Dimerization, Translocation and Localization of Ku70 and Ku80 Proteins

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Chromosomal localization / GFP / Ku70 / Ku80 / Nuclear translocation

The Ku protein is a complex of two subunits, Ku70 and Ku80, and was originally identified as an autoantigen recognized by the sera of patients with autoimmune diseases. The Ku protein plays a key role in multiple nuclear processes, e.g., DNA repair, chromosome maintenance, transcription regulation, and V(D)J recombination. The mechanism underlying the regulation of all the diverse functions of Ku is still unclear, although it seems that Ku is a multifunctional protein that works in nuclei. On the other hand, several studies have reported cytoplasmic or cell surface localization of Ku in various cell types. To clarify the fundamental characteristics of Ku, we have examined the expression, heterodimerization, subcellular localization, chromosome location, and molecular mechanisms of the nuclear transport of Ku70 and Ku80. The mechanism that regulates for nuclear localization of Ku70 and Ku80 appears to play, at least in part, a key role in regulating the physiological function of Ku \textit{in vivo}.

INTRODUCTION

Ku is a nuclear protein originally identified by Mimori \textit{et al.} (1981) as an autoantigen that was recognized by the sera of a Japanese patient with scleroderma polymyositis overlap syndrome.¹ The name Ku was derived from the first two letters of the family name of this patient. Autoantibodies against Ku are also found in patients with other autoimmune diseases, e.g., systemic lupus erythematosus and scleroderma. The human Ku protein is a complex composed of two protein subunits of about 70 and 80 kDa (designated as Ku70 or Ku p70 and Ku80, Ku86 or Ku p80, respectively); various investigators have reported proteins with molecular weights ranging from 67 to 75 kDa and from 80 to 87 kDa, respectively². Here I refer to these proteins as Ku70 and Ku80.

The Ku plays a key role in multiple nuclear processes, e.g., DNA repair, chromosome maintenance, transcription regulation, and V(D)J recombination. The nonhomologous DNA-end-joining (NHEJ) repair process, which is responsible for repairing a major fraction of DNA double strand breaks (DSBs) in somatic cells of all multicellular eukaryotes, is thought to begin with the binding of Ku³. The mechanism of Ku-end recognition has been shown with the recently reported crystal structure of Ku bound to DNA⁴. Evidence for the importance of Ku in the NHEJ repair process has also been reported⁵,⁶. Cells genetically deficient for either Ku70 or Ku80 are sensitive to ionizing radiation. Heterodimerization between Ku70 and Ku80 is essential for Ku-dependent DNA repair \textit{in vivo}, although its role is poorly understood⁷. Some lines of evidence suggest that the heterodimerization is required for the stabilization of Ku70 and Ku80⁸–¹⁰. Ku also plays an important role in V(D)J recombination which is a physiologic DNA double strand breakage and rejoining process restricted to lymphoid cells.

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It was shown that Ku is the DNA-binding component of a DNA-dependent protein kinase (DNA-PK) that phosphorylates several nuclear proteins in vitro, e.g., p53, c-fos, Sp1, XRCC4, DNA-PKcs, or Ku itself, and DNA-PK is involved in NHEJ repair and V(D)J recombination\textsuperscript{6,11–14}. In vivo substrates for DNA-PK have yet to be clearly identified and the role of DNA-PK in the NHEJ repair and V(D)J recombination remains unclear.

Each Ku and DNA-PKcs can bind independently to DNA ends\textsuperscript{15–17}, although upon Ku binding to a DNA end, Ku improves the affinity of DNA-PKcs for the DNA end by 100-fold\textsuperscript{16}. Recently, the gene (Artemis) responsible for a novel human form of scid (radiosensitive severe combined immune deficiency) was cloned\textsuperscript{18}. Mutation in the Artemis protein in human results in hypersensitivity to DNA double-strand break-inducing agents and absence of B and T lymphocytes. Most recently, Ma et al. (2002) have reported that DNA-PKcs and Artemis form a complex in which DNA-PKcs efficiently phosphorylates Artemis in the absence of DNA or DNA termini\textsuperscript{19}. In addition, they showed that the Artemis:DNA-PKcs complex endonuclease activity cleaves 5’ and 3’ overhangs and has hairpin-opening activity. Interestingly, Ku did not interact with Artemis in vitro. Furthermore, Ma et al. (2002) were not able to detect a role for Ku in the overhang processing or in the hairpin opening by the Artemis:DNA-PKcs complex\textsuperscript{19}. Thus, it remains unclear whether Ku is involved in the Artemis:DNA-PKcs-dependent process or not.

In mammalian nuclei, Ku also plays a role in telomeres. Ku has been reported to bind to telomeric sequences\textsuperscript{20,21} and to prevent end-to-end fusions in mammals\textsuperscript{22,23}. Localization of Ku to the telomere does not depend on the DNA-PKcs\textsuperscript{24}, although the absence of DNA-PKcs also results in an increased frequency of telomeric fusions\textsuperscript{25}. Samper et al. (2000) have reported that Ku80 deficiency in mice does not result in telomere shortening or in deregulation of the G-strand overhang, although Ku deficiency in yeast leads to telomere shortening and deregulation of the G-strand overhang\textsuperscript{23}. On the other hand, d’Adda di Fagagna et al. (2001) have demonstrated that inactivation of mouse Ku70 or Ku80 results in telomeric shortening in various primary cell types at different developmental stages\textsuperscript{26}. They have also shown that the telomere length is not altered in cells impaired in two other components, i.e., XRCC4 or DNA ligase IV, indicating that Ku70, Ku80, XRCC4, and DNA ligase IV are all necessary for the NHEJ repair process, but only defects in Ku70 or Ku80 lead to telomeric shortening\textsuperscript{26}. Most recently, it has been reported that mouse Ku80 is the negative regulator of telomerase-mediated telomere elongation\textsuperscript{27}. Further studies will be necessary in order to define the exact role of Ku in the mammalian telomere.

Although Ku and DNA-PKcs belong to the same enzymatic complex, several studies have demonstrated that Ku plays some role in growth regulation and/or senescence independent of the function of DNA-PK activity. Both Ku70- and Ku80-knockout mice exhibited not only deficiencies in DNA DSB repair but also growth retardation\textsuperscript{8,28}. In addition, Ku70- and Ku80-mutant embryo fibroblasts in primary cultures have longer doubling time than that of normal embryo fibroblasts due to the rapid loss of proliferating cells and they show signs of senescence\textsuperscript{8,28}. However, these appear not to be the case for DNA-PKcs-knockout mice\textsuperscript{29}.

In general, Ku is abundantly present in the nucleus, consistent with their functions as subunits of DNA-PK. On the other hand, several studies have reported cytoplasmic or cell surface localization of Ku in various cell types\textsuperscript{30–34}. Thus, Ku may be a multifunctional protein that works in not only nuclei but also cytoplasm and cell surface.

How do these Ku-related pathways function, and how are they regulated? The mechanism underlying the regulation of all the diverse functions of Ku is still unclear. In this review, I focus on the subcellular localization and the molecular mechanisms of nuclear transport of Ku70 and Ku80. To clarify the fundamental characteristics of Ku, we have also examined the expression, heterodimerization, and chromosome location of Ku70 and Ku80. Hence, this review also introduces our studies on the fundamental characteristics of Ku proteins.
DIMERIZATION AND LOCALIZATION OF Ku

CHROMOSOME LOCATION OF Ku70 AND Ku80

DNA damage induced by ionizing radiation results in measurable endpoints such as cell death, mutation, and cell transformation. In particular, double-strand breaks in DNA due to radiation could induce a lethal effect. The repair of this genotoxic lesion can be undertaken via two major pathways: homologous recombination (HR) and NHEJ. It is known that the DSB repair pathways are conserved from yeast to human, but that the preferred pathway utilized differs. Yeast and chicken cells repair the majority of its breaks by HR, whereas mammalian cells utilize predominantly a Ku-dependent NHEJ repair process. For example, disruption of the Ku70 gene in mouse embryonic stem (ES) cells results in a severely increased sensitivity to ionizing radiation, whereas disruption of the same gene in the chicken-B cell line DT40 or yeast does not result in radiosensitivity. The difference may not only be in the relative importance but also in the mechanism of NHEJ. Five proteins (Ku70, Ku80, DNA-PKcs, XRCC4, and DNA ligase IV) are all necessary for NHEJ in vertebrates. In yeast, homologues of Ku70, Ku80, XRCC4 and DNA ligase IV have been identified and shown to participate in NHEJ. However, there is no obvious homologue of DNA-PKcs in yeast.

A chromosome map of functional genes provides information about the evolution of the genome and the identity of candidate genes. In humans, Ku70 was localized to chromosome 22q13, and Ku80 was localized to chromosome 2q35. To clarify the fundamental characteristics of Ku, we determined the chromosomal location of rodent Ku70 (G22p1) and Ku80 (Xrc5) genes by in situ hybridization and/or molecular linkage analysis. Both genes were mapped to a region conserved between the two species, i.e., the C4 band of mouse chromosome 1 and the q34 band of rat chromosome 9. This conserved region is syntenic with human chromosome 2q34-q36, where the human Ku80 was located. In the study of DNA repair, defective hamster mutant cells are a powerful tool for both the identification of genes involved in repair processes and the characterization of repair pathways. In addition, the Chinese hamster is an animal model for studies of rodents, i.e., hereditary diseases and chromosomal aberrations. However, genetic studies (particularly mapping studies) of functional genes are very limited for both the Syrian and Chinese hamster. Thus, we examined the chromosome location of hamster Ku70 and Ku80 genes by in situ hybridization. Ku70 was localized to Syrian hamster chromosome 4qa4.1-qa4.2 and Chinese hamster chromosome 2p3.1, while Ku80 was localized to Syrian hamster chromosome 4qb5-qb6.1 and Chinese hamster chromosome 2p3.5-p3.6. These regions have conserved linkage homologies between the mouse and hamster (Mouse Genome Database [MGD]; available at http://www.informatics.jax.org). Based on these data, we concluded that Ku70 and Ku80 are located within the chromosome regions that are conserved among human, mouse, rat, and the two hamster species (Table 1). We also succeeded in cytogenetically mapping the gene coding for Ku70 in the chicken (KU70) on chromosome 1p2.3 by direct R-banding fluorescence in situ hybridization (FISH) (Table 1). This locus position is included in the chromosome region homologous to human chromosome 22q13 to which the human homologue of Ku70 has been localized. The search for chromosomal homology by comparative mapping provides a clue for clarifying the process of chromosome rearrangements during evolution after divergence of the species. The Ku70 and Ku80 genes are evolutionarily conserved, and homologues were identified in a number of species, e.g., yeast, insect, and vertebrates. Recently, Doherty et al. (2001) have reported the existence of prokaryotic homologues of Ku. On the other hand, the Ku70 and Ku80 proteins share sequence homologies and they display marked structural
Dimerization of Ku70 and Ku80 Proteins

Ku has been generally believed to always exist and function as a heterodimer. Heterodimerization between Ku70 and Ku80 is essential for DNA DSB repair in vivo and is also important in activating DNA-PK, which is one of the main functions of Ku. The X-ray crystal structure of Ku shows that it binds to DNA through a channel that is formed by the heterodimer. Thus, the heterodimer formation might be important in modifying the function of Ku. The heterodimerization is, in part, required for the stabilization of each Ku subunit. Loss of one of the Ku subunits results in a significant decrease in the steady-state level of the other. For example, the absence of the Ku80 protein in xrs-6 cells has been shown to result in the loss of the Ku70 protein. Reintroduction of the Ku80 gene restores the expression of the Ku70 protein. Recently, we confirmed that the exogenous human Ku80 tagged with enhanced green fluorescent protein (EGFP) can also stabilize hamster Ku70.

Both Ku70 and Ku80 contain leucine zipper-like motifs. In early research, it was thought that these regions mediate dimerization, although the role of these motifs remains unknown. During the past five years, the interaction regions of Ku70 and Ku80 have been reported by our and other research groups, but the role of this interaction in the Ku functions remains uncertain. As shown in Fig. 1, we have identified homology, suggesting that the Ku heterodimer evolved from a homodimeric ancestor. A comparative mapping data of both Ku70 and Ku80 genes might provide information about the evolution of the genome and the structure of both genes.

Comparison of the chromosome map of the hamster with those of "map-rich" species, such as mouse and human, enable integration of genomic information obtained from these species with that obtained from hamster. Dmc1, which is required for the homologous synopsis of chromosomes in meiosis, and Ku70 are located on mouse chromosome 15E and human chromosome 22. The two genes that are thought to be involved in the cascade of recombination have been mapped to a region of conserved linkage homology among the five organisms (i.e., mouse, rat, Chinese hamster, Syrian hamster and human). Information regarding the location of the hamster Ku70 gene can be useful in identifying a hamster Dmc1 homologue, although it is not clear whether any biological relationship exists between the function of the two genes and their chromosome location. For example, based on the proximity of Ku70 and Dmc1, it may be assumed that the Dmc1 gene is also located on Syrian hamster chromosome 4q.

DNA-PKcs (PRKDC) is localized to a region of conserved linkage homology among four species, i.e., the mouse, rat, chicken, and human and that MCM4 and DNA-PKcs are close neighbors located on these chromosomes. Chromosomal locations of these genes described above are summarized in Table 1.

### Table 1. Localizations of Ku70, Ku80, DNA-PKcs and their homologues on vertebrate chromosomes

<table>
<thead>
<tr>
<th>Organism</th>
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<td></td>
<td>Ku70</td>
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<td>Human</td>
<td>22q13</td>
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<tr>
<td>Mouse</td>
<td>15E</td>
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<tr>
<td>Rat</td>
<td>7q34</td>
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<tr>
<td>Syrian hamster</td>
<td>4qa4.1</td>
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<tr>
<td>Chinese hamster</td>
<td>2p3.1</td>
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<tr>
<td>Chicken</td>
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DIMERIZATION AND LOCALIZATION OF Ku

Identified using the yeast two-hybrid system that the stretches from amino acid (aa) 378 to 482 of human Ku70 and from aa 374 to 502 of Ku80 participate in the heterodimerization\(^5^9\). Some types of amino acid substitution in these regions prevent the interaction between these two proteins. For example, mutation of Ku70 at aa 385 or 413 significantly impairs its ability to interact with Ku80 in mouse and human cells\(^7,5^4,6^0\). Furthermore, mutation(s) of Ku80 at aa 410 or 453 and 454 significantly impair(s) its ability to interact with Ku70 in human cells\(^5^4,5^7,6^1\). These data are useful for analyzing not only this interaction but also the individual roles of Ku70 and Ku80. On the other hand, these interaction regions do not contain any previously recognized protein-protein interaction motifs. Furthermore, there are questions that need to be answered.

Fig. 1. Schematic representation of functional regions of Ku70 and Ku80 (A) The top panel is a diagram of the full-length Ku70 protein. The location of the region involved in dimerization with Ku80 (amino acids 378–482) is indicated as Ku80-binding. The bottom panel is a diagram of the full-length Ku80 protein. The location of the region involved in dimerization with Ku70 (amino acids 374–502) is indicated as Ku70-binding. NLS, nuclear localization signal. (B, C) Comparison of NLS of human Ku70 (B) or Ku80 (C) with the corresponding regions of other species. Areas in yellow indicate conserved basic amino acids.
Where is the subcellular localization of Ku70 and Ku80? Is the heterodimer always formed?

**SUBCELLULAR DISTRIBUTION OF Ku70 AND Ku80 DURING THE CELL CYCLE**

There are many reports about the subcellular localization of Ku70 and Ku80, which remains controversial, although it seems that Ku is a multifunctional protein that works in nuclei, as mentioned above. Purely nuclear, membrane, cytoplasmic, and both nuclear and cytoplasmic localization of Ku have been reported. The discrepancy between these studies might be due to differences in the use of detection methods, type of cells, species, phase of the cell cycle, phase of the development and/or phase of differentiation. For instance, Bakalkin et al. (1998) have reported that in the embryonic rat brain, Ku was associated with cell nuclei, but was predominantly located in the cytosol in the adult rat cerebellum and hippocampus. It has been reported that the subcellular localization of Ku is different between humans and rodents. It has been shown that the subcellular localization of Ku70 and Ku80 changes during the cell cycle. We have also demonstrated that both Ku70 and Ku80 are distributed throughout both the nucleus and cytoplasm in late telophase as determined by immunological staining.

Ku70- or Ku80-knockout mice exhibit additional defects in certain processes such as the cell cycle and growth control, which were not observed in the absence of DNA-PKcs. It has been reported that Ku plays a role in the G2 and M phases of the cell cycle. Thus, in order to better understand the function of Ku proteins, it is important to determine the cellular localization of both proteins during the course of the cell cycle. We have examined the subcellular localization of Ku70 and Ku80 as a heterodimer complex in interphase and mitotic cells. Our immunocytochemical studies using confocal laser scanning microscopy (CLSM) showed that Ku70 and Ku80 are confined to the nucleus of interphase cells in several cultured human cell lines of tumor-derived and normal origins, e.g., HeLa-S3 and MRC-5. Thus, in interphase nuclei, some of the Ku protein might be bound to chromatin DNA and might be involved in the physical organization of interphase chromatin, as was suggested previously. Most recently, it was reported that in fluorescence photobleaching experiments, the mobility of Ku is characterized by a transient, high-flux association with DNA. By extending confocal analysis to the analysis of mitotic nuclei in cells from early prophase to late telophase, we found that (a) a fraction of Ku was detected at the periphery of condensing chromosomes, (b) most of the Ku protein was found to be diffusely distributed in the cytoplasm, (c) the Ku proteins was not found in the chromosome interior. In early studies using conventional microscopy, Reeves (1987) and Li and Yeh (1992) showed independently that the Ku protein dissociates from chromatin at metaphase. In early studies using CLSM, Higashiura et al. (1992) reported that Ku70 was localized at the periphery of condensed chromosomes at metaphase. On the other hand, by confocal analysis, Reeves et al. (1997) have reported that Ku was associated with condensing chromosomes in early prophase cells, but not later in mitosis, i.e., from late prophase to late telophase. Although the reason for this discrepancy remains unclear, this may be due to the differences in sensitivity of the confocal imaging system and/or to the different antibodies used. We have demonstrated that Ku70 and Ku80 as a heterodimer complex exist at the periphery of condensed chromosomes. The presence of Ku70 and Ku80 at the periphery of mitotic chromosomes might be relevant to their recognition and response to DNA damage that occurs during mitotic processes. Alternatively, Ku70 and Ku80 might participate in sustaining the chromosome structure during metaphase. Although some of the Ku protein is located at the periphery of the chromosomes, most of the protein is scattered throughout the cell during the early prophase to late telophase. The unbound Ku protein could be reused later during assembly of intact chromatin in newly formed daughter nuclei.

Do Ku70, Ku80, and DNA-PKcs always work...
together during the cell cycle in human cells? Evidence obtained from knock-out experiments of mice deficient in either Ku or DNA-PKcs, supports the idea that the Ku protein is implicated not only in DNA-DSB repair through a DNA-PKcs-dependent mechanism, but also in chromatin organization, telomere maintenance, the cell cycle and growth regulation through DNA-PKcs-independent pathways (See above). For example, fibroblasts derived from Ku80-null mice have a prolonged G2/M phase.\(^{28}\) Using CLSM, we observed that the subcellular localization of DNA-PKcs was not perfectly identical to that of Ku in human mitotic cells: no DNA-PKcs staining was detected at the periphery of condensed chromosomes at metaphase, which were stained otherwise with Ku-specific antibodies.\(^{63}\) The association of Ku complexes devoid of DNA-PKcs with metaphase chromosomes indicates that during mitosis, these complexes might perform a function that does not require DNA-PK activity. Munoz et al. (1998) have shown that the DNA topoisomerase II inhibitor induces G2 arrest in Ku80-deficient rodent cells.\(^{72}\) Moreover, bypass of this G2 arrest blockade revealed defective chromosome condensation in Ku80-deficient cells, but not in DNA-PKcs-deficient cells. Taken together, the localization of Ku at the periphery of metaphase chromosomes might very important for a novel function of Ku in the G2/M phase that does not require DNA-PKcs.

**MOLECULAR MECHANISMS OF NUCLEAR TRANSPORT OF Ku70 AND Ku80**

Do Ku70 and Ku80 always form a heterodimer complex? These proteins have been generally believed to always form and function as a heterodimer complex. However, Ku70 and Ku80 may have unique functions that are independent of each other. For example, Ku70 has been reported to show Ku80-dependent and -independent DNA binding, whereas Ku80 requires association with Ku70 for DNA binding.\(^{55}\) As described above, mice that are defective in Ku70 or Ku80 have been generated to examine the physiological roles of Ku.\(^{8,28,70,71}\) Consistent with biochemical and cell biological studies, each gene disruption causes fundamentally similar phenotypes and that Ku70- and Ku80-deficient mice share some features with DNA-PKcs-knockout mice. In addition, there are some phenotypic differences between Ku70- and Ku80-knockout mice. For example, Ku70-knockout mice have small populations of mature T lymphocytes and a significant incidence of thymic lymphoma, but Ku80-knockout mice do not. The differences between them are difficult to explain with available data.\(^5\)

It is possible that Ku70 and Ku80 do not always colocalize and/or the nuclear translocation of Ku70 and Ku80 is independently regulated in mammalian cells. We examined the heterodimerization and molecular mechanisms of nuclear transport of Ku proteins to understand their functional regulation mechanism.\(^{54,59–61,63,64}\) By immunocytochemical analysis, we found that Ku70 and Ku80 are colocalized in the nucleus in interphase. Interestingly, we also found that the staining pattern of Ku70 did not completely coincide with that of Ku80 during the cell cycle.\(^{61}\) The distribution of both Ku70 and Ku80 varied depending on the stage of the cell cycle and the nuclear translocation of Ku70 precedes that of Ku80 in late telophase/early G1 cells.\(^{61}\) These indicate that the time of nuclear translocation of Ku70 and Ku80 is not always simultaneous. In addition, we have also shown that the nuclear transport of free Ku80 starts after the nuclear membrane is formed in late telophase/early G1 cells, suggesting that the nuclear transport of Ku across nuclear pore complexes is selective and depends on the stage of the cell cycle. On the other hand, Fewell and Kuff (1996) reported that the subcellular localization of Ku70 is affected by somatostatin treatment in CV-1 cells, but that of Ku80 is not.\(^{67}\) These results indicate that Ku70 and Ku80 do not always colocalize in mammalian cells. On the basis of these findings, we also speculated the possibility that some of the Ku70 and Ku80 proteins are transported to the nucleus, independent of the translocation of the other.

How is the subcellular localization of Ku70 and Ku80 regulated? We speculated that the nuclear trans-
location of Ku70 and Ku80 could be independently regulated and that the \textit{in vivo} function of Ku may be partially regulated by the control of its transport. Generally, it is known that nuclear proteins containing an intrinsic nuclear localization signal (NLS) enter the nucleus associated with NLS receptors (e.g., importin $\alpha / \beta$) through their own NLSs\textsuperscript{78}). Thus, we searched for the nuclear targeting domain and identified NLSs of Ku70 and Ku80 to clarify the molecular mechanisms of nuclear transport of Ku70 and Ku80\textsuperscript{61,64)} (Fig. 1). NLS of human Ku70 was located at a region composed of 18 amino acid residues (positions 539 to 556). Ku70-NLS consisted of two basic subregions and a nonbasic intervening region. NLS of human Ku80 was located at a region composed of nine amino acid residues (positions 561 to 569). The structures of NLSs of the two Ku protein subunits are different. NLSs of Ku80 and Ku70 belong to the single-basic type and the variant bipartite-basic type, respectively\textsuperscript{61,64)}. Moreover, Ku70- and Ku80-NLSs were recognized and were mediated to target the nuclear rim by NLS receptors. Thus, the nuclear translocation of Ku70 and Ku80 may be regulated by binding with different NLS receptors. Indeed, a number of NLS receptors have been identified\textsuperscript{79)} and the existence of at least three structurally and functionally distinct NLS receptors was reported in a human single-cell population\textsuperscript{80)}. The nuclear translocation of Ku proteins might be controlled (at least in part) at the NLS-recognition step, and that this might be regulated by NLS receptors with various specificities \textit{in vivo}. We have also demonstrated that both Ku70 and Ku80 can translocate to the nucleus without forming into a heterodimer complex using their own NLS\textsuperscript{60,61)}, supporting the possibility that Ku70 and Ku80 could have unique functions independent of each other. Each Ku may have a functional NLS that performs unique functions independent of each other, although further studies will be necessary to confirm this. Interestingly, all of the basic amino acid residues in each NLS are conserved not only among mammalian homologues but also amphibian and avian homologues, suggesting their importance in the mechanism of Ku70 and Ku80 nuclear translocation\textsuperscript{61,64)} (See Fig. 1). Last year, Bertinato \textit{et al.} (2001) have also reported that Ku70 and Ku80 monomers could localize independently to the nucleus\textsuperscript{81}). Searching for \textit{in vivo} receptors of Ku70- and Ku80-NLSs would lead to a better understanding of the physiological functions of the Ku protein.

As described above, heterodimerization is essential for Ku-dependent DNA-repair \textit{in vivo}, but the role of this interaction in Ku functions remains unknown\textsuperscript{7)}. Some lines of evidence suggest that heterodimerization is required for the stabilization of Ku70 and Ku80\textsuperscript{8–10)}. Recently, we have shown a novel role of the heterodimerization of Ku70 and Ku80\textsuperscript{54)}. We examined the subcellular localization of chimeric constructs of green fluorescent protein (GFP) variants and Ku proteins to which mutations were introduced by site-directed mutagenesis, and found that the heterodimerization of Ku is important for their nuclear entry. Thus, Ku70 and Ku80 can translocate to the nucleus not only through their own NLS but also through their heterodimerization and that this heterodimerization is useful for efficient nuclear translocation of each Ku. It is not understood how a cell controls the choice among many Ku functions. We consider that the heterodimerization of Ku is important for their nuclear entry and functional regulation. Ku70 and Ku80 appear to have multiple functions as a monomeric form and a heterodimeric form. The Ku proteins may use the NLS-dependent nuclear translocation pathway to perform some function(s) independent of each other, and Ku subunits may use the nuclear translocation pathway through heterodimerization to perform the same functions dependent on each other.

In addition to our research, some reports may support the idea that the control mechanism for subcellular localization of Ku70 and Ku80 plays an important role in regulating the function of Ku, although the mechanism is poorly understood. For example, changes in the subcellular localization of Ku could be controlled by various external growth-regulating stimuli\textsuperscript{67,82)}. In addition, CD40L treatment of myeloma cells induces the translocation of Ku from the cytoplasm to the cell surface, and that cell surface Ku can mediate both homotypic and heterotypic adhesions\textsuperscript{34)}. Tai \textit{et al.} (2002) have reported the func-
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The functional significance of Ku translocation to the cell membrane of CD40-activated human multiple myeloma cells. Moreover, Morio et al. (1999) have reported that DNA-PK activity of human B cells is, at least in part, regulated by the nuclear translocation of Ku. Most recently, Okui et al. (2002) have reported that the reduced level of repair of DSBs in fibroblasts from a hyper-radiosensitive mutant strain of LEC rat is due to the defect in nuclear accumulation of Ku70 and Ku80 after X-irradiation. The control mechanism for heterodimerization and/or nuclear localization of Ku70 and Ku80 appear to play, at least in part, a key role in regulating the physiological function of Ku in vivo. On the other hand, we also consider that the Ku70 localization may be dependent on not only the nuclear import mechanism but also the nuclear export mechanism, because Ku70 contains a nuclear export signal similar to a leucine-rich sequence. Further studies will be necessary to understand the mechanism of Ku70 and Ku80 nuclear localization in vivo. We hope that further studies to elucidate the molecular mechanisms of the nuclear transport of the Ku subunits will lead to a better understanding of the regulation mechanism of these proteins. The subcellular localization and nuclear translocation mechanism of Ku during the cell cycle are summarized in Fig. 2.

Fig. 2. Subcellular localization and nuclear translocation of Ku during the cell cycle in human cells. The distribution of Ku70 and Ku80 varies depending on the stage of the cell cycle. Unexpectedly, the localization of Ku80 does not completely coincide with that of Ku70 in some cells in late telophase to early G1 phase. In interphase (G1/S/G2) cells, Ku70 and Ku80 colocalize as a heterodimer complex in the nucleus. During mitosis (M), most Ku70 and Ku80 diffusely distributed throughout the cytoplasm. Some of the Ku complexes localized at the periphery of mitotic chromosomes, but DNA-PKcs did not. The nuclear translocation of each Ku starts during the late telophase/early G1 phase after the nuclear envelope is formed. In this case, each Ku translocates to the nucleus through its own NLS, although each Ku can also translocate to the nucleus independent of its own NLS. Ku70-NLS and Ku80-NLS are recognized and are mediated to target the nuclear rim by NLS receptors. Ku70 and Ku80 can be also present as heterodimers, some in complex with DNA-PKcs during the cell cycle. Where green (Ku protein image) and red (PI-stained DNA image) signals overlap, a yellow pattern is seen in interphase cells.
**PERSPECTIVE**

Two major pathways exist in mammalian cells for the repair of DSBs: NHEJ and HR. It is not understood how a cell controls the choice between the two repair pathways. Differences in the timing and destination of translocations of protein are used to provide fine spatial and temporal control of protein-complex formation and function within the cell. Several proteins involved in DNA DSB repair have been found to change their localization within the cell nucleus after exposure to DNA-damaging agents, e.g., ionizing radiations. In particular, Mre11, Rad50, Nbs1, Brca1, and Rad51 form many foci on the nuclei of culture cells exposed to ionizing radiations. Most recently, it was shown that histone H2AX is required for irradiation-induced Nbs1, 53bp1, and Brca1 focus formation, but not for the assembly of Rad51 foci using B cells and fibroblasts derived from an H2AX-knockout mouse. As described in this review, the control mechanism for hetrodimerization and/or subcellular localization of Ku70 and Ku80 appear to play a key role in regulating the physiological function of Ku. Thus, it is important to clarify the timing and destination of translocation of Ku70 and Ku80 within a cell in order to obtain clues that will lead to the understanding of not only the control mechanism of the physiological functions of these proteins but also the choice of the DSB repair pathway.

Accumulating evidence suggests a causal relationship between defects in the two major DNA DSB repair pathways and the development of cancer. Ku80 is a caretaker gene that maintains the integrity of the genome by a mechanism involving the suppression of chromosomal rearrangements. The combined loss of p53 and a DNA repair gene of the genome caretaker class accelerates genomic instability and tumorigenesis. Double mutant mice (i.e., Ku80−/−/p53−/−) develop early onset pro-B-cell lymphomas, in contrast to p53−/− mutant mice. In addition, Ku70 mutant mice have a high incidence of T-cell lymphomas and have increased rates of fibroblast transformation. Thus, it seems that increased knowledge of the Ku-dependent DNA repair pathway will lead to not only obtaining some exciting biological information but also developing more effective cancer therapies. On the other hand, defects in Ku can lead to hypersensitivity to ionizing radiation regardless of the presence or absence of the p53 function in cells. In addition, cells derived from p53−/−/Ku80−/− double mutant mice show higher radiosensitivity than cells derived from p53−/− mutant mice. Thus, it is expected that Ku70 and Ku80 would be important candidate targets for combined gene therapy and radiotherapy.

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