The T cell receptor (TCR) is internalized following activation of protein kinase C (PKC) via a leucine (Leu)-based motif in CD3γ. Some studies have indicated that the TCR is recycled back to the cell surface following PKC-mediated internalization. The functional state of recycled TCR and the mechanisms involved in the sorting events following PKC-mediated internalization are not known. In this study, we demonstrated that following PKC-induced internalization, the TCR is recycled back to the cell surface in a functional state. TCR recycling was dependent on dephosphorylation of CD3γ, probably mediated by the serine/threonine protein phosphatase-2A, but independent on microtubules or actin polymerization. Furthermore, in contrast to ligand-mediated TCR sorting, recycling of the TCR was independent of the tyrosine phosphatase CD45 and the gand-mediated TCR sorting, recycling of the TCR was actin polymerization. Furthermore, in contrast to phosphatase-2A, but independent on microtubules or probably mediated by the serine/threonine protein kinase C.1

The T cell receptor (TCR) constitutes the clonotypic Τεβ heterodimer, the CD3γε and CD3εε dimers, and the εζ homodimer (3–7). The Τεβ heterodimer is responsible for recognition of antigen (8, 9), and the associated CD3 and ε chains (10) are responsible for the signaling events that follow antigen recognition. It has recently been described that the TCR is internalized and sorted to a degradative compartment after antibody or antigen stimulation (11–13). In contrast, some studies have indicated that the TCR is not degraded following PKC-mediated internalization (14, 15). Furthermore, ligand-mediated TCR sorting is dependent on tyrosine phosphorylation but independent of PKC and the CD3γ Leu-based sorting motif (16, 17). In contrast, PKC-mediated TCR internalization is dependent on the Leu-based sorting motif in CD3γ but independent of tyrosine phosphorylation (16, 18, 19). Thus, two independent pathways for TCR sorting seem to exist. The physiological role of PKC-mediated TCR internalization and recycling is not known.

The aim for this study was to examine the functional state of recycled TCR and the mechanisms involved in TCR sorting following PKC-induced internalization. We find that following PKC-induced internalization, the TCR recycles to the cell surface in a functional state. Recycling was absolutely dependent on dephosphorylation of CD3γ but was independent upon actin polymerization, microtubules, and tyrosine phosphorylation. Even though the phosphorylation state of CD3γ controlled TCR recycling, no recycling signal was contained within the cytoplasmic tail of CD3γ. In addition, our results indicated that not only TCR cycling but also T cell responsiveness can be regulated by the phosphorylation state of CD3γ.

**EXPERIMENTAL PROCEDURES**

**Cells, Antibodies, and Chemicals—**JGN cells, a TCR cell surface negative variant of the human T cell line Jurkat that synthesizes no CD3γ (20), were cultured in RPMI 1640 medium supplemented with penicillin 2 × 10^5 units/liter (Leo Pharmaceutical Products, Ballerup, Denmark), streptomycin 50 mg/liter (Merck, Darmstadt, Germany), and 10% (v/v) fetal calf serum (FCS) (Life Technologies, Inc., Paisley, UK) at 37°C in 5% CO2. Purified and phycoerythrin (PE)-conjugated UCHT1 mouse mAb directed against human CD3ε was obtained from Dakopatts (Glostrup, Denmark). The anti-TCR mAb F101.01 was produced in our own laboratory (21). PE-conjugated Fab(ab)2 fragments of buffered saline; FCS, fetal calf serum; FACS, fluorescence-activated cell sorter; Ca2+1, intracellular calcium; mAb, monoclonal antibody.
CD3γ Controls TCR Cycling and Affects T Cell Responsiveness

24233
goat anti-mouse IgG H+L were obtained from Jackson ImmunoResearch (West Grove, PA). The anti-phosphotyrosine mAb 4G10 was from Upstate Biotechnology, Inc. (Lake Placid, NY). The phorbol ester phorbol 12,13-dibutyrate (PDBu) was from Sigma. The following inhibitors with the concentrations indicated were used: 1 μM cycloheximide (Sigma; 5 μg/ml), 50 μM cycloheximide (Cytochalasin D, Sigma), 10 μM cycloheximide (Calbiochem), 50 μM cycloheximide (Calbiochem), 130 μM cycloheximide (Calbiochem), 10 μM cycloheximide (Calbiochem), 10 μM cycloheximide (Calbiochem).

Constructs, Transfection, and TCR Down-regulation—All CD3γ mutations were constructed as described previously (7, 18) by the polymerase chain reaction (PCR) using Vent DNA polymerase containing 3′→5′-reading exonuclease activity (New England Biolabs, Beverly, MA) and the plasmid pJG3-γ (22) as template. Chimeric CD4-CD3γ constructs were produced as described previously (19) using the plasmid pC-D-γT4.25 (23) as template. The PCR products were digested with XhoI and EcoRI and subcloned into the expression vector pMHC-Neo (24). Mutations were confirmed by DNA sequencing using the ABI 310 (Perkin-Elmer). Transfections were performed using the Bio-Rad Gene Pulser at a setting of 270 V and 960 microfarads with 40 μg of plasmid per 2 × 10⁷ cells. After 3–4 weeks of selection, G418-resistant clones were expanded and maintained in medium without G418. For TCR down-regulation, cells were adjusted to 2 × 10⁶ cells per ml of medium (RPMI 1640 + 10% FCS) and incubated at 37 °C with different concentrations of F101.01 for 45 min. At the indicated times, aliquots were transferred to ice-cold PBS containing 2% FCS and 0.1% NaN₃ and washed twice. The cells treated with PDBu were stained directly with PE-conjugated UCHT1 (Dakopatts A/S). Cells stimulated with F101.01 were stained with PE-conjugated F(ab)₂ fragments of goat anti-mouse Ig H + L. Cells were analyzed in a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA). Mean fluorescence intensity (MFI) was recorded and used in the calculation of percent antibody binding: (MFI of phorbol ester-treated cells) divided by (MFI of untreated cells) × 100%. For each construct at least three different clones were analyzed.

Phosphorylation and Intracellular Calcium—Phosphorylation assays were performed as described previously (18, 25). The phosphorylated CD3γ chain with a molecular mass of 26–30 kDa was co-precipitated with CD8α (20 kDa) using the anti-CD8α mAb UCHT1. Following stimulation with 1 μg/ml F101.01, intracellular calcium ([Ca²⁺]ᵢ) of cells was measured with the intracellular fluorescent indicator fura-2/AM (Sigma) as described previously (26).

Surface Biotinylation, Immunoprecipitation, and Phosphotyrosine Blots—Phosphotyrosine blots were performed as described previously (26). Surface biotinylation was performed as described previously with Blots—Phosphotyrosine blots were performed as described previously (26). Surface biotinylation was performed as described previously. 

RESULTS

The TCR Recycles to the Cell Surface in a Functional State following PKC-mediated Internalization—In contrast to ligand-mediated sorting of the TCR (11), the TCR is not sorted to a degradative compartment following PKC-induced internalization (14). To analyze whether the TCR recycled to the cell surface in Jurkat cells, cells were pretreated with cycloheximide to stop protein synthesis and then incubated with PDBu for 60 min. The cells were subsequently washed to remove PDBu and incubated in medium without PDBu but with cycloheximide. At different times, aliquots of cells were analyzed for TCR surface expression (Fig. 1A). These experiments showed that following PKC-mediated TCR internalization, the TCR was recycled to the cell surface. Approximately 60–90 min after removal of PDBu, TCR surface expression reached the same level as untreated cells (Fig. 1A). Omitting cycloheximide did not change TCR internalization or recycling significantly (data not shown). As receptor stimulation and internalization have been shown to result in receptor desensitization of some receptors (28, 29), we next analyzed whether the internalized and recycled TCRs were desensitized or were able to signal when re-expressed at the cell surface. Jurkat cells were either left untreated, treated with PDBu for 45 min, or treated with PDBu for 45 min and washed to remove the PDBu followed by incubation in medium without PDBu for 120 min to allow TCR recycling. The cells were then analyzed for TCR surface expression as well as for [Ca²⁺]ᵢ, responses after stimulation with an anti-TCR antibody. Treatment of the cells with PDBu for 45 min resulted in down-regulation of the TCR and an almost abolished [Ca²⁺]ᵢ response (Fig. 1A, B). However, after 120 min of recycling, the responsiveness of the T cell changed from a state of almost non-responsiveness to that of untreated cells (Fig. 1B). To examine whether the lack of responsiveness following PDBu treatment was due to a reduction in cell-surface expression of the TCR or to an unknown effect of PDBu, JGNγ-S123/126V cells were analyzed. In JGNγ-S123/126V cells the CD3δ phospho-acceptor group Ser-123 and Ser-126 have been mutated to valines. Consequently CD3δ is not phosphorylated upon activation of PKC, and TCR internalization is almost abolished (18) (Fig. 1A). In correlation with a minimal internalization of the TCR upon PKC activation, TCR signaling in JGNγ-S123/126V cells as measured by [Ca²⁺]ᵢ, after stimulation with an anti-TCR antibody was only slightly affected by the PDBu treatment (Fig. 1C). The difference in PDBu-induced internalization and TCR signaling following PDBu treatment of JGNγ-S123/126V cells compared with JGNγ-WT cells was not due to a general defect in JGNγ-S123/126V cells. Thus antibody stimulation leads to TCR internalization as well as increased tyrosine phosphorylation, in both JGNγ-S123/126V and JGNγ-WT cells (Fig. 1, D and E). These results demonstrated that activation of PKC induced internalization of the TCR with subsequent sorting to a non-degradative compartment from where the TCR recycled back to the cell surface in a functional state. Thus, the TCR is not desensitized following PKC-induced internalization and recycling. Furthermore, the effect of PKC on T cell responsiveness was solely due to the
CD3γ-dependent internalization of the TCR.

**Recycling of the TCR Requires PP-2A-mediated Dephosphorylation of CD3γ**—We have previously shown that PKC-mediated internalization of the TCR requires phosphorylation of CD3γ S126 (18). To analyze whether CD3γ was dephosphorylated after PKC-mediated phosphorylation, Jurkat cells were loaded with 32P and incubated with PDBu for 10 min. The cells were then washed and transferred to medium without PDBu. At different times, aliquots of cells were lysed, and immunoprecipitation was performed with an anti-CD3e mAb. The precipitates were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. CD3γ was phosphorylated upon PKC activation, but 45 min after removal of PDBu, most CD3γ had been dephosphorylated (Fig. 2A, upper panel). This showed that phosphorylated CD3γ is quickly dephosphorylated in vivo, probably by serine/threonine protein phosphatases in agreement with other studies (30). Furthermore, the kinetics of CD3γ dephosphorylation correlated with that of TCR recycling, as most of the PKC-induced internalized TCRs were re-expressed at the cell surface after 60–90 min (Fig. 2B). To analyze whether recycling of the TCR subsequent to PKC-mediated TCR internalization was dependent on serine/threonine protein phosphatases, Jurkat cells were treated with PDBu and subsequently with different serine/threonine protein phosphatase inhibitors. After incubation with PDBu and phosphatase inhibitor for 45 min, the cells were washed to remove PDBu and transferred to medium with phosphatase inhibitor. At different times, aliquots of cells were analyzed for TCR surface expression. The inhibitors calyculin A and okadaic acid strongly inhibited TCR recycling, whereas tautomycin only had a minor effect on TCR recycling (Fig. 2B). Calyculin A most markedly inhibited TCR recycling. However, incubation with PDBu and calyculin A for 4 h did not lead to degradation of the TCR (Fig. 2C). Thus, the lack of recycling in the presence of calyculin A was not due to degradation of internalized TCR. To determine whether calyculin A inhibited dephosphorylation of CD3γ, 32P analysis was performed with or without treatment with calyculin A, as described above. Calyculin A strongly inhibited dephosphorylation of CD3γ (Fig. 2A, lower panel), correlating with calyculin A-mediated inhibition of TCR recycling. From these studies, it may be suggested that dephosphorylation of CD3γ following PKC-mediated internalization of the TCR is required for TCR recycling.

**TCR Recycling Is Not Dependent on Microtubules or Actin Polymerization**—Actin polymerization has recently been shown to be involved in antigen-mediated T cell activation (31). Furthermore, both actin filaments and microtubules have been shown to be involved in post-endocytic sorting (32). To analyze whether TCR recycling was dependent upon actin polymerization or microtubules, Jurkat cells were treated with PDBu and subsequently with either cytochalasin D, an inhibitor of actin polymerization (32), or nocodazole, an agent that disrupts microtubules (33). After incubation with PDBu and the inhibitor for 45 min, the cells were washed to remove PDBu and transferred to medium with the inhibitor. At different times, aliquots of cells were analyzed for TCR surface expression. TCR recycling was not affected by cytochalasin D, and nocodazole only slightly reduced TCR recycling (Fig. 2D). This indicates that actin polymerization or microtubules are not important for TCR recycling.

**Recycling of the TCR Does Not Require CD45 or the Src Kinases**—Recent experiments have indicated a role for CD45...
with PDBu and calyculin A for 0, 1, 2, and 4 h. CD3ɛp56Lck, or the inhibition of Src kinases did not prevent recycling was analyzed as described above. Lack of CD45, treated for 30 min with PP1, an Src kinase inhibitor (36). TCR above. After PKC-mediated internalization the TCR containing cell surface and were subsequently analyzed as described information involved in the recycling event. CD3γ was mediated by a specific recycling signal in the cytoplasmic tail of CD3γ was not degraded (Fig. 4C). This indicated that the residues

A Lysosomal Sorting Signal in CD3γ Controls TCR Recycling—Since PKC-induced internalization of the TCR required phosphorylation of CD3γ and TCR recycling required dephosphorylation of CD3γ, we next analyzed whether recycling TCR was mediated by a specific recycling signal in the cytoplasmic tail of CD3γ. We first analyzed whether the residues C-terminal to the Leu-based motif in CD3γ contained any sorting information involved in the recycling event. CD3γ was truncated at Pro-133 (CD3γ-tP133). This construct was transfected into the Jurkat CD3γ negative (JGN) cell line (20). G418-resistant clones were isolated that expressed the TCR at the cell surface and were subsequently analyzed as described above. After PKC-mediated internalization the TCR containing the CD3γ-tP133 chain was recycled to the cell surface (Fig. 4B) and was not degraded (Fig. 4C). This indicated that the residues C-terminal to the Leu-based motif in CD3γ did not play a role in the recycling of the TCR. To analyze whether the residues comprising the remaining part of the cytoplasmic tail of CD3γ (Gln-117–Leu-132) contained a recycling signal, a chimeric CD4-CD3γ construct in which the amino acids Gln-117–Leu-132 of CD3γ substituted for the cytoplasmic tail of CD4 (CD4-

FIG. 2. TCR recycling requires PP-2A-mediated dephosphorylation of CD3γ. A, phosphorylation analyses of CD3γ from JGNγ-wt after PDBu treatment for 10 min in the presence or absence of the phosphatase inhibitor calyculin A (Cal.) and a chase period of 0, 15, 30, and 45 min. B, Jurkat cells were treated with PDBu and subsequently with different serine/threonine protein phosphatase inhibitors. After incubation with PDBu and the phosphatase inhibitor, the cells were washed to remove PDBu and transferred to medium with the phosphatase inhibitor but without PDBu. At different times aliquots of cells were analyzed for TCR surface expression by flow cytometry comparing MFI of PDBu-treated cells with MFI of untreated cells, and percent anti-CD3ε antibody binding was determined. C, Western blot of surface-biotinylated cells treated with PDBu and calyculin A for 0, 1, 2, and 4 h. CD3ε was immunoprecipitated and detected by ECL using streptavidin conjugated with HRP. Positions of the CD3 chains are indicated. D, cells were treated with PDBu and subsequently with an inhibitor of actin polymerization (cytochalasin D) or microtubules (nocodazole). After incubation with PDBu and the inhibitor the cells were washed to remove PDBu and transferred to normal medium with the inhibitor but without PDBu. At different times aliquots of cells were analyzed for TCR surface expression by flow cytometry comparing MFI of PDBu-treated cells with MFI of untreated cells, and percent anti-CD3ε antibody binding was determined.

FIG. 3. TCR recycling does not require CD45 or the Src kinases. A, J76, J76 pretreated with PP1, J.Cam 1.6, and J45.01 cells were treated with PDBu for 45 min, washed to remove PDBu, and transferred to medium without PDBu. At different times aliquots of cells were analyzed for TCR surface expression. Flow cytometry comparing MFI of PDBu-treated cells with MFI of untreated cells was used to calculate percent anti-CD3ε antibody binding. B, J76, J76 pretreated with PP1, J.Cam 1.6, and J45.01 cells were stimulated with different concentrations of anti-TCR mAb (F101.01) for 1 h and then incubated with different inhibitors for 24 h. Flow cytometry comparing MFI of PDBu-treated cells with MFI of untreated cells was used to calculate percent F101.01 binding. The cell lines are indicated below each histogram.

3Gln-117–Leu-132γ was made. In this construct, CD3γ Asp-127 was changed to an alanine to allow surface expression of the chimera, as we have previously shown that CD4 containing the CD3γ SDXXXLL motif is spontaneously sorted to the lysosomes...
Role of Protein Serine/Threonine Phosphatases in Recycling of the TCR—Ligand stimulation of T cells leads to TCR internalization and sorting to the lysosomes (11). In contrast, PDBu-induced activation of PKC leads to sorting to a non-degradative compartment, presumably the early endosomes, from where the TCR is recycled back to the cell surface (Fig. 1). The rate of TCR recycling correlated with that of CD3γ dephosphorylation indicating that TCR recycling was controlled by the phosphorylation state of CD3γ. Thus, the phospho-acceptor group in CD3γ plays at least two roles, a role in TCR internalization and a role in TCR recycling. In agreement with previous studies (30, 37) we found that the serine/threonine protein phosphatase acting on CD3γ probably belongs to the phosphatase-2A group as the inhibitors of CD3γ dephosphorylation and TCR recycling, calyculin A and okadaic acid, primarily inhibit this group of phosphatases (38, 39). A similar phosphorylation/dephosphorylation-dependent cycling behavior has been described for the β-adrenergic (40), the C5a anaphylatoxin (41), the neurokinin 1 (42), and the chemokine CXCR4 (43) receptors. Interestingly, like the TCR chain CD3γ, the C5a receptor contains a cytoplasmic di-Leu motif preceded by an N-terminal potential PKC site containing a serine. In fact, this serine has been shown to be phosphorylated following ligand binding or phorbol ester treatment (44), and as for the TCR (Fig. 2B), treatment of cells with okadaic acid delayed the recycling of the C5a receptor to the cell surface. Furthermore, the CXCR4 receptor also contains a di-Leu-like sequence (IL) preceded by a potential PKC site which may be involved in PKC-mediated CXCR4 receptor internalization (43). Thus, it seems likely that these receptors share some of the mechanisms that mediate and regulate their intracellular sorting.

TCR Can Be Sorted by Different Independent Sorting Routes—The polymerization of actin or microtubules was not important for TCR recycling or PKC-mediated internalization of the TCR. In agreement with our results, inhibition of actin polymerization did not affect recycling of the transferrin receptor (45). In contrast, actin polymerization has been shown to be required for ligand-mediated receptor internalization and T cell activation (31, 45) indicating that the mechanisms involved...
in PKC-induced internalization/recycling and ligand-mediated T cell activation/TCR internalization are different. In agreement with recent results (16, 17) CD45 and the Src tyrosine kinases were required for efficient ligand-induced internalization of the TCR (Fig. 3B), demonstrating that these proteins play an important role in the internalization process that leads to lysosomal sorting of the TCR. In contrast, TCR recycling was not dependent upon CD45 or the Src kinases p56Lck and p59Fyn. Likewise, PKC-induced internalization of the TCR is not dependent upon CD45, the Src kinases p56Lck and p59Fyn, or tyrosine phosphorylation (Fig. 3) (16). This indicates that the PKC-dependent and the ligand-dependent TCR sorting routes represent two different pathways of receptor sorting. Stimulation of the TCR with ligand activates both tyrosine kinases and PKC but leads to lysosomal sorting of the TCR. This implies that ligand/tyrosine kinase-mediated TCR sorting to lysosomes of the TCR is dominant over PKC-induced TCR sorting. Thus, sorting of TCR via the recycling pathway may only apply to unstimulated TCR and consequently may predominantly occur prior to ligand stimulation. Regarding the sorting of the TCR following PKC-induced internalization, we found that CD3γ does not contain a cytoplasmic recycling signal. In contrast, it was shown that the Leu-based motif in CD3γ, Ser-126 to Leu-132, possessed the ability to mediate lysosomal sorting of CD4-CD3Gln-117–Leu-132 (Fig. 4). Why the TCR is recycled and not sorted to the lysosomes following PKC-mediated internalization despite the potential of the CD3γ Leu-based motif to mediate lysosomal sorting is still not known. Following PKC-mediated TCR internalization CD3γ was dephosphorylated (Fig. 2). It has been suggested that phosphorylation exposes the Leu-based internalization/degradation motif in CD3γ (19). According to this suggestion, dephosphorylation of CD3γ might lead to masking of the CD3γ Leu-based motif which may explain the lack of lysosomal sorting following PKC-induced internalization of the TCR. However, the present results argue against this explanation. Thus, TCRs were not sorted to a degradative compartment despite being located in an intracellular compartment for 4 h in a state in which CD3γ was most likely phosphorylated due to the presence of the phosphatase inhibitor calyculin A (Fig. 2C). This indicates that irrespective of an exposed lysosomal targeting signal in CD3γ other factors and/or TCR chains prevent the sorting to the lysosomes. In line with these experiments, other phosphorylation-dependent recycling receptors have also been shown to be retained, but not degraded, in an intracellular compartment in a phosphorylated state (43, 44). Thus, in these receptors, as well as in the TCR, phosphorylation leads to internalization and localization in an intracellular compartment but not to degradation.

A Physiological Function of CD3γ and TCR Cycling—It is not known why the TCR is sorted to the lysosomes following stimulation with antigen. It could be speculated that the TCR is desensitized after antigenic stimulation and that these stimulated/desensitized TCRs influence the activation threshold of the T cell due to competition for the ligand with unstimulated TCRs. In contrast, the present results showed that the PKC-induced cycling event does not lead to TCR desensitization (Fig. 1). Thus, the internalized TCRs are able to signal when re-expressed at the cell surface. PKC-induced internalization of the TCR and the following recycling to the cell surface probably reflects the spontaneous cycling of the TCR (16). Since the signaling ability of the TCR is not affected by the cycling event, the responsiveness of the T cell can be quickly altered by changing the kinetic parameters in the cycling process. T cell activation requires a certain amount of ligand and surface-expressed TCR to enable an activation signal that is above the threshold for T cell activation (46, 47). Regarding the amount of surface-expressed TCR, our results showed that this amount, and thereby the activation threshold of the T cell, can be controlled solely by the activities of PKC, a phosphatase that is probably PP-2A, and the CD3γ SDXXXXL motif (Fig. 1 and 2). Concerning the physiological role of CD3γ and TCR cycling, the mechanisms involved in TCR cycling may be targets for immune regulatory factors, e.g. certain cytokines, which may modulate the activity of PKC or PP-2A and thereby the phosphorylation state of CD3γ. This in turn will affect TCR surface expression and T cell responsiveness as shown in Fig. 1. Indeed several cytokines, some of which are secreted by professional antigen-presenting cells, have been reported to affect the activities of PP-2A or PKC and may also affect T cell responsiveness toward antigenic stimulation (48–50). Apart from playing a role in setting the activation threshold of the T cell, it could also be suggested that TCR cycling may ensure that only functional TCR is expressed at the cell surface. Thus, a role for the constitutive cycling of the TCR or other receptors may be to serve as a quality check of the receptors as only fully functional and assembled receptors can proceed through the entire cycling event.

In conclusion, these studies have identified a specific role for the phosphorylation state of CD3γ in the internalization and recycling of the T cell receptor and in determining the responsiveness of the T cell. Although controlling both internalization and recycling of the TCR, CD3γ did not contain a recycling signal in its cytoplasmic tail. Thus, whether TCR recycling is a signal-mediated event involving a recycling signal in other TCR chains or is a default process is still unknown. Likewise, the signal mediating ligand-induced lysosomal sorting of the TCR has not yet been identified. We are presently addressing these questions as well as whether the mechanisms involved in TCR cycling are targets for immune regulatory factors.

Acknowledgments—We thank Dr. M. J. Crompton for plasmid pJ6T3γ-2 and Dr. D. R. Littman for plasmid pCD-L3T4.25. The technical help of Bodil Nielsen and Maria Schmidt is gratefully acknowledged.

REFERENCES

1. Sandowal, I. V., and Balke, O. (1994) Trends Cell Biol. 4, 292–297
2. Mellman, I. (1996) Annu. Rev. Cell Dev. Biol. 12, 575–625
3. Koning, F., Maloy, W. L., and Coligan, J. E. (1990) Eur. J. Immunol. 20, 299–305
4. Manolios, N., Letourneur, F., Bonifacino, J. S., and Klausner, R. D. (1991) EMBO J. 10, 1643–1651
5. Kuhlmann, J., and Geisler, C. (1993) Scand. J. Immunol. 37, 271–275
6. Hou, X., Dietrich, J., Kuhlmann, J., Wegener, A.-M., and Geisler, C. (1994) J. Immunol. 153, 2628–2633
7. Dietrich, J., Neissig, A., Hou, X., Wegener, A.-M., Kajhede, M., and Geisler, C. (1996) J. Cell Biol. 132, 299–310
8. Dembic, Z., Haas, W., Weiss, S., McClure, J., Kiefer, H., von Boehmer, H., and Steinmetz, M. (1986) Nature 320, 232–238
9. Saito, T., Weiss, A., Miller, J., norcross, M. A., and Germain, R. N. (1987) Nature 325, 125–130
10. Weiss, A., and Littman, D. R. (1994) Cell 76, 263–274
11. Valitutti, S., Muller, S., Salio, M., and Lanazavecchia, A. (1997) J. Exp. Med. 185, 1599–1604
12. Luton, P., Bofenter, M., Davoost, J., Schmitt-VeRulst, A. M., and Boer, C. (1994) J. Immunol. 153, 63–72
13. Luton, P., Legendre, V., Gorvel, J. P., Schmitt-VeRulst, A. M., and Boer, C. (1997) J. Immunol. 158, 3140–3147
14. Minami, Y., Samelson, L. E., and Kuhlmann, R. D. (1987) J. Biol. Chem. 262, 13342–13347
15. Ruegg, C. L., Rajasekar, S., Stein, B. S., and Engleman, E. G. (1992) J. Biol. Chem. 267, 18837–18843
16. Lauritsen, J. P. H., Christensen, M. D., Dietrich, J., Kastrup, J., Oudem, N., and Geisler, C. (1997) J. Immunol. 161, 260–267
17. D’Oro, U., Varchio, M. S., Weissman, A. M., and Ashwell, J. (1997) Immunity 7, 619–628
18. Dietrich, J., Hou, X., Wegener, A.-M., and Geisler, C. (1994) EMBO J. 13, 2156–2166
19. Dietrich, J., Kastrup, J., Nielsen, B. L., Oudem, N., and Geisler, C. (1997) J. Cell Biol. 138, 271–281
20. Geisler, C. (1992) J. Immunol. 148, 2437–2445
21. Geisler, C., Pleasler, T., Falesen, G., Skipol, K., Oudem, N., and Larsen, J. K. (1988) Scand. J. Immunol. 27, 685–696
22. Krissansen, G. W., Owen, M. J., Verbi, W., and Crompton, M. J. (1986) EMBO J. 5, 1799–1808
23. Littman, D. R., and Gettner, S. N. (1987) Nature 325, 453–455
24. Hahn, W. C., Menzin, E., Saito, T., Germain, R. N., and Bierer, B. E. (1993) Gene (Amst.) 127, 267–268
25. Dietrich, J., Hou, X., Wegener, A.-M. K., Pedersen, L. O., Odum, N., and Geisler, C. (1996) J. Biol. Chem. 271, 11441–11448
26. Hou, X., Dietrich, J., Odum, N., and Geisler, C. (1996) J. Biol. Chem. 271, 22815–22822
27. Backstrom, T., Milia, A., Peter, B., Jaureguiberry, C., Baldari, T., and Palmer, E. (1996) Immunity 5, 437–447
28. Countaway, J. L., Nairn, A. C., and Davis, R. J. (1992) J. Biol. Chem. 267, 1129–1140
29. Hausdorff, W. P., Campbell, P. T., Ostrowski, J., Yu, S. S., Caron, M. G., and Lefkowitz, R. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2979–2983
30. Alexander, D., Goris, J., Marais, R., Rothbard, J., Merlevede, W., and Crumpton, M. J. (1989) J. Biol. Chem. 264, 695–701
31. Valitutti, S., Dessing, M., Aktories, K., Gallati, H., and Lanzavecchia, A. (1997) J. Exp. Med. 181, 55–65
32. Maples, C. J., Ruiz, W. G., and Apodaca, G. (1997) J. Biol. Chem. 272, 6741–6751
33. Cole, N. B., Sciaky, N., Marotta, A., Song, J., and Lippincott-Schwartz, J. (1996) Mol. Biol. Cell 7, 631–650
34. Koretzky, G. A., Picos, J., Schultz, T., and Weiss, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2037–2041
35. Straus, D. B., and Weiss, A. (1992) Cell 70, 585–593
36. Hanke, J. H., Gardner, J. P., Dow, R. L., Changelian, P. S., Brissette, W. H., Weringer, E. J., Pollok, B. A., and Connelly, P. A. (1996) J. Biol. Chem. 271, 695–701
37. Alexander, D. R., Brown, M. H., Tutt, A. L., Crumpton, M. J., and Shivnan, E. (1992) Biochem. J. 288, 69–77
38. Cohen, P., Holmes, C. F., and Tsukitani, Y. (1990) Trends Biochem. Sci. 15, 98–102
39. Hori, M., Magae, J., Han, Y. G., Hartshorne, D. J., and Karaki, H. (1991) FERS Lett. 283, 145–148
40. Pippig, S., Andexinger, S., and Lohse, M. J. (1995) Mol. Pharmacol. 47, 666–676
41. Garland, A. M., Grady, E. F., Lovett, M., Vigna, S. R., Frucht, M. M., Krause, J. E., and Brunnett, N. W. (1996) Mol. Pharmacol. 49, 438–446
42. Pippig, S., Andexinger, S., and Lohse, M. J. (1995) Mol. Pharmacol. 47, 666–676
43. Garland, A. M., Grady, E. F., Lovett, M., Vigna, S. R., Frucht, M. M., Krause, J. E., and Brunnett, N. W. (1996) Mol. Pharmacol. 49, 438–446
44. Giannini, E., and Boulay, F. (1995) J. Immunol. 154, 4055–4064
45. Garcia, M., Grady, E. F., Lovett, M., Vigna, S. R., Frucht, M. M., Krause, J. E., and Brunnett, N. W. (1996) Mol. Pharmacol. 49, 438–446
46. Signoret, N., Oldridge, J., Pelchen-Matthews, A., Klasse, P. J., Tran, T., Brass, L. F., Rosenkilde, M. M., Schwartz, T. W., Homes, W., Luther, M. A., Wells, T. N. C., Hoxie, J. A., and Marsh, M. (1997) J. Cell Biol. 139, 651–664
47. Giannini, E., Brouchet, L., and Boulay, F. (1995) J. Biol. Chem. 270, 19166–19172
48. marking, D. H. (1997) Curr. Opin. Immunol. 9, 390–395
49. Viola, A., and Lanzavecchia, A. (1996) Science 273, 104–106
50. Lu, Y., Tremblay, R., Joussouff, H., Chakravarthy, B., and Durkin, J. P. (1994) J. Immunol. 152, 1495–1504
51. Ganz, M. B., Saks, B., Sexena, R., Hawkins, K., and Sedor, J. R. (1996) Am. J. Physiol. 271, 108–113
52. Kolesnick, R., and Golde, D. W. (1994) Cell 77, 325–328