Mechanisms for Management of Biological Genetic Information

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Genomic DNA that bears biological genetic information is kept in a cellular nucleus that is positioned at the center of cell. Ciliates, a group of single-celled organism, have two distinct cellular nuclei, which individually contain differentiated genomic DNA. Each one is functionally specialized for “storage” or “usage”. We consider the biological advantages of the nuclear dualism, and introduce our research toward fully understanding and application of this prominent system.

1 Introduction

All organisms have genetic information that is unique to each species. The expression of unique shapes, behavioral patterns, and sometimes even illnesses and death are based on this genetic information. Damage to or deletion of genetic information is extremely harmful to biological activity, so organisms have a variety of systems to carefully maintain and manage this information. This system potentially holds clues on how to efficiently handle the huge volumes of data accumulated by human society. In this paper we will take a look at an actual example of how an organism ingeniously stores, uses and passes on genetic information. We will also report on our research to try and elucidate the principles behind these mechanisms.

2 Where and how genetic information is stored

The genetic information of organisms is contained in the DNA of the genome. The DNA has the shape of a strand, and in humans the genome is made up of 46 strands of DNA (23 are inherited from each parent), which have a total length of around 2 meters (with a diameter of around 2 nanometers). These DNA strands contain all the information necessary for life, and individual units of information are known as genes. All cells making up a body contain the same 46 strands of DNA. Appropriate genetic information is conveyed as necessary by the messenger RNA (through transcription), and proteins with all kinds of functions are synthesized based on the messenger RNA (known as translation of genetic information). In other words, DNA is a medium for storage of information, and proteins are what drive biological activity.

In bacteria, known as prokaryotes, the place for storing genes and where transcription and translation take place are not clearly separated within the cell. By comparison, DNA strands in the cells of animals and plants known as eukaryotic organisms are ingeniously folded and stored in a single bag-like structure known as the cellular nucleus in the center of the cell. The messenger RNA transcribed within the nucleus is transported out of the nucleus, and translation takes place within the cytoplasm. In this way, genetic information is stored and used in different places within the eukaryotic cell, enabling genetic information to be stored safely and used efficiently.

The nucleus of the eukaryotic cell is enclosed in a nuclear envelope made up of a double-layered lipid membrane isolating it from the cytoplasm. Substances are transported between the cytoplasm and nucleus through nuclear pores on the surface of the nuclear envelope (Fig. 1). The transcribed messenger RNA passes through the nuclear pore to move from the nucleus to the cytoplasm. Proteins used within the nucleus are synthesized in the cytoplasm after which they are transported to the nucleus through the nuclear pore.

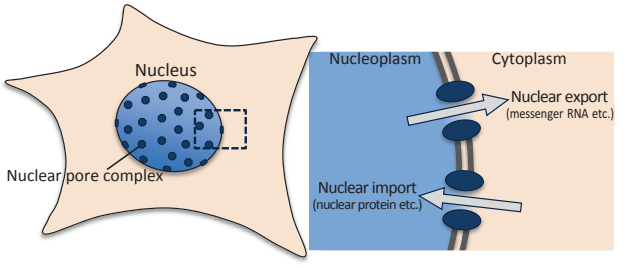

Fig. 1 (Left): The cellular nucleus and nuclear pore complex of a eukaryotic cell. (Right): Substance transport between the nucleus and cytoplasm through the nuclear pore.

Transportation of information and substances through the nuclear pore is strictly controlled both qualitatively and quantitatively. This transportation control of substances between the nucleus and cytoplasm is regarded as one of the most important molecular mechanisms pertaining to the life of the eukaryotic cell.

3 Ciliates have further evolved the method of both storage and usage of genetic information

3.1 Two types of cellular nuclei with different functions

A group of single cellular organisms known as ciliates include the well-known *Paramecium*, *Stentor* and *Vorticella*[1]. They swim through water by moving the numerous cilia on the surface of their cells. Ciliates are peculiar cells that have two types of cellular nuclei (Fig. 2). One is the macronucleus, which functions in the same way as the nucleus of the somatic cell, which makes up our body. The other is the micronucleus, which functions in the same way as the nucleus of the germ cell such as in sperm and ovum[2]. The somatic cells of multicellular organisms are responsible for carrying out all functions necessary to maintain life, while germ cells are not directly involved in maintaining the life of an individual. Instead they are responsible for giving birth to the next generation of individuals. In other words, somatic cells function only to support the life of the individual, and the genetic information they carry is used for only one generation. On the other hand, genetic information carried by germ cells is not used for supporting the life of the individual, but it is passed onto the next generation. Because ciliates are single cellular organisms, they are not differentiated into somatic cells and germ cells, but they have divided the functions of the nucleus within a single cell, into those that are the equivalent of somatic cells and germ cells (Fig. 3).

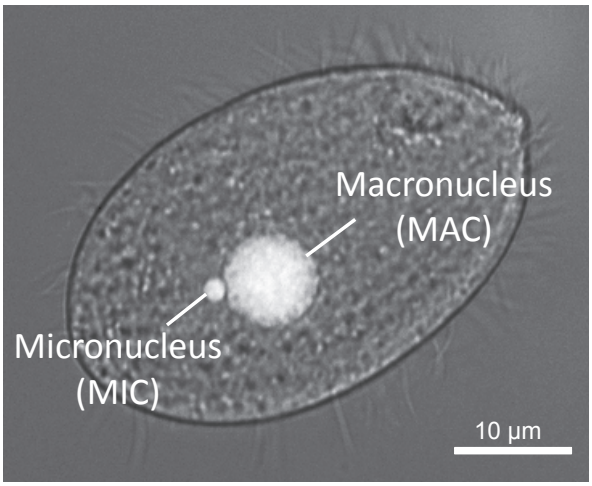

Fig. 2 *Tetrahymena*, a species of ciliate
The DNA has been made visible using a special dye.

Fig. 3 The somatic cell lineage (green) and germ cell lineage (red) of higher animals and ciliates.

3.2 The advantages of differentiating the cellular nucleus and genetic information

Both the macronucleus and micronucleus originate from a single fertilization nucleus formed during the process of sexual reproduction. The genome of the micronucleus consists of DNA passed on directly from the fertilization nucleus, but the DNA of the macronucleus has been heavily edited and amplified[2]. Its DNA is not passed onto the next generation. It is discarded after one generation, so its genetic information is modified irreversibly for greater efficiency. In editing the macronuclear DNA, the unnecessary parts of the genome DNA of the fertilization nucleus (= genome DNA of the micronucleus) are removed. The necessary information is then amplified several dozen to several thousand times (differs between ciliate species). Ciliates succeeded in becoming large single cellular organisms by evolving the macronucleus for making full use of genetic information.

DNA normally exists wrapped around basic globular proteins called histones, in a form known as a nucleosome. But in reading genetic information and duplicating DNA, the nucleosome structure is temporarily loosened. DNA is most susceptible to damage during this phase. DNA has to be duplicated prior to each cell division, so neither the macronucleus nor the micronucleus can avoid this state. However, the reading of genetic information in ciliates is carried out exclusively in the macronucleus, allowing the DNA of the micronucleus to remain in a highly condensed, dormant state at times other than during cell division. This minimizes the risk of genetic information being damaged

before it is passed onto the next generation.

Single cellular organisms other than ciliates only have one nucleus, so the genetic information used for daily life, and that passed onto the next generation are one and the same. This means that unlike ciliates they are unable to edit or amplify their genetic information, and they cannot store their DNA in condensed form. They are constantly exposed to the danger of damaging their one and only set of genetic information.

3.3 A system for controlling the two types of genetic information

In the cell of a general eukaryotic organism, numerous molecular structures interact in a complicated way to control the functions of the single nucleus. But this mechanism is further complicated in ciliates that have two types of cellular nuclei. We questioned whether there are two separate mechanisms and sets of factors involved in controlling the functions of the two types of nuclei.

To investigate, we decided to use *Tetrahymena* (*Tetrahymena thermophila*) (Fig. 2), a species of ciliate often used as a model in biological research. We examined various proteins associated with controlling nuclear functions to see which nucleus they were acting on. First we studied the protein group responsible for forming the nuclear pore complex (Fig. 4), which forms a passageway for the transport of substances between the nucleus and cytoplasm. The nuclear pore complex is known to consist of around 30 different types of proteins from studies carried out on other model organisms. But nothing was known about the proteins making up the pore complex of *Tetrahymena*. From the genetic information database on *Tetrahymena* (from which information can be obtained on DNA sequences, predicted genes, and the structure of proteins coded for by the genes), we decided to focus on proteins similar in structure to nuclear pore proteins of other organisms, to see which nucleus they could be found in within the *Tetrahymena* cell. As a result, it was discovered that the nuclear pore complexes of the *Tetrahymena* nuclei are not made up of two separate sets of 30 different proteins each, but they have many proteins in common. It is only a few specific proteins that differ between the two types of nuclei[3][5].

One such family of proteins was nucleoporin 98 (Nup98). Both the macronucleus and micronucleus were found to have two different types of this protein each. We succeeded in artificially incorporating these proteins into the nuclear pore complexes of opposite nuclei by modifying parts of their molecular structures controlling their selectivity of localization. We expected the transfer of Nup98 to different locations to prompt the transportation of substances to and from opposite nuclei, but this did not happen. Instead, what we observed was the unexpected but obvious obstruction of substance transport by miss-locating Nup98 within both the micronucleus and macronucleus. This suggests that Nup98s act to prevent the wrong directional transport from entering the nuclei (Fig. 5). We succeeded in experimentally demonstrating for the first time that it is this type of partial difference in nuclear pore proteins, which enables the exclusive

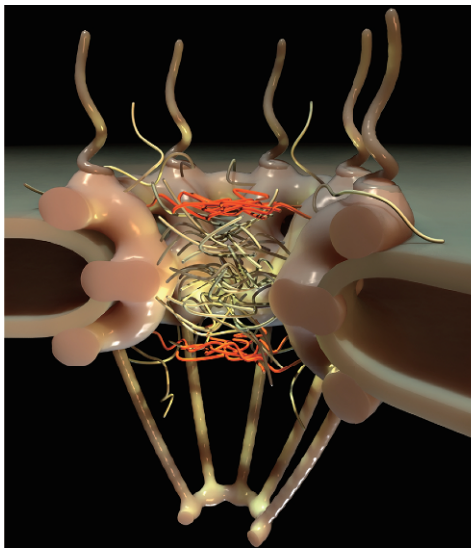

Fig. 4 A model of the nuclear pore complex
The cytoplasm is on the upper side, and the nucleus is below. The orange parts represent Nup98, the proteins that differ between the macronucleus and micronucleus.

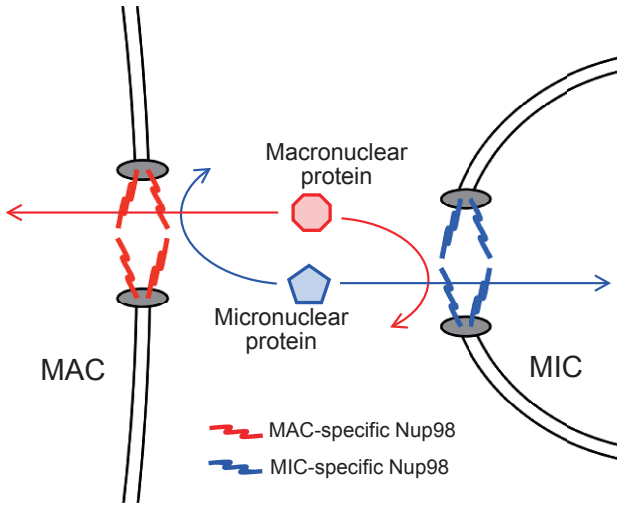

Fig. 5 The relationship between nucleus-specific Nup98 and selective transport of substances to the nucleus

transportation of nucleus-specific substances through the nuclear pore. *Tetrahymena* does not waste unnecessary effort in preparing two sets of completely different nuclear pore proteins, but it achieves differentiation of the permeability of nuclear pores to different substances, and differentiation of nuclear functions by altering minimal kinds of proteins between the two nuclei.

4 The direction of future research and the prospects for practical application

Ciliates store genetic information specialized for utilization in the macronucleus. They make full use of this information in their daily lives, but the macronucleus has a set time limit (life span), and its performance deteriorates after a long time of use. Ciliates spawn new generations of cells before their macronucleus functions break down by using the micronucleus of the parent cell to generate new nuclei. This allows them to generate a brand new macronucleus with a reset time limit. These simple organisms acquired this way of handling genetic information in ancient times, and they have regenerated themselves over and over again passing along their genetic information. It is not clear how ciliates alone acquired this system among eukaryotic single cellular organisms, but the fact that ciliates have survived and remained unchanged for hundreds of millions of years despite violent changes in the terrestrial environment testifies to the merits and durability of the system.

We currently do not have adequate knowledge to thoroughly understand the processes involved in how ciliates acquired this system, and the principles behind it, and further research is required. The differences between the nuclear pore complexes of the macronucleus and micronucleus were limited to substances such as Nup98. But how these proteins are able to position themselves selectively on their respective nuclei that both have the same nuclear pore framework, and the structural reasons behind this phenomenon remain a complete mystery. Understanding this mechanism will require the clarification of all *Tetrahymena* nuclear pore proteins, and a complete comparison of the structures of the macronucleus and micronucleus pore complexes. We also aim to identify the key substances that form the structural bases of the pore complexes giving rise to the differences between the nuclei. Elucidating this will allow the artificial modification of the structures and functions of the nuclear pore complexes of not only ciliates, but also a variety of other cells. The nuclear pore complex and transport of substances through it is one of the most important systems located at the center of a complex intracellular molecular function network. For this reason, great expectations are held for technology to control the structures and functions of nuclear pore complexes, to help in creating artificial cells with a variety of added or modified functions.

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