to lane *e*. When these lanes are compared in isolation, the presence of the transferrin receptor in the immunoprecipitate appears to be *ras*-specific.

Transferrin-laden normal rat serum is clearly an inappropriate control for monoclonal antibodies collected by salt precipitation from ascites fluid or from culture fluid of hybridoma cells grown in medium with or without 10% fetal bovine serum[11]. Finkel and Cooper[12] also observed that transferrin added to the lysate eliminated the transferrin receptor band from anti-*ras* immune precipitates and proposed a transferrin-mediated conformational change in the receptor that disrupted the interaction with p21. They also suggested that a disruption of the p21-receptor complex by an anti-transferrin receptor monoclonal antibody was responsible for the fact that p21 was not co-precipitated by anti-receptor. The effect of transferrin and the asymmetrical co-precipitation are both explained more simply by the inadvertent affinity chromatography of the receptor demonstrated here. Loss of co-precipitation of the receptor on 'exhaustive precipitation' with anti-*ras*[12] may be due to the difference in the nature of the binding of the transferrin receptor to the resin in the absence and presence of anti-receptor antibodies. Only biologically active receptor can be bound by virtue of its affinity for the transferrin present on coated protein A-Sepharose. No such dependence on activity applies, however, in the binding of receptor by anti-receptor antibody. Thus, any loss of biological receptor activity during extraction or handling would result in transferrin receptor that could no longer become associated with coated protein A-Sepharose by affinity chromatography but which would still be precipitated with anti-receptor antibody. Partial inactivation of the receptor may also account for the high variability (5-50%) in the molar amounts of transferrin receptor apparently co-precipitated by anti-*ras*[12]. It is unsurprising that several anti-*ras* monoclonal antibodies apparently co-precipitated the transferrin receptor[12] because the association of the receptor with the coated proteinA-Sepharose is independent of the presence of the anti-*ras* (Fig. 1, lanes *b–d*). Using the methodologies of Finkel and Cooper[12] with normal rat serum as a control, addition of other monoclonal antibodies against any protein in the lysate would yield results suggesting that the transferrin receptor is co-precipitated specifically.

The results of Finkel and Cooper[12] have been reproduced here but additional controls support the lack of any molecular complex between p21 and the transferrin receptor. Although the 90,000 MW polypeptide observed[12] is the transferrin receptor, its presence in immunoprecipitates is demonstrated to be independent of anti-*ras*. I have shown here that it is due to inadvertent affinity chromatography resulting from the presence of transferrin on the coated protein A-Sepharose used in immunoprecipitation.

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FINKEL AND COOPER COMMENT—Since learning of the above results we have repeated our experiments with additional controls. We concur with Harford that our findings are explained by the artefact he describes and, therefore, do not indicate an interaction between transferrin receptor and *ras* proteins.

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Primary structure of human transferrin receptor deduced from the mRNA sequence

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In vertebrates all iron is taken up via the carrier protein transferrin[1]. The carrier first binds its receptor and the receptor–ligand complex is then internalized via coated pits[2–4]. The transferrin receptor is a transmembrane glycoprotein (apparent molecular weight (MW) 180,000) composed of two disulphide-bonded subunits (each of apparent MW 90,000)[1,5,6]. It contains three N-linked glycan units and is post-translationally modified with both phosphate and fatty acyl groups[5,7]. Here we have determined the nucleotide sequence of the coding region of the human transferrin receptor mRNA and from this deduced the amino acid sequence of the protein. The receptor does not contain an N-terminal signal peptide but there is a membrane-spanning segment 62 amino acids from the N-terminus. It therefore has a somewhat unusual configuration with a small N-terminal cytoplasmic domain and a C-terminal extracellular domain of 672 amino acids.

The clone pTR48 contains a 2-kilobase (kb) cDNA insert derived from the human transferrin mRNA[8]. Using this clone as a probe, a set of five overlapping cDNA clones was isolated containing, in total, 5 kb of sequence derived from the human transferrin receptor mRNA (Fig. 1). The mRNA is slightly over 5 kb long (ref. 9 and C.S., unpublished) thus this set of clones probably spans most of the length of the mRNA. We have used primer extension (Fig. 2) to show that the insert in clone pTR36 terminates only 20(±2) nucleotides from the 5′ end of the mRNA. The nucleotide sequence of the inserts in the set of cDNA clones was determined and is presented in Fig. 3. There is an open reading frame which extends from nucleotide 33 to nucleotide 2,544. The first available initiation codon in this reading frame is at position 264 and translation using this codon

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