The IκB kinase (IKK) complex, composed of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (IKKγ), is the key enzyme in activation of nuclear factor κB (NF-κB). To study the mechanism and structure of the complex, we wanted to recombinantly express IKK in a model organism that lacks IKK. For this purpose, we have recombinantly reconstituted all three subunits together in yeast and have found that it is biochemically similar to IKK isolated from human cells. We show that there is one regulatory subunit per kinase subunit. Thus, the core subunit composition of IKKα-β-γ complex is αβγ, and the core subunit composition of IKKβ-γ is βγ. The activity of the IKK complex (α+β+γ or β+γ) expressed in yeast (which lack NF-κB and IKK) is 4–5-fold higher than an equivalent amount of IKK from nonstimulated HEK293 cells. In the absence of IKKγ, IKKβ shows a level of activity similar to that of IKK from nonstimulated HEK293 cells. Thus, IKKγ activates IKK complex in the absence of upstream stimuli. Deleting the γ binding domain of IKKβ or IKKα prevented IKKγ induced activation of IKK complex in yeast, but it did not prevent the incorporation of IKKγ into IKK and large complex formation. The possibility of IKK complex being under negative control in mammalian cells is discussed.

Nuclear factor κB (NF-κB) comprises a family of dimeric transcription factors that regulate the expression of over 150 genes involved in immune, stress, and antiapoptotic processes (1–4). Under normal circumstances, NF-κB is tightly regulated so as to prevent inappropriate inflammation while allowing a rapid response to infection or stress. In unstimulated cells, NF-κB is found predominantly in the cytoplasm in a complex with IκB proteins (a family of inhibitory subunits including IκBα, IκBβ, IκBγ, IκBε, and Bcl-3), which sequester NF-κB and prevent its migration to the nucleus (5, 6). Diverse stimuli, including cytokines, bacterial and viral products, oxidants, and mitogens, lead to phosphorylation of two regulatory serine residues on IκBs, which targets it for polyubiquitination and proteolytic degradation. This frees NF-κB to move to the nucleus, where it binds to and stimulates the transcription of target genes (7).

This phosphorylation is catalyzed by a large kinase complex, IκB kinase (IKK) (8–10). IKK is composed of two homologous kinase subunits, IKKα and IKKβ (85 and 87 kDa, respectively) and a 52-kDa regulatory subunit IKKγ (8, 10, 11), also called NEMO (NF-κB essential modulator) (12). IKKγ is required for activation of IKK in response to TNF and other stimuli (13). IKKα and IKKβ each contain an N-terminal protein kinase domain (containing a canonical mitogen-activated protein kinase activation loop (9)), a leucine zipper, and a helix-loop-helix motif toward the C terminus (10). The catalytic subunits are associated with each other via their leucine zippers (11), and the helix-loop-helix domains are required for full IKK activation (14, 15). It has been suggested that intramolecular interaction of the helix-loop-helix with the kinase domain is involved in IKK activation (14, 15). Studies of recombinant IKKα and IKKβ in insect cells indicate that the catalytic subunits are capable of forming both homodimers and heterodimers (11).

Despite the high degree of sequence similarity between IKKα and IKKβ (52% overall identity and 65% identity in the kinase domains (10)), the two proteins differ. Whereas IKKβ is essential for induction of NF-κB by cytokines, IKKα is essential for limb development and skin differentiation (16–18). Moreover, IKKβ homodimer has ~30-fold higher activity toward IκBα than IKKα (19). Other homologs of IKKα and IKKβ have been isolated, including TBK1/NAK (20, 21) and IKKβ (22, 23).

Based on gel filtration analysis, IKK predominantly forms a 700–900-kDa complex containing IKKα, IKKβ, and IKKγ, but some IKK also elutes at 230 kDa (6, 8). The stoichiometry of IKK subunits in the large complex is still not known. The 230-kDa complex appears to be dimers containing only IKKα and IKKβ, because IKKα and IKKβ expressed in insect cells and purified to homogeneity elute at 230 kDa (11) and because, in IKKγ-deficient cells, IKKα and IKKβ elute at this size (12). The large IKK complex contains a roughly stoichiometric amount of IKKα and IKKβ and an unknown amount of IKKγ (6, 8, 13).

IKKγ is required for the stimulation of IKK activity by upstream signals such as TNF, Tax, lipopolysaccharide, phorbol 12-myristate 13-acetate, and interleukin 1 (12, 13). An α-helical region toward the N terminus of IKKγ interacts with six amino acids at the very C terminus of IKKα and IKKβ (24); interfering with this interaction by means of a peptide inhibitor

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in cells diminishes stimulation of IKK by TNFα (24).

The effect of IKKγ on basal IKK activity is less clear. One report indicated that IKKβ (lacking the C-terminal region, where it binds to IKKγ) was able to activate NF-κB 1.5–2 times more than wild-type IKKβ, and expression of IKKβ that contains point mutations to prevent IKKγ binding was able to activate NF-κB to a greater extent than IKKγ that is capable of binding IKKγ (24). Moreover, May et al. (Ref. 24; see their Fig. 4F) showed that the peptide that diminished interaction of IKKγ with IKKβ increased basal NF-κB activity 2-fold (24). From these experiments, the authors suggested that interfering with the interaction of IKKγ and IKKβ increases basal intrinsic activity of IKK (24). By contrast, another report indicated that expressed IKKβ in COS cells alone had low activity but that its activity was stimulated by co-expression of IKKγ, suggesting that IKKγ stimulates IKKβ (in the absence of stimuli) (25). To better understand whether the presence of IKKγ has a stimulatory or inhibitory effect on IKKβ in the absence of stimulation and to ascertain the role of the IKKγ binding domain (γBD) on basal IKK activity, reconstitution of the full IKK complex in a model system lacking endogenous IKK and its upstream signaling pathways would be very helpful.

In this paper, we demonstrate that human IKK can be reconstituted in yeast and forms a complex that is the same size as IKK isolated from human cells. The activity of this complex was 4–5-fold higher than the IKK activity from nonstimulated HeLa cells. We used this reconstituted system to study the role of the interaction of IKKγ with IKKα and IKKβ (on the level of kinase activity) and also to study the stoichiometry of subunits.

EXPERIMENTAL PROCEDURES

Cloning and Expression of IKK in Yeast—All IKK subunits were expressed with an influenza hemagglutinin (HA) tag at the N terminus. HA-IKKγ was subcloned into the p425 methionine-inducible yeast expression vector, which contains a LEU2 selection marker (26). The promoter regions of pESC-ura and pESC-trp (Stratagene) were removed and replaced with the promoter, multiple cloning site, and transcription termination sequence from p425, and HA-IKKα and HA-IKKβ were subcloned into these vectors, respectively, to generate pESC uralIKKα and pESC trp-met IKKβ. The mutant IKKβBHBD was generated by PCR using Pfu polymerase (Stratagene) and the primers 5'-GTATGAGGGCACAACATTGG and 5'-TCATGAAGCCTGTCAGCCGACTTCTCTCCCTCGTGGCACCGTTACTGCTG to loop out the 18 nucleotides corresponding to the γBD (24); the PCR product was digested and subcloned into the vector pESC trp met HA-IKKβ. IKKβΔ54 was constructed using PCR to truncate the last 8 amino acid residues that could be phosphorylated nonspecifically (27). The reaction was terminated by the addition of SDS-PAGE sample buffer and heating at 95 °C. The reaction products were run on a 10% gel, transferred to nitrocellulose, and probed with an antibody directed against HA (USC/Norris core facility) followed by horseradish peroxidase-linked anti-mouse IgG antibodies (Amersham Pharmacia Biotech). For HeLa cell extracts, IKK was concentrated by Q Sepharose chromatography prior to gel filtration. Samples were loaded on a Superose 6 gel filtration column (Amersham Pharmacia Biotech). For gel filtration procedures, up to 0.3 ml of sample (0.3–1 mg of yeast extract) was injected onto a Superose 6 gel filtration column (Amersham Pharmacia Biotech). For immunoprecipitation, kinase assays, and western blotting, lysates (100 μg) from nonstimulated or TNF-stimulated HeLa cells were prepared as previously described (8). IKKγ with a hexahistidine tag was expressed in Escherichia coli and purified by nickel affinity chromatography as described previously (13). IKKβ with a hexahistidine tag was expressed in Sf9 cells and purified by nickel affinity chromatography as described (11).

Extracts or fast protein liquid chromatography fractions from HeLa cells and yeast were immunoprecipitated using 1 μg of monoclonal anti-IKKα antibodies (B78–1, Pharmingen) followed by binding to protein G-Sepharose beads (Amersham Pharmacia Biotech). Immunocomplexes were pelleted and washed once with lysis buffer and once with 20 mM Tris (pH 7.6), 20 mM MgCl2.

For kinase assays, 5–15 μl of fast protein liquid chromatography fraction or washed immune complexes was incubated for 30 min at 30 °C with a 30-μl reaction mixture containing 20 mM Tris (pH 7.6), 20 mM MgCl2, 20 μM cold ATP, 2 mM dithiothreitol, 35 μM GST-IκBα, and γ32P-ATP (ICN). (GST-IκBα was expressed in bacteria and purified using glutathione-Sepharose beads (Amersham Pharmacia Biotech), was used as the substrate because it contains the regulatory serines but lacks other residues that could be phosphorylated nonspecifically (27)). The reaction was terminated by the addition of SDS-PAGE sample buffer and heating for 5 min at 97 °C. After SDS-PAGE and transfer (see below) radioimaging was detected by PhosphoImager (Molecular Dynamics).

RESULTS

Reconstitution of Human IKK in Yeast—Endogenous and recombinantly expressed IKK has been characterized from mammalian cells as well as insect cells (Sf9 cells with the baculovirus system), but the yeast system may have some advantages for biochemical studies. The baculovirus expression system in Sf9 cells has been successfully used to reconstitute catalytically inactive subunits (11, 13). However, a complete reconstitution has not been shown in Sf9 cells and is not practiced due to the concerns associated with viral infection of Sf9 cells. Mechanistic analysis is also complicated in Sf9 and mammalian cells by the presence of endogenous proteins because expressed mutated forms of IKK are directed into heterocomplexes containing endogenous proteins (11). Recent development of IKK knockout cell lines partially resolves this problem, but there are also newly discovered IKK homologs that may...
have some redundant and overlapping functions.

Many of these potential pitfalls can be overcome by using a reconstituted system. Current knowledge indicates that S. cerevisiae lacks NF-κB activity (28) and therefore is unlikely to contain NF-κB or its upstream signaling molecules. Therefore, exogenously expressed proteins (such as IKK subunits) probably would not be affected by yeast signaling pathways.

The three subunits of IKK were subcloned into plasmids (each with a different selection marker: uracil, tryptophan, or leucine) containing HA tags and methionine-inducible promoters and transformed into yeast. (The inducible system was used in order to grow the yeast to a sufficient density before induction in case the expressed proteins were toxic.) The yeast were grown in selective liquid media prior to induction. After 10–12 h induction, the yeast were washed and lysed, and the S100 was obtained (see under “Experimental Procedures”).

As indicated by Western blot (Fig. 1A), yeast that were not transformed did not contain IKKα, IKKβ, or IKKγ (see the far right lane (YPD)); however, these yeast do contain a protein recognized by the αHA antibody that runs below IKKγ (data not shown). Yeast were transformed with IKKα, IKKβ, or IKKγ in various combinations, and clones expressing the IKK proteins at high levels were chosen for further study. In most clones transformed with multiple subunits, the IKKγ expression was higher than the expression of α or β (as assessed by Western analysis with their identical HA tag). The level of IKKα was slightly lower than the level of IKKβ in the IKKKαβγ clone shown (which was used for further studies).

Because IKK expressed in bacteria forms large aggregates that are not native (data not shown), we needed first to determine whether IKK reconstituted in yeast formed a complex that was similar in size to IKK isolated from human cells. Extracts from untransformed yeast and yeast expressing human IKKβ or IKKαβγ or mutant IKKβH41γ were fractionated on a Superose 6 gel filtration column, and IKK activity toward GST-IκBα was assessed in each fraction. As shown in Fig. 1B, IKKβ (alone) produced in yeast runs at 158–300 kDa; this is the same size as dimers of IKKβ (without IKKγ) from mammalian or Sf9 cells (11). The predominant peak of IKK from TNF-stimulated HeLa cells elutes at about 900 kDa. IKKαβγ produced in yeast produces two peaks, one the size of the full IKK complex from human cells and the other around 158–300 kDa (the size of the catalytic subunit dimers). Extracts from untransformed yeast and from yeast expressing mutant IKKβH41γ do not have significant IKK activity in any fraction (compared with an equal amount of fractions 10–11 taken from yeast expressing IKKαβγ). Similar results were obtained when IKK was isolated from each fraction by immunoprecipitation for kinase assay. These results indicate that the IKK that we have expressed in yeast is native and that, most likely, the 900-kDa complex contains no additional proteins. To demonstrate that it behaves the same as in mammalian cells, we expressed a mutant of IKK in which the critical lysine in the catalytic site is mutated to alanine (βK41A); this IKK was inactive as assessed by immunoprecipitation/kinase assay (Fig. 1C).

**Stoichiometry of the IKK Complex**—The IKKα, IKKβ, and IKKγ that were used for yeast expression have identical HA tags at their N termini. This allowed us to determine the ratio of regulatory to catalytic subunits in the complex. Supernatant from yeast co-expressing human IKKβ and IKKγ was partially purified by gel filtration to remove any subunits that were not incorporated into the large complex. The 900-kDa fraction was analyzed by Western blot using antibodies directed against HA. As shown in Fig. 2A, there is roughly an equal amount of IKKβ and IKKγ in this complex. Densitometric analysis indicates that the ratio of γ to β is between 1.2 and 1.5.

Similarly, when IKKαβγ was partially purified by gel filtration and analyzed by Western using antibodies against HA, the ratio of IKKαβγ to IKKγ was 1:1. We attempted to use the HA immunoblot to quantify the ratio of IKKα to IKKβ, but unfortunately, the tagged proteins are inseparable, even with a large 7.5% SDS-PAGE gel (Fig. 2B). It was previously shown (by Coomassie Blue staining) that the IKK complex contains...
FIG. 2. IKK has a 1:1 ratio of regulatory to catalytic subunits. A, HA-IKKβ and HA-IKKγ were co-expressed in yeast, and the 900-kDa complex was isolated by gel filtration. There is an equal amount of both subunits in this complex as assessed by Western blot against their identical HA tag. Similarly, when HA-IKKα, HA-IKKβ, and HA-IKKγ were co-expressed in yeast and isolated by gel filtration, the total amount of catalytic subunit (HA-IKKα + HA-IKKβ) was equal to the total amount of regulatory subunit (HA-IKKγ). Therefore, the ratio of regulatory to catalytic subunits is 1:1. B, HA-IKKαβγ complex (partially purified by gel filtration) was electrophoresed through a large 7.5% SDS-PAGE gel and transferred to polyvinylidene difluoride, and parallel lanes were probed using antibodies directed against IKKβ, IKKα, and HA. Because the Western bands for HA-IKKα and HA-IKKβ directly overlap, it is not possible to discern the ratio of HA-IKKα to HA-IKKβ. roughly equal amounts of IKKα and IKKβ (13). Therefore, the core subunit composition of IKKαβγ complex is αβγ, and the core subunit composition of IKKβγ is βγ.

Activity of Human IKK Expressed in Yeast—In terms of activity, we predicted two possible scenarios: 1) that the complex would be low activity (similar to or lower than IKK activity from nonstimulated HeLa cells), or 2) that the complex would have high activity (similar to IKK from TNF-stimulated cells). IKK activity from yeast expressing IKKαβγ (partially purified by gel filtration) was compared with nonstimulated and TNF-stimulated HeLa cell extracts (S100); for these studies, the complexes were all immunoprecipitated using specific antibodies against IKKα (the subunit that was limiting in the yeast). The results (Fig. 3A) indicate that the activity of yIKKαβγ is intermediate to nonstimulated and TNF-stimulated HeLa cells. The activity of TNF-stimulated HeLa cells was ~15–20-fold higher than the activity from nonstimulated HeLa cells, and the activity of IKKαβγ expressed in yeast was ~4-fold higher than the activity from nonstimulated HeLa cells (Fig. 3B). To verify that the IKK complex reconstituted in yeast is specific for the regulatory serines in IKKα, we tested the activity of this enzyme toward a mutant form of IKKβ in which the regulatory serines are substituted with alanines (AA). Similar to the enzyme from HeLa cells, IKKαβγ made in yeast phosphorylates wild-type 1xβαβ54 but not the AA mutant (Fig. 3C).

We also compared the various recombinant IKK complexes expressed in yeast to each other. Fig. 3D compares the activities of IKKα, IKKαγ, and IKKαβγ. The 900-kDa complexes of IKKαγ and IKKαβγ were partially purified by gel filtration before immunoprecipitation to eliminate complexes not containing γ, whereas IKKα was immunoprecipitated directly from the S100. Samples were adjusted to contain similar amounts of IKKα in this experiment. Because the stoichiometry of IKKαβγ is 1:2, and the stoichiometry of αγ is 2:2, the IKKαβγ sample contained approximately twice as many total IKK complexes as IKKα alone. The results indicate that IKKα and IKKαγ have very low kinase activity toward GST-1xβαβ54 whereas IKKαβγ has much higher kinase activity. The activity of IKKαγ was over twice the activity of IKKα alone. The activity of IKKαβγ was 10–13-fold higher than that of IKKαγ.

Next, we compared the activities of IKKβ, IKKβγ, and IKKαβγ complexes reconstituted in yeast and partially purified by gel filtration. As shown in Fig. 3E, the activity of IKKβ was lower than the IKK activity of the complexes containing IKKβγ or IKKαβγ. The activity of IKKβγ and IKKαβγ was ~7–15-fold higher than that of IKKβ alone. These data suggest that IKKγ plays a role in allowing the kinase to self-activate. The kinase-stimulating effect of co-expression of IKKγ with IKKβ was observed in completely different yeast clones and
preparations, indicating that the effect is a general phenomenon (data not shown). Moreover, the higher activities of IKKβγ and IKKαβγ than IKKβ alone was observed over a range of IκBα concentrations, indicating that the substrate was not limiting (Fig. 3F). Finally, we compared the activity of IKKβγ expressed in yeast to IKKβ expressed in Sf9 cells by immunoprecipitation/kinase assay; the results (Fig. 3G) indicate that the enzyme expressed in Sf9 cells is over twice as active as the enzyme expressed in yeast.

Role of IKKγ and γ Binding Domain in IKK Activity—To further explore the role of IKKγ on the activity of IKK, we generated IKKα and IKKβ constructs in which the γBD at the C terminus (24) has been deleted. IKKβΔγBD was transformed alone and along with IKKγ and IKKγ plus IKKαΔγBD into S. cerevisiae and the interaction of IKKγ with these mutants was assessed by immunoprecipitation and by gel filtration. As previously shown by affinity pull-down analysis (24), the interaction of IKKγ with IKKβΔγBD was very weak compared with the interaction of IKKγ with wild-type IKKβ as assessed by immunoprecipitation (data not shown). However, the interaction of IKKγ with IKKβΔγBD or with IKKαΔγBD + IKKβΔγBD was not entirely abolished as assessed by gel filtration. As shown in Fig. 4A, IKKβΔγBD expressed alone elutes from the Superose 6 gel filtration column at 158–300 kDa (the same as wild-type IKKβ). However, when co-expressed with IKKγ in the yeast, some of the IKKβΔγBD forms a complex with IKKγ and elutes as a high molecular weight complex. Similarly, some of the IKKαΔγBD+IKKβΔγBD forms a >700-kDa complex with IKKγ.

Whereas wild-type IKKβγ and wild-type IKKαβγ elute predominantly in fractions 10 and 11 (~900 kDa), the IKKαΔγBDβΔγBDγ and IKKβΔγBDγ complexes eluted predominantly in fractions 11 and 12, suggesting that the size or shape of the complex may be slightly different from wild-type IKK.

To investigate the role of the γBD in IKK activity, we compared the activity of these mutant forms to the corresponding wild-types (Fig. 4B). IKKβΔγBD alone had a level of activity similar to that of IKKβ wild-type, and as shown previously, the activity of IKKβ alone was much lower than that with IKKγ. We looked at two gel filtration fractions from the IKKβΔγBDγ extract, fraction 11, in which IKKβΔγBDγ was complexed with IKKγ, and fraction 14, which was devoid of IKKγ. Fraction 11 had very low activity, indicating that the association of IKKβΔγBDγ with IKKγ was not enough for IKKγ to allow IKK to self-activate, suggesting that the γBD is required for the self-activation of IKKβ in the absence of stimulation. Fraction 14 had a level of activity that was similar to that of wild-type IKKβ and that of IKKβΔγBD alone.

Similar effects were observed when we compared the activity of IKKβγ with wild-type to IKKβΔγBDβΔγBDγ. Association of IKKγ with the IKKαΔγBD and IKKβΔγBD mutants was not sufficient to allow the complex to self-activate. It appears that the presence of the γBD is needed for IKKγ to allow IKK to self-activate even in the absence of upstream signaling. This may suggest that this interaction is inhibited in resting mammalian cells.

Finally, we wanted to investigate whether we could activate IKKβ by the addition of purified IKKγ in vitro. IKKβ (partially...
purified by gel filtration) was incubated with 0, 10, and 50 ng of pure IKKγ for 30 min on ice before assessment of IKK activity. As shown in Fig. 4C, addition of IKKγ could not activate the kinase. Similarly, incubation of IKKγ with IKKβ2BD did not change the kinase activity. This suggests that the IKKγ must form a complex with IKKβ in vivo in order to facilitate self-activation.

DISCUSSION

Previous research indicated that S. cerevisiae lacks NF-κB activity (28), and this report indicates that yeast do not contain IKK subunits as assessed by Western blot and also lack the ability to phosphorylate the regulatory serines on IkBa. Reconstitution of IKK complex containing α, β, and γ subunits turned out to be a useful tool because it allowed production of a large quantity of native complex for structural and mechanistic studies. Similar to mammalian and insect cells, IKK catalytic subunits expressed alone in yeast form relatively small 158–300-kDa complexes, whereas the catalytic subunits co-expressed with IKKγ elute at ~900 kDa. This indicates that the IKK reconstituted in yeast is native and most likely contains no additional proteins. Through the use of the identical HA tag on each subunit, we were able to show that there is approximately a 1:1 ratio of IKK catalytic subunits to IKKγ. Therefore, the core subunit composition of IKKαβγ is αβγ. Both IKKα and IKKαγ reconstituted in yeast had a much lower level of kinase activity toward GST-IκBα–574 than IKKαβγ when adjusted for equal amounts of IKKα. This was a predicted result because it was previously shown that IKKβ is a more effective kinase for IkBα than IKKα (19).

The activity of reconstituted IKKαβγ was higher than an equivalent amount of IKK from TNF-simulated HeLa cells. In mammalian cells, IKK is regulated by phosphorylation and dephosphorylation, but the exact mechanisms of regulation are still not known. IKK activity is inhibited by PP2A in vitro, indicating that the kinase is activated by phosphorylation (8). Phosphorylation of two sites in the activation loop of IKKβ is essential for activation of IKK by itself or after stimulation with TNF or interleukin 1, although the kinase responsible is unknown (14). Putative upstream kinases of IKK include NF-κB-inducing kinase (29), mixed-lineage kinase (30), NF-κB-activating kinase (20), and DNA-dependent protein kinase (31). There is also evidence to suggest that the phosphorylation of T-loop residues may occur through autophosphorylation (28), and this report indicates that yeast do not contain this activity toward GST-IκBα–574. Therefore, the yeast data suggest that this dynamic interaction is somehow prevented in resting mammalian cells. In addition, the 4–5-fold higher activity from TNF-stimulated cells over IKK expressed in yeast suggests that interaction of IKKγ with the C terminus of IKKα and IKKβ is a dynamic interaction required for activation. The yeast data suggest that this dynamic interaction is somehow prevented in resting mammalian cells. In addition, the 4–5-fold higher activity from TNF-stimulated cells over IKK expressed in yeast suggests that interaction of IKKγ with the C terminus of IKKα and IKKβ is a dynamic interaction required for activation. The yeast data suggest that this dynamic interaction is somehow prevented in resting mammalian cells. In addition, the 4–5-fold higher activity from TNF-stimulated cells over IKK expressed in yeast suggests that interaction of IKKγ with the C terminus of IKKα and IKKβ is a dynamic interaction required for activation.

The yeast reconstitution system will provide a useful tool for further structural and mechanistic analyses of IKK. Human IKK expressed in yeast can be used for clean mechanistic analysis because there is no background of endogenous IKK proteins. It is also useful for biochemical and regulatory studies, because when the IKK is expressed in yeast and isolated, it is simple to test whether a single molecule or subcellular fraction changes the activity of the enzyme. Finally, it can be used to study the structure and composition of the IKK complexes.

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