We have cloned a novel protein kinase from human cerebellum and named it LZK (leucine zipper-bearing kinase). The LZK cDNA encoded a 966-amino acid polypeptide that contains a kinase catalytic domain and double leucine/isoleucine zippers separated by a short spacer region. The amino acid sequence of the kinase catalytic domain was a hybrid between those in serine/threonine and tyrosine protein kinases, indicating that LZK belongs to the subfamily of the mixed lineage kinase (MLK) family. The kinase catalytic domain of LZK was most similar to DLK (Holtzman, L. B., Merritt, S. E., and Fan, G. (1994) J. Biol. Chem. 269, 30808–30817), MUK (Hirai, S., Izawa, M., Osada, S., Spyrou, G., and Ohno, S. (1996) Oncogene 12, 641–650), and ZPK (Reddy, U. R., and Presure, D. (1994) Biochem. Biophys. Res. Commun. 202, 613–620), which belong to the same subfamily of the MLK family. However, besides the kinase catalytic domain and double leucine/isoleucine zippers, there was no significant homology with known proteins. The recombinant LZK autophosphorylated in the presence of ATP and divalent cations, and exhibited serine/threonine kinase catalytic activity. Northern blot analysis revealed that LZK is expressed most strongly in the pancreas, with a pattern that differs from other MLKs. Expression of LZK in COS7 cells induced phosphorylation of c-Jun and activation of JNK-1, indicating the association of LZK in the c-Jun amino-terminal kinase/stress-activated protein kinase pathway. The expressed LZK was detected primarily in the membrane fraction, suggesting that LZK interacts with other cellular components in vivo.

**EXPERIMENTAL PROCEDURES**

**cDNA Library Screening and Sequence Determination of LZK—A 826-bp rat cDNA clone with unknown functions, which had been iso-
lated by screening of the zAP rat brain cDNA library with an antibody raised against a soluble fraction of rat brain, was labeled with [α-32P]dCTP by a random primer DNA labeling kit (Takara), and the radiolabeled cDNA was used as a probe to screen approximately 5 × 10^6 plaques of a human cerebellum cDNA library (CLONTECH). Hybridization and washing were done in the buffer containing 50% formamide, 50 mM sodium phosphate, 0.1% SDS, 50 mM NaCl, 1.5 mM MgCl2, 1.5 mM EDTA, 1% Triton X-100, 10% glycerol, 0.1% sodium orthovanadate, and protease inhibitors. The filter was finally washed at 65 °C in 0.1 × SSC and 0.1% SDS, and analyzed by BAS 2000 image analyzer to ensure the integrity and the quantity of RNA per lane, the blot was rehybridized to radiolabeled β-actin cDNA.

Construction of Epitope-tagged LZK—The cDNA fragment encoding the LZK coding sequence was engineered with the Xba1 and dIII restriction sites, and the product was amplified by long and accurate (LA) polymerase chain reaction (oligonucleotides: 5′-GCTCTAGATGCGCACCATT-TCAGGAGCACCTGAGCTGCTCCT-3′; 5′-GCTCTAGATCATTACCA-GTATCGAGTCTAGTTTATGTTG-3′). The digested fragment and a double-strand oligonucleotide linker (oligonucleotides: 5′-AGCT-TCACCATGGGACAGCATGCTCGACACATCCATCACCAGCT-3′; 5′-CTAGGATTGCTAGATGCGCATGCTCGACACATCCATCACCAGCT-3′) were inserted into the cytomegalovirus expression plasmid pCDM8, which had been double-digested with HindIII and XhoI. The linker presented above was engineered with HindIII (5′) and XhoI (3′) restriction sites, and contained the typical Kozak’ consensus sequence and coding sequence for the “MRGSHis” epitope (Met-Arg-Gly-Ser-His8). The MRGSHis8 tag was inserted into the amino terminus of the LZK coding sequence. The construct was sequenced to confirm that the polymerase fidelity and maintenance of the appropriate reading frame.

Expression of the LZK Constructs—COS7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and kanamycin. Cells (2 × 10^6) plated onto a 10-cm tissue culture dish were grown overnight and transiently transfected with 5 μg of the eukaryotic expression plasmid using LipofectAMINE (Life Technologies, Inc.) according to the manufacturer’s protocol. After 48 h, cells were washed twice in ice-cold phosphate-buffered saline, and then lysed by adding 1 ml of lysis buffer (50 mM Hepes, pH 7.5, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 1% Triton X-100, 1 mM sodium orthovandate, 50 mM NaF, 20 mM β-glycerophosphate, 1 mM phenylmethylsulfon fluoride, 10 μg/ml leupeptin, 2 μg/ml aprotinin, and 2 μg/ml pepstatin). After removal of insoluble materials by ultracentrifugation, 2 μg of anti-JNK1 antibody (Santa Cruz Biotechnology) and 200 μl of protein G-Sepharose (Sigma) (10% v/v) were added to the supernatant of the cell lysate, and the mixture was incubated at 4 °C overnight. Beads were washed twice in buffer consisting of 50 mM Hepes, pH 7.5, 150 mM NaCl, 1.5 mM MgCl2, 1 mM EGTA, 0.1% Triton X-100, 1 mM sodium orthovandate, 50 mM NaF, 20 mM β-glycerophosphate, 1 mM phenylmethylsulfon fluoride, 10 μg/ml leupeptin, 2 μg/ml aprotenin and 2 μg/ml pepstatin, and subsequently washed four times in buffer consisting of 20 mM Hepes, pH 7.5, 15 mM MgCl2, 15 mM β-glycerophosphate, 0.1 mM sodium orthovandate, and 2 mM DTT. The immunoprecipitates were incubated for 30 min at 30 °C in 30 μl of the same buffer containing 25 μM ATP, 10 μCi of [γ-32P]ATP (3000 Ci/mmol, Amersham), and 2.5 μg of glutathione S-transferase-c-Jun. Reactions were terminated by addition of the Laemmli sample buffer. The samples were boiled, resolved by SDS-PAGE, and then analyzed by using a BAS 2000 image analyzer.

RESULTS

Isolation of a LZK cDNA and Its Deduced Amino Acid Sequence—A 826-bp rt cDNA fragment with unknown function was used as a probe to screen a human cerebellum cDNA library. Three independent clones were isolated, and their inserts were sequenced. The nucleotide sequence of the longest insert is shown in Fig. 1. The cDNA extends over 3450 nucleotide bases and contains 272 bp of 5'-untranslated nucleotides, a continuous open reading frame of 2898 bp, and 399 bp of 3'-untranslated nucleotides. The putative initiation codon was assigned at nucleotide 273. This methionine codon is located within a sequence context favorable for the Kozak’s rule and is preceded by an in-frame stop codon beginning at base 234. Within the 3'-untranslated region, putative polyadenylation signals are found at 3318 bp (AATAA), at 3354 bp (AATAA), at 3370 bp (AATTA), and at 3517 bp (AATTA) upstream from the poly(A) tract. The longest open reading frame of the cDNA encodes a polyprotein of 966 amino acids, with a calculated molecular mass of 108 kDa. Hydropathy analysis revealed that the protein contains no obvious signal sequence or transmembrane domain (data not shown). Comparison of the sequence with other known proteins revealed that the protein can be divided into several structural domains: a kinase catalytic domain, a double leucine/isoleucine zipper separated by a short spacer region, and an acidic domain at its carboxyl-
terminal end (Fig. 2). Because of this characteristic amino acid sequence, the novel protein was designated as H-LZK (human leucine zipper-bearing kinase).

The kinase catalytic domain extends 249 amino acids from amino acids 166 through 414 (Fig. 3). All 11 subdomains (8), which are typical and common motifs in protein kinases, were conserved in the LZK kinase catalytic domain. This kinase catalytic domain had both features of serine/threonine kinases and tyrosine kinases. Thus, Trp344-Glu345 and Pro352-Tyr353 in subdomain IX and Trp319 (GTVAWMAPE) in subdomain VIII are characteristic for tyrosine kinases. However, Lys281 (HRDLKSPN) in subdomain VIb and Thr316 (GTVAWMAPE) in subdomain VIII are strong indicators of serine/threonine specificity, and Trp319-Met320 (GTVAWMAPE) is often found in Raf family proteins (8).

Following the kinase catalytic domain, LZK contained two...
heptad repeats of nonaromatic hydrophobic amino acids separated by a 25-amino acid spacer. By Chou and Fasman analysis (32), this amino acid sequence formed an α-helix structure, indicating that these regions of LZK are composed of two leucine/isoleucine zipper motifs (Figs. 2 and 3), which may promote homo- or heterodimerization of proteins through hydrophobic interactions. As shown in Fig. 4, hydrophobic residues are conserved at the d position in zipper 1 and 2, forming a hydrophobic stripe on the face of the helix. Except for the d position, these regions are comparatively rich in charged amino acids. In particular, position b (EETE) and position f (KSRR) in zipper 1, and position g (IRRK) in position 2 were primarily composed of negatively or positively charged amino acids, suggesting that they are involved in intra- or intermolecular electrostatic interactions (33, 34).

The regions containing the kinase catalytic domain and leucine zipper domain of this protein have 86.4% and 84.4% identity, respectively, to previously reported proteins DLK (dual leucine zipper-bearing kinase) (21) and ZPK (leucine zipper protein kinase) (22) (see Fig. 3). In addition, the sequence of this region was homologous to MLK1 (15), MLK2 (16, 17), and MLK3 (18–20) by 40.2%, 40.4%, and 39.5%, respectively (Figs. 3 and 5), suggesting that LZK, together with DLK/ZPK, belongs to the MLK (mixed lineage kinase) family, although no strong similarity was found outside this region. However, in contrast to the other members of MLKs, which have a SH3 domain at their amino-terminal ends, LZK (as well as DLK/ZPK) did not contain such a structure (Fig. 5). In addition, LZK and DLK/ZPK have a single invariant Glu at 7 amino acid residues downstream from the invariant Lys in subdomain II, but this is not the case with ordinary MLKs. This Glu residue is believed to play an important role in stabilizing ATP in the ATP-binding site from the crystallographic study (35). These results suggest that LZK, together with DLK and ZPK, belongs to the secondary subgroup of MLK. In addition, LZK and DLK/ZPK share a unique sequence, Ser-Ser-Glu-Glu-Glu-Gly-Glu-Val-Asp-Ser-Glu-Val-Glu (Ser152-Glu262 in LZK) (Figs. 5 and 6). However, the glycine/proline-rich region present in DLK/ZPK at the carboxyl- and amino-terminal ends was not detected in LZK (Fig. 5). It should be noted that the sequence of the LZK kinase catalytic domain is 94.6% identical with that of a partial putative serine/threonine protein kinase (36), implying that these proteins are identical or closely related (Fig. 3).

Tissue Distribution of LZK mRNA—Expression of LZK mRNA was examined by Northern blotting mRNA from several human tissues. The probe used for this analysis was corre-
sponded to nucleotides 1895–3174 (See Fig. 1). Three bands at about >9.5, 8.7, and 6.5 kb were found with pancreas mRNA at the highest level. These bands were also markedly detected in the brain, liver, and placenta, and no positive signal was detected in the heart, lung, skeletal muscle or kidney (Fig. 7A). The expression levels of these three transcripts varied among the tissues. The 8.7-kb band was detected only in mRNA from pancreas. Similarly, the >9.5-kb band was detected only with pancreas and brain. After initial probing with LZK cDNA, the blot was rehybridized with β-actin cDNA to confirm the integrity of the RNA from different tissues (Fig. 7B).

Expression of LZK cDNA in COS 7 Cells and in Vitro Phosphorylation of the Recombinant LZK—To facilitate the detection and immunoprecipitation of the LZK, MRGSHi6 epitope was incorporated at the amino terminus of LZK (see “Experimental Procedures”). The epitope-tagged full-length LZK cDNA was incorporated into the eukaryotic expression vector pCDM8, and the resulting plasmid was transfected into COS 7 cells. Upon immunoblot analysis of LZK transfectants following the SDS-PAGE under reducing conditions, a protein with a molecular mass of 135–150 kDa, which is in good agreement with the predicted mass of the epitope-tagged LZK, was detected, while no band was detected for the non-transfectant (Fig. 8A). In addition, a protein of 135–150 kDa was specifically immunoprecipitated with a MRGSHi6 antibody from the lysate of the transfectant (data not shown).

To study the subcellular localization of LZK, COS7 cells expressing LZK were homogenized in the absence of detergent. The homogenate was fractionated into the soluble and the membrane fractions, and the respective fractions were subjected to SDS-PAGE and followed by immunoblot analysis. Strong immunoreactive bands were detected in the membrane fraction, while only weak bands were found in the soluble fraction (Fig. 8B), suggesting that LZK protein binds to some membrane components probably through interaction with some other cellular components such as lipid and/or anchor protein.

To confirm that LZK is an active protein kinase, MRGSHi6 antibody immunoprecipitates of the LZK transfectants were incubated with [γ-32P]ATP in the presence of Mn2+, Mg2+, and Na2VO4 (protein-tyrosine phosphatase inhibitor), and then the proteins were separated by SDS-PAGE under reducing conditions followed by transfer onto PVDF membranes. Upon autoradiography, immunoprecipitates from the transfectants revealed radioactive bands of 135–150 and 50 kDa, but no detectable bands in non-transfectants (Fig. 9B). The radioactive band of 50 kDa comigrated with the band of heavy chain of IgG, indicating that LZK not only autophosphorylated itself but also phosphorylated heavy chain of IgG.

The radioactive 135–150-kDa band of LZK from the in vitro kinase assay was excised and subjected to partial acid hydrolysis. The resulting materials were separated by one-dimen-
sional electrophoresis on a cellulose plate (25). Analysis by autoradiography and comparison to ninhydrin-stained phosphoamino acid standards revealed only phosphoserine and phosphothreonine (Fig. 9C), indicating that LZK has a serine/threonine kinase activity. However, the present experiment cannot completely exclude the possibility that LZK has a tyrosine kinase activity.

Activation of JNK Pathway by LZK—Recent studies show that some MLKs activate JNK pathway (23, 26, 27). JNK pathway is believed to be predominantly activated by cellular stresses such as UV radiation, inflammatory cytokines, and osmotic shock (28, 29), which results in the activation of transcriptional factors such as c-Jun and ATF2 (30, 31). Because the amino acid sequence of LZK showed high homology to DLK/MUK, which were known to activate JNK pathway, we tested whether or not LZK activates the phosphorylation of c-Jun. COS7 cells were transiently transfected with the expression vector harboring an epitope-tagged LZK, after which the mobility delay of endogenous c-Jun was monitored by immuno-
blot analysis with anti-c-Jun antibodies. As shown in Fig. 10, expression of LZK induced the mobility delay of c-Jun as much as
as was observed with UV radiation. Because the mobility delay is caused by the phosphorylation of c-Jun, these results suggested that expressed LZK activates the endogenous JNK pathway (28). Then to confirm that the phosphorylation of c-Jun observed was really caused by activation of JNK, endogenous JNK1 was immunoprecipitated from the cell lysate and JNK1 activity was determined by in vitro kinase assay using soluble glutathione S-transferase-c-Jun as substrate. As shown in Fig. 11, expression of LZK elevated the JNK1 kinase activity. The extent of JNK1 activation by expression of LZK was comparable to that caused by UV radiation. These results taken together indicated that LZK can effectively activate JNK pathway.

**DISCUSSION**

We examined the cDNA cloning, expression, and characteristics of a novel protein kinase, which is expressed in a spatially regulated fashion in adult human tissues. This protein kinase...
contains a kinase catalytic domain, followed by two leucine/isoleucine zipper motifs, which are separated by a short spacer region. We designated this protein kinase as LZK. The LZK cDNA encodes a protein with an apparent molecular mass of 135–150 kDa, and has serine/threonine kinase activity. LZK is most similar to DLK and ZPK. DLK was identified by Holzman (21) as a novel protein kinase with a unique kinase catalytic domain, the expression of which is regulated spatially and developmentally. ZPK is cloned and identified as a novel putative protein kinase, which is up-regulated in retinoic acid-treated NT2 cells (22). When the region containing the kinase catalytic domain and the leucine/isoleucine zipper domain of LZK was aligned to DLK and ZPK, homology was 86.4% and 86.4%, respectively, with no insertion and/or deletion. Like DLK and ZPK, LZK had invariant Glu at the specific location 7 amino acids downstream from invariant Lys in subdomain II. From crystallographic study and structure-function analysis of other protein kinases, the invariant Glu in subdomain III and invariant Lys in subdomain II are believed to play an important role in stabilizing ATP in the ATP-binding site. The amino acid sequence WMAPE in subdomain VIII is often found in Raf family protein kinases, suggesting that LZK has a MAPKKK-related activity. It is interesting to note that Hirai et al. (23) recently identified MUK, which corresponds to rat homologue of DLK (mouse) and/or ZPK (human), as a MAPKKK-related protein kinase such as c-Raf and MAPK/ERK kinase kinase (MEKK) (37). They showed that MUK phosphorylates and activates JNKK in vivo and in vitro. JNKK (38, 39) can be phosphorylated and activated by the MAPKKK-related kinase, MEKK (40, 41), and acts on Jun kinases, resulting in activation of c-Jun (29, 42). MUK-transfected cells induced hyperphosphorylation of c-Jun, suggesting that MUK can regulate the JNK/SAPK pathway in vivo. The induction of JNK was also observed in a truncated MUK consisting of the kinase catalytic domain and leucine/isoleucine zipper motifs, the amino acid sequence of which was 86.4% identical to that of LZK. As might be expected from this high homology with MUK, LZK was in fact shown to induce phosphorylation of c-Jun and activation of JNK1, indicating that LZK stimulates the JNK/SAPK pathway. The extent of JNK1 activation by LZK expression was comparable to that caused by UV radiation. Consideration of the structures of LZK and other MLKs. SH3 domains, glycine-rich regions, proline-rich regions, glycine/proline-rich regions, kinase domains, leucine zippers, Rac/Cdc42 binding motifs, and SSEESEEVDSEVE sequences are shown.

**Fig. 4.** Helical wheel representation of the leucine/isoleucine zippers of LZK. The residues of putative leucine/isoleucine zippers of LZK were arrayed on a helical wheel. The spokes of the wheel show the relative positions of the amino acids in an a-helix, and the positions a–d correspond to the location of the amino acid residues. In an ideal a-helix, amino acid residues appear on one side of the helix in every two turns. In this model, conserved hydrophobic amino acids were located at the d position.

**Fig. 5.** Schematic representation of the structures of LZK and other MLKs. SH3 domains, glycine-rich regions, proline-rich regions, glycine/proline-rich regions, kinase domains, leucine zippers, Rac/Cdc42 binding motifs, and SSEESEEVDSEVE sequences are shown.

**Fig. 6.** The amino acid sequence SSEESEEVDSEVE were commonly conserved in the carboxyl-terminal domain of LZK and ZPK/DLK/MUK. Besides the regions of kinase catalytic domain and leucine/isoleucine zippers, LZK does not show strong similarity with other protein kinases of the MLK family (see the text). However, the short amino acid sequence SSEESEEVDSEVE is completely conserved in the carboxyl-terminal domain of LZK and DLK/ZPK. The amino acid numbers are indicated on the left side of the sequences.
ering the efficiency and cytotoxicity of the transfection procedure, it seems reasonable to speculate that LZK directly phosphorylates and activates the main components of JNK pathway, such as JNKK and MEKK in vivo.

When expressed in COS7, LZK was present in both cytosol and membrane fractions. Because LZK contains no obvious signal sequence or transmembrane domain, LZK should first be synthesized in cytosol and then translocated to membranes. It has been thought that subcellular compartmentalization is crucial in providing specificity in the regulation and function of protein kinases (43). Some protein kinases were targeted in a given compartment in the cell, and following various stimulations, they translocated to new sites within the cell, where they associated with anchor proteins, regulated by other protein and/or lipid, to gain access to their physiological substrates. Mata et al. (44) recently reported that DLK also exists in both cytosolic and membrane-bound form. They showed that each form of DLK has different biochemical characteristics. The membrane-bound form of DLK is not phosphorylated and forms high molecular complexes, and the cytosolic form of DLK is phosphorylated and exists as monomers. Since LZK, unlike other related protein kinases, does not contain a SH3 domain or a proline-rich region that is a presumed SH3-binding motif, it remains to be clarified what kind of interaction induces the translocation of LZK and regulates the functions of LZK. Recently, MLK-3 was shown to interact specifically with the GTP-bound form of Rac and Cdc42 (45), which regulates the JNK signaling pathway leading to the c-Jun post-transcriptional

![FIG. 7. Expression of LZK mRNA in adult human tissues. The Northern blot was purchased from CLONTECH. In each lane, 2 μg of poly(A)+ RNA from human tissues were loaded. A, the blot was hybridized to a radiolabeled probe corresponding to LZK nucleotides 1895–3174. Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas. B, the blot was rehybridized with a radiolabeled actin probe to confirm the integrity of the RNA.](image)

![FIG. 8. Expression and cellular localization of epitope-tagged LZK in COS7 cells. A, the MRGSHis6-tagged LZK was transiently transfected into COS7 cells. Transfectants and non-transfectants were lysed in the presence of detergent (see “Experimental Procedures”), and the lysate was resolved by SDS-PAGE under reducing conditions, followed by immunoblot analysis with a MRGSHis6 antibody. Lane 1, non-transfectant; lane 2, transfectant. B, COS7 cells transiently transfected with epitope-tagged LZK were lysed in detergent-free lysis buffer (see “Experimental Procedures”) and fractionated by ultracentrifugation. The resulting supernatant (soluble fraction) and pellet (insoluble fraction) were resolved by SDS-PAGE under the reducing conditions, followed by immunoblot analysis with MRGSHis6 antibody. The loaded materials in each lane corresponded to the equal numbers of the cells. Lane 1, soluble fraction; lane 2, insoluble fraction.](image)

![FIG. 9. Immunoprecipitation, autophosphorylation, and phosphoamino acid analysis of LZK. A, expressed epitope-tagged LZK was immunoprecipitated from cell lysate as described under “Experimental Procedures.” Immunoprecipitates were subjected to SDS-PAGE, followed by immunoblot analysis with MRGSHis6 antibody. Lane 1, non-transfectant; lane 2, transfectant. B, immunoprecipitates were incubated in kinase assay buffer containing [γ-32P]ATP and divalent cations, subjected to SDS-PAGE, transferred onto the PVDF membrane, and analyzed by BAS 2000. Lane 1, immunoprecipitates from non-transfectant; lane 2, immunoprecipitates from LZK-transfected cells. C, radioactive band of 130–140 kDa that corresponds to autophosphorylated LZK was excised and subjected to partial acid hydrolysis. Resultant material was resolved by one-dimensional electrophoresis. pS, phosphoserine; pT, phosphothreonine; pY, phosphotyrosine; Pi, free phosphate.](image)

![FIG. 10. Hyperphosphorylation of c-Jun by LZK. Cells were lysed with the Laemmli sample buffer and then subjected to SDS-PAGE under reducing conditions, followed by immunoblot analysis with anti-c-Jun antibodies. Lane 1, control cells (non-transfectants); lane 2, cells stimulated by UV radiation (100 J/m²); lane 3, cells transfected with pCDM8-LZK.](image)
signals regulating the compartmentalization of LZK. Phosphorylated and non-phosphorylated forms of LZK and the studies must be done on the biochemical difference between mechanism which might regulate the function of LZK, further tion of a small GTP-binding protein. However, to clarify the mitogen-activated protein kinase pathways under the regula-
activation (46–48). Considering that structurally related MUK activated the JNK pathway, LZK might be associated with activation (46–48). Considering that structurally related MUK activated the JNK pathway, LZK might be associated with

**FIG. 11. Activation of JNK-1 by LZK.** Endogenous JNK1 was immunoprecipitated from COS7 cells and incubated with glutathione S-transferase-c-Jun in the presence of [γ-32P]ATP (see "Experimental Procedures"). The reaction was stopped by the addition of the Laemmli sample buffer, and the samples were separated by SDS-PAGE and then analyzed by using the BAS 2000 image analyzer. The data shown in the upper panel were quantified and are shown in the graph. Data represent the mean ± S.E. of three independent experiments and are expressed as JNK1 relative activity (lower panel).

activation (46–48). Considering that structurally related MUK activated the JNK pathway, LZK might be associated with mitogen-activated protein kinase pathways under the regulation of a small GTP-binding protein. However, to clarify the mechanism which might regulate the function of LZK, further studies must be done on the biochemical difference between phosphorylated and non-phosphorylated forms of LZK and the signals regulating the compartmentalization of LZK.

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