

The nuclear pore complex acts as a master switch for nuclear and cell differentiation

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Cell differentiation is associated with the functional differentiation of the nucleus, in which alteration of the expression profiles of transcription factors occurs to destine cell fate. Nuclear transport machineries, such as importin- α , have also been reported as critical factors that induce cell differentiation. Using various fluorescence live cell imaging methods, including time-lapse imaging, FRAP analysis and live-cell imaging associated correlative light and electron microscopy (Live CLEM) of *Tetrahymena*, a unicellular ciliated protozoan, we have recently discovered that type switching of the NPC is the earliest detectable event of nuclear differentiation. Our studies suggest that this type switching of the NPC directs the fate of the nucleus to differentiate into either a macronucleus or a micronucleus. Our findings in this organism may provide new insights into the role of the NPC in controlling nuclear functions in general in eukaryotes, including controlling cell fate leading to cell differentiation in multicellular metazoa.

In multicellular organisms, including humans, cells differentiate into various cell types to constitute different tissues and organs during early developmental stages. Such cell differentiation is governed by epigenetic chromatin remodeling involving histone modification¹ and DNA methylation² to establish cell lineage-specific gene expression without changing the genomic sequence. Thus, cell differentiation is destined by the functional differentiation of the nucleus.

Several factors are known to be involved in the nuclear differentiation that

in turn causes cell differentiation. One set of such factors are transcription factors. It is known that expression of a specific set of transcription factors can reprogram the differentiation state of fully differentiated cells obtained from an adult animal back to a pluripotent state;³ Oct4 (Pou5f1), Sox2, c-Myc, and Klf4 have been identified as key factors that cause nuclear reprogramming in mice and humans.^{3,4} Thus, transcription factors act as a molecular switch to change the cellular state of differentiation.

Because such transcription factors have to be transported into the nucleus, the nuclear transport machinery that transports transcription factors into the nucleus is likely to be another factor involved in nuclear differentiation. In support of this premise, it has been reported that subtype switching of importin- α leads to cellular differentiation of embryonic stem (ES) cells into neuronal cells;⁵ importin- α is an adaptor protein that connects the cargo protein to the transporter protein, called importin- β , by binding to the amino acid residues known as the nuclear localization signal (NLS) of the cargo protein. Distinct subtypes of importin- α that transport a distinct set of neural transcription factors into the nucleus cause neural differentiation in ES cells.⁶

Another factor that participates in the nuclear transport of transcription factors, and consequently likely to be involved in nuclear differentiation, is the nuclear pore complex (NPC). All nuclear proteins, including transcription factors, must travel from the cytoplasm through the NPC to reach the nucleus. The NPC structure is well conserved among the eukaryotes.^{7–11} It is composed of

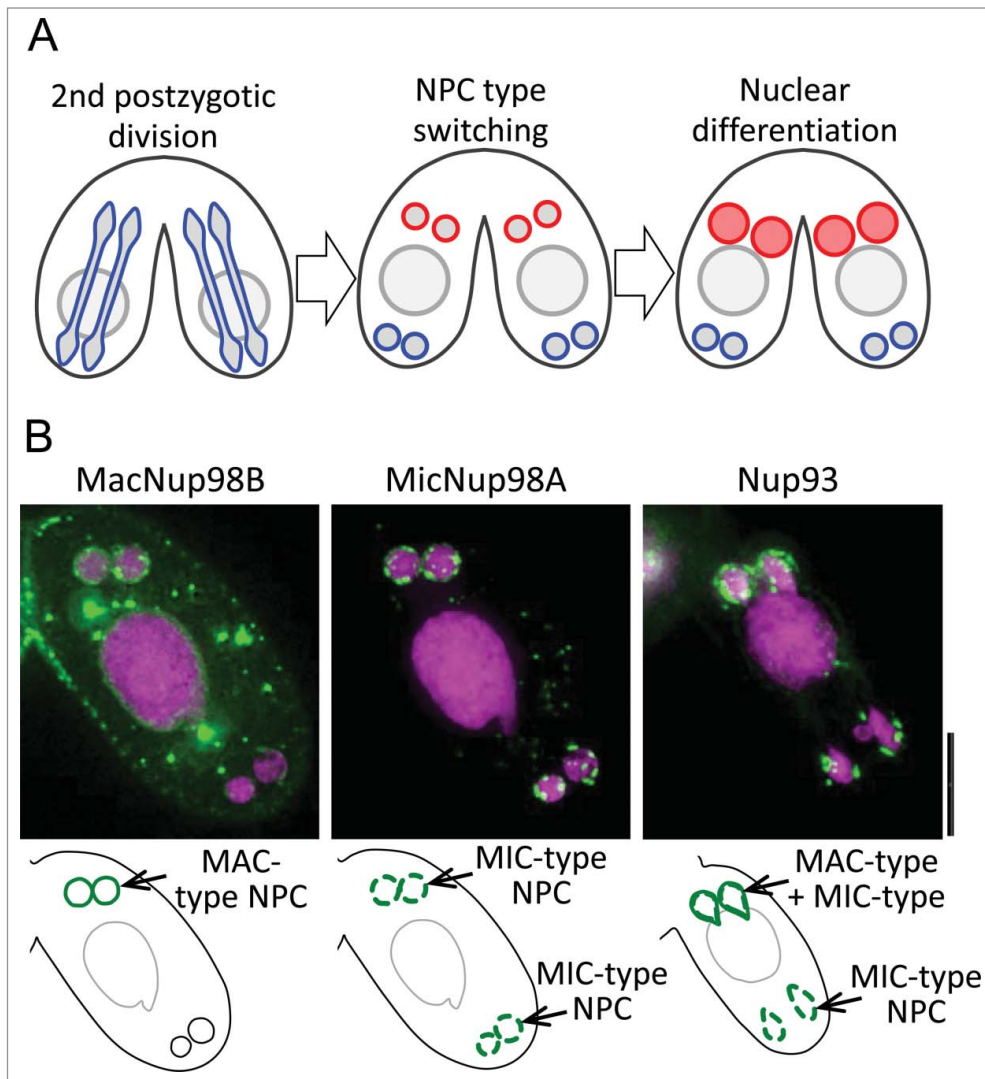


Figure 1. De novo assembly of the macronuclear NPC occurs only in presumptive new macronuclei. **(A)** Schematic representation of nuclear differentiation of *Tetrahymena*. NPC type switching from MIC-type (represented by blue) to MAC-type (represented by red) occurs only in the two anterior-located daughter nuclei of the second postzygotic division (PZD) of the MIC-like zygotic nucleus in each mating partner. The two nuclei that acquire MAC-type NPCs differentiate to new macronuclei. **(B)** Localization of fluorescent protein-tagged nucleoporins early in nuclear differentiation. Methanol-fixed mating pairs were observed. MacNup98B (representing the MAC-type NPC) appeared only in the anterior daughter nuclei of the second PZD (left panel). The pre-existing MicNup98A (representing the MIC-type NPC) is evenly segregated to both daughter nuclei (center panel). Nup93 that commonly exists in all NPCs (representing both types of NPC) exhibits biased distribution to the anterior nuclei (right panel). Left and center panels show the same mating partner of the pair expressing GFP-MacNup98B and mCherry-MicNup98A. mCherry fluorescence in the center panel is shown in green. In the right panel, the single partner of the GFP-Nup93-expressing pair is different from that shown in the other panels. In all panels, the upper side is the anterior of the cell and magenta represents DAPI staining. Bar is 10 μm .

multicopies of almost 30 kinds of the protein components, called nucleoporins (Nup). Recent studies have revealed that the composition of NPC components varies between various cell lines that have a different state of cell differentiation and also changes during cell

differentiation.¹²⁻¹⁶ However, it remains unclear whether such differences in NPC composition is a cause or a consequence of cell differentiation, because no experimental system that directly links active changes of NPC composition to cell differentiation exists.

Tetrahymena is a fascinating model organism that has 2 functionally and structurally distinct nuclei, the macronucleus (MAC) and the micronucleus (MIC), within a single cell. The MAC is a somatic nucleus, in which gene expression is fully active during all life cycle stages. The MIC is a germline nucleus, which is used for sexual reproduction; gene expression in the MIC is kept inactive in all life cycle stages.¹⁷ The MAC and MIC are generated from a single zygotic nucleus that originates from the MIC. Thus, in *Tetrahymena* the mitotic daughters of a single nucleus differentiate into 2 functionally and structurally distinct nuclei.

We used *Tetrahymena* to study the relationship between nuclear structure and function and to investigate the possibility that the NPC may be involved in nuclear differentiation. The NPC is composed of distinct sets of nucleoporins in the MAC and MIC. The well-conserved NPC component Nup98 has 4 homologs in *Tetrahymena thermophila*.^{18,19} Two of the 4 Nup98 homologs are specifically localized in the MAC NPC and the other 2 are specifically localized in the MIC NPC.^{18,20} The MAC-specific Nup98s possess Gly-Leu-Phe-Gly (GLFG) repeats in their N-terminal regions, while the 2 MIC-specific Nup98s possess Asn-Ile-Phe-Asn (NIFN) repeats in their corresponding N-terminal regions.¹⁸ These distinct repeat regions of the Nup98s function in the transport of nucleus-specific linker histones exclusively to the correct nucleus.¹⁸ Thus, the MAC-type NPC and the MIC-type NPC can direct nuclear transport of particular proteins to either the MAC or the MIC in *Tetrahymena*.

To delineate the process of nuclear differentiation, we observed conjugating *Tetrahymena* cells using various fluorescence live-cell imaging methods, including

time-lapse observation, FRAP analysis and Live CLEM (live-cell imaging associated correlative light and electron microscopy).²¹ We found that biased assembly of the MAC-type NPCs occurs immediately after the last post-zygotic division, which generates anterior-posterior polarized nuclei. MAC-specific NPCs assemble in anterior nuclei (presumptive MACs), but not in posterior nuclei (presumptive MICs) (Fig. 1). MAC-specific NPC assembly in the anterior nuclei occurs much earlier than transport of Twi1p,²¹ which is required for MAC genome rearrangement.²² This result indicates that type switching of the NPC is the first event to determine/direct the fate of the nucleus.

Tetrahymena may be regarded as an extreme and singular representative of an organism in which the NPC is involved in the nuclear differentiation process. However, the involvement of the NPC in nuclear differentiation is difficult to detect in typical mononucleated cells since nuclear transport is only directed to a single nucleus. Interestingly, Nup210 (also called gp210) is required for myogenic and neuronal differentiation¹⁶ and Nup133 is required for neural differentiation,¹³ suggesting that a role of the NPC in controlling nuclear differentiation may be common in mammalian cells. In addition, tissue-specific expression of the nucleoporins¹² and disease related mutations of the nucleoporins¹⁴ also support our idea that the NPC is one of determinants controlling the state of nuclear differentiation. Thus, our finding in *Tetrahymena*, revealing a role of the NPC and/or nucleoporins in nuclear differentiation, opens the possibility that the NPC is a master switch in determining the fate of cells during differentiation generally in multicellular eukaryotes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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