Characterization of Active and Inactive Forms of the JAK2
Protein-tyrosine Kinase Produced via the Baculovirus
Expression Vector System*

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Three forms of rat JAK2 (type 2) anus tyrosine kinase were produced via the baculovirus expression vector system. Recombinant baculoviruses encoded either the full-length rat jak2 cloned from the Nb2-SP cell line (rJAK2), a carboxyl-terminal deletion mutant lacking the putative catalytic domain (rJAK2(CΔ795)), or an amino-terminal deletion mutant containing the putative catalytic domain (rJAK2(NΔ661)). The proteins produced in infected SF21 cells were assayed for phospho-tyrosine content and autophosphorylation activity. Tyrosine phosphorylation of rJAK2 was not observed 1 day postinfection when rJAK2 was initially produced but was apparent 2 or more days postinfection when the rJAK2 level had significantly increased. Tyrosine phosphorylation of rJAK2(CΔ795) was not observed; further, coproduction of rJAK2(CΔ795) with rJAK2 blocked tyrosine phosphorylation of rJAK2, consistent with previously published results (Zhuang, H., Patel, S. V., He, T.-C., Sonstbye, S. K., Niu, Z., and Wojchowski, D. M. (1994) J. Biol. Chem. 269, 21411-21414). Mutant (NΔ661)rJAK2 exhibited a robust tyrosine phosphorylation signal. A second 62-kDa tyrosine phosphoprotein co-immunoprecipitated with (NΔ661)rJAK2 but not with rJAK2 or rJAK2(CΔ795). Both rJAK2 and (NΔ661)rJAK2 incorporated phosphate under in vitro kinase assay conditions, but rJAK2(CΔ795) did not. A JAK2 oligomer with interacting catalytic sites and/or inhibitory sites would provide a simple model to describe these results.

The JAK family of protein-tyrosine kinases (JAK1, JAK2, JAK3, and TYK2; EC 2.7.1.112) appears to play a crucial role in the signal transduction cascade initiated by activation of numerous cytokine receptors (2). Cytokines that signal through a common γ chain, such as interleukin-2, interleukin-4, and interleukin-7, trigger the tyrosine phosphorylation of JAK3 and may also induce the activation of other Janus kinases (3-5). Interferons α and β require both JAK1 and TYK2 in their signaling cascade (6), and interferon γ signaling requires both JAK1 and JAK2 (7). A number of cytokine receptors are associated with JAK2, and activation of these receptors by their cognate ligands results in the rapid tyrosine phosphorylation of JAK2. Such receptor systems include those for erythropoietin (8), interleukin-3 (9), interleukin-6 (10), leukemia inhibitory factor, ciliary neurotrophic factor, oncostatin M (11), granulocyte-macrophage colony-stimulating factor (12), growth hormone (13), and prolactin (14, 15). It is noteworthy that deletion of the membrane-proximal domains of most of these cytokine receptors abrogates not only the transduction of a cytokine-initiated proliferation but also the cytokine-triggered activation of JAK2 (8, 10, 12, 16, 17). The specific mechanism by which cytokine receptor activation converts these kinases from a latent to an active state remains unclear, but in systems involving the prolactin receptor (14, 15), the leukemia inhibitory factor receptor β chain, and the interleukin-6 signal transducer gp130 (11), JAK2 is associated with receptor before ligand stimulation of the receptor. Thus, it does not appear that receptor association per se is sufficient to activate the kinase. The detailed enzymologic behavior of the “activated” Janus kinases has yet to be characterized, and site-directed mutagenesis studies assessing the functional importance of some of the structural domains of murine JAK2 (1) and of human TYK2 (18) have only recently appeared in the literature.

As an initial step toward understanding the mechanism of enzymatic activation of the JAK protein-tyrosine kinases, we overexpressed the rat jak2 cDNA clone in SF21 cells via the baculovirus expression vector system. We also overexpressed two deletion mutants of JAK2 that were predicted to be active and inactive forms based on the retention and deletion, respectively, of a highly conserved tyrosine kinase motif. The characterization of the resultant protein products is described.

MATERIALS AND METHODS

Construction of Recombinant Baculoviruses—The transfer vector PBacPAK9(rJAK2) was created by first subcloning a 3.0-kilobase EcoRI fragment from the 3′-end of the rat jak2 clone pBK-CMV:RA3.17 (19) into the EcoRI site of PBacPA9 (Clontech), isolating a plasmid containing the jak2 insert in the correct orientation, then substituting a 2.0-kilobase BamHI fragment from another EcoRI site of the rat jak2 clone pBK-CMV:RA3.17 into this plasmid. The transfer vector PBacPAK9(rJAK2(CΔ795)) was created by digesting PBacPAK9(rJAK2) with the restriction endonuclease Stul, religating the plasmid digest mixture, then isolating a plasmid that lacked the Stul fragment at the 3′-end of the jak2 coding region. The transfer vector PBacPAK9(NΔ661)rJAK2 was created by digesting PBacPAK9(rJAK2) with the restriction endonuclease BamHI, religating the plasmid digest mixture, then isolating a plasmid that lacked the BamHI fragment at the 5′-end of the jak2 coding region. Recombinant baculoviruses were generated by Lipofectin-mediated cotransfection of SF21 cells with Bsu36 I-digested BacPAK6 viral DNA (20) (Clontech no. 6144-1) and the appropriate transfer vector. Recombinant baculoviral clones were isolated from the cotransfection supernatant via limiting dilution (21) and identified by dot-blot hybridization (22) against a radiolabeled probe derived from base pairs 2050-2640 of the rat jak2 cDNA. The viral isolate was amplified and the titer ascertained by dot-blot hybridization of DNA from SF21 cells infected in a limiting dilution assay.

Solubilization and Immunoprecipitation of Recombinant Proteins—
SF21 cells, originally derived from Spodoptera frugiperda, were grown at 27 °C in IPL-41 media supplemented with 10% heat-inactivated fetal calf serum, 0.1% penicillin F-68, 50 units of penicillin/mL, and 50 μg of streptomycin/mL. SF21 cells were infected with recombinant baculovirus at a defined multiplicity of infection (m.o.i.),1 then harvested (10-min centrifugation, 3000 × g) at various times post-infection (p.i.) as indicated in the figure legends. Cell pellets were washed in cold phosphate-buffered saline, pH 7.4, and then frozen and stored at −80 °C. Typically, 4 × 10^6 cells were solubilized by resuspension and incubation in 1 ml of lysis buffer (1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM sodium fluoride, 0.2 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, 1 μg/ml leupeptin, 10 μg/ml Tris-Cl, pH 7.6) for 1 hr at 4 °C. Nuclei and insoluble material were removed by centrifugation for 10 min at 3000 × g. The lysate was incubated with 0.1 volume of 10% protein A-Sepharose CL-4B (Pharmacia LKB Biotech Inc.) for 1 hr at 4 °C and centrifuged 15 min at 18000 × g; the supernatant was then transferred to new vials. In some experiments, aliquots were taken at this point for SDS-PAGE and immunoblot analysis. Samples were immunoprecipitated by addition of rabbit anti-mouse JAK2 antisera (Upstate Biotechnology Inc., catalog no. 06-255) and overnight incubation at 4 °C. One-tenth volume of 10% protein A-Sepharose CL-4B was added, and samples were incubated 30 min at 4 °C and then centrifuged 10 min at 18,000 × g. The pellet was washed three times with 1 ml of lysis buffer, then boiled in SDS-PAGE sample buffer before electrophoretic analysis.

In Vitro Kinase Assay—Infected SF21 cells were harvested as above, but not washed with phosphate-buffered saline, then lysed and pre-cleared as above. The lysates were incubated with rabbit anti-mouse JAK2 antisera for 2 hr at 4 °C, then, 0.1 volume of 10% protein A-Sepharose CL-4B was added; samples were incubated another 30 min, centrifuged 15 min at 18,000 × g, and then washed twice with 1 ml of lysis buffer. The immunoprecipitated proteins were resuspended with 0.05% sodium azide for 30 min, then washed three times in 50 mM Tris-Cl, pH 7.6, 200 mM NaCl, 0.25% Tween 20. The membrane was then incubated with secondary antibody (peroxidase conjugated to either goat anti-mouse IgG (KPL, catalog no. 074-1806)) or to goat anti-mouse JAK2 (Upstate Biotechnology Inc., catalog no. 06-255) or murine monoclonal anti-phosphotyrosine (catalog no. 06-321), diluted in blocking buffer for 2 hr, and washed three times in 50 mM Tris-Cl, pH 7.6, 200 mM NaCl, 0.25% Tween 20. The membrane was then incubated with secondary antibody (peroxidase conjugated to either goat anti-mouse IgG (KPL, catalog no. 074-1506) or goat anti-mouse JAK2 (Upstate Biotechnology Inc., catalog no. 06-255)) diluted in blocking buffer for 2 hr, and washed three times in 50 mM Tris-Cl, pH 7.6, 200 mM NaCl, 0.25% Tween 20. The immunodecorated bands were visualized using the ECL system (Amersham).

RESULTS

A recombinant baculovirus was created with a transfer vector (pBacPAK9::rJAK2) that contained approximately 150 base pairs between the polyhedron promoter and the initiation codon of rat JAK2. The mutants (NΔ661) rJAK2 and rJAK2(CΔ795) were derived from restriction endonuclease digests and religation of the transfer vector pBacPAK9::rJAK2. A schematic diagram showing the JAK homology domains retained in each of the three forms, rJAK2, (NΔ661) rJAK2, and rJAK2(CΔ795), is presented in Fig. 1.

A time course of protein production in SF21 cells infected

1 The abbreviations used are: M.O.I., multiplicity of infection; p.i., post-infected; rJAK2(CΔ795), rat JAK2, rJAK2(CΔ795), rat JAK2 mutant initiating at Met661; rJAK2(CΔ795), rJAK2 mutant terminating after Arg795; PAGE, polyacrylamide gel electrophoresis.

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rJ2AK contained a 120-kDa protein that was recognized by both polyclonal anti-J2AK antisera and by the monoclonal antiphosphotyrosine antibody. When Sf21 cells were co-infected with equal M.O.I. levels of both baculoviruses, the production of both rJ2AK and rJ2AK(C795) was detected by anti-J2AK immunodecoration (Fig. 3, panel A), but neither form was recognized by antiphosphotyrosine antibody (Fig. 3, panel B). One interpretation of these data is that the inactive rJ2AK(C795) and rJ2AK form an inactive oligomer, an interpretation originally proposed by Zhuang et al. (1), to account for similar observations obtained in a COS cell expression system. Another concept that derives from such an interpretation is that the amino-terminal portion of the enzyme may contain an inhibitory or regulatory domain.

We sought to test this concept, as well as to determine if sufficient information was contained in the J1H1 J2H2 domains to confer catalytic activity, by constructing the recombinant baculovirus expressing rJ2AK and then harvested 27, 51, 71, 97, or 123 h post-infection as indicated. Control plates were left non-infected or were infected with recombinant baculovirus expressing human prolactin receptor (hPRLR) and harvested 27, 51, 71, 97, or 123 h post-infection. Cellular contents were solubilized and immunoprecipitated with anti-J2AK as described under “Materials and Methods.” Sample aliquots equivalent to approximately 6.7 × 10⁵ cells were subjected to SDS-PAGE and transferred to Immobilon-P membranes and immunodecorated with anti-J2AK (panel A) or with anti-phosphotyrosine (panel B).

been immunoprecipitated before electrophoresis (Fig. 4, panel A). Under these conditions, an endogenous cross-reactive 97-kDa protein was also observed. More importantly, the (N661)J2AK protein was detected as a 56-kDa protein (or as a doublet of protein bands) between 27 and 49 h post-infection (Fig. 4, panel A) and became tyrosine phosphorylated by 49 h p.i. (Fig. 4, panel B). As the infection progressed, a distinct 62-kDa protein in the anti-J2AK immunoprecipitation complex was detected by anti-phosphotyrosine immunodecoration at 72 and 93 h p.i. (Fig. 4, panel B). A Coomassie-stained band was observed at this mobility in immunoblots obtained from cells infected with baculoviruses producing rJ2AK, rJ2AK(C795), or (N661)J2AK (data not shown), but tyrosine phosphorylation of the 62-kDa protein was only observed in immunoblots from cells infected with baculovirus producing (N661)J2AK. It is not yet clear whether the common Coomassie-detectable band, which was not detectable by anti-J2AK (data not shown), was a non-phosphorylated version of the tyrosine-phosphorylated 62-kDa protein or whether it coincidentally comigrated with it.

To demonstrate the relative intensities of the anti-phosphotyrosine signal of rJ2AK and of (N661)J2AK, as well as to test whether co-expression of these two forms would influence each other’s phosphotyrosine state, co-infection studies were carried out (Fig. 5). Sf21 cells were infected with varying pro-
portions of baculoviruses producing rJAK2 and (N\_661)rJAK2 AK2, then harvested and detergent-solubilized 88 h post-infection. The presence of rJAK2 was determined via Western blot analysis of proteins that had not been immunoprecipitated before electrophoresis (Fig. 5, panel A). As the proportion of baculovirus producing rJAK2 increased, so did the intensity of the 120-kDa rJAK2 protein band. The decrease in intensity of the 56-kDa (N\_661)rJAK2 protein band did not correlate as well with the decrease in the proportion of (N\_661)rJAK2 protein band did not correlate as well as phosphotyrosine bands as described under "Materials and Methods." Panel A, following sample pre-clearance with protein A-Sepharose CL-4B but prior to immunoprecipitation, aliquots equivalent to 1.5 x 10^6 cells were subjected to SDS-PAGE. The resolved proteins were transferred to Immobilon-P membrane and then immunodecorated with anti-JAK2. The black arrow points to the (N\_661)rJAK2 AK2. Panel B, cellular contents were immunoprecipitated with anti-JAK2, aliquots equivalent to 2 x 10^6 cells subjected to SDS-PAGE, transferred to Immobilon-P membrane, and then immunodecorated with anti-phosphotyrosine. The black arrow points to (N\_661)rJAK2 AK2, and the white arrow points to an unidentified 62-kDa protein.

Two plausible explanations for this is that the (N\_661)rJAK2 AK2-producing baculovirus produces (N\_661)rJAK2 AK2 more efficiently than does the rJAK2 AK2-producing baculovirus due to differences in the 5'-untranslated region. Since the rJAK2 AK2-producing baculovirus and the rJAK2(C\_795)-producing baculovirus do not differ in the 5'-untranslated region, one would not anticipate such a discrepancy in protein production levels when cells are infected at equal M.O.I. levels. Indeed, the data in Fig. 3, panel A, support this contention.

The tyrosine phosphorylation of JAK2 overexpressed via baculovirus expression vector system has been previously reported (12), and it appeared that the tyrosine phosphorylation occurs constitutively in such an overexpression system. By observing the time course of rJAK2 AK2 protein production (Fig. 2) and by observing JAK2 AK2 production at a fixed post-infection harvest time under varied multiplicities of infection (Fig. 5),

**DISCUSSION**

The tyrosine phosphorylation of JAK2 overexpressed via baculovirus expression vector system has been previously reported (12), and it appeared that the tyrosine phosphorylation occurs constitutively in such an overexpression system. By observing the time course of rJAK2 AK2 protein production (Fig. 2) and by observing JAK2 AK2 production at a fixed post-infection harvest time under varied multiplicities of infection (Fig. 5),
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Fig. 6. Autophosphorylation of immunoprecipitated rJ AK2 forms. Four plates of 4 x 10^6 SF21 cells were infected at a 20-fold M.O.I. with recombinant baculoviruses expressing one of the following: human prolactin receptor (hPRLR), (N\(\Delta\)661)rJ AK2, rJ AK2(C\(\Delta\)795), or rJ AK2. Infected cells were harvested 112 h p.i. and then assayed for in vitro kinase activity as described under "Materials and Methods." Immunoprecipitated equivalents of 2 x 10^6 SF21 cells were loaded in each gel lane the dried gel was exposed to film for 9 h. The black arrow points to the full-length (120 kDa) rJ AK2 in the gel autoradiograph, and the white arrow points to the (56 kDa) (N\(\Delta\)661)rJ AK2 mutant.

one can amend this interpretation. It appears that once the JAK2 production exceeds a critical level, tyrosine autophosphorylation occurs spontaneously.

A simple, plausible explanation of this observation is that monomers of JAK2 begin to form catalytically competent oligomers (possibly simple dimers) once the JAK2 concentration approaches a value that shifts the JAK2(oligomer)/JAK2(monomer) equilibrium to a detectable amount of oligomer. This explanation is currently plausible because it is consistent with several pieces of information. First, the observation that inactive JAK2 mutants are capable of inhibiting autophosphorylation of wild-type JAK2 (initially observed by Zhuang, et al. (1) and demonstrated here in Fig. 3) could be explained by the formation of an inactive hetero-oligomer. Second, it has also been shown that JAK2-related receptors such as growth hormone receptor (26, 27) dimerize subsequent to ligand binding. Activation of tyrosine kinase activity is mediated by dimerization of JAK2-related receptors such as prolactin receptor (28) and gp130 (29), which are associated with JAK2 before ligand binding (11, 14, 15), leading to the presumption that bringing the JAK2 proteins into close physical proximity is crucial for the "activation" of the JAK2. Finally, there is evidence that correlates a significant increase in jak2 mRNA (19) in the Nb2-SP pre-T lymphoma cell line (as compared to the parental Nb2-1IC cell line) to an increase of tyrosine-phosphorylated JAK2 protein, a phenomenon that may be relevant to the loss of absolute prolactin dependence in the Nb2-SP cell line (30).

The concept that the oligomeric form of JAK2 is the catalytically active form must be verified by additional independent experiments before it is acceptable as a part of the mechanism of enzymatic activation. It should be noted that there is no evidence yet to support transphosphorylation or inter-JAK2 phosphorilation; the full-length rJ AK2 does not phosphorylate the inactive rJ AK2(C\(\Delta\)795), nor does the "hyperactive" (N\(\Delta\)661)rJ AK2 appear to phosphorylate rJ AK2. These data are admittedly inconclusive since we have not shown that rJ AK2(C\(\Delta\)795) contains suitable phosphorylation sites nor have we demonstrated that (N\(\Delta\)661)rJ AK2 and rJ AK2 can form hetero-oligomers. Without a "transphosphorylation mechanism," many scenarios remain that would link oligomerization to activation. These include cooperativity between multiple catalytic sites (e.g. the proton-translocating ATP synthase (31)), catalytic sites formed at subunit interfaces (e.g. lipoamide dehydrogenase (32)), or relief of interactions between inhibitory domains and catalytic domains (e.g. CaMK kinase II (33)). The last scenario may also help to explain the apparent hyperactivity of the (N\(\Delta\)661)rJ AK2 mutant.

There are three pieces of evidence that suggest that the (N\(\Delta\)661)rJ AK2 mutant is both hyperphosphorylated and hyperactive. These data are as follows: 1) the exaggerated anti-phosphotyrosine signal intensity of this form relative to that of the full-length protein (Fig. 5), 2) the significant increase in radiolabel incorporation observed in in vitro kinase assay autoradiography (Fig. 6), and 3) the appearance of a novel 62-kDa protein that is recognized by anti-phosphotyrosine, but not by anti-j AK2, on Western blots (Figs. 4 and 5). Although the variance in the 5'-untranslated region (which does not vary between rJ AK2 and rJ AK2(C\(\Delta\)795)) may have slightly increased expression of (N\(\Delta\)661)rJ AK2, the apparent equal intensity of the anti-j AK2 signal in samples that have obvious differences in anti-phosphotyrosine signal intensities (Fig. 5) leads to the conclusion that (N\(\Delta\)661)rJ AK2 is hyperphosphorylated relative to rJ AK2. One plausible explanation for hyperactivity is that an inhibitory or regulatory domain is contained within the amino-terminal portion of the enzyme. This model is consistent with data from Zhuang, et al. (1) that show that kinase-deficient JAK2 mutants inhibit autophosphorylation of the wild-type JAK2 in the COS cell expression system, as we also show in the baculoviral expression vector system (Fig. 3). The concept of such an inhibitory or regulatory domain requires additional experimental support, since alternative interpretations for the "trans-inhibitory" properties of an inactive JAK2 still exist.

If such inhibitory or regulatory domains exist in JAK2, one might expect other Janus kinases to possess them as a consequence of the high degree of sequence conservation in these proteins. Expression of TYK2 mutants \(\Delta\)TK (lacking H1) and \(\Delta\)KL (lacking H2) in cells derived from the 2fTGH cell line resulted in the generation of inactive kinases (18), which is consistent with the presence of an inhibitory domain in the amino-terminal domains J H3 through J H7. A useful test of the inhibitory/regulatory hypothesis will be to determine if expression of only the TK (H J1), KL (H J2), or combined KL/TK (H J2) H1 domains is sufficient to produce an active kinase.

The nature and relevance of the 62-kDa band observed in Fig. 4 pose intriguing questions. Although identification of this protein is in progress, the identification process is not yet complete. Thus, there remains some ambiguity as to whether this protein is virally encoded, is produced in response to viral infection, or whether it is a constitutive cellular product. It is also unknown whether the appearance of an intense phosphotyrosine signal associated with the 62-kDa protein in (N\(\Delta\)661)rJ AK2 (but not rJ AK2) immunoprecipitation complex is due to an alteration in substrate specificity or whether the apparent increase in catalytic turnover of the (N\(\Delta\)661)rJ AK2 mutant increased the phosphotyrosyl forms of the 62-kDa protein beyond the capacity of endogenous phosphatases.

The dual hypotheses that the oligomeric form of JAK2 is the catalytically active form and that there is an inhibitory or regulatory domain in the amino terminus of the enzyme are currently under investigation in our laboratory.

\(^3\) H. Rui and W. L. Farrar, unpublished results.

\(^4\) R. J. Duhé, R. J. Fisher, and W. L. Farrar, unpublished results.
es do, however, allow for the speculative prediction that dysfunctional regulation of JAK2 expression may be responsible for factor-independent and receptor-independent proliferation and hence may predict circumstances for JAK2-mediated oncogenesis.

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REFERENCES

1. Zhuang, H., Patel, S. V., He, T., Sonstedy, S. K., Niu, Z., and Wojcikowski, D. M. (1994) J. Biol. Chem. 269, 21411–21414
2. Ihle, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., Thierfelder, W. E., Kreider, B., and Silvennoinen, O. (1994) Trends Biochem. Sci. 19, 222–227
3. Johnston, J. A., Kawamura, M., Kirken, R. A., Chen, Y.-Q., Blake, T. B., Shibuya, K., Ortaldo, J. R., McVicar, D. W., and O’Shea, J. J. (1994) Nature 370, 151–153
4. Witthuhn, B. A., Silvennoinen, O., Miura, O., Lai, K. S., Cwik, C., Liu, E. T., and Ihle, J. N. (1994) Nature 370, 153–157
5. Zeng, Y.-X., Takahashi, H., Shibata, M., and Hirokawa, K. (1994) FEBS Lett. 353, 289–293
6. Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A. G., Barbieri, G., Witthuhn, B. A., Schindler, C., Pellegrini, S., Wilks, A. F., Ihle, J. N., Stark, G. R., and Kerr, I. M. (1993) Nature 366, 127–135
7. Watling, D., Guschin, D., Müller, M., Silvennoinen, 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
8. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., and Ihle, J. N. (1993) Cell 74, 227–236
9. Silvennoinen, O., Witthuhn, B. A., Quelle, F. W., Cleveland, J. L., Yi, T., and Ihle, J. N. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8429–8433
10. Narazaki, M., Witthuhn, B. A., Yoshida, K., Silvennoinen, O., Yasukawa, K., Ihle, J. N., Kishimoto, T., and Taka, T. (1994) FEBS Lett. 344, 810–817
11. Stahl, N., Boulton, T. G., Farruggella, T., Ip, Y. N., Davis, S., Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Barbieri, G., Pellegrini, S., Ihle, J. N., and Yancopoulos, G. D. (1994) Science 263, 92–95
12. Quelle, F. W., Sato, N., Witthuhn, B. A., Inhorn, R. C., Eder, M., Miyajima, A., Griffin, J. D., and Ihle, J. N. (1994) Mol. Cell. Biol. 14, 4335–4341
13. Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N., and Carter-Su, C. (1993) Cell 74, 237–244
14. Rui, H., Kirken, R. A., and Farrar, W. L. (1994) J. Biol. Chem. 269, 5364–5368
15. Campbell, G. S., Argetsinger, L. S., Ihle, J. N., Kelly, P. A., Rillaena, J. A., and Carter-Su, C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5232–5236
16. VanderKuur, J. A., Wang, X., Zhang, L., Campbell, G. S., Allcovetz, G., Billestrup, N., Norstedt, G., and Carter-Su, C. (1994) J. Biol. Chem. 269, 21709–21717
17. DaSilva, L., Howard, O. M. Z., Rui, H., Kirken, R. A., and Farrar, W. L. (1994) J. Biol. Chem. 269, 18267–18270
18. Velazquez, L., Mogensen, K. E., Barbieri, G., Fellous, M., Uze, G., and Pellegrini, S. (1995) J. Biol. Chem. 270, 3327–3334
19. Duhé, R. J., Rui, H., Greenwood, J. D., Garvey, K., and Farrar, W. L. (1995) Gene (Amst.) 158, 281–285
20. Kitts, P. A., and Possee, R. D. (1993) Biotechniques 14, 810–817
21. Pen, J., Welling, G. W., and Welling-Wester, S. (1989) Nucleic Acids Res. 17, 451
22. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1995) Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley-Interscience, John Wiley and Sons, Inc., New York
23. Rillaena, J. A., Campbell, G. S., Lawson, D. M., and Carter-Su, C. (1992) Endocrinology 131, 973–975
24. Rui, H., Djeu, J. Y., Evans, G. A., Kelly, P. A., and Farrar, W. L. (1992) J. Biol. Chem. 267, 24076–24081
25. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) Science 241, 42–52
26. Cunningham, B. C., Ultsch, M., De Vos, A. M., Mulkerrin, M. G., Clauser, K. R., and Wells, J. A. (1991) Science 255, 24076–24081
27. De Vos, A. M., Ultsch, M., and Kossiakoff, A. A. (1992) Science 255, 306–312
28. Rui, H., Lebrun, J.-J., Kirken, R. A., Kelly, P. A., and Farrar, W. L. (1994) Endocrinology 135, 1299–1306
29. Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Taga, T., and Kishimoto, T. (1993) Science 260, 1808–1810
30. Gertler, A., Walker, A., and Friese, H. G. (1985) Endocrinology 116, 1636–1644
31. Boyer, P. D. (1989) FASEB J. 3, 2164–2178
32. Mattevi, A., Schierbeek, A. J., and Hol, W. G. J. (1991) J. Mol. Biol. 220, 975–994
33. Soderling, T. R. (1990) J. Biol. Chem. 265, 1823–1826
34. Harpur, A. G., Andrews, A.-C., Ziemiecki, A., Aston, R. R., and Wilks, A. F. (1992) Oncogene 7, 1347–1353
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