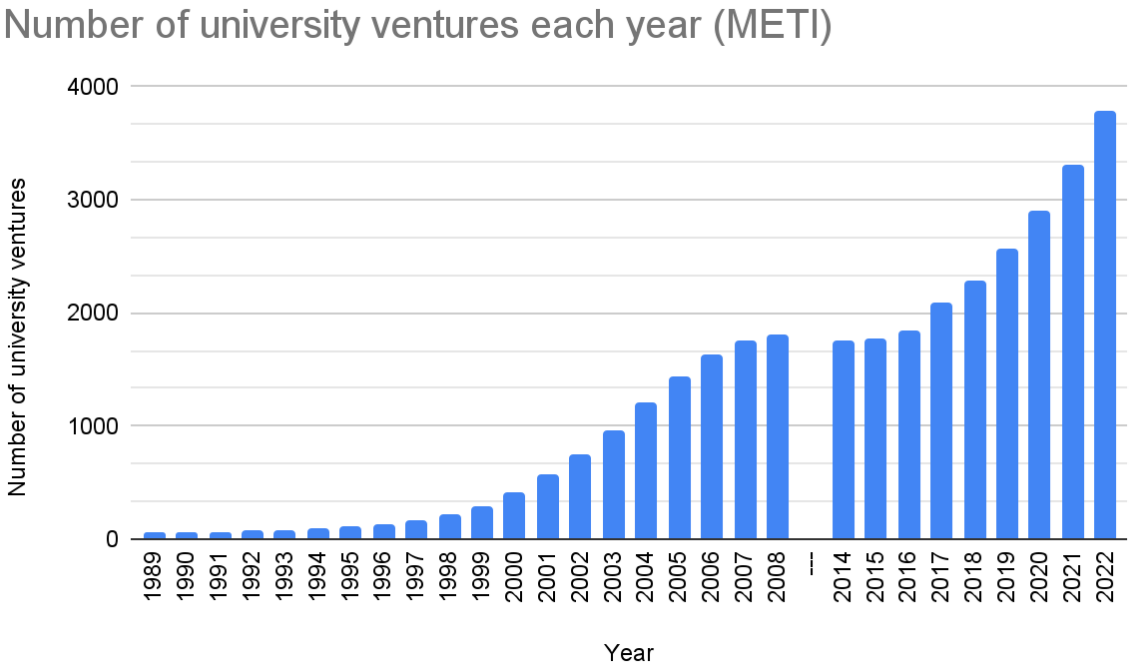
Data

METI
Ministry of Economy, Trade and Industry

University venture survey:

- Run since 1989
- Last edition 2022:
  3782 companies
- Receive data from universities and then send survey to companies,

Academic Entrepreneurship Survey (Total)

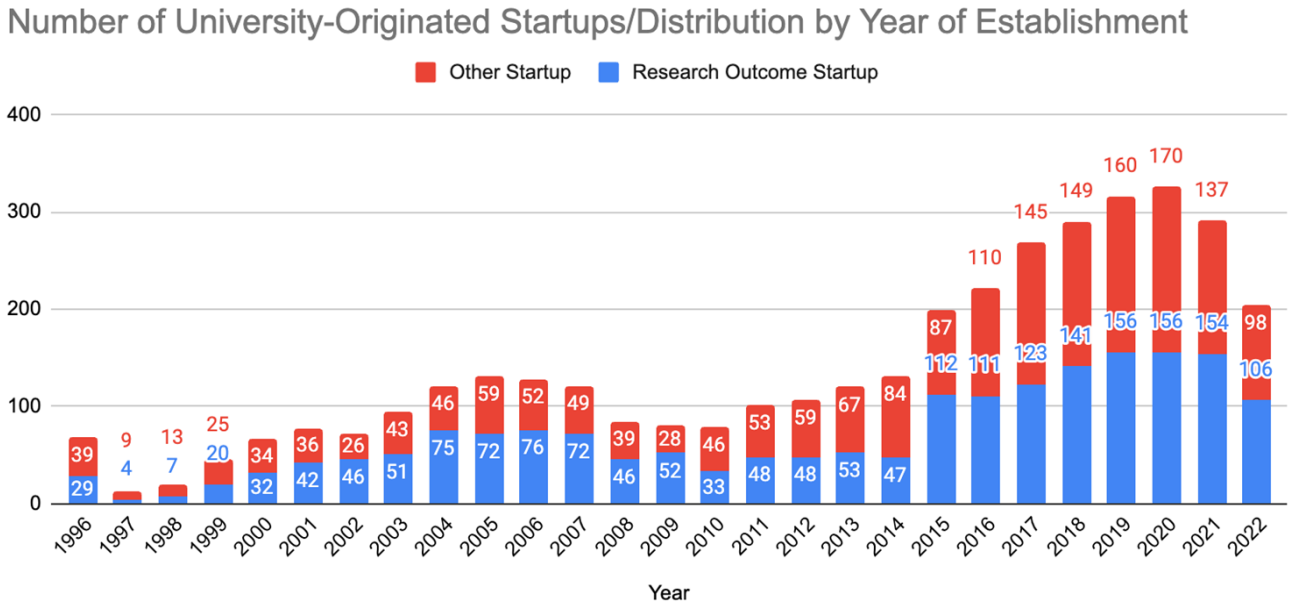


2001 **Hiranuma Plan**, to introduce **1,000** university ventures was met in 2004.

In 2022, there is a total of 3782 university ventures.

Source: MEITI (2022)

Academic Entrepreneurship Survey (Per year)



Starting off with a total of **68 university based ventures** ventures established in 1996, up to **326 in 2020** and **204 in 2022.**

50.71% were based on **research results**

Source: METI (2022)

Methodology

Researcher Database

METI
Ministry of Economy, Trade and Industry

JSPS 独立行政法人 日本学術振興会
Japan Society for the Promotion of Science

researchmap

OpenAlex

特許庁
JAPAN PATENT OFFICE

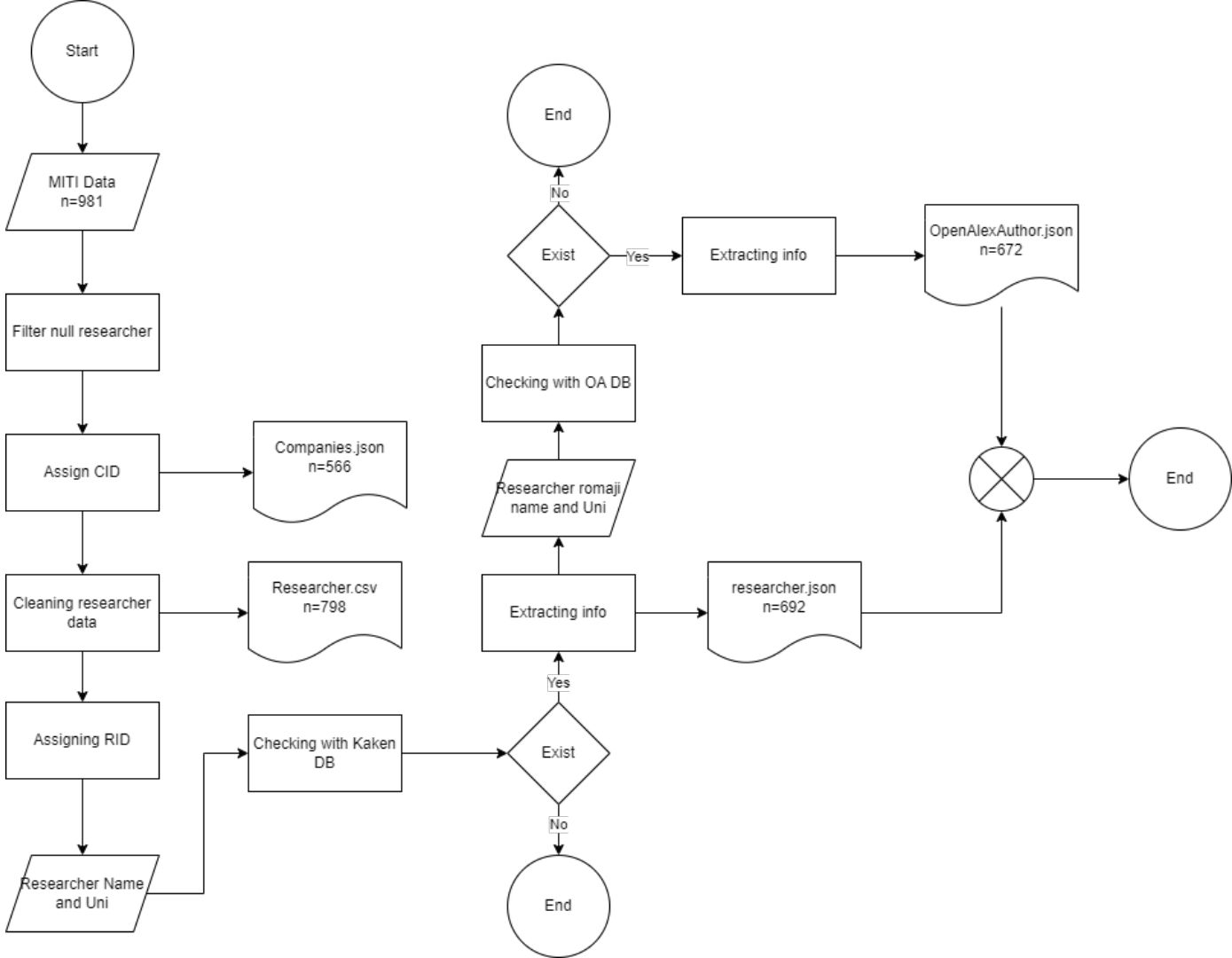
Extraction of academic entrepreneurs from METI database

- 798 individuals

Creating a database linking their information with:

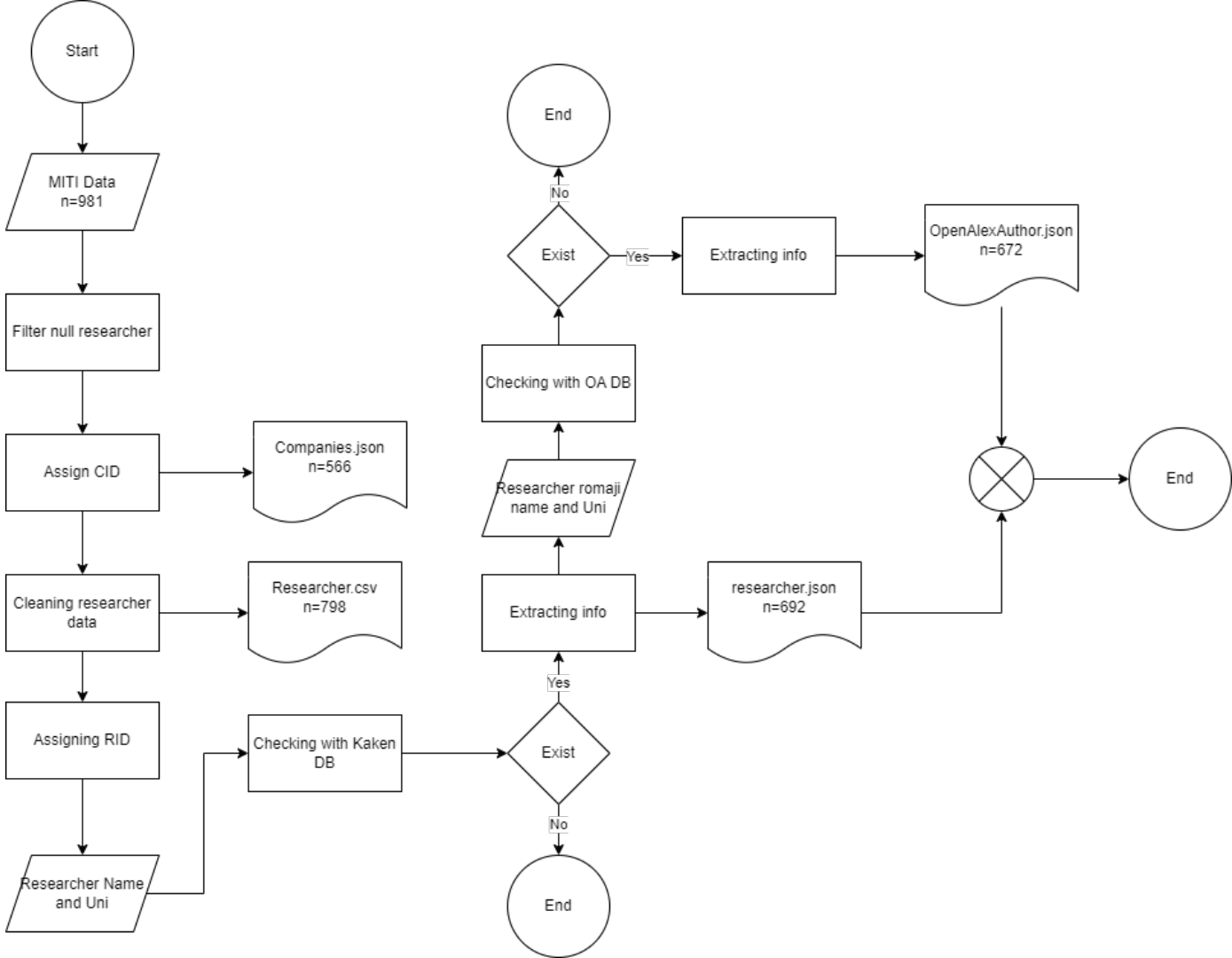
- Kakenhi (JSPS) | *Funding*
- Researchmap | *Career*
- OpenAlex | *Publication*
- JPO | *Patent*
- METI |

Flowchart Diagram



981 Companies

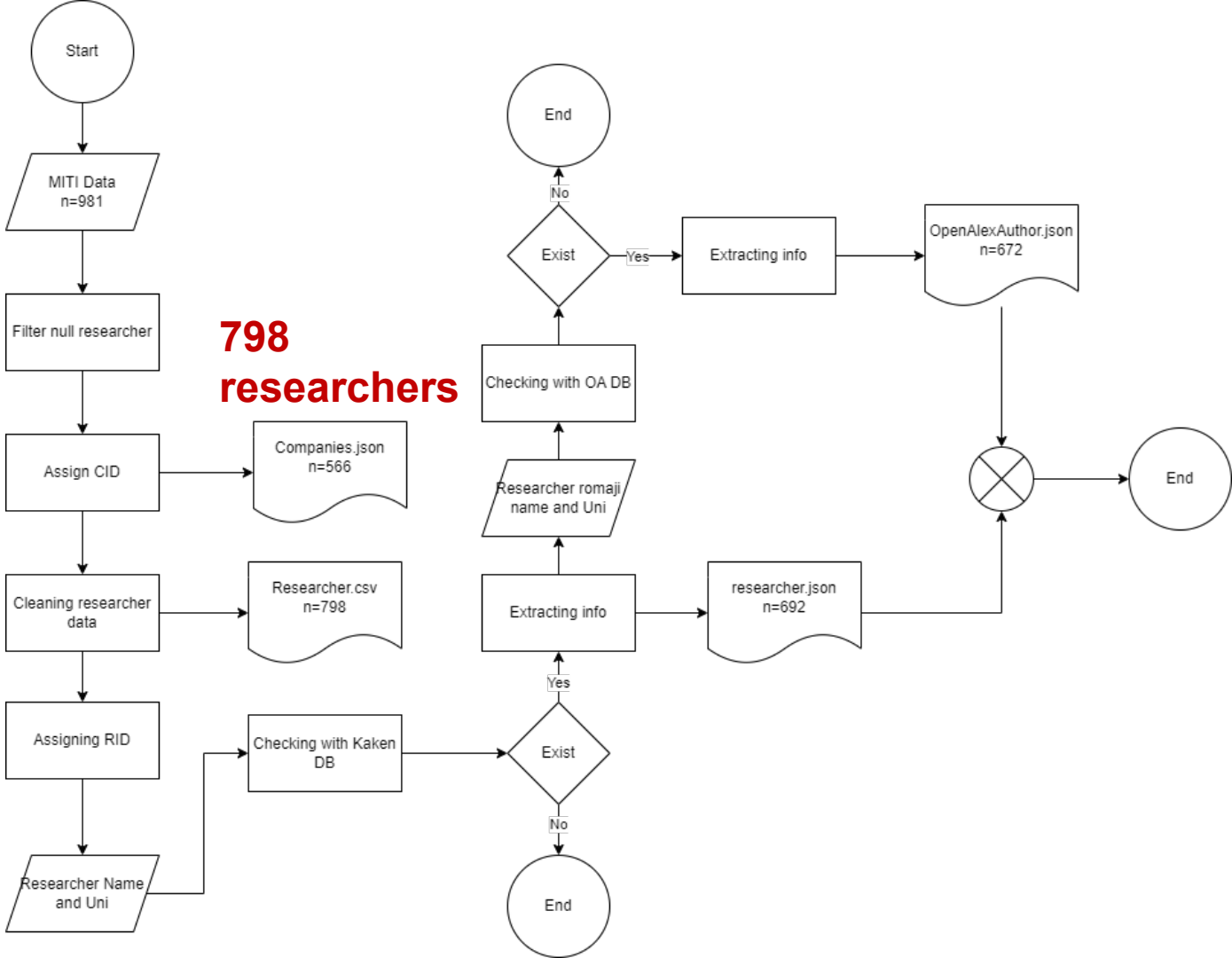
Flowchart Diagram



981 Companies

798 researchers

Flowchart Diagram

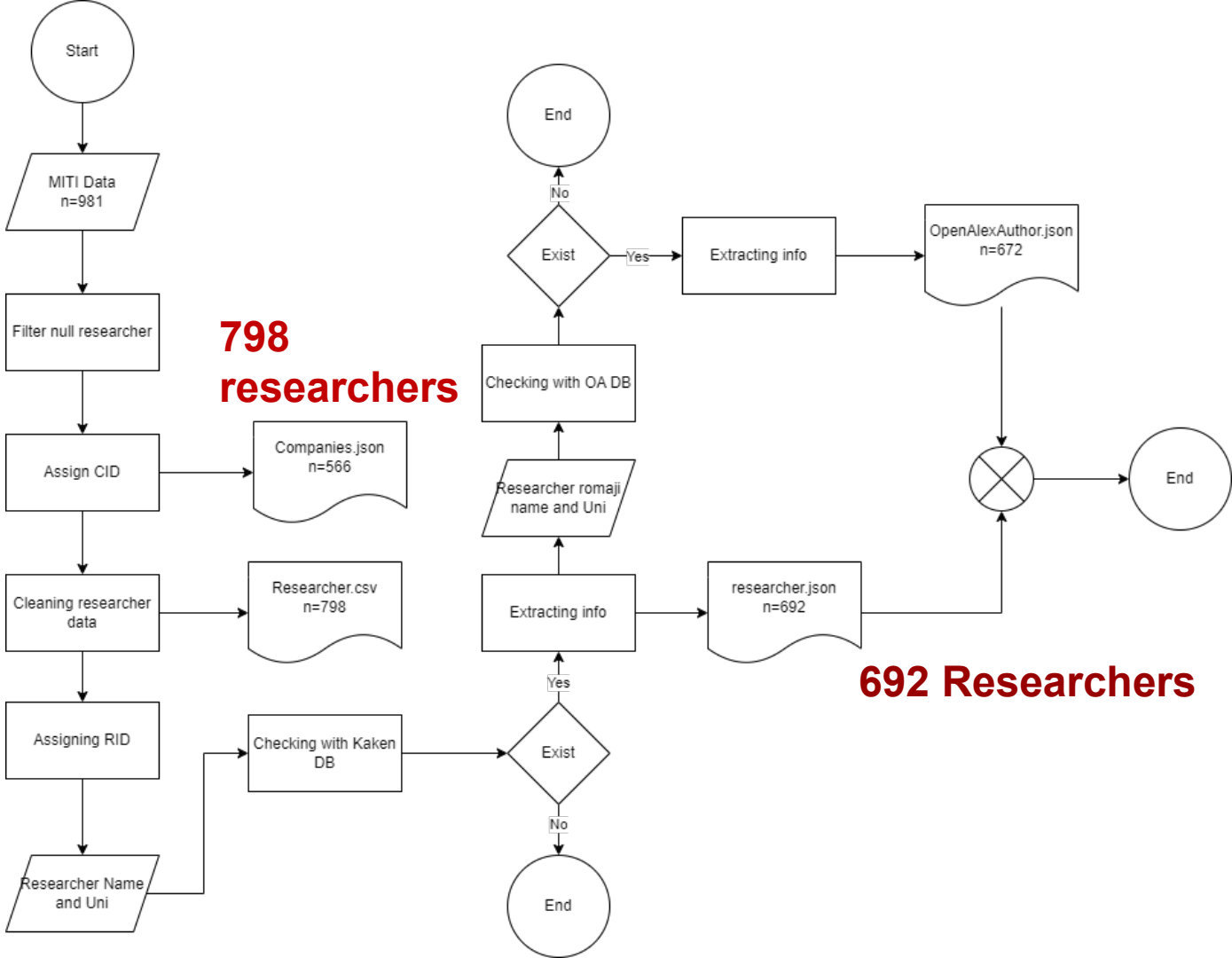


981 Companies

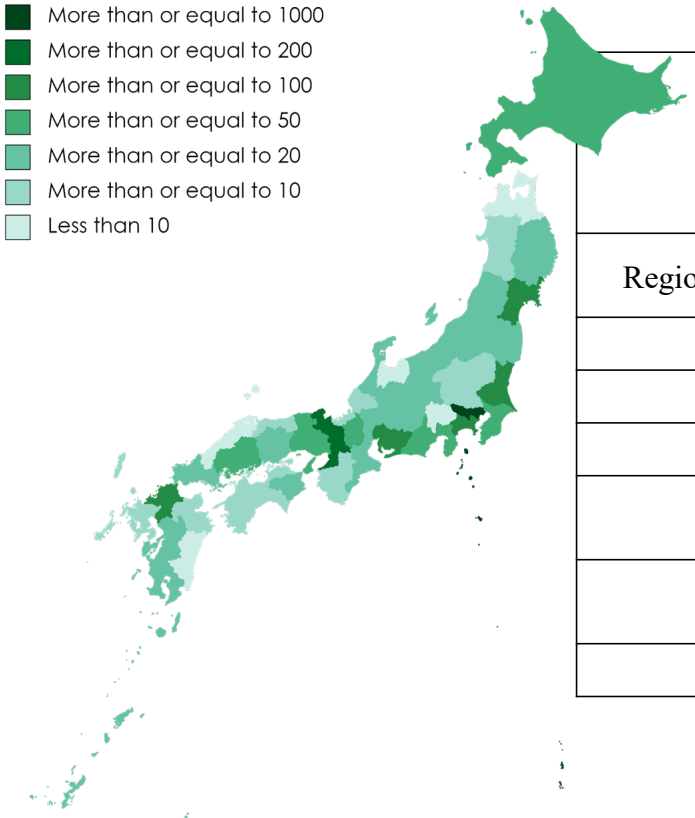
798 researchers

692 Researchers

Flowchart Diagram

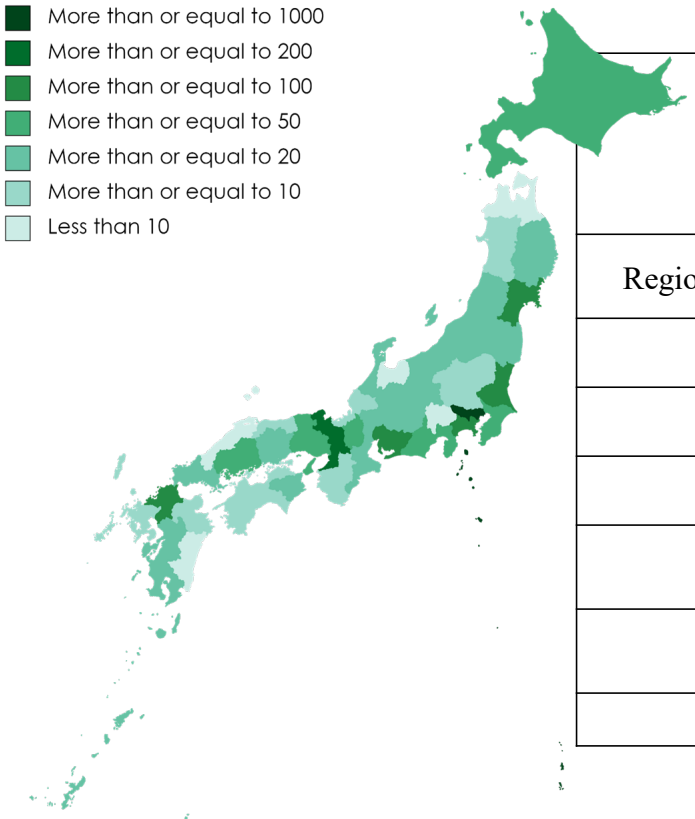


Representativeness of our Sample



Variable		Population Percentage (n=3792)	Database Percentage (n=979)	Academic Inventor Percentage (n=508)
Region	Tohoku-Hokkaido	8.45%	11.63%	12.60%
	Kanto	51.52%	36.73%	33.07%
	Chubu	6.66%	12.76%	13.39%
	Kinki	18.44%	20.92%	20.08%
	Chugoku-Shikoku	5.97%	4.90%	7.28%
	Kyushu-Okinawa	8.16%	10%	10.83%
	Unknown	0.79%	3.06%	2.76%

Representativeness of our Sample



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	Kinki	**18.44%**	20.92%	**20.08%**
	Chugoku-Shikoku	5.97%	4.90%	7.28%
	Kyushu-Okinawa	8.16%	10%	10.83%
	Unknown	0.79%	3.06%	2.76%

Representativeness of our Sample (2)

Variable		Population Percentage (n=3792)	Database Percentage (n=979)	Academic Inventor Percentage(n=508)
Types of Relationship	Research Outcome Venture	50.60%	51.58%	69.49%
	Joint Research Venture	9.30%	14.50%	14.17%
	Technology Transfer Venture	2%	1.32%	1.57%
	Student Venture	24.60%	11.54%	2.17%
	Related Venture	12.70%	6.13%	4.13%
	Unknown	0.80%	14.91%	8.46%

Representativeness of our Sample (2)

Variable		Population Percentage (n=3792)	Database Percentage (n=979)	Academic Inventor Percentage(n=508)
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	Student Venture	**24.60%**	11.54%	**2.17%**
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Academic Startup Age

Variable		Population Percentage (n=3792)	Database Percentage (n=979)	Academic Inventor Percentage (n=508)
Age of Company	0-3 years	-	17%	11%
	4 - 10 years	-	43%	46%
	11- 20 years	-	26%	28%
	21 - 30 years	-	8%	7%
	>31 years	-	1%	0%
	Unknown	-	5%	8%

Academic Startup Age

Variable		Population Percentage (n=3792)	Database Percentage (n=979)	Academic Inventor Percentage (n=508)
Age of Company	0-3 years	-	17%	11%
	4 - 10 years	-	43%	**46%**
	11- 20 years	-	26%	28%
	21 - 30 years	-	8%	7%
	>31 years	-	1%	0%
	Unknown	-	5%	8%

Area and Field of Business

Variable		Population Percentage (n=3792)	Database Percentage (n=979)	Academic Inventor Percentage(n=508)
Field	Other	35.38%	16.96%	14.17%
	Manufacturing (excluding IT hardware)	16.14%	7.35%	8.46%
	Natural science such as chemistry and materials (excluding bio-tech field)	7.32%	5.41%	6.89%
	IT (Hardware)	8.59%	12.67%	12.40%
	Bio/Healthcare Medical devices	30.43%	31.36%	37.99%
	Environmental Technology Energy	8.51%	6.84%	7.68%
	IT (applications, software)	30.97%	9.91%	5.31%
	Unknown	-	9.50%	7.09%

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	Bio/Healthcare Medical devices	**30.43%**	**31.36%**	**37.99%**
	Environmental Technology Energy	8.51%	6.84%	7.68%

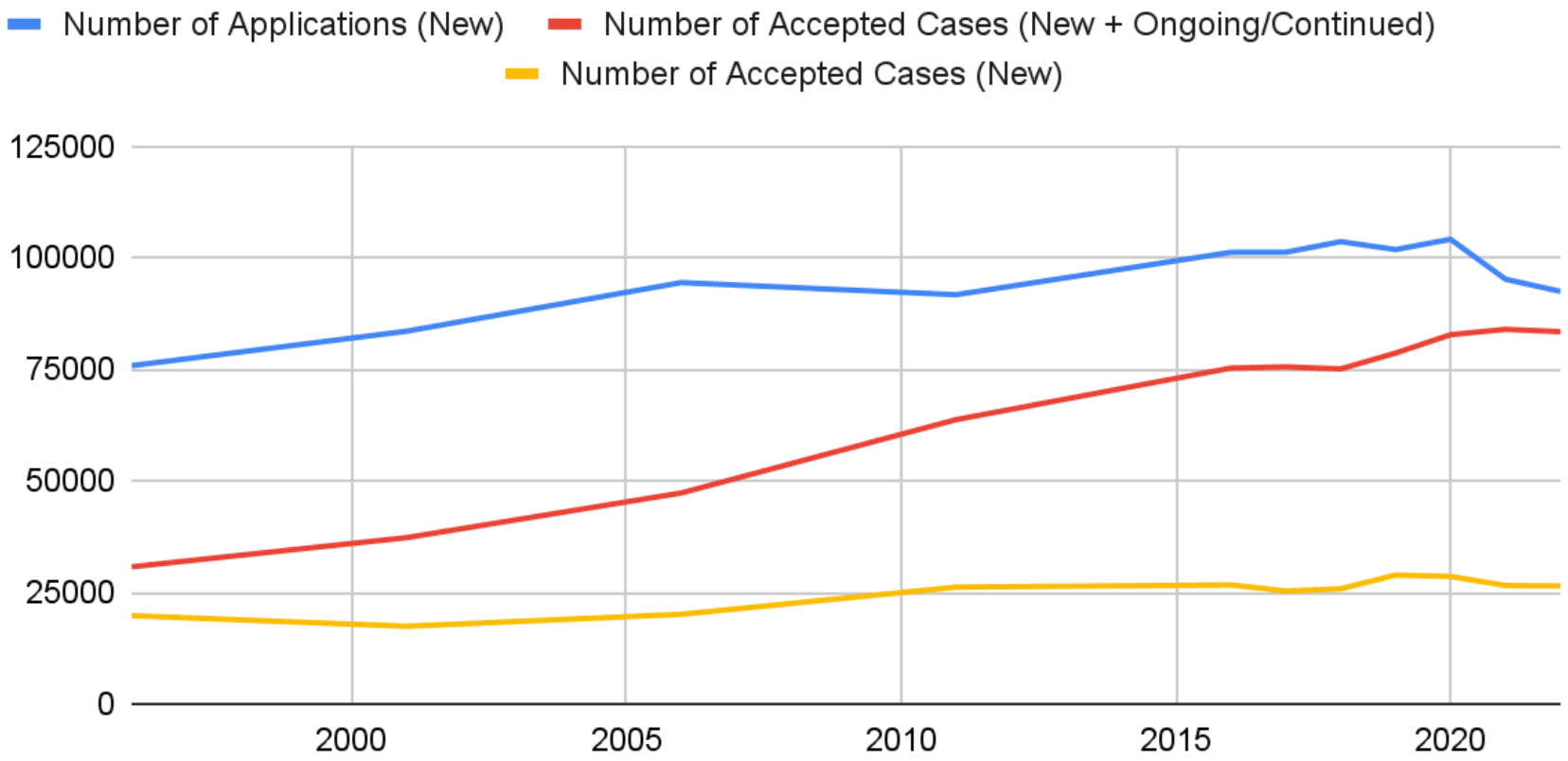
Descriptive Statistics

Descriptive Statistics

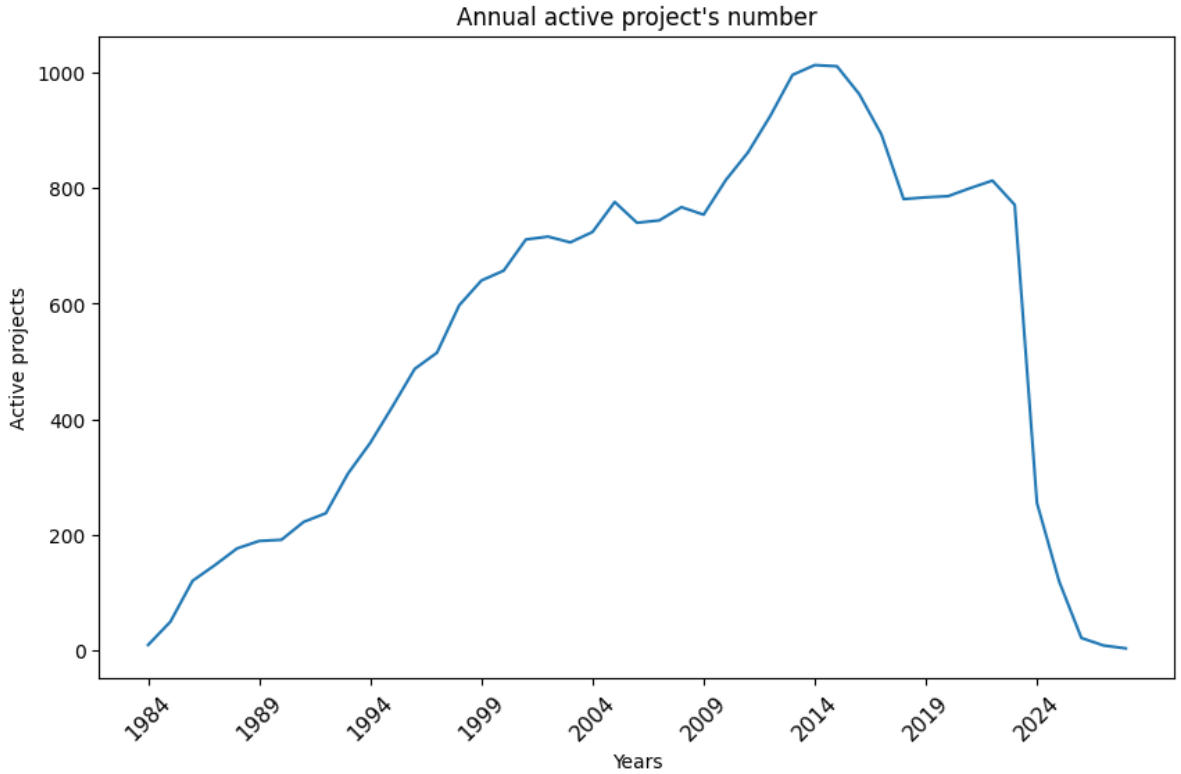
Grant-in-Aid for Scientific Research (Kaken Grants)

Money (1 euro = 160 Yen)

Trends in the number of applications, the number of applications adopted for Grants-in-Aid for Scientific Research



Kaken Grants Annual Active Project



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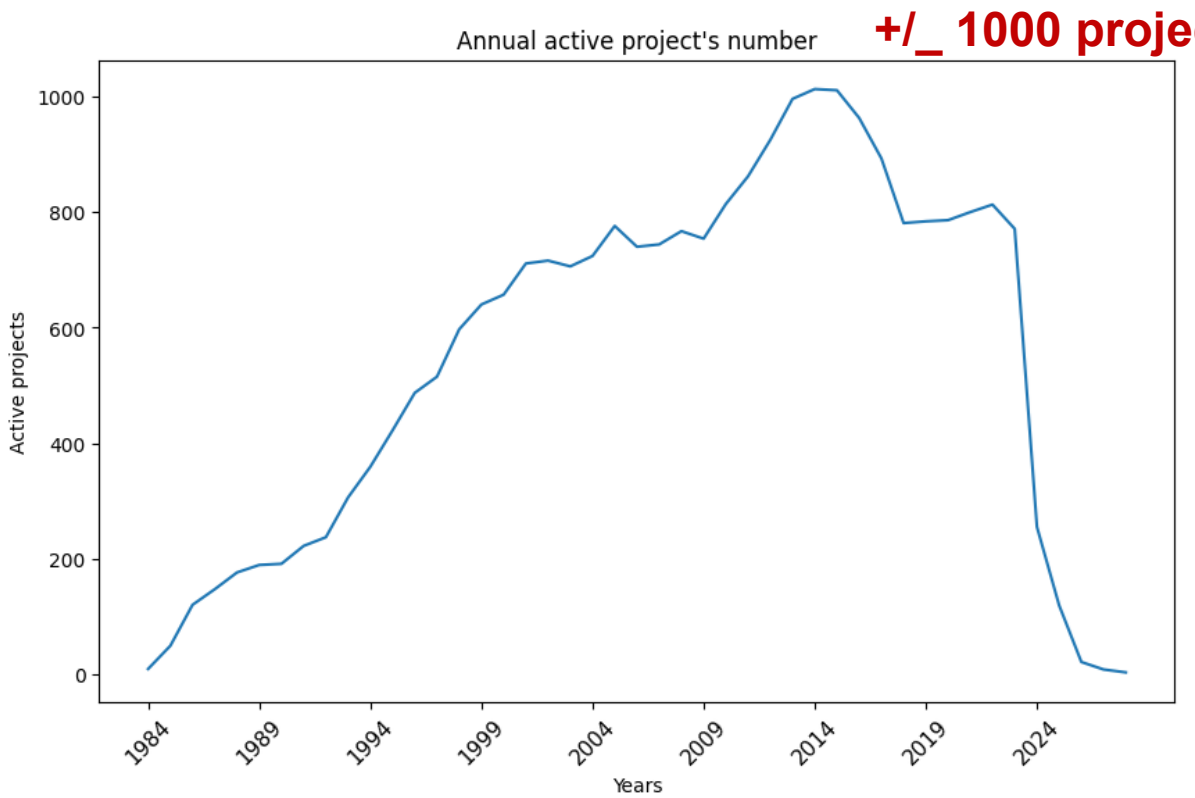
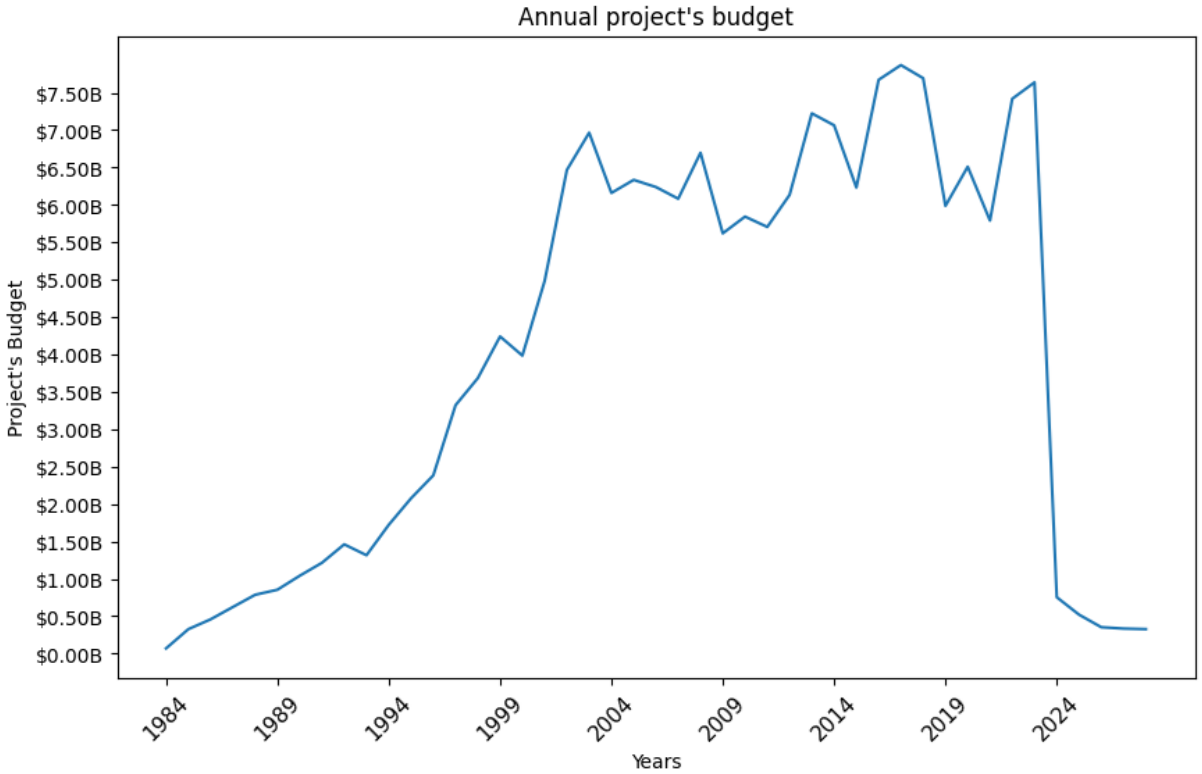
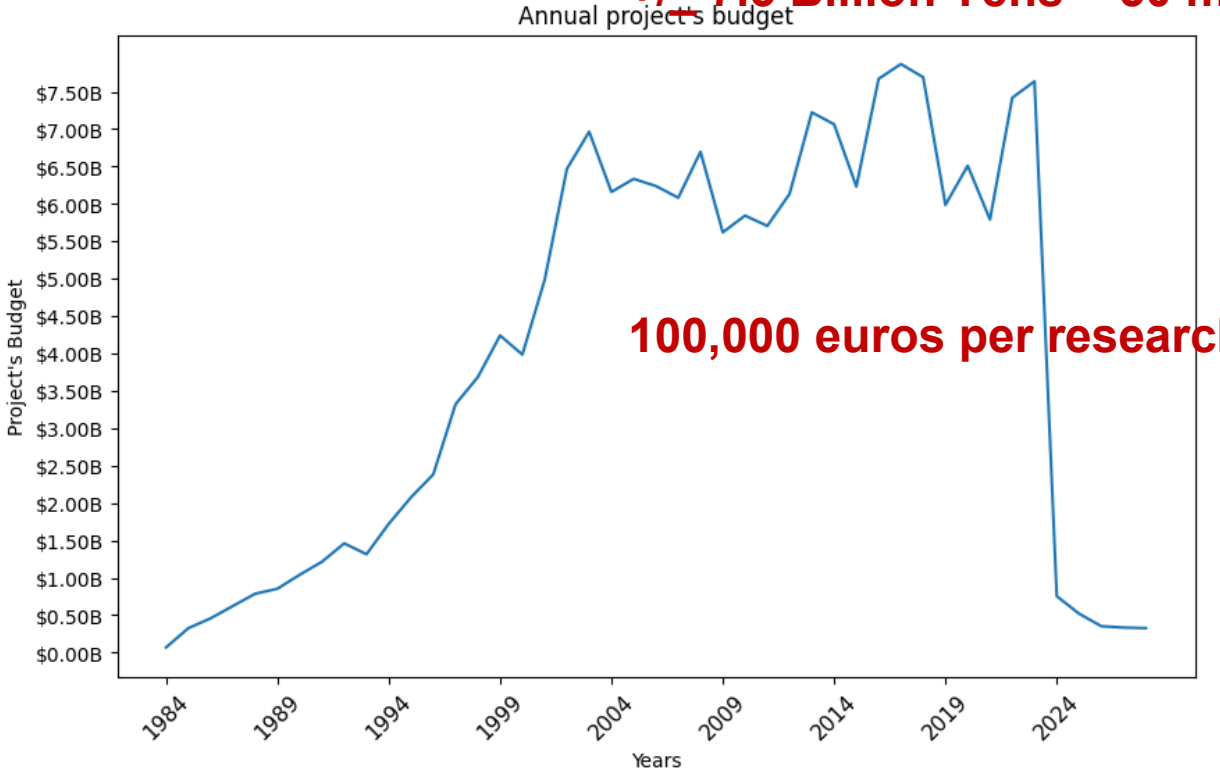


Kaken Grants: Annual Project Budget



Kaken Grants: Annual Project Budget

+/_ 7.5 Billion Yens = 50 million euros



100,000 euros per researcher

Network: Number of co-investigators

Total number of co-investigators	Average number per Academic Inventor	Standard Deviation	Min	Max
15 495	26.71	33.71	0	321

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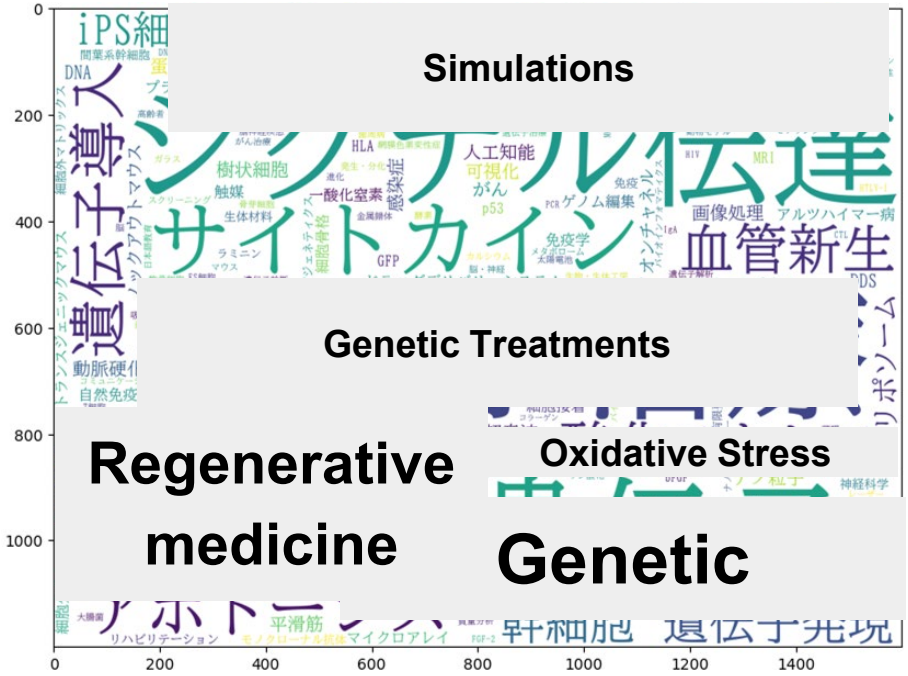
Kaken Grants Word Cloud

Kaken Word Cloud



Descriptive Statistics

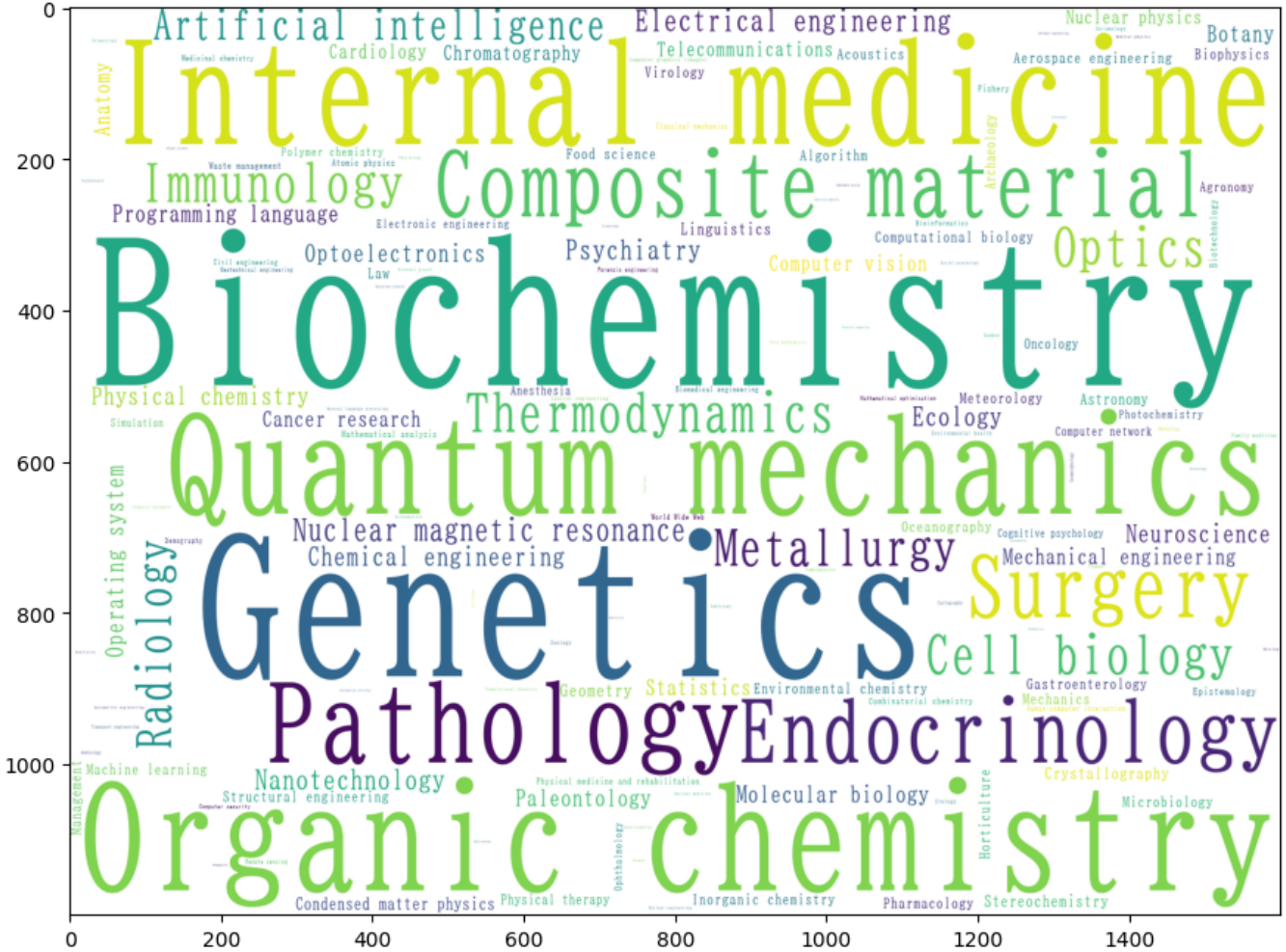
OpenAlex _ Open source bibliographic database

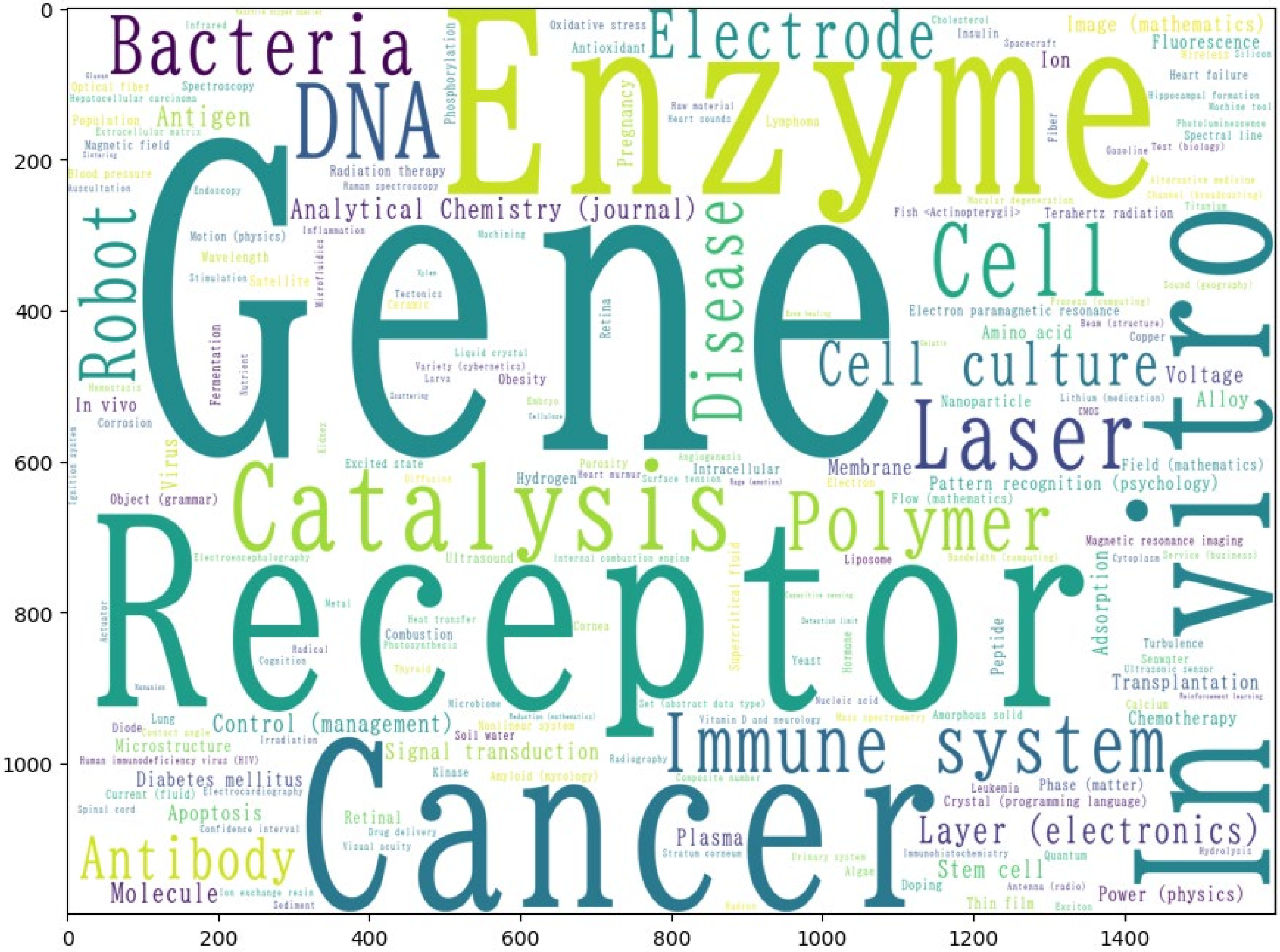
Publications

Year	Works	AVG Work (n=672)
2012	8070	12.01
2013	8410	12.51
2014	7970	11.86
2015	7789	11.59
2016	9075	13.50
2017	8881	13.22
2018	8131	12.10
2019	8552	12.73
2020	8214	12.22
2021	7787	11.59
2022	6477	9.64
2023	7110	10.58

Field	Frequency
Biology	627
Physics	588
Chemistry	578
Medicine	484
Engineering	391
Computer science	365
Materials science	317
Mathematics	257
Psychology	222
Geology	192
Geography	92
Economics	74
Philosophy	70
Environmental science	38
Political science	32
Sociology	23
History	22
Business	17
Art	9
Total	4398

Field	Frequency
Biology	14.26%
Physics	13.37%
Chemistry	13.14%
Medicine	11.01%
Engineering	8.89%
Total	100%





Very productive researchers: 12+ publications per year

Biology/Medecine/Materials Science/ Simulation/AI

Well funded: 100,000 euros per year

Big research teams: +/- 30 co-investigators (kaken)

Preliminary results

Source: METI (2022)

3 strategies

1. Interview of researchers
2. Productivity of researchers pre & post creation of a company
3. Patent-Paper Pairs

Interviews

Researchers	Duration	Average	Period
17 *(31)*	544 min	32 min	December 22 - Mai 23

Interview Analysis

Qualitative coding method (Eisenhardt 1989 and Saldaña 2009) :

- **Identified keywords** in transcriptions related to research questions and literature review
- Summarized keywords into **categories** such as **scientific productivity**, **commercial activity**, and **trade-off** between **entrepreneurial activities** and **academic activities.**
- **Organized categories** into themes informed by literature, providing a framework for data analysis.

Researcher Quadrant

		Considerations of use?	
		Local	Global
Quest for scientific understanding?	Global		
	Local		

Source: Adapted from Donald E. Stokes, *Pasteur's Quadrant*, p. 73

Researcher Quadrant

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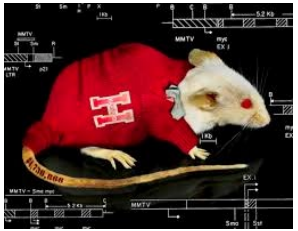
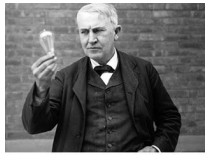
Quest for scientific understanding?	Global		
	Local		  Thomas Alva Edison & the lightbulb

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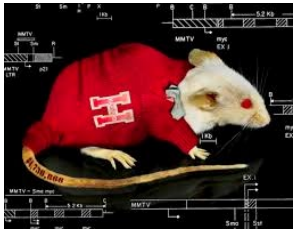
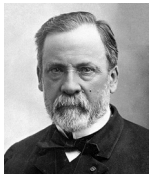
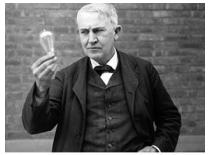
Quest for scientific understanding?	Global		  
	Local		  Thomas Alva Edison & the lightbulb

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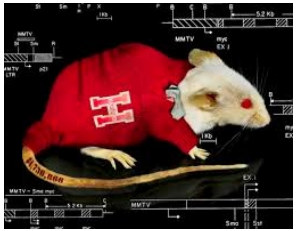
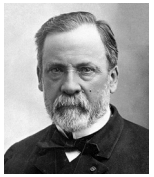
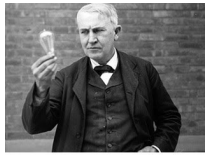
Quest for scientific understanding?	Global		  
	Local		  Thomas Alva Edison & the lightbulb

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Researcher Quadrant: Scientific Productivity

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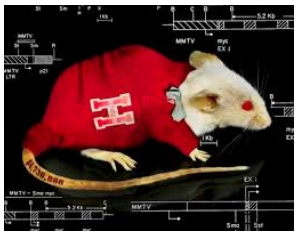
Quest for scientific understanding?	Global		
	Local		

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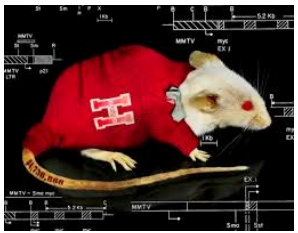
Quest for scientific understanding?	Global		
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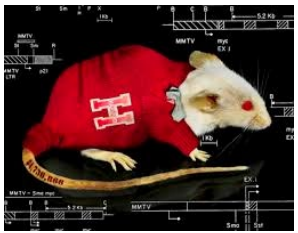
Quest for scientific understanding?	Global		
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Researcher Quadrant: Scientific Productivity

		Considerations of use?	
		Local	Global
Quest for scientific understanding?	Global		
	Local	ESCAPE GAME	

Source: Adapted from Donald E. Stokes, *Pasteur's Quadrant*, p. 73

Productivity Before/After Startup Involvement

Paired sample *T* tests

	Average Before	Average After	Pr > |t|
Publications 2 years	12.117	12.009	0.71
Publications 5 years	11.761	10.689	0.0001
IF 2 years	1.201	0.774	0.0001
IF 5 years	1.268	0.597	0.0001
CIF 2 years	0.010	0.006	0.0001
CIF 5 years	0.012	0.005	0.0001

Productivity Before/After Startup Involvement

Paired sample *T* tests

	Average Before	Average After	Pr > |t|
Publications 2 years	12.117	12.009	**0.71**
Publications 5 years	11.761	10.689	0.0001
IF 2 years	1.201	0.774	0.0001
IF 5 years	1.268	0.597	0.0001
CIF 2 years	0.010	0.006	0.0001
CIF 5 years	0.012	0.005	0.0001

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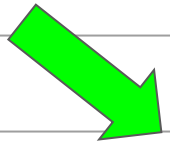

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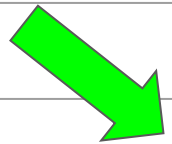


Bikard and Marx (2020)

Follow-up research

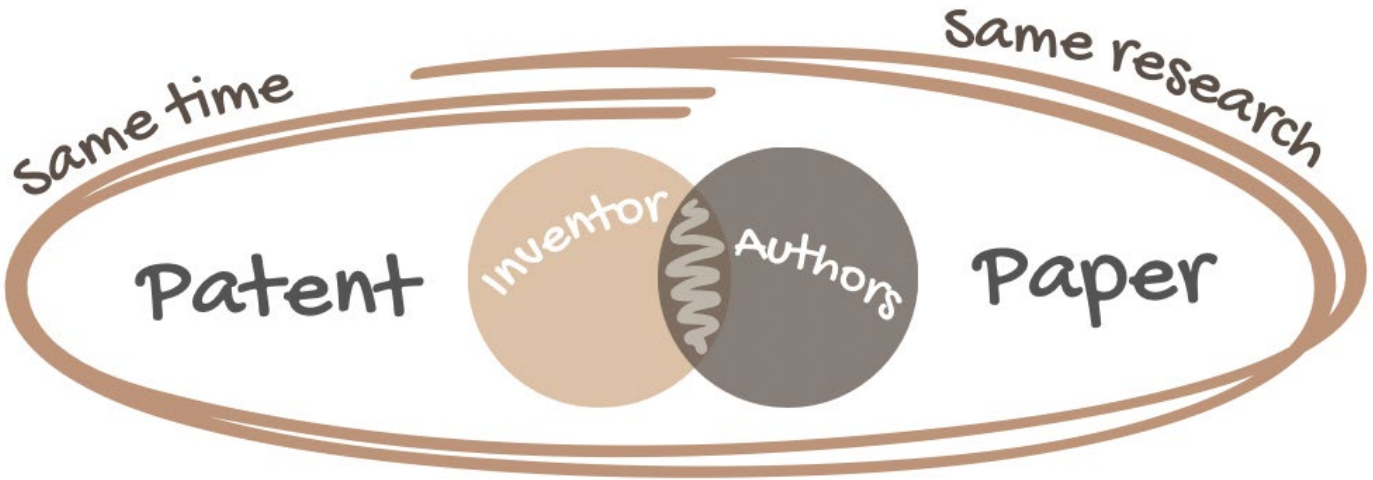
Negative impact on research productivity

But…need to perform ..

Multivariate analysis: Finance; IF&CIF; Gender/Position; Discipline; Type of companies; Patent Activity

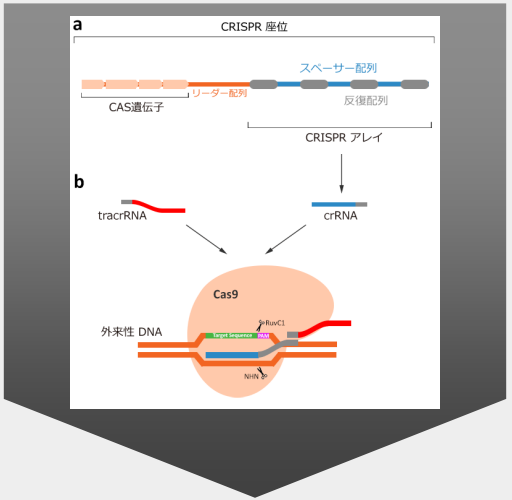
Patent-Paper-Pairs (PPP): In a Nutshell

PPPs are formed when ***a scientific discovery*** described in a ***research paper*** is at the ***same time*** ***granted as patent***, *thus indicating the convergence of scientific and technical concepts.*



Jennifer Doudna

Discovery - 2011
Nobel Prize - 2020



CRISPR-Cas9 - a technology for editing DNA with unprecedented precision and efficiency. It opened up novel and wide-ranging possibilities across medicine, biology and agriculture.

RNA-guided complex from a bacterial immune system enhances target recognition through seed sequence interactions

Blake Wiedenheft[a,b], Esther van Duijn[c,1], Jelle B. Bultema[d,1], Sakharam P. Waghmare[e,1], Kaihong Zhou[a,1], Arjan Barendregt[c], Wiebke Westphal[b], Albert J. R. Heck[c], Egbert J. Boekema[d], Mark J. Dickman[e], and Jennifer A. Doudna[a,b,f,g,2]

[a]Howard Hughes Medical Institute; [b]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720; [f]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; Department of Chemistry, University of California, Berkeley, CA 94720; [d]Department of Chemistry, University of California, Berkeley, CA 94720; [e]Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research, Utrecht Institute for Pharmaceutical Sciences, and The Netherlands Proteomics Center, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands; [c]Electron Microscopy Group, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands; and [g]Department of Chemical and Biological Engineering, ChELSI Institute, University of Sheffield, Mappin Street, Sheffield S1 3JD, United Kingdom

Contributed by Jennifer A. Doudna, February 24, 2011 (sent for review January 5, 2011)

Prokaryotes have evolved multiple versions of an RNA-guided adaptive immune system that targets foreign nucleic acids. In each case, transcripts derived from clustered regularly interspaced short palindromic repeats (CRISPRs) are thought to selectively target invading phage and plasmids in a sequence-specific process involving a variable cassette of CRISPR-associated (*cas*) genes. The CRISPR locus in *Pseudomonas aeruginosa* (PA14) includes four *cas* genes that are unique to and conserved in microorganisms harboring the Csy-type (CRISPR system yersinia) immune system. Here we show that the Csy proteins (Csy1–4) assemble into a 350 kDa ribonucleoprotein complex that facilitates target recognition by enhancing sequence-specific hybridization between the CRISPR RNA and complementary target sequences. Target recognition is enthalpically driven and localized to a "seed sequence" at the 5′ end of the CRISPR RNA spacer. Structural analysis of the complex by small-angle X-ray scattering and single particle electron microscopy reveals a crescent-shaped particle that bears striking resemblance to the architecture of a large CRISPR-associated complex from *Escherichia coli*, termed Cascade. Although similarity between these two complexes is not evident at the sequence level, their unequal subunit stoichiometry and quaternary architecture reveal conserved structural features that may be common among diverse CRISPR-mediated defense systems.

Cmr | RNA interference | RNA silencing | Argonaute | surveillance system

Clustered regularly interspaced short palindromic repeats (CRISPRs) are the genetic record of an RNA-based adaptive immune system that is prevalent among prokaryotes. Each CRISPR locus consists of a series of short repeats that are separated by nonrepetitive spacer sequences derived from foreign genetic elements (1, 2). These repetitive elements rapidly expand in response to phage challenge by site-specifically integrating short fragments of the foreign DNA at one end of the evolving CRISPR (3–5). CRISPR adaptation results in sequence-specific resistance to genetic parasites containing a complementary sequence (4, 5).

The genes flanking CRISPRs encode proteins that have been implicated as mediators of these diverse immune systems. Genetic experiments in *Streptococcus thermophilus* provided initial evidence for the role of CRISPR-associated (Cas) proteins in adaptive immunity, but assigning function to *cas* genes in other organisms has been challenging due to a lack of primary sequence conservation (4). Phylogenetic analyses have identified distinct subfamilies of the CRISPR system, which are named using three letter abbreviations (reviewed in ref. 6). Each immune system includes a distinct set of 4–10 *cas* genes that are associated with a particular CRISPR repeat sequence type (7–9).

CRISPR loci are transcribed as long precursor RNAs that are recognized and processed into short CRISPR RNAs (crRNAs) by CRISPR-specific endoribonucleases. The high-resolution crystal structure of a crRNA bound to Csy4, the CRISPR-specific endoribonuclease from *Pseudomonas aeruginosa* (PA14), revealed a unique combination of sequence- and structure-specific interactions that explain this highly selective association (10). A CRISPR-specific endoribonuclease in *Escherichia coli* (Cse3, also known as CasE) performs an analogous function, although this enzyme is structurally distinct from Csy4 (11, 12).

In the *E. coli* system, CasE and its associated crRNA are essential components of a multisubunit macromolecular complex termed Cascade (CRISPR-associated complex for antiviral defense). Cascade is composed of an unequal stoichiometry of five subfamily-specific Cas proteins (Cse-type) that have been designated CasA-CasE (11). Protection against phage challenge requires both the Cascade complex and an additional protein (Cas3), which is predicted to function as a helicase and nuclease (11, 13). Although the mechanism of CRISPR-mediated phage interference in *E. coli* is not currently known, phage challenge experiments have shown that crRNAs complementary to phage DNA are significantly more effective at reducing phage titers than are crRNAs that target the corresponding RNA sequence (11). CRISPR-mediated DNA targeting also occurs in *Staphylococcus epidermidis* (Csm-type) and *S. thermophilus* (Csn-type) (5, 14). By contrast, biochemical evidence in *Pyrococcus furiosus* (Cmr-type) indicates that the Cmr proteins form a complex that specifically cleaves RNA targets at a fixed distance from the 3′-end of the crRNA (15).

Here we report the discovery of a CRISPR-associated complex from the PA14 strain of *P. aeruginosa*. The complex is composed of a unique set of proteins, which have previously been shown to be exclusive to and conserved in the Csy subfamily (CRISPR system yersinia) of CRISPR-mediated immune systems (7, 9). We show that this complex participates in target recognition by facilitating sequence-specific hybridization between the crRNA and complementary targets. Similar to mRNA recognition by Argonaute proteins during RNA interference (RNAi) in eukar-

Author contributions: B.W., A.J.R.H., E.J.B., M.J.D., and J.A.D. designed research; B.W., E.v.D., J.B.B., S.P.W., K.Z., A.B., and W.W. performed research; B.W., E.v.D., J.B.B., S.P.W., A.B., A.J.R.H., E.J.B., M.J.D., and J.A.D. analyzed data; and B.W. and J.A.D. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

[1]E.v.D., J.B.B., S.P.W., and K.Z. contributed equally to this work.

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(12) United States Patent
May et al.

(10) Patent No.: US 9,260,752 B1
(45) Date of Patent: Feb. 16, 2016

(54) COMPOSITIONS AND METHODS OF NUCLEIC ACID-TARGETING NUCLEIC ACIDS

(71) Applicant: **CARIBOU BIOSCIENCES, INC.**, Berkeley, CA (US)

(72) Inventors: **Andrew Paul May**, San Francisco, CA (US); **Rachel E. Haurwitz**, Kensington, CA (US); **Jennifer A. Doudna**, Berkeley, CA (US); **James M. Berger**, Baltimore, MD (US); **Matthew Merrill Carter**, North Granby, CT (US); **Paul Donohoue**, Berkeley, CA (US)

(73) Assignee: **Caribou Biosciences, Inc.**, Berkeley, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/416,338**

(22) PCT Filed: **Mar. 12, 2014**

(86) PCT No.: **PCT/US2014/023828**
§ 371 (c)(1),
(2) Date: **Jan. 22, 2015**

(87) PCT Pub. No.: **WO2014/150624**
PCT Pub. Date: **Sep. 25, 2014**

Related U.S. Application Data

(60) Provisional application No. 61/781,598, filed on Mar. 14, 2013, provisional application No. 61/818,386, filed on May 1, 2013, provisional application No. 61/818,382, filed on May 1, 2013, provisional application No. 61/822,002, filed on May 10, 2013, provisional application No. 61/832,690, filed on Jun. 7, 2013, provisional application No. 61/845,714, filed on Jul. 12, 2013, provisional application No. 61/858,767, filed on Jul. 26, 2013, provisional application No. 61/859,661, filed on Jul. 29, 2013, provisional application No. 61/865,743, filed on Aug. 14, 2013, provisional application No. 61/883,804, filed on Sep. 27, 2013, provisional application No. 61/899,712, filed on Nov. 4, 2013, provisional application No. 61/900,311, filed on Nov. 5, 2013, provisional application No. 61/902,723, filed on Nov. 11, 2013, provisional application No. 61/903,232, filed on Nov. 12, 2013, provisional application No. 61/906,211, filed on Nov. 19, 2013, provisional application No. 61/906,335, filed on Nov. 16, 2013, provisional application No. 61/907,216, filed on Nov. 21, 2013, provisional application No. 61/907,777, filed on Nov. 22, 2013.

(51) **Int. Cl.**
C12Q 1/68 (2006.01)
A61K 38/46 (2006.01)
A61K 47/48 (2006.01)

(52) **U.S. Cl.**
CPC ***C12Q 1/6869*** (2013.01); ***A61K 38/465*** (2013.01); ***A61K 47/48092*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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5,767,367	A	6/1998	Dudits et al.
5,968,738	A	10/1999	Anderson et al.
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8,546,553	B2	10/2013	Terns et al.
8,685,737	B2	4/2014	Serber et al.
8,697,359	B1	4/2014	Zhang
8,771,945	B1	7/2014	Zhang
8,795,965	B2	8/2014	Zhang
8,865,406	B2	10/2014	Zhang et al.
8,871,445	B2	10/2014	Cong et al.
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8,895,308	B1	11/2014	Zhang et al.
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8,921,332	B2	12/2014	Choulika et al.
8,932,814	B2	1/2015	Cong et al.

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(Continued)

Primary Examiner — Jim Ketter
(74) *Attorney, Agent, or Firm* — Gary R. Fabian; Barbara G. McClung

(57) **ABSTRACT**

This disclosure provides for compositions and methods for the use of nucleic acid-targeting nucleic acids and complexes thereof. Genome engineering can refer to altering the genome by deleting, inserting, mutating, or substituting specific nucleic acid sequences. The altering can be gene or location specific. Genome engineering can use nucleases to cut a nucleic acid thereby generating a site for the alteration. Engineering of non-genomic nucleic acid is also contemplated.

12 Claims, 73 Drawing Sheets

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]† Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

Bacteria and archaea have evolved RNA-mediated adaptive defense systems called clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) that protect organisms from invading viruses and plasmids (*1*–*3*). These defense systems rely on small RNAs for sequence-specific detection and silencing of foreign nucleic acids. CRISPR/Cas systems are composed of *cas* genes organized in operon(s) and CRISPR array(s) consisting of genome-targeting sequences (called spacers) interspersed with identical repeats (*1*–*3*). CRISPR/Cas-mediated immunity occurs in three steps. In the adaptive phase, bacteria and archaea harboring one or more CRISPR loci respond to viral or plasmid challenge by integrating short fragments of foreign sequence (protospacers) into the host chromosome at the proximal end of the CRISPR array (*1*–*3*). In the expression and interference phases, transcription of the repeat-spacer element into precursor CRISPR RNA (pre-crRNA) molecules followed by enzymatic cleavage yields the short crRNAs that can pair with complementary protospacer sequences of invading viral or plasmid targets (*4*–*11*). Target recognition by crRNAs directs the silencing of the foreign sequences by means of Cas proteins that function in complex with the crRNAs (*10*, *12*–*20*).

There are three types of CRISPR/Cas systems (*21*–*23*). The type I and III systems share some overarching features: specialized Cas endonucleases process the pre-crRNAs, and once mature, each crRNA assembles into a large multi-Cas protein complex capable of recognizing and cleaving nucleic acids complementary to the crRNA. In contrast, type II systems process pre-crRNAs by a different mechanism in which a trans-activating crRNA (tracrRNA) complementary to the repeat sequences in pre-crRNA triggers processing by the double-stranded (ds) RNA-specific ribonuclease RNase III in the presence of the Cas9 (formerly Csn1) protein (fig. S1) (*4*, *24*). Cas9 is thought to be the sole protein responsible for crRNA-guided silencing of foreign DNA (*25*–*27*).

We show here that in type II systems, Cas9 proteins constitute a family of enzymes that require a base-paired structure formed between the activating tracrRNA and the targeting crRNA to cleave target dsDNA. Site-specific cleavage occurs at locations determined by both base-pairing complementarity between the crRNA and the target protospacer DNA and a short motif [referred to as the protospacer adjacent motif (PAM)] juxtaposed to the complementary region in the target DNA. Our study further demonstrates that the Cas9 endonuclease family can be programmed with single RNA molecules to cleave specific DNA sites, thereby raising the exciting possibility of developing a simple and versatile RNA-directed system to generate dsDNA breaks for genome targeting and editing.

Cas9 is a DNA endonuclease guided by two RNAs. Cas9, the hallmark protein of type II systems, has been hypothesized to be involved in both crRNA maturation and crRNA-guided DNA interference (fig. S1) (*4*, *25*–*27*). Cas9 is involved in crRNA maturation (*4*), but its direct participation in target DNA destruction has not been investigated. To test whether and how Cas9 might be capable of target DNA cleavage, we used an overexpression system to purify Cas9 protein derived from the pathogen *Streptococcus pyogenes* (fig. S2, see supplementary materials and methods) and tested its ability to cleave a plasmid DNA or an oligonucleotide duplex bearing a protospacer sequence complementary to a mature crRNA, and a bona fide PAM. We found that mature crRNA alone was incapable of directing Cas9-catalyzed plasmid DNA cleavage (Fig. 1A and fig. S3A). However, addition of tracrRNA, which can pair with the repeat sequence of crRNA and is essential to crRNA maturation in this system, triggered Cas9 to cleave plasmid DNA (Fig. 1A and fig. S3A). The cleavage reaction required both magnesium and the presence of a crRNA sequence complementary to the DNA; a crRNA capable of tracrRNA base pairing but containing a noncognate target DNA-binding sequence did not support Cas9-catalyzed plasmid cleavage (Fig. 1A; fig. S3A, compare crRNA-sp2 to crRNA-sp1; and fig. S4A). We obtained similar results with a short linear dsDNA substrate (Fig. 1B and fig. S3, B and C). Thus, the trans-activating tracrRNA is a small noncoding RNA with two critical functions: triggering pre-crRNA processing by the enzyme RNase III (*4*) and subsequently activating crRNA-guided DNA cleavage by Cas9.

Cleavage of both plasmid and short linear dsDNA by tracrRNA:crRNA-guided Cas9 is site-specific (Fig. 1, C to E, and fig. S5, A and B). Plasmid DNA cleavage produced blunt ends at a position three base pairs upstream of the PAM sequence (Fig. 1, C and E, and fig. S5, A and C) (*26*). Similarly, within short dsDNA duplexes, the DNA strand that is complementary to the target-binding sequence in the crRNA (the complementary strand) is cleaved at a site three base pairs upstream of the PAM (Fig. 1, D and E, and fig. S5, B and C). The noncomplementary DNA strand is cleaved at one or more sites within three to eight base pairs upstream of the PAM. Further investigation revealed that the noncomplementary strand is first cleaved endonucleolytically and subsequently trimmed by a 3′-5′ exonuclease activity (fig. S4B). The cleavage rates by Cas9 under single-turnover conditions ranged from 0.3 to 1 min^{-1}, comparable to those of restriction endonucleases (fig. S6A), whereas incubation of wild-type (WT) Cas9-tracrRNA:crRNA complex with a fivefold molar excess of substrate DNA provided evidence that the dual-RNA–guided Cas9 is a multiple-turnover enzyme (fig. S6B). In

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May et al.

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(45) Date of Patent: Feb. 16, 2016

(54) **COMPOSITIONS AND METHODS OF NUCLEIC ACID-TARGETING NUCLEIC ACIDS**

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(51) **Int. Cl.**
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A61K 38/46 (2006.01)
A61K 47/48 (2006.01)

(52) **U.S. Cl.**
CPC ***C12Q 1/6869*** (2013.01); ***A61K 38/465*** (2013.01); ***A61K 47/48092*** (2013.01)

(58) **Field of Classification Search**
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See application file for complete search history.

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Primary Examiner — Jim Ketter
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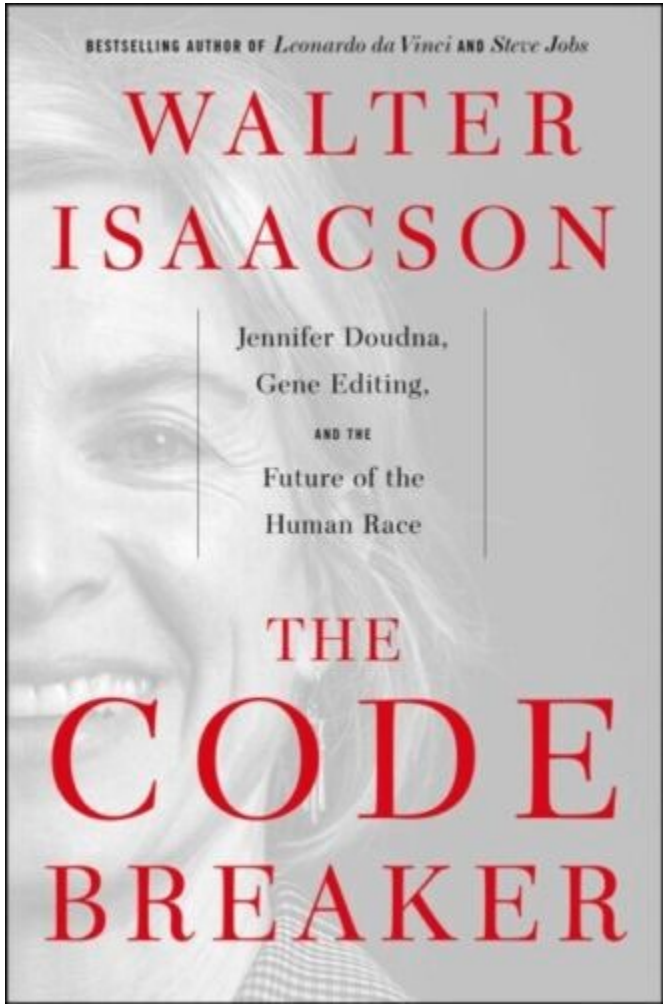

Chapter 31

Patents

"Useful arts"

Ever since the Republic of Venice in 1474 passed a statute giving the inventors of "any new and ingenious device" the exclusive right to profit from it for ten years, people have been wrestling over patents. In the United States, they are enshrined in Article 1 of the Constitution: "The Congress shall have power to . . . promote the progress of science and useful arts by securing for limited times to authors and inventors the exclusive right to their respective writings and discoveries." A year after ratification, Congress passed an act that allowed patents on "any useful art, manufacture, engine, machine, or device, or any improvement thereon not before known."

As courts came to realize, it's complicated to apply such concepts, even to things as simple as a doorknob. In the 1850 case *Hotchkiss v. Greenwood*, which involved a patent application for the manufacture of doorknobs out of porcelain rather than wood, the U.S. Supreme Court began the process of defining what was "obvious" and "non-obvious" in assessing whether an invention was "not before known." Deciding on patents was particularly difficult when it involved biological processes. Nevertheless, biological patents have a long history. In 1873, [illegible] the French biologist Louis Pasteur was awarded the first

PPPs 14 top Japanese universities & 3 national research labs

We obtained the patent data from the United States Patent and Trademark Office (USPTO) through *PatentsView* covering the period from 2003 to 2015.

This resulted in a set of **7,766 USPTO patents**. (17 institutions)

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We identified **3,177** ***True Matches*** out of the **12,627** potential matches considered.

PPPs originating from our sample

Out of 3,177 PPPs, **546** come from this set of researchers

17% of all the PPPs

78 researchers (11% of the sample)

Researcher Quadrant

		Considerations of use?	
		Local	Global
Quest for scientific understanding?	Global		
	Local		

Source: Adapted from Donald E. Stokes, *Pasteur's Quadrant*, p. 73

Researcher Quadrant

Chihaya Adachi

H-127 - thermally activated delayed fluorescence (TADF).

Adachi has had over 515 papers published in the field of organic electronics.

Adachi's lab in Kyushu University has filed over 180 patents since 1989

Kyolux: Market cap: $0.26 Billion

TADF is like a glow-in-the-dark effect where materials light up after being warmed up.

Conclusion

Very rich sample to analyse **academic entrepreneurship** in Japan. | **Ongoing**

Funding, publication, patent, company data…

Conclusion

Very rich sample to analyse academic entrepreneurship in Japan. | Ongoing

Look at the **microfoundations** of their research and tech transfer activity | **Econometric analysis**

Analyse the impact of entrepreneurial activities on research productivity

Conclusion

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Look at the microfoundations of their research and tech transfer activity | Econometric analysis

Analyse the different **patterns** of researchers and how the involvement in an academic startup influence their activities | **Interview based - more interviews are planned**

Qualitative appraisal and categorization of different type of researchers ⇒ adapted policy recommendations

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Look at the microfoundations of their research and tech transfer activity | Econometric analysis

Analyse the different patterns of researchers and how the involvement in an academic startup influence their activities | Interview based - more interviews are planned

PPPs a powerful tool to spot researchers in the Pasteur_Doudna_Yamanaka Quadrant | **categorisation**

Automatic tool to spot researchers who can combine entrepreneurial/commercial activities and research projects

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Thank you

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