J. Membrane Biol. 88, 205–215 (1985)

The Journal of
Membrane Biology

Topical Review

Transferrin Receptor: Its Biological Significance

W. Stratford May, Jr., and Pedro Cuatrecasas†
The Johns Hopkins Oncology Center, The Johns Hopkins School of Medicine, Baltimore, Maryland 21205, and
†The Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

Introduction

The transferrin receptor (TR) has been characterized biochemically (Sutherland et al., 1981) and the gene for the human TR has been localized to chromosome 3 (Enns et al., 1982; Goodfellow et al., 1982) and has been cloned and sequenced revealing important structural information about this transmembrane protein (McClelland, Kuhn & Ruddle, 1984; Schneider, Owen, Banville & Williams, 1984). The TR is a crucial surface membrane component which is present in high levels on most rapidly proliferating normal and transformed cells (Larrick & Cresswell, 1979; Shindleman, Ortmeyer & Sussman, 1981; Sutherland et al., 1981; Trowbridge & Omary, 1981) and drastically diminishes when cells are induced to terminally differentiate (Rovera et al., 1982; Yeh, Papamichael & Faulk, 1982; Pan, Blotstein & Johnstone, 1983). Thus, the TR is appreciated as a specific surface marker for rapidly growing cells and its expression is closely linked to the proliferation status of the cell. The TR performs the major function of binding and internalizing its specific iron-loaded ligand, transferrin (van Brockxmeer, Hammaplardh & Morgan, 1975; Seligman, Schleicher & Allen, 1979; Wada, Hass & Sussman, 1979; Karin & Mintz, 1981; Ciechanover, Schwartz, Dautry-Varsat & Lodish, 1983; Klausner et al., 1983*b*). It is the iron and possibly transferrin and/or the TR itself that is necessary for rapid cell proliferation to occur (Tormey, Imrie & Mueller, 1972; Hutchings & Sato, 1978; Barnes & Sato, 1980; Dillner-Centerlind, Hammarstram & Perlman, 1980; Trowbridge & Lopez, 1982). Much important information has been published recently with transferrin and the TR that elucidates the receptor mediated process by which receptor and ligand can be recycled (Dautry-Varsat, Ciechanover & Lodish, 1983; Yamashiro, Tycko, Fluss & Maxfield, 1984; Williham & Pastan, 1985) instead of being degraded as are most ligands which have been studied (Ashwell & Morell, 1974; Carpenter & Cohen, 1976; Goldstein & Brown, 1977; Ascoli & Puett, 1978; Tsai & Seeman, 1981; Bridges, Harford, Ashwell & Klausner, 1982). While the precise mechanism operating to trigger receptor internalization is not known, recent studies with the TR have suggested that ligand occupancy (Enns et al., 1983; Klausner et al., 1983*b*; Klausner, Harford & van Renswoude, 1984), possibly receptor phosphorylation (Klausner, Harford & van Renswoude, 1984; May, Jacobs & Cuatrecasas, 1984; Testa et al., 1984; Hebbert & Morgan, 1985; May et al., 1985) or spontaneous internalization (Watts, 1985) may play a role. These features concerning the TR will be explored here with an attempt to delineate certain controversial areas involving the biological significance of the TR.

Since iron metabolism (Aisen & Litowsky, 1980) and the TR (Testa, 1985) has been reviewed in detail recently, this work will primarily concentrate on the following areas: role of the TR in cellular iron delivery, the nature of the signal(s) necessary to induce receptor endocytosis, involvement of the TR in regulation of cell proliferation, and the possible clinical role for the TR in anti-tumor therapy. Other areas of TR characterization such as structure, biosynthesis, intracellular iron metabolism, and receptor turnover will be touched on only briefly.

Significance of Iron and Transferrin for Cell Growth

All living systems require iron for growth and survival (Aisen & Litowski, 1980). Indeed, it has been stated that "life in any form without iron is in all

Key Words transferrin · transferrin receptor · growth factors · cellular differentiation · receptor phosphorylation · receptor endocytosis · receptor cycling · iron

likelihood impossible" (Neilands, 1972). The basis for iron's importance is revealed by the understanding that iron is stable in both an oxidized ferric (Fe^{3+}) and reduced ferrous (Fe^{2+}) state and it is this property which allows iron to play an integral role in crucial biological reactions. Such key reactions include those involving electron transfer and energy transduction by oxidative cytochromes, activation of molecular oxygen, nitrogen and hydrogen, the generation of inactive derivatives from reactive superoxide and hydroxyl radicals with their deleterious effects, and complexing with oxygen in the form of specialized transport proteins like hemoglobin and myoglobin (Aisen & Litowski, 1980). However, since the hydrolytic properites of the trivalent iron ion are so great at physiological pH, free Fe^{3+} iron is only present at exceedingly low concentrations (Spiro & Saltman, 1969). Thus, in order to retain soluble iron for bioavailability, organisms have evolved complex iron-chelating molecules. In vertebrates, iron transport is mediated by the transferrin proteins (Feeney & Komatsu, 1964; Aisen & Litowski, 1980), while cellular iron is stored chiefly by ferritin, a protein iron sink (Fischback & Anderegg, 1965; Aisen & Litowski, 1980). The physiochemical and functional properties of these proteins have been reviewed in detail recently (Aisen & Litowksy, 1980). Thus, only a brief overview of the general properties of transferrin, the protein responsible for intracellular iron transport and which interacts specifically with the cell surface (transferrin) receptor, is indicated here.

Transferrins are of three major types named according to their primary location within the vertebrate host (Feeney & Komatsu, 1964). Serum transferrin is the prototype iron binding protein which mediates the transport of iron from sites of absorption by the intestinal epithelium and synthesis and storage to sites of utilization. It is this protein that will be discussed in some detail below. Lactoferrin is the distinctive iron binding protein found in milk, granulocytes and certain other body secretions (Metz-Boutigue et al., 1978). Ovotransferrin is synthesized and secreted in the oviduct of birds and is a major protein found in egg white (Thibodeau, Lee & Palmiter, 1978). The latter two transferrins may function in cell survival by restricting the availability of iron during microbial infection (Arnold, Cole & McGhee, 1977), by damaging phagocytosed bacteria (McCord & Day, 1978), and by regulating granulocyte differentiation (Broxmeyer et al., 1980).

Human serum transferrin is a glycoprotein synthesized primarily in the liver and consisting of a single polypeptide chain with a molecular weight between 77,000 and 80,000 daltons (Feeney & Komatsu, 1964; Williams, 1982). Each molecule of transferrin is folded into two globular domains, each containing a binding site for one trivalent atom of iron (Aisen, Leibman & Zweirer, 1978; Williams, Evans & Moreton, 1978). At physiologic pH the ferric iron complexes independently to either site with such high affinity (K_{eq} in the range of $1–6 \times 10^{22}$ M^{-1}) (Aisen & Leibman, 1978) that there are virtually no free iron ions remaining in serum. Such a mechanism is convenient for cells that can specifically bind the iron-loaded ferrotransferrin and accumulate iron. Delivery of iron to precise intracellular locations occurs following receptor-mediated endocytosis of bound ferrotransferrin and separation of the iron complexed with transferrin (Hemmaplardh & Morgan, 1977; Karin & Mintz, 1981; Octave, Schneider, Crichton & Trouet, 1981), and will be discussed in a later section.

Biochemical Characterization of Membrane Transferrin Receptors

Transferrin receptors (TR) are cell surface proteins, which can bind specifically and facilitate entry of ferrotransferrin (transferrin) into cells via a process known as receptor mediated endocytosis (RME). TR's have been identified and isolated from primary cell types, including reticulocytes, placental trophoblast and kidney cells (Hu & Aisen, 1978; Escarot-Charrier, Grey, Wilczynska & Schulman, 1980; Fernandez-Pol & Klos, 1980; Ward, Kushner & Kaplan, 1982*a*; Stein & Sussman, 1983) as well as from many transformed cells (Rovera et al., 1982; Schneider, Sutherland, Newman & Greaves, 1982; Ward, Kushner & Kaplan, 1982*a*,*b*; Stein & Sussman, 1983). The human TR isolated from various primary and transformed cell types appear to be identical (Schneider et al., 1982; Stein & Sussman, 1983), even when compared by peptide mapping (Stein & Sussman, 1983), in spite of any functional differences between cell types. TR's have been identified on most rapidly proliferating cells (Sutherland et al., 1981) and specialized nondividing cells like reticulocytes that have particular needs for high levels of iron. TR's are greatly diminished or absent on the surface of quiescent, nonproliferating and differentiated cells (Rovera et al., 1982; Yeh et al., 1982; Pan et al., 1983; Weiel & Hamilton, 1984). This finding suggests that the TR is a specific surface marker for rapid proliferation by both normal and malignant cells. The TR has been characterized biochemically (Enns & Sussman, 1981; Schneider et al., 1982) and also the gene for the human TR has been cloned recently and the primary structure determined (McClelland et al., 1984; Schneider et al., 1984). While the gene for both the human TR and

transferrin has been localized to chromosome 3 (Goodfellow et al., 1982; Enns et al., 1982), this coincidence appears to be of no particular functional significance.

The TR is a transmembrane, homodimeric, glycoprotein with a molecular weight of 180,000 daltons (Figure; Schneider et al., 1982). Each identical 90,000-dalton subunit is covalently attached through a single disulfide bridge (Trowbridge & Omary, 1981). The TR is synthesized in the endoplasmic reticulum, but post-translational modifications are made and the mature TR is actually a glyco-phosphoprotein with covalently attached fatty acids (Omary & Trowbridge, 1981; Schneider et al., 1982). The primary sequence contained in each functional monomer consists of about 760 amino acids (McClelland et al., 1984; Schneider et al., 1984). The major portion of the TR is a 70,000-dalton fragment that faces the extracellular environment and is sensitive to proteolysis by exogenously added trypsin (Figure; Schneider et al., 1981; Newman et al., 1983). This fragment contains the transferrin-ligand binding domain as determined by studies using cross-linking agents to covalently bind the radiolabeled transferrin ligand to the receptor (Enns & Sussman, 1981; Schneider et al., 1982). Following ligand binding and cross-linking, trypsin treatment of cells yields two molecules of labeled transferrin, which are recovered per molecule of receptor dimer, indicating that each monomer subunit binds a single transferrin molecule. Thus, two moles of transferrin can be internalized by each mole of receptor. Further, since two moles of iron can bind to each mole of transferrin, up to four moles of iron can be accumulated for each internalized receptor. This is an incredibly efficient system for delivery of intracellular iron to cells and accounts for the huge accumulation rates of iron by active hemoglobin synthesizing reticulocytes (i.e., $\sim 1 \times 10^6$ atoms per cell per minute, with each cell containing 3×10^5 receptors; van Bockxmeer & Morgan, 1979).

The transmembrane orientation of the TR as described above demonstrates that the majority of the polypeptide is facing the extracellular environment. This fact was substantiated when the gene for the human TR was cloned and sequenced (McClelland et al., 1984; Schneider et al., 1984). In addition, sequencing revealed some unsuspected properties for a membrane-spanning protein. For instance, the C-terminus region of the polypeptide was found to be facing the extracellular environment and the N-terminus domain, consisting of approximately 62 amino acids, represents the cytoplasmic tail of the receptor (Figure). In general, secreted and transmembrane proteins studied thus far have been

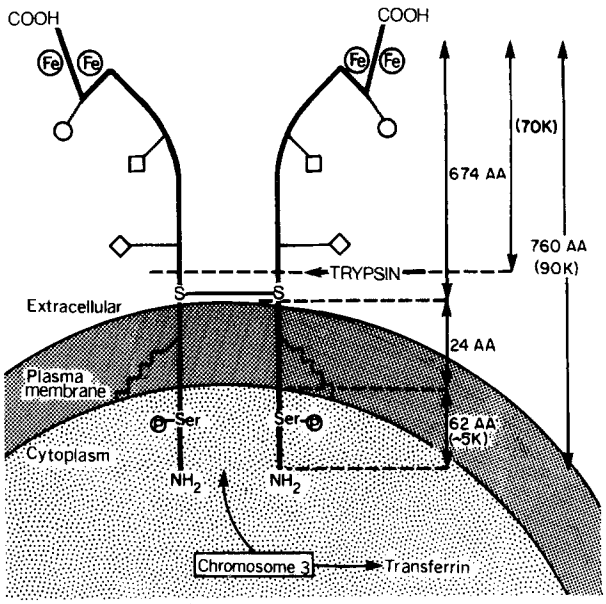

Fig. Hypothetical representation of the plasma membrane bound transferrin receptor in its homodimeric state. This cartoon is a modification after Newman et al., 1982. It represents a compilation of information published by Sutherland et al. (1981), Trowbridge and Omary (1981), Enns, Suomalainen et al. (1982), Goodfellow et al. (1982), Schneider et al. (1982, 1984), McClelland, Kuhn and Ruddle (1984). ◇, High mannose oligosaccharide chain; ○, complex oligosaccharide chain; ⁓ acylated fatty acid moiety; ( ), estimated mol wt in kilodaltons; *AA*, approximate number of amino acids

found to be synthesized with an N-terminus region facing the extracellular environment. This is believed to occur because the N-terminus end usually contains some 15–30 hydrophobic amino acids which are thought to be required for translocation of the nascent protein across the hydrophobic lipid membrane (Sabatini, Kreibich, Morimoto & Adesnik, 1982). Usually this so-called leader sequence is cleaved proteolytically once the proper transmembrane position is achieved and the mature N-terminus end revealed. From cloning and sequence analysis the TR does appear to contain a stop-transfer sequence located as predicted at the cytoplasmic border of the hydrophobic transmembrane spanning portion of the receptor, but the deduced sequence also indicates that a leader or signal peptide is missing from the N-terminus region (McClelland et al., 1984). Thus, the exact mechanism for membrane insertion of the newly synthesized TR cannot be predicted easily. While the C-terminus extracellular orientation of the TR is somewhat unsuspected for surface membrane receptor proteins it is not the only example. The hepatic asialoglycoprotein receptor is also oriented in the membrane in a similar manner to the TR with the C-

terminus facing the extracellular environment (Drickamer, Mamon, Binns & Leung, 1984). On the other hand, two other recently cloned surface receptors [i.e., for the epidermal growth factor (EGF) and low density lipoprotein (LDL)] both conform to orientation where the N-terminus faces the extracellular side and the C-terminus the cytoplasmic side of the plasma membrane (Russell et al., 1984; Ullrich et al., 1984). Thus, perhaps some of the post-translational modifications of the TR, which include fatty acid addition and phosphorylation (Schneider et al., 1982) may be involved in directing, inserting, and/or anchoring the transmembrane protein (Omary & Trowbridge, 1981; Schneider et al., 1982).

Role of the Transferrin Receptor in Endocytosis of Transferrin and Iron Delivery

The role of the TR in mediating rapid iron delivery to actively metabolizing dividing and specialized nondividing cells is well established (Wada et al., 1979; Karin & Mintz, 1981; Ciechanover et al., 1983; Klausner et al., 1983). Intracellular delivery of iron that is bound to transferrin is facilitated by the same general process, termed receptor-mediated endocytosis (RME), that mediates cellular entry of certain other soluble substances such as growth factors, hormones, plasma proteins, viruses, and bacterial toxins (Anderson, Brown & Goldstein, 1977; Pastan & Willingham, 1981; Dickson, Hanover, Willingham & Pastan, 1983; Helenius, Mellman, Wall & Hubbard, 1983).

By carrying specifically bound ligand during endocytosis, surface receptors are capable of providing cells with a continuous supply of nutrients and metabolites. The original description of RME was made following studies with the cholesterol nutrient carrying ligand, LDL. LDL was found to be internalized within intracellular vesicles (i.e., endosomes) while remaining bound to their specific receptors (Anderson, Goldstein & Brown, 1976; Anderson et al., 1977). It is now clear that most recycling receptors are internalized within specific microdomain invaginations of the cell surface membrane which are supported by clathrin, hence the term clathrin-coated pits. Clathrin-coated pits bud intracellularly and lose their associated clathrin to produce prelysosomal endosomes termed receptosomes, since they contain complexes of ligand and receptor. These endosomes ferry their cargo into the cell. The rate of formation of these endosomes may be so rapid that the surface of each cell may contain thousands of such coated-pits which can occupy an estimated 1–2% of the entire surface area (Pastan & Willingham, 1983). With this much of the cell's surface being used for coated-pit formation, the importance of the internalization mechanism to cell viability is easily understood.

Many different types of ligands enter cells by the RME process, indicating a common initial route of entry. Once internalized within endosomes, two well-defined destinations await ligands and their receptors. After formation internally, endosomes and their contents move by saltatory motion within the cytoplasm probably along microtubule tracts (Pastan & Willingham, 1981, 1983; Freed & Leibowitz, 1970; Herman & Albertini, 1980), until they fuse with trans-reticular golgi elements to form a vesicle compartment within which ligand and receptor can dissociate and become segregated for their separate fates (Willingham & Pastan, 1982). From this station, many separated ligands and their unoccupied receptors are destined to be delivered to lysosomes and be degraded (Geuze et al., 1983) and so are not returned to the cell surface with their unoccupied receptors (Tanabe, Pricer & Ashwell, 1979; Tolleshaugh & Berg, 1979; Steer & Ashwell, 1980; Kaplan, 1981; Ciechanover, Schwartz & Lodish, 1983). Notable among exceptions to the general finding that ligands and receptors may have a rather brief intracellular fate is transferrin and the TR. Transferrin has been demonstrated to be recycled by remaining bound with its receptor (Brown, Anderson & Goldstein, 1983; Ciechanover et al., 1983*a*; Harding, Heuser & Stahl, 1983; Hopkins, 1983*a*; Hopkins & Trowbridge, 1983; Klausner et al., 1983*b*; Lamb et al., 1983; Pastan & Willingham, 1983; Willingham, Hanover, Dickson & Pastan, 1984). Instead of becoming separated, transferrin, after dissociating from its bound iron, remains tightly coupled with its receptor within the endosome. The mechanism that is operating is most fascinating. Since the environment within the endosome is mildly acidic (i.e., pH 5–6.5; Tycko & Maxfield, 1982; van Renswoude, Bridges, Harford & Klausner, 1982), this generally favors dissociation of other receptor-ligand pairs. Following separation the individual components are segregated into specialized vesicular compartments in preparation for their different destinations (Hopkins, 1983*b*; Helenius et al., 1983). However, the acidic pH differential within the endosome does not promote separation of transferrin from its receptor (Ciechanover, Schwartz, Dautry-Varsat & Lodish, 1983*a*; Karin & Mintz, 1981; Klausner et al., 1983*a*). Instead, the iron complexed to transferrin is exquisitely sensitive to the acidic pH and rapidly dissociates (Princiotto & Zapolski, 1975; Lestas, 1976), leaving apotransferrin coupled with the TR. The two components are then returned together to the cell surface within a microtubule-associated en-

dosome (Willingham et al., 1984; Willingham & Pastan, 1985). Upon exposure to the relatively alkaline but physiological pH of the external milieu, the dissociation constant for apotransferrin is markedly increased in the absence of bound iron and dissociation from the TR is more rapid and leaves apotransferrin free in solution to re-bind with iron and any unoccupied TR.

Since many different surface receptors follow this route of endocytosis, these "migrant proteins" may be expected to share a common regulatory signal (Brown et al., 1983). It was thought that with cloning and sequencing of the genes for receptors that some common structural similarity might be revealed. However, the genes have been cloned and sequenced for four different surface receptor proteins [i.e., EGF (Ullrich et al., 1984), transferrin (McClelland et al., 1984; Schneider et al., 1984), LDL (Russell et al., 1984), and ascialoglycoprotein (Drickamer et al., 1984)] and there has been no common feature obvious to suggest the existence of such a signal at the level of the surface receptor.

Thus, while the mechanism of intracellular iron accumulation has been worked out as described above, the precise signal(s) necessary to induce endocytosis of the TR are not known. Is binding of transferrin a sufficient signal or even a necessary signal to stimulate receptor internalization? In fact, the fundamental question of what stimulus is operative to trigger RME of any ligand remains unanswered. Perhaps studies with transferrin and the TR can provide a clue.

On the question of whether transferrin binding is necessary to induce internalization of the TR, the available evidence does not provide a definitive answer. There is evidence to suggest that the TR in K562 human leukemic cells is mobilized to cycle only in the presence of transferrin and that the surface receptor is otherwise static in the absence of this ligand (Enns et al., 1983; Klausner et al., 1983*b*; Karin & Mintz, 1981). However, incubation of cells with phorbol ester tumor promoters, which are structurally unrelated to transferrin, can also induce internalization of the TR even in the absence of added ligand (Rovera et al., 1982; Klausner et al., 1984; May et al., 1984, 1985; Testa et al., 1984). The nature of this signal is not known precisely. It has been suggested that the internalization signal might result from phosphorylation of the TR (Klausner et al., 1984; May et al., 1984, 1985; Schulman, Wilczynska & Ponka, 1984; Testa et al., 1984; Hebbert & Morgan, 1985) since active phorbol esters are known to mediate their effects by stimulating the Ca^{2+}, phospholipid-dependent protein kinase, protein kinase C (Castagna et al., 1982; Niedel, Kuhn & Vandenbark, 1983). Indeed, there is evidence that these activators of protein kinase C can induce internalization of the TR in association with increased phosphorylation of the receptor and also that phorbol ester activated protein kinase C can directly mediate the phosphorylation of the TR in an in vitro system (May et al., 1984, 1985). Thus the TR appears to be a substrate for activated protein kinase C. From these data a model was proposed whereby phosphorylation of the TR itself was postulated to act as an initial trigger for receptor internalization. This concept can be tested experimentally. For such a phosphorylation mechanism to be generally operative during TR internalization, however, it would be expected that transferrin might also be able to induce receptor phosphorylation, particularly if ligand binding initiates endocytosis. Preliminary evidence reported in a recent abstract suggests that this might be the case (Schulman et al., 1984). Other investigators have been unable to demonstrate transferrin-sensitive phosphorylation of the TR (Johnstone, Mohammed, Turbide & Larrick, 1984; Johnstone, Adam & Pan, 1984; May et al., 1984). Other recent reports provide evidence that general inhibitors of protein kinases can also block endocytosis of transferrin and phosphorylation of the TR, findings which tend to support the concept that a phosphorylation mechanism might be operative (Hebbert & Morgan, 1985; Besterman et al., 1985). One further bit of evidence which supports a role for receptor phosphorylation is the recent finding that the immunopurified TR may possess intrinsic protein kinase activity (Johnstone et al., 1984). Unfortunately, under conditions employed, transferrin was not able to stimulate this activity. While it remains unresolved whether transferrin can induce stimulation of phosphorylation of the TR, even if this were the case, phosphorylation does not appear to be sufficient by itself to bring about receptor internalization (May et al., 1985). Using inhibitors of cytoskeleton assembly these agents were found to inhibit phorbol ester-induced internalization of the surface TR but did not simultaneously inhibit receptor phosphorylation, indicating that the internalization process is probably a complex one and may be preceded by receptor phosphorylation (May et al., 1985).

Alternatively, there is experimental evidence that supports the idea that the TR can cycle in mature macrophages (Hopkins, 1985*b*) and in K562 leukemic cells (Watts, 1985) and in reticulocytes (Sullivan, Grasso & Weintraub, 1976) independent of bound transferrin. In the first case, this finding may have more to do with the surface area requirements of the motile macrophage rather than specific needs for TR receptor internalization. However, for K562 transformed cells it has been shown that TR cycling can proceed without apparent ligand occupancy (Watts, 1985). These results suggest that any

function for ligand in receptor endocytosis and cycling may concern intracellular trafficking of membrane receptors to different destinations following internalization and play no fundamental role in triggering endocytosis (Watts, 1985). Other surface receptor molecules such as the LDL (Anderson et al., 1976, 1977; Anderson, Brown, Beisiegel & Goldstein, 1981; Basu, Goldstein, Anderson & Brown, 1981) and the ascialoglycoprotein receptors (Wall & Hubbard, 1981) have been found also which can apparently cycle independently of added ligand.

Thus, it is possible that more than one type of stimulus can induce surface receptor internalization. Whether receptor occupancy by ligand and/or biochemical modification (perhaps in the form of phosphorylation) or a combination of these or an as-yet-unidentified trigger mechanism is operating is not certain. Whatever the internalization mechanism for ligand and receptor may be, it does appear to be a complex one and may even vary according to the receptor-ligand system and the functional cell type studied.

Role of the Transferrin Receptor in Cellular Proliferation

While the TR serves a major function in delivering ferrotransferrin for intracellular iron sequestration, an alternate role in regulation of cellular growth has been suggested. Such a notion has developed from reports that demonstrate that transferrin may serve a role as a growth factor independent of its iron carrying properties (Tormey, Imrie & Mueller, 1972; Tormey & Mueller, 1972; Barnes & Sato, 1980; Dillner-Centerlind, Hammarstrom & Perlman, 1980). If so, this effect is likely mediated through specific interaction with the TR. Several findings indicate that transferrin and/or the TR serves such a role in growth regulation. First, transferrin has been demonstrated to be a necessary addition for cultured cells grown under serum-free conditions (Barnes & Sato, 1980). Second, transferrin or a molecule with transferrin-like activity has been found to be produced by both normal activated T-cells and transformed lymphoma cells which is necessary for cell growth (Imrie & Mueller, 1968; Nishiya, Chaio & deSousa, 1983). Third, transformed melanoma cells, but not normal cultured cells, express a surface glycoprotein antigen (p 97) which is homologous to transferrin (Brown et al., 1981; Plowman et al., 1983). This antigen is not reported to have retained iron. Fourth, a chicken B-cell lymphoma cell contains an activated oncogene (*ChBLYM*-1) which has sequence homology to transferrin (Goubain et al., 1983) and is capable of transforming NIH 3T3 fibroblast cells upon transfection (Cooper & Nieman, 1980; Cooper, 1982). Fifth, deprivation of cultured cells for transferrin or blocking the TR with a monoclonal antibody specific for the transferrin binding site can inhibit proliferation of mitogen-stimulated lymphocytes and arrest cell growth (Trowbridge & Lopez, 1982; Mendelsohn, Trowbridge & Castagnola, 1983; Neckers, 1984). The inhibitory effect can be overcome only partially by addition of soluble iron. Sixth, in addition to an inhibitory effect on cell proliferation, antibody blockade of the TR can inhibit erythroid differentiation induced by hemin in cultured K562 leukemic cells (Gambari, 1984). And seventh, lymphokine-induced surface expression of the TR is required for cellular proliferation (Neckers & Cossman, 1983). In this case the presence of the surface TR has been demonstrated to be linked stringently to cell proliferation and DNA synthesis following mitogen stimulation of resting T-lymphocytes. Mitogenic action is thought to be involved in induction of surface growth factor receptors (Morgan, Ruscetti & Gallo, 1976; Ruscetti, Morgan & Gallo, 1977; Lotze, Strausser & Rosenberg, 1980; Mier & Gallo, 1980). Specifically for T-cell activation, mitogen stimulation induces the expression of the T-cell growth factor receptor (i.e., IL-2 receptor) so that T-cell growth factor (IL-2) can interact and help to mediate cell proliferation (Robb, Munck & Smith, 1981). The permissive role for the TR in this activation process has been recently described (Hamilton, 1982; Neckers & Cossman, 1983). Kinetic studies have suggested that there is a sequential induction of expression of IL-2 receptors followed by TR's and that this sequence is absolutely necessary in order to initiate cell proliferation in quiescent T-lymphocytes (Hamilton, 1982; Neckers & Cossman, 1983). The relationship between any interaction of the lymphokine receptor and the TR is not clear, but monoclonal antibody blockade of expression of IL-2 receptors appears to prevent both TR expression and cellular proliferation, while treatment of cells with anti-TR antibody blocks only DNA synthesis without affecting IL-2 receptor expression (Neckers & Cossman, 1983; Weiel & Hamilton, 1984). These findings support the idea that the growth promoting effects assigned to certain lymphokines may be mediated, in part at least, through expression of the TR. Indeed constitutive expression of the surface TR may be a key finding in such a mechanism and represents the central concept in a recently proposed model for leukemonogenesis (Neckers, 1984). In this model transformed cells are postulated to have bypassed this sequential control system, normally operative for induction of cellular proliferation, by maintaining constitutive expres-

sion of the surface TR. This feature is thought to be responsible, in part at least, for the continued growth of such cells. Collectively these findings are compelling and suggest that transferrin or a transferrin-like activity may play a role in regulating cellular growth which may not depend entirely on its iron carrying properties.

If indeed transferrin and the TR does possess growth factor properties independent of its iron carrying function, it would be expected that such properties would result from the propagation and amplification of a signal(s) generated by specific binding and/or stimulation of the TR. How then might the interaction of transferrin or transferrin-like activity with the TR generate such growth signals? Three possible mechanisms for iron-independent transmembrane signalling can be readily conceived and have been suggested as mechanism(s) for the EGF growth factor receptor (Das & Fox,1979). First, transferrin could act directly on its receptor leading to internalization and subsequent metabolic processing of one or both components by some (lysosomal?) intracellular mechanism with generation of a "second messenger" (peptide?) which initiates cell growth and DNA synthesis. Second, transferrin could be internalized by its receptor and transferrin or a metabolic product might act directly as a "second messenger" to stimulate growth. Third, transferrin could act directly on its receptor to activate or convert it to a form which could stimulate production of a "second messenger," perhaps by stimulating an enzyme catalyzed process. Since transferrin and the TR are known to be recycled following RME, the first two possibilities do not seem likely. However, data exist which could give some credence to the third possibility listed. Recently investigators have uncovered evidence that the TR isolated from reticulocytes may possess intrinsic protein kinase activity (Johnstone et al., 1984). The role for any such enzymatic activity is unknown, but other growth factor receptors (e.g., EGI , insulin, somatomedin C and PDGF) have been found which possess intrinsic protein kinase activity (Cohen, Carpenter & King, 1980; Denton, Brownsey & Belsham, 1981; Cooper et al., 1982; Zick, Kasuga, Kahn & Roth, 1983). This activity is stimulated by specific ligand or antibody binding. The intrinsic enzymatic activity associated with these other receptors is similar in substrate specificity (i.e., tyrosine) to that activity reported for a number of oncogene products (Hunter & Sefton, 1980; Collett, Erikson & Erikson, 1980). The obvious presumed connection between growth factor receptors and these oncogene products, with respect to their intrinsic protein kinase properties, is that events necessary for stimulating cellular growth may be regulated, in part at least, by the interaction of growth factor and its receptor to activate the receptor-associated kinase. Thus cellular transformation resulting from oncogene expression may result from the unregulated activity of the oncogene protein kinase. Since the enzymatic activity reported to be associated with the TR has substrate specificity for serine (Johnstone et al., 1984) and not tyrosine, already one major difference between the TR and other growth factor receptors has been revealed. In addition, binding of transferrin could not be demonstrated in this same study to stimulate the TR-associated protein kinase activity found in the partially purified or immunoaffinity-purified receptor preparations. Thus, it is difficult then to understand what significance, if any, the TR-associated protein kinase activity may have since its regulation has not been determined. There is one recent report (in an abstract) which suggested that the addition of transferrin to intact reticulocytes, which had been rigorously depleted of bound transferrin, could induce phosphorylation of the TR (Schulman et al., 1984). Thus, perhaps intrinsic protein kinase activity can be regulated by ligand binding, but such regulation is inactivated somehow during isolation of the TR and so transferrin induced enzyme activation does not occur. Therefore, the possibility exists that the TR may possess intrinsic protein kinase activity and this exciting finding must be investigated further. Thus, one mechanism by which transferrin or a transferrin-like activity could possibly initiate growth-related transmembrane signalling, which could be unrelated to iron delivery, would be by activating a TR-related enzyme activity.

Role of Transferrin and the Transferrin Receptor in Iron Deficiency Disorders

The hematology literature is replete with examples of iron deficiency resulting in anemia. Since iron is required for heme synthesis the lack of iron from a dietary source, blood loss, liver insufficiency leading to decreased transferrin synthesis or acquired hypotransferrinemia can obviously precipitate this disorder. Two other rare conditions have been found that lead to decreased cellular iron uptake and anemia. One is congenital atransferrinemia, a deficiency in production of serum transferrin (Goya, Miyazaki, Kodata & Ushio, 1972). This disorder has been described in only a small number of cases, probably because absolute atransferrinemia is incompatible with life and such afflicted children may die in utero or shortly after birth. The resulting

anemia has been demonstrated to respond to infusions of transferrin (Goya et al., 1972).

The second rare cause of iron deficiency anemia involving the TR has been reported recently in a patient who produced IgM autoantibodies against the TR. These antibodies blocked the interaction of the TR with transferrin and prevented iron accumulation by the erythron (Larrick & Hyman, 1984). As is frequently the case with autoimmune disorders the cause of immunoglobulin production was not determined. However, in this instance, autoimmune antibody production and anemia responded to immunosuppressive therapy.

Role of the Transferrin Receptor as a Target for Anti-Tumor Therapy

Because the TR is expressed in high density on rapidly proliferating cells including malignant cells, two intriguing methods can be envisioned for specifically attacking such cells. The first is based in principle on the fascinating possibility that the surface TR may be an actual target for lymphocyte-like cells with so-called natural killer (NK) activity (Herberman & Holden, 1978; Herberman, 1980). NK cells are thought to exist as nonimmune surveillance cells which are capable of mediating rapid killing of unwanted malignant or diseased cells (Herberman, 1980). One way for NK cells to recognize these deleterious cells while they are proliferating would be by targeting the surface TR which is a specific marker for proliferation (Vodinelich et al., 1983; Alarcon & Fresno, 1985). This idea was conceived essentially because it was recognized that NK target cells had particularly high levels of surface TR (Vodinelich et al., 1983). Results of experiments done to test this hypothesis revealed a correlation between surface TR density and NK cell killing activity (Vodinelich et al., 1983). Further, preincubation of NK cells with the purified extracellular 70,000-dalton fragment of the TR was found to partially block NK cell recognition and killing of target cells (Vodinelich et al., 1983). Additional support for this concept came when it was discovered that addition of specific anti-transferrin antibody and complement to these effector cells prior to incubation with target cells could partially block the killing (Alarcon & Fresno, 1985). The reasoning for this was that the receptor on the NK cell which recognizes the TR on the target cell should have some similarity with the transferrin molecule and hence might be recognized by an anti-transferrin antibody.

Theoretically, at any rate, it should be possible to exploit therapeutically this notion that NK cells recognize their target cells by binding tot he TR on such cells. That is, by recognizing and eliminating cells with high density of surface TR, it might be possible to treat tumors. One situation in which to consider this sort of therapy would be in an attempt to remove occult tumor cells from harvested autologous bone marrow known to be contaminated with such tumor. This sort of clearing of contaminating cells from autologous bone marrow is thought to be necessary prior to reinfusion of the marrow into a patient undergoing therapy. Such a procedure would be performed in the case where a suitable allogeneic bone marrow donor is not available. Unfortunately, this type of situation where an allogeneic donor is not available is all too common and provisions must be made where harvested autologous marrow can be effectively treated so that reinfusion of tumor is avoided. Several forms of therapy are currently being used to try to rid infected autologous bone marrow of occult tumor. The methods employed include incubating the harvested marrow with specific anti-tumor monoclonal antibodies (Rizt & Schlossman, 1982; Bast et al., 1983) and direct incubation of the marrow with cytotoxic chemotherapeutic agents (Kaiser et al., 1985; Sharkis, Santos & Colvin, 1981). This sort of approach to such a removal process using NK cells also seems possible. However, certain potential problems must be recognized and include: nonspecific killing of nonmalignant hematopoietic progenitor cells containing high levels of surface TR, and addition of adequate numbers of NK cells to be of value. These problems would, of course, have to be dealt with before such an approach using NK cells could be expected to be successful.

The second method is based on the principle of selectively indentifying and delivering adquate cytotoxic therapy to malignant cells possessing high levels of TR. A role for the TR in such drug delivery seems possible in spite of the fact that the TR is not a tumor-specific antigen. At certain stages tumor cells are proliferating rapidly and express high levels of the TR (Sutherland et al., 1981; Trowbridge & Domingo, 1981). Since the TR can be specifically identified by both transferrin and many specific monoclonal anti-TR antibodies (Reinherz et al., 1980; Haynes et al., 1981; Trowbridge & Omary, 1981; Trowbridge & Lopez, 1982), if these agents are coupled with a specific toxin, preferential delivery of the toxin to cells with a high density of surface TR can be effected (Trowbridge & Domingo, 1981; Fitzgerald, Trowbridge, Pastan & Willingham, 1983). Coupling of such agents as the A chain of ricin (Raso & Basala, 1984) or diptheria fragment A toxin (Trowbridge & Domingo, 1981) to transferrin or anti-TR-antibody has been successful

and conjugates have retained their biological potency against tumor cells at low concentration *in vitro*. Coupling of other agents for S-phase (i.e., DNA synthesis phase) specific chemotherapy might also be considered since TR's are expressed maximally during the proliferation phase of the cell cycle (Chitambar, Massay & Seligman, 1983). While these delivery methods have been shown to be effective *in vitro* against cultured tumor cells, their *in vivo* efficacy in inhibiting the growth of implanted tumor melanoma cells in nude mice was no greater than anti-receptor antibody activity alone (Trowbridge & Domingo, 1981). While this may reflect some instability of these conjugates *in vivo*, it also suggests that unaltered anti-TR antibodies may have important therapeutic properties in inhibiting tumor expansion. Thus, this same technique or one employing unaltered antibodies could also be considered in conjunction with other methods used to eliminate occult tumor cells from harvested bone marrow as discussed above.

Conclusions

The TR is a vital surface component which has been demonstrated to be involved in processes critical for cell metabolism and growth. This review has attempted to briefly touch on the more well understood aspects of study of the TR. These aspects include the biochemical characterization of the TR and the functional studies concerning the central role of the TR in binding transferrin for the purpose of internalization and accumulation of intracellular iron. Other less well-understood and controversial aspects surrounding our present knowledge of the TR have been highlighted and discussed. These include: the nature of the biochemical signal involved in triggering receptor endocytosis; the role for the transferrin-TR interaction or the TR alone in regulation of cellular growth processes; and the possible clinical role(s) for the TR in anti-tumor therapy.

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Received 19 June 1985