Activated Release of Membrane-anchored TGF-α in the Absence of Cytosol

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Abstract. The ectodomain of proTGF-α, a membrane-anchored growth factor, is converted into soluble TGF-α by a regulated cellular proteolytic system that recognizes proTGF-α via the C-terminal valine of its cytoplasmic tail. In order to define the biochemical components involved in proTGF-α cleavage, we have used cells permeabilized with streptolysin O (SLO) that have been extensively washed to remove cytosol. PMA, acting through a Ca²⁺-independent protein kinase C, activates cleavage as efficiently in permeabilized cells as it does in intact cells. ProTGF-α cleavage is also stimulated by GTPγS through a mechanism whose pharmacological properties suggest the involvement of a heterotrimeric G protein acting upstream of the PMA-sensitive Ca²⁺-independent protein kinase C. Activated proTGF-α cleavage is dependent on ATP hydrolysis, appears not to require vesicular traffic, and acts specifically on proTGF-α that has reached the cell surface. These results indicate that proTGF-α is cleaved from the cell surface by a regulated system whose signaling, recognition, and proteolytic components are retained in cells devoid of cytosol.

The extracellular domains of certain transmembrane proteins are released from the cell surface by proteolytic cleavage. Various membrane-anchored growth factors, receptors, the β-amloid precursor protein (β-APP), cell adhesion proteins and ectoenzymes share this property (Ehlers and Riordan, 1991). This process converts the localized activity of membrane-anchored growth factors and receptors to the diffusible activities of soluble growth factors (Massagué, 1990) and binding proteins (Fernández-Botran, 1991; Gordon, 1991) Alterations in the balance between membrane-anchored and soluble forms of these proteins may be pathological, as deficiency in β-APP ectodomain cleavage is thought to lead to β-amloid plaque formation characteristic of Alzheimer's disease (Esch et al., 1990; Hardy and Allsop, 1991; Sisodia et al., 1990).

Several growth factors have been shown to be functional in their membrane-anchored forms, including the precursors of TGF-α (proTGF-α), EGF, heparin-binding EGF-like growth factor, colony stimulating factor-1 (CSF-1), and tumor necrosis factor-α (TNF-α) (Wong et al., 1989; Brachmann et al., 1989; Dobashi and Stern, 1991; Naglich et al., 1992; Stein et al., 1990; Perez et al., 1990). The membrane-anchored forms of the c-kit receptor ligand (KL) are not only active, but are required for proper murine development and cannot be replaced by soluble KL (Bernstein, 1960; Branan et al., 1991; Flanagan et al., 1991; Dolci et al., 1991; Huang et al., 1992).

Proteolytic cleavage and release of some cell surface protein ectodomains is a regulated process. Ectodomain cleavage of proTGF-α (Pandiella and Massagué, 1991a), the c-kit ligands KL-1 and KL-2 (Huang et al., 1992), CSF-1 (Stein and Rettenmier, 1991), the CSF-1 receptor (Downing et al., 1989), the p55 and p75 TNF-α receptors (Porteu et al., 1991; Porteu and Nathan, 1990), the β-APP protein (Buxbaum et al., 1990), and the cell adhesion receptor L-selectin (Kishimoto et al., 1989) is slow in unstimulated cells, but occurs within minutes of treatment with activators of protein kinase C such as PMA. The protease(s) responsible for regulated cleavage appear able to cleave a wide variety of primary sequences, as shown by the need to extensively mutate the regions proximal to the cleavage sites of membrane TGF-α (Wong et al., 1989; Pandiella and Massagué, 1991), TNF-α (Perez et al., 1990), and β-APP (Sisodia, 1992; Sahasrabudhe et al., 1992) to reduce or prevent cleavage.

Cleavage of proTGF-α is strictly dependent on the presence of a valine residue at the COOH-terminus of the cytoplasmic tail, providing an example of how some transmembrane proteins may be recognized as substrates by the ectodomain cleavage system (Bosenberg et al., 1992). ProTGF-α is not susceptible to cleavage until it has reached the cell surface (Bosenberg et al., 1992). Agents that block lysosomal function have little effect on the regulated cleavage of proTGF-α (Bosenberg et al., 1992) or β-APP (Capporaso et al., 1992).

We are using proTGF-α cleavage as a model system to define the mechanism that activates cleavage of membrane
protein ectodomains. Having observed that activated cleavage of proTGF-α occurs inefficiently if at all in isolated membrane preparations, we have implemented a permeabilized cell system that is devoid of many cytosolic components yet leaves cells relatively intact and allows biochemical characterization. The bacterial cytolsin streptolysin O (SLO) has been used to permeabilize various cell types (Ahnert-Hilger et al., 1989), including CHO cells (Miller and Moore, 1991), the cell line used in this study. SLO forms stable pores in the plasma membrane that allow rapid diffusion of cytosolic components of <150 kD (Ahnert-Hilger et al., 1989).

In this study, we show that activation of proTGF-α ectodomain cleavage is readily achieved in permeabilized and extensively washed cells, and has the characteristics of a process activated via a G-protein acting through a Ca2+-independent protein kinase C.

**Materials and Methods**

**Materials**

SLO was purchased from Burroughs-Wellcome (Research Triangle Park, NC) and was reconstituted in Milli-Q purified water to a 20 U/ml dilution and rapidly frozen at -70°C in 50-μl aliquots. Immediately before use, aliquots were rapidly thawed and diluted. ATP, adenylylimidodiphosphate (App[NH4]p), GTP, guanosine 5′-diphosphate (GDP), guanosine 5′-O-(2-thiodiphosphate) (GDP3S), guanosine 5′-O-3-thiotriphosphate (GTPγS), and guanylylimidodiphosphate (Gpp[NH4]) were from Boehringer Mannheim Biochemicals (Indianapolis, IN). [35S]Cysteine was purchased from Dupont-New England Nuclear (Boston, MA). Pertussis toxin was from List Biological Labs. Inc. (Campbell, CA). Staurosporine was from Kamiya Biomedical Co. (Thousand Oaks, CA). H7 was from Seikagaku America, Inc. (St. Petersburg, FL). Bovine brain cytosol was kindly donated by Dr. David Palmer (Memorial Sloan-Kettering Cancer Center, New York). All other reagents were from Sigma Immunochemicals (St. Louis, MO). EGTA purity was determined by titrating an EGTA solution against a standard calcium chloride solution to a potassium oxalate precipitation endpoint (Tatham and Gomperts, 1990).

**Cell Permeabilization**

CHO cells were grown to near-confluence in six-well cell culture plates. Cells were washed once with cold PBS, transferred to 4°C and washed once with 20 mM Hepes-KOH, pH 7.2, 125 mM K-glutamate, 15 mM KCl, 5 mM NaCl, 2 mM MgCl2 (buffer A) and 3 mM EGTA. An aliquot of SLO was diluted at 4°C to a final concentration of 0.2 U/ml in buffer A containing 3 mM EGTA. 500 μl of this solution was added per well and SLO was allowed to bind to cells on ice for 10 min. Cells were washed with 1 ml buffer A/EGTA and warmed to 37°C for 5 min, which allows SLO to oligomerize and form stable pores (Ahnert-Hilger et al., 1989). The plates were again placed on ice and washed with buffer A/EGTA. The above procedure has been shown to selectively permeabilize the plasma membrane (Ahnert-Hilger et al., 1989; Miller and Moore, 1991). The efficiency of permeabilization was tested by adding 10 μg/ml of the membrane-impermeant nucleophilic dye propidium iodide to permeabilized cells and to mock permeabilized cells (to which no SLO was added) and scoring for fluorescent nuclei. In agreement with previous observations (Miller and Moore, 1991), cell permeability assays showed that 100% of the permeabilized cells stained with propidium iodide whereas <3% of the control intact cells did. To demonstrate the diffusion of cytosolic components out of SLO permeabilized cells, CHO cells were grown in six-well plates, metabolically labeled with 500 μl of 300 μCi/ml [35S]cysteine for 2 h, and washed with PBS. Some cells were permeabilized as described above and after excess unbound SLO had been washed off on ice, 500 μl of buffer A/EGTA was added to cells, which were warmed to 37°C to initiate pore formation. 20-μl aliquots were removed at various times and added to an equal volume of electrophoresis buffer. Media from mock permeabilized cells (no SLO added) was also collected. All samples were boiled and analyzed by 3-10% gradient gel SDS-PAGE and fluorography.

**Metabolic Labeling and Cleavage Assay**

CHO cells stably transfected with wild-type rat proTGF-α (Bosenberg et al., 1992) were grown to 80% confluence in six-well plates. Cells were washed twice for a total of 40 min with cysteine-free MEM at 37°C before labeling with 500 μl of 300 μCi/ml [35S]cysteine (in cysteine-free MEM) for 10 min. Cells were chased for 40 min in MEM and placed on ice. Permeabilization was then carried out as described above, followed by three washes with 1 ml of Buffer A/EGTA (free Ca2+/EGTA) for a total of 1 h at 4°C. A Ca2+/EGTA buffering system (Tatham and Gomperts, 1990) was used in experiments requiring a particular concentration of free Ca2+. An ATP-regenerating system (40 IU/ml phosphocreatine kinase, 2 mM creatine phosphate, 500 μM ATP) was used in all experiments unless otherwise noted, although 500 μM ATP alone was found to be sufficient. In experiments requiring the absence of ATP, an ATP depleting system (50 IU/ml hexokinase, 1 mM glucose) was used. The cleavage assays were carried out by adding 500 μl of buffer A containing the proper concentration of free Ca2+ and ATP system to cells at 4°C. Other reagents were then added and cells were warmed to 37°C for 15 min. When protein kinase C inhibitors, GDPβS, or protease inhibitors were tested, these reagents were added to permeabilized cells for 10 min at 4°C before other agonists were added. For chronic treatment with PMA, cells were incubated for 24 h with 1 μM PMA before metabolic labeling. For treatment with pertussis toxin, cells were incubated 18 h with 0.1 ng/ml pertussis toxin before labeling. After a 15-min incubation with agonists at 37°C, cells were washed with PBS, lysed, and immunoprecipitated with a polyclonal antiserum against the COOH-terminal region of the proTGF-α cytoplasmic tail as previously described (Bosenberg et al., 1992; Teixido et al., 1987). Samples were analyzed by SDS-PAGE and fluorography. Samples from cells treated with pertussis toxin analyzed by SDS-PAGE were loaded at twice the normal concentration to compensate for reduction in the efficiency of metabolic labeling. Dried gels were quantitated on a PhosphorImager using ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA).

**Results**

**Activated proTGF-α Cleavage in Permeabilized Cells**

ProTGF-α is synthesized as an O- and N-linked glycoprotein (Bringman et al., 1987; Teixido et al., 1990). Proteolytic cleavage at the NH2-terminus of the mature factor is rapid after transport to the cell surface of CHO cells (τa < 15 min) and removes all glycosylated sites (Pandiella and Massagué, 1991a; Teixido et al., 1990). The resulting form of proTGF-α contains the mature growth factor, a short linker region, the transmembrane domain, and a 38 amino acid cytoplasmic tail. Under basal conditions, cleavage at the C-terminus of the mature factor is relatively slow (τa ~ 3 h) and much of the membrane-anchored form turns over without release of the soluble growth factor. Proteolytic cleavage and release of soluble TGF-α are rapid (τa < 5 min) when cells are treated with the protein kinase C activator PMA, Ca2+ ionophores, or serum (Pandiella and Massagué, 1991a,b). ProTGF-α is susceptible to cleavage after it has reached the cell surface and only if it contains the proper determinant at the COOH-terminus of its cytoplasmic tail (Bosenberg et al., 1992).

We sought to activate proTGF-α cleavage in permeabilized cells. CHO cells stably transfected with rat proTGF-α cDNA (Lee et al., 1985; Bosenberg et al., 1992) were permeabilized with SLO using conditions under which stable pores form rapidly and selectively in the plasma membrane of CHO cells and render the constitutive transport from the trans-Golgi network to the plasma membrane dependent on added cytosol (Miller and Moore, 1991). Cell permeability assays showed that 100% of the SLO-treated cells stained with propidium iodide whereas <3% of the control cells did.
Retention of activated proTGF-α cleavage in SLO-permeabilized CHO cells. (A) Diffusion of metabolically labeled cytosolic proteins out of SLO-CHO cells. CHO cells were metabolically labeled with [35S]cysteine for 2 h with or without 0.2 U/ml SLO at 37°C. Aliquots of media were removed from permeabilized and mock permeabilized cells (no SLO) at the indicated times after pore formation and were analyzed by SDS-PAGE and fluorography. (B) Samples of stably transfected CHO cells denoted SLO after chase were metabolically labeled with [35S]cysteine for 10 min and chased at 37°C for 40 min. Cells were cooled, permeabilized with 0.2 U/ml SLO and washed on ice for 1 h. Cleavage was assayed in a buffer containing an ATP regenerating system, an ATP depleting system, or a depleting system supplemented with the nonhydrolyzable ATP analogue App(NH)p. PMA induced proTGF-α cleavage only in permeabilized CHO cells, but only to a limited extent (data not shown). Collectively, these results indicated that the mechanism for activation of proTGF-α cleavage by PMA is retained in permeabilized CHO cells, acts on proTGF-α located on the cell surface, and is independent of cytosolic components and vesicular traffic.

Biochemical Requirements for Activated ProTGF-α Cleavage

To determine the biochemical requirements of activated proTGF-α cleavage and the effect of various agents on this process, CHO cells were metabolically labeled, chased for 40 min, permeabilized, washed, and exposed to the test agents of interest. Using these conditions, we tested the ability of PMA to activate cleavage in the presence of an ATP regenerating system, an ATP depleting system, or a depleting system supplemented with the nonhydrolyzable ATP analogue App(NH)p. PMA induced proTGF-α cleavage only in cells incubated with the ATP regenerating system (Fig. 2). Thus cleavage activated by PMA required the presence of hydrolyzable ATP.

ProTGF-α cleavage can also be activated by Ca2+-ionophores in intact cells (Pandiella and Massagué, 1991b). Permeabilized CHO cells were incubated in buffer containing ATP and free Ca2+ levels ranging from 1 nM to 10 μM. High concentrations of Ca2+ alone activate proTGF-α cleavage in permeabilized cells, but only to a limited extent (data not shown), suggesting that permeabilized cells lose components required for cleavage activated by Ca2+. Cleavage activated by PMA was nearly maximal at 1 nM Ca2+ and did not increase significantly with higher Ca2+ concentrations (data not shown).

Involvement of a G Protein Acting through a Ca2+-independent Protein Kinase C

One approach to probing the possible involvement of vesicular transport events in cellular processes involves the use of the nonhydrolyzable GTP analogue, GTPγS, which perturbs...
ATP hydrolysis is required for regulated pro-TGF-α cleavage. CHO cells stably transfected with rat pro-TGF-α were metabolically labeled with [35S]cysteine for 10 min and chased for 40 min at 37°C. Cells were cooled, permeabilized with SLO, and washed for 1 h at 4°C. Permeabilized cells were incubated in buffer containing an ATP regenerating system (+), an ATP depleting system (−), or with 1 mM App(NH)p and an ATP depleting system (NH), in the absence or presence of 1 μM PMA, 50 μM AlCl₃ and 20 mM NaF (AlF₄⁻), or 100 μM GTPγS for 15 min at 37°C. Cell lysates were immunoprecipitated with anti-proTGF-α antisera and analyzed with SDS-PAGE and fluorography.

The activity of GTP-binding proteins involved in many steps of vesicular transport and inhibits this process (Pfeffer, 1992; Miller and Moore, 1991). In initial experiments following this rationale, we observed that GTPγS addition did not inhibit pro-TGF-α cleavage in permeabilized cells but, instead, activated cleavage in a concentration-dependent manner (EC₅₀ ~40 μM) (Fig. 3, A and B).

In addition to providing further evidence against the involvement of known vesicular traffic events, this result raised the possibility that GTPγS might activate pro-TGF-α cleavage through one of the various heterotrimeric G proteins that are involved in signal transduction processes (Gilman, 1987). We investigated this further by testing the effect of various activators and inhibitors of heterotrimeric G proteins (Sternweis and Gilman, 1982; Gilman, 1987). AlF₄⁻ activated pro-TGF-α cleavage with EC₅₀ ~5 mM. The nonhydrolyzable GTP analog Gpp(NH)p activated cleavage only slightly, and GTP, GDP, or GDPβS were without effect. A 10-fold molar excess of GDPβS specifically blocked GTPγS-activated cleavage of pro-TGF-α (Fig. 4). These observations are consistent with cleavage of pro-TGF-α being activated through a heterotrimeric G protein.

Both GTPγS and AlF₄⁻ required hydrolyzable ATP for activation of pro-TGF-α cleavage (Fig. 2). In the presence of ATP, cleavage was nearly complete in response to either agonist at free [Ca²⁺] <1 nM and increasing the free [Ca²⁺] to 10 μM did not result in any further stimulation of cleavage (data not shown). These results suggest that activation of heterotrimeric G proteins stimulates pro-TGF-α cleavage in a Ca²⁺-independent manner.

Certain heterotrimeric G proteins are coupled to activation of protein kinase C (Nishizuka, 1992; Rhee and Choi, 1992). Prolonged stimulation of CHO cells with PMA, a treatment that down-regulates various members of the protein kinase C family (Nishizuka, 1988), prevents pro-TGF-α cleavage activated by further addition of PMA (Pandiella and Massagué, 1991a). Permeabilized CHO cells that were pretreated with 1 μM PMA for 18 h before permeabilization, failed to show activation of pro-TGF-α cleavage in response to PMA or GTPγS, although they cleaved pro-TGF-α in response to AlF₄⁻ (Fig. 5 A). Furthermore, the protein kinase inhibitors staurosporine and H7, which inhibit various protein kinase C family members (Hidaka et al., 1984; Kawai-moto and Hidaka, 1984; Nakadate et al., 1988) prevented activation of pro-TGF-α cleavage by PMA and GTPγS, but not by AlF₄⁻ (Fig. 5, A and B). This suggested that GTPγS acted upstream of a protein kinase C family member whereas AlF₄⁻ acted at least in part through a different mechanism.

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Various Activating Mechanisms Converge on a Common Cleavage System

To determine if the same proteolytic system was being activated by PMA, GTP$\gamma$S, and AIF$_4^-$, cleavage activated by these agents was tested in the presence of a panel of protease inhibitors that were previously characterized for their ability to interfere with proTGF-$\alpha$ cleavage in intact cells (Pandiella et al., 1992). Cleavage activated by PMA, GTP$\gamma$S, or AIF$_4^-$ in SLO permeabilized CHO cells was inhibited by 30 $\mu$M 3,4-dichloroisocoumarin (3,4-DCI) and by 5 mM diisopropylfluorophosphate (DFP), but not by 2 mM PMSF, or 1 mg/ml soybean trypsin inhibitor (data not shown), which is similar to the effect of these reagents on proTGF-$\alpha$ cleavage activated by PMA in intact CHO cells (Pandiella et al., 1992). Earlier work has shown that mutation of the COOH-terminal amino acid of proTGF-$\alpha$ from Val to Phe (V$^{199}$F) renders proTGF-$\alpha$ resistant to cleavage activated by PMA in intact CHO cells (Bosenberg et al., 1992). V$^{199}$F-proTGF-$\alpha$ was also resistant to cleavage in the presence of PMA, GTP$\gamma$S, or AIF$_4^-$ in permeabilized cells (data not shown).

Discussion

Regulated proteolytic cleavage of the ectodomains of transmembrane proteins occurs in a variety of proteins, and we have focused on proTGF-$\alpha$ as a model for studying this process. Earlier work has shown that cleavage of proTGF-$\alpha$ can be activated by PMA, Ca$^{2+}$-ionophoroes, or serum (Pandiella and Massagué, 1991a,b). Cleavage appears to be mediated by a serine protease (Pandiella et al., 1992), occurs outside of Golgi or lysosomal locations, and does not appear to require extensive membrane traffic (Bosenberg et al., 1992). Cleavage of proTGF-$\alpha$ is absolutely dependent on the presence of a cytoplasmic determinant containing the COOH-terminal valine (Bosenberg et al., 1992).

In this study, we have used a SLO-permeabilized cell system to further define the properties of the membrane protein ectodomain cleavage system. SLO has been used to form stable pores in many cell lines and the properties of SLO-permeabilized CHO cells have been described (Miller and Moore, 1991). The pores are selectively and rapidly formed in the plasma membrane, and allow essentially complete diffusion of ions, nucleotides, soluble cytosolic proteins $<150$ kD (Ahmert-Hilger et al., 1989; Bhakdi et al., 1985; Howell and Gomperts, 1987) and significant diffusion of cytosolic components as large as urease (483 kD) (Buckingham and Duncan, 1983). The diameter of pores formed with SLO is dependent on the cell line being permeabilized and the concentration of SLO used (Ahmert-Hilger et al., 1989; Buckingham and Duncan, 1983). Cytosolic components of at least 120 kD rapidly diffuse out of CHO cells under the conditions used for permeabilization in this study (Fig. 1 A). Constitutive secretion from the TGN to the plasma membrane in SLO-permeabilized cells is dependent on exogenously added cytosolic extracts (Miller and Moore, 1991), presumably due to loss of essential endogenous cytosolic factors.

Using this permeabilized cell system, we show that once proTGF-$\alpha$ has reached the cell surface, it is cleaved in response to various effectors. Activated proTGF-$\alpha$ cleavage does not require the addition of exogenous cytosol, is Ca$^{2+}$-independent, and is not inhibited by extraction with 2.5 M NaCl, treatment with 2 mM N-ethylmaleimide, GTP$\gamma$S or AIF$_4^-$. According to an extensive body of evidence (Melançon et al., 1987; Miller and Moore, 1991; Rothman and Orci, 1992), these observations collectively argue against the involvement of vesicular transport in the process of activated proTGF-$\alpha$ cleavage. Furthermore, proTGF-$\alpha$ retained in the ER, the Golgi apparatus, or TGN is not susceptible to activated cleavage (Bosenberg et al., 1992; Fig. 1 B). In light of this evidence, we conclude that proTGF-$\alpha$, the protease that cleaves its ectodomain, the components that bring them together, and those required for activation of the cleavage process, are all associated, directly or indirectly, with the plasma membrane.

Activation of cleavage by PMA is mediated by protein kinase C (Pandiella and Massagué, 1991a; and present results). The results with permeabilized cells indicate that a Ca$^{2+}$-independent member of this family can mediate activated cleavage. ProTGF-$\alpha$ cleavage in permeabilized cells is also stimulated by the G protein activator GTP$\gamma$S. Cell treatments that suppress protein kinase C activity prevent GTP$\gamma$S-activated cleavage as effectively as they prevent PMA-activated cleavage. However, GDP$\beta$S blocks cleavage stimulated by GTP$\gamma$S, but not cleavage stimulated by PMA. These results provide evidence that the Ca$^{2+}$-independent protein kinase C that signals proTGF-$\alpha$ cleavage acts downstream of a heterotrimeric G protein.

The identity of the G protein that signals proTGF-$\alpha$ cleavage, and the mechanism that mediates the effect of AIF$_4^-$...
are unknown at present. Addition of GTPyS to membranes activates heterotrimeric G proteins that contain Gaq (Rhee and Choi, 1992) which in turn can activate phospholipase C-β, resulting in diacylglycerol formation and protein kinase C activation (Nishizuka, 1992). However, the involvement of a phospholipase C-β in proTGF-α cleavage is unlikely because all the known forms of this enzyme are inactive in <1 mM Ca²⁺ (Rhee and Choi, 1992) whereas proTGF-α cleavage is efficiently activated by GTPyS at this Ca²⁺ concentration. Pretreatment of cells with pertussis toxin, an inhibitor of Gaαi proteins (Gilman, 1987), does not prevent proTGF-α cleavage (our own unpublished observation), and thus none of the agonists that activate cleavage act solely through Gaαi.

In contrast to the effect of GTPyS and PMA, activation of proTGF-α cleavage by AIF4- is not prevented by staurosporine, GDPβS or chronic PMA treatment, implying that AIF4- is acting at least in part through a mechanism distinct from that of GTPyS and PMA, or through the same mechanism but at a level downstream of protein kinase C. However, a mutant proTGF-α (V129F), which is resistant to cleavage activated by PMA (Bosenberg et al., 1992), is also resistant to cleavage activated by GTPyS or AIF4-. Additionally, the same protease inhibitors block cleavage activated by PMA, GTPyS or AIF4- in permeabilized cells. These results suggest that the mechanisms activated by these three agonists converge to either promote exposure of proTGF-α to the same protease or increase the catalytic activity of this protease. The ability to retain all the components required for signaling, recognition and cleavage of membrane proTGF-α in permeabilized cells provides a basis to further characterize the properties of these components and establish their identity.

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References


