December 15, 2025

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NEWS RELEASE

A Breakthrough Technology That Reveals the Unique Identity of Every Single Cell

【Key points】

- A novel single-cell technology, scRepli-RamDA-seq (scRR-seq), has been developed, enabling simultaneous analysis of genomic DNA and gene expression within individual cells.
- scRR-seq provides high-quality analysis of both DNA and RNA.
- Using scRR-seq, the team can reveal a direct relationship between DNA and RNA in single cells, uncovering insights not possible with previous methods.
- scRR-seq is a powerful and versatile tool with broad applications, from studying disease mechanisms to early embryonic development, offering new opportunities across diverse research fields.

【Overview】

A collaborative research group, including Professor Shin-ichiro Takebayashi (Graduate School of Biological Resources Science, Mie University), Assistant Professor Rawin Poonperm, Graduate students Taiki Yoneda and Taito Imada (at the time of the research), Dr. Ichiro Hiratani (Team Director, RIKEN), and Professor Itoshi Nikaido (Team Director, RIKEN; Institute of Science Tokyo), has developed a novel single-cell analysis technology[1] called scRepli-RamDA-seq (scRR-seq). This method allows high-resolution, simultaneous analysis of both genomic DNA[2] and RNA within a single cell. By directly linking changes in DNA to changes in gene expression, scRR-seq is expected to help solve problems that have been difficult to address with conventional techniques.

In recent years, advances in single-cell analysis have revolutionized biology by revealing cell-to-cell variability that cannot be captured in analyses of cell populations, leading to discoveries such as the identification of rare abnormal cells. However, many existing single-cell methods analyze DNA and RNA separately, limiting our understanding of how these molecules are functionally connected. To overcome this limitation, scRR-seq was designed to analyze DNA and RNA simultaneously from a single cell.

This new method was made possible by combining two cutting-edge single-cell analysis techniques previously established by the research team: scRepli-seq, which allows high-resolution analysis of DNA copy number, and RamDA-seq, a highly sensitive RNA sequencing method. The integration of these approaches resulted in scRR-seq, a groundbreaking single-cell analysis technology.

Using scRR-seq, the research team was able to investigate the direct relationship between DNA and gene activity in individual cells. This allowed them to address previously unresolved, long-standing questions in biology and led to the discovery of new markers of cell cycle progression. The study also revealed that the relationship between DNA copy number and gene activity is complex and not always a simple positive correlation.

This research was published in the scientific journal *Nature Communications* (issue dated December 15, 2025).

【Background】

Until now, most studies of DNA and RNA have analyzed large populations of cells together. While this approach has been useful, it only provides averaged information across the population and cannot capture the unique differences between individual cells, known as cellular heterogeneity (Fig. 1a). With the advent of technologies such as next-generation sequencing (NGS)[3], single-cell isolation, and highly sensitive nucleic acid amplification technologies[4], we can now study DNA and RNA at the level of a single cell. This allows researchers to better understand the function of genomic DNA and the differences in gene activity between individual cells.

Professor Shin-ichiro Takebayashi and his collaborative research group have previously developed two advanced single-cell analysis techniques applicable to animal cells:

- **scRepli-seq**: This method amplifies extremely small amounts of genomic DNA extracted from individual cells using whole-genome amplification (WGA)[5] and measures DNA copy number[6] at high resolution (Takahashi et al., *Nature Genetics* 2019; Miura et al., *Nature Protocols* 2020). It can be used to detect DNA copy number variations (CNVs)[7] and predict the state of DNA replication[8] (Fig. 1b).
- **RamDA-seq**: A highly sensitive method that captures full-length RNA[9], including non-coding RNAs[10] (Hayashi et al., *Nature Communications* 2018) (Fig. 1c).

Single-cell technologies are powerful, but without analyzing DNA and RNA together from the same cell, it is impossible to fully understand how the genome and gene activity are linked. Existing methods for simultaneous DNA/RNA analysis, however, lacked sufficient sensitivity and resolution, making it difficult to clearly address this relationship. To close this gap, the team combined their technologies, scRepli-seq and RamDA-seq, to develop a new single-cell simultaneous analysis technique called scRepli-RamDA-seq (scRR-seq).

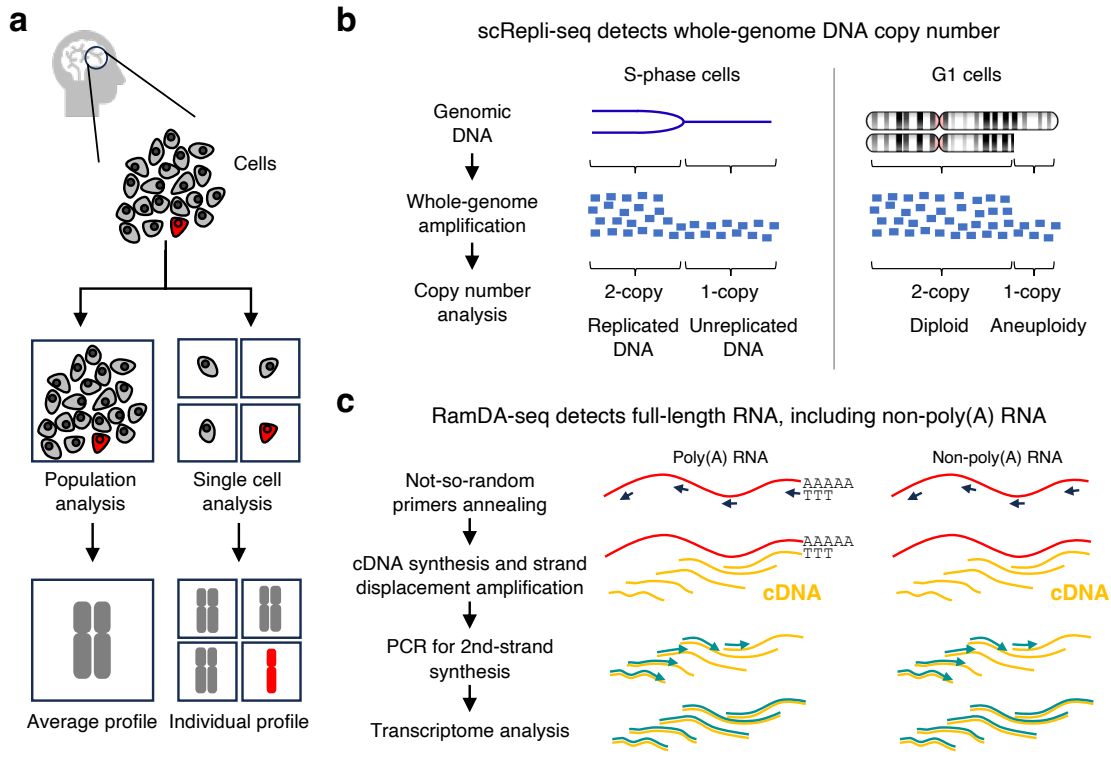

Figure 1. Single-cell technologies: scRepli-seq and RamDA-seq.
(a) Comparison of bulk versus single-cell profiling. Bulk methods mask cell-to-cell differences, whereas single-cell approaches capture variation between individual cells.
(b) Overview of scRepli-seq. This method uses whole-genome amplification (WGA), which is relatively uniform across the genome and allows DNA copy number analysis. In S-phase cells, copy number profiles can be used to infer replication status at specific loci (2-copy = replicated; 1-copy = unreplicated). In G1-phase cells, the same approach can reveal copy number variations and genome instability.
(c) Overview of RamDA-seq. This method uses not-so-random primers (arrows) for cDNA synthesis and strand displacement amplification to increase cDNA yield, allowing detection of even small amounts of RNA. The not-so-random primers also enable full-length RNA sequencing and capture both poly(A) RNAs and non-poly(A) RNAs, the latter of which cannot be captured by conventional RNA-seq methods.

【Research content】

To establish this new method, the team first explored a way to cleanly separate DNA and RNA from a single cell. As a result, they found that magnetic beads containing carboxyl groups can be used to selectively capture genomic DNA (Fig. 2).

When a single cell is lysed in the presence of these beads, the DNA binds to the beads and can be pulled out with a magnetic stand. The RNA, meanwhile, remains in the solution. Following this separation, scRepli-seq can be applied to the DNA and RamDA-seq to the RNA from the same cell.

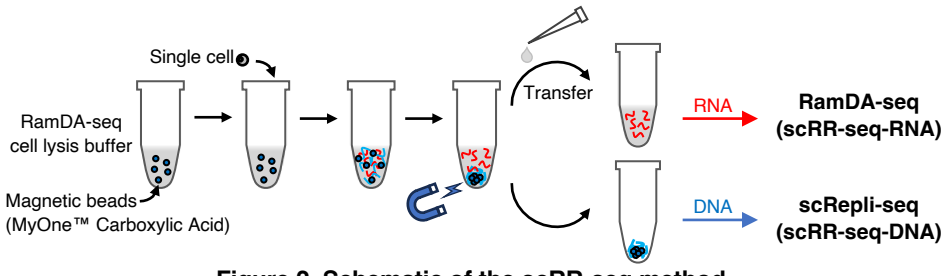

Figure 2. Schematic of the scRR-seq method.

One of the important points when evaluating a new technology is the quality of the results it produces. To benchmark scRR-seq, the team compared its results with those obtained using conventional scRepli-seq and RamDA-seq individually.

For this, they used human retinal pigment epithelial cells (hTERT-RPE1) collected from mid-S phase[11] of the cell cycle by FACS[12] (Fig. 3a). Applying scRR-seq DNA sequencing (scRR-seq-DNA hereafter), they examined genome-wide DNA copy number changes associated with DNA replication (Fig. 3b). Because DNA copy number reflects the replication status during S phase (1-copy = unreplicated DNA, 2-copy = replicated DNA), this analysis can determine early- and late-replicating regions across the genome. The replication timing (RT) patterns obtained with scRR-seq-DNA were highly consistent with those obtained with conventional scRepli-seq.

In parallel, scRR-seq RNA sequencing (scRR-seq-RNA hereafter) achieved coverage of gene transcripts comparable to RamDA-seq (Fig. 3c), detecting about 80% of genes identified by RamDA-seq alone. Moreover, it was able to detect both poly(A) and non-poly(A) RNAs[13], similar to RamDA-seq. These results confirm that the method can sensitively and efficiently profile gene expression within a single cell.

Taken together, these results demonstrate that scRR-seq is a powerful new technology that can simultaneously obtain high-quality DNA and RNA information from the same single cell, showing its usefulness for understanding cellular states.

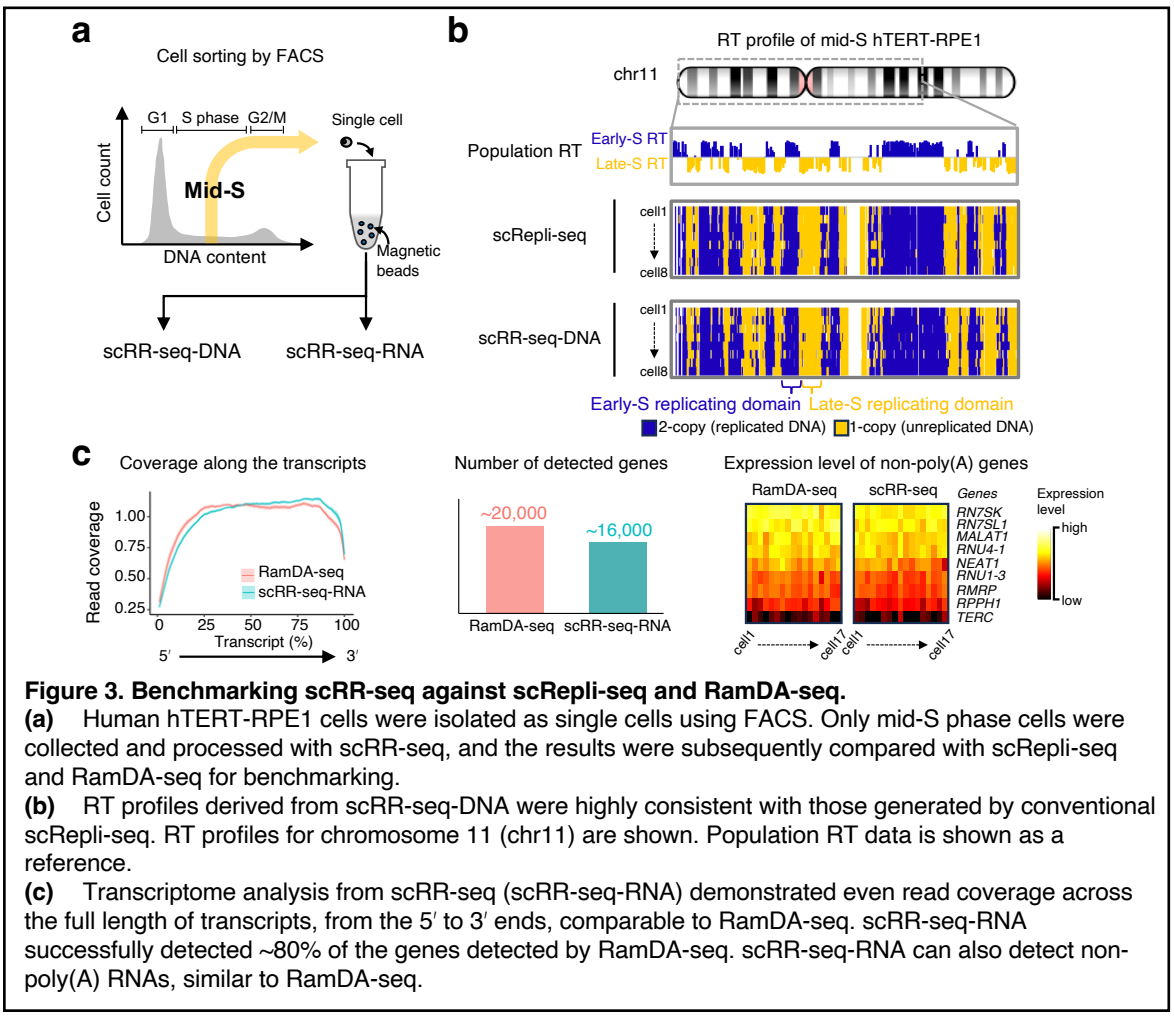

Figure 3. Benchmarking scRR-seq against scRepli-seq and RamDA-seq.
(a) Human hTERT-RPE1 cells were isolated as single cells using FACS. Only mid-S phase cells were collected and processed with scRR-seq, and the results were subsequently compared with scRepli-seq and RamDA-seq for benchmarking.
(b) RT profiles derived from scRR-seq-DNA were highly consistent with those generated by conventional scRepli-seq. RT profiles for chromosome 11 (chr11) are shown. Population RT data is shown as a reference.
(c) Transcriptome analysis from scRR-seq (scRR-seq-RNA) demonstrated even read coverage across the full length of transcripts, from the 5′ to 3′ ends, comparable to RamDA-seq. scRR-seq-RNA successfully detected ~80% of the genes detected by RamDA-seq. scRR-seq-RNA can also detect non-poly(A) RNAs, similar to RamDA-seq.

Using scRR-seq DNA copy number data, the stage of S phase for each cell can be estimated. This can be done by calculating the proportion of genomic regions that have DNA copy number increased to two copies (Fig. 4a; see % replication score on the right). By combining and sorting data from many cells according to this replication score, the progression of DNA replication throughout S phase can be tracked in detail (Fig. 4a).

At the same time, because scRR-seq allows simultaneous analysis of DNA and RNA, it is also possible to track changes in gene expression during the S phase progression. This suggests the possibility of identifying genes that serve as markers of the S phase. Using mouse embryonic stem cells (ES cells), the team successfully identified genes whose expression changes dynamically during the S phase. These were defined as “S phase progression markers” (Fig. 4b).

Further analysis revealed that these genes change their expression patterns in a way that corresponds to the progression of S phase (Fig. 4c). Remarkably, when these markers were applied to publicly available scRNA-seq data from mouse ES cells, it was possible to predict the S phase stage of each cell and reconstruct the progression of S phase, even without direct DNA replication data (Fig. 4d).

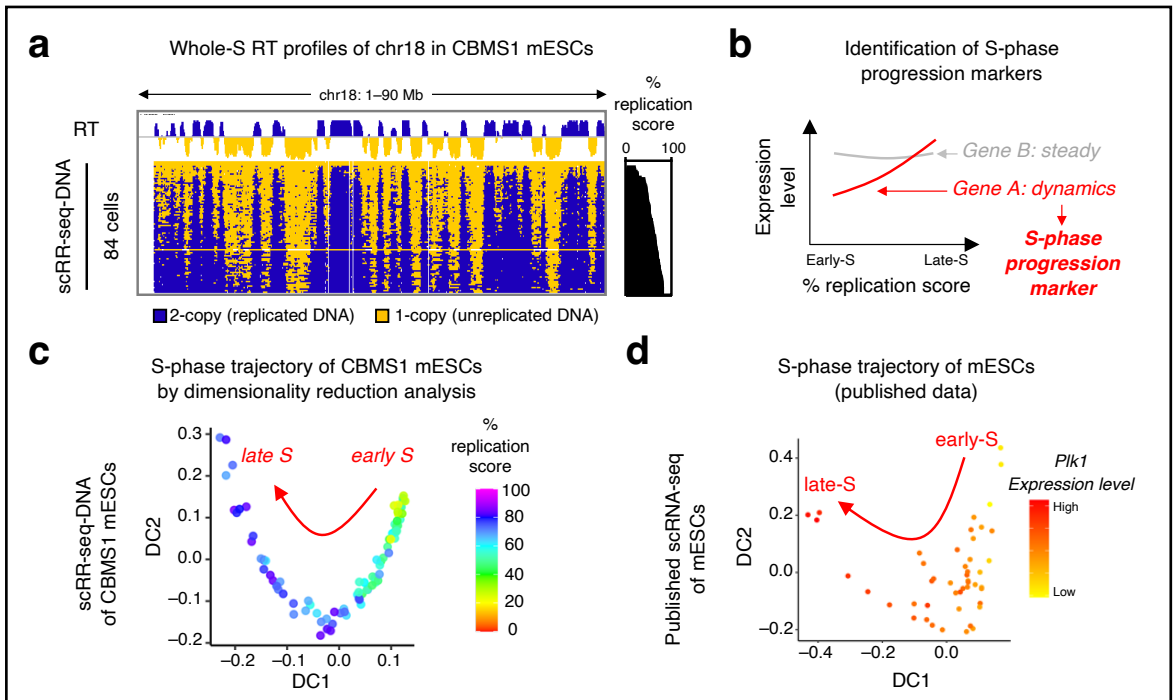

Figure 4. Novel S-phase progression markers of CBMS1 mESCs identified by scRR-seq.
(a) Whole-S scRT profiles of CBMS1 mESCs derived from scRR-seq-DNA. Chromosome 18 (chr18) is shown. Population RT data is shown as a reference. The right panel shows the percentage replication score.
(b) To identify S-phase progression markers, genes with dynamically changing expression levels across S phase were selected and defined as “S-phase progression markers.”
(c) Using these markers from CBMS1 scRR-seq, a diffusion map (a dimensionality reduction analysis[14]) analysis was performed. This revealed the trajectory of S-phase progression in CBMS1 mESCs. Each cell is color-coded according to its percentage replication score.
(d) Applying the newly identified S-phase progression markers to published scRNA-seq data of mESCs (Hayashi et al., *Nature Communications* 2018), a trajectory of S-phase progression was observed. Here, *Plk1* was used as a reference gene, as its expression increases during S-phase progression. Each cell is color-coded by the expression level of *Plk1*.

Using this technology, the team next tackled a question that had been difficult to study until now. In the field of DNA replication, it has long been unclear how changes in DNA copy number during replication affect gene expression in animal cells. In bacteria, gene expression is positively correlated with DNA copy number, but it was unknown whether the same applies to animal cells with larger and more complex genomes.

To investigate this, scRR-seq was performed on human HAP1 cells, which have only a single copy of each chromosome. Focusing on cells in S phase, some genomic regions had been replicated (2-copy) while others remained unreplicated (1-copy) (Fig. 5a). This enabled direct comparison of gene expression between 1-copy and 2-copy states to test whether increased copy number leads to increased transcription.

Surprisingly, for most genes, there was no significant increase or decrease in expression after replication (Fig. 5b). This suggests that, unlike in bacteria, animal cells may possess a robust regulatory mechanism that buffers gene expression against changes in DNA.

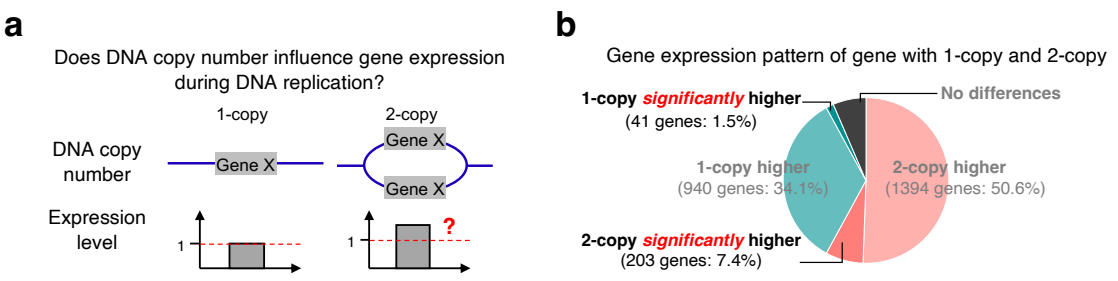

Figure 5. The relationship between HAP1 DNA replication status and gene expression.
(a) During mid-S phase, a gene may exist in either a 1-copy or 2-copy state. Using expression data from each cell, the team tested whether a higher copy number is associated with higher gene expression.
(b) Pie charts show gene expression patterns of 1-copy and 2-copy genes during mid-S-phase in HAP1 cells. Only 8.9% of the total informative genes showed statistically significant differences in gene expression levels between the two groups. The other 50% of the informative genes showed a trend toward higher expression in the 2-copy gene group, although the difference was not statistically significant.

Next, the team investigated how CNVs and genomic instability in human cells influence gene expression. They used IMR-90 cells, a normal diploid fibroblast cell line, and induced CNVs by treating the cells with a drug that inhibits DNA synthesis enzymes.

Using scRR-seq-DNA to examine G1-phase[15] cells, they observed both increases and decreases in DNA copy number in some cells (Fig. 6a). They then analyzed gene expression in regions with altered copy number and found that changes in DNA copy number do not necessarily lead to changes in gene expression (Fig. 6b).

These findings demonstrate that the relationship between DNA copy number and gene activity is not always directly linked, highlighting the importance of simultaneously analyzing DNA and RNA within a single cell.

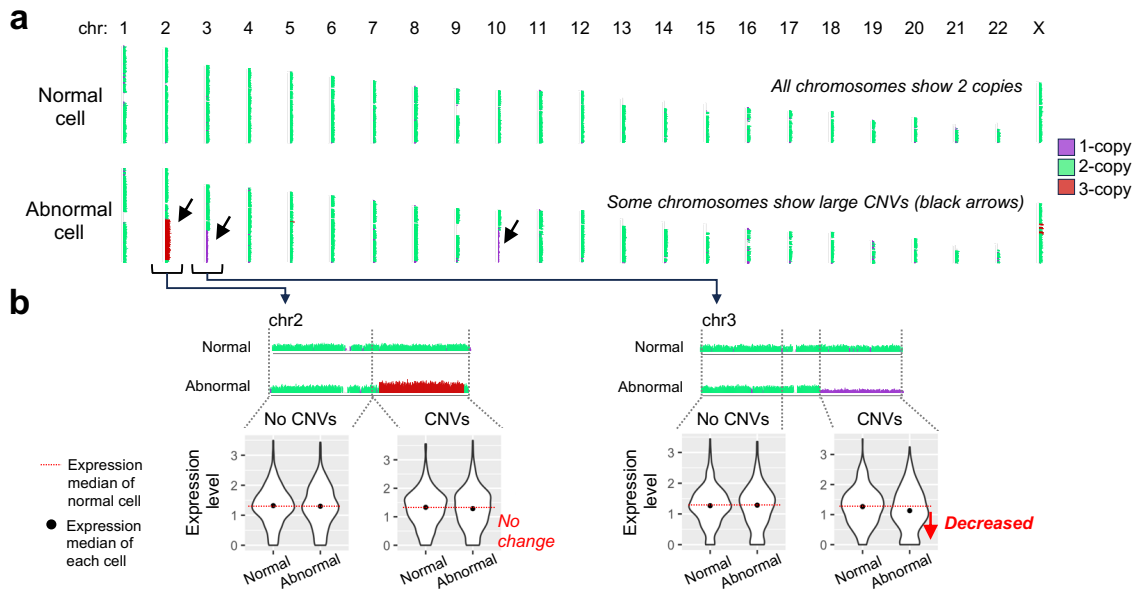

Figure 6. CNVs in IMR-90 cells treated with low-dose aphidicolin.
(a) Whole-genome karyogram plots of human IMR-90 cells show DNA copy number across chromosomes, with colors indicating the copy number state of each genomic region. Chromosome numbers are shown at the top. While a normal cell showed a uniform 2-copy state across the genome, an abnormal cell treated with aphidicolin to induce CNVs exhibited large chromosomal abnormalities (black arrows).
(b) Two representative CNV-affected chromosomes in abnormal IMR-90 cells are shown. Violin plots display gene expression for genes in these regions. Black dots indicate the median expression of genes in each cell, while red lines represent the median expression in normal cells. A cell with a large 3-copy CNV on chromosome 2 (chr2) showed median gene expression levels comparable to the normal cell (left). In contrast, a cell with a large 1-copy CNV on chromosome 3 (chr3) exhibited reduced median gene expression relative to the normal cell (right). These findings highlight the complex nature of gene regulation in mammalian cells with chromosomal abnormalities.

【Future Prospects】

The newly developed scRR-seq is expected to have applications across a wide range of research fields and may also be extended to future clinical studies.

In cancer research, for example, tumors often contain a mix of cells with different gene expression patterns and CNVs, which drive disease progression and treatment resistance. scRR-seq is a powerful tool for detecting such cellular heterogeneity and is expected to aid in the development of diagnostics and therapies.

Moreover, scRR-seq is ideal for studying early-stage embryos, where it is often difficult to obtain sufficient cell numbers for conventional experiments. It can reveal how DNA copy number and gene expression are coordinated in these early stages, helping to uncover the origins of developmental disorders. In addition, it may also be applied to detect abnormalities in the earliest stages of pregnancy.

Taken together, scRR-seq represents a next-generation single-cell technology for integrated DNA and RNA analysis, with broad potential applications ranging from basic biology to clinical research.

【Glossary】

1. **Single-cell analysis technology**: A set of techniques that study the DNA, RNA, proteins, etc., of individual cells rather than averaging over a population of cells. This allows the detection of differences between individual cells that would be hidden in bulk analysis.
2. **Genomic DNA**: The complete set of DNA contained in a cell, including both genes and non-coding regions, representing all the genetic information of the organism.
3. **Next-generation sequencing (NGS)**: Advanced technology that can read DNA sequences in large quantities and at high speed.
4. **Nucleic acid amplification technology**: Methods for increasing small amounts of DNA or RNA to quantities sufficient for analysis, which is especially important in single-cell studies.
5. **Whole-genome amplification (WGA)**: A method to amplify the entire genome from a small amount of DNA, such as from a single cell.
6. **DNA copy number**: The number of copies of a specific DNA region or gene in a cell. Typically, diploid cells have 2 copies.
7. **Copy number variation (CNV)**: A condition in which the number of copies of a DNA region differs from the usual two copies, appearing as duplications or deletions.
8. **DNA replication**: The process by which DNA is accurately copied so that complete genetic information is passed to new cells during cell division.
9. **Full-length RNA**: RNA molecules that are complete from the 5′ end to the 3′ end.
10. **Non-coding RNA**: RNA that is not translated into protein but plays important roles, such as regulating gene expression.
11. **S phase**: The DNA synthesis phase of the cell cycle, during which DNA is copied.
12. **FACS (Fluorescence-Activated Cell Sorting)**: A technique for analyzing and sorting cells using fluorescent markers or other labels.
13. **PolyA RNA and non-polyA RNA**: RNAs with (mainly mRNA) or without a continuous sequence of adenine nucleotides at the tail end.
14. **Dimensionality reduction analysis**: Computational methods that simplify large, complex datasets by reducing the number of variables while retaining the main information; commonly used in genomics and single-cell studies.
15. **G1 phase**: The first stage of the cell cycle, during which the cell grows and prepares for DNA replication.

【Publication information】

Title: scRepli-RamDA-seq: a multi-omics technology enabling the analysis of gene expression dynamics during S-phase

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Journal: *Nature Communications* (DOI: 10.1038/s41467-025-64688-1)

Publication Date: December 15, 2025

【Grant supports】

This research was supported by the following grants: JST CREST (Grant Number JPMJCR20S5) to Professor Shin-ichiro Takebayashi and Dr. Ichiro Hiratani; JSPS KAKENHI (Grant Numbers 23K23862, 24K21835, 25K02254, and 25H01445) to Professor Shin-ichiro Takebayashi; RIKEN BDR intramural grants, RIKEN Pioneering Project ‘Genome Building from TADs’ and JSPS KAKENHI (Grant Number 20K20582) to Dr. Ichiro Hiratani; JST CREST (Grant Numbers JPMJCR21N6 and JPMJCR1926), and the Japan Agency for Medical Research and Development (AMED, Grant Numbers JP21bm0404073) to Professor Itoshi Nikaido.