Homodimerization and intermolecular tyrosine phosphorylation of the Tyk-2 tyrosine kinase

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Abstract The Jak kinases and Stat transcription factors play a major role in signaling of various cytokines including IFNα. In this report we show a ligand-independent interaction between Tyk-2 and Jak-1 kinases. We also demonstrate that the Tyk-2 kinase forms a homodimer that has the ability to undergo intermolecular tyrosine phosphorylation. The formation of the Tyk-2 homodimer is independent of both tyrosine phosphorylation and the presence of the tyrosine kinase domain.

Key words: Type I IFN-R; Jak kinase; Signal transduction

1. Introduction

Tyrosine phosphorylation plays a central role in signaling by different cytokine and growth factors including type I interferons (IFNs). Type I IFNs (IFNα/β/ω) bind to a cell surface receptor (IFNαR or type I IFN-R) composed of two chains: the α and β subunits [1–3]. The α subunit corresponds to the cDNA termed IFNAR cloned by Uzé et al. [4,5]. The IFNαβR cDNA recently cloned by Novick et al. [6] corresponds to a short form of the β subunit (termed β_S), while a long form (termed β_L) has been recently cloned in our laboratory and is reported elsewhere [7]. The cytoplasmic domain of the α subunit directly associates with Tyk-2 [5,8]. Similarly, Jak-1 appears to coprecipitate with the β subunit [6], although a direct interaction has not been demonstrated. Type I IFN binding to the receptor induces rapid tyrosine phosphorylation of the Tyk-2 [5,9] and Jak-1 kinases [9–11], the components of the transcription factor ISGF3t (Stat1α, Stat1β, and Stat2) [12–16] (reviewed in [17]), and the α and β subunits of the IFNαR [18–20], (E.N. Constantinescu et al., Proc. Natl. Acad. Sci. USA 91 (1994) 9602–9606).

Genetic complementation studies have had a cornerstone role in elucidating the first steps of the type I IFN signaling pathway [9,21,22]. It has been demonstrated that expression of the Jak-1 kinase in mutant U4 cells restores the IFNγ and IFNα responses indicating that Jak-1 plays a role in signaling by IFNα and IFNγ [9]. A different mutant cell line, U1D which lacks the expression of Tyk-2, fails to phosphorylate Jak-1 in response to IFNα indicating that these kinases are interdependent [9]. Interestingly, both U4 and U1D mutant cells showed impaired IFNα binding ability indicating that Tyk-2 and Jak-1 must play a role in the assembly of the IFNαR [9,23]. Velazquez et al. [23] have demonstrated that mutant U1D cells bind IFNα with low affinity, and that transfection of Tyk-2 restores high affinity binding. The low affinity binding observed in U1D cells [23] resembles the low affinity receptors observed in mouse transfectants that only express the human β subunit of the type I IFN-R [7]. These findings led us to hypothesize that an interaction, either direct or through adapter proteins, between the Tyk-2 and Jak-1 kinases brings together the α and β subunits to form the high affinity receptor. In this report we demonstrate that: (i) there is an interaction between the Jak-1 and Tyk-2 kinases; (ii) this interaction does not appear to require tyrosine phosphorylation of the kinases; and (iii) the Tyk-2 kinase forms a homodimer that can undergo intermolecular tyrosine phosphorylation.

2. Materials and methods

2.1. IFNs and antibodies

Human recombinant IFNα2 was kindly provided by Dr. M. Brunda (Hoffman-La Roche). The antiphosphotyrosine and anti-JAK-1 antibodies were obtained from UBI (Lake Placid, NY) and the anti-tyk2 monoclonal antibody was purchased from Transduction Laboratories. The rabbit sera anti-Tyk2 have been previously described [8].

2.2. Immunoblotting

Cells were treated as indicated or left untreated, rapidly centrifuged at 2000 × *g* for 30 s in an Eppendorf microfuge, and subsequently solubilized in lysis buffer (1% Triton X-100/150 mM NaCl/25 mM HEPES (pH 7.5)/1 mM EDTA/200 μM sodium orthovanadate/100 mM NaF/1 mM $MgCl_2$/1 mM phenyl methylsulfonyl fluoride/10 μg/ml aprotinin /10 μg/ml leupeptin) at 4°C for 30 min. Protein complexes were precipitated from cell lysates with the indicated antibodies and protein-G Sepharose (Pharmacia). The immunoprecipitates were analyzed by SDS-PAGE, and transferred to polyvinyldenedilfluoride filters (Immobilon, Millipore). Non-specific binding sites on the filter were blocked with 10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween 20 containing 5–8% bovine serum albumin for 1 h at room temperature. Immunoblots were subsequently incubated with the indicated primary antibodies and appropriate secondary antibodies (linked to horseradish peroxidase) and developed using an enhanced chemiluminescence kit (Amersham).

2.3. Expression of Jak kinases using the vaccinia virus/T7 polymerase system

The cDNAs encoding the different constructs for the Tyk-2 and Jak-1 kinases were subcloned into the pGem vector under the control of the T7 promoter. Cos 7 cells were infected with vaccinia virus carrying the T7 polymerase gene as previously reported [24]. Thirty minutes after infection, cell were transfected using the lipofectamine reagent (Gibco-BRL) following manufacturers' procedures, and incubate for 16 h at 37°C. Cells were lysed as described above for the immunoblotting procedure.

3. Results and discussion

3.1. Interaction between Jak-1 and Tyk-2

To look for an interaction between the Tyk-2 and Jak-1

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Abbreviations: IFN, Interferon; IFNaR, Interferon a receptor; Stat, signal transducer and activator of transcription; mAb, monoclonal antibody.

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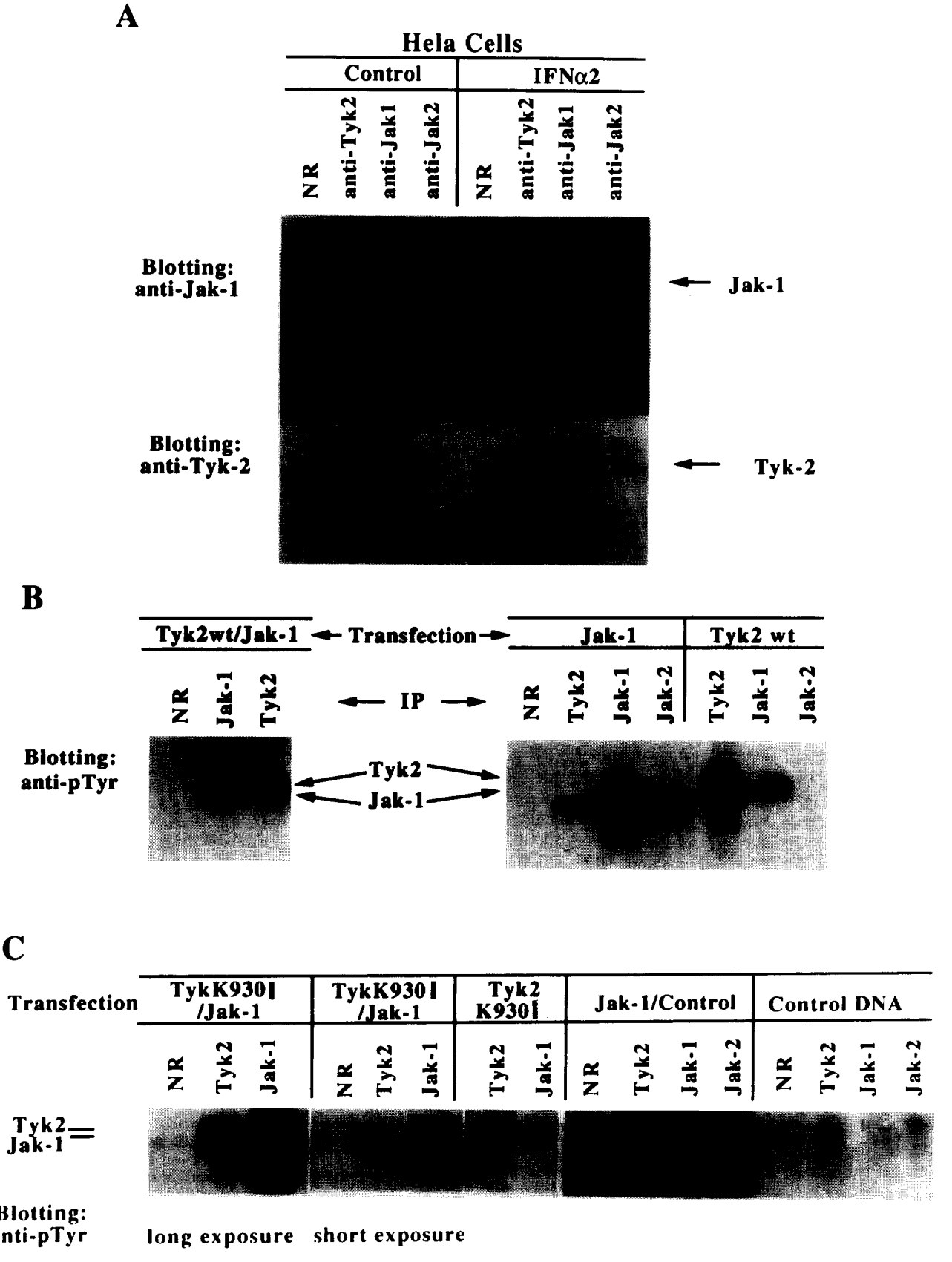
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Hela Cells
Control
IFNα2
NR
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anti-Jak1
anti-Jak2
Blotting: anti-Jak-1
Jak-1
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Tyk-2
B
Tyk2wt/Jak-1
Transfection
Jak-1
Tyk2 wt
IP
Blotting: anti-pTyr
Tyk2
Jak-1
C
Transfection
TykK930I /Jak-1
Tyk2 K930I
Jak-1/Control
Control DNA
Tyk2
Jak-1
Blotting: anti-pTyr
long exposure
short exposure

tyrosine kinases we first performed coprecipitation experiments using Hela cells that have been treated with IFNα2 or left untreated. Fig. 1A (upper panel) shows that the Tyk-2 C-terminal serum coprecipitates a protein that comigrates with Jak-1 in anti-Jak-1 immunoblots. Similarly, stripping and reprobing of the same filter with an anti-Tyk-2 antibody showed that the anti-Jak-1 serum coprecipitates Tyk-2 (Fig. 1A, lower panel). These results suggests that there may be a ligand independent interaction of the Jak-1 and Tyk-2 kinases.

Since the stoichiometry of the Tyk-2/Jak-1 interaction was fractional, we sought an independent way to demonstrate the interaction between these Jak kinases. We overexpressed the Jak tyrosine kinases, alone or in combination, using the vaccinia virus system. The Jak-1 and Tyk-2 cDNAs were subcloned into the pGEM vector under the control of the T7 promoter, and were transfected into COS7 cells that had been previously infected with a vaccinia virus strain carrying the T7 polymerase gene [24]. Cell lysates were immunoprecipitated with the different anti-Jak kinase antibodies followed by immunoblotting with an anti-phosphotyrosine monoclonal antibody. We selected immunoblotting with antiphosphotyrosine antibodies because the transiently overexpressed Jak kinases are baseline tyrosine phosphorylated [5,8,10,25] allowing us to discriminate the recombinant protein from the non-phosphorylated endogenous kinases. More over, tyrosine phosphorylation of the endogenous Jak kinases resulting from type I IFNs in the conditioned medium of vaccinia virus infected cells is not possible, as vaccinia virus produces the B18R protein that binds IFNs and completely blocks IFNα signaling [26]. Fig. 1B shows that the anti-Tyk-2 and anti-Jak-1 sera coprecipitate tyrosine phosphorylated Jak-1 and Tyk-2, respectively, in COS 7 cells cotransfected with the wild type Jak-1 and Tyk-2 constructs. Furthermore, overexpression of only the Tyk-2 kinase shows that the recombinant Tyk-2 protein interacts with the endogenous (non-phosphorylated) Jak-1 protein as it can be coprecipitated by the anti-Jak-1 serum (Fig. 1B). Similarly, the overexpressed Jak-1 kinase interacts with the endogenous Tyk-2 protein (Fig. 1B). Immunoprecipitation with an anti-Jak-2 serum also shows coprecipitation of tyrosine phosphorylated Jak-1, indicating that Jak-1 may also interact with the Jak-2 tyrosine kinases.

3.2. *Tyrosine kinase activity of Tyk-2 is not required for association with Jak-1*

The experiments performed with Hela cells (Fig. 1A) suggested that the interaction between the Jak-1 and Tyk-2 kinases does not require tyrosine phosphorylation, as it is detected in the absence of IFNα2 treatment. To demonstrate that tyrosine kinase activity of Tyk-2 is not necessary for interaction with Jak-1, we coexpressed the tyrosine kinase deficient mutant Tyk2(K930I) [8] with the wild type Jak-1 tyrosine kinase. Fig. 1C shows that no tyrosine phosphorylation of the mutant Tyk-2 is observed when Tyk-2(K930I) is expressed alone. However, the anti-Tyk-2 serum coprecipitates tyrosine phosphorylated Jak-1 when the wild type Jak-1 kinase is coexpressed with the Tyk-2(K930I) (Fig. 1C, long and short exposures). Interestingly, tyrosine phosphorylation of the mutant Tyk-2 protein was detected when this mutant kinase was coexpressed with wild type Jak-1, suggesting that Jak-1 could be responsible for at least part of tyrosine phosphorylation of Tyk-2. The association of Tyk-2(K930I) with Jak-1 was also detected by immunoblotting with anti-Tyk-2 and -Jak-1 antibodies (data not shown). These data clearly demonstrate that (i) there is an association between the Tyk-2 and Jak-1 tyrosine kinases, (ii) this interaction does not require tyrosine kinase activity of the Tyk-2 tyrosine kinase (Fig. 1C), and (iii) a similar interaction is likely to occur between Jak-1 and Jak-2 tyrosine kinases (Fig. 1B, right panel). The finding of tyrosine phosphorylation of the Tyk-2(K930I) mutant further supports the data obtained by genetic complementation studies [9,22] which shows interdependence in the activation (tyrosine phosphorylation) of the Jak-1 and Tyk-2 kinases in IFNα signaling. However, the level of tyrosine phosphorylation of the Tyk-2(K930I) protein observed was lower than when Tyk-2 wild type is overexpressed alone suggesting that the Tyk-2 kinase may also undergo intramolecular and/or intermolecular tyrosine phosphorylation (see below).

3.3. *Homodimerization of Tyk-2*

To test whether the Tyk-2 tyrosine kinase forms a homodimer, we coexpressed the wild type Tyk-2 kinase and a Tyk-2 construct in which the whole kinase domain has been deleted (Tyk-2KD$^-$). The anti-Tyk-2 mAb raised against the N-terminal region of Tyk-2 recognizes both the wild type and the KD$^-$ forms of Tyk-2 (Fig. 2A, lanes 2, 4, 6, and 8). The rabbit serum raised against the C terminal part of Tyk-2, deleted in the KD$^-$ construct, only recognizes the wild type form (Fig. 2A, lane 6, compare blotting with anti-Tyk-2 C-terminal and anti-Tyk-2 mAb). Fig. 2A (lower panel, blotting with Tyk-2 mAb) shows that the Tyk-2 C-terminal serum coprecipitates Tyk-2KD$^-$ with Tyk-2 wild type in cells cotransfected with wild type Tyk-2 and Tyk-2KD$^-$ constructs (lane 8), indicating that Tyk-2 forms a homodimer. Figure 2A (*right side, upper* and *lower panels*) also shows intense tyrosine phosphorylation of wild type Tyk-2 when it is transfected alone (lanes 10 and 12); however, there is a decrease in tyrosine phosphorylation of wild type Tyk-2

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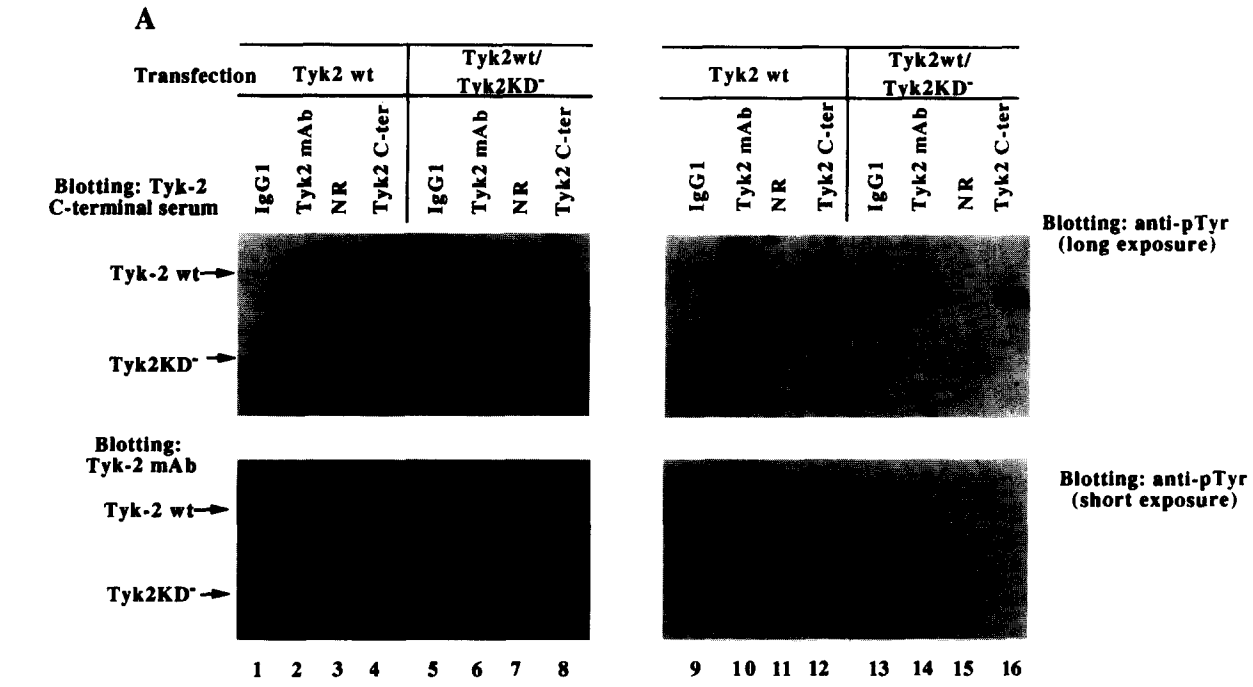
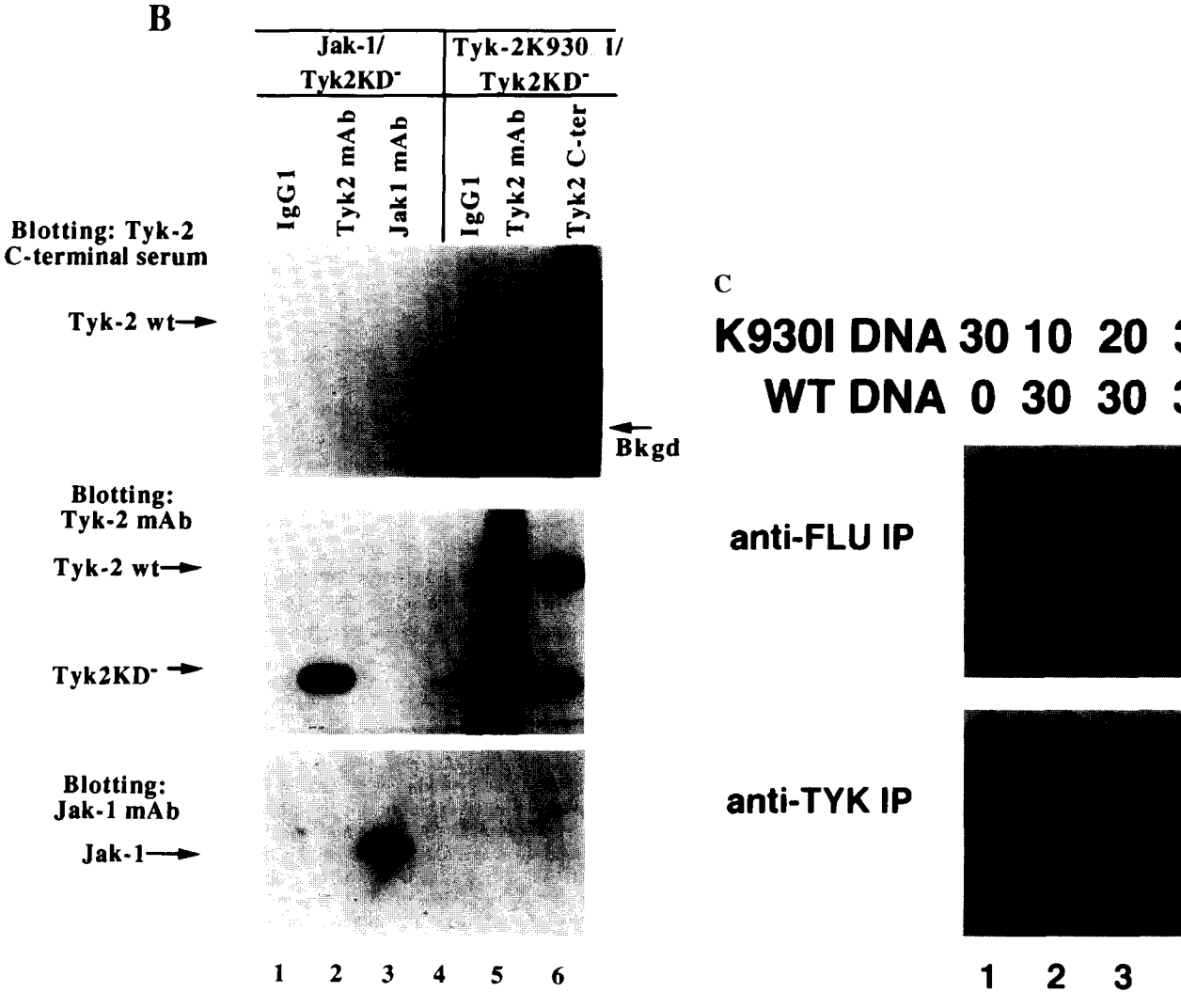
Fig. 1. Interaction between the Jak-1 and Tyk-2 kinases. (A) Hela cells were treated with 10,000 U/ml of IFNα2 for 10 min at 37°C or left untreated, lysed in lysis buffer (1% Triton X-100/ 150 mM NaCl/ 25 mM HEPES pH 7.5/1 mM EDTA/100 μM sodium orthovanadate/100 mM NaF, 1 mM $MgCl_2$/1 mM phenyl methylsulfonyl fluoride/0.15 U/ml aprotinin, 10 μg/ml leupeptin), and immunoprecipitated with the anti-Jak-1 and -Jak-2 rabbit sera (UBI), or anti-Tyk-2 serum [8]. Immunoblotting was performed using anti-Jak-1 and -Tyk-2 monoclonal antibodies (Transduction Laboratories). The filter was stripped with 2% SDS/5% 2-mercaptoethanol/62.5 mM Tris pH 6.8 at 50°C for 30–60 min. Weak coprecipitation of Tyk-2 by the anti-Jak2 antibody was observed in some immunoblots and it could be explained by interaction of these Jak kinases in other cytokine systems [27]. (B) Overexpression of the Jak kinases using the vaccinia virus system. The cDNAs encoding the wild type Jak-1 and Tyk-2 tyrosine kinases and the kinase deficient mutant Tyk-2(K930I) were subcloned into the pGem vector under the control of the T7 promoter [24]. No IFNα treatment was performed in the vaccinia virus experiments because vaccinia virus blocks binding and signaling through the type I IFN-R [26]. A weak tyrosine phosphorylated band observed in anti-Tyk-2 immunoprecipitates after transfection with Tyk-2(K930I) does not correspond to Tyk-2 since it is not detected in anti-Tyk-2 immunoblots (data not shown). (C) Overexpression of Tyk-2(K930I) and wild type Jak-1. Coexpression of the Tyk-2(K930I) and wild type Jak-1 was achieved as described in (B). Immunoblotting was performed with the anti-phosphotyrosine antibody 4G10 (UBI).


A
Transfection
Tyk2 wt
Tyk2wt/ Tyk2KD-
IgG1
Tyk2 mAb
NR
Tyk2 C-ter
Blotting: Tyk-2 C-terminal serum
Tyk-2 wt
Tyk2KD-
Blotting: anti-pTyr (long exposure)
Blotting: Tyk-2 mAb
Blotting: anti-pTyr (short exposure)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16


B
Jak-1/ Tyk2KD-
Tyk-2K930 I/ Tyk2KD-
IgG1
Tyk2 mAb
Jak1 mAb
Tyk2 C-ter
Blotting: Tyk-2 C-terminal serum
Tyk-2 wt
Bkgd
Blotting: Tyk-2 mAb
Tyk2KD-
Blotting: Jak-1 mAb
Jak-1
1 2 3 4 5 6
C
K930I DNA 30 10 20 30
WT DNA 0 30 30 30
anti-FLU IP
anti-TYK IP
1 2 3 4

when it is cotransfected with the KD$^-$ mutant (lanes 14 and 16). This result could not be completely explained by lower levels of expression of Tyk-2 wild type in cotransfection experiments (see below).

3.4. Homodimerization of Tyk-2 does not require kinase activity

To examine whether tyrosine kinase activity of Tyk-2 is necessary for homodimerization, we coexpressed both kinase deficient forms of Tyk-2, Tyk-2KD$^-$ and Tyk-2(K930I), in COS 7 cells. Fig. 2B shows that the kinase deficient forms can form a dimer (lane 6) indicating that tyrosine kinase activity and tyrosine phosphorylation are not required for dimerization. Interestingly, coexpression of Tyk-2KD$^-$ with wild type Jak-1 did not result in an interaction between Tyk-2KD$^-$ and Jak-1 (Fig. 2B) suggesting that the interaction between Tyk-2 and Jak-1 may require the tyrosine kinase domain of Tyk-2.

3.5. Intermolecular tyrosine phosphorylation of the Tyk-2 dimers

We next determined whether the homodimer undergoes intermolecular tyrosine phosphorylation by coexpressing wild type Tyk-2 and a Tyk-2(K930I) construct tagged with the HA epitope in 293T cells. When these constructs are cotransfected in a ratio of 3:1 (wt/mutant) specific tyrosine phosphorylation of Tyk-2(K930I) is observed (Fig. 2C, lane 2). Decreasing the ratio to 1:1 (lane 4) impaired tyrosine phosphorylation as expected when a larger number of Tyk-2(K930I) homodimers are formed. Similar results are observed when an anti-Tyk-2 serum was used for immunoprecipitation; however, the levels of tyrosine phosphorylated Tyk-2 are higher due to detection of both wild type and K930I forms by the Tyk-2 serum (Fig. 2C, lower panel). These result demonstrates that the Tyk-2 homodimer undergoes intermolecular phosphorylation. More over, the lack of tyrosine phosphorylation of Tyk-2KD$^-$ in a Tyk-2KD$^-$/Tyk-2 wild type homodimer (Fig. 2A) also indicates that the tyrosine residue(s) that is (are) phosphorylated are in the kinase domain.

The data presented here demonstrate that the Jak-1 and Tyk-2 tyrosine kinases interact constitutively and after IFNα treatment in Hela cells. This interaction does not require Tyk-2 kinase activity, but may require the presence of the kinase domain of Tyk-2 since the Tyk-2KD$^-$ mutant failed to associate with Jak-1. Second, the Tyk-2 kinase is constitutively present as a homodimer with the ability of intermolecular tyrosine phosphorylation. The interaction between the Tyk-2 and Jak-1 kinases plays a role in the assembly of the high affinity IFNαR explaining the finding that mutant cells that lack either Tyk-2 or Jak-1 bind IFNα with low affinity [9,23]. Altogether these data lead us to propose a model in which activation of the IFNα system can be separated in different stages (Fig. 3). First, binding of type I IFNs to the α and β subunits leads to activation of the Tyk-2 and Jak-1 tyrosine kinases first by cross-phosphorylation between the kinases followed by intermolecular phosphorylation of the homodimers. This sequence of events is supported by genetic complementation studies [9] indicating that no phosphorylation is obtained when either Jak kinase is missing and by the data presented here (Fig. 1). The role of intermolecular phosphorylation of the homodimer is not clear, but it is possible that it serves as an amplification system which activates neighboring receptors. In a second stage, Tyk-2 and Jak-1 tyrosine phosphorylate the α and β subunit of the receptor, respectively [7,8], and the Stat1 and Stat2 transcription factors. Our preliminary studies indicate that the Stat1 and Stat2 factors are docked to the β_L subunit in a ligand-independent fashion forming a constitutive complex with the receptor and Jak kinases (Domanski et al., manuscript in preparation). Finally, a third stage would involve the formation of the ISGF3α complex, translocation to the nucleus an activation of gene transcription.

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References

[1] Colamonici, O.R., D'Alessandro, F., Diaz, M.O., Gregory, S.A., Neckers, L.M. and Nordan, R. (1990) Proc. Natl. Acad. Sci. USA 87, 7230–7234.
[2] Colamonici, O.R., Pfeffer, L.M., D'Alessandro, F., Platanias, L., Rosolen, A., Nordan, R., Cruciani, R.A. and Diaz, M. O. (1992) J. Immunol. 148, 2126–2132.
[3] Colamonici, O.R. and Domanski, P. (1993) J. Biol. Chem. 268, 10895–10899.
[4] Uzé, G., Lutfalla, G. and Gresser, I. (1990) Cell 60, 225–234.
[5] Colamonici, O.R., Uyttendaele, H., Domanski, P., Yan, H. and Krolewski, J.J. (1994) J. Biol. Chem. 269, 3518–3522.
[6] Novick, D., Cohen, B. and Rubinstein, M. (1994) Cell 77, 391–400.
[7] Domanski, P., Witte, M., Kellum, M., Menachen, R., Larner, A., Pitha-Rowe, P. and Colamonci, O.R. (1995) J. Biol. Chem., 270, 21606–21611.
[8] Colamonici, O.R., Yan, H., Domanski, P., Handa, R., Smalley, D., Mullersman, J., Witte, M., Krishnan, K. and Krolewski, J. (1994) Mol. Cell. Biol. 14, 8133–8142.

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Fig. 2. (A) Homodimerization of the Tyk-2 tyrosine kinase. Expression of the different constructs was achieved using the vaccinia virus/T7 polymerase system as described in Fig. 1B. The construct for Tyk-2KD$^-$ was made by deleting a Pst I fragment between nucleotide 2461 in the Tyk-2 cDNA and a *Pst*I site in the polylinker of the pGem vector containing the entire tyrosine kinase domain. The anti-Tyk-2 monoclonal antibody (Transduction Lab.) recognizes an epitope between amino acids 46–258 present in wild type Tyk-2 and Tyk-2KD$^-$, while the anti-Tyk-2 rabbit serum recognizes the last 15 carboxy terminal residues only present in wild type Tyk-2 [8]. The predicted migration of the Tyk-2KD$^-$ is indicated with an arrow in the *upper panel*. The same filter was sequentially probed with anti-phosphotyrosine, anti-Tyk-2 C terminal and anti-Tyk-2 mAb. Stripping of the filter was achieved by incubation with 62.5 mM Tris pH 6.8/2% SDS/5% 2-mercaptoethanol for 1 h at 42°C. (B) Coexpression of the Tyk-2KD$^-$ mutant with the kinase deficient Tyk-2(K930I) and wild type Jak-1. Filters were sequentially probed with the indicated antibodies. The background (bkgd) band observed in some immunoblotting experiments performed with the anti-Tyk-2 C-terminal antibody has slower electrophoretic mobility than Tyk-2KD$^-$ as demonstrated by superimposing autoradiograms obtained with anti-Tyk-2 C terminal serum and anti-Tyk-2 monoclonal antibody (mAb). The background band is not consistently observed in all experiments and it cannot correspond to Tyk-2KD$^-$ because it is not observed after blotting with the C-terminal anti-Tyk2 serum in immunoprecipitates obtained with the N-terminal anti-Tyk-2 mAb (lane 5, upper panel). (C) Coexpression of Tyk-2(K930I) tagged with the HA epitope (K930I DNA) and wild type Tyk-2 (wt DNA). Cells (293T) were transfected using the calcium phosphate method with the HA epitope-tagged Tyk-2(K930I) and wild type Tyk-2 (wt DNA). The amount of DNA (in μg) transfected are indicated on top of each lane. The *upper panel* and *lower panel* correspond to immunoprecipitations with anti-FLU and Tyk-2 antibodies, respectively. Immunoblotting was performed with the antiphosphotyrosine antibody 4G10 (UBI). The anti-Tyk-2 antibody precipitates both wild type Tyk-2 and Tyk-2(K930I), while the anti-FLU antibody precipitates only Tyk-2(K930I).

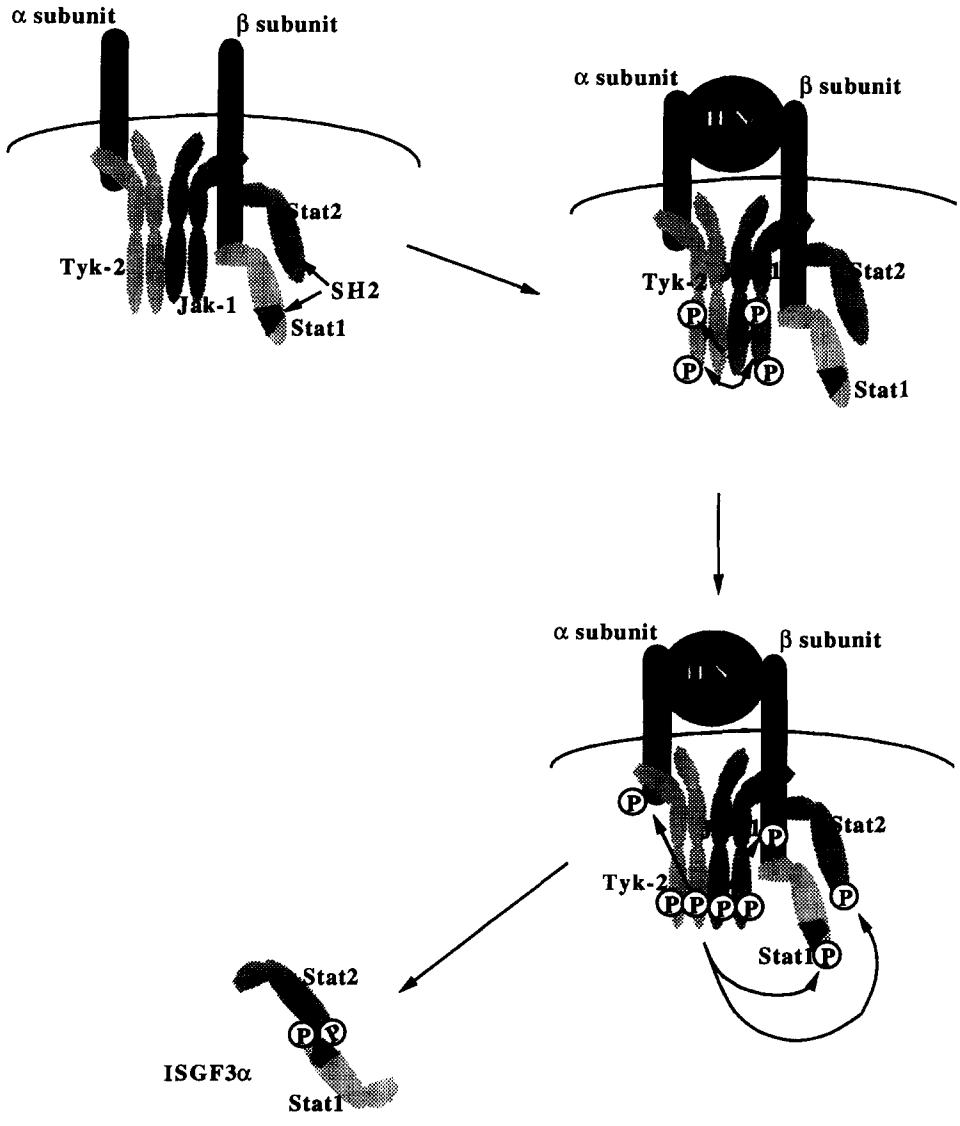

Fig. 3. Model for activation of the IFNα system. The IFNαR is represented by α and β_L subunits. The Tyk-2 tyrosine kinase is shown forming a homodimer. This model also postulates that Jak-1 homodimerizes because of the similarities between the Jak- kinases. The Tyk-2 and Jak-1 kinases associate with each other and with the α and β subunits, respectively. The transcriptional factors Stat1 and Stat2 are docked by the β subunit (β_L) (Domanski et al., manuscript in preparation). The arrows indicate the crossphosphorylation of the Jak kinases and the intermolecular phosphorylation of the Jak homodimers. The model does not speculate on tyrosine phosphorylation of a particular Stat factor by a specific Jak kinase.

[9] Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A.G., Barbieri, G., Witthunh, B.A., Schindler, C., Pellegrini, S., Wilks, A.F., Ihle, J.N., Stark, G.R. and Kerr, I.M. (1993) Nature 366, 129–135.
[10] Silvennoinen, O., Ihle, J.N., Schlessinger, J. and Levy, D.E. (1993) Nature 366, 583–585.
[11] Silvennoinen, O., Schindler, C., Schlessinger, J. and Levy, D.E. (1993) Science 261, 1736–1739.
[12] Fu, X.-Y. (1992) Cell 70, 323–335.
[13] Schindler, C., Shuai, K., Prezioso, V.R. and Darnell, J.E.J. (1992) Science 257, 809–812.
[14] Gutch, M.J., Daly, C. and Reich, N.C. (1992) Proc. Natl. Acad. Sci. USA 89, 11411–11415.
[15] David, M. and Larner, A. (1992) Science 257, 813–815.
[16] Shuai, K., Horvath, C.M., Tsai Huang, L.H., Qureshi, S.A., Cowburn, D. and Darnell Jr, J.E. (1994) Cell 76, 821–828.
[17] Darnell, J.J.E., Kerr, I.M. and Stark, G.R. (1994) Science 264, 1415–1418.
[18] Platanias, L.C. and Colamonici, O.R. (1992) J. Biol. Chem. 267, 24053–24057.
[19] Platanias, L.C., Uddin, S. and Colamonici, O.R. (1994) J. Biol. Chem. 269, 17761–17764.
[20] Abramovich, C., Shulman, L.M., Ratovitski, E., Harroch, S., Tovey, M., Eid, P. and Revel, M. (1994) EMBO J. 13, 5871–5877.
[21] Velazquez, L., Fellous, M., Stark, G.R. and Pellegrini, S. (1992) Cell 70, 313–322.
[22] Watling, D., Guschin, D., Müller, M., Silvenoinnen, O., Witthuhn, B.A., Quelle, F.W., Rogers, N.C., Schindler, C., Stark, G.R., Ihle, J.N. and Kerr, I.M. (1993) Nature 366, 166–170.
[23] Velazquez, L., Mogensen, K.E., Barbieri, G., Fellous, M., Uzé, G. and Pellegrini, S. (1995) J. Biol. Chem. 270, 3327–3334.
[24] Fuerst, T.R., Niles, E.G., Studier, F.W. and Moss, B. (1986) Proc. Natl. Acad. Sci. USA 83, 8122–8126.
[25] Yamamoto, K., Quelle, F.W., Thierfelder, W.E., Kreider, B.L., Gilbert, D.J., Jenkins, N.A., Copeland, N.G., Silvennoinen, O. and Ihle, J.N. (1994) Mol. Cell. Biol. 14, 4342–4349.
[26] Colamonici, O.R., Domanski, P.D., Sweitzer, S.M., Larner, A. and Buller, R.M.L. (1995) J. Biol. Chem. 270, 15974–15978.
[27] Bacon, C.M., Mc Vicar, D.W., Ortaldo, J.R., Rees, R. C., O'Shea, J.J. and Jonhston, J.A. (1995) J. Exp. Med. 181, 399–404.